Julie Sørbø Stang

Expired nitric oxide after high intensive exercise at altitude

Master thesis in Sport Sciences
Department of Sports Medicine
Norwegian School of Sport Sciences, 2010

Abstract

Nitric oxide has, as an exhaled biomarker, become an accepted tool in the management of inflammatory airway diseases, with its non-invasive, quick and easy measurement procedures. However, its use is still discussed and NO have currently only been assigned a complementary value. Other studies have reported reduced partial pressure of expired [NO] (PE_{NO}) at altitude (Hemmingsson and Linnarsson, 2009) and acute hypoxia is shown to down-regulate NO synthesis (Brown et al., 2006). Exercise alter values of expired NO concentration (FE_{NO}) (Shin et al., 2003). A large individual variance of FE_{NO} has also been reported in the literature (Olivieri et al., 2006). Different methods (i. e. flow rate) and measurement equipment may account for some of the variety of available research results and materials today. The purpose of this study was to examine changes in and the course of expired [NO] (FE_{NO} and PE_{NO}) after high intensive exercise, during exposure to reduced barometric pressure.

We measured FE_{NO} in 20 healthy subjects at reduced ambient pressure (543 mmHg, corresponding to 2800 meters above sea level [masl]) and in normobaric conditions (300 masl), while performing an 8 min high intensive (>90%HF_{peak}) exercise test on a treadmill. Measurements of FE_{NO} were obtained with a standard procedure defined by the American Thoracic Society and the European Respiratory Society (2005). FE_{NO} readings are reported as partial pressure of expired [NO] (PE_{NO}) to correct for gas density effects.

Individual differences were observed in sea level control values for PE_{NO} (range 0.46-2.41 mPa). Average PE_{NO} levels were reduced (p=<0.001) in both climates 5 minutes after exercise. In normobaric environment the decrease was -1% (±35) [mean ±sd] after warm up and -28% (±19) 5 minutes after completion of the exercise test. Hypobaric exposure resulted in a -21% (±19) and -31% (±22) reduction after warm up and exercise test, respectively. These alterations sustained until 5 min and 15 min after end of test in normobaric and hypobaric environment, respectively, when PE_{NO} returned to baseline levels.

A decreased PE_{NO} was shown after high intensive exercise. The severity of decline was similar in both barometric pressures, with the exception of a faster reduction at altitude (-21(±19)% after warm up). The inter-individual differences found coincide with earlier studies. There is still a need for more research on this subject in order to better understand the nature of expired NO, as well as a consensus on the use of terms and methods available.

Keywords

Expired nitric oxide, high intensive exercise, altitude, healthy humans.

Preface

I am grateful to the subjects for their participation in the study. The testing was physically challenging and time consuming. Thank you for your high spirits and excellent performance. You are all responsible for the completion of this thesis.

A special thanks to you, Trine, for being my counsellor and also my friend. Thank you for the good companionship, conversations, encouragements and smiles. I will miss your daily presence after this year.

Thank you, Vesla, for your never-ending good spirits and guts. Two heads think better than one! We have proven to be a good team. From now on, I am always ready for new challenges, as we can do everything, obviously.

Thank you Einar Thorsen. You are a living encyclopaedia with good wits. Thanks for your help on “all the hard stuff”.

Thanks to my peer master-students. Especially Olav; for taking me running in the forest, Ruth; for making me seem like the positive thinker, Håvard and Knut Eirik; just for being in the same boat, for friendship and good talks.

Thanks to my parents, for always believing in me and feeding me when I am poor. Thanks to my sister, Marie, for being my best friend.

Last, I would like to thank “my personal chauffeur”, Niels. Thank you for being you.

Abbreviations

		Unit
aNO	nitric oxide concentration in ambient air	ppb
AT	anaerobic threshold	-
EIA	exercise-induced asthma	-
EIB	exercise-induced bronchoconstriction	-
FE_{NO}	fractioned expired nitric oxide	ppb
PE_{NO}	partial pressure of expired nitric oxide	mPa
FEV_1	forced expiratory volume in one second	$l \cdot min^{-1}$
FEV_{pred}	predicted value of FEV_1	%
FVC	forced vital capacity	$l \cdot min^{-1}$
FVC_{pred}	predicted value of FVC	%
Hb	hemoglobin	-
HR_{peak}	highest recorded heart rate during pretest	$beats \cdot min^{-1}$
masl	meters above sea level	m
NOS	nitric oxide synthase	-
PO_2	partial pressure of oxygen	mPa/kPa
RER	respiratory exchange ratio	$VCO_2 \cdot VO_2^{-1}$
RET	repetitive exercise test	-
S_aO2	oxygen saturation in arterial blood	%
V_A/Q	ventilation-perfusion matching	-
VO_{2max}	maximal oxygen uptake recorded during pretest	$l \cdot kg^{-1} min^{-1}$
V_{Emax}	maximal oxygen uptake recorded during pretest	$l \cdot min^{-1}$

Table of Contents

1. Introduction

1.1 Background

Since NO first was discovered in expired air in humans and animals (Gustafsson, Leone, Persson, Wiklund and Moncada, 1991), the gas has been intensively investigated and the use of NO is today extensive. Still, more research is required as many fundamental mechanisms considering NO are still undescribed in the literature, in example site of NO production and stimuli of NO release. Information of healthy humans is particularly lacking, as most studies only include healthy subject as controls because of the role NO possesses as an inflammatory biomarker (Torre, Olivieri, Barnes and Kharitonov, 2008).

Measurements of expired NO concentrations (FE_{NO}) are highly affected by sampling procedure, especially considering expiratory flow rate (Silkoff et al., 1997). In 2005, the American Thoracic Society and the European Respiratory Society collaborated in developing standard measurement procedures of expired NO concentration (ATS/ERS, 2005). Before that, no consensus in measurement technique existed, which have resulted in misinterpretation and difficulties in comparing data reported in the literature. Recently, difficulties considering sampling at different barometric pressures have been reported (Hemmingsson and Linnarsson, 2009), resulting in a suggestion of expressing expired [NO] in partial pressure of NO (PE_{NO}) to account for discrepancies in gas density.

There are reasons to believe that NO plays a role in the physiological response of exercise in healthy humans (Sheel, Road and McKenzie, 1999). However, the effect and relevance of exercise upon NO is not yet completely understood. Strong indications imply that FE_{NO} is reduced after exercise (Chirpaz-Oddou et al., 1997; Maroun, Mehta, Turcotte, Cosio and Hussain, 1995; Persson, Wiklund and Gustafsson, 1993). Differences in exercise intensity, as well as exercise duration, have been shown to produce differences in FE_{NO} values (Verges et a., 2006).

Oxygen (O_2) is a substrate in NO synthesis, and is proposed to be of importance in production of NO (Dweik et al., 1998). FE_{NO} depends on the partial pressure of oxygen (PO_2) in inspired air (Schmetterer et al., 1997), and studies have shown reduced FE_{NO}

during exposure of both hyperbaric and hypobaric pressure (Kjelkenes and Thorsen, 2008; Gustafsson et al, 1991). The relationship between oxygen and expired [NO] is still uncertain, and there have not yet been developed a proper dose-response relationship between FE_{NO} and PO_2.

During exercise in altitude, pulmonary gas exchange is influenced by the severity of altitude and exercise intensity, ventilation-perfusion matching (V_A/Q) in particular (Gale, Torre-Bueno, Moon, Saltzman and Wagner, 1985). Reduced FE_{NO} is associated with increased pulmonary artery pressure during exposure to reduced barometric pressure at rest (Busch et al., 2001). NO has been proposed to be an important factor regulating hypoxia-induced pulmonary vasoconstriction (Blitzer, Lee and Creager, 1996).

In this thesis, expired nitric oxide concentrations will be variously addressed as both expired [NO], FE_{NO} or PE_{NO}, because of differences in measurement methods and units used in the literature.

1.2 Purpose

The purpose of this study is to contribute to the understanding of how physical exercise combined with exposure to reduced barometric pressure will affect NO concentrations in expired air in healthy humans. To our knowledge, few studies have assessed the effect from exercise during exposure to reduced barometric pressures upon expired [NO].

1.3 Hypothesis

1. Intensive physical exercise, in hypoxic conditions and/or at sea level, alter expired NO concentrations in healthy humans.
    a. Different barometric pressures affect the expiration of [NO].
    b. Altered FE_{NO} levels will sustain for several minutes post exercise and hypobaric exposure.
2. Exercise at sub-maximal and maximal intensities will affect FE_{NO} levels at sea level.

2. Theory

2.1 History of nitric oxide

Nitric oxide (NO) is a free radical gas detectable in the expired air of human subjects (Corradi et al. 1998). Originally, NO was considered an atmospheric pollutant produced by smog and cigarette smoke. In 1987, two research groups separately identified NO as endothelium derived relaxing factor (EDRF) (Palmer, Ferrige and Moncada, 1987; Ignarro, Buga and Wood, 1987). The importance of NO started to become apparent in the regulation of physiological functions and pathophysical processes.

2.2 NO synthesis

Production of NO occurs endogenously in different cells in both humans and animals. NO is produced in epithelial cells, airway nerves, inflammatory cells (macrophages, mast cells and neutrophils), and vascular endothelial cells (Barnes and Belvisi, 1993). Palmer and co-workers initially described the NO synthesis in 1988. Studies of expired [NO] show that [NO] accumulates exponentially to a plateau, a finding that indicates a balance between NO production and consumption in the lung (Dweik et al., 1998).

NO synthesis occurs by the conversion of the amino acid L-arginine into L-citrulline, a process requiring an enzyme called nitric oxide synthase (NOS) and oxygen (figure 2-1). The nitrogen atom is derived from the terminal guanidino nitrogen from L-arginine, by reduction of inorganic nitrate (Sheel et al., 1999). In addition, others cofactors such as nicotinamide adenine dinucleotide phosphate (NADPH), flavin adenine dinucleotide (FAD), flavin mononucletide (FMN) tetrahydrobiopterin (BH4) calcium (Ca^{2+}) and calmodulin are essential (Ricciardolo, 2003).

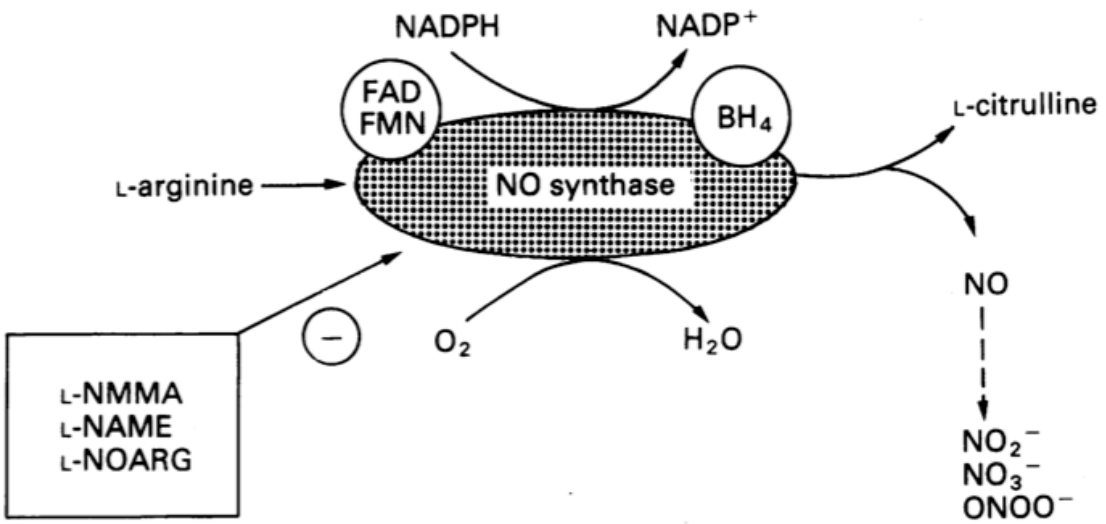


Figure 2-1 NO synthase: Conversion of L-arginine to L-citrulline[1].

2.2.1 NO synthase in the lungs

Three distinct enzyme isoforms of NOS are identified in human lung (Michel and Feron, 1997): (1) neural NOS (nNOS), (2) inducible NOS (iNOS), and (3) endothelial NOS (eNOS). NO synthase can be classified as either constitutive or inducible. The constitutive isoforms (nNOS and eNOS) are Ca^{2+} and calmodulin dependent. nNOS and eNOS release small amounts of NO for short periods of time in response to receptor or physical stimulation. These constitutive isoforms are the more active enzymes in healthy subjects, and can be instantly activated. eNOS acts as a signalling molecule in blood flow regulation, platelet reactivity, non-adrenergic non-cholinergic (NANC) neurotransmission, as well as memory. nNOS originates from neural tissue, but is widely distributed within various tissues. nNOS has been found to be of importance in skeletal muscle function, among others (Michel and Feron, 1997). The inducible form is independent of Ca^{2+}, requires BH4 and calmodulin, and generates NO in large amounts for long periods of time. Increased NO production induced by iNOS occurs hours after stimulation, and is activated through gene transcription and after exposure to endotoxin and to certain cytokines (interferon γ, interleukin β, tumor necrosis factor α) (Dweik et al., 1998). iNOS acts as a cytotoxic and cytostatic defensive mechanism against tumors and pathogens, and are present in macrophages, fibroblast, smooth muscle cells, endothelial cells and neutrophils (Moncada and Higgs, 1993; Barnes and Belvisi, 1993).

[1] From Barnes and Belvisi (1993).

2.2.2 NOS inhibitors

To assess inhibitors of NOS is useful in research with the purpose of determining the roles of NO in various processes. The NOS inhibitor NG-monomethyl L-arginine (L-NMMA) is used in several studies (Sartori et al., 1999; Yates, Kharitonov, Thomas and Barnes, 1996; Vaughn, Huang, Kuo and Liao, 2000), especially in experiments studying the effects of inhalation and excretion of L-NMMA in order to assess the site of NO production.

2.2.3 Site of production

The exact source of expired NO has not yet been completely identified. Research have indicated that NO is produced at different locations within the airways (Barnes and Belvisi, 1993). The precise site of production has proven to be difficult to identify. Expired NO may originate from the upper respiratory tract, in particular the nasal epithelium, the lower airways and terminal bronchioles, the alveolar capillaries and the endothelium of pulmonary arteries. Dweik et al. (1998) measured NO synthesis in the static lung, through bronchiolar gases in an expiratory breath-hold in healthy subjects, and suggest that NO in expired air originates from within the respiratory tract. Persson et al. (1993) compared excretion of carbon dioxide (CO_2) and NO after a breath-hold and suggest that the major site of formation is in, or close to, the small airways epithelium. Byrnes, Dinarevic, Busst, Bush and Shinebourne (1997) concluded that NO is not produced in alveolar levels like CO_2, because of differences between NO and CO_2 in time to peak levels during expiration. However, Sartori et al. (1999) rapports that the majority of NO is released by the surface epithelium of lower airways and alveoli, and that less than 10% of NO excretion from the lower respiratory tract arises from the vascular endothelium. Yates et al. (1996) and Vaughn et al. (2000) found that expired NO from the lower respiratory tract is, by large part, synthesized by eNOS found in both airways and vessels, whose activity is influenced by intracellular [Ca^{2+}].

Schedin et al. (1995) showed that the upper airways, in particular nasal NO, makes a stronger contribution to FE_{NO} compared to lower airways and lungs. The authors suggest that the lower airways account for 10% of expired NO produced in the airways and lungs, while the upper airways are responsible for 90%. The proposal of nasal NO being the major contributor to expired NO at rest has been confirmed by other authors (Alving et al., 1993). However, studies have suggested that the NO contribution from

the lower airways is approximately 50% (Sheel 1999). Separating pulmonary air from air originated in the nasopharynx using balloon occlusion, Phillips, Giraud and Holden (1996) proposed that half of the NO in mixed expired air originate from a lower airway source.

It is likely that the NO detected in expired air is formed within the respiratory system and that the site of production is close to the pulmonary air space, given the rapid fixation of NO to hemoglobin (Chirpaz-Oddou et al., 1997). Part of the NO produced in the lung may diffuse across the alveolocapillary membrane to bind hemoglobin. Hyde et al. (1997) estimated that 94% of the NO produced in the lower airways diffuses into blood. The remaining 6% will diffuse into airway lumen to be expired.

2.3 Stimuli for NO release

The activity of NO release depends of many local factors, such as the amount and activity of the NOS enzymes, oxidant stress and the rate of NO uptake by antioxidant molecules such as hemoglobin and glutathione (Ricciardolo, 2003). Physical stimuli on the vasculature have been proposed as probable stimuli for NO release (Persson, Gustafsson, Wiklund, Moncada and Hedquist, 1990). Deformation of endothelial cells through frictional forces caused by blood flow on the blood vessel wall is proposed to activate a NO-cGMP signal transduction system (figure 2-2) (Barnes and Belvisi, 1993). However, if a similar reaction in the pulmonary vasculature is reflected in expired NO concentrations (FE_{NO}) is debatable, as shear stress-responded vasodilatation of the pulmonary vessels is not as understood as in the peripheral vasculature (Sheel et al., 1999). The NO released by iNOS is essentially generated in inflammatory cells, or in other cells such as endothelial and smooth muscle cells, in response to endotoxin and some cytokines (Michel and Feron, 1997).

2.4 NO function

NO is the only gaseous signalling molecule and it can diffuse freely across membranes (Gaston et al., 1994). The NO molecule possesses a small dipole because of the equal electonegativity of oxygen and nitrogen atoms, making it essentially hydrophobic. This eliminates the need for extracellular receptors. Nitric oxide is highly reactive, having a lifetime of a few seconds and is possibly involved in important biological roles within the cell, as well as in interaction with nearby cells and molecules (Michel and Feron,

1997). NO strongly interacts with molecular oxygen, to form nitrite (NO_2^-), nitrate (NO_3^-) in the presence of hemoglobin (Hb), or with superoxide (O_2^-) to form peroxynitrite ($ONOO^-$) (Ricciardolo, 2003). NO has also been shown to react with thiol to form S-nitrosothiols that may act as a NO stabilizer or carrier (Ignarro, Ross and Tillisch, 1991). It is believed that NO contributes to the regulation of functions within the circulatory, pulmonary, nervous and immune systems (Sheel et al. 1999).

Nitric oxide contributes to the regulation of blood flow and oxygen delivery as a vasodilator (Brown, Beall, Strohl and Mills, 2006). Reduced NO values have been observed in patients with heart disease, who experience an enhanced vasoconstriction of pulmonary blood vessels (Sumino et al., 1998). Inspirations of large concentration of NO has been shown to dilute bronchioles (De Gouw et al., 2001; Barnes and Belvisi, 1993) Thus, NO may be involved in control of bronchial tone, in example to reduce airway resistance at high levels of ventilation during exercise. NO production causes relaxation of smooth muscle tissue, possibly through a signal transduction system (figure 2-2). NO enters pulmonary smooth muscle cells through diffusion and activates soluble guanylate cyclase (CG) to produce cGMP (Güzel, Sayan and Erbas, 2000; Sheel et al., 1999). Guanylate cyclase (GC) is an enzyme that stimulates the conversion of 3′,5′-cyclic guanosine triphosphate (GTP) to 3′,5′-cyclic guanosine monophosphate (cGMP), resulting in decreased concentration of free Ca^{2+} in cytocol.

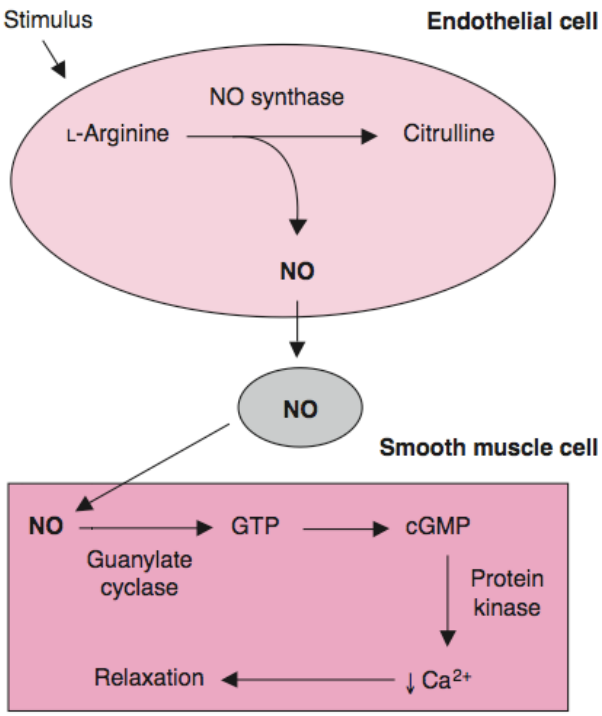


Figure 2-2 *NO synthesis in endothelial cells and mechanisms in smooth muscle cells*[2].

As NO is though to be an important vasodilator and is continuously released in the systemic circulation (Güzel et al. 2000), it has been proposed that NO produced in the lungs and/or airways is involved in regulation of pulmonary gas exchanges, in particular ventilation-perfusion matching (V_A/Q) (Phillips et al., 1996). Increased NO production can enhance perfusion of well-ventilated lung areas through vasodilation, and improve the ventilation-perfusion distribution and enhance pulmonary O_2 exchange during exercise. Inspired NO has shown to improve V_A/Q relationships through inducing pulmonary vasodilation (Persson et al., 1990). In response to physical factors such as shear stress, increased NO production may decrease pulmonary vascular resistance and participate to the recruitment of pulmonary capillaries during exercise. Blitzer and co-workers (1996) found increased systemic and pulmonary vascular resistance after insertion of L-NMMA in healthy subjects. Vasoconstriction of the pulmonary vasculature is a hypoxic-induced mechanism that occurs in order to minimize perfusion to poorly oxygenated parts of the lung and thus improving V_A/Q (Blitzer et al., 1996). Considering NO being an important factor in maintaining the low pressure in the

[2] From Sheel et al. (1999).

pulmonary circulation, NO is believed to have a significant role in the regulation of pulmonary function and pulmonary diseases (Barnes & Belvisi, 1993).

NO activated by cytokines (through iNOS) may play a role in the pathophysiology of pulmonary diseases; halting viral infections, eliminating various pathogens independent of guanylate cyclase and cGMP, and protecting the lungs from toxic effect from inhaled pollution and allergens (Ricciardolo, 2003; Michel and Feron, 1997).

2.5 Expired NO

The publication of the article by Gustafsson et al. (1991) identifying the presence of NO in expired air marks the beginning of the interest in NO, followed by publications reporting high FE_{NO} in patients with pulmonary diseases compared to healthy controls (Kharitonov and Barnes, 2006). Expired [NO] are shown to be elevated in patients with asthma (Kharitonov et al., 1994), bronchiectasis and upper respiratory tract inflammation (Bonsignore et al., 2001). Reduced FE_{NO} are found in patients with cystic fibrosis (Stark, Purokivi, Kiviranta, Randell and Tukiainen, 2007), smokers (Kharitonov et al., 1994) and hypertensive subjects (Sumino et al. 1998) compared to healthy subjects (table 1). Alving, Weitzberg and Lundberg (1993) were the first to report an increased production of NO in asthmatics compared to healthy subjects. Since then, FE_{NO} has been extensively investigated in studies on asthma. Kharitonov and Barnes (2006) observed increased FE_{NO} during spontaneous or induced asthma exacerbations and decreases after anti-inflammatory treatment in asthmatics. FE_{NO} has proved to correlate with predominantly eosinophilic airway inflammation, and to be reduced by corticosteroid therapy (Kharitonov and Barnes, 2006). Elevated FE_{NO} values in patients with asthma can be caused by an increased activity of iNOS that may be activated by inflammatory cytokines (Kharitonov and Barnes, 2006; Olivieri et al. 2006). As iNOS produces NO in large quantities for long periods of time, NO in the lungs have shown to correlate with predominantly eosinophilic airway inflammation (Ricciardolo, 2003). In healthy subjects, eNOS is known to be the more dominant isoform in the airway walls, rather than iNOS (Kharitonov et al., 2000).

Table 2-1 *Physiological, pathophysiological conditions and habits affecting expired nitric oxide measurements*[3].

	Increased NO	Decreased NO
Habits		
	Allergen and/or pollen exposure	Menstruation
	Air pollution	Smoking
		Acute alcohol ingestion
	Occupational exposure (ozone)	Mouth washing
	Arginine ingestion, nitrite/nitrite enriched food	
Conditions		
	Asthma	Nonasthmatic chronic cough
		Pulmonary hypertension
	Unstable/severe COPD	Kartagener`s syndrome
	Allergic rhinitis	Primary cilia diskynesia
	URTI	Cystic fibrosis
	Influenza vaccination	
	LPS administration	
	Bronchiectasis	
	Ulcerative colitis	
	Tuberculosis	
	Lung cancer	
	Active pulmonary sarcoidosis	

COPD: chronic obstructive pulmonary disease. UTRI: upper respiratory tract infection. LPS: lipopolysaccharide.

Chemical products of NOS in the lung vary with disease. Involvement in pulmonary neurotransmission, host defence and relaxation in airway and vascular smooth muscle have linked NO to inflammatory/pulmonary disease. Therefore, NO measurements of expired air have been proposed as a method of monitoring airway diseases (Corradi et al. 1998). However, it is important to consider the extent of which FE_{NO} reflect inflammatory processes that occurs in the lungs and airways (Sheel. 1999).

2.5.1 NO as an inflammatory biomarker

A biomarker is defined as ”an objective measure and evaluated indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention[4]”. Assessment of inflammation in the lung and airways is important in order to understand different mechanisms underlying inflammatory diseases (Shin, Rose-Gottron, Cooper, Hill and George, 2003). Measurements of biomarkers in the

[3] From Kharitonov and Barnes (2000)
[4] From Shin et al. (2003)

breath are non-invasive and can provide an assessment of the severity of the disease, contribute to early recognition of the disease, as well as it can be used for monitoring patients response to treatment (Shin et al. 2003; Kharitonov and Barnes, 2006). The evidence that breath analysis may have an important role in clinical management of asthma and COPD is increasing (Kharitonov and Barnes, 2006). As an expired biomarker, NO has today become an accepted tool in the management of inflammatory airway diseases (Taylor, Pijnenburg, Smith and De Jongste, 2006; Hemmingsson & Linnarsson, 2009), with its non-invasive, quick, easy and reproducible measurement procedures. However, its use is still discussed and it have currently only been assigned a complementary value.

2.5.2 Measurement procedures

Because NO is a gaseous molecule produced in the airways it is possible to measure the fractional expired nitric oxide (FE_{NO}) in human air non-invasive. The most common method is known as chemiluminescence (Palmer et al., 1987). The advantages considering the chemiluminescence method is that it provides few technical challenges, along with the possibility to perform repetitive measurements within short periods of time. In addition, the measure is sensitive enough to detect small amounts of gas present in expired air (down to 1 part per billion [ppb]). Chemiluminescence can easily be used in assessment of NO in children and patients with severe airway obstruction, because the procedure is easy and physically non-demanding.

Measurements of FE_{NO} assessed by chemiluminescence are accomplished by conversion of NO to nitrite (NO_2) in the presence of an excess of ozone (Busch et al., 2001). The chemiluminescence measurement occurs through a photochemical reaction between NO and ozone generated in the analyzer, resulting in the conversion of NO to NO_2. The intensity of this chemical reaction, related to the production of infrared radiation, is used as a indicator of NO concentration: When NO_2 return to its basal level it emits a photon, whereas the total number of photons produced is proportional to the NO concentration in the expired air (Borland, Cox & Higenbottam, 1993; Busch et al., 2001). FE_{NO} assessed with chemiluminescence is performed using a single-breath technique, where the sample can be managed either online or offline. Measures conducted online consist of a single flow-controlled exhalation against a given resistance, connected to the analyzer. Results are presented graphically on a monitor connected to a computer.

Offline measurement is conducted using the same breath technique, only with a hand-held kit, which collects expired air into a reservoir (i. e. a Mylar bag) for later analysis of the content.

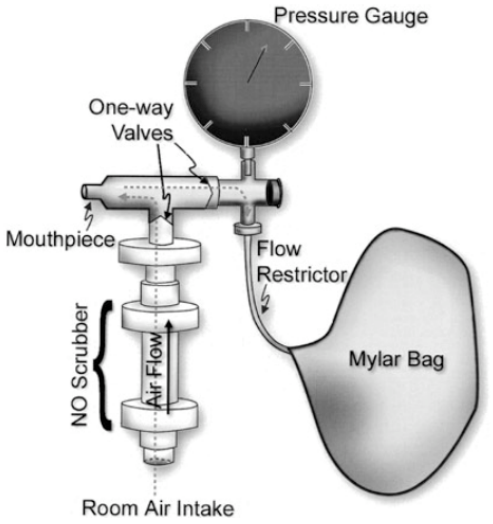


Figure 2-3 *Apparatus for the collection of expired gas for offline NO measurements. Inspired air is scrubbed for NO and expiratory flow rate and pressure are controlled by means of an in-line pressure gauge and flow resistor*[5].

FE_{NO} measured at a controlled expiratory flow, reflects the balance between its local production within the airways and its continuous transformation and/or elimination by endogenous pathways or by ventilation (Hyde et al., 1997). An important consideration associated with measurements of NO in expired air is the fact that FE_{NO} is highly dependent on and inversely related to the expiration flow rate (Silkoff et al., 1997). This is because NO exchange occurs in both alveolar and airway compartments (Silkoff et al., 1997), which separates the exchange dynamics of NO from other respiratory gases (O_2 and CO_2) whose exchange occurs predominantly in the alveolar region (ATS/ERS, 2005). The relationships between FE_{NO} and expired flow rate are presented graphically in figure 2-4.

[5] From ATS/ERS, 2005.

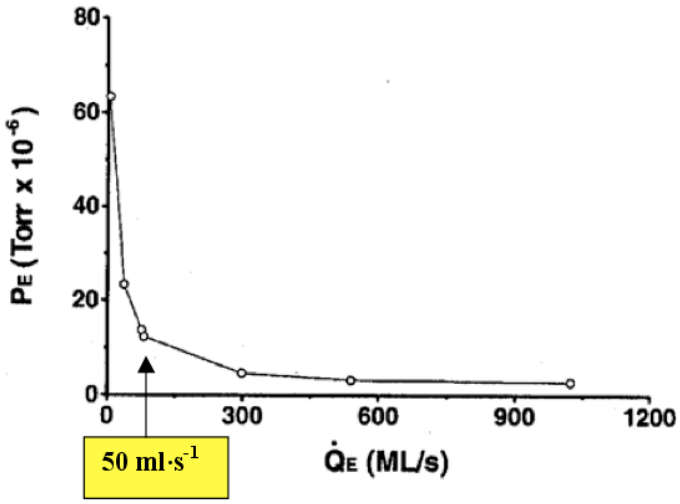


Figure 2-4 *Relationship between expired flow rate (x-axis) and expired [NO] (y-axis)*[6].

Studies using various flow-rates in measurements of NO have confounded interpretation of NO in several of settings (Shin et al. 2003), resulting in standard recommendations to determine expired NO defined by the American Thoracic Society and the European Respiratory Society (ATS/ERS). The most updated FE_{NO} standard procedure to determine expired NO is from 2005. The technique involves clear instructions of inspirations of NO-free air to total lung capacity and standard expiration flow rates of 50 $ml \cdot s^{-1}$ for 10 seconds against a counter pressure of 5-10 cm H_2O. Increased airway pressure during expiration ensures closure of the soft palate and excludes nasal air from the sample (Kharitonov, Alving and Barnes, 1997). Prolonged constant flow induces a plateau in NO concentration (Jörres, 2000). The recommended exhalation flow of 50 $ml \cdot s^{-1}$ is based on the assumption that this flow results from the region defined as "of most interest for NO excretion[7]", at the lower non-cartilaginous portion of the airways. However, more peripheral airway sections close to and within the alveolar space are excluded using this flow rate (Silkoff et al., 1998). Additionally, according to figure 2-4, it seems that a flow rate of 50 $ml \cdot s^{-1}$ is the rate that is most sensible to changes on FE_{NO} values.

[6] From Thorsen, E. (2010)
[7] ATS/ERS (2005)

2.6 NO and exercise

The exact effects on, and relevance of, NO and exercise are not completely understood, but NO is believed to have a profound effect on the physiological response to exercise (Sheel et al., 1999). Alterations in FE_{NO} during exercise have been reported by multiple studies. Most studies report of decreased expired [NO] after exercise (Verges, Flore, Favre-Juvin, Lévy and Wuyam, 2005; Persson et al., 1993; Maroun et al., 1995). Studies showing increased FE_{NO} (Bauer, Wald, Doran and Soda, 1994; Bonsignore et al., 2001) or no change in expired [NO] levels (Iwamoto, Pendergast, Suzuki and Krasney, 1994; Maroun et al., 1995) have also been published. There exist a great variability of response from exercise between subjects (Iwamoto et al., 1994; Maroun et al., 1995). De Gouw et al. (2001) found no effect in expired [NO] after treatment of a NOS inhibitor (L-NMMA) or a NOS substrate (L-arginine) on the airway response to exercise in healthy controls. The authors conclude that their results indicate that NO does not play a role in the airway response to exercise in healthy subjects. Shin et al. (2003) found decreased [NO] within the bronchial wall, but unchanged FE_{NO}, 3 minutes after intense exercise for 20 minutes. Additionally, no change FE_{NO} after 3-min exercise at 30%, 60% and 90% of VO_{2max} has also been reported (St. Croix, Wetter, Pegelow, Meyer and Dempsey, 1999). St. Croix et al. (1999) reports of a small, but significant, increase in expired NO concentration immediately after high intensive exercise (60% and 90% VO_{2max}), measured at a constant flow rate (46 $ml \cdot s^{-1}$). However, these measures of NO were conducted on the first breath after exercise.

It is today established that the amount of expired NO is altered during exercise (Sheel et al. 1999). Most research report of reduced expired [NO] after exercise, and that FE_{NO} decreases as workload increases (Phillips et al., 1996). The fundamental mechanisms causing this reduction are not yet understood.

2.6.1 Production rate of NO

Production rate of NO (NO output, V_{NO}) has been extensive investigated in the literature. V_{NO} is calculated by multiplying VE ($l \cdot min^{-1}$) with expired [NO] (ppb) and is often expressed in $nmol \cdot l^{-1}$ (Kippelen, Caillaud, Robert, Masmoudi and Préfaut, 2002; Phillips et al.,1996; Verges et al., 2006). A wide selection of studies have found reduced levels of FE_{NO} and increased V_{NO} during exercise (Bauer et al., 1994; Chirpaz-Oddou et al., 1997; Iwamoto et al., 1994; Phillips et al., 1996).

Hyde et al. (1997) have predicted, using mathematical models, that if NO production in the airways remains constant during exercise, the NO concentration in the airway lumen will decrease because of large increases in expiratory flow rates. This will result in a reduced concentration gradient for NO between the alveolar space and the pulmonary capillary blood, which will result in a decrease in the fraction of NO taken up by the blood and an increase in the volume of NO recovered in the expired air. Hyde et al. (1997) proposes that it may not be the exercise that increase NO release by the airway epithelial cells, but may merely shift the rate of elimination from the alveolar capillary blood to the expired gas, owing largely to increases in alveolar expiration. The close relationship observed between V_{NO} and variables related to the magnitude of metabolism suggests that the origin of expired NO is linked to structures dependent on functions that become involved progressively as metabolisms increases, such as VO_2, VE and HR. The literature is divided in the aim to understand the factors that influences V_{NO} during exercise, between two main hypotheses: increased VE (Iwamoto et al., 1994; Phillips et al., 1996; St. Croix et al., 1999; Kippelen et al., 2002) and increased cardiac output (Bauer et al., 1994; Chirpaz-Oddou et al., 1997; Maroun et al., 1995). These theories will be further discussed in chapter 5.9.2.

2.7 NO and altitude

When exposed to hypobaric hypoxia a number of adaptive responses in the cardiopulmonary system immediately initialize to maintain adequate oxygen delivery in the body (i. e. oxygen saturation, increased heart rate and ventilation) (Gale et al., 1985). I has been hypothesized that pulmonary NO may play an important role in the physiological response to acute hypobaric hypoxia (Brown et al., 2006). Studies have shown reduced expired [NO] at altitude (Hemmingsson & Linnarsson, 2009; Busch et al., 2001) and acute hypoxia is shown to down-regulate NO synthesis (Brown et al., 2006), due to oxygen-sensitive enzyme kinetics (Dweik et al., 1998; Le Cras and McMurtry, 2001). Reduced expired [NO] under hypoxic conditions is though to be caused by an adaptive pulmonary vasoconstriction, to reduce hypoxic stress by redistribute blood flow from poorly to temporarily better oxygenated areas of the lung (Brown et al., 2006). The end metabolic product of NO is nitrite and nitrate (St. Croix et al., 1999). Güzel et al. (2000) showed a contradictory increase in plasma nitrite levels after a stay of 7 days at moderate altitude (1300 masl).

Reduced ambient pressure is associated with proportionally reduced gas density. These physical characteristics of air at altitude may have an important impact that has confounded several of the previous studies of expired NO at altitude. Hemmingsson and Linnarsson (2009) found no change in PE_{NO} from normobaric normoxia to normobaric hypoxia (10.7% O_2) measured at an expired flow of 50 $ml \cdot s^{-1}$. St. Croix et al. (1999) found no change in expired [NO] values during exercise and breathing of hypoxic gas mixtures, compared to normoxic exercise at the same intensity.

2.7.1 Hypoxia limits substrate (O_2) availability

Oxygen is an essential substrate for NO synthesis, as it regulates NO levels through effects on NOS enzyme kinetics (Ricciardolo, 2003). Thus, limitations of O_2 (i. e. hypoxia) may limit NO production (Le Cras and McMurtry, 2001). Pulmonary NO decrease rapidly and proportionately to the amount of oxygen in inspired air (Brown et al., 2006). Dweik and co-workers (1998) reduced the mean level of expired NO by 20% in healthy subjects breathing an inspired gas mixture of 15% oxygen (corresponding to about 2900 meters above sea level [masl]) for just 60 seconds, compared with sea-level room air (21% O_2).

The mechanisms by which oxygen regulates pulmonary vascular tone are not completely understood. Reduced expired NO levels can be a result of decreased NO production in the lung through reduced/inhibited NOS activity or decreased substrate availability (Dweik et al. 1998). Rapid inactivation/scavenging of NO by Hb is another potential mechanism for hypoxia quickly decreasing NO (Dweik et al. 1998).

2.8 Physiological response to hypoxia

In healthy humans, systemic vasodilation and pulmonary vasoconstriction occurs as a result of acute hypoxia, in order to maintain the metabolic demand of systemic organs and improve V_A/Q matching (Blitzer, Lee and Creager, 1996). Peripheral resistance vessels dilate during hypoxia when metabolic supply is compromised (Gale et al., 1985). Blitzer et al. (1996b) showed increases in blood flow and vascular resistance decrements in the forearm of healthy humans during hypoxic exposure. Both systemic and local mechanisms have been proven to account for these alterations, among them chemoreceptors via activation of sympathetic efferent neurons and local release of vasodilator substances, such as NO (Blitzer et al., 1996b).

2.8.1 Exercise at altitude

It is known that physical activity at altitudes of 1300 masl affects the human body due to reductions in ventilation (Güzel et al., 2000). Endurance exercise is more affected by altitude than anaerobic exercise (Åstrand, Rodahl, Dahl and Strømme, 2003; Gale et al., 1985). Verges et al. (2005) showed a greater reduction in FE_{NO} after exercise in normoxia (-27.8% ±22.8) compared to hypoxia (-23.8% ±17.5) at an intensity of 90% P_{max} (power output). Güzel et al. (2000) found decreased NO levels after staying and exercising at high altitude as compared to baseline values.

2.8.2 Gas laws and axial backdiffusion

For a given gas, for example air, reduced ambient pressure is associated with proportionally reduced gas density. At the same time, the binary diffusion coefficient for NO in air is increased in inverse proportion to the pressure (Chang, 1985). Hemmingsson and Linnarsson (2009) found an approximate linear association between the binary diffusion coefficient for NO and air and the reduction of PE_{NO} at simulated altitude, compared to an equivalent hypoxia at sea level (figure 2-5). Shin, Condorelli and George (2006) have demonstrated the impact of the binary diffusion coefficient of NO in a breathing gas for the intrapulmonary NO transport. By using a helium-oxygen breathing gas mixture with a binary diffusion coefficient of more than 0.50 cm^2s^{-1} (as compared to air with 0.22 cm^2s^{-1}) they showed signs of enhanced axial backdiffusion of NO from conducting airways into the alveolar compartment. Since NO has a very high affinity to the Hb of pulmonary-capillary blood, backdiffusion of NO into the alveolar compartment was found to be associated with less NO being expired, due to an increase uptake of NO into the blood (Hyde et al., 1997). Therefore, the partial pressure of NO in the capillaries is extremely low, and the concentration gradient between the cells lining the airway and the capillary blood is high (St Croix et al., 1999).

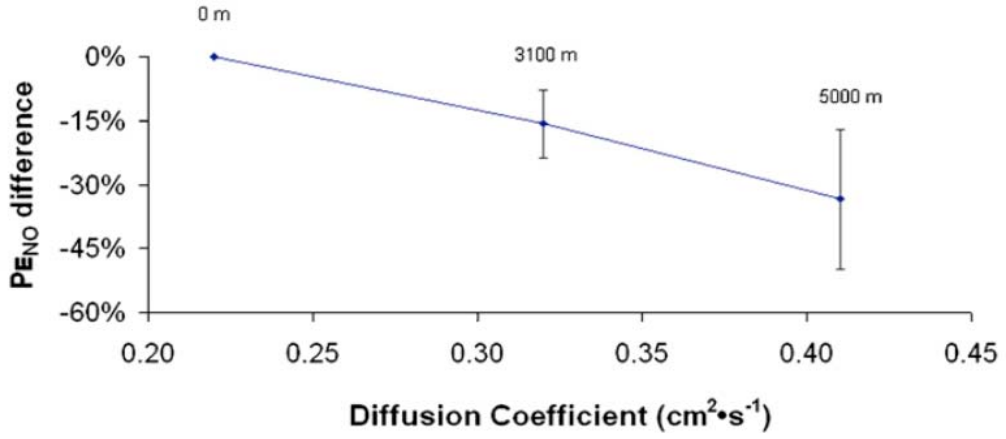


Figure 2-5 *PE_{NO} difference vs. binary diffusion coefficient for NO and air. Differences are between normobaric and hypobaric conditions with equivalent inspired oxygen partial pressures. Values are means with SD*[8].

Table 2-2 *Differences in ambient conditions on PO_2, gas density and the binary diffusion coefficient for NO in air*[9].

	Ambient pressure (hPa)	PO_2 (kPa)	Relative gas density	Binary diffusion coefficient for NO in air (cm^2s^{-1})
Normobaric	1013	19.9	1.0	0.22
	1013	13.8	1.0	0.22
	1013	10.7	1.0	0.22
Hypobaric	846	16.4	0.84	0.26
	693	13.2	0.68	0.32
	540	10	0.53	0.41

Inspired oxygen partial pressures (PO_2) are given as BTPS. Binary diffusion coefficients were calculated according to Chang (1985).

Further support for the importance of axial backdiffusion of NO in the lungs has been provided by Van Muylem, Noël, and Paiva (2003) and Kerckx and Van Muylem (2009). Kerckx et al. (2008) have shown a 30% reduction of FE_{NO} after equilibrium with an 80% helium/20% oxygen mixture in comparison to air. Hemmingsson and Linnarsson (2009) hypotizes that enhanced axial backdiffusion and increased NO uptake in the blood are the reasons for reduced PE_{NO} values at reduced ambient air pressure.

[8, 9] From Hemmingsson and Linnarsson (2009)

2.9 Individual differences

Great individual variations are described in the literature (Busch et al., 2001; Olivieri et al., 2006). Airways and alveolar vessels present a large endothelial surface area. As NO is generated by endothelial cells (Sheel et al., 1999), differences in body size has been proposed to influence expired [NO] values. Phillips et al. (1996) found a correlation between expired [NO] and body surface area (m^2). It should be noted that measurements of the fraction of expired NO (FE_{NO}) are independent of volume size, as this is a relative unit measuring the concentration of NO in expired air. Area of diffusion, body size and surface area are not likely to explain differences in FE_{NO}. Others have tried to explain possible discrepancies with differences in NOS genotypes, hormone production or other biochemical factors (Olivieri et al., 2006). Taylor et al. (2006) discuss age difference in FE_{NO} levels, emphasizing that a difference between children and adults may be related to increasing airway surface area with age. Also age dependent induction of NOS, immunological stimulation or the progressive reduction over time of a constant flow rate, which is relatively high in younger children, cannot be excluded (Taylor et al., 2006).

Brown et al. (2006) measured expired NO concentrations in 47 subjects at sea level, 2800 masl and 4200 masl, and found that although average PE_{NO} decreased with increasing altitude, both the degree and direction of change expired NO values varied among subjects. At 4200 masl 23% of the subject experience higher PE_{NO} values compared to sea level. After 2 and 3 hours 26% measured elevated PE_{NO}. Previous studies have shown both differences (Olivieri et al., 2006) and no difference (Verges et al., 2006) between genders.

Discrepancies between studies can be attributed to methodological concerns, including differences in expired flow rate. Varieties of expiration manoeuvres, use of a nose clip or a facemask, direct sampling vs. mixing chamber, online vs. offline and positive end expiratory pressures will affect the result of the measurements. Mixing of nasal and lower airway air gases can significantly elevate NO concentrations (Schedin et al., 1995). Given the range of NO values reported in the literature, it is important to be aware of the measurement technique when interpreting and comparing results (Sheel et al., 1999).

Despite of number of publications on FE_{NO}, reference values in healthy adults are few. The connection between NO and pulmonary diseases might explain this lack of available data as, in large part, experimental studies of FE_{NO} mainly consider healthy subjects as controls. Therefore, due to small sample sizes, various subject characteristics and the wide variety of measurements methods used, it has been (and still is) difficult to generalize current FE_{NO} data to a population as a whole. Olivieri and co-workers (2006) described FE_{NO} values in a large group of healthy men and women (n=204), and found great individual differences in expired NO levels, reporting values ranging from 2 to 80 ppb.

2.10 Ambient NO

Environmental air is contaminated with various amounts of NO. Ambient NO (aNO) can change over different days, depending on atmospheric pressure and traffic conditions (Corradi et al., 1998). Corradi et al. (1998) found a significant correlation between expired NO (ppb) and atmospheric NO ($r=0.38$, $p=0.001$). However, the correlation disappeared with atmospheric NO concentrations lower than 35 ppb. The authors state that their results indicate a relationship between atmospheric NO and NO levels measured in expired air, and therefore expired NO should not be measured on very high atmospheric days.

3. Methods

3.1 Design

The present study used an open randomized crossover design. Drawing lots carried out the randomization. The study was part of a project comparing changes in expired [NO] after high intensive exercise in three different environmental climatic conditions (normobaric, hypobaric and cold environment). In the present thesis only two of the climatic conditions will be presented: a normobaric environment (741.3 mmHg) and a hypobaric environment (543.3 mmHg). All subjects conducted a pretest and two similar exercise tests. All tests were optimally completed within 14 days after pretest, with a minimum of 48 hours between each test. The 14 days limit was not always maintained carefully due to practical limitations of carrying out the study. All tests were performed in the respiratory laboratory at Norwegian School of Sport Sciences, in the period of October 2009 to January 2010.

3.2 Subjects

Twenty healthy, non-smoking and "non-snuffing" men and women (12 males and 8 females), primarily students or staff at the Norwegian School of Sport Sciences, volunteered to participate in the study. All subjects maintained an active lifestyle throughout the study, and were engaged in various physical activities (cross-country skiing, triathlon, soccer, orienteering or running) at different levels, both competitive and non-competitive. All participants had normal lung function as measured by maximal expiratory flow volume loops. Demographics and baseline lung function, expired [NO] and VO_{2max} are presented in table 3-1.

Table 3-1 *Subject characteristics. Values are given as means (±sd).*

	Males	Females	Total
	(n=12)	(n=8)	(n=20)
Age (years)	22.5 (±2.4)	22.8 (±3.2)	22.6 (±2.7)
Height (cm)	178.2 (±5.4)	164.6 (±4.7)*	172.8 (±8.5)
Weight (kg)	74.5 (±7.0)	57.5 (±7.9)*	67.7 (±11.1)
BMI ($kg \cdot (m^2)^{-1}$)	23.4 (±1.4)	21.1 (±2.3)*	22.5 (±2.1)
VO_{2max} ($ml \cdot kg^{-1}min^{-1}$)	52.6 (±5.8)	61.9 (±4.0)*	58.2 (±6.6)
Baseline FEV_{1pred} (%)	108 (±7.9)	99 (±8.5)*	104 (±8.9)
Baseline FVC_{pred} (%)	106.6 (±9.3)	104.1 (±3.8)	105.6 (±7.7)

Two-tailed *t*-tests, gender differences: *P<0.05

Exercise and intake of any food or drink containing nitrite and nitrate (such as salad, milk or smoked food) was prohibited on the day of experiments, as was any food and drink (except water) within one hour before exercise testing (ATS/ERS, 2005). Inclusion criterion was a maximum oxygen uptake > 40 $mL \cdot kg^{-1}min^{-1}$ (females) and > 55 $mL \cdot kg^{-1}min^{-1}$ (males). Exclusion criteria were FE_{NO} >30 ppb and any acute or chronic illnesses interfering with the possibility to perform the study, in addition to upper-respiratory tract infections during the last 14 days before testing.

3.3 Test protocols

3.3.1 Anthropometric measurements

Total body weight and height were measured in the laboratory before each test with the subjects dressed in light clothing and without shoes. Height was measured to the nearest cm and body mass to the nearest 0.5 kg.

3.3.2 Pretest

The pre-test consisted of an anaerobic threshold (AT) test followed by assessment of maximum oxygen uptake ($VO_{2\ max}$). Measurements of ventilation (VE) and fractioned expired nitric oxide concentration (FE_{NO}) were collected online before exercise, between bouts of exercise and 5, 10, 15, 20 and 60 minutes after the exercise test. VE was measured after exercise until the values had returned to baseline. Lung function was measured before and 6 -10 minutes after the exercise test to exclude subjects with exercise-induced asthma (EIA). Heart rate (HR) was measured during the test. A complete test protocol of the pretest, including all sample collections, is illustrated in a timeline below (figure 3-1).

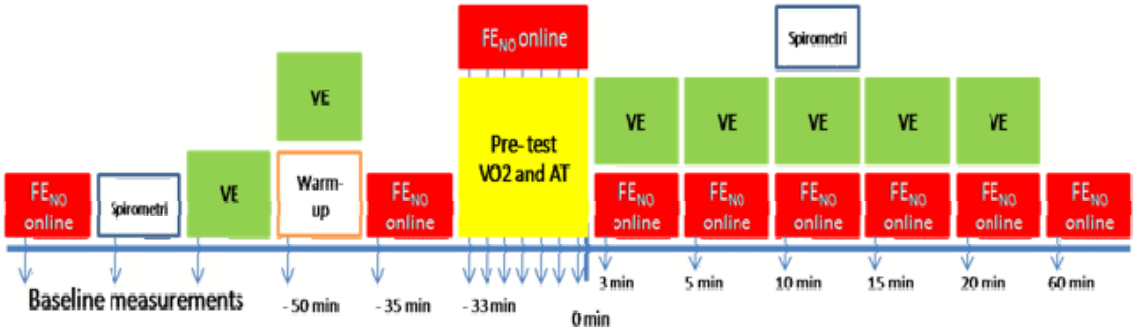


Figure 3-1 *Protocol of pretest with time of sample collections.*

3.3.3 Anaerobic threshold

Anaerobic threshold (AT) was determined using an indirect measurement of maximal lactate steady state (MLSS) (Helgerud, Ingjer and Strømme, 1990). The test was performed on a treadmill (Woodway, Germany) with an inclination of 5.3 %. After a 10-minute standard warm-up at a workload corresponding to 50-60% of estimated $VO_{2\ peak}$, 5-minute sub-maximal running bouts were performed separated by 2 min of rest. For each bout, the speed was increased in steps of 1 $km \cdot h^{-1}$. Measurements of oxygen uptake (VO_2), respiratory exchange ratio (RER) and ventilation (V_E) were obtained between 2½ - 4 minutes of each step (between 6½ -9 minutes during the warm up) (Champion Jeager Inst, Germany). HR was registered electronically using Polar Vantage^{TM} (Polar Electro, Finland) at the end (4 -5 minutes) of each bout. During rest periods, a fingertip capillary blood sample (50 µl) was obtained for the assessment of lactate concentration. (1500 SPORT Lactate Analyzer, Yellow Springs Inc, USA) and measures of FE_{NO} were conducted. The lactate analyzer has a measurement uncertainty of ±2% (YSI 23L Operational Manual Yellow Springs Instruments, 1995). Calibration of the lactate analyzer was conducted with injection of 5 $mmol \cdot l^{-1}$ (±0.1 $mmol \cdot l^{-1}$) lactate standard solution, further checked with a 15 $mmol \cdot l^{-1}$ standard solution every morning before test.

3.3.4 Maximal oxygen uptake ($VO_{2\ max}$)

A $VO_{2\ max}$-test followed immediately (<2 minutes) after the lactate threshold was attained. The test was performed using a stepwise protocol according to the procedure described by Hermansen (1973) and Åstrand et al. (2003), with increasing velocity for every minute, preferably by 1 $km \cdot h^{-1}$. Considering the subject's state, through physiological values and the test leaders subjective observation on signs of exhaustion,

velocity could remain constant at the last minutes of the protocol. Expired gas was assessed (Champion Jeager Inst, Germany) throughout the test. The subjects wore a nose-clip and breathed through a low resistance mouthpiece (2700 Series: Hans Rudolph Inc, USA). Gas samples of O_2 and CO_2 were collected from a mixing chamber with average values obtained over 30 second periods and used for analysis. Heart rate was monitored electronically and registered manually every minute (Polar Electro, Kempele, Finland) throughout the test. Polar Electro has showed measurements uncertainty of ±1% (Mcfarlane et al. 1989). The subjects were encouraged to maintain maximal running speed until exhaustion. A blood sample was collected shortly after the test to assess lactate acid concentration, as an additional criterion for reached $VO_{2\ max}$. The volume of the VO_2 analyzer was calibrated manually with a 3 L pump and oxygen and gas (95% nitrogen and 5% carbon dioxide). The equipment has a measurement uncertainty of ±3% (Åstrand et al., 2003). VO_{2max} was defined as the highest average over the range of 1 minute. The highest recorded values of VE, RER, HR and running speed were expressed as peak values. The HR_{peak} and running speed during $VO_{2\ max}$ was used as a reference to maintain the workload at the repetitive exercise tests.

3.3.5 Repetitive exercise tests

Each participant performed two repetitive exercise tests (RET) in a low-pressure climatic chamber (Norwegian Sub diving Technology A/S, Haugesund, Norway), in which the temperature, altitude, relative humidity and air velocity were monitored continuously. The subjects were running on a motor-driven treadmill (Bodyguard Cardionics 2313, Cardionics AB, Sweden) with an inclination of 5.3 % and a wind velocity of 0.5 $m \cdot sec^{-1}$. The RETs were conducted in regular indoor environment (18ºC, 35 % relative humidity, with a barometric pressure corresponding to 300 masl) and in a hypobaric environment (18ºC, 35 % relative humidity and ambient pressure corresponding to 2800 masl). The RETs were separated by at least 24 hours. After a 15-minute warm-up at a workload corresponding to 65-75 % of HR_{peak}, gas samples of the expired air and FE_{NO} were obtained in Douglas and Mylar bags, respectively. The RET consisted of an eight minute exercise bout at a sub-maximal workload. The velocity was adjusted during the first four minutes to achieve a work load corresponding to 90% of HR_{peak}, followed by four minutes at a an intensity of >90% of HR_{peak} (Fredriksen, Ingjer, Nystad and Thaulow, 1998). Heart rate was monitored electronically and registered manually every minute (Polar Electro OY, Kempele, Finland). The subjects

were cheered on and encouraged throughout the test. After each, RET the participants remained in the current environment for 20 minutes where samples of expired air and FE_{NO} was collected 0, 10 and 20 minutes after RET, and 5, 10, 15 and 20 minutes after RET, respectively. The subjects were allowed to exit the climatic chamber 20 minutes post exercise. A complete test protocol for the RET is illustrated in a timeline below (figure 3-2).

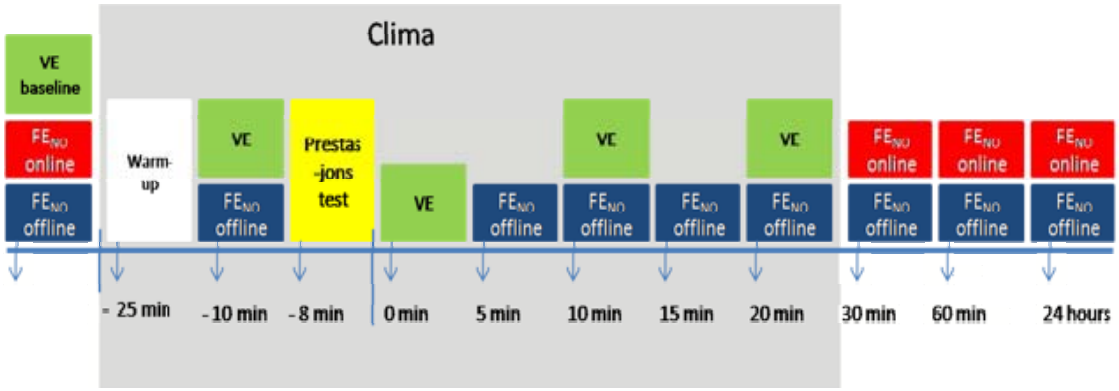


Figure 3-2 *Protocol with sample collection and exposure to different barometric environments (Clima) during RET.*

3.4 Measurement procedures

3.4.1 Expired nitric oxide sampling

FE_{NO} online measurements were conducted before and 30, 60 minutes and 24 hours after each RET and during pretest (all measurements). Offline measures of FE_{NO} were assessed inside the climatic chamber; after warm-up and 5, 10, 15 and 20 minutes after RET, as well as before and 30, 60 minutes and 24 hours after the RET. Ambient NO (aNO) concentration were registered during each breath, as recommended (Corradi et al., 1998; Hemmingsson, Horn and Linnarsson, 2009).

Online

For nitric oxide analysis, online samples of FE_{NO} were measured using a NO chemiluminescence analyzer (Eco Medics, Duerten, Switzerland) according to the ATS/ERS recommendations (2005). The subjects were standing and instructed to expire normally, and then inspire to total lung capacity through a mouthpiece connected to the NO analyzer. A breathing filter provided NO-free air during inspiration. The subjects were to expire through the mouthpiece in a steady flow rate of 50 $mL \cdot sec^{-1}$ for 12

seconds. A visual feedback system helps to maintain a positive pressure between 10 and 20 cm H_20 to ensure closure of the soft palate (Silkoff et al., 1997). FE_{NO} was recorded as the mean value from three successive measurements. A value is approved when 3 recordings are within ±10%, or 2 measures within 5%. Both gas and volume calibrations were carried out each day before testing with 3 ppm Kvävemonoksid shallow gas nitrogen (AGA gas) and 100 ml cal syringe (Hans Rudolph, Inc, K.C.MO., U.S.A). The spirette for dead space reducer was renewed weekly.

Picture 3-1 *NO sampling online*

Offline

Samples of expired air were collected in Mylar bags using a Collector Kit with flow restrictor (EcoMedics – Offline Collection Kit, Duerten, Switzerland) for NO analysis offline. The procedure is similar the online sample collection. Participants expire before inspiration of NO free air through a NO-filter with a one-way valve, followed by a full expiration, until the Mylar bag is filled. A visible needle indicates the pressure gauge and helps maintain the counter pressure between 10 and 20 cm H_2O. The test leader signalizes when bag is filled. After the Mylar bag was filled it was disconnected from the kit and closed with a cap. The bags were analyzed within 4 hours by the same chemiluminescence analyzer used during online sampling (EcoMedics, Duerten, Switzerland).

Picture 3-2 and 3-3 *NO sampling offline*

3.4.2 Lung function measurements

Lung function was measured by maximum expiratory flow volume loops (Masterlab, Erich Jaeger®, Germany), forced expiratory volume in one second (FEV_1), forced vital capacity (FVC) and forced expiratory flow at 50% of FVC (FEF_{50}). Lung function measurements were carried out in a regular indoor environment before and 6-10 min after the pretest to exclude subjects with exercise-induced bronchoconstriction (EIB) or exercise-induced asthma (EIA). All manoeuvres complied with the general acceptability criteria of the European Respiratory Society (ERS, 1997).

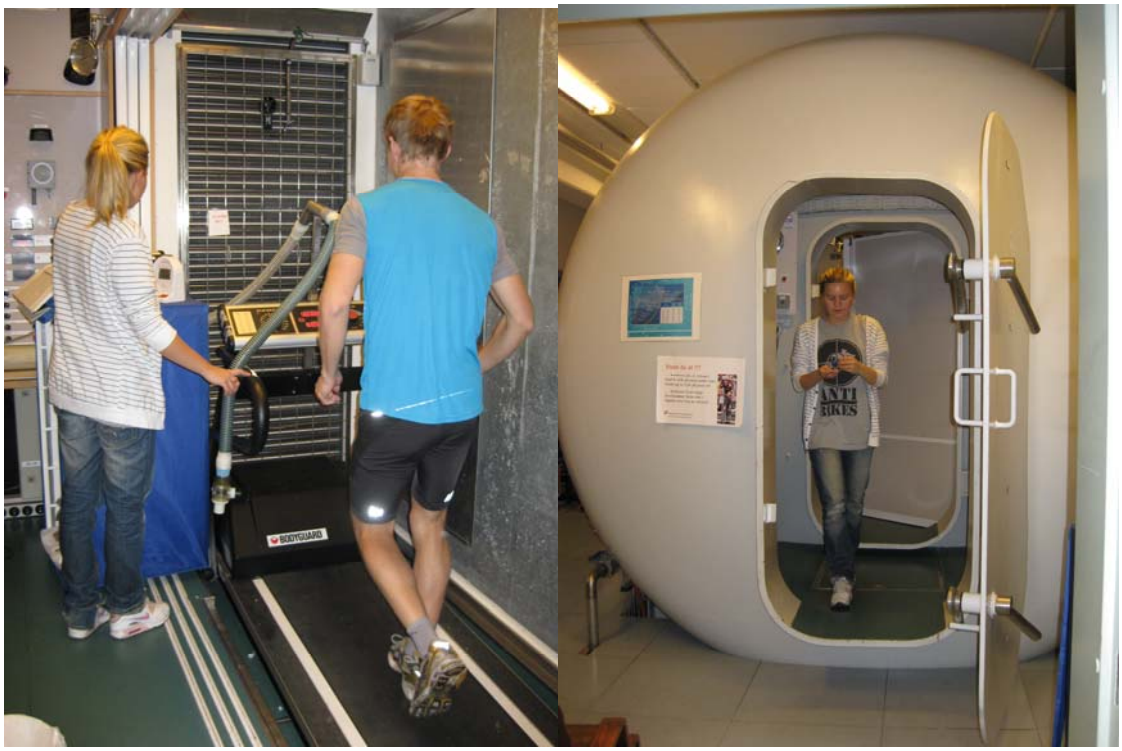
3.4.3 Expired gas sampling

Douglas bags were used for collecting gas samples of expired air before and immediately after warm-up, immediately after RET, and 5, 10 and 20 minutes after RET. The variations reported of the Douglas bag method are 2.3 -2.5% for daily variations and 3.3 -5.1% for between day variations (Carter and Jeukendrup, 2002). The subjects wore a nose clip and breathed through a mouthpiece (2700 series; Hans Rudolph Inc, USA). Expiratory gas samples were collected for 60 seconds and analyzed for O_2 and CO_2 content (Oxygen analyzer model S-3A/1 and Carbon dioxide analyzer model CD-3A; Ametek Inc, USA). The volume, temperature and barometric pressure of the expired gas were measured at the same time the air was analyzed ("Ventilation

measuring system", model S-430, KL-Engineering, Northridge, California, USA). The Douglas bags were deflated and checked for any damage every morning before use.

3.5 Ambient conditions

RETs were conducted in a low-pressure climatic chamber (Norwegian Sub diving Techniques A/S, Haugesund, Norway) according to identical procedures. Temperature, humidity and hypobaric pressure were controlled from a control panel on the outside of the chamber.



Picture 3-4 and 3-5 *The climatic chamber viewed from both the inside (left) with test set-up, and from the outside (right).*

Picture 3-6 *Control panel of the climatic chamber*

3.6 Conversions of expired [NO] values

NO concentration in expired air is influenced by the partial pressure of oxygen (PO_2) (Hemmingsson et al., 2009). FE_{NO} is therefore converted to the partial pressure of NO (PE_{NO}) to express the molar concentration of NO, which allows comparison of FE_{NO} samples collected at different barometric environments (Hemmingsson et al., 2009). Our PE_{NO} values are calculated as FE_{NO} (ppb)*(barometric pressure (mPa)/water vapor pressure (mPa)/1000000000, and expressed in mPa. Water vapor pressure at 37° Celsius equals 47 mmHg or 6266134 mPa.

3.7 Pilot study

A pilot study was conducted, including six voluntary students from the Norwegian School of Sport Sciences, in September 2009. The aim of the pilot study was to become familiar with the test protocols, as well as gather useful information and expectations about the future research. The main study followed the pilot study protocol with minor changes. The results from the pilot study are not presented in the present study.

3.8 Ethical considerations

The present study was performed according to the principles stated in the Declarations of Helsinki. The subjects were informed about the testing procedures (Appendix 1) and that they could withdraw at any time during the study. All subjects signed a written

consent form (Appendix 2). The Regional Medical Ethics Committee approved the study (Appendix 3). All results were immediately filed and stored in a lockable cabinet after testing. Test forms were coded with the subjects own identification number.

3.9 Statistical analyses

Results are listed as means with 95% confidence intervals unless otherwise stated. Demographics are given as means with standard deviations. Paired t-tests are used to assess differences between changes in FE_{NO} and PE_{NO} in normobaric and hypobaric environments. Possible associations are assessed by Pearson's correlation coefficient. Coefficient of variation (CV) are calculated as = (SD/mean)*100, and expressed in percent (%). The level of statistical significance was set as P=0.05. Statistical analyses were performed with Statistical Package of Social Science (SPSS) version 15.0 and Microsoft Excel version 11.5.6 (2004), Microsoft Corporation. All figures and tables were done in Microsoft Excel. A number of 20 subjects were found to be sufficient to assess differences in FE_{NO}, based on a power of 80% with a significance level of 0.05.

4. Results

4.1 Main findings

The results from this study showed a significant decline in FE_{NO} from baseline to 5 minutes after exercise ($p<0.001$) in a normobaric environment. No change was seen in FE_{NO} measures 5 min after exercise during exposure of reduced barometric pressure, compared to baseline. However, when conforming FE_{NO} to PE_{NO}, a significant reduction in expired [NO] after the exercise during hypobaric exposure ($p<0.001$) occurred after warm up ($p=0.001$) and 5 minutes post exercise ($p<0.001$). This reduction sustained significant throughout measures of 10 and 15 min after exercise in hypobaric hypoxia. After the normobaric test, PE_{NO} declined significantly 5 min after end of exercise ($p=0.001$). A difference between the two barometric pressures was seen after warm up ($p=0.04$) only.

All the 20 subjects performed the test correctly without discomfort. There were found significant gender-related differences ($p<0.05$) in body mass (weight), height, body mass index (BMI), VO_{2max} and lung function (measured as forced expiratory volume at the first second, FEV_1).

RET intensity

In the hypobaric environment, RET intensity (% HR_{peak}) was reduced ($p<0.01$) compared to RET intensity at sea level. A difference in running velocity ($km \cdot h^{-1}$) in the hypobaric RET compared to normobaric RET was observed, as velocity was significantly lower in hypobaric environment compared to normobaric environment ($p<0.001$). No difference was seen in maximal ventilation measured immediately (0 min) after end of exercise between the two barometric pressures, or ventilation measured at baseline, after warm up or 10 and 20 minutes after exercise (figure 4-4).

Table 4-1 *HRpeak (%) and velocity (km·h^{-1}) during repetitive exercise tests (RETs) in hypobaric (hypo) and normobaric (normo) environments, and ventilation (VE) (l·min^{-1}) immediately after end of exercise tests. Results are given as means (±sd). *Significant difference between RETs.*

RET intensity	Normo	Hypo	p
Velocity (km h^{-1})	12.7 (±1.1)	11.3 (±0.9)	0.0000004*
VE (l min^{-1})	83 (±27.1)	86 (±29.1)	0.8
HRpeak (%)	91 (±5.5)	89 (±5.5)	0.004*

4.2 Alterations in concentration of expired NO

A significant reduction in FE_{NO}, measured offline, after exercise in normobaric pressure occurred 5 min (p=0.001) and 10 minutes (p=0.01) after exercise (figure 4-1). After hypobaric exposure and exercise, FE_{NO} values (offline) showed alterations 20 minutes after exercise (p=0.006) and borderline 15 minutes (p=0.06) after the exercise test. Online measures showed a significant change 24 hours after exercise test in both normobaric (p=0.05) and hypobaric (p=0.003) pressures.

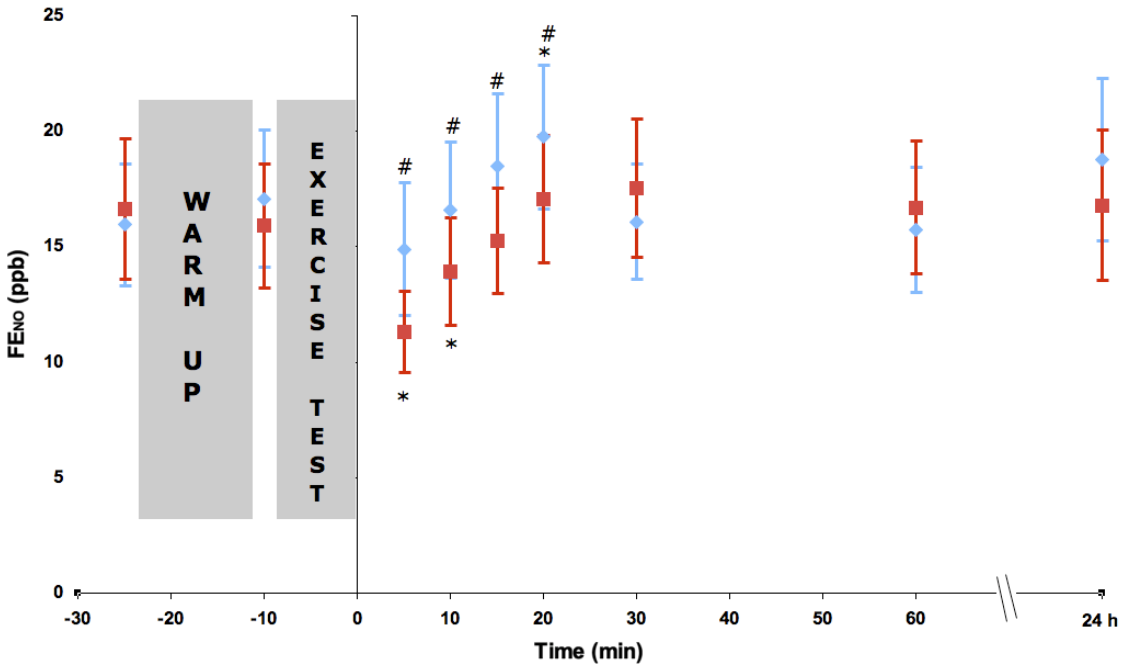


Figure 4-1 *FE_{NO} expiration at baseline (-25 min), after warm up (-10 min), and after exercise (5 min, 10 min, 15 min, 20 min, 30 min, 60 min and 24 hours) in hypobaric hypoxia corresponding to 2800 meters above sea level (blue) and normobaric normoxia (red). Results are given as means with 95% confidence intervals. *Significantly different from baseline. #Significant difference between the two barometric pressures.*

No difference was seen between baseline values between the two RETs. Comparing the course of FE_{NO} after RET, a significant difference (p=0.003) was found between

normobaric and hypobaric exposure during exercise. Additionally, differences were found in measures made 5 min (p=0.002), 10 min (p=0.006) 15 min (p=0.002) and 20 min (p=0.006) after end of exercise test.

4.3 $FE_{NO} \rightarrow PE_{NO}$

FE_{NO} values measured in hypobaric hypoxia after warm up and 5, 10, 15 and 20 min after exercise test, are elevated (p=0.003) compared to normobaric FE_{NO} measured at the same time of test (figure 4-1). This difference is not present after conforming the values into PE_{NO} (figure 4-2).

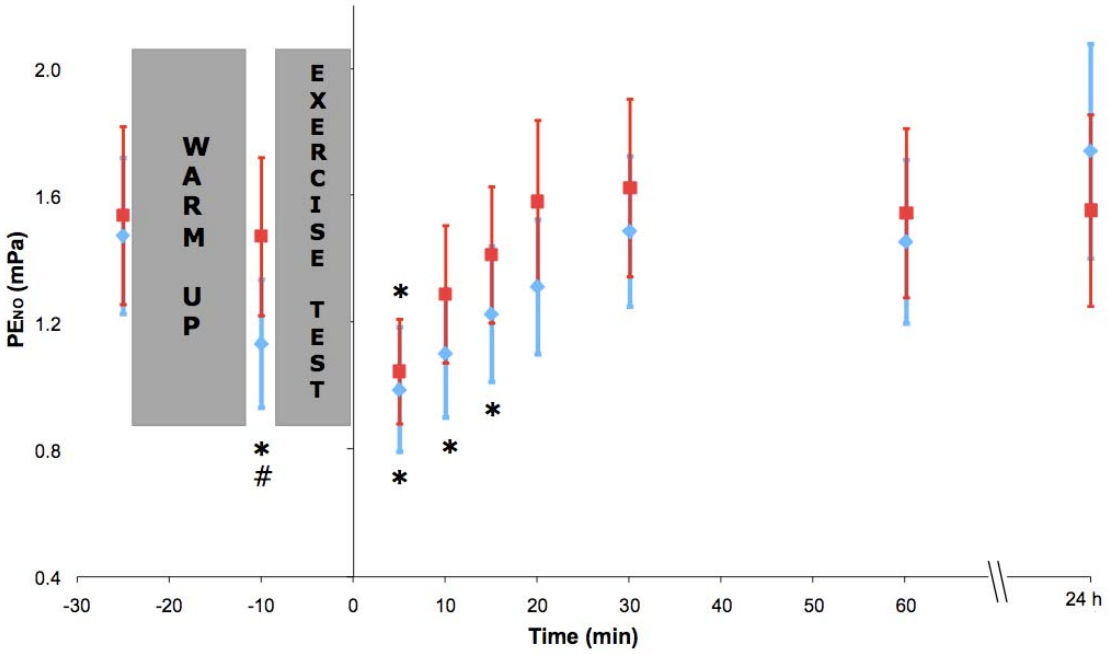


Figure 4-2 *PE_{NO} before, after warm up and after high intensive exercise (RET) in hypobaric (blue) and normobaric (red) pressures. Results are given as means with 95% confidence intervals. *Significantly different from baseline. #Significant difference between the barometric pressures.*

Reductions in offline PE_{NO} values were significant different from baseline after warm up (p=0.001), 5 min (p<0.001), 10 min (p=0.001) and 15 minutes (p=0.01) after exercise and hypobaric exposure. A decrease occurred 5 min after RET (p=0.001), and borderline (p=0.06) 10 min after exercise in a normobaric environment. A difference between the two barometric pressures was apparent after warm up (p=0.04).

Online measurements showed a change in PE_{NO} from baseline to 24 hours after test (p<0.01), and from 60 minutes to 24 hours after test (p<0.05) after the RET in

hypobaric pressure, and from baseline to 24 hours after RET ($p<0.05$) in normobaric pressure. No other significant changes were seen in the online measures.

Different relationships between FE_{NO} and PE_{NO}, measured in the two barometric environments, are observed. The correlation between FE_{NO} and PE_{NO} measured at sea level are strong and approximately linear ($r=0.999$). At simulated altitude (hypobaric hypoxia) this relation does not occur ($r=0.01$).

4.4 Relative change (%) in expired [NO]

PE_{NO} levels were reduced by 31% (9, 53 [mean (95% confidence intervals)]) and 28% (9, 47) 5 minutes after the hypobaric and normobaric RET, respectively, which was the maximal reduction of FE_{NO} after RET. A reduction of 21% was seen after warm up in the hypobaric climate, whilst no change (-1% (-36, 34)) was seen after warm up in normobaric climate (figure 4-3). Differences between the two environments were significant after warm up ($p=0.03$) and 20 min post exercise ($p=0.04$).

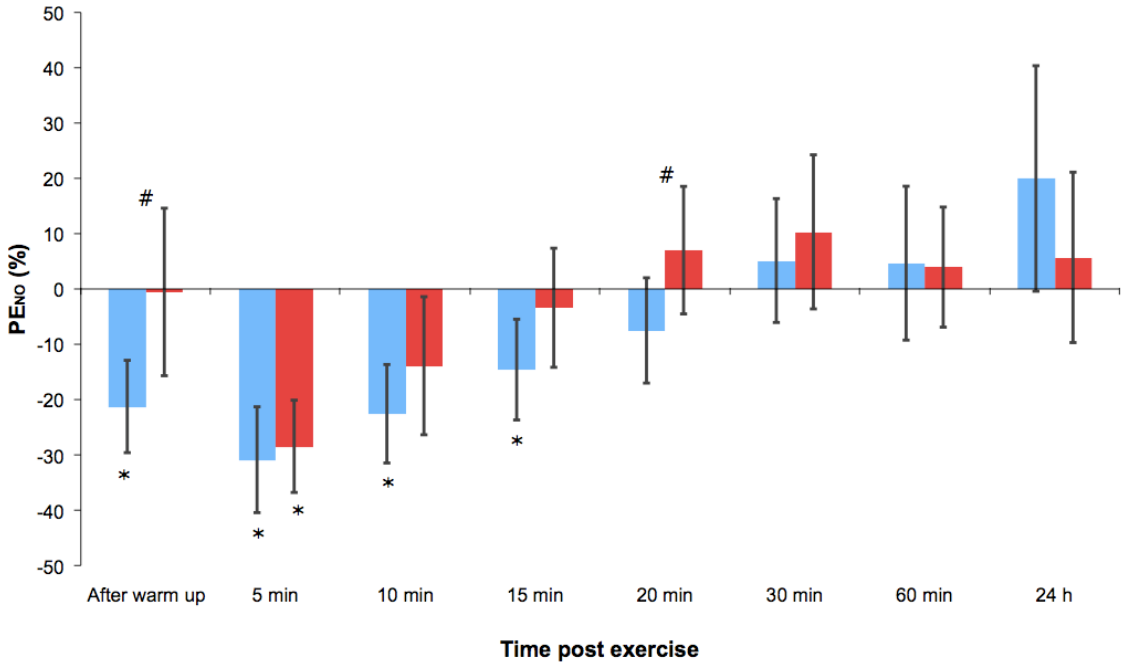


Figure 4-3 *Change in PE_{NO} (%) from baseline to after warm up and after exercise tests (5, 10, 15, 20, 30 and 60 minutes, and 24 hours) in two different barometric pressures; hypobaric (blue) and normobaric (red). Results are given as means with 95% confidence intervals. *Significantly different from baseline. #Significant difference between barometric pressures.*

During pretest, the largest drop in FE_{NO} occurred 3 minutes after end of exercise (-37.3% (26.5, 48.1)). Pretest values sustained significantly reduced throughout measurements (<60 min post exercise) compared to baseline ($p<0.004$). Data from the pretest are illustrated in figures 4-5 and 4-11.

4.5 Ventilation

No differences were found between ventilation during the exercise test in hypobaric and normobaric pressure (figure 4-4). A correlation of 0.998 was found comparing the expiration of the two barometric climates.

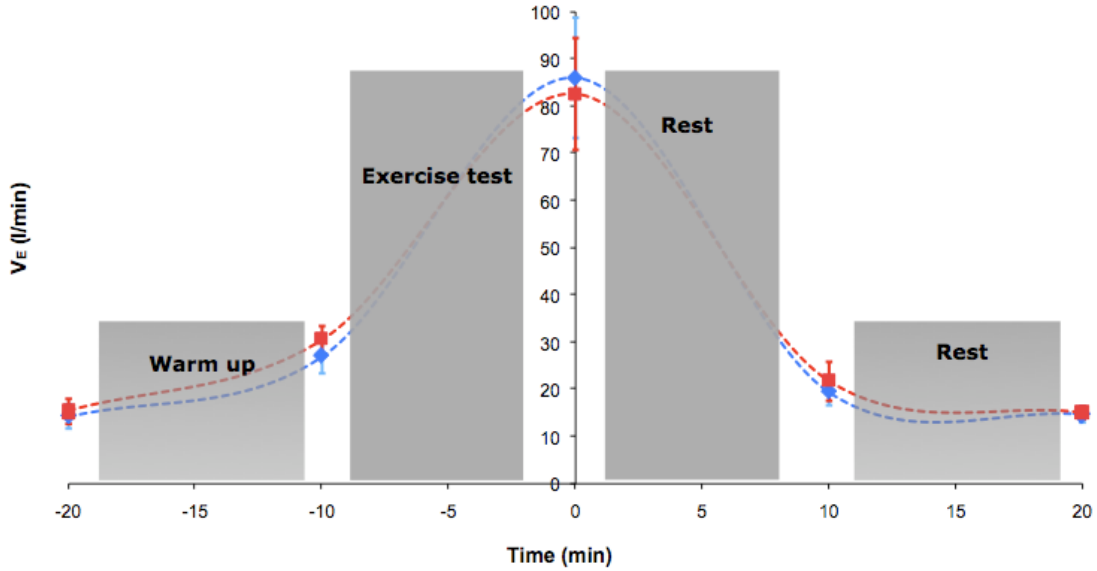


Figure 4-4 *Ventilation (VE) at baseline (-20 min), after warm up (-10 min), immediately after exercise tests (0 min) and post exercise (10 min and 20 min), in hypobaric (blue) and normobaric (red) pressures. Grey areas represent degree of physical activity; warm up, exercise test and rest, respectively.*

Negative correlations between ventilation and the course of PE_{NO} from baseline to the period after the exercise test were found in both hyperbaric (r=-0.71) and normobaric (r=-0.83) pressures. However, ventilation did not correlate with neither of the NO measurements (baseline, after warm up or 0 min, 10 minutes and 20 minutes after RET) offline or online nor at the two different barometric pressures.

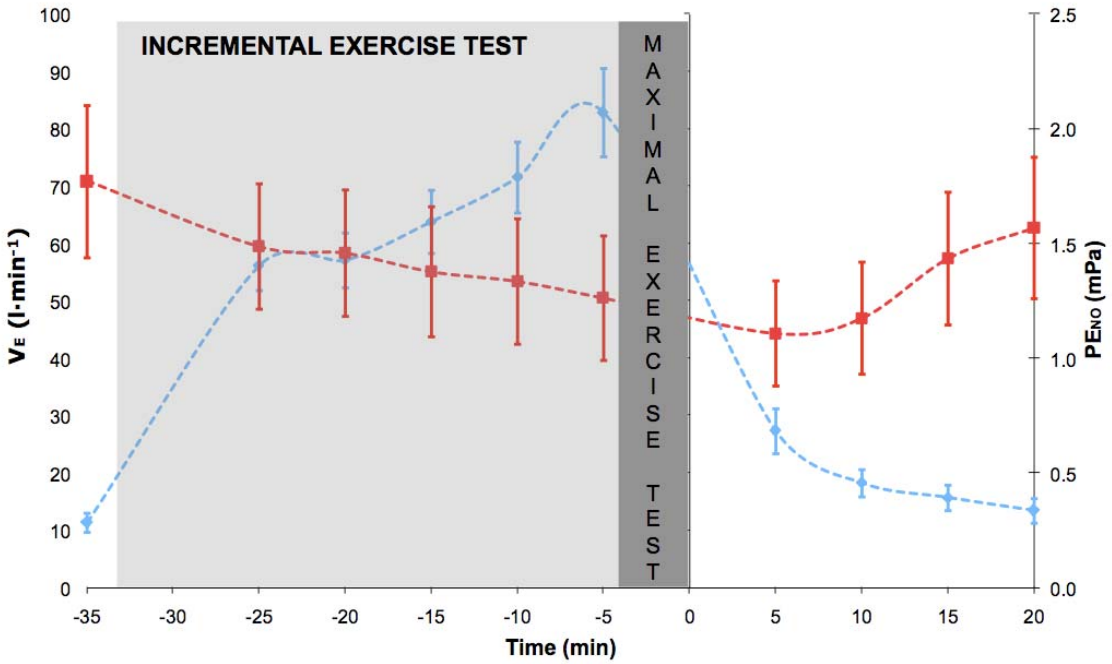


Figure 4-5 *Relationship between ventilation (VE) (blue) and expired nitric oxide (PE_{NO}) (red) during and after physical activity with increasing intensity (pretest). Values are given as means with 95% confidence intervals.*

4.6 Production rate of NO (V_{NO})

V_{NO} increased significantly after warm up ($p<0.005$) and after exercise test ($p<0.001$) in hypobaric and normobaric pressures (figure 4-6). Significant differences ($p<0.05$) were observed in V_{NO} between the two barometric pressures at all sample times, with the exception of baseline values. Relatively, percent change in V_{NO} were significant different in the two barometric pressures after warm up ($p<0.01$) as well as after exercise ($p<0.03$) (figure 4-7). No difference was observed 10 and 20 minutes after exercise.

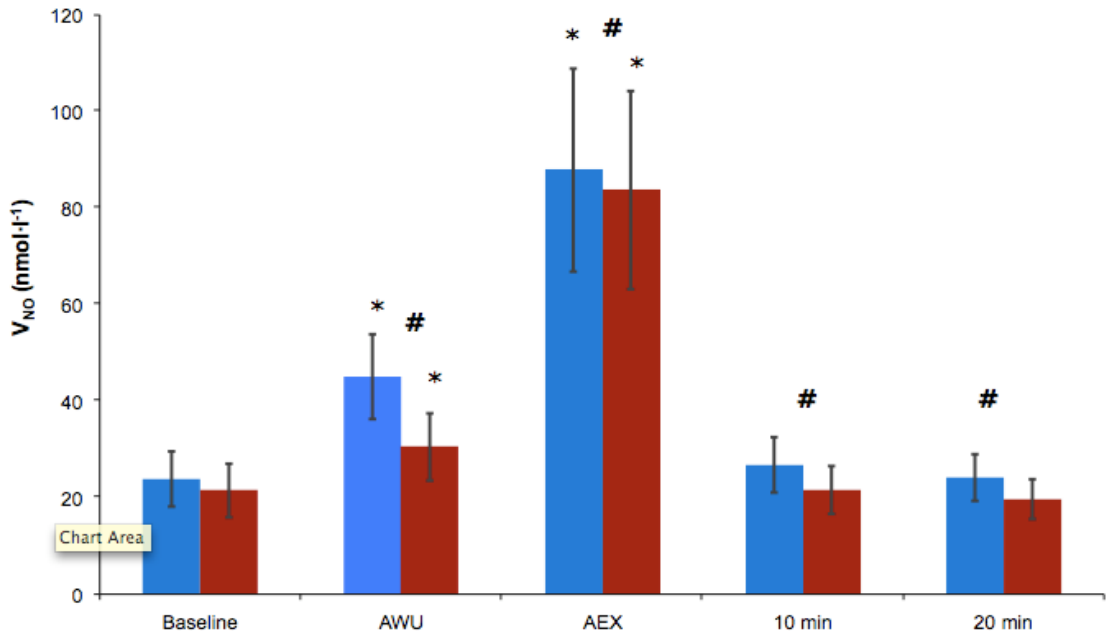


Figure 4-6 *NO output (V_{NO}) (nmol·min^{-1}) before and after exercise test (RET) in hypobaric (red) and normobaric (blue) pressure. After warm up (AWU), immediately after exercise (AEX), 10 min and 20 min after exercise. Results are given as mean with 95% confidence interval. *Significantly different from baseline. #Significant difference between the two barometric pressures.*

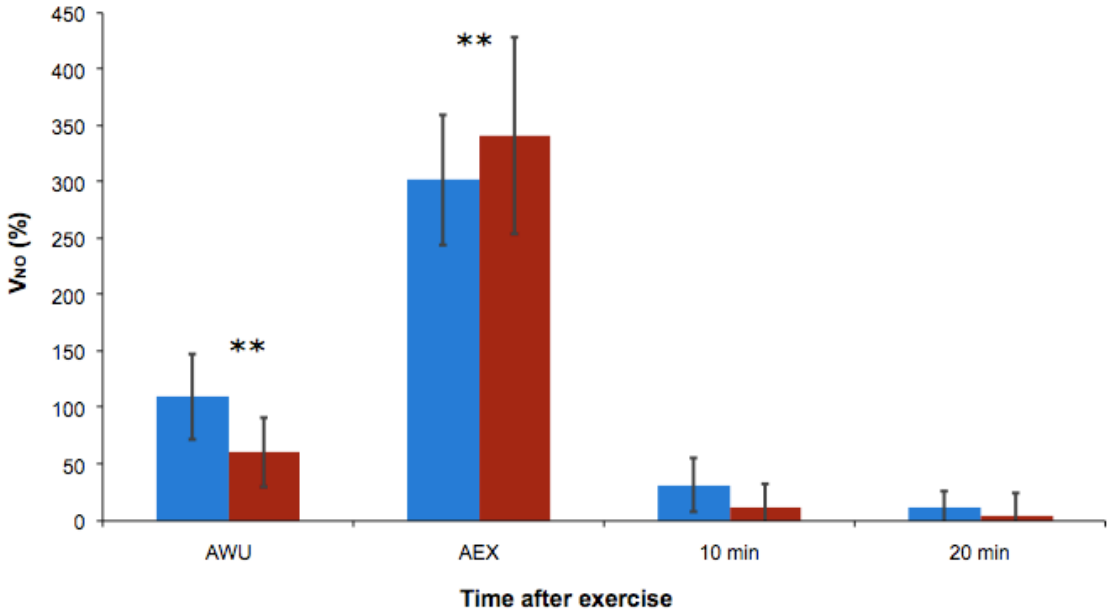


Figure 4-7 *Change in NO output (V_{NO}) (%) after warm up (AWU), immediately after exercise (AEX), 10 min and 20 min after exercise in hypobaric (red) and normobaric (blue) pressure. Results are given as means with 95% confidence intervals. **Significantly different from baseline and between the two barometric pressures.*

Table 4-2 *Relative change (%) in V_{NO} after RETs. Values are given as means with 95% confidence intervals.*

Barometric pressure	After warm up	After RET	Post RET (10min)	Post RET (20min)
Hypobaric	61% (30, 92)	341% (254, 248)	12% (-9, 33)	5% (-15, 25)
Normobaric	110% (72, 150)	302% (244, 360)	32% (8, 56)	12% (-2, 26)

Significant correlations were found between V_{NO} and VO_{2max} and VE_{max} in absolute values ($p<0.05$) at baseline ($r=0.47$), after warm up ($r=0.50$), after exercise test ($r=0.62$) and 20 minutes post exercise ($r=0.63$) No correlations were significant when change in V_{NO} was expressed in percent, with the exception of a weak correlation V_{NO} 10 minutes post exercise ($r=0.44$).

4.7 Individual values and variety

Baseline FE_{NO}, measured offline, was 16.3 ppb (14.3, 18,3), while the mean value for online measurements was 18.5 ppb (16.7, 20.4). PE_{NO} baseline values were 1.51 mPa (1.32, 1.69) and 1.71 mPa (1.54, 1.88), measured offline and online, respectively. FE_{NO} values measured offline and online ranged between 4.5-34.0 and 3.9-38.3, respectively. Ranges in PE_{NO} values were 0.4-3.1 offline, and 0.4-3.6 online. Offline measures were not conducted during pretest.

Table 4-3 *Baseline FE_{NO} and PE_{NO} measures, online and offline.*

	Offline		Online	
	FE_{NO} (ppb)	PE_{NO} (mPa)	FE_{NO} (ppb)	PE_{NO} (mPa)
Hypobaric RET	15.9 (±6.5)	1.47 (±0.56)	17.7 (±7.2)	1.64 (±0.63)
Normobaric RET	16.6 (±6.9)	1.54 (±0.64)	18.7 (±7.1)	1.73 (±0.66)
Pretest	-	-	19.1 (±8.2)	1.77 (±0.76)

The mean variability coefficients (CV) among the separate days of measurements (interday variability) were 40.7% for online and 26.6% for offline PE_{NO} measurements, and 17% and 14.7% for FENO measured online and offline, respectively. No differences were seen between the baseline measurements offline. FE_{NO} measured online and offline experienced a significant correlation ($r=0.9$, $p=0.01$). No correlation were found between online and offline PE_{NO}.

Great varieties of PE_{NO} levels were seen within the subjects (figure 4-8). Baseline value range was excessive (0.46-2.81) after exercise in hypobaric environment (figure 4-8). In general, reductions of PE_{NO} are seen after exercise in all subjects, reaching its lowest value immediately after end of high intensive exercise (5 min). However the extent of reduction varies widely. A tendency of increase (return to baseline levels) is observed after exercise in some subjects, although the intra-individual differences are great here as well. There is a tendency that subjects inhabiting large FE_{NO} and PE_{NO} values tend to have a larger drop, compared to subject expiring more moderate concentrations of NO. However, relatively speaking the difference is not of probable importance.

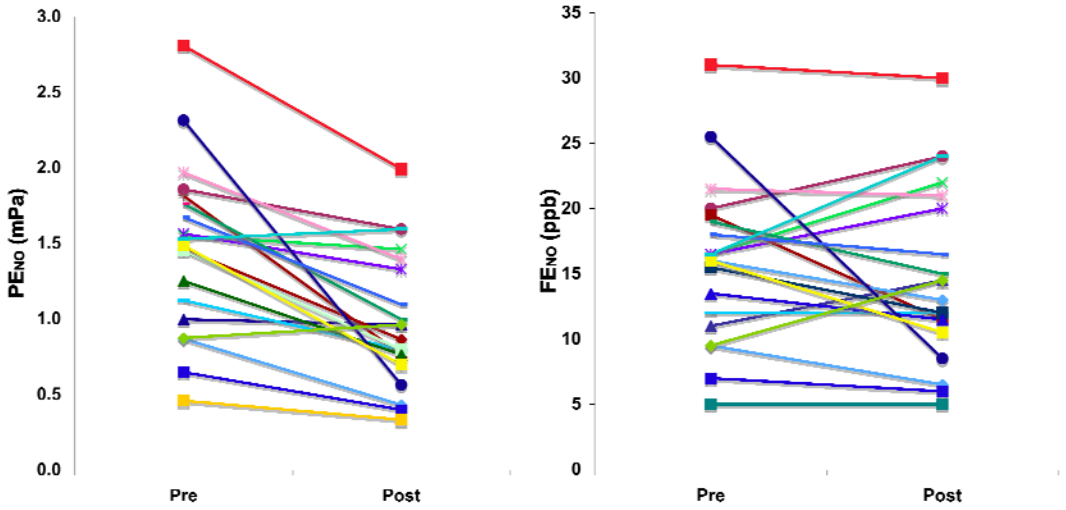


Figure 4-8 *(left). Individual PE_{NO} levels (mPa) before (pre) and 5 minutes after (post) high intensive exercise in hypobaric hypoxia.*
Figure 4-9 *(right). Individual FE_{NO} values pre and post exercise in hypobaric hypoxia*

Neither age nor mass (weight) correlated with baseline PE_{NO} offline measures conducted before each RET. However, a borderline correlation ($p=0.07$) was seen comparing height with PE_{NO}. Lung function measures (FEV_1, FVC and MEF_{50}) did not correlate with PE_{NO} baseline measurements. However, PE_{NO} baseline conducted before the hypobaric RET correlated significantly with VE_{max} ($p=0.04$). VE_{max} correlated also with PE_{NO} after warm up during the hypobaric RET ($p=0.03$).

4.8 Gender

Gender-related differences in baseline expired [NO] values, expressed in both FE_{NO} and PE_{NO}, were found (p=0.004). Significant differences (p=0.002) were seen in baseline values conducted before the hypobaric RET, the normobaric RET, as well as the pretest, both online and offline (n=200). Borderline significance was seen between NO in hypoxia and gender (r=0.06) and no difference was found between genders in normobaric conditions (p=0.1).

4.9 Online and offline measures

Strong correlations (p<0.01) were assessed between all online and offline measurements; baseline (r=0.82), 30 min (r=0.97), 60 min (r=0.96) and 24 hours (r=0.98) after hypobaric RET, and baseline (r=0.92), 30 min (r=0.96), 60 min (r=0.96) and 24 hours (r=0.98) after normobaric RET. Comparing baseline values, the level of significance (p) was stronger between online (p=0.00001) compared to offline measurements (p=0.001). Measurements assessed 24 hours after RET completion showed a significant change compared to baseline online measures, and not offline, in both hypobaric (p=0.003) and normobaric (p=0.046) pressures.

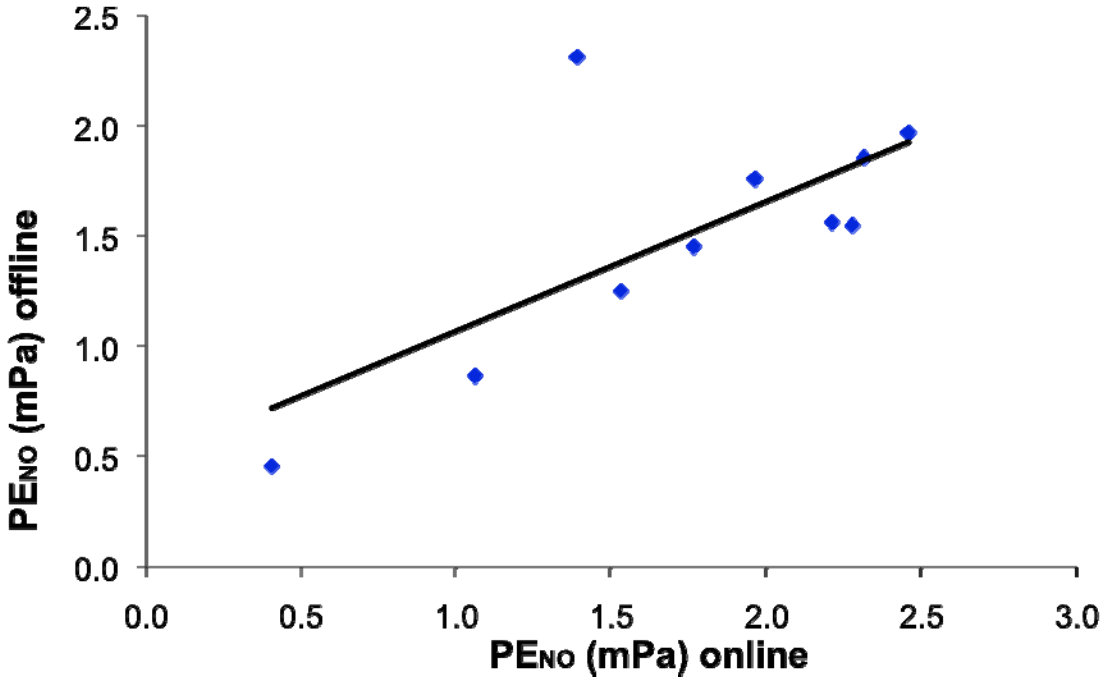


Figure 4-10 *Correlation (r=0.82) between PE_{NO} baseline measures, online and offline.*

4.10 Ambient NO

Average ambient NO (aNO) before each test was 4.4 ppb (-5.6, 14.4). The range of the NO concentration was from 0.00 to 90.00 ppb, however with only five outlying registrations with elevated NO levels above 30 ppb (32.00, 40.00, 40.00, 50.00 and 90.00 ppb). No correlation was seen between PE_{NO} and the aNO level, even when including the outlying values.

4.11 Pretest

Maximal reductions of FE_{NO} were greater immediately after end of pretest, -37,3% (32.5, 42.1), compared to RETs, -31% (-21.4, -40.6), and -28% (-19.7, -36.3) in hypobaric and normobaric exposure, respectively. PE_{NO} levels decreased as exercise intensity increased, reaching its lowest point after end of high intensive exercise (figure 4-5 and 4-11). Values increased towards baseline levels 5 minutes post exercise, yet still being significantly lower compared to baseline 60 minutes post exercise. As the pretest was conducted at the same barometric pressure, the course of PE_{NO} and FE_{NO} is similar (figure 4-12). Comparing PE_{NO} with V_E and HR during pretest (exercise with increasing exercise intensity) resulted in correlations of 0.65 and 0.42, respectively.

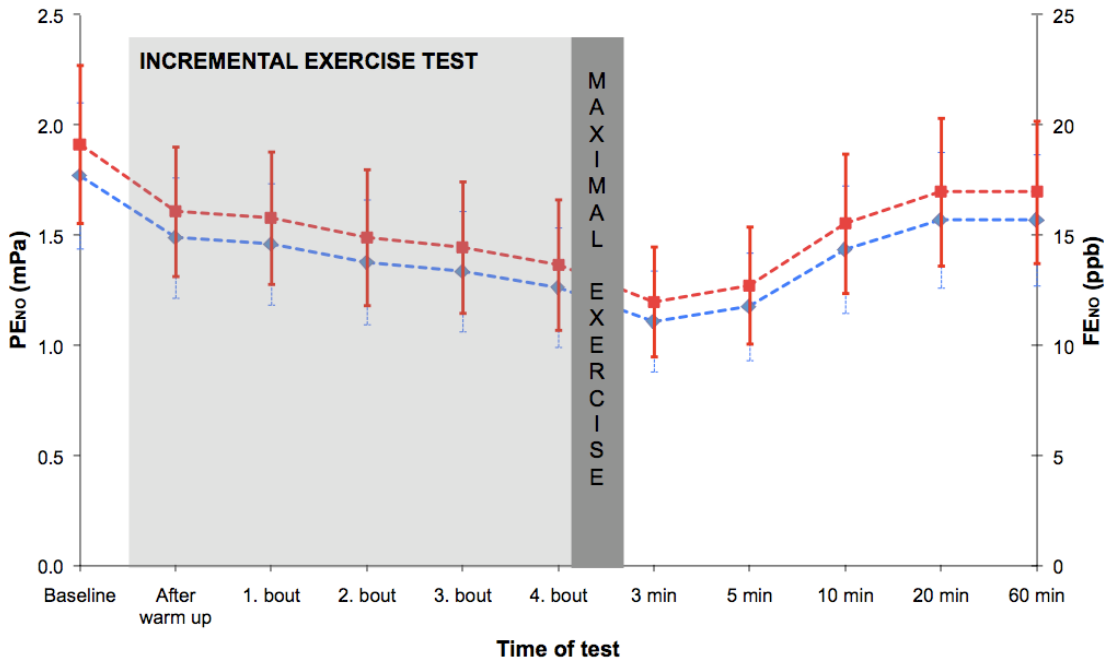


Figure 4-11 *FE_{NO} (red) and PE_{NO} (blue) values measured pre, during and post exercise performed as 5 minute running bouts with increasing intensity on a treadmill. Results are given as means with 95% confidence interval.*

5. Discussion

5.1 Main findings

The main results show that expired [NO] (FE_{NO}, PE_{NO}), measured at a standardized flow, decreases from rest to exercise, in proportion to exercise intensity, both in normobaric and hypobaric conditions. This alteration sustained for several minutes after end of exercise, in both environments. The study considered possible effects upon PE_{NO} from altitude and exercise combined, an approach that, to our knowledge, few have examined previously (Verges et al., 2005).

In a group of healthy adults our data are in agreement with those of Verges et al. (2005) who reported reduced FE_{NO} levels in athletes after high intensive exercise a hypobaric environment. De Gouw et al. (2001) and (Persson et al., 1993) observed reduction in expired [NO] after exercise, while Hemmingsson et al. (2009) and Brown et al. (2006) found reductions in PE_{NO} after exposure to hypobaric pressures. However, other authors report contradicting results. St. Croix et al. (1999) did not find a decrease, rather an increase of [NO] in expired air, after cycling at different intensities, in agreement with Bauer et al. (1994). Iwamoto et al. (1994) reported no change in expired [NO] levels after exercise. De Gouw et al. (2001) found no effect upon expired NO after treatment of a NO synthase inhibitor (L-NMMA) or a NO synthase substrate (L-arginine) on the airway response to exercise in healthy controls. Discrepancies within the literature may be explained by the lack of consistency in measurement procedures (i. e. standardized flow rates), differences in exercise intensity, altitude severity and/or duration of either interventions. To exclude possible differences in NOS activity based on airway inflammation and atopy, we included a homogeneous group of non-smoking, "non-snuffing" healthy subjects with equal level of fitness (defined as VO_{2max}), without a recent history of respiratory viral infection.

5.2 RET intensity

Heart rate (HR) and velocity ($km \cdot h^{-1}$) during RETs are lower in hypobaric pressure compared to normobaric pressure. However, no change is seen in ventilation ($l \cdot min^{-1}$) between the two exercise tests, which suggests that an equal amount of stress is applied the pulmonary system. Thus, one can argue that there is no difference in pulmonary strain/stress between the two exercise tests.

5.3 Methodological considerations

Measurement procedures were standardized according to the current recommendations (ATS/ERS, 2005), to ensure a valid comparison with other studies, as well as between rest and exercise. Flow rate and sample collection assessment (i. e. single breath vs. tidal breathing) will influence expired [NO] values (Silkoff et al., 1997) and must be taken into consideration during interpretation of data and more importantly comparison of data.

Assumptions that NO measured in expired gas is of pulmonary origin (and not nasal) must be taken into consideration in the interpretation of data from this study and studies discussed in general, as the exact origin of expired [NO] is not yet understood (Busch et al., 2001). Changes in expired [NO] does not necessarily reflect changes in vascular NO production, and differences in response of cardiac output, pulmonary capillary blood volume and circulating catecholamines can also alter NO production and/or scavenging (Busch et al., 2001). Busch et al. (2001) found no direct relationship between NO production at the vascular endothelial level and expired [NO], and only a moderate correlation between changes in expired [NO] and hypoxic response in pulmonary artery systolic pressure.

5.3.1 NO measurements in a hypobaric environment

It has recently been proposed that the barometric pressure in the location of data collection can influence flow rate. Hemmingsson and Linnarsson (2009) point out how important the awareness of how equipment and measurement procedures can be influenced by reduced barometric pressures (i. e. at altitude). Reduced gas density causes deviations from the standard expired flow rate, as well as in the detector sensitivity of the equipment. During NO sampling at different altitudes, Brown et al. (2006) standardized the rate of expiration to control for altitude-associated variation in resting ventilation: "*The expired air collected for measurement passed through the airways at the same rate at all altitudes so that NO was transferred from the airway wall to the airway lumen for the same period of time at all altitudes*[9]". Hemmingsson et al. (2009) studied the altitude effect upon two NO analysers and suggest that to avoid

[9] From Brown et al. (2006)

methodological errors, the flow regulator should be readjusted to give a volume flow of 50 $ml \cdot s^{-1}$ at the altitude of interest.

In the present study, FE_{NO} samples were assess in a hypobaric environment and collected (offline) in Mylar bags, which were later analyzed in normobaric conditions. An acclimatization of the equipment to altitude (through correcting readings according to the change in gas density on the flow regulator and on the NO detector sensitivity) was not conducted in the present study. As discrepancies in flow rate are only avoidable if the equipment is calibrated according to the current barometric pressure (Hemmingsson and Linnarsson, 2009), probable alterations in flow rate must be taken into account when interpreting the results from this study. Although, our NO measuring system use a visible needle system for the subject to control flow rate (picture 2 and 3), which has been recommended by a research group reporting that flow rate is independent of gas density (Laskowski et al., 2010). Additionally, baseline measures at the current barometric pressure should have been conducted immediately after entering the low-pressure chamber, before start of exercise. As we have no baseline value of PE_{NO} at the altitude of interest, it is hard to control the severity of alterations, from either climatic intervention or exercise, that occurred, which makes it challenging to compare the values to the normobaric RET.

It is important to take into account that the measurements made at baseline, and 30 min, 60 min and 24 hours after the hypobaric RET are executed in a normobaric pressure, outside of the climatic chamber. Another part of the present study included assessment of FE_{NO} and exercise in a cold environment. Therefore, the duration of climatic exposure needed to be adjusted, for both ethical and practical considerations. Data from the cold experiment are not presented in this thesis. Measurement conducted after warm up is the first measurement conducted at altitude. Considering this, a slight elevation, yet of no significant importance, of expired [NO] during hypoxia is observed when measures are expressed in FE_{NO} (figure 4-1). This is an alteration that is not present in the PE_{NO} values (figure 4-2). FE_{NO} values conducted 30 and 60 minutes after hypobaric exercise showed a drop. This change is not seen after the normobaric RET, and the reduction could be addressed the difference in barometric pressure at the time of sample collection. However, this theory is debatable, as no other studies, of our awareness, indicate that a change in barometric pressure would induce such a long-term alteration

in PE_{NO} (60 min samples are conducted 30 minutes after exit of the chamber). However, the 30 min post exercise measurement is conducted only minutes after exit and could be addressed the change in barometric exposure (Caspersen, Thorsen and Stensrud, 2010).

5.3.2 Sample time

Our pilot study show that PE_{NO} measures were not possible to sample during exercise, due to implications with the measurement procedure and exercise at such high intensity. Subjects were obligated to perform a single breath hold, at a standard expiratory flow, for the sufficient amount of time needed to fill the collector bag, which proved not to be possible shortly after such heavy exercise. St. Croix et al. (1999) showed that FE_{NO} measured in the first breath after the end of exercise was similar to values obtained during exercise at 60% VO_{2max}, using a constant flow technique similar to the one used in our study. This result implies that FE_{NO} measured at rest and immediately at the end of exercise can assess exercise-induced changes in pulmonary NO. In the present study, PE_{NO} sampling was assessed 5 minutes post RETs. During pretest, NO samples were conducted immediately after sub-maximal exercise bouts, and 3 minutes after maximal exercise. It is important to acknowledge that the measures sampled 5 minutes post exercise does not reflect the condition during exercise. As observed during our study, difficulties in maintaining constant mouth pressure immediately after heavy exercise is an issue. In the study by St. Croix et al. (1999) only five (out of nine) subjects completed NO sampling after exercise at 60% VO_{2max} and no one completed the breath procedure after exercise at 90% VO_{2max}. Kippelen et al. (2002) conducted a study using an exercise protocol of similar intensity (90% VO_{2max}), yet with longer duration (15 min) compared to the present study, assessing expired [NO] during exercise through tidal breathing. The results showed that the main reduction occurred during the last minutes of exercise. However, shortly after exercise (3 min) expired [NO], though still significantly reduced, was higher than the levels during exercise. As we did not measure PE_{NO} during exercise, Kippelen et al. (2002)`s finding suggest that our PE_{NO} decrement could have been greater if the measures had been conducted during or immediately after exercise. However, measurements sampled through tidal breathing, and not single breath, implies cautiousness in comparisons with our data.

5.3.3 Subjects

The subjects included in the present study were mainly derived from the Norwegian School of Sport Science (both students and staff) and turned out to be a fairly homogenous group according to age (range= 18-28 y. o.) and physical fitness level (range VO_{2max}= 47.6-64.9 ml·kg^{-1}min^{-1} and 51.7-66.9 ml·kg^{-1}min^{-1} for female and male subjects, respectively), which reduces the possibility for generalization of the results. The absence of atopy, diet and exercise on days of testing was not objectively documented in our study. However, clinical history was negative for symptoms or signs of asthma, as well as no reduction in FEV_1 was present after strenuous exercise during the pretest. The inclusion criterion of an oxygen uptake >40 ml·kg^{-1}min^{-1} for female and >50 ml·kg^{-1}min^{-1} for male, were set in order to exclude possible effects of low fitness levels or low aerobic capacity. It is speculated that high intensive exercise will produce discrepancies in FE_{NO} between athletes and sedate subjects. Endurance athletes have an increased prevalence of bronchial hyperreactivity and exercise-induced asthma (Larsson et al., 1993; Heir, Aanestad, Carlsen and Larsen, 1995), which can imply that FE_{NO} values will be of a different character considering NO's role in inflammatory diseases. Expired [NO] has found to be unchanged during exercise in highly trained athletes (Maroun et al., 1995). Chirpaz-Oddou et al. (1997) found a similar expired [NO] decline, at exhaustion, between athletes and sedate subjects. Although a higher exercise intensity was required to get significant decreased FE_{NO} in trained men compared to sedentary, values did not differ between groups for a given exercise intensity. Additionally, Chirpaz-Oddou et al. (1997) report of similar V_{NO} values between three groups of sedentary men, sedentary women and trained men, exercising at the same sub-maximal intensity, while VE was different. Differences in exercise intensity, aerob capacity, maximal ventilation, cardiac output and perfusion rate in the lung can induce different FE_{NO} values.

5.3.4 Oxygen saturation

Average O_2 saturation (S_aO_2) has been shown to decrease at altitudes similar to the one used in the present study, both during rest (Brown et al., 2006) and exercise (Verges et al., 2005). St. Croix et al. (1999) found decreased S_aO_2 during resting hypoxia (PO_2 = 14%). During hypoxic exercise at 90% VO_{2max}, S_aO_2 fell to 85,4 %. Conductions of S_aO_2 measures would probably have provided useful information, especially as the subjects in the present study were fairly good endurance trained (mean VO_{2max}=58.2

$ml \cdot kg^{-1}min^{-1}$ ±6.6) and that the study considered high intensive exercise combined with exposure to hypoxia, two of which are well-known factors of S_aO_2 decrement (Åstrand et al., 2003; Gale et al., 1985). Decreased pulmonary NO may contribute to pulmonary gas exchange abnormalities in athletes, probably through its effect on V_A/Q matching and/or pulmonary tension (Verges et al., 2005).

5.4 NO and exercise in hypoxia

To our knowledge, only one other study has previously assessed expired [NO] after exercise in hypoxia. Our results are in agreement with those of Verges et al. (2005) who showed that expired NO concentration (FE_{NO}), measured at a standardized flow rate, decreased (in proportion to exercise intensity) in both normoxic and hypoxic conditions. Important differences between these studies are that Verges et al. (2005) used inhalation of hypoxic gas, whilst in the present study, subjects exercised in reduced barometric pressure. Also, Verges et al. (2005) used a lower exercise intensity (60% P_{max}) compared to the present study. Verges et al. (2005) found a greater NO decrease in hypoxia compared to normoxia in healthy subjects. Our study showed a similar decline in both barometric pressures, with the exception of a faster reduction after warm up (21% ±19 in hypobaric environment vs. 1% ±35 at sea level, respectively). Higher cardiac output and higher ventilation are physiological alterations associated with altitude (Gale et al., 1985), which could affect NO levels by increasing NO scavenging by pulmonary blood and/or increased NO output (see chapter 5.9.2). In the present study, heart rate was significantly reduced during exercise in hypobaric environment compared to normobaric, as was running velocity, and no change was seen in VE. Verges et al. (2005) assessed FE_{NO} levels at a flow rate of 170 $ml \cdot s^{-1}$, and report 16.6% (-31.2, -2) decrease, in healthy subjects exercising at 90% P_{max} for 5 minutes in normobaric conditions (Verges et al., 2005). This decrement is slightly smaller compared to the one observed in our study. However, our protocol was of a longer duration and it was performed on a treadmill, compared to on a cycle ergometer.

5.5 Course of PE_{NO} post exercise

A decline in PE_{NO} occurred shortly after end of exercise, and sustained reduced for several minutes in both environments. PE_{NO} values returned to baseline values 15 minutes post exercise during reduced barometric pressure, and 5 minutes post exercise at sea level. In a study by Chirpaz-Oddou et al. (1997), post exercise reduction in FE_{NO}

levels ceased to be significantly reduced 5 minutes after end of exercise compared to baseline values. De Gouw et al. (2001) observed a small, but significant, decrease in expired NO concentration shortly after the exercise (<5 min) and an increase >20 min after exercise, in healthy adults. In the present study, measures assessed 30 and 60 minutes is not significant different from baseline, which suggests that the time interval is sufficient to allow complete NO recovery. This corresponds to the findings by Verges et al. (2006). Kippelen et al. (2002) found that decreased level of expired [NO] had returned to baseline values 1 hour after heavy exercise (90% VO_{2max}) in a study including both trained and untrained subjects. As we conducted PE_{NO} samples more frequently, Kippelen et al. (2002) could hypothetically experience a similar expiration by including more frequent measures.

In relative terms (%), a difference in PE_{NO} between the two environments 20 minutes post exercise is apparent (figure 4-3). After exercise in hypoxia, PE_{NO} is still reduced, though not significantly different from baseline, whilst a shift from negative to positive change (%) in PE_{NO} levels has occurred after exercise in normoxia. This shift is clearly illustrated in figure 4-3, reaching a peak 24 hours after hypobaric exercise, though not of significant importance. It is difficult to explain these differences between RETs. It seems that exercise combined with hypoxic exposure result in greater stress on the pulmonary vascular system and thus causes greater reduced PE_{NO} values that last longer in time.

5.6 Effect from exercise

The exercise protocol of high intensive exercise (>90% HR_{max}) used in this study, resembles a test protocol developed to provoke exercise-induced bronchoconstriction (EIB-test) (Carlsen, Eng and Mørk, 2000) in both duration and intensity. This protocol was chosen with the purpose to apply maximal amount of strain upon the pulmonary vascular system in order to assess potential differences in PE_{NO} values. Exercise of such high intensity (>90%HF_{peak}) as conducted in our study decreased PE_{NO} significantly, a finding supported by others (Kippelen et al., 2002; Verges et al., 2005). FE_{NO} has been found to decrease when exercise intensity exceeds 65% VO_{2peak} (Chirpaz-Oddou et al., 1997). It could seem that exercise of such high intensity is necessary to induce a significant decrease in FE_{NO} and PE_{NO}. Verges et al. (2005) found greater decrements after exercise at 60% and 90% of P_{max} compared to 40%.

In the present study, mean maximal reduction in FE_{NO} occurred after the pretest (-37,3%). As the pretest lasted for a longer period of time compared to the RET, the decrement observed in PE_{NO} values may be related to the duration of the exercise performed. Verges et al. (2006) report of a -41.5% decrease in FE_{NO} after exercise for 100 min (five bouts of 10 min at 60% of maximal power output followed by 2 min at an intensity of 25% P_{max}). In the present study, the pretest consisted of incremental exercise (AT-test) closing at a VO_{2max} test. The intensity at the VO_{2max} test was not significantly different than the intensities during the RET, and were similar in duration (data not presented). However, the preceding incremental exercise consisted of multiple bouts (mean = 4 bouts) of 5 minutes. We can only speculate that the greater reduction occurred due to the difference in duration, as the mechanisms underlying these alterations are not understood in the literature today.

The pretest showed a decline in FE_{NO} proportional to increasing exercise intensity. A result that is in agreement with other data provided by St. Croix et al. (1999), Chirpaz-Oddou et al. (1997) and Verges et al. (2005). Maroun et al. (1995) found declining FE_{NO} with increasing VO_2, and Bauer et al. (1994) found that decreased NO excretion rate correlated with HR.

5.7 FE_{NO} vs. PE_{NO}

When expressed in PE_{NO}, an expired [NO] measurement is adjusted for gas density effects (Hemmingsson et al., 2009). This is a necessity when sampling at different barometric pressures. In our data, there is a clear change in PE_{NO} when exposed to hypobaric pressure, compared to corresponding FE_{NO} values (figure 4-1 vs. 4-2). When expressed in FE_{NO}, no change is seen in expired [NO] values after exercise during hypobaric exposure compared to baseline, a difference that is apparent in PE_{NO} values. Also, FE_{NO} levels are significantly elevated in the hypobaric climate compared to normobaric. Hemmingsson et al. (2009) measured expired [NO] at different altitudes and recommend that expired [NO] should always be reported as partial pressure of NO (PE_{NO}), and not as volume fraction of NO (FE_{NO}). This ensures comparison between measurements at different altitudes. Although, Hemmingsson et al. have experienced critique from colleagues regarding methodological issues (Laskowski et al., 2010), expired [NO] was decided to be expressed in PE_{NO} in this study, as recommended by Hemmingsson et al. (2009) and Brown et al. (2006).

5.8 Altitude effect

The chosen severity of altitude in this study was considered to be sufficient to achieve alterations in PE_{NO} levels, as well as for subjects to be able to sustain the high exercise intensity required. Dweik et al. (1998) showed a reduction of 20% in FE_{NO} in resting subjects breathing a gas mixture (15% oxygen) corresponding to about 2900 masl.

An interesting difference between PE_{NO} after exercise in the two barometric pressures is the reduction of PE_{NO} observed after warm up in hypoxia, a reduction not seen after the normobaric RET. Verges et al. (2006) found a similar progressive decrease in FE_{NO} after warm up (-24 %). However, their warm up lasted 30 minutes, at 25% maximal power output. In the present study, PE_{NO} reduction sustained for a longer period of time after exercise in hypobaric climate (15 min) compared to sea level (5 min). It is difficult to explain the discrepancies between the two exercise tests, as one can argue that exercise intensity (HR and running speed) was higher during exercise in normoxia, and not the contrary. Still, the subject's individual feeling of exhaustion combined with the test leaders impression of exhaustion were similar, or even greater, in hypoxia compared to normoxia.

5.9 Possible hypotheses of reduced NO

The exact mechanisms explaining the reduction in PE_{NO} after exercise and exposure to reduced hypobaric pressure are not completely understood. Verges et al. (2005) reviews reasons for decreases in expired NO concentrations during hypoxic exercise as an increase in NO elimination through expiration (V_{NO}, NO output), blood circulation and haemoglobin scavenging, and/or decreased NO production within the airways. These potential mechanisms cannot be explained from the data collected from this study, as no such measurements were conducted.

5.9.1 Ventilation

Exposure to both barometric pressures induced a similar V_E during exercise, suggesting that NO elimination through expired air was also similar. Still, there is a significant difference in NO output (V_{NO}) between exercise in normoxia and hypoxia. A difference is observed in HR during the exercise test, which can suggest elimination through blood, and can thus account for differences in V_{NO}. However, V_{NO} during hypobaric hypoxia experience a greater change in percent, but the HR is notably lower compared

to normoxia. In absolute values, V_{NO} is larger during exercise at sea level compared to simulated altitude.

Inspired air is being warmed up and saturated with humidification before reaching the alveoli (Åstrand et al., 2003). Increased ventilation (i. e. during exercise) can thus cause increased heat and water loss from the respiratory tract, when both heat and water are provided to the airway epithelium by increased blood flow and vasodilation. As NO induces vasodilation of bronchial vessels (Barnes and Belvisi, 1993), an increase in V_{NO} during exercise may be related to pulmonary vasodilation. However, as a protective response, vasoconstriction of airway lumen and pulmonary vessels occurs in attempt to reduce this loss (Åstrand et al., 2003). Reduced NO production, due to the known dilating effect of NO, has been proposed to explain reduced expired NO concentrations post exercise.

5.9.2 V_{NO}

NO output (V_{NO}) is the product of expired [NO] (ppb) and VE ($l \cdot min^{-1}$), and is often expressed in $nmol \cdot min^{-1}$ (Kippelen et al., 2002; Phillips et al., 1996; Verges et al., 2006). In the current study, differences between V_{NO} after exercise in normobaric and hypobaric environments have been found (figure 4-6). In normoxia, a larger V_{NO} is experienced immediately after exercise, but the percent increase is larger during hypobaric exposure (figure 4-7). As no change was seen in VE between the two exercise tests in our study, it is difficult to argue that the differences observed between the RETs were correlated with ventilation. Especially since documented changes occurred in HR and running speed. In the literature, an increased elimination rate of NO from the lungs (V_{NO}) is thought to be due to an increased ventilation rate. This can either remove NO from tissue stores or decrease the relative fraction of endogenous NO lost to the blood in the pulmonary circulation (Shin et al., 2003). Still, PE_{NO} reductions were seen in both environments, as well as increases in VE and V_{NO}. It should be stated that conductions of PE_{NO} and VE measures were not executed at the same time during the present study. PE_{NO} was assessed immediately after VE, with the exception of immediately post exercise test, where PE_{NO} measures were conducted 5 minutes after exercise, whilst VE was assessed during the first minute after exercise.

Several studies have considered the effect of exercise upon levels of expired [NO] in healthy subjects, demonstrating an increased release of NO during and shortly after exercise (Chirpaz-Oddou et al., 1997; Iwamoto et al., 1994; Maroun et al., 1995; Bauer et al., 1994). V_{NO} increases in proportion to exercise intensity and decreases upon recovery (Chirpaz-Oddou et al., 1997). A linear relationship between NO output and increasing exercise has been described by Phillips et al. (1996). NO output increases in proportion to exercise intensity in both athletes and sedentary subjects (Kippelen et al., 2002; Maroun et al., 1995; Phillips et al., 1996). Two physiological hypotheses are dominant in the literature, trying to explain the well-documented increase in NO output during exercise: increased V_E and increased blood flow. High levels of V_E during exercise have been proposed to explain the changes in V_{NO}, as resting isocapnic hyperventilation has shown to induce an increase in V_{NO} (Phillips et al., 1996; St. Croix et al., 1999; Kippelen et al., 2002). However, contradictory evidence has also been reported (Iwamoto et al., 1994; Bauer et al., 1994). Phillips et al. (1996) suggest that increased V_{NO} is related to increased ventilation through the pulmonary circulation during exercise. Increase in V_{NO} during exercise can reflect a rise in NO production by airway epithelial cells related to increased or turbulent airflow (Phillips et al., 1996; St. Croix et al., 1999). Others suggest that increased V_{NO} is caused by exercise-induced pulmonary inflammations (Bonsignore et al., 2001). Exercise-induced increases in V_{NO} have been linked to increases in cardiac output, through increased shear stress on the pulmonary vascular endothelium (Bauer et al. 1994; Chirpaz-Oddou et al. 1997; Maroun et al. 1995). As endothelial release of NO can result from vascular shear stress, both the pulmonary and systemic vasculature can cause increased NO by augmentation of CO during exercise (St. Croix et al., 1999; Bauer et al., 1994; Maroun et al., 1995). However, attempts to try to modify pulmonary blood flow in humans by injection of dopamine have failed to alter V_{NO} measured in expired air (Phillips et al., 1996).

V_{NO} correlated with VO_2 and VE in the present study. Similar correlations have been reported by Chirpaz-Oddou et al. (1997), Maroun et al. (1995) and Bauer et al. (1994). As these are parameters that increase during exercise, this data suggests that V_{NO} can be related to physiological mechanisms linked to the magnitude of the aerobic energy expenditure during exercise. Maroun et al. (1995) found a higher NO output in athletes, which may be attributed the higher VE or cardiac output in athletes during exercise. Moreover, NO output has been found to correlate with VO_2 and HR in athletes, but not

in sedentary and intermediate subject (Maroun et al., 1995). As all of these variables correlate with one another, it is difficult to know how they relate to NO.

Differences in NO measurements can also explain differences seen in V_{NO} values in the literature. St. Croix et al. (1999) calculated V_{NO} as: VE * $C_{ET}NO$ (whereas $C_{ET}NO$ represent the last part of each tidal breath). Still, St. Croix et al. (1999) report of increased V_{NO} during exercise, but no change in $C_{ET}NO$ after exercise. Similar NO measurements were conducted by Chirpaz-Oddou et al. (1997). In the present study, V_{NO} was calculated from PE_{NO} values, expressed in mPa, as expired NO measures were conducted in different barometric pressures. This makes the results difficult to compare with other studies in absolute values.

5.9.3 NO in blood

NO has a high affinity for hemoglobin (Gaston et al., 1994). During exercise HR is increased. Thus, blood flow in the pulmonary circulation increases, which can increase the amount of NO fixed by the Hb and explain reduced FE_{NO} levels after exercise. Due to the high diffusing capacity of the lungs for NO, NO might be transported into the pulmonary capillary blood (Hyde et al., 1997), thus the partial pressure of NO in the capillaries will be extremely low and the concentration gradient between the cells lining the airway and the capillary blood will be high (St Croix et al., 1999). On the contrary, Gaston et al. (1994) argues that because of the short half-life of NO in physiological systems combined with the high affinity of NO for Hb, it is unlikely that NO from the vascular compartment (capillary blood or endothelium) diffuses into the airways.

5.9.4 Nasal NO contribution, during rest and exercise

The nasal cavities contain the highest concentration of NO in the respiratory organs (Schedin et al., 1995). Research show that the contribution from nasal NO on FE_{NO} decreases during exercise, whilst non-nasal (from lungs and lower airways) increases (Phillips et al., 1996). A change of ventilation from the nose to the mouth during exercise can explain the reduction seen in FE_{NO} during/after exercise. The reduction of FE_{NO} during exercise can be attributed this respiratory change, as it has been shown that nasal NO contributes as much as 50% to expired NO concentrations (Phillips et al, 1996). Removal of contribution from nasal NO, through balloon occlusion, has shown to reduce FE_{NO} during both rest and exercise (Phillips et al, 1996).

5.9.5 Increased inflammatory drive after exercise.

Physical activity, especially repeated prolonged exercise, may cause changes in airway cells and cause pulmonary inflammation and release of cytokines, induced by high levels of ventilation associated with exercise (Bonsignore et al., 2001). Increased inflammatory drive after exercise could confound interpretation of data on NO and exercise. However, usually a decreased FE_{NO} is observed after exercise, and not an increase as would be expected during elevated inflammatory activity. Athletes often experience a different kind of asthma (Stensrud, 2007). Some athletes develop symptoms from exercise that often adhere when the athlete quit competing. Bonsignore et al. (2001) found increased FE_{NO} values after a marathon race. However, NO measurements were conducted several hours after exercise, which weakens the strength of this study. Other factors such as pollution and duration of exercise, could affect FE_{NO} during a marathon race. In the present study, no increase in PE_{NO}, measured offline, was observed 24 hours after the RETs. It seems unlikely that heavy exercise induces a large inflammatory reaction, a finding confirmed by Kippelen et al. (2002). Verges et al. (2006) found that five repetitive prolonged exercise sessions, separated by 24 hours in minimum, did not result in significant differences at baseline FE_{NO} or greater post-exercise values.

5.9.6 Reduced barometric pressure

Hemmingsson and Linnarsson (2009) suggest that there are physical effects of the reduced ambient pressure, rather than metabolic effects of hypoxia that are the primary factors behind decreased PE_{NO} at altitude. In their study PE_{NO}, values were decreased at altitude, while no change was seen at sea level with subjects breathing different hypoxic gases. This is in agreement to a recent published study showing that PE_{NO} was decreased during exposure to hypobaric environment (Caspersen et al., 2010), and also to observations by Dweik (1998), who found limited effects of PO_2 on expired [NO] within a PO_2 range of 15-30 kPa, but a marked reduction below a PO_2 of 10kPa. Busch et al. (2001) showed no effect of inspired PO_2 down to 12 kPa in healthy subjects. Verges et al. (2005) found even a slight increase in expired NO in moderate hypoxia (15% O_2).

5.10 NO and lung function

A post-exercise bronchodilation, measured by increased FEV_1 after strenuous exercise, could increase the surface area for diffusion of NO (Shin et al., 2003). This contradicts the theory of a reduced NO production response to minimize the loss of heat and water from the airways, during high ventilation levels related to exercise. Lung function tests were only assessed before and after the pretest as a subject exclusion criterion, and not during RETs. Our study did not find a relationship between PE_{NO} and lung function during pretest. Sachs-Olsen et al. showed that lung function tests (FEV_1, FEF_{50} and FVC, % predicted values) were strongly associated with FE_{NO} in non-asthmatic athletes. The authors speculate that the discrepancies possible might be due to the difference in airway surface area. On the other hand, Olivieri et al. (2006) did not find any significant correlation between FE_{NO} and lung function in 204 healthy non-smoking adults, as did Verges et al. (2006).

5.11 Individual differences

Because of the large inter-individual and intra-individual variations, FE_{NO} values are expressed in percentage of resting values in most studies, ours among them.

Our data is in agreement with the literature, whereas a wide variety in individual FE_{NO} values is reported. Corradi et al. (1998) measured expired NO concentrations in 78 subjects and found ranges between 5-58.5 ppb, 5-54.7 ppb and 4.4-52 ppb in three different samples. This grand range of values in expired [NO] levels of healthy subjects could create some difficulties when comparing healthy subjects with patients. Taylor et al. (2006) suggest that group mean data may not be helpful in determining clinically relevant changes in individual patients, citing a study (Jones et al., 2001) who found a very large range (-10 to + 141 ppb). In our study we also experienced large ranges, which in turn resulted in large standard deviations (percent change in FE_{NO} after warm up = -1% ±35, [range= -32 to +96 %]).

Great day-to-day differences were observed in baseline values, in both online and offline measures. Different factors affect levels of expired [NO], as discussed earlier in this thesis. Discrepancies in diet, exercise or inflammation could possible interfere, though participants did not report any symptoms or changes in diet. This emphasizes the

need for long-term monitoring of individual FE_{NO} levels to use in pathophysiology, in example asthma monitoring.

5.12 Gender

There is still some disagreement about whether gender affects FE_{NO} levels (Taylor et al., 2006). Results from the present study showed a significant difference in baseline PE_{NO} ($p=0.01$) and FE_{NO} ($p<0.01$) between men and women. Values from female subjects occurred to be lower than males, a relationship also shown by Olivieri et al. (2006). Olivieri et al. (2006) measured FE_{NO} (n=178) at three different flow rates (50, 100 and 200 $ml \cdot s^{-1}$), compared to our one (50 $ml \cdot s^{-1}$). After adjusting for age, height, weight, body mass index (BMI) and body surface area (BSA), a gender specific difference was found apparent. Olivieri et al. (2006) therefore suggests, in agreement with Sachs-Olsen et al. (unpublished), that the difference in airway surface area and internal diameter of airways is causing this variation. Thus, similar flow rate in airways of various diameters may differently dilute NO.

5.13 The use of NO as a predictor of disease

During the test period (Sept, 2009-Jan, 2010), we used ourselves as biological controls for repetitive measurements to ensure valid and stable measuring methods. Through this repetitive sampling, we experienced that inter-individual FE_{NO} levels are stable on a long-term basis. From our regular testing we experienced sudden discrepancies during periods of disease (pulmonary, inflammatory), i. e. colds and viral infections. FE_{NO} levels increased dramatically, even before registration of symptoms, and were elevated in a period of about 2 weeks post illness. This alteration occurred without exception, and we consider this an important finding that underlines the usage of NO as a biomarker. NO is today vastly used as an inflammatory biomarker and we suggest future research to enhance assessments of the use of NO as a predictor of non-chronic disease or illness, along with the importance of research including healthy subject, in regards to be able to understand the fundamental mechanisms of nitric oxide.

5.14 Possible negative effects of reduced NO

The physiological or pathophysiological effects of such a decrease in NO availability remains to be clarified. Verges et al. (2006) speculate that a prolonged decrease in NO availability can be damaging, especially for an endurance athlete, because of the

involvement of NO in several biological functions of the respiratory system. Gaston et al. (1994) proposes that decreased FE_{NO} may contribute to alterations of the immune system.

5.15 Ambient NO concentration

We found no correlation between FE_{NO} and atmospheric NO concentration (aNO), in contrast to the observations made by Corradi et al. (1998). Therefore, aNO cannot be proposed as a potential confounding factor in the present study. Our measurements were primarily conducted on days with low levels of aNO, with the exception of five days where aNO levels exceeded 30 ppb. Corradi et al. (1998) states that the correlations between aNO and FE_{NO} disappear when aNO is lower than 35 ppb. Although Corradi et al. (1998) did not measure FE_{NO} following an standardized inspiration with NO free air.

5.16 Conclusion

The results from this study show that PE_{NO} decreases after high intensive exercise during hypobaric hypoxia corresponding to an altitude of 2800 masl. Differences between exercise at altitude and at sea level are apparent after warm up. Exercise during exposure to reduced barometric pressure induces a faster response in PE_{NO} decrement compared to normoxia and sustains for a longer period of time. An inverse relationship between FE_{NO} and exercise intensity were established during pretest. FE_{NO} levels decreased as exercise intensity increased, reaching its lowest point shortly after end of high intensive exercise. Values started to increase towards baseline levels 5 minutes post exercise, and still being significantly lower compared to baseline 60 minutes post exercise.

The study confirms the importance of standardized measurement procedures, as well as correcting for gas density during measurements at different barometric pressures, to ensure correct results.

References

ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide. (2005). *Am. J. Respir. Crit. Care Med., 171*(8), 912–930.

Alving, K., Weitzberg, E. and Lundberg, J. M. (1993). Increased amount of nitric oxide in exhaled air of asthmatics. *Eur Respir J., 6*, 1368-1370.

Barnes, P. J. & Belvisi, M. G. (1993). Nitric oxide and lung disease. *Thorax, 48*, 1034-1043.

Bauer, J. A., Wald, J. A., Doran, S. and Soda, D. (1994). Endogenous nitric oxide in expired air: effects of acute exercise in humans. *Life Sci., 55*(24), 1903-1909.

Blitzer, M. L., Loh, E., Roddy, M., Stamler, J. S. and Creager, M. A. (1996). Endothelium-derived nitric oxide regulates systemic and pulmonary vascular resistance during acute hypoxia in humans. *JACC, 28*(3), 591-596.

Blitzer, M. L., Lee, S. D. and Creager, M. A. (1996). Endothelium-derived nitric oxide mediates hypoxic vasodilation of resistance vessels in humans. *Am J Physiol., 271*(40), 1182-1185.

Bonsignore, M. R., Morici, G., Riccobono, L., Insalaco, G., Bonanno, A., Profita, M., Paternò, A., Vassalle, C., Mirabella, A. and Vignola, A. M. (2001). Airway inflammation in nonasthmatic amaterur runners. *Am J Physiol Lung Cell Mol Physiol., 281*, 668-676.

Borland, C., Cox, Y. and Higenbottam, T. (1993). Measurement of exhaled nitric oxide in man. *Thorax, 48*(11), 1160-1162.

Brown, D. E., Beall, C. M., Strohl, K. P. and Mills, P. S. (2006). Exhaled nitric oxide decreases upon acute exposure to high-altitude hypoxia. *American Journal of Human Biology, 18*, 196-202.

Busch, T., Bärtsch, P., Pappert, D., Grünig, E., Hildebrandt, W., Elser, H., Falke, K. J. and Swenson, E. R. (2001). Hypoxia decreases exhaled nitric oxide in mountaineers susceptible to high-altitude pulmonary oedema. *Am J Crit Care Med, 163*, 368-373.

Byrnes, C. A., Dinarevic, S., Busst, C., Bush, A. and Shinebourne, E. A. (1997). Is nitric oxide in exhaled air produced at airway or alveolar level? *Eur Respir J., 10*(5), 1021-1025.

Carlsen, K-H., Engh, G. and Mørk, M. (2000). Exercise-induced bronchoconstriction depends on exercise load. *Respir Med., 94*(8), 750-755.

Carter J. and Jeukendrup A. E. (2002). Validity and reliability of three commercially available breath-by-breath respiratory systems. *Eur J Apply Physiol., 86*(5), 435-441.

Caspersen, C., Thorsen, E. and Stensrud, T. (2010). FE_{NO} and PE_{NO} at 2800 m altitude. *In abstract*, ATS. University of Bergen, Norway.

Chang, H. K. (1985). *General concepts of molecular diffusion*. In: In engel, L.A., Paiva, M. (Eds.), Gas Mixing and Distribution in the Lung. Marcel Dekker, Inc., New York, USA, p. 1-22.

Chirpaz-Oddou, M. F., Favre-Juvin, A., Flore, P., Eterradossi, J., Delaire, M., Grimbert, F. and Therminarias, A. (1997). Nitric oxide response in exhaled air during an incremental exhaustive exercise. *J Appl Physiol, 82*(4), 1311-1318.

Corradi, M., Pelizzoni, A., Majori, M., Cuomo, A., Munari, E. M. and Pesci, A. (1998). Influence of atmospheric nitric oxide concentration on the measurement of nitric oxide in exhaled air. *Thorax, 53*, 673-676.

De Gouw, H. W. F. M., Marshall-Partridge, S. J., Van Der Veen, H., Van Den Aardweg, J. G., Hiemstra, P. S. and Sterk, P. J. (2001). Role of nitric oxide in the airway response to exercise in healthy and asthmatic subjects. *Journal of Applied Physiology, 90*, 586-592.

Dweik, R. A., Laskowski, D., Abu-Soud, H. M., Kaneko, F. T., Hutte, R., Stuehr, D. J. and Erzurum, S. C. (1998). Nitric oxide synthesis in the lung. *J. Clin. Invest., 101*(3), 660-666.

ERS (European Respiratory Society). (1997). Clinical exercise testing with reference to lung diseases: indications, standardization and interpretation strategies. ERS Task Force on Standardization of Clinical Exercise Testing. *Eur Respir., 10*(11), 2662-2689.

Fredriksen, P. M., Ingjer, F., Nystad, W. and Thaulow, E. (1998). Aerobic endurance testing of children and adolescents – a comparison of two treadmill-protocols. *Scand J Med Sci Sports, 8*(4), 203-207.

Gale, G. E., Torre-Bueno, J. R., Moon, R. E., Saltzman, H. A. and Wagner, P. D. (1985). Ventilation-perfusion inequality in normal humans during exercise at sea level and simulated altitude. *J Appl Physiol, 58*(3), 978-988.

Gaston, B., Drazen, J. M., Loscalzo, J. and Stamler, J. S. (1994). The biology of nitrogen oxides in the airways. *Am J Respir Crit Care Med., 149*, 538-551.

Gustafsson, L. E., Leone, A. M., Persson, M. G., Wiklund, N. P. and Moncada, S. (1991). Endogenous nitric oxide is present in the exhaled air of rabbits, guinea pigs and humans. *Biochemical and Biophysical Research Communications, 181*(2), 852-857.

Güzel, N. A., Sayan, H. and Erbas, D. (2000). Effects of moderate altitude on exhaled nitric oxide, erythrocytes lipid peroxidation and superoxide dismutase levels. *Japanese Journal of Physiology, 50*, 187-190.

Heir, T., Aanestad, G., Carlsen, K-H. and Larsen, S. (1995). Respiratory tract infection and bronchial responsiveness in elite athletes and sedentary control subjects. *Scand J Med Sci Sports, 5*, 94-99.

Helgerud, J., Ingjer, F. and Strømme S. B. (1990). Sex differences in performance matched marathon runners. *Eur J Appl Physiol., 61*, 433-439.

Hemmingsson, T., Horn, A. and Linnarsson, D. (2009). Measuring exhaled nitric oxide at high altitude. *Respiratory Physiology and Neurobiology, 167*(3), 292-8.

Hemmingsson, T. and Linnarsson, D. (2009). Lower exhaled nitric oxide in hypobaric than in normobaric acute hypoxia. *Respiratory Physiology and Neurobiology, 169,* 74-77.

Hemmingsson, T. E. (2010). Reply to the letter to the editor. Response to Beall et al. Re: "Lower exhaled nitric oxide in hypobaric than normobaric acute hypoxia" by T. Hemmingson and D. Linnarsson [Respir. Physiol. Neurobil. 169 (2009) 74-77] *Respiratory Physiology & Neurobiology, 170*(3), 213-214.

Hermansen, L. (1973). Oxygen transport during exercise in human subjects. *Acta Physiol Scand Suppl., 399*, 1-104.

Hyde, R. W., Geigel, E. J., Olszowka, A. J., Krasney, J. A., Forster, R. E. 2nd, Utell, M. J. and Frampton, M. W. (1997). Determination of production of nitric oxide by lower airways of humans—theory. *J Appl Physiol., 82*(4), 1290-1296.

Ignarro, L. J., Buga, G. M. & Wood, K. S. (1987) Endothelium-derived relaxing factor is nitric oxide. *Proc Natl Acad Sci USA, 84*, 9265-9269.

Ignarro, L. J., Ross, G. and Tillisch, J. (1991). Pharmacology of endothelium derived nitric oxide and nitrovasodilators. *West J Med., 154*(1), 51-62.

Iwamoto, J., Pendergast, D. R., Suzuki, H. and Krasney, J. A. (1994). Effect of graded exercise on nitric oxide in expired air in humans. *Respiration Physiology, 97*, 333-345.

Jörres, R. A. (2000). Modelling the production of nitric oxide within the human airways. *Eur Respir J., 16*(3), 555-560.

Kerckx, Y. and Van Muylem, A. (2009). Axial distribution heterogeneity of nitric oxide airway production. *J Appl Physiol., 106*, 1832-1839.

Kharitonov, S. A., Yates, D., Robbins, R. A., Logan-Sinclair, R., Shinebourne, E. A. and Barnes, P. J. (1994). Increased nitric oxide in exhaled air of asthmatic patients. *The Lancet, 343*(8890), 133-135.

Kharitonov, S. A., Alving, K. & Barnes, P. J. (1997). Exhaled and nasal nitric oxide measurements: recommendations. *Eur Respir J., 10*, 1683-1693.

Kharitonov, S. A. and Barnes, P. J. (2000). Clinical aspects of exhaled nitric oxide. *Eur Respir J., 16*(4), 781-792.

Kharitonov, S. A. and Barnes, P. J. (2006). Exhaled biomarkers. *Chest, 130*, 1141-1146.

Kippelen, P., Caillaud, C., Robert, E., Masmoudi, K. and Préfaut, C. (2002). Exhaled nitric oxide level during and after heavy exercise in athletes with exercise-induced hypoxemia. *Eur J Physiol., 222*, 397-404.

Kjelkenes, I. and Thorsen, E. (2008). Time course of the reduction in nitric oxide concentration in exhaled gas after exposure to hyperbaric hypoxia. *Proceedings of XXXIVth Annual meeting of European Undersea & Baromedical Society (eds. Smolle-Juettner, F., Ratzenhofer, B. and Beuster, W. Gras*, 14-18.

Larsson, K., Ohlsen, P., Larsson, L., Malmberg, P., Rydstrom, P. O. and Ulriksen, H. (1993). High prevalence of asthma in cross-country skiers. *BMJ, 307*, 1326-1329.

Laskowski, D., Beall, C. M., Dweik, R., Strohl, K. P., Hutte, R. and Erzurum, S. C. (2010). Letter to the editor. Response to Hemmingsson, Horn and Linnarsson article "Measuring exhaled nitric oxide at high altitude" Reps. Physiol. Neurobiol. 167(3), 292-298. *Respiratory Physiology & Neurobiology, 170*, 1-2.

Le Cras, T. D. and McMurtry, I. (2001). Nitric oxide production in the hypoxic lung. *American journal of physiology – Lung Cellular and Molecular Physiology, 280*, 575-582.

Macfarlane, P. W., Coleman, E. N., Pomphrey, E. O., McLaughlin, S., Houston, A. and Aitchison, T. (1989). Normal limits of the high-fidelity pediatric ECG. Preliminary observations. *J Electrocariol., 22*(suppl.), 162-168.

Maroun, M. J., Mehta, S., Turcotte, R., Cosio, M. G. and Hussain, S. N. A. (1995). Effects of physical conditioning on endogenous nitric oxide output during exercise. *J Appl Physiol., 79*(4), 1219-1225.

Michel, T. & Feron, O. (1997). Nitric oxide synthases: Witch, Were, How, and Why? *J Clin Invest., 100*, 2146-52.

Moncada, S. & Higgs, E. A. (1993). The L-arginine-nitric oxide pathway. *N Engl J Med., 329*, 2002-12.

Olivieri, M., Talamini, G., Corradi, M., Perbellini, L., Mutti, A., Tantucci, C. and Malerba, M. (2006). Reference values for exhaled nitric oxide (reveno) study. *Respiratory Research, 7*(94).

Palmer, R. M., Ferrige, A. G. & Moncada, S. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. [Abstract]. *Nature, 327*, 524-526.

Palmer, R. M., Ashton, D. S. and Moncada, S. (1988). Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature, 333*, 664-666.

Persson, M. G., Gustafsson, L. E., Wiklund, N. P, Moncada, S. and Hedqvist, P. (1990). Endogenous nitric oxide as a probable modulator of pulmonary circulation and hypoxic pressor response in vivo. *Acta Physiol Scand., 140*(4), 449-457.

Persson, M. G., Wiklund, N. P. and Gustaffson, L. E. (1993). Endogenous nitric oxide in single exhalations and the change during exercise. *Am Rev Respir Dis., 148*(5), 1210-1214.

Phillips, C. R., Giraud, G. D. and Holden, W. E. (1996). Exhaled nitric oxide during exercise: site of release and modulation by ventilation and blood flow. *J Appl Physiol., 80*(6), 1865-1871.

Quanjer P. H., Tammeling G.J., Cotes J. E,, Pedresen O. F., Peslin R. and Yernault J. C. (1993). Lung volumes and forced ventilator flows. Report Working Partly Standardization of Lung Functions Test, European Community for Steel and Cole. Official Statement of the European Respiratory Society. *Eur Respir J – Supplements, 16*, 5-40.

Ricciardolo, F. L. M. (2003). Multiple roles of nitric oxide in the airways. *Thorax, 58*, 175-182.

Sachs-Olsen, C., Stensrud, T., Hem, J. E., Mowinckel, P. and Carlsen, K-H. (**unpublished**). Exhaled nitric oxide and lung function in elite cross country skiers and speed skaters.

Sartori, C., Lepori, M., Busch, T., Duplain, H., Hildebrandt, W., Bärtsch, P., Nicod, P., Falke, K. J. and Scherrer, U. (2000). Exhaled nitric oxide does not provide a marker of vascular endothelial function in healthy humans. *Am J Respir Crit Care Med., 161*(6), 2113-2114.

Schedin, U., Frostell, C., Persson, M. G., Jakobsson, J., Andersson, G. and Gustafsson, L. E. (1995). Contribution from upper and lower airways to exhaled endogenous nitric oxide in humans. *Acta Anaesthesiol Scand, 39*(3), 327-332.

Schmetterer, L., Strenn, K., Kastner, J., Eichler, H. G. and Wolzt, M. (1997). Exhaled NO during graded changes in inhaled oxygen in man. *Thorax, 52*(8), 736-738.

Sheel, A. W., Road, J. and McKenzie, D. C. (1999). Exhaled Nitric Oxide During Exercise. *Sports Medicine, 28*(2), 83-90.

Shin, H. W., C. M. Rose-Gottron, D. M. Cooper, N. Hill, and S. C. George. (2003). Impact of high-intensity exercise on nitric oxide exchange in healthy adults. *Medicine and Science in Sports and Exercise, 35*(6), 995-1003.

Shin, H. W., Condorelli, P. and George, S. C. (2006). Examining axial backdiffusion of nitric oxide in the lungs using heliox and breath hold. *J Appl Physiol,, 100*, 623-630.

Silkoff, P. E., McClean, P. A., Slutsky, A. S., Furlott, H. G., Hoffstein, E., Wakita, S., Chapman, K. R., Szalai, J. P. and Zamel, N. (1997). Marked flow-dependence of exhaled nitric oxide using a new technique to exclude nasal nitric oxide. [Abstract]. *Am J Respir Crit Care Med., 155*(1), 260-267.

St. Croix, C. M., Wetter, T. J., Pegelow, D. F., Meyer, K. C. and Dempsey, J. A. (1999). Assessment of nitric oxide formation during exercise. *Am J Respir Crit Care Med., 159*, 1125-1133.

Stark, H., Purokivi, M., Kiviranta, J., Randell, J. and Tukiainen. H. (2007). Short-term and seasonal variations of exhaled and nasal NO in healthy subjects. *Respiratory medicine, 101*, 265-271.

Stensrud, T. (2008). *Asthma and exercise: Climatic effects upon exercise-induced bronchoconstriction and exercise capacity in asthmatic subjects*. Dissertation from the Norwegian School of Sport Sciences.

Sumino, H., Sato, K., Sakamaki, T., Masuda, H., Nakamura, T., Kanada, T. and Nagai, R. (1998). Decreased basal production of nitric oxide in patients with heart disease. *Chest, 113*(2), 317-322.

Taylor, D. R., Pijnenburg, M. W., Smith, A. D. and De Jongste, J. C. (2006). Exhaled nitric oxide measurements: clinical application and interpretation. *Thorax, 61*, 817-827.

Torre, O., Olivieri, D., Barnes, P. J. and Kharitonov, S. A. (2008). Feasibility and interpretation of FE(NO) measurements in asthma patients in general practice. *Respir Med, 102*(10), 1417-1424.

Van Muylem, A., Noël, C. and Paiva, M. (2003). Modelling of impact of gas molecular diffusion on nitric oxide expired profile. *J Appl Physiol., 94*(1), 119-127.

Vaughn, M. W., Huang, K. T., Kuo, L. and Liao, J. C (2000). Erythrocytes possess an intrinsic barrier to nitric oxide consumption. *J Biol Chem., 275*(4), 2342-2348.

Verges, S., Flore, P., Favre-Juvin, A., Lévy, P. and Wuyam, B. (2005). Exhaled nitric oxide during normoxic and hypoxic exercise in endurance athletes. *Acta Physiol Scand., 185*, 123-131.

Verges, S., Tonini, J., Flore, P., Favre-Juvin, A., Lévy, P. and Wuyam, B. (2006). Exhaled nitric oxide in single and repetitive prolonged exercise. *Journal of Sports Sciences, 24*(11), 1157-1163).

Yates, D. H., Kharitonov, S. A., Thomas, P. S. and Barnes, P. J. (1996). Endogenous nitric oxide is decreased in asthmatic patients by an inhibitor of inducible nitric oxide synthase. *Am J Respir Crit Care Med., 154*(1), 247-250.

Åstrand P. O., Rodahl K., Dahl H. A. and Strømme S. B. (2003). *Textbook of Work Physiology*. (Revised edition). New York: McGraw Hill.

List of tables

List of figures

Appendixes

Forespørsel om deltakelse i en forskningsstudie:

Ekspirert nitrogenoksid etter et intensivt fysisk utholdenhetsarbeid i ulike klimatyper, normobart (normalt innendørsklima), hypobart klima (tilsvarende 2500 m.o.h.) og kaldt klima (-10^{o}C).

Forespørselen om å delta i forskningsprosjektet ekspirert nitrogenoksid (FeNO) målt før og etter et maksimalt arbeid i normalt innendørs klima, hypobart og kald klima går til en gruppe friske personer i alderen 18 – 35 år. Undersøkelsene vil bli utført ved respirasjonsfysiologisk laboratorium og i trykk og klimakammeret på Norges idrettshøgskole på fire adskilte dager. Ansvarlig for prosjektet er Trine Stensrud, 1.amanuensis, Norges idrettshøgskole. Veslemøy Bråten og Julie Stang, masterstudenter ved Norges idrettshøgskole, vil utføre de praktiske undersøkelsene. Hvis du bestemmer deg for å ikke delta i prosjektet, får dette selvfølgelig ingen følger for deg ved senere undersøkelser og/eller medisinsk behandling ved NIH. Det samme gjelder naturligvis dersom du først sier ja, men så velger å trekke deg ut etter at prosjektet har startet opp. Du har full rett til når som helst å si at du ikke ønsker eller har anledning til å være med i prosjektet videre.

Prosjektets innhold

Studien består av fire undersøkelsesdager i løpet av to uker høsten 2009 med minimum 24 timer mellom testene. Alle testene utføres ved løp på tredemølle. En pretest med måling av laktatprofil, maksimalt oksygenopptak og maksimal hjertefrekvens for å undersøke om FeNO forandrer seg etter løp på ulike belastninger og for fastsettelse av belastning under de tre prestasjonstestene i ulike klimatyper. Ekspirert nitrogenoksid måles før test og videre etter hver belastningsøkning. Videre vil FeNO bli målt umiddelbart etter test og videre 5, 10, 20 og 30 minutter etter avsluttet test. I tillegg vil lungefunksjon ved maksimale flow-volum kurver måles før test og ved de samme måletidspunkt som FeNO.
Måling av FeNO måles i denne studien på to ulike måter, online ved at du først fyller lungene med nitrogenfri luft fra et lite instrument, deretter skal du puste ut med en jevn luftstrøm i 10 sekunder. Resultatet foreligger umiddelbart. Når testene utføres inne i trykk og klimakammeret utføres målingene offline ved at du først fyller lungene med nitrogenfri luft, deretter skal du puste ut med en jevn luftstrøm i ca 20 sekunder (til oppsamlingsposen er fylt). Posene med luft blir analysert i etterkant i det samme instrumentet som online målingene gjøres.

De tre andre undersøkelsesdagene innebærer en maksimal løpetest på tredemølle i 8 minutter med en hjertefrekvens (HF) på 90-95% av maksimal hjertefrekvens (HF_{max}) med 15 minutters oppvarming på forhånd med en belastning tilsvarende ca 50-60 % av HF_{max}. Oksygenopptak måles kontinuerlig under løpetestene. Måling av FeNO før oppvarming, etter oppvarming, umiddelbart etter avsluttet test og videre etter 5, 10, 15 og 20 minutter. Testene skal utføres i normalt innendørsklima, i høyde (2800 m.o.h.) og i kaldt klima (-10^{o}C). Rekkefølgen av testene blir avgjort ved loddtrekning.

Hvorfor gjør vi denne studien?
Nitrogenoksid er en gass som dannes normalt i kroppen og som er med å regulere blodgjennomstrømningen lokalt i vevene. Nitrogenoksid er også med å regulere betennelsesprosesser. Nye enkle målemetoder for ekspirert nitrogenoksid er utviklet i den senere tid og det har gjort det mulig å studere denne gassen nærmere. Det er få undersøkelser som har sett på FeNO i forhold til fysisk aktivitet generelt og enda færre som har sett på FeNO i forbindelse med fysisk aktivitet i ulike klimatyper.

Hva er målet med prosjektet?
Hovedhensikten med studien er å undersøke om det er endring i ekspirert nitrogenoksid fra før til etter et maksimalt arbeid i ulike klimatyper. I tillegg vil vi se på forløpet av eventuell forandring i FeNO inntil 20 minutter etter arbeidet i de ulike klimatypene.

Hvilken fordeler kan du ha ved å være med i prosjektet?
Det utbetales intet honorar for å være med på undersøkelsen. Deltakelse i undersøkelsen medfører en grundig undersøkelse av lungefunksjon og luftveistilstand. Du vil i tillegg få målt laktatprofil (anaerob terskel), maksimalt oksygenopptak (VO_{2max}) og maksimal hjertefrekvens (HF_{max}).

Personvern og frivillig deltakelse
All informasjon som samles inn i løpet av prosjektet er konfidensielle opplysninger som lagres forskriftmessig. Ditt navn oppevares ikke sammen med resultatene. Hver forsøksperson får et forsøksnummer, og koblingen mellom navn og forsøksnummer blir oppbevart innlåst i et arkivskap. Bortsett fra test personell og prosjektansvarlig, får ingen andre innsyn i resultatene vedrørende den enkelte forsøksperson. Personopplysningene og dataene vil bli oppbevart til utgangen av 2022? og deretter slettet

Du kan på et hvilket som helst tidspunkt trekke deg ut av undersøkelsen uten å oppgi grunn. Dette vil ikke få noen konsekvenser for ditt videre forhold til respirasjonsfysiologisk laboratorium ved Norges idrettshøgskole. Du har også rett til innsyn i data registrert om deg og dersom du trekker deg fra studien, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner.

Sikkerhet
Under alle forsøkene stilles strenge krav til din sikkerhet. Behandling for eventuelt respirasjonsbesvær vil kunne gis umiddelbart. Måling av ekshalert FeNO er helt ufarlig og medfører ingen risiko.

Videre behandling av forsøksresultatene
Dine resultater fra undersøkelsene formidles direkte til deg. I tillegg samles resultatene (uten personlige pasientdata) og utgis som to masteroppgaver, og muligens publisert i en eller flere forskningsartikler.

Har du spørsmål?
Da kan du kontakte:
Veslemøy Bråten, på telefon 41564049 eller mail veslemoy.braten@student.nih.no
Julie Stang, på telefon 98411440 eller mail julie.stang@student.nih.no.

SAMTYKKE

Til forsøkspersonen

Jeg har lest informasjonsskrivet om prosjektet : ”Ekspirert nitrogenoksid etter et intensivt fysisk utholdenhetsarbeid i ulike klimatyper, normobart (normalt innendørsklima), hypobart klima (tilsvarende 2500 m.o.h.) og kaldt klima (-10^{o}C)”, og gir min tilslutning til deltagelse i undersøkelsen. Jeg er kjent med at jeg når som helst kan trekke meg fra studien uten å måtte oppgi grunn for det. Jeg er klar over at de innsamlede data utelukkende brukes til forskning.

Jeg samtykker i at følgende kan måles:

* Måling av ekspirert nitrogenoksid
* Måling av lungefunksjon
* Måling av laktatprofil, maksimalt oksygenopptak og maksimal hjertefrekvens
* Maksimal løpetest i høyde, kulde og normalt innendørsklima

Forsøkspersonens navn:_____

Jeg nås på telefon:_____

Dato:_____ Underskrift:_____

Samtykke returneres til Norges idrettshøgskole før studiestart.

UNIVERSITETET I OSLO

DET MEDISINSKE FAKULTET

1.amanuensis dr.scient Trine Stensrud
Norges idrettshøgskole
PB 4014 Ullevål stadion
0806 Oslo

Regional komité for medisinsk og helsefaglig forskningsetikk Sør-Øst A (REK Sør-Øst A)
Postboks 1130 Blindern
NO-0318 Oslo

Telefon: 22 84 46 66
Telefaks: 22 85 05 90
E-post: jorgen.hardang@medisin.uio.no
Nettadresse: http://helseforskning.etikkom.no

Dato: 18.09.09
Deres ref.:
Vår ref.: 2009/944a

2009/944a Ekspirert nitrogenoksid før og etter et intensivt fysisk arbeid i ulike klimatyper

Vi viser til søknad om forhåndsgodkjenning av ovennevnte forskningsprosjekt. Søknaden ble behandlet av Regional forskningsetisk komité for medisinsk og helsefaglig forskningsetikk i møtet 26. august 2009. Søknaden er vurdert i henhold til lov av 20. juni 2008 nr. 44, om medisinsk og helsefaglig forskning (helseforskningsloven) kapittel 3, med tilhørende forskrift om organisering av medisinsk og helsefaglig forskning av 1. juli 2009 nr 0955.

Prosjektleder: 1.amanuensis dr.scient Trine Stensrud, Norges idrettshøgskole

Formålet med studien er å undersøke eventuell endring i ekspirerte nitrogenoksider før og etter en maksimal løpetest på tredemølle under ulike klimatiske forhold. 20 friske fysisk aktive ikke-røkende voksne i alderen 18-35 år vil bli inkludert i studien. Det vil bli målt lungefunksjon og oksygenopptak, samt at deltakerne må besvare et spørreskjema utarbeidet for idrettsutøvere i forbindelse med astma og allergi.

Frivillighet er ivaretatt ved at samtykke innhentes og deltakelse er frivillig. Data er avidentifiserte og skal oppbevares i innelåst i en safe adskilt fra personidentifikasjon

Komiteen ber om tilbakemelding på følgende merknader:

1. Spørsmål 1c om forskningsansvarlig er ikke besvart. Ifølge helseforskningsloven skal det oppgis en forskningsansvarlig for et forskningsprosjekt. Til vanlig er det en forskningsinstitusjon. Det skal oppgis en kontaktperson som vil få kopi av all korrespondanse om prosjektet.
2. Det skal gjennomføres en provokasjonstest med metakolin. Komiteen ønsker en redegjørelse for hvordan provokasjonstesten skal gjennomføres og hva slags beredskap det legges opp til.
3. Samtykkeerklæringen bør kun inneholde samtykket. Deltakerne skal ved sin underskrift ikke behøve å stadfeste annet enn å ha mottatt informasjon om prosjektet og at de ønsker å delta.

Vedtak:

Vedtak i saken utsettes. Det bes om tilbakemelding om de merknader som er anført før endelig vedtak kan fattes. Komiteens leder tar stilling til godkjenning av prosjektet etter mottatt svar.

Vi ber om at alle henvendelser sendes inn via vår saksportal: http://helseforskning.etikkom.no eller på e-post til: post@helseforskning.etikkom.no
Vennligst oppgi vårt saksnummer/referansenummer i korrespondansen.

Med vennlig hilsen

Gunnar Nicolaysen (sign.)
Professor
Leder

Jørgen Hardang
Komitésekretær