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Exhaled nitric oxide

Changes after high intensive exercise in cold and normobaric climates among healthy adults

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Summary

Background: Despite extensively research of the effect of exercise upon fractional exhaled nitric oxide ($F_{E_{NO}}$) little is known about the effect of exercise upon $F_{E_{NO}}$ in a cold climate. The primary objective of the present theses was therefore to assess $F_{E_{NO}}$ before and after high intensity exercise in two different climates, a normobaric climate compared to a cold climate. Secondly: to assess the changes in $F_{E_{NO}}$ during sub maximal exercise and to add more data of exhaled nitric oxide values among healthy subjects according to ATS/ERS guidelines.

Subjects and methods: Twenty well trained ($58.2 \pm 6.6 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) healthy non-smoking, non-snuffing males ($n=12$) and females ($n=8$), aged 18-28 (22.8) years were included. They performed three tests; one incremental exercise tests (pretest) in a normobaric environment, temperature (20°C) and two eight minutes repeated high intensity exercise tests (RET) in a climatic chamber in two different climatic conditions, normobaric (18°C) (RET_N) and cold (-10°C) (RET_C) ambient temperature at random. Ventilation (V_E) and $F_{E_{NO}}$ were measured before and after the RET and $F_{E_{NO}}$, V_E and heart rate (HR) were monitored continuously during the pretest. Lung function measurements (forced expiratory volume in one second; FEV_1 and forced vital capacity; FVC) were conducted before and after pretest.

Results: The $F_{E_{NO}}$ decreased from before (baseline) to after exercise in all three tests and peak reduction was; [mean (\pm SD)] 30.7 % (± 18.3), 28.5 % (± 19.0) and 37.3 % (± 10.8) in RET_C , RET_N and pretest, respectively. $F_{E_{NO}}$ was lower in RET_C after warm up and 20, 30 and 60 min post exercise compared to RET_N . The subjects returned to baseline $F_{E_{NO}}$ approximately 10 min and 15 min after RET_C and RET_N , respectively. A reduction in $F_{E_{NO}}$ was seen after 20 min and a further reduction was seen 30 and 60 min post exercise in the cold climate. $F_{E_{NO}}$ was significantly lower 5 and 10 min post exercise in the normobaric climate. Increased $F_{E_{NO}}$ from baseline (online measure) was seen 24 hours post exercise in the normobaric climate.

The subjects did not reach baseline $F_{E_{NO}}$ values 60 min after pretest. The baseline $F_{E_{NO}}$ value was in average 17.2 ± 7.2 ppb, with a 95th percentile of 31.6 ppb. A significant gender difference was seen, with higher $F_{E_{NO}}$ values among the males both in baseline and post exercise.

Conclusion: $F_{E_{NO}}$ is decreased after exercise both in a normobaric and cold climate. A cold ambient climate induces an initial decrease in the $F_{E_{NO}}$ values that was reduced until 60 minutes post exercise compared to a normobaric climate. $F_{E_{NO}}$ values among healthy males and females aged 18-28 years are 17.2 ± 7.2 ppb with a 95th percentile of 31.6 ppb.

Keyword: Exhaled nitric oxide, healthy, cold climate, exercise

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Veslemøy Bråten

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Abbreviations

| | |
|--------------------------|---|
| NO | nitric oxide |
| NOS | nitric oxide synthases |
| ppb | part per billion |
| mPa | milli Pascal |
| F _{ENO} | fractional exhaled nitric oxide |
| P _{ENO} | partial pressure of exhaled nitric oxide |
| Online F _{ENO} | F _{ENO} measured on a stationary NO analyzer |
| Offline F _{ENO} | exhaled air collected in a reservoir and analyzed for F _{ENO} later |
| V _{NO} | production of nitric oxide in the lung: minute ventilation (l·min ⁻¹) multiplied with the rate of exhaled [NO] (ppb or nanoliters). |
| NO _A | measured nitric oxide in the ambient environment |
| FEV ₁ | forced expiratory volume in one second (l) |
| FVC | forced vital capacity (l) |
| VO ₂ | oxygen uptake (ml·kg ⁻¹ ·min ⁻¹) |
| VO _{2max} | highest recorded oxygen uptake during pretest (ml·kg ⁻¹ ·min ⁻¹) |
| V _E | minute ventilation (l·min ⁻¹) |
| V _{Epeak} | highest recorded minute ventilation during pretest (l·min ⁻¹) |
| HR | heart rate (beats·min ⁻¹) |
| HR _{peak} | highest recorded heart rate during pretest (beats·min ⁻¹) |
| RET | repetitive exercise test with duration of eight minutes |
| RET _C | repetitive exercise test in a cold ambient climate |
| RET _N | repetitive exercise test in normal indoor climate |
| PaO ₂ | partial pressure of oxygen |
| PaCO ₂ | partial pressure of carbon dioxide |

Cold climate cold ambient temperature (-10°C)

Normobaric climate normal indoor ambient temperature (21°C)

1 INTRODUCTION

1.1 Background

Approximately 30 years ago nitric oxide was identified as a biological agent in the cardiovascular and nervous system and a period of intensive interest and research in the biology of nitric oxide followed. In 1987 it was demonstrated that nitric oxide accounted for the biological properties of endothelium-derived relaxing factor and opened up a new area of biological research. Nitric oxide is one of the most investigated molecules in the recent history and was named the “Molecule of the Year” in 1992 by the journal Science.

Nitric oxide in exhaled air of healthy subjects was originally reported by Gustafsson and colleagues (Gustafsson et al., 1991) using chemiluminescence. Extensive research of nitric oxide’s biology in the pulmonary and immune system followed.

Chemiluminescence is a non-invasive method measuring the fraction of exhaled nitric oxide (FE_{NO}) quite simple and enables measurements of the inflammatory response despite the lack of functional response (Habib, 2008). Due to this simple method, FE_{NO} in exhaled human air, is today an accepted complementary tool in the physiological and respiratory test battery.

The effects of exercise upon FE_{NO} values are extensively investigated (Sheel et al., 1999). There are strong indicators that FE_{NO} are reduced during and after exercise (Chirpaz-Oddou et al., 1997; Maroun et al., 1995; Persson et al., 1993; Trolin et al., 1994). Some investigators have reported no changes (Bauer et al., 1994; Iwamoto et al., 1994; Shin et al., 2003) and others report an increased FE_{NO} (Bonsignore et al., 2001). Furthermore, most of the previous reports show the effect of exercise in a normobaric climate. To my knowledge, few studies have investigated the exposure to cold climate in healthy human and only one of them is conducted in healthy subjects in a cold ambient environment (Therminarias et al., 1998). The change in FE_{NO} during exercise is also widely studied but alteration in FE_{NO} values after exercise are lacking. As far as I know the alteration in FE_{NO} values for more than 15 minutes exposed to the cold has not previously been reported. Finally, FE_{NO} values reported after ATS/ERS recommendations is lacking in healthy well trained subjects aged 18-30 years.

1.2 Aims of the theses

The aims of the present master theses are:

Primarily:

1. To assess the effect of intensive exercise in cold climate compared to normobaric climate upon fractional exhaled nitric oxide ($F_{E_{NO}}$) in healthy males and females
2. To observe any changes in $F_{E_{NO}}$ until 24 hours after exercise in cold and normobaric climate

Secondly:

3. To add more data to reference values in healthy, well trained males and females aged 18-28 years

1.3 Literature search

The theoretical basis of this study was gathering mainly by search of the PubMed database. Initial searches included combination of the terms “nitric oxide”, “exercise” and “cold climate” (MeSH terms). Extensive hand searches of article reference list were also a major means of identifying relevant literature.

3 THEORY

3.1 Nitric oxide

3.1.1 Nitric oxide formation and synthase

Nitric monoxide, named nitric oxide in the present thesis, is a simple gas composed of nitrogen (N) and oxygen (O₂) atoms and was mainly considered as an atmospheric pollutant produced by smog and cigarette smoke. In 1987 two groups independently identified nitric oxide (NO) as endothelium derived relaxing factor (EDRF) (Palmer et al., 1987; Ignarro et al., 1987) and its importance in the regulation of physiological functions and pathophysiological processes become apparent. NO is a free radical with a short half-life (1-5 seconds) synthesized from the amino acid L-arginine by the action of an enzyme nitric oxide synthase (NOS). NO is the only gaseous signaling molecule and it is produced when L-arginine convert into L-citrulline by NOS, first time described in 1988 of Palmer and colleagues (Palmer et al., 1988). The nitrogen of nitric oxide is derived from the terminal guanidino nitrogen from L-arginine and the oxygen is derived from molecular oxygen. In addition, others cofactors as nicotinamide adenine dinucleotide phosphate (NADPH), flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN) tetrahydrobiopterin (BH₄) calcium (Ca²⁺) and calmodulin are required (Barnes & Belvisi, 1993) (figure 3.1).

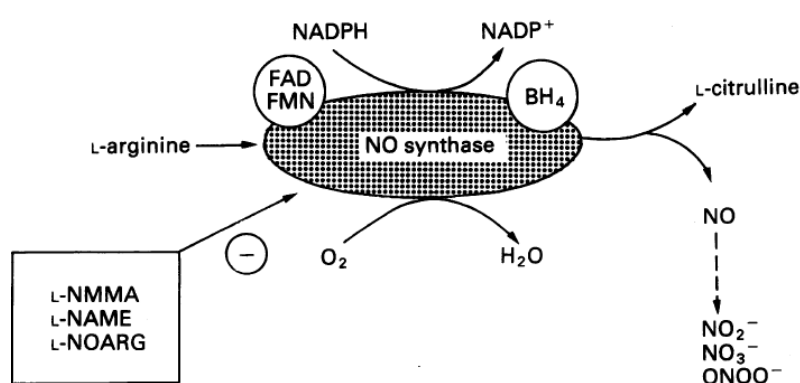


Figure 3.1: Schematic representation of the nitric oxide (NO) synthase involves conversion of L-arginine to L-citrulline and the cofactors necessary for enzyme activity and inhibition; NADPH, nicotinamide adenine dinucleotide phosphate; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; BH₄, tetrahydrobiopterin; L-NMMA, N^G-monomethyl L-arginine; L-NAME, N^G-nitro-L-arginine methyl ester; L-NOARG, N^G-nitro-L-arginine (Barnes & Belvisi, 1993)

The activity of NO depends on many local factors, like amount and activity of the enzymes responsible for producing NO, oxidant stress and its rate of uptake by antioxidant molecules (Ricciardolo 2003). In addition, NO may carry out important biological roles within the cell, as well as in interaction with nearby cells and molecules (Michel & Feron, 1997).

NOS can mainly be classified as either constitutive or inducible, and three distinct enzyme isoforms of NOS are identified by protein purification and molecular cloning: (1) constitutive neural NOS (nNOS) and constitutive endothelial NOS (eNOS); (2) inducible NOS (iNOS). The constitutive form release low amounts of NO for short periods in response to receptor or physical stimulation and are Ca^{2+} and calmodulin dependent. Constitutive NOS acts as a signaling in blood flow regulation, platelet reactivity, non-adrenergic non-cholinergic (NANC) neurotransmission and memory (Al-Sa`Doni & Ferro, 2000). The inducible form, independent of Ca^{2+} , requires BH_4 and calmodulin, generates NO in large amounts for long periods after exposed to certain cytokines, include interferon γ (IFN γ), interleukin β (IL-1 β) and tumor necrosis factor α (TNF α), and endotoxin (Barnes & Belvisi, 1993). Inducible forms are described in macrophages, fibroblast, smooth muscle cells, endothelial cells and neutrophils (Moncada & Higgs, 1993) (figure 3.2). The cellular localization of NOS isoforms and are not quite understood. When NOS are expressed in different tissue they are distinct, but pathway outlined in one tissue might not be the same in another cell (Michel & Feron, 1997).

3.1.2 NOS inhibitors

The inhibitors of NOS are useful to determining the roles of NO in various processes. Moncada and colleagues showed in 1989 by in vitro experiments that NOS is blocked by N^{G} -monomethyl L-arginine (L-NMMA) induced by acetylcholine and histamine (Moncada et al., 1989). N^{G} -nitro-L-arginine (L-NOARG), N^{G} -nitro-L-arginine methyl ester (L-NAME) and N^{G} -iminoethyl-L-ornithine (L-NIO) are others NOS inhibitors, and all of them are stereospecific (Barnes & Belvisi, 1993) (figure 3.1).

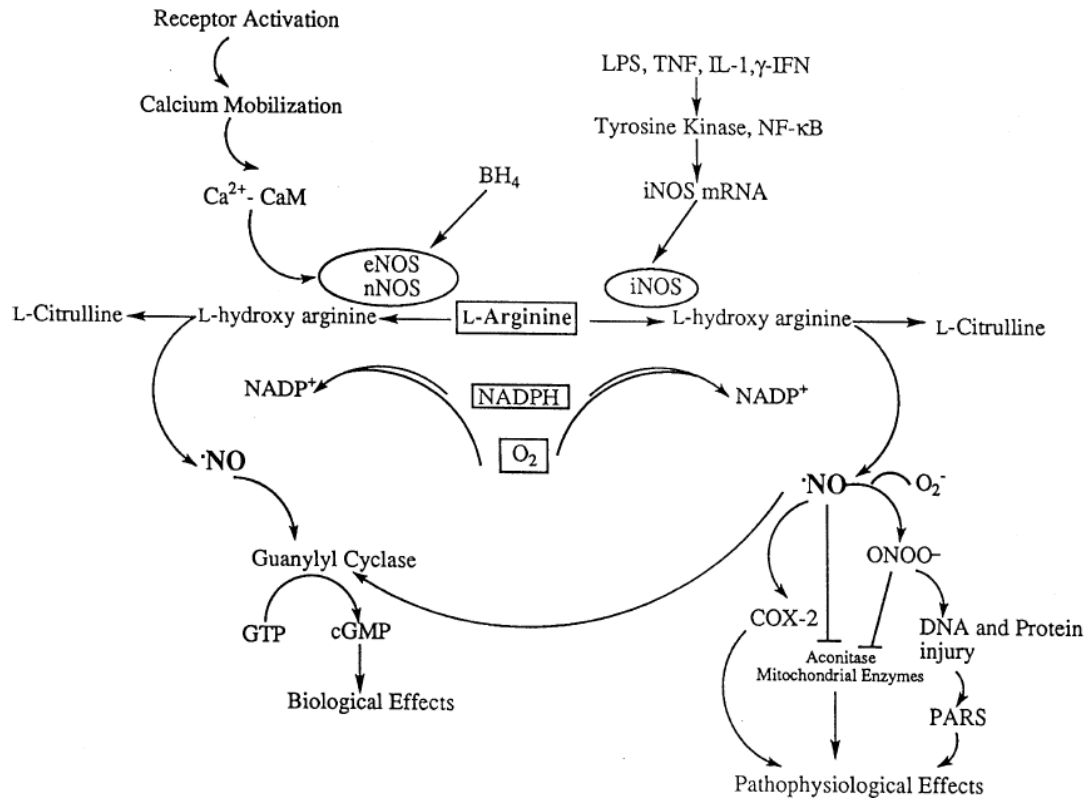


Figure 3.2: Schematic representation of the mechanism of activation of different NOS isoforms, and subsequent effects of NO generated. BH₄, tetrahydrobiopterin; CaM, calmodulin; COX-2, cyclo-oxygenase 2; γ -IFN, gamma interferon; IL-1, interleukin-1; LPS, lipopolysaccharide; NF- κ B, nuclear factor κ B; PARS, poly-adenosine diphosphate synthase; TNF- α , tumor necrosis factor- α ; NADPH, nicotinamide adenine dinucleotide phosphate; nNOS, iNOS and eNOS refer to neural, inducible and endothelial isoforms of NOS, respectively (Al-Sa`Doni & Ferro, 2000).

3.1.3 Cellular effects of nitric oxide

As stated above, NO can interact with other cells and molecules, by permeate cell membranes. The NO molecule possesses a small dipole because of the same electronegativity of oxygen and nitrogen, making it essentially hydrophobic. Thus, eliminate the need of extracellular receptors. The principal effects of NO are related to the activation of guanylate cyclase, stimulating conversion of intracellular guanosine-5'-triphosphate (GTP) to cyclic guanosine 3`5`- monophosphate (cGMP) in target cells, resulting in smooth muscle relaxation (Katsuki et al., 1977). The exact mechanism behind the rise in cGMP is still unknown. In addition, iNOS released NO act as an immune effector molecule killing tumor cells, halting viral infections and eliminating various pathogens, independent of guanylate cyclase and cGMP (Ricciardolo, 2003).

NO strongly interacts with molecular oxygen, to form nitrite (NO_2^-), nitrate (NO_3^-) in the presence of hemoglobin, or free radicals like superoxide (O_2^-) and peroxynitrite (ONOO^-) (Al-Sa`Doni & Ferro., 2000), see figure 3.2. The latter is a potential cytotoxic molecule due to oxidizing properties, thus able to damage a wide array of molecules in the cells and cause inflammation and edema in the lung (Gaston et al., 1994). NO is also been shown to react with thiol to form S-nitrosothiol (RS-NO) (Ignarro et al., 1981). RS-NO may act as a NO stabilizer or carrier, facilitating transport in tissues and contribute in important mechanism in the lung (Gaston et al., 1994; Al-Sa`Doni & Ferro., 2000).

3.1.4 Summary

Endogenous NO apparently have important roles in host defenses, neurotransmission, and smooth muscle relaxation in the human body, but the complexity of NO is a two-edged sword, in terms of toxic effect during certain physiological condition.

3.2 Nitric oxide in the airways

As stated previously, NO is important in the regulation of body function including the respiratory tract. In 1991 Gustafsson and colleagues determine NO in exhaled air in several species, including human (Gustafsson et al., 1991). This discovery was followed by a period of intensive research of NO role in respiratory pathophysiology and was shown to have an important role in regulation of pulmonary function and pulmonary diseases (Barnes & Belvisi, 1993). NO concentration in the lung arise both from the alveolar and airway regions of the lung (Gerlack et al., 1994; Tsoukias et al., 2001). NO are produced by a wide variety of cell types in the airways, including epithelial cells (Asano et al., 1994), airway nerves (Rand 1992), inflammatory cells (macrophages, mast cells and neutrophils) (Gaston et al., 1994), and vascular endothelial cells (Ignarro et al., 1987).

3.2.1 Inflammatory defense mechanism of nitric oxide in the airways

The chemical products of NOS in the lung vary with disease states, and are involved in pulmonary neurotransmission, host defense, and airway and vascular smooth muscle relaxation. The iNOS, cNOS and eNOS are constantly expressed in the human airway (Barnes & Belvisi, 1993). Lane and colleagues found that epithelial iNOS activity is the

major determinant of FE_{NO} , with little contribution from other isoforms (Lane et al., 2004). When iNOS are activated by certain cytokines (figure 3.2), NO is generated in large quantities and for a long period, which differ from eNOS and cNOS. It is seen in healthy subjects, free from airway inflammation, cNOS rather than iNOS is predominant in airway walls (Kharitonov & Barnes, 2000). The concentration of NO that appears in the lung has been shown to correlate with predominantly eosinophilic airway inflammation (Ricciardolo, 2003). Increased production of NO is seen in asthmatic subjects, first time reported by Alving and colleagues in 1993 (Alving et al., 1993).

NO play a role in the non-specific defense mechanism against pathogens, both as an enzyme working intracellular killing pathogens, and as a substances working as an inflammatory mediator, including chemokine (Widmaier et al., 2006). Some of the chemokines are known as CD4 + T helper (Th) cells, and are further divided in Th1 or Th2 cells according to their cytokine secretion patterns. Th1 cells release IL-2, IFN- γ and TNF- β , whereas TH2 cells produce IL-3, IL-4, IL-5, IL-6 and IL-10 (Ricciardolo, 2003), followed by activating B cells and macrophages among others – and an increased NO concentration in the airways.

3.2.3 Ventilation-perfusion (V/Q) in the lung and nitric oxide

Adequate gas exchanges between alveoli and blood are required matching alveolar ventilation to blood perfusion, and the ratio of alveolar ventilation to pulmonary blood flow is termed ventilation-perfusion ratio (V/Q) (McArdle et al., 2006). There is always a small mismatch of V/Q in the lung, and partial pressure of oxygen (PaO_2) is the most important regulator minimizing this action through vasoconstriction of pulmonary vessels to decrease the blood flow in gas exchange areas with low ventilation. A reduced perfusion in a lung region leads to local decreased partial pressure of carbon dioxide ($PaCO_2$) and causes a bronchoconstriction (Widmaier et al., 2006). These mechanism leads to an optimal gas exchange ratio.

The role of NO in the pulmonary vasculature has been studied extensively because of the early observation that EDRF is NO, and therefore the possibility that it may be involved in the regulation of the pulmonary circulation. There is evidence that NO contributes to systemic vasodilatation and acts as a breaking mechanism to reduce the

pulmonary vasoconstriction (Blitzer et al., 1996), thus redistribution of blood flow in well ventilated areas in the lung, via the cGMP pathway. It is known that an injection of L-NMMA in the pulmonary artery causes an increased pulmonary resistance (Gaston et al., 1994), suggesting that NOS activity regulates the perfusion. In a recent review, Dias-Junior and colleagues (2008) propose that NO modulates the vasoconstrictive response mainly associated with hypoxia, and it has been reported that oxygen tension regulate NO levels (Schmetterer et al., 1997; Dweik et al., 1998; Tsuchiya et al., 2000).

The lungs have a high diffusion capacity and a large fraction of NO produced in the lung might be transported in the pulmonary capillary blood (Hyde et al., 1997). The NO delivered from the lung into circulatory are stabilized by thiols such as S-nitroso-albumin and S-nitrosol-hemoglobin, in addition to nitrite (NO_2^-) in the blood (Gladwin et al., 2000). NO inhalation has shown to inhibit pulmonary vasoconstriction in both animals and humans (Barnes & Belvisi, 1993).

There is reason to believe that NO also may act as a bronchodilator, when nitro-vasodilators, such as nitroglycerin and nitroprusside, relax airway smooth muscle via cGMP pathway in animals (Barnes & Belvisi, 1993). It is reported a small bronchodilator effect in asthmatic patient, but NO has no effect on airway resistance in normal subjects (de Gouw et al., 2001). However, RS-NO possess a bronchodilator effect independent of cGMP pathway (Gaston et al., 1994), additionally, NO act as a neurotransmitter that inhibits a non-adrenergic non-cholinergic (NANC) neural system driving the bronchomotor tone (Rand, 1992), leading to relaxation in central and peripheral airways.

3.3.5 Summary

NO may have a key role in many physiological and pathophysiological events in the lung, including immune defense, neural bronchodilator, vasodilator and pulmonary blood flow. cNOS seems to have a generally beneficial role, while iNOS in large amounts may results in increased inflammatory response and tissue damaging. The non-invasive measurement of NO seems to correlate with the airways inflammation status.

3.2 Measurements of the fractional exhaled nitric oxide (F_ENO)

The fact that NO is a pro-inflammatory mediator in the airways makes it a useful marker of airway inflammation. There are several methods for direct measurements of NO, both spectroscopic and electroanalytical. The fact that NO is a gaseous molecule produced endogenous in the airways makes it possible to measure the fraction of nitric oxide in exhaled breath (F_ENO) in human air noninvasive. The most common method is known as chemiluminescence (Palmer et al., 1987). This method has few technical challenges with the possibility to perform repetitive measurements in a short period of time without affecting the NO production or causing discomfort for the subject. In addition, it is able to detect small amounts present, down to 1 part per billion (ppb), which is equivalent to nanoliters ($10^{-9} \cdot l$), and the exhaled gas is analyzed immediately and the results are available within few minutes. Another great advantage of chemiluminescence is that it can be used in children and patient with severe airway obstruction.

The chemiluminescence approach is based on photochemical reaction between NO and ozone generated in the analyzer, leads to the product nitrite (NO₂⁻). When NO₂⁻ return to its basal level it emits a photon, and total number of photons produced is proportional to the NO concentration in the exhaled air (Borland et al., 1993). There are two main approaches measuring F_ENO with chemiluminescence: single-breath online and offline technique. Online is conducted during a single flow-controlled exhalation against a resistance, and the data are presented graphically on a monitor via a computer. Offline measurement is conducted, using the same breath technique, with a hand-hold kit collecting exhaled air into a reservoir, and later analyze the content at the online analyzer (ATS/ERS, 2005). This makes it possible to test at any location.

3.2.1 Recommendations for F_ENO measurements

Since the first guidelines on exhaled and nasal NO measurement was established in 1997 by the European Respiratory Society (ERS) (Kharitonov et al., 1997), there have until today been two more recommendations. In 1999, the second recommendation for standardized procedure for exhaled and nasal NO in adults and children was published by the American Thoracic Society (ATS) (Slutsky & Drazen, 1999). Both reports recommended a constant exhalation flow rate, but ERS recommends 250 ml·sec⁻¹ and

the ATS recommends $50 \text{ ml}\cdot\text{sec}^{-1}$. Exhalation flow rate is shown to affect the FE_{NO} measurements (Silkoff et al., 1997; St Croix et al., 1999), explained by faster flow rates minimize the transit time of alveolar gas in the airway, and therefore reduce the amount of NO transferred. In view of this flow dependency the latest standard procedure to determine FE_{NO} is defined by the American Thoracic Society and the European Respiratory Society in 2005 (ERS/ATS, 2005), and they suggest a standard flow rate of $50 \text{ ml}\cdot\text{sec}^{-1}$. This flow rate is shown to be acceptable and reproducible for children and adults, healthy and sick. This last standardization of techniques makes it possible to compare the results of clinical trials between different centers.

3.2.3 Physiological condition and habits affecting FE_{NO} values

There are several reported condition affecting FE_{NO} , some increase and other decrease the fraction. Some are listed in table 3.1 (Lim et al., 2008; Kharitonov & Barnes, 2000; ATS/ERS, 2005).

Table 3.1: *Physiological, pathophysiological conditions and habits affecting FE_{NO} measurements.*

| Increased NO | Decreased NO |
|------------------------------------|----------------------------------|
| Age | After spirometry |
| Ambient NO level | Cigarette smoking |
| Breath holding | Exercising 30 min before testing |
| Height | Cystic fibrosis |
| Upper respiratory tract infection | Bronchoprovocation study |
| Low exhaled flow rate | High exhaled flow rate |
| Allergen and/or pollutant exposure | Inhaled NOS inhibitors |
| Air pollution | Inhaled glucocorticosteroids |
| Occupational exposure | Menstruation |
| Nitrite/nitrate-enriched food | Smoking |
| Asthma | Acute alcohol ingestion |
| COPD | Mouth washing |
| URTI | Pulmonary hypertension |
| LPS | Primary cilia dyskinesia |
| | Cystic fibrosis |

COPD: chronic obstructive pulmonary disease, URTI: upper respiratory tract infection, LPS: lipopolysaccharide

3.2.4 Reference values of fractional expired nitric oxide (FE_{NO})

Despite of number of publications of FE_{NO} , reference values according to ATS/ERS guidelines (2005) in healthy adults are few. This might be due to the connection between NO and pulmonary diseases. Small samples size, different age and gender mixed in study, variety of measurements methods and technique, are all factors severely

limiting the generalization of the data. There are attempted to provide guidelines of “normal” reference values for adults and children between 10-20 ppb ranges, but there are individuals with higher and lower values. Lately, three studies have suggesting reference values after ATS/ERS recommendation. Lim and colleagues consider FE_{NO} levels from 10-35 ppb as normal, and >35 ppb as elevated levels (Lim et al., 2008), and a review article by Taylor and colleagues suggest FE_{NO} level at 5-25 ppb as low, at 30-45 ppb as intermediate and >50 as high (Taylor et al., 2006). Olivieri and colleagues obtained FE_{NO} values at exhaled flow rate of $50 \text{ ml}\cdot\text{sec}^{-1}$ ranged from 2.6-28.8 ppb in men and 1.6-21.5 ppb in women.

3.2.5 Summary

FE_{NO} measurement is a “patient friendly” clinical tool that quickly and simply marks inflammation and oxidative stress in the lung. The latest standardization of FE_{NO} measurements makes it possible comparing data from different intuitions; however, more data on intermediates values according to ATS/ERS standardization in healthy are needed.

3.3 Exercise and cold climate

The main function of the respiratory system is gas exchanges. In addition, the lung has non-respiration functions, including humidify and heat the inspired air and host defense (Sue-Chu, 2000). Although, these mechanisms are categorized as non-respiratory functions, they are often associated with gas exchange.

3.3.1 The lung function as a thermo regulator

Inspired air is conditioned as it is warmed to body temperature and humidified in the airways (Giesbrecht, 1995). Condition of the inspired air is fulfilled in the top of trachea to the first 6-7 bronchial generation during rest and more in the peripheral part of the airways during increased ventilation and when a switch from nasal to mouth breathing occurs (Giesbrecht, 1995; Widmaier et al., 2006). In addition, since the relationship between temperature and humidity is non-linear, the cooling and drying of the airways becomes greater as temperature and humidity decrease (figure 3.3).

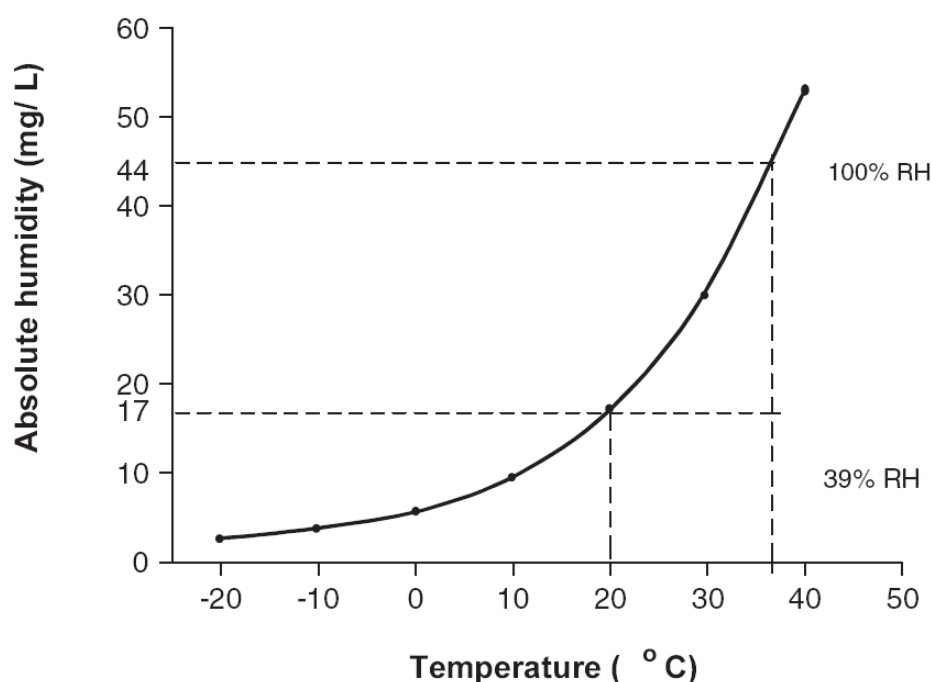


Figure 3.3: Non-linear relationship between temperature and humidity. Water content in 1 l inspired air that is 100 % humidified is > 5 mg in -10°C, 17 mg in 20°C and 44 mg at body temperature (37°C) (Sue-Cha, 2000).

At rest human breathe about $6 \text{ l}\cdot\text{min}^{-1}$ and during high intensity exercise the minute ventilation (V_E) can increase to more than $200 \text{ l}\cdot\text{min}^{-1}$ cover the oxygen require (McArdle et al., 2007). A shift from nose to combined nose and mouth breathing takes place when the V_E level exceeds approximately $30 \text{ l}\cdot\text{min}^{-1}$ (Koskela, 2007). Souhrada & Souhrada (1981) showed that inspired air temperature directly influence the airway smooth muscle and cause a bronchoconstriction. Thus, exercise in cold climate magnifies the cooling and drying of the airways, thus a bronchoconstriction. The exact mechanism behind bronchoconstriction in the cold is not known. In addition, it is not clear if it is the low temperature, low water content of the inspired air or both that is responsible for the bronchoconstriction effect. In a review of Koskela (2007) it was suggested that cooling of the airway leads to dehydration of the airway surface liquid, followed by a respond in mast cells or eosinophilic cells. Mast cells are shown to synthesis NO in rats (Barnes & Belvisi, 1993).

3.3.2 Host defense

The respiratory system maintains a constant interaction with the external environment, making the respiratory system vulnerable against microbes. Despite this the lung is quite sterile, because of nonspecific and specific immune defense mechanism (Sue-Chu, 2000). NO is shown to have a important impact on the mucuciliary clearance, protecting the lungs from toxic effect from inhaled pollution, allergens and pathogens, activated by cytokines (Ricciardolo, 2003). The oral and nasal cavities trap airborne particles, while smaller particles are removed by the mucuciliary clearance system (Widmaier et al., 2006). Particles and microbes that reach the alveoli are destroyed by macrophages via phagocytosis (Widmaier et al., 2006), or the macrophages secrete cytokines (TNF- γ , IL-6, IL-1 and leukotriene B₄ (LTB₄)) that activates an inflammatory process (Sue-Chu, 2000).

3.3.3 The interaction of nitric oxide and exercise in the cold

An increased prevalence of asthma in cross-country skiers is shown (Larsson et al., 1993), probable due to the increased prolonged ventilation in cold air. Higher levels in FE_{NO} among asthmatics are reported (Alving et al., 1993; Taylor et al., 2006). Davis and colleagues (2005) found an increased cytokines profile (IL-4, IL-5 and IL-10) in horses 5 hours after completing 15 minutes exercise (increasing intensity) in -5°C. An increased cytokine expression may lead to iNOS synthesis (figure 3.2). Larsson and colleagues (1998) investigated the effect of inhalation of cold air in healthy subjects and found an increase in inflammatory cells (granulocytes and macrophages) in the lungs after running in -23°C in four exercise-rest cycles of 15 minutes each. Together these studies indicate a strong relationship between NO and exercise in the cold among both healthy and asthmatics.

3.4.2. Summary

The respiratory system has many task and host defensive and humidified and heat the inspired air is some of them. Exercise in the cold magnifies the drying and cooling of the airways that leads to a bronchoconstriction and increased cytokine expression.

4 SUBJECTS AND METHODS

4.1 Design and subjects

4.1.1 Design

The present study has an open randomized crossover design. Randomizing was carried out by drawing lots. The study was part of a larger project comparing changes in exhaled NO after strenuous exercise in three different climatic conditions, normobaric, hypobaric and cold climate. In the present thesis two of the climatic conditions will be presented, a normobaric environment (mean \pm SD), 741.3 mmHg (\pm 12.0) with a temperature of 18°C (\pm 1.0) and a relative humidity of 40.0 % (\pm 3.3) and a cold environment, 732.7 mmHg (\pm 13.5) with a temperature of -10°C (\pm 1.0) and a relative humidity of 39.2 % (\pm 3.8). All subjects conducted a pretest and two repetitive exercise tests (RET) in random order within 14 days after pretest with a minimum of 48 hours between each test. All tests were conducted at the laboratory at the Norwegian School of Sport Sciences. Pretest is performed in a normal indoor environment with a temperature of 19.9 (\pm 1.3) °C and a barometric pressure of 736.9 \pm 8.6 mmHg. The RET`s are performed in a low-pressure climatic chamber (Norwegian Sub diving Techniques A/S, Haugesund, Norway). The subjects performed RET`s according to identical test procedures in the period of October to January 2009 -2010.

The study was performed according to the principles stated in the Declaration of Helsinki. All participants were informed about the testing procedures and signed a written consent form (appendix 1). The Regional Medical Ethics committee approved the study.

4.1.2 Subjects

Twenty healthy subjects (♂ =12 / ♀ =8), non-smoking or snuffing, were included. All subjects participated in various physical activities (cross-country skiing, triathlon, football, orienteering or running) and were recruited from the Norwegian School of Sport Sciences. Demographics and physiological variables are listed in table 4.1. The subjects were not allowed to exercise, drink or eat food with nitrite and nitrate on the day of experiments, neither to drink nor eat <1 hour before testing. They were free to

drink water before and during the experiments. Inclusion criterion was oxygen uptake of $> 40 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (females) and $> 55 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (males). Exclusion criteria were $\text{FE}_{\text{NO}} > 30$ ppb, any acute or chronic illnesses interfering with the possibility to perform the study, exercise induced asthma (EIA) and to upper-respiratory tract infections during the last 14 days before testing.

Table 4.1: Demographic data and baseline maximal oxygen uptake ($\text{VO}_{2\text{max}}$), peak heart rate (HR_{peak}), peak minute ventilation (VE_{peak}) and lungfunction (% predicted) ($n=20$) from the pretest.

| | Male | Female | Total |
|--|--------------|--------------|----------------|
| Age (years) | 22.5 ± 2.4 | 22.8 ± 3.2 | 22.6 ± 2.7 |
| Weight (kg) | 74.5 ± 7.0 | 57.5 ± 7.9 | 67.69 ± 11.1 |
| Height (cm) | 178.2 ± 5.4 | 164.6 ± 4.7 | 172.8 ± 8.5 |
| Body mass index (kg/m^2) | 21.3 ± 2.3 | 21,25 ± 2,34 | 22.5 ± 2.1 |
| $\text{VO}_{2\text{max}}$ ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) | 61.9 ± 4.0 | 52.6 ± 5.8 | 58.2 ± 6.6 |
| HR_{peak} ($\text{beats}\cdot\text{min}^{-1}$) | 194.3 ± 5.8 | 197.0 ± 4.5 | 195.3 ± 5.3 |
| VE_{peak} ($\text{l}\cdot\text{min}^{-1}$) | 160.9 ± 23.6 | 106.4 ± 14.7 | 139.1 ± 33.9 |
| Baseline FEV1 (% predicted) | 105.7 ± 10.0 | 96.1 ± 9.1 | 102.17 ± 10.55 |
| Baseline FVC (% predicted) | 106.6 ± 9.3 | 104.1 ± 3.8 | 105.7 ± 7.7 |

Data are given as mean ± SD

4.2 Test procedures

4.2.1 Anthropometric measurements

Total body weight and height were measured in the laboratory before each test (pretest and repetitive climatic exercise test) with the subjects dressed in light clothing and without shoes. Height was measured to the nearest 0.5 cm and body mass to the nearest 0.1 kilo (Seca, Hamburg, Germany).

4.2.2 Visit 1: Pretest

Fractional exhaled nitric oxide (FE_{NO}) and lung function was measured before all subjects performed a stepwise exercise test, assessing anaerobic threshold (AT) and maximal oxygen uptake ($\text{VO}_{2\text{max}}$). Minute ventilation (VE), respiratory exchange ratio (RER) and heart rate (HR) was measured during exercise. Lung function was conducted 6-10 minutes after exercise to exclude EIA (figure 4.1).

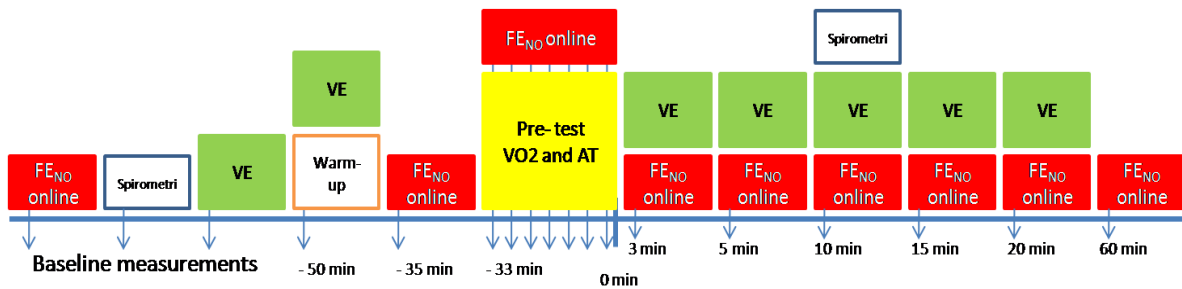


Figure 4.1: Flow chart of the pretest with repetitive increased exercise intervals and VO_{2max} . V_E , minute ventilation; AT, anaerobic threshold; VO_{2max} , maximal oxygen uptake; FE_{NO} , fraction of exhaled nitric oxide.

4.2.3 Visit 2 and 3: Repetitive exercise test (RET) in cold and normobaric environment

The RET was conducted in a low-pressure climatic chamber (Norwegian Sub diving Technology A/S, Haugesund, Norway). The subjects were running on a motor-driven treadmill (Bodyguard Cardionics 2313, Cardionics AB, Sweden) with an inclination of 5.3 % and with a $0.5 \text{ m}\cdot\text{sec}^{-1}$ air velocity. The warm up lasted for 15 minutes at a workload corresponding to 65-75 % of HR_{peak} estimated from the pretest. The RET consisted of eight minutes run at a sub-maximal to maximal work load. The running speed was increased the first four minutes to achieve a work load corresponding to 95 % of HF_{peak} the last four minutes. HR were monitored electronically and registered every minute (Polar Electro OY, Kempele, Finland) during exercise. The subjects were cheered at and encouraged to keep on running in eight minutes or until exhaustion. After RET the participants remained in the current climate for 20 minutes before exiting to normal indoor climate.

FE_{NO} was measured offline and online before exercise, offline measurement was conducted inside the climatic chamber after warm up and 5, 10, 15 min and 20 min after RET. Then the subjects exit the chamber and both offline and online measurements was performed 30 and 60 min and in addition 24 hours after exercise outside the chamber (figure 4. 2).

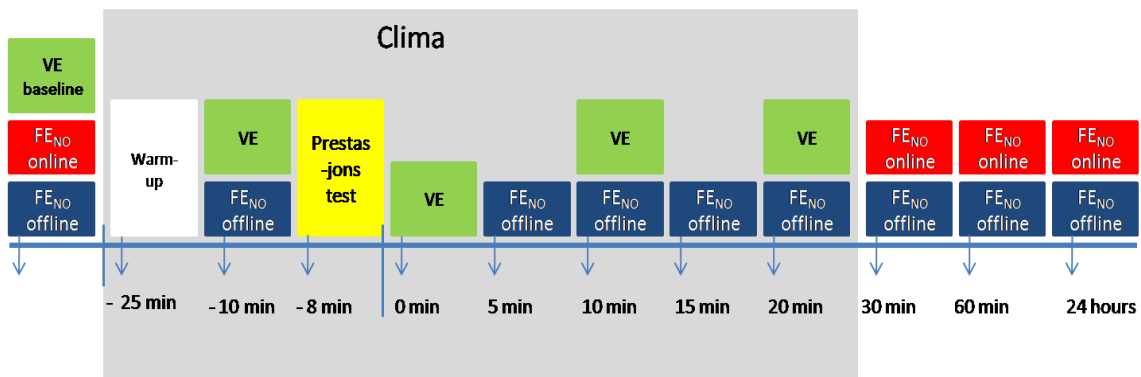


Figure 4.2: Flow chart of the repetitive exercise test (RET). V_E , minute ventilation; FE_{NO} , fraction of exhaled nitric oxide.

4.2.4 Exhaled nitric oxide

For analysis of exhaled nitric oxide (NO) online, fractional exhaled nitric oxide (FE_{NO}) were measured using a NO chemiluminescence analyzer (DENOX 88, Eco Medics, Duerten, Switzerland) according to American Thoracic Society and the European Respiratory Society recommendations (ATS/ERS, 2005). The method has a satisfactory reproducibility of about 10% (Kharitonov et al., 2003). Each participants exhaled before inhaling NO-free air to total lung capacity and then slowly exhaled against a constant flow of $50 \text{ ml} \cdot \text{sec}^{-1}$ against a counter pressure of 10-20 cm H_2O in 12 seconds. The procedure was carried out with a visual feed-back system helping the participants to maintain the pressure during the expiration. FE_{NO} was recorded as the mean value from three successive measurements, all within 10 % deviation. Gas and volume calibration was carried out according to the user manual before each experiment and the spirette for dead space reducer was renewed weekly.

For analyzing NO offline, samples of exhaled air were collected in Mylar bags using a Collector Kit with flow restrictor (EcoMedics – Offline Collection Kit, Duerten, Switzerland). The participants exhaled before inhaling NO-free air to total lung capacity from a NO-filter with one way valve, followed by a slowly expiration until the Mylar bag was filled. A visible needle indicated the pressure gauge and helped maintaining the pressure between 10-20 cm H_2O . The filled Mylar bag was then disconnected from the Collector kit, closed with a cap and analyzed by connecting it with the luer connector to the sample line of the online chemiluminescence analyzer for analyzing FE_{NO} within 4

hours. Comparability of online and offline chemiluminescence based NO measurements are previously showed to be satisfactorily (Schiller et al., 2009).

FE_{NO} online measurements were conducted before, during and after the pretest, and before and after the subjects had exiting the climatic chamber after performing RET. FE_{NO} offline measurements were conducted before, after warm-up and 5, 10, 15, 20 minutes after the RET in the climatic chamber. In addition, all FE_{NO} measurements after exiting the chamber were sampled both online and offline 30 and 60 minutes and 24 hours after the RET. The concentration of NO in exhaled gas are expressed both as fraction (parts per billion, ppb) and as an amount exhaled per unit (V_{NO}), using the minute ventilation (BTPS) in 1 min, expressed as ppb·l·min⁻¹.

FE_{NO} has been validated against invasive measurements of inflammatory by bronchoscope (r=0.91) (Kharitonov et al., 1996), and induced sputum (non-invasive marker) (r=0.48) (Jatakanon et al., 1998). The reproducibility of FE_{NO} measurements is high, with a standard deviation for repeated measurements of 2.1 ppb in adults (Kharitonov et al., 2003).

4.2.5 Lung function measurements

Lung functions was measured by maximum forced expiratory flow volume loops (Masterlab, Erich Jaeger®, Germany), forced expiratory volume in one second (FEV₁), forced vital capacity (FVC) and forced expiratory flow at 50% of FVC (FEF₅₀). In the present study lung functions measurements were carried out before and 6-10 min after pretest to exclude subjects with EIA. All maneuvers complied with the general acceptability criteria of the European Respiratory Society (ERS 1997). Predicted values are according to Quanjer PH et al. (1993).

4.2.6 Anaerobic threshold (AT) and maximum oxygen uptake (VO_{2max})

To assess the effect of increased workloads on FE_{NO}, each subject performed an anaerobic threshold test followed by a VO_{2max}-test. The tests were performed on a motor driven treadmill (Woodway, Germany) at an inclination of 5.3 %. After a standard warm-up of 10 minutes at a workload corresponding to 50-60% of estimated VO_{2 max}, AT test with five minutes sub-maximal running bouts with increasing intensity were

performed with 2 min breaks. The subjects wore a nose-clip and breathed through a low resistance Hans Rudolph's mouthpiece (2700 Series: Hans Rudolph Inc, USA). The speed was increased in steps of $1 \text{ km}\cdot\text{h}^{-1}$ and measurements of oxygen uptake (VO_2), respiratory exchange ratio (RER) and minute ventilation (V_E) were obtained between $2\frac{1}{2}$ - 4 minutes (between $6\frac{1}{2}$ -9 minutes during the warm up) by use of Champion Jeager gas analyzer. 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) daily. Heart rate (HR) was registered electronically by Polar VantageTM (Polar Electro, Finland) between 4 -5 minutes. Polar Electro has showed measurement uncertainty of $\pm 1\%$ and correlates with ECG (Mcfarlane et al. 1989). Between each of the bout, a fingertip capillary blood sample (50 ul) was obtained for the assessment of lactate concentration. (1500 SPORT Lactate Analyzer, Yellow Springs Inc, USA) and a sample of F_{ENO} was conducted. The lactate analyzer has a measurement uncertainty of $\pm 2\%$ (YSI 23L Operational Manual Yellow Springs Instruments, 1995). Calibration of the lactate analyzer was conducted with injection of $5 \text{ mmol}\cdot\text{l}^{-1}$ ($\pm 0.1 \text{ mmol}\cdot\text{l}^{-1}$) lactate standard solution, further checked with $15 \text{ mmol}\cdot\text{l}^{-1}$ standard solution every morning before test. The correlation between blood and muscle lactate is good, despite a linear relationship (Spurway & Jones, 2007). The Lactate analyzer has a measurements uncertainty of $\pm 2\%$ (YSI 23L Operational Manual Yellow Springs Instruments, 1995). The protocol continued until the blood lactate concentration was $>1.5 \text{ mmol}\cdot\text{l}^{-1}$ relative to the average of the first two lactate measurements (after warm-up and the first bout).

A $\text{VO}_{2 \text{ max}}$ -test, according to the procedure described by Hermansen (1973) and Åstrand and Rodahl et al. (2003), followed immediately (<2 minutes) after the lactate threshold was attained. The speed increased every minute, preferably by $1 \text{ km}\cdot\text{h}^{-1}$, but also $0.5 \text{ km}\cdot\text{h}^{-1}$ or remained constant, considering the subject's condition. HR was registered every minute and the subjects were encouraged to keep on running until exhaustion. A lactate acid blood sample was collected shortly after the test and used as an additional criterion for reached $\text{VO}_{2 \text{ max}}$. $\text{VO}_{2 \text{ max}}$ was defined as the highest average measures over the range of one minute. The highest recorded values of V_E , RER, HR and running speed were expressed as peak values. The HR_{peak} and running speed during $\text{VO}_{2 \text{ max}}$ was used as a reference to maintain the workload at the repetitive exercise tests.

4.2.7 Expired gas sampling in Douglas bags

Douglas-bags were used for collecting gas samples of expired air before and immediately after warm-up, immediately after RET, in addition, 4.-5. and 9.-10. minutes after RET. The variations reported of the Douglas bag method used with cycle ergometer are 2.3 -2.5% for daily variations and 3.3 -5.1% for between day variations (Carter & Jeukendrup, 2002). The Douglas bag system was chosen because measurements using the automatic, electronic equipment may give unstable and not reproducible results in the cold environment. The Douglas bags were stored in an isolated box with a thermal blanket to avoid any complications with the bags in the cold environment. This process was also conducted during testing in hypobaric and normobaric climate to make sure identical procedures. The subjects wore a nose clip and breathed through a Hans Rudolph mouthpiece (2700 series; Hans Rudolph Inc, USA). Expiratory gas samples were taken for 60 seconds and analyzed for O₂ and CO₂ content (Oxygen analyzer model S-3A/1 and Carbon dioxide analyzer model CD-3A; Ametek Inc, USA). In the cold and normobaric environment the Douglas bags were carried out of the climatic chamber immediately after finishing the expiratory gas sampling. The volume, temperature and 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). Ventilation was expressed as l·min⁻¹. The Douglas bags were emptied for air and checked for any damage every morning before use.

4.3 Conversion of F_{E_{NO}} to partial pressure of nitric oxide (P_{E_{NO}})

As stated previously oxygen is a substrate in the NO synthesis, and the concentration of exhaled nitric oxide is influenced by the partial pressure of oxygen (PaO₂). This study is part of a larger project comparing changes in the fraction of exhaled nitric oxide (F_{E_{NO}}) after strenuous exercise also in a hypobaric climate, in addition to cold and normobaric climate. To be able to compare the results of these three climates, the F_{E_{NO}} has to be converted to partial pressure of nitric oxide (P_{E_{NO}}) to express the molar concentration of NO (Hemmingsson et al., 2009). The actually ambient pressure was measured before every test, and the results were converted from F_{E_{NO}} and ppb into P_{E_{NO}} and mPa.

$$(\text{Ambient pressure} - \text{Water pressure}) * F_{E_{NO}} / 1\ 000\ 000\ 000 = P_{E_{NO}}$$

The FE_{NO} is expressed in part per billion (ppb) and PE_{NO} is expressed as millipascal (mPa).

4.4 Nitric oxide output (V_{NO})

Nitric oxide output (V_{NO}) is a product of minute ventilation ($l \cdot \text{min}^{-1}$) and rate of exhaled NO (ATS/ERS, 2005) and are expressed in different units, including $nl \cdot \text{min}^{-1}$, $nmol \cdot \text{min}^{-1}$ or $ppb \cdot l \cdot \text{min}^{-1}$. V_{NO} is generally assumed to reflect the NO production by cells within the respiratory tract. ATS/ERS suggest V_{NO} as a useful tool when exhaled NO is measured over longer periods of time and varying flow situations. In this thesis the expression of V_{NO} will be reported as $ppb \cdot l \cdot \text{min}^{-1}$.

4.5 Ethical considerations

The present study was performed according to the principles stated in the Declarations of Helsinki. All subjects signed a written consent form, and they were informed that they could whatever time or reasons withdraw from the study. The Regional Medical Ethics Committee approved the testing protocol (appendix 2). All the print outs were immediately stored in lockable cabinet after testing. Test forms were coded with the subjects own identification number.

4.6 Statistical analyses

Demographics are given as mean with standard deviation (SD) and results as mean with 95% confidence intervals (CI). Difference between two measurements were analyzed by Student`s paired t-test if the data were normally distributed, if not a non parametric test (Wilcoxon) was used. The FE_{NO} response following exercise was measured as fractional exhaled nitric oxide: FE_{NO} (ppb) and partial pressure of nitric oxide, PE_{NO} (mPa).

Correlation was calculated by Parson`s correlation coefficients for normally distributed and Spearman`s correlation for not normally distributed. Power calculation was conducted prior to the study, and a sample of 20 participants was found to be sufficient to obtain differences with a significance level of 5% and a power of 80%. Statistical analyses were performed with Statistical Package of Social Science (SPSS) version 15.0 and Microsoft Excel, version 2007.

4.7 Pilot study

A pilot study was conducted with six voluntary students from the Norwegian School of Sport Sciences in September 2009. The aim of the pilot study was to practice, get used to the test protocols and gather useful information and expectations about the present research. The main study followed the pilot study, with the exceptions of a few changes. The results from the pilot study are not included in the present thesis.

5 RESULTS

5.1 Subjects

All the 20 subjects performed the tests without discomfort. There were found significant gender-related differences ($p < 0.05$) in body mass (weight), height, body mass index (BMI), VO_{2max} , VE_{peak} and lung function (measured forced expiratory volume at the first second, FEV_1) (table 5.1). One healthy male with FE_{NO} baseline value >30 ppb before one of the RET was not excluded due to baseline value at pretest (inclusion test) was <30 ppb.

Table 5.1: Demographic and physiological data ($n=20$). Values are given as mean \pm SD.

| | Male n=12 | Female n=8 | Total n=20 |
|---|-------------------|-------------------|--------------------|
| Age (years) | 22.5 \pm 2.4 | 22.8 \pm 3.2 | 22.6 \pm 2.7 |
| Weight (kg) | 74.5 \pm 7.0* | 57.5 \pm 7.9* | 67.69 \pm 11.1 |
| Height (cm) | 178.2 \pm 5.4* | 164.6 \pm 4.7* | 172.8 \pm 8.5 |
| Body mass index (kg/m ²) | 21.3 \pm 2.3* | 21.25 \pm 2.34* | 22.5 \pm 2.1 |
| VO_{2max} (ml·kg ⁻¹ ·min ⁻¹) | 61.9 \pm 4.0* | 52.6 \pm 5.8* | 58.2 \pm 6.6 |
| HR_{peak} (beats·min ⁻¹) | 194.3 \pm 5.8 | 197.0 \pm 4.5 | 195.3 \pm 5.3 |
| VE_{peak} (l·min ⁻¹) | 160.9 \pm 23.6* | 106.4 \pm 14.7* | 139.1 \pm 33.9 |
| Baseline FEV_1 (% predicted) | 105.7 \pm 10.0* | 96.1 \pm 9.1* | 102.17 \pm 10.55 |
| Baseline FVC (% predicted) | 106.6 \pm 9.3 | 104.1 \pm 3.8 | 105.7 \pm 7.7 |

Two-tailed t-test, gender differences: * $p < 0.05$.

5.2 FE_{NO} and PE_{NO} baseline and post exercise values

There was a significant difference between males and females ($p < 0.05$) both in baseline and post exercise FE_{NO} and PE_{NO} values.

Table 5.2: Baseline and post exercise FE_{NO} and PE_{NO} values ($n=20$). Results are given as mean \pm SD.

| | Male n=12 | Female n=8 | Total n=20 |
|---------------------------|------------------|-------------------|------------------|
| Baseline FE_{NO}^1 | 19.69 \pm 7.09 | 13.53 \pm 5.57* | 17.23 \pm 7.15 |
| Baseline PE_{NO}^1 | 1.80 \pm 0.65 | 1.25 \pm 0.51* | 1.58 \pm 0.65 |
| Post exercise FE_{NO}^2 | 12.81 \pm 4.61 | 8.80 \pm 3.51* | 11.27 \pm 4.63 |
| Post exercise PE_{NO}^2 | 1.17 \pm 0.43 | 0.81 \pm 0.32* | 1.04 \pm 0.43 |

¹ Mean of baseline values in the cold, normobaric and pretest. ² Post exercise values 5 min after RET_N and RET_C and 3 min after pretest.

* $p < 0.05$

5.3 Repetitive exercise test in a cold and a normobaric climate

5.3.1 Intensity

The average running velocity, measured in $\text{km}\cdot\text{h}^{-1}$, was significantly different between the cold and the normobaric climate. There was no difference in intensity, measured by % HR or VE_{peak} measured immediately (0 minutes) after end of exercise (table 5.2).

Table 5.3: The intensity (% HR_{peak}), running velocity ($\text{km}\cdot\text{h}^{-1}$) and highest minute ventilation (VE_{peak}) during eight minute exercise in cold and normobaric climate ($n=20$). Results are given as mean \pm SD

| Intensity RET | Normobaric | Cold | p |
|--|----------------|-----------------|-------|
| HR_{peak} (%) | 91 \pm 5.5 | 91 \pm 5.3 | 0.8 |
| Velocity ($\text{km}\cdot\text{h}^{-1}$) | 12.7 \pm 1.1 | 11.4 \pm 0.8 | 0.006 |
| VE_{peak} ($\text{l}\cdot\text{min}^{-1}$) | 83 \pm 27.1 | 76.7 \pm 21.9 | 0.07 |

5.3.2 FE_{NO} values

Offline

FE_{NO} values measured offline decreased significantly 5 min ($p<0.001$) and 10 min ($p<0.01$) after exercise in normobaric climate. After exercise in the cold climate FE_{NO} values decreased significantly after warm-up ($p<0.001$) and 5 min after RET_{C} ($p<0.001$). Tendency of decreased FE_{NO} levels was seen 10 min ($p=0.052$) and 15 min ($p=0.058$) after RET_{C} . There was no significant difference in baseline FE_{NO} between cold and normobaric climate. Comparing the FE_{NO} values of the two climates, a significant difference was seen after warm-up ($p<0.05$), 20 min after RET ($p<0.01$), 30 min and 60 min after RET ($p<0.05$) (figure 5.1).

Online

Online measurements shows a change in FE_{NO} from baseline when exposed to cold climate at 30 min ($p<0.01$) and at 60 min ($p<0.05$) post exercise. When the subjects were exposed to normobaric climate a significant difference from baseline was seen 24 hours post exercise ($p<0.05$). There was a significant difference between the two climates 30 min ($p<0.01$) and 60 min ($p<0.05$) after exercise (figure 5.2).

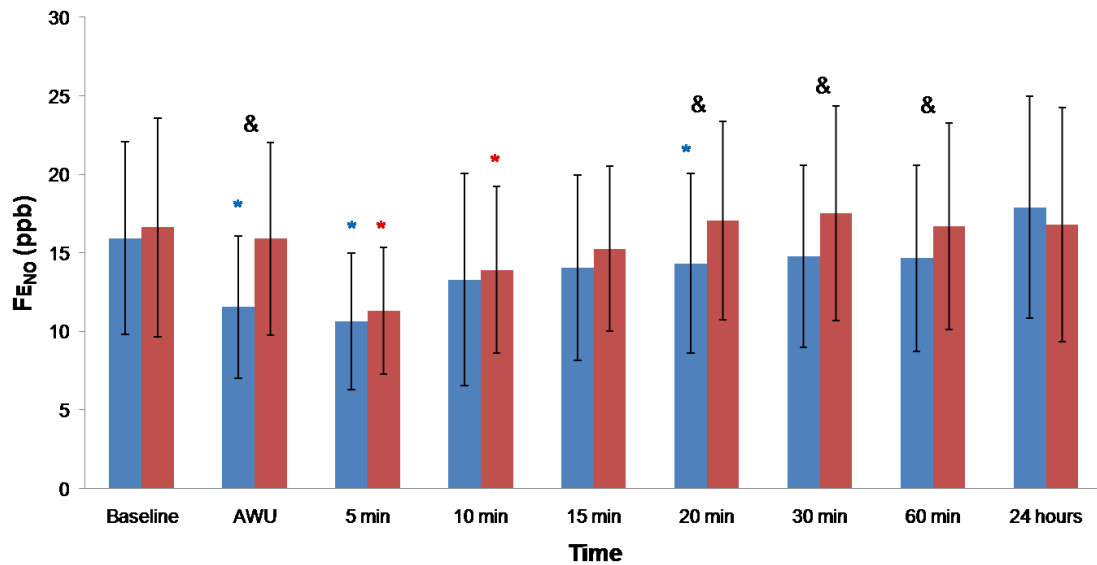


Figure 5.1: FE_{NO} values offline pre exercise (baseline), after warm up (AWU) and post exercise (5 min, 10 min, 20 min, 30 min, 60 min and 24 hours) in two different climates; normobaric (red bars) and cold (blue bars). Results are given as mean with 95 % confidence intervals ($n=20$).
 * significant difference from baseline, & significant difference between the two climates.

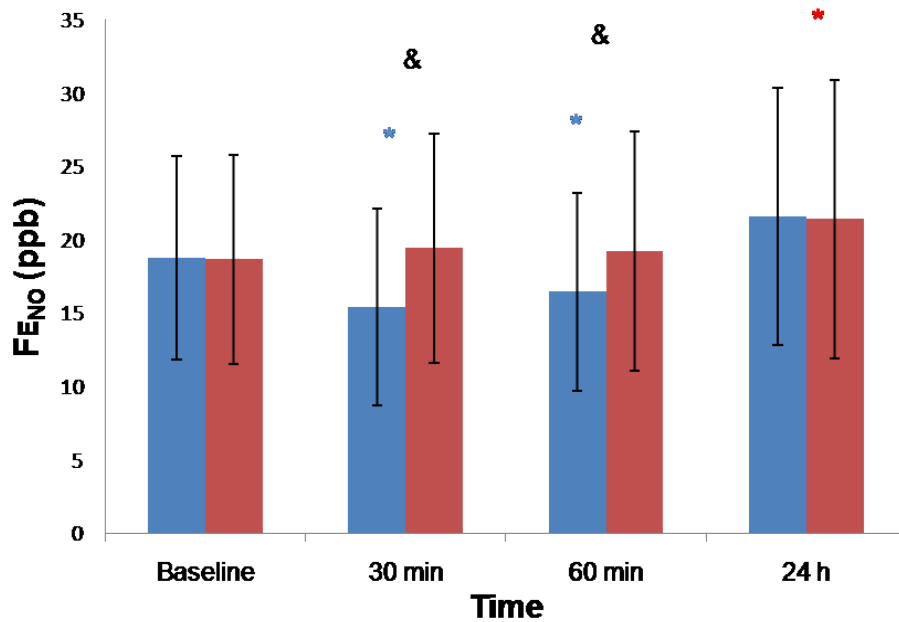


Figure 5.2: Baseline and post exercise FE_{NO} values (30 min, 60 min and 24 hours) measured online in two different climates; normobaric (red bars) and cold (blue bars)($n=20$). Results are given as mean with 95 % confidence interval.
 * significant difference from baseline, & significant difference between the two climates.

5.3.3 Relatively (%) changes in FE_{NO}

FE_{NO} levels (measured offline) were maximally reduced by 30.7 % (± 18.3 %) and 28.5 % (± 19.0 %) after warm up and 5 minutes, respectively, after cold and normobaric exposure. Significant difference between the two climates was seen after warm up ($p < 0.05$) (figure 5.3).

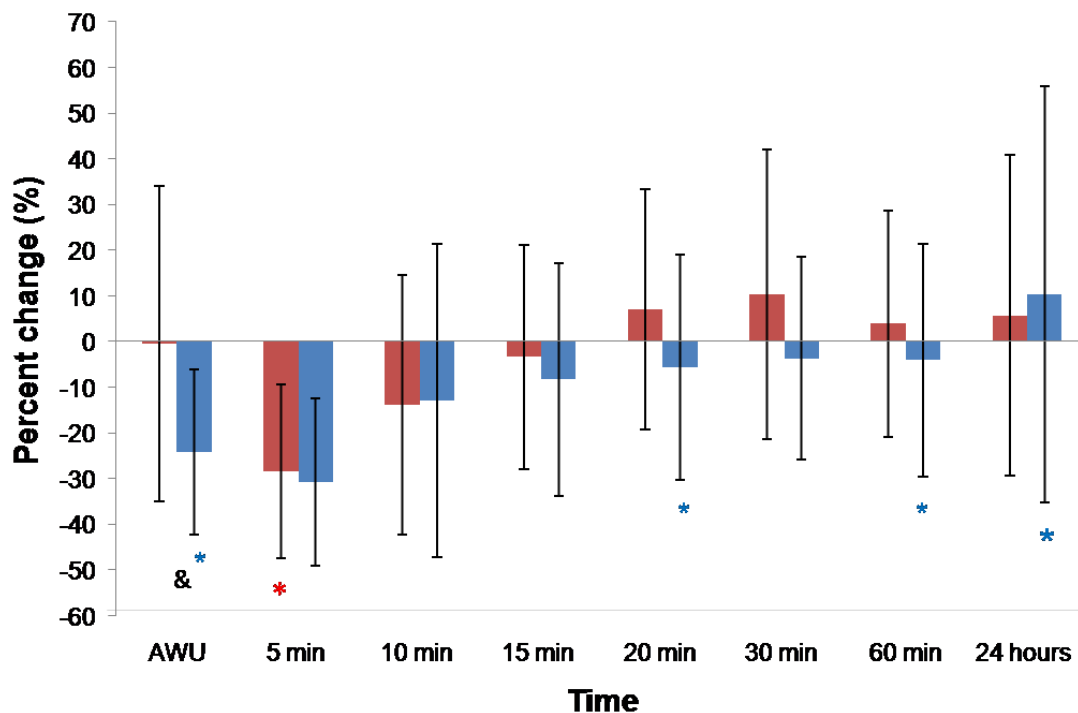


Figure 5.3: Change in FE_{NO} (%) from baseline after warm up (AWU) and after exercise test (5 min, 10 min, 15 min, 20 min, 30 min, 60 min and 24 hours) exposed to normobaric (blue bars) and cold (red bars) climates. Results are given as mean with 95 % confidence intervals ($n=20$). * Significant different from baseline, & significant difference between the two climates.

5.3.4 Ventilation

No differences in minute ventilation ($l \cdot \text{min}^{-1}$) were found between cold and normobaric climate (figure 5.4).

5.3.5 Nitric oxide output (V_{NO})

V_{NO} increased significantly after warm up ($p < 0.001$) and immediately after RET ($p < 0.000$) from baseline in both climates, and a significant difference between cold and normobaric climate occurred after warm up ($p < 0.001$) and after 20 minutes ($p < 0.05$)

(figure 5.5). Relatively (%) change in V_{NO} was significantly different between the two climates after warm up ($p < 0.05$) and V_{NO} increased $260.9 \pm 133.3 \%$ and $281.9 \pm 156.4 \%$ in the cold and the normobaric climate, respectively (figure 5.6).

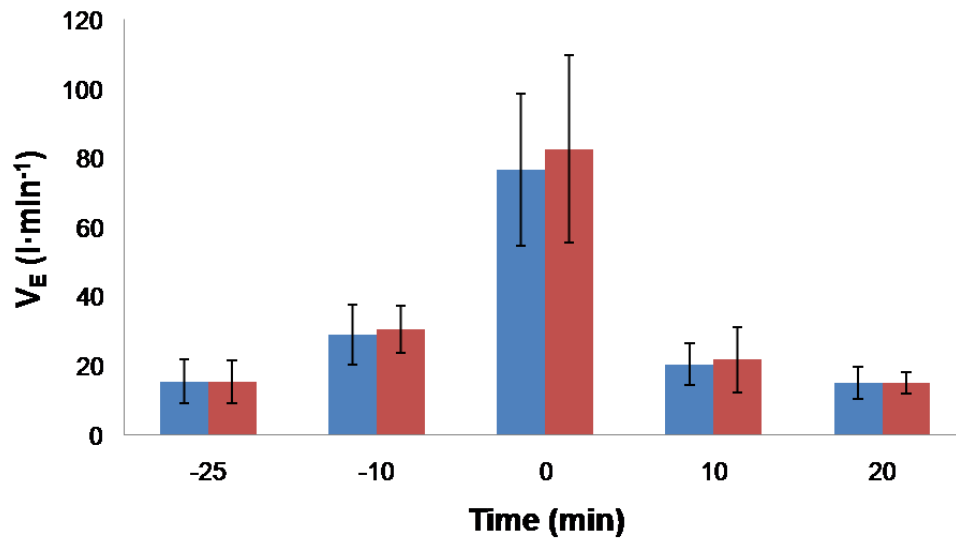


Figure 5.4: Ventilation (V_E) pre exercise (-25 min), after warm up (-10 min), immediately after exercise (0 min) and post exercise (10 and 20 minutes), in cold (blue bars) and normobaric (red bars) climate. Results are presented as mean with 95 % confidence interval ($n=20$).

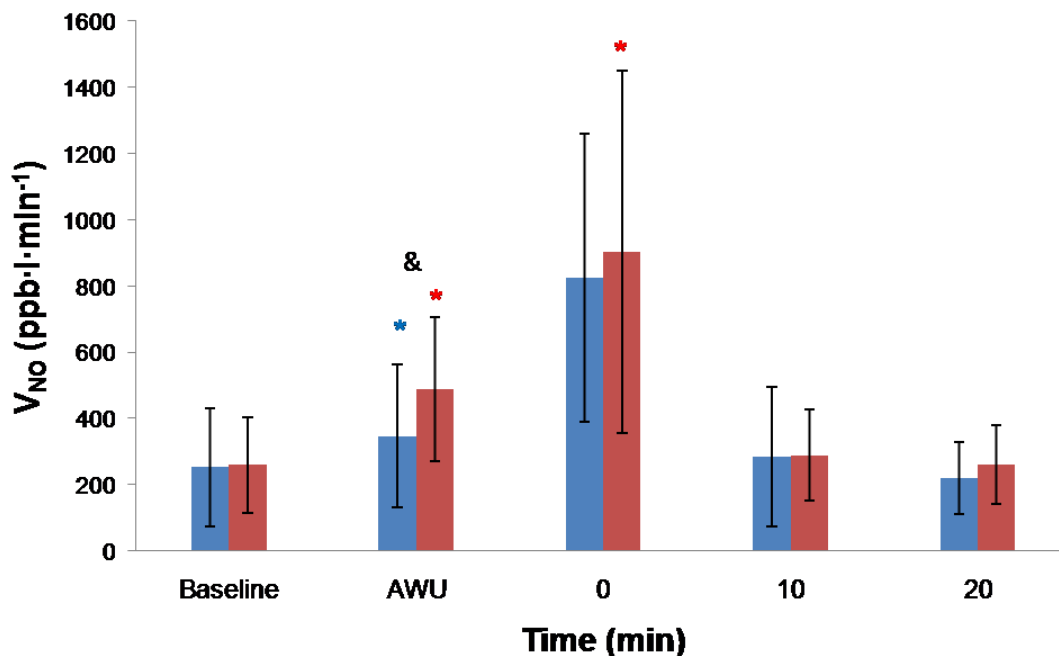


Figure 5.5: V_{NO} ($ppb \cdot l \cdot min^{-1}$) after warm up (AWU), after exercise (0 min, 10 min and 20 min) in cold (blue bars) and normobaric (red bars) climate. Results are given as mean with 95 % confidence interval ($n=20$). * Significant difference from baseline, & significant different between the two climates

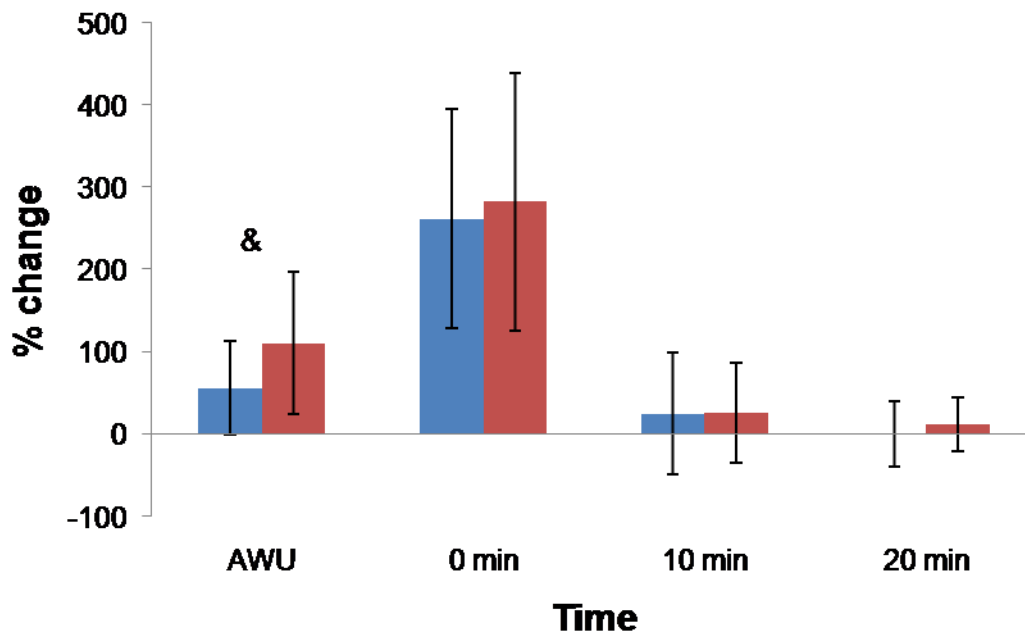


Figure 5.6: Change (%) in V_{NO} ($ppb \cdot l \cdot min^{-1}$) after warm up (AWU), after exercise (0 min, 10 min and 20 min) in cold (blue bars) and normobaric (red bars) climate. Results are given as mean with 95 % confidence interval ($n=20$).
& significant difference between climates

5.4 Pretest

5.4.1 Intensity

The anaerobic threshold test lasted in average in 5 bouts of 5 min each for the 20 subjects, with a variance of 3-7 bouts. Duration of the VO_{2max} test was in average 6 min with a variance of 4-7 min with an average HR of 184.4 ± 12.6 beats \cdot min $^{-1}$. The HR increased in average from 85 % to 99 % of HR_{peak} from 1.-7.min during the VO_{2max} test. Total exercise time during the pretest was approximately 41 min (10 min warm up, 25 min AT and 6 min VO_{2max}). There was no significant difference between the intensity, measured as percent of HR_{peak} , between AT and RET_C and RET_N .

5.4.2 Absolute and relatively (%) changes in FE_{NO}

FE_{NO} values decreased during the pretest and the reduction remained significant different from baseline throughout the measurements (<60 min) ($p < 0.03$). The lowest FE_{NO} value was seen 3 minutes post exercise, thus peak relatively reduction was -37.3 ± 10.8 % and the absolute values are presented in table 5.7. There was a correlation

between FE_{NO} and VE ($r=0.229$) ($p<0.02$) but there was no correlation between FE_{NO} and HR. Ventilation and FE_{NO} levels before, during and after pretest (AT and VO_{2max}), are present in figure 5.8

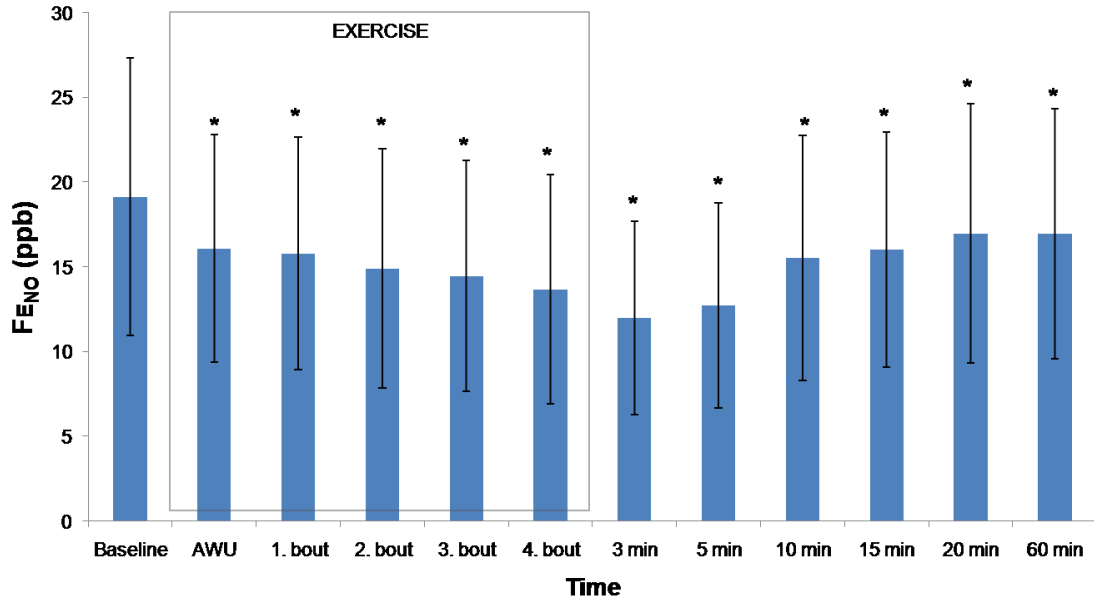


Figure 5.7: FE_{NO} values measured pre, during and post exercise (5 minutes bouts with increased intensity running at a treadmill and VO_{2max}). Results are given as mean \pm SD ($n=20$). * Significant from baseline.

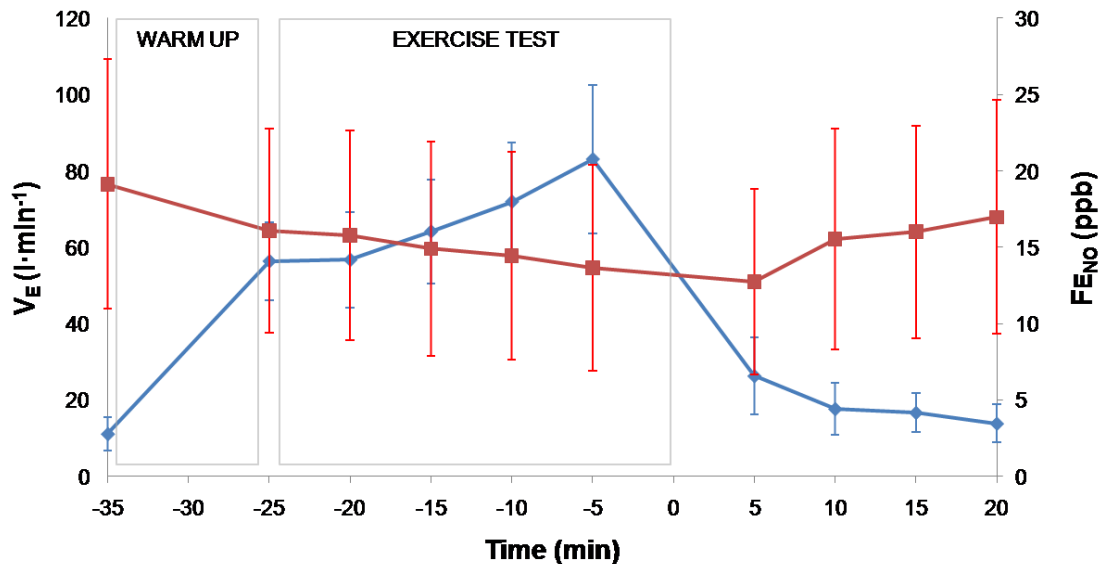


Figure 5.8: Relationship between ventilation (V_E) (blue line) and FE_{NO} (ppb) (red line) from baseline (-35 min), after warm up (-25), during increased intensity (AT) (-20 min, -15 min, -10 min, -5 min), after VO_{2max} (0 min), and pre exercise (5 min, 10 min, 15 min and 20 min). Values are presented as mean with 95 % confidence interval ($n=20$).

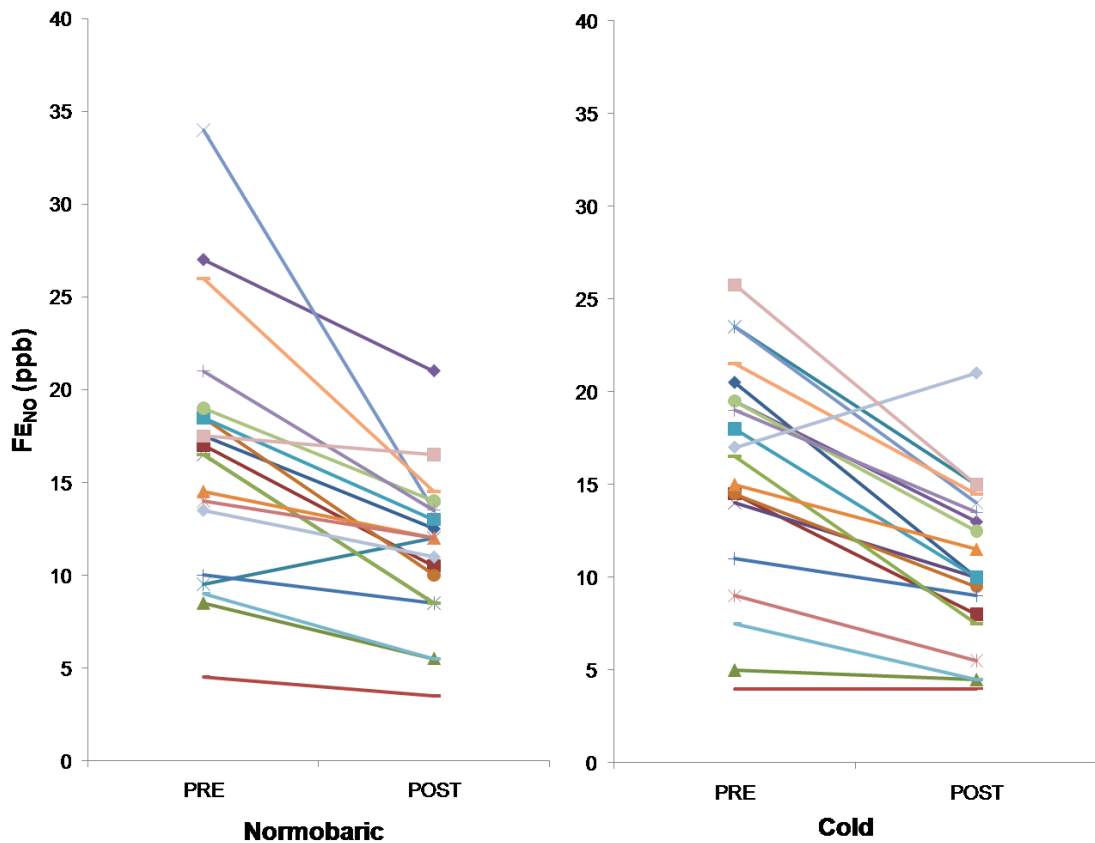


Figure 5.9: Individual levels of $F_{E_{NO}}$ (ppb) before (PRE) and 5 min after (POST) exercise test in a normobaric and a cold climate ($n=20$).

5.5 Individual $F_{E_{NO}}$ values and variety

There was no difference in baseline $F_{E_{NO}}$ and $P_{E_{NO}}$ (online and offline) measurements between cold climate, normobaric climate and pretest (table 5.3). $F_{E_{NO}}$ measured online and offline showed a significant correlation ($r=0.9$, $p<0.01$). A significant difference between gender was seen in baseline $F_{E_{NO}}$ values ($p<0.001$) and pre exercise ($p<0.001$) in total (cold, normobaric and pretest) (table 3.4). The main trend was a reduction in $F_{E_{NO}}$ after exercise in cold and normobaric climate (figure 5.9).

5.6 Ambient nitric oxide (NO_A)

Average ambient NO (NO_A) before each test was $4.4 \text{ ppb} \pm 10$. The NO concentration range from 0.0-90 ppb, however, with only five NO_A registrations with elevated NO values above 30 ppb (32 ppb, 40 ppb, 50 ppb and 90 ppb). No correlation was seen

between FE_{NO} and NO_A , even after excluding the extreme values. The NO_A registration was conducted by the online NO chemiluminescence analyzer.

5.7 Overview of FE_{NO} and PE_{NO} values pre and post exercise

Table 5.4: Baseline and post exercise values of FE_{NO} and PE_{NO} measured online and offline in cold climate, normobaric climate and pretest of 20 subjects. Results are given as mean \pm SD (range).

| | | FE_{NO} (ppb) | | PE_{NO} (mPa) | |
|----------------|----------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
| | | Online | Offline | Online | Offline |
| Baseline | RET_C | 18.77 \pm 6.93 (5.3-29.7) | 15.93 \pm 6.15 (4.0-25.8) | 1.68 \pm 0.63 (0.49-2.74) | 1.46 \pm 0.56 (0.38-2.14) |
| | RET_N | 18.66 \pm 7.12 (3.9-29.5) | 16.63 \pm 6.95 (4.5-34.0) | 1.73 \pm 0.66 (0.36-2.70) | 1.54 \pm 0.64 (0.42-3.11) |
| | Pretest | 19.13 \pm 8.18 (7.0-38.3) | | 1.76 \pm 0.75 (0.64-3.50) | |
| After exercise | RET_C^1 | | 10.63 \pm 4.3* (4.0-21.0) | | 0.97 \pm 0.4* (0.38-1.96) |
| | RET_N^1 | | 11.3 \pm 4.0* (3.5-21.0) | | 1.0 \pm 0.38* (0.32-1.96) |
| | Pretest ² | 11.98 \pm 5.7* (3.8-25.5) | | 1.1 \pm 0.52* (0.36-2.33) | |

¹ 5 min after exercise test.

² 3 min after exercise test.

* Significant different from baseline $p < 0.05$.

6 DISCUSSION

The most important finding was that FE_{NO} measured at a standardized flow of $50 \text{ ml}\cdot\text{sec}^{-1}$ decreased after exercise both in a normobaric and in a cold climate in healthy trained males and females aged 18-28 years. In the cold climate, the reduction in FE_{NO} was significant after warm up, 5 and 20 min after RET (offline measure) and 30 and 60 min (online measure) compared to a significantly reduced FE_{NO} 5 and 10 min (offline measure) after exercise in the normobaric climate. Furthermore, a significant difference between the two climates was seen after warm up and 20, 30 and 60 min post exercise. FE_{NO} was lower in the cold compared to the normobaric climate at these points (both measured online and offline). Twenty four hours after exercise, the FE_{NO} values were similar to baseline in both climates measured offline. Online measurements 24 hours post exercise RET_N showed a significantly higher FE_{NO} value from baseline. A significant decrease in FE_{NO} during and after exercise was seen in the pretest conducted in a normobaric climate, and the change remained to the last measurement 60 minutes post exercise.

The baseline FE_{NO} in average of pretest, RET_C and RET_N was 17.2 ± 7.2 ppb. There was no significant difference between the FE_{NO} values baseline between the three tests. Moreover, there was a significant difference in FE_{NO} between gender, both at baseline and post exercise.

6.1 Intensity of RET in the cold and normobaric climate

The average running velocity during eight minutes exercise for the 20 subjects was significant higher in the normobaric climate compared to the cold climate. However, no significant difference in heart rate (HR) and minute ventilation ($\text{l}\cdot\text{min}^{-1}$) was seen between the two climates, indicating an equal stress on the pulmonary system during the two testes. During the pretest the subjects exercised with similar intensity, but for a longer period of time.

6.2 Nitric oxide during and after exercise in normobaric climate

Our findings of reduced FE_{NO} during and after exercise in normobaric climate are in agreement of previous findings according to Sheel et al. (1999) concluding that most of

the existing data points to a reduced FE_{NO} value from rest to after exercise. Several of the published articles of FE_{NO} and exercise are listed in table 6.1. A closer look to the studies examined the response of FE_{NO} during exercise show disagreement to our findings. The term FE_{NO} is a relatively new expression which refers to the fraction of nitric oxide during a constant flow rate. The early investigation of exhaled NO and exercise used different methods without a constant air flow rate and exhaled gas collection for analyses of NO. Therefore the term exhaled concentration of nitric oxide, [NO], will be used in this chapter if the comparing studies have used this term to avoid wrong expression.

In contrast to a decreased FE_{NO} during exercise, Bauer and colleagues (1994) found a slightly increase in exhaled [NO] in four males after 20 min (2 x 10 min) of stationary bicycling with increasing intensity of 25 % to 50 % beyond resting HR. The exercise experiment was repeated two to three times by each individuals with >2 days apart. An increased exhaled [NO] was also seen after a marathon run, with a duration of 175 ± 50 min, in nine middle aged healthy males (Bonsignore et al., 2001), in fact the exhaled [NO] was twice as high after the competition (26.9 ± 8.6 ppb) compared to the baseline values (12.0 ± 3.8 ppb). Iwamoto et al. (1994) report no change in the exhaled [NO] in eight young subjects after a treadmill test. The treadmill test contained of walking or running with increased treadmill grade of 2.5 % every 3 min until exhaustion. Six members of the Canadian Olympic Rowing Team showed an increased exhaled [NO], followed by a slight decrease, after a constant workload of 1, 2 and 4 liters VO_2 (Maroun et al., 1995). In contrast, the trained and sedate males, six in each category, in the same study experienced a significant decrease in exhaled [NO]. Shin et al. (2003) report in their article an unchanged FE_{NO} in five males and five females 3 min after finishing 20 min. running on a treadmill of 90 % HR_{peak} (predicted: 220 beats per min - age in years). The reason why these studies are conflicting with the others are uncertain, but might be attributed to the methods, exercise duration and intensity and the number of subjects included.

When it comes to methodological differences the exhalation flow rate and gas collection are two main factors that vary between the different studies. Some of the studies used a mixed exhaled measurements for calculating NO (Iwamoto et al., 1994; Maroun et al., 1994), one study has used an end-exhaled sampling (Bauer et al., 1994) and another

study has used a constant air flow rate of $50 \text{ ml}\cdot\text{sec}^{-1}$ and $250 \text{ ml}\cdot\text{sec}^{-1}$ (Shin et al., 2003). This variation of gas collection might affect the measured exhaled [NO] values, although all studies have measured the concentration. A comparison of mixed expired and end-tidal measurement of [NO] shows a significantly higher [NO] in mixed compared to end-tidal expired measurement, but the same tendency of decreased [NO] from rest to exercise was seen (St.Croix et al., 1999). Persson and colleagues (1993) report an early peak of exhaled [NO] and thereafter a decreased [NO] to a plateau during tidal breathing. This may be due to a mixture of exhaled NO and nasal NO during the first seconds of exhalation, and since the nasal release of NO is 40-120 times the release from rest of the respiratory tract without resistance this may lead to an increased [NO] during tidal breathing (Kharitonov et al., 1997). However, due to increased V_E during exercise a higher exhaled air flow is performed and this have been reported to give significant reduced [NO] (Kharitonov et al., 1997), and no change in [NO] can be seen.

A constant flow technique ensures valuable comparisons of FE_{NO} within the lung between rest and exercise. St Croix and colleagues (1999) was the first group investigating the effect of exercise up on exhaled [NO] at a constant airflow rate, both $46 \text{ ml}\cdot\text{sec}^{-1}$ and $950 \text{ ml}\cdot\text{sec}^{-1}$. They report a small but significant decrease in [NO] in the two different airflow rates, represent alveolar ($950 \text{ ml}\cdot\text{sec}^{-1}$) and airway sources ($46 \text{ ml}\cdot\text{sec}^{-1}$) of exhaled NO in five participants. Bonsignore et al. (2001) measured the exhaled [NO] with a constant airflow rate in their study and still they reported increased exhaled [NO] levels. The reason for this may be attributed to an airway infection after strenuous exercise in 8°C outdoor rather than an effect of exercise. Still, some of the studies report a decrease in exhaled [NO] despite in bag collection of exhaled air (Trolin et al., 1994; Phillips et al., 1996; Chirpaz-Oddou et al., 1997; Therminarias et al., 1998). In all these experiments the subjects inhaled NO free air before exhaling and this may not interfere the exhaled [NO], therefore it should not be any difference in [NO] between collecting mixed exhaled air and one tidal breath (Kharitonov et al., 1997).

Table 6.1: Published articles that have measured nitric oxide before and after exercise in normobaric climate in healthy adults.

| Author | Exercise type, intensity and duration | Exhaled flow rate | Subjects | Basal NO ³ | Post NO ⁴ |
|--------------------------------------|---|---|---|--|--|
| Sachs-Olson et al 2009 (unpublished) | Roller ski, bicycle and running maximal | 50 ml·sec ⁻¹ | n = 31 Age 20-40 A M = 23 F = 8 | 20.90 ppb | 1.2-1.3 ppb reduction |
| Mantiore et al 2007 | Climbing stairs 2 min | | n = 24 Age 21-45 S M = 7 F = 17 | 22.8 ± 4 ppb | 13.0 ± 2 ppb |
| Verges et al 2006 | Ergometer cycling A: 100 min increased intensity (25-60 % max power output) B: 5 x 90-120 min 75-85 % max power output(24 and 48 h between test) | 170 ml·sec ⁻¹ | n = 18 Age 27 ± 2 T/A M = 10 F = 8 | A: 15.1 ± 2.1 ppb B: 25.7 ± 5.1ppb ² | A: 9.5 ± 1.7 ppb B: 18.8 ± 4.0 ppb ² |
| Verges et al 2005 | 3 x 10 min 40 % of max power output at VO _{2max} (P _{max}) 10 min 60 % P _{max} 5 min 90 % P _{max} | 170 ml·sec ⁻¹ | n = 20 Age 31.2 ± 10.7 T/A M | 27.7 ± 28.5 ppb | 23.5 ± 27.6 ppb |
| Shin H-W et al 2003 | Running (indoor) 90 % HR _{max} (HR _{max} : 220-age in years) 20 min | A: 50 ml·sec ⁻¹ B: 250 ml·sec ⁻¹ | n = 10 Age 21-37 T M = 5 F = 5 | A: 9.91 ± 6.02 ppb B: 3.46 ± 2.43ppb | A: 9.25 ± 5.86 ppb B: 3.46 ± 2.47 ppb |
| Kippelen et al 2002 | Ergometer cycling 10 min 60 % VO _{2max} 15 min 90 % Vo _{2max} 3 min 60 W recovery | Mixed exhaled | n = 22 Age 21 ± 0.5 A = 12 S = 10 M | A = 7.9 ± 3.7 ppb S = 13 ppb ¹ A = 4 nmol·min ⁻¹ S = 5 nmol·min ⁻¹ | A = 6 ppb ¹ S = 8 ppb ¹ A = 18 nmol·min ⁻¹ S = 18 nmol·min ⁻¹ |
| Bonsignore et al 2001 | Running (outdoor) marathon competition 179 ± 24 min | 100 ml·sec ⁻¹ | n = 8 Age 40.1 ± 10.7 A M | 12.0 ± 3.8 ppb | 26.9 ± 8.6 ppb |
| de Gouw et al 2001 | Ergometer cycling 40-50 % VE _{max} 6 min | 100 ml·sec ⁻¹ | n = 12 Age: 19-37 | 6.7 ± 0.7 ppb | Reduction of 10 % ¹ |
| St Croix et al 1999 | Cycling submaximal 30,60 & 90 % VO _{2max} 3 min x 3 constant loaded (total 9 min) | A: 46 ml·sec ⁻¹ B: 950 ml·sec ⁻¹ | n = 5 Age 27-59 S/T M = 6 F = 3 | A: 36.9 ± 32.3 ppb B: 4.6 ± 2.7 ppb | A: 33.6 ± 29.0 ppb B: 3.2 ± 2.0 ppb |
| Therminarias et al 1998 | 30 W increase every 3.min until exhaustion | Mixed exhaled 400 ml·sec ⁻¹ | n=8 Age 31.2±2 T M | 17 ppb ¹ | 8.77 ± 1.01 ppb 41.52 ± 5.83 nmol·min ⁻¹ |

| | | | | | |
|--------------------------|--|---|--|---|---|
| Pogliaghi et al 1997 | Ergometer cycling 0-50 W and increased 50 W every 3 min until exhaustion | Mixed exhaled | n = 10 Age 22.1 ± 2.1 T M | 15.3 ± 8.0 ppb 137.7 ± 57.8 ppb·min ⁻¹ | 6.1 ± 4.0 ppb 544.7 ± 387.0 ppb·min ⁻¹ |
| Chirpaz-Oddou et al 1997 | Cycling submaximal increased 30 W every 3 min until W _{max} , 2 min 50 % W _{max} recovery | Mixed exhaled 400 ml·sec ⁻¹ | n=22 Age 31±2 (MA), 31±4 (MS), 37±7 (FS) A/S M=14 (A=8, S=7) F=7 (8) | S M=14.8 ppb S F=16.2 ppb A M=15.9 ppb | S M=9.0 ppb S F=10.5 ppb A M=10.5 ppb |
| Phillips et al 1996 | Ergometer bicycle 0-150 W (increasing 25 W each step) 6 min each step | Mixed exhaled | n = 8 Age 20-53 | 15.1 ± 2 ppb 57 ± 10 nl·min ⁻¹ ·m ⁻² | 6.8 ± 1.5 ppb 171 ± 30 nl·min ⁻¹ ·m ⁻² |
| Maroun et al 1995 | Ergometer bicycle 55 (S/T/A), 120 (S/T/A) and 310 W (A) ≥10 min | Mixed exhaled 45-50 ml·sec ⁻¹ | n = 18 Age 20-31 S/T/A | S/T/A: 12 ppb ¹ | S/T: 5ppb ¹ A: 9 ppb ¹ |
| Matsumoto et al 1994 | Cycling maximal Mean duration 11.3 ± 1.3 min | | n = 5 Age 21-40 M | 10 ± 4 ppb 121 ± 53 nl·min ⁻¹ | 6 ± 2 ppb 398 ± 164 nl·min ⁻¹ |
| Iwamoto et al 1994 | Treadmill exercise (graded intensity) T:6 mph run S:3.5 mph walk Increased (2.5 %) treadmill grade 3. min until exhaustion | Mixed exhaled | n = 8 young S/T M=6 F=2 | 26.3 ± 19.0 ppb 12.3 ± 9.9 nl·min ⁻¹ | Increased 297 ± 94 % nl·min ⁻¹ |
| Trolin et al 1994 | Ergometer bicycle F:30,60 & 90 W M:50, 100 & 150 W 6 min each step | Mixed exhaled | n = 8 Age 25-46 M=4 F=4 | 9.52 ± 1.35 ppb | 4.25 ± 0.55 ppb |
| Bauer et al 1994 | Ergometer bicycle Increase 25 % - 50 % HR _{max} 20 min | Mixed exhaled 40 ml·sec ⁻¹ | n = 4 Age 27-47 | 12.9 ± 2.4 ppb | 14.3 ± 2.8 ppb |
| Persson et al 1993 | Ergometer bicycle 50 & 100 W 6 min each level | Mixed exhaled 500 ml·sec ⁻¹ | n = 5 Age 30-42 | 9 ± 2 ppb 69 ± 14 nl·min ⁻¹ | 5 ppb ¹ 225 ± 40 nl·min ⁻¹ |

A, athlete; T, trained; S, sedate; M, male; F, female; ppb, part per billion; nl·min⁻¹ and ppb·min⁻¹, product of exhaled nitric oxide output and minute ventilation (l·min⁻¹); nl·min⁻¹·m², product of exhaled nitric oxide output per square meter of body surface area per minute;

¹ absolute value of NO is not reported and the values reported are based on figure, average value; ² mean of 5 repetitive exercise session conducted with 24 and 48 hours between ; ³ resting values of exhaled nitric oxide concentration; ⁴ the first post exercise or last measured during exercise values of exhaled nitric oxide concentration; NO, nitric oxide.

There are differences in the duration and intensity of the exercise protocol used in the different studies. In the present study a greater relatively reduction in percent after pretest ($-37.3 \pm 10.8 \%$) compared to RET_N ($-28.5 \pm 19.0 \%$) was found, although there was no difference in the intensity measured in % of HR_{peak} . During the pretest the subjects exercised over a longer period of time compared to the RET_N , and it may appear that duration can affect the FE_{NO} levels. The duration of the exercise protocols in the reported studies vary from 20 min to 154-221 min and the activities are running, cycling and running marathon, respectively. However, Verges et al. (2006) did not find a correlation between change in FE_{NO} and duration in eight subjects after outdoor cycling for 90-120 min duration with an intensity of 75-85 % of HR_{max} .

There is a great variation in the exercise intensity among the studies, from 50 % to 90 % of HR_{max} . In the present study the intensity during the exercise test was similar in all three tests. However, a significantly reduction in FE_{NO} from baseline after warm up in pretest was found but stalled after warm up in the RET_N . This may be due to different intensity during warm up but the intensity in warm up during pretest was in average 71 % of HR_{peak} and the intensity of the warm up during the RET_N was 65-75 % of HR_{peak} . Chirpaz-Oddou and colleagues (1997) reported that the intensity had to exceed 65 % of VO_{2peak} to be able to see a significant alteration in exhaled [NO] during exercise. This result may explain the unchanged results in exhaled [NO] in Bauers group. On the other hand, St. Croix et al. (1999) report a small but significantly decrease in FE_{NO} after 30 % of VO_{2max} , with no difference between the exercise intensity levels of 60 and 90 % of VO_{2max} . It is not clear in the literature if intensity influences the exhaled [NO] or FE_{NO} values during exercise.

6.2.1 FE_{NO} values after exercise in a normobaric climate

The FE_{NO} values after the RET_N in the present study reached values that did not differ significant from baseline 15 min post exercise measured offline. Despite this, a significant difference from baseline 24 hours after RET_N measured online was seen. This was not found in the offline measurement 24 hours after RET_N . The FE_{NO} levels after the pretest had not returned to resting values at the last measurement performed 60 min post exercise. It should be mention that the relatively changes of FE_{NO} (offline) in percent was shown to be significantly lower 5 min after RET_N . Published studies show

that FE_{NO} returns to its baseline values 5 min (Cirpaz-Oddou et al., 1997; St Croix et al., 1999;), 10 min (de Gouw et al., 2001; Bauer et al., 1994), 15 min (Verges et al., 2006; Verges et al., 2005;), and 1 hour (Kippelen et al., 2002) post exercise. The reasons for these differences are difficult to explain due to the variation in methods and exercise protocols.

6.2.2 Nitric oxide output before and after exercise in normobaric climate

Nitric oxide output (V_{NO}) is a product of minute ventilation ($l \cdot \text{min}^{-1}$) and rate of NO exhaled and are expressed in different units, including $nl \cdot \text{min}^{-1}$, $nmol \cdot \text{min}^{-1}$ or $ppb \cdot l \cdot \text{min}^{-1}$. V_{NO} is generally assumed to reflect the NO production by cells within the respiratory tract. In accordance with previous studies (Persson et al., 1993; Iwamoto et al., 1994; Matsumoto et al., 1994; Phillips et al., 1996; Pogliaghi et al., 1997; Kippelen et al., 2002) an increased V_{NO} during exercise in normobaric climate was found in the present study. Since V_{NO} is based on the values of exhaled [NO], diverse gas collection methods and collection duration is performed. Due to the lack of consistent units, these data should be interpreted with caution.

6.2.3 Summary

When measuring exhaled NO during and after exercise in normobaric climate in line with the latest guidelines, the FE_{NO} decrease. Most of the studies conducted before 2000 did not include a standardized method for FE_{NO} measurements, in addition to a mixture of intensity, duration and type of exercise may explain the variance in the exhaled NO modulating when exposure to exercise. Overall, V_{NO} increases during exercise and the exhaled NO values return to resting values approximately within one hour.

6.3 Nitric oxide before and after exercise in the cold climate

We observed a significant reduction in the FE_{NO} after exercise in -10°C and it was significantly lower exposed to a cold climate compared to a normobaric climate after warm-up, 20, 30 and 60 min post exercise. It is not known if the FE_{NO} values between the two climates differs during exercise because absent of measured FE_{NO} during RET. To our knowledge there is only one study that has examined the changes in exhaled NO and V_{NO} in a cold ambient climate (Therminarias et al., 1998). In addition, Pendergast et

al. (1999) explored the effect of immersion in cool water on exhaled NO at rest and during exercise. The main results from these two studies are shown in table 6.2.

Therminarias et al. (1998) investigated the effect of graded stationary bicycling until exhaustion on exhaled [NO] in eight trained males in an ambient temperature of -10°C and 22°C. Accordingly to our findings they report a decrease in exhaled [NO] in the cold (-10°C). A significantly lower exhaled [NO] was report at an intensity of 30, 60, 90 and 120 W (approximately until 50% of VO_{2peak}) in the cold climate; thereafter no differences between the two climates were seen. Pendergast et al. (1999) found a significantly decrease in exhaled [NO] when ten male participants cycled immersed in water temperature of 20, 35 and 30°C. They report a significantly lower exhaled [NO] in 20°C compared to 30 and 35°C at 50 and 100 W. Similar to Pendergast and Therminarias groups we experienced a significant difference between the two climates after running at a low intensity in the present study.

Table 6.2: An overview of the published articles that have measured exhaled nitric oxide before and after exercise in a cold climate in healthy adults.

| Author | Exercise type, intensity and duration | T _c | Subjects | Basal NO ² | Post NO ³ |
|-------------------------|--|----------------------------|--------------------------------|---|---|
| Pendergast et al 1999 | Ergometer cycling in water Start: 25-50 W increased with 50 W every 4 min until exhaustion | Water: 35°C, 30°C and 20°C | n = 10 Age 22 ± 2 T M | 35°C = 11 ppb ¹ 30°C = 10.5 ppb ¹ 25°C = 8 ppb ¹ | 35°C = 6.5 ppb ¹ 30°C = 5 ppb ¹ 25°C = 4 ppb ¹ |
| Therminarias et al 1998 | 30 W increase every 3.min until exhaustion | Air: -10°C | n=8 Age 31 ± 2 T M | 12 ppb ¹ 5 nmol·min ⁻¹ ¹ | 9.99 ± 1.95 ppb 44.99 ± 11.11 nl·mol ⁻¹ ¹ |

T_c, temperature during exercise; T, trained; M, male; ¹ absolute value of NO is not reported, therefore are the value reported here based on figure, average value. ² resting values of exhaled nitric oxide concentration; ³ the first post exercise or last measuring during exercise values of exhaled nitric oxide concentration; NO, nitric oxide; nmol·min⁻¹, product of exhaled nitric oxide output and the minute ventilation (l·min⁻¹)

Our results shows a suddenly drop in FE_{NO} after warm up in the cold climate and it was significantly lower than the normobaric climate at the same time. The baseline value of exhaled [NO] was significant different between the climates in both Therminarias and Pendergast. The different baseline values in exhaled [NO] may be due to the resting period before measuring baseline values. Measurements of exhaled [NO] was conducted after 5 min rest in water temperature of 35°C and after 30 min rest in water temperature

of 30 and 20°C (Pendergast et al., 1999) and 5 min in a cold ambient climate (Therminarias et al., 1998) before start exercising. Perhaps the drop in FE_{NO} after warm up in the cold climate not had been seen if the measured baseline FE_{NO} had been performed resting in the cold but rather a significant difference in baseline values.

6.3.1 FE_{NO} level post exercise in cold climate

After RETs we observed a parallel relationship between the climates in the change of FE_{NO} at 5, 10 and 15 min after test. FE_{NO} was significant different from baseline 5 and 20 min (offline measurement) and 30 and 60 min (online measurement) post exercise. By adding more subjects we might have seen these results 10 and 15 min offline post exercise. Moreover, a significantly lower FE_{NO} after RET_C compared to RET_N was seen 20, 30 and 60 min post exercise both measured offline and online. FE_{NO} 24 hours after test was similar to baseline value and not different from the normobaric climate. Therminarias and colleagues (1998) report a similar exhaled [NO] in a temperate ambient temperature 15 min after exercise, whether exercise was performed at -10 or 22°C, and they did not report any further measurement post exercise after 15 min. No other studies have followed the development of FE_{NO} after exercise in a cold climate as in this present study.

Subjects in the present thesis were exposed to the cold climate approximately in 45 min, hence total 23 min of exercise and 20 min resting after the exercise. Compared to Therminarias et al. their subjects were exercising in 29 ± 1 min, followed by a 5 min rest period before leaving the climatic chamber our subjects were exposed by the cold climate for a longer period. Despite these inequalities I cannot find other explanation for the small differences seen between our results and Therminarias et al. Perhaps Therminarias et al. would see a further change in FE_{NO} if they had preceded the measurement longer than 15 min post exercise.

6.3.2 Nitric oxide output before and after exercise in cold climate

Like in the normobaric climate an increased V_{NO} was found in the cold climate of both Therminarias and Pendergast, expressed as $ppb \cdot \text{min}^{-1}$, and V_{NO} increased progressively as a function of workload. This result is in agreement to our finding of an increased V_{NO} from baseline (before warm up) to immediately (0 min) after exercise, a relatively

change of 260.9 ± 133.3 %. A significantly lower V_{NO} in the RET_C compared to the RET_N was seen after warm up, and a tendency to lower V_{NO} immediately after test in the cold climate was found, but did not reach a significant level. Significantly lower V_{NO} was also reported by Therminarias in the cold compared to normobaric climate at rest and at a work load of 30, 60, 90, 120, 150 and 180 W, peak work load was 270 W. Additionally, Pendergast and colleagues found a significantly lower V_{NO} in the water of $20^\circ C$ than 30 and $35^\circ C$, and at peak workload (250 W) this difference reached 59 %.

6.3.3. Summary

It is likely to believe that a cold climate influences the FE_{NO} level and may lead to a greater reduction in the FE_{NO} than a normal indoor climate at the same exercise intensity and duration. The reduced FE_{NO} in the cold remained reduced until 60 min post exercise measured online. The V_{NO} seems to increase less in a cold climate, but more data are required to underline these findings. The further alteration in FE_{NO} after exercise needs to be investigated more.

6.4 Mechanism modulating V_{NO} outcome and FE_{NO}

The mechanism underlying the alteration in V_{NO} and FE_{NO} during exercise is not entirely understood and the sources of NO in exhaled breath can originate from the epithelial and nerve cells in the lung or derived to the lung through the circulating blood (Gaston et al., 1994). Some hypothesis is proposed to explain the increased V_{NO} and decreased FE_{NO} / exhaled [NO] during exercise, include vascular shear stress and increased airway flow.

6.4.1 Normobaric climate

As a result of increased pulmonary blood flow during exercise it is thought that the alter lung V_{NO} outcome is due to vascular shear stress. Release of endothelial NO results in a smooth muscle relaxation via cGMP pathway (Katsuki et a., 1977) and a fall in vascular resistance, thus vasodilatation. Because of the close relationship between endothelial cells and alveolar space it is suggested that some of the exhaled NO is derived from pulmonary vascular endothelium (Mauron et al., 1995). Bauer and colleagues (1994) suggested changes in V_{NO} was due to cardiovascular factors on the background of decreased V_{NO} during hyperventilation and a parallel change in NO and HR. This is

supported by Maroun et al. (1997) and Iwamoto et al (1994), showing a rise in V_{NO} correlating with VO_2 and HR and an unchanged exhaled [NO] during voluntary increased V_E . However, we did not find a correlation between HR and FE_{NO} levels during exercise in the pretest. Because FE_{NO} values were measured before and after exercise in the climatic chamber we don't have any data on HR and FE_{NO} values during RET_N .

On the other hand, Phillips et al. (1996) did not find any increased V_{NO} output during dobutamine infusion in attempt to increase pulmonary blood flow. Yet, a small increase in V_{NO} output during dobutamine infusion was observed, although the change was not significant. Overall, they conclude that increased ventilation rather than blood flow stimulate increased exhaled V_{NO} during exercise. It is shown that V_{NO} is not increased simultaneously with pulmonary blood flow during water immersion or increased gravity in absence of changes in V_E and VO_2 (Pendergast et al., 1997). There is a possibility that experimental maneuvers of dobutamine, water immersion and gravity did not alter vascular shear stress enough to affect the NO release. Additionally, given the rapid uptake and inactivation of NO by hemoglobin and short half-life of NO in the physiological system (Gaston et al., 1994) it is suggested to be unlikely that changes in systemic and/or pulmonary vascular formation of NO would be detectable in the exhalation.

The first group that investigated the effect of exercise on exhaled [NO] in humans reported a similar increase in V_{NO} ($nl \cdot min^{-1}$) during hyperventilation and exercise with similar V_E output (Persson et al., 1993). They claimed that an increased V_{NO} is due to the increased ventilation, and not an increased cardiac output or endothelial shear stress. Trolin and colleagues (1994), propose an endogenous production of NO since exhaled NO always was higher than [NO] of room air and they suggest increased blood flow and increased NO release from the airway contribute to increased exhaled [NO].

In 1999 a group of researchers measured exhaled [NO] at a constant airflow for the first time and this was the first FE_{NO} measurement (St. Croix et al., 1999), thus the [NO] within the airways. They found a small but significant decrease in FE_{NO} both at a constant airflow rate of $46 ml \cdot sec^{-1}$ and $950 ml \cdot sec^{-1}$ and reports an increased V_{NO} during exercise. Additionally, they saw no changes in measured levels of nitrate or nitrite in venous plasma. They conclude that the increased V_{NO} is not due to systemic

and/or airway production, but rather a function of high airflow rates followed by a reduced luminal [NO]. This is explained by Hyde and colleagues (1997) with a mathematical model. Exercise results in an increased diffusion capacity of NO into the blood and increased ventilation in the lower airways followed by a reduced NO tension, if the production in the lung is constant. These mechanisms cause a decrease in the concentration gradient and less NO diffuses into the blood and leads to an increase in the volume NO recovered in the exhaled air. In fact, exercise leads to a fall in NO taken up by the blood from normally 94 % to 76 % and NO in exhaled air increase fourfold (Hyde et al., 1997). This is showed by Shin et al (2005) who investigated the mechanism of NO exchange after high-intensity exercise among ten healthy males and females by a two-compartment model. They did not see any changes in FE_{NO} measured at $50 \text{ ml}\cdot\text{sec}^{-1}$ and $250 \text{ ml}\cdot\text{sec}^{-1}$ constant airflow rates after 20 min of 90 % of predicted HR_{peak} but decreased [NO] in the bronchial wall. They conclude that high-intensity exercise enhanced the ability of NO to diffuse between the airway tissue and the gas phase. In contrast to the results of Shin et al. Verges et al. (2006) reported a decreased FE_{NO} in 18 healthy subjects throughout prolonged exercise session performed on a cycle with duration of 100 min. They exclude these effects from increased blood flow. Verges results are supported by others (Sachs-Olsen et al., 2009 (unpublished); Mantiore et al., 2007). Our results of reduced FE_{NO} during exercise (pretest) and reduced FE_{NO} after exercise (pretest and RET_N) are according to Verges et al. (2006). Moreover, Cirpaz-Oddou and colleagues (1997) suggest as an effect of increased V_E increased heat and waterloss from the airways due to an increased blood flow that leads to a reduced FE_{NO} during exercise.

6.4.2 Cold climate

The V_{NO} and the FE_{NO} levels in the present study were significantly lower exposed to -10°C compared to a normobaric climate and may be due to the cold climate exposure. In addition to mechanisms that contribute to increased V_{NO} and decreased FE_{NO} or exhaled [NO] in normobaric climate there may be additional factors that contributes in the cold. Pendergast et al. (1999) suggest that the reduction in NO in a cold environment could contribute to bronchoconstriction and pulmonary vasoconstriction. An airway obstruction, shown by a decreased FEV_1 by 5 % in healthy well-trained males, was seen in Therminarias et al. (1998). Inhalation of cold air is shown to decrease V_E rate at rest and sensitivity to various ventilator stimuli (Giesbrecht, 1995). Quirion et al., (1989)

reports a reduced VO_{2max} , time to exhaustion and workload during incremental cycling in a climatic chamber with a temperature of $-20^{\circ}C$ in 18 males. The cold stimulus may lead to a greater loss of heat and water in the airway tract than normobaric climate and despite lower V_E this may be due to a greater FE_{NO} reduction after exercise in the cold climate. Koskela (2007) suggested a respond in mast cells and eosinophilic cells and are shown to synthesis NO in rats (Barnes and Belvisi, 1993) and an increased FE_{NO} after exercise in the cold climate was therefore likely to be seen. Additionally, an increased cytokines profile is seen in animals after exercise in $-5^{\circ}C$ and this might lead to an increased iNOS expression. iNOS release NO several hours after exposure of cytokines (Riccardolo, 2003). On the other hand, NO works as an inflammatory mediator and intracellular enzyme (Widmaier et al., 2006). Yoshihara et al. (1998) exposed guinea pigs to cold air after NOS inhibition that lead to a bronchoconstriction. They suggest that the cold air leads to an inflammation and a release of kinins that excite sensory nerves, thus releasing the proinflammatory tachykinins and when NO release is blocked, tachykinins cause airway smooth muscle contraction. They believe kinins also modulate the release of NO, thus inhibition of bronchoconstriction effect of tachykinins (Yoshihara et al., 1998). This might be due to the decreased FE_{NO} levels seen immediately after exercise in the cold climate. It should be mention that relative change of FE_{NO} in percent increased significant 24 hours post exercise and SD was large. The alteration 24 hours post exercise of FE_{NO} varied greatly, some increased 75 % and other decreased 46 %.

Pendergast and colleagues (1999) discuss a possible left-shift of the hemoglobin dissociation curve, because of the cold hemoglobin keeps on the oxygen (McArdle et al., 2006). The reduced partial pressure of oxygen (PaO_2) is followed by decreased oxyhemoglobin saturation and it is suggested to be caused by ventilation perfusion (V/Q) mismatching (Sheel et al., 2000). Decreased SaO_2 is observed in well trained males ($VO_{2max} >55$ ml/kg/min) and females during heavy workloads and are termed exercise-induced hypoxemia (EIH). However, a significant difference between exhaled [NO] in a group with EIH compared to non EIH is lacking (Sheel et al., 2000; Kippelen et al., 2002). Recently it has been suggested that a reduced exhaled [NO] post exercise is due to a greater V_E and oxygen utilization and therefore a lower PaO_2 in arterial blood immediately after exercise followed by a reduced NOS modulating (Mantione et al., 2007). FE_{NO} is shown to be sensitive to PaO_2 (Hemmingsson et al., 2009) and O_2 is the

main component in the NOS synthase. The subjects in the present study had a tendency of lower V_E and a significantly lower running speed in the cold climate despite similar exercise intensity, measured in % of HR_{peak} , throughout the two climatic tests. Despite a lower V_E in the cold a significantly lower FE_{NO} value was seen after warm up.

The mechanism that results in a further reduction in FE_{NO} is unknown and the lacking of measured physiological variables in our study, like PaO_2 , $PaCO_2$, FEV_1 , V_E and FE_{NO} measurements during exercise and monitoring of cytokines, makes it impossible to add further indications.

6.4.3 Summary

Overall, it seems that the changes in FE_{NO} and V_{NO} in a normobaric climate are predominately explained by increased ventilation induced by exercise but may not be the sole stimulus. It seems like the term “production of NO” or “output of V_{NO} ” may be misleading. Moreover, I must emphasize different exercise protocols and methods make it presumptuous to draw a conclusion. The greater decreased FE_{NO} and exhaled [NO] in the cold climate may be due to a greater loss of heat and water from the airways. Cold climatic exposure seems to decrease FE_{NO} levels for a longer duration in the post exercise period. Furthermore, more research needs to confirm our data.

6.5 FE_{NO} values in healthy adults after ATS/ERS standard

The average total baseline FE_{NO} value in the present theses was 17.2 ± 7.2 ppb in 20 healthy well trained males and females aged in average 22.6 (18-28) years, measured after /ERS (2005) standardization using $50 \text{ ml}\cdot\text{sec}^{-1}$ as the standard flow rate. Despite current guidelines there are not yet specified “normal” values. There are several studies attempted to provide these reference values. Because there are several factors that contributes the FE_{NO} values (table 3.1) there may be more useful to express the “normal” FE_{NO} values by upper normal values as mean plus two standard deviations (95th percentiles) (Taylor et al., 2006). According to this, our result shows an upper normal FE_{NO} limit of 31.6 ppb. In a study of 30 healthy subjects aged 18-60 years a mean FE_{NO} of 16.3 ppb was reported, that gave a 95th percentiles of 33.1 (Kharithonov et al., 2003). These values are according to the suggested “normal” FE_{NO} values reported by others (Lim et al., 2008; Olin et al., 2007; Taylor et al., 2006). However,

our finding is slightly higher than 19.7 ppb suggested by Olivieri et al. (2006) and Taylor et al. (2007), and lower than mean $F_{E_{NO}}$ of 25.8 ± 29.5 ppb and 48.3 ± 46.4 ppb among 20-29 years old female and male, respectively, reported by Tsang and colleagues (2001). It is obvious need for more data on normal $F_{E_{NO}}$ values in healthy adults to confirm these findings.

6.5.1 Individually difference in $F_{E_{NO}}$

A large range in $F_{E_{NO}}$ levels measured in the three tests was found (table 5.4). Other investigators have also reported a large range in their $F_{E_{NO}}$ values (Oliveieri et al., 2006; Olin et al., 2007). By selecting a homogeneous group of non-smoking, non-snuffing, healthy subjects with intermediate to high level of fitness and with no recent history of respiratory viral infection we tried to limit the variety in $F_{E_{NO}}$. Despite strictly inclusion and exclusion criteria there are individuals with unexplained higher or lower $F_{E_{NO}}$ levels.

Our results show significant differences between male and female in $F_{E_{NO}}$ values, with higher values among the males. Gender related differences were first reported by Jilma and colleagues (1996). Male appears to have higher $F_{E_{NO}}$ levels than women (Tsang et al., 2001; Grasemann et al., 2003; Taylor et al., 2007). However, there are variable reports regarding gender. Verges and colleagues (2006) did not find a gender difference among 18 participants, and this is supported by others (Sachs-Olsen et al., 2009 (unpublished); Mantione et al., 2007). The gender differences seen in $F_{E_{NO}}$ may be due to differences in surface area of the airway epithelium, thus height. The fact that $F_{E_{NO}}$ is a measure of the fraction of nitric oxide within the lung, the size of the lungs should not affect the $F_{E_{NO}}$ value. Olin and colleagues (2007) report a higher $F_{E_{NO}}$ among males compared to females, but when they compared males and females with similar height and age no gender differences was seen. The same group of investigators predicted that 50-70 % of the variations are explained by height, age and sex and they suggest that additionally factors like airway inflammation, nutritional history and genetic may be involved together with methodological factors which may contribute to this variation. Tsang and colleagues (2001) found a significant correlation between $F_{E_{NO}}$ and body mass index (BMI), weight and body surface area. It is suggested that genetic factors in nNOS in females may contribute to the $F_{E_{NO}}$ values (Grasemann et al., 2003). A

Norwegian study showed that genetic effects accounted for 57 % of the variance in FE_{NO} together with nonshared environmental effects on twins (Lund et al., 2007). In a cross sectional study recently published, there was no gender differences in FE_{NO} measured 2200 subjects (1111 females and 1089 males) (Olin et al., 2006).

Habib (2008) state that there is no consistent relationship between FE_{NO} and adults, but in children FE_{NO} increases with age. This statement is supported by Tsang and colleagues (2001) who were unable to find a correlation between age and FE_{NO} among 120 nonsmoking subjects in addition to Olivieri et al. (2006). However, Olin et al. (2007) reports a mean FE_{NO} of 36.9 ppb among subjects >60 years and 18.7 ppb among subjects <30 years. The subjects in the present theses were all about the same age and difference in age cannot likely be proven.

6.5.2 Summary

There is a large individual variance in FE_{NO} levels and it might be explained by weight, height, age, genetic and nutritional factors rather than gender; further studies should be carried out to verify these factors at FE_{NO} .

6.6 PE_{NO} values

PE_{NO} values are presented in the present thesis and this makes it possible to compare the effect of exercise upon FE_{NO} at different altitudes. There was a significantly decrease in PE_{NO} from baseline to after exercise in RET_N , RET_C and pretest and the baseline values of PE_{NO} was not significant different between the three tests.

6.7 Methodological consideration

6.7.1 Design and subjects

By using a cross over design it was possible to investigate the physiological differences of individuals, rather than groups. A limitation of a cross over design is a lack of a control group not exposed to the experiment, since the subjects are their own control. We selected a homogenous group of non smoking healthy, well trained males and females aged 18-28 years to avoid age and fitness related differences. The

generalization of the FE_{NO} results are therefore reduced, anyway, we add more information of FE_{NO} values in this particular group.

6.7.2 Strength and limitation of the study

There has been reported an increased FE_{NO} after ingestion of nitrate or nitrate-containing food (ATS/ERS, 2005). To avoid any influence of food to the FE_{NO} measurements we asked the subjects to refrain from certain food and eat and drink at least one hour before testing. The result of the present study is comparable with studies conducted in altitude because converting FE_{NO} values to PE_{NO} values.

It is shown both short term and seasonal variation of exhaled and nasal NO in healthy subjects. Stark and colleagues (2006) revealed lower FE_{NO} levels in the mornings than afternoon values. However, day-to-day, week-to-week and seasonal levels of FE_{NO} was found to be highly reproducible. Unfortunately, it was impossible for us to implement the test so each person had the same measurement time for each test. That may affect the results; anyway, there was no significantly difference between the baseline FE_{NO} values. Moreover, during the test period we regularly conducted FE_{NO} measurements on our selves – and the FE_{NO} values proved to be stable throughout the period from August to January.

The subjects had to be well from any viral infection at least 14 days before they were included in the study. The fact that the subjects self reported this information may have reduced the validity. Additionally, the subject intake of food and beverage before test was self reported and the subjects had the opportunity to drink s much water they needed throughout the test, and the subjects were allowed to exercise the day before test. These factors may have contributed the measurements. To include as many as 20 subjects we were required to allow these factors.

Corradi and colleagues (1998) reports a relationship between ambient NO (NO_A) and exhaled [NO] levels. They recommend not to measuring exhaled [NO] on days with high NO_A levels. We recorded NO_A during the online measurements and found a NO_A of 4.4 ± 10 ppb in average and we did not see any correlation between NO_A and FE_{NO} . The registration of NO_A is lacking when we were testing in the climatic chamber.

Therefore we cannot confirm a low NO_A during RET, and this may contribute to the values.

Four tests in all of the 20 subjects were performed. The testing period lasted both in the fall and winter 2009. Stark et al. (2006) report no seasonal influence on $\text{F}_{\text{E}_{\text{NO}}}$ values. However, if the subjects exercised in a cold climate outdoor before test this may have affected the $\text{F}_{\text{E}_{\text{NO}}}$ value. In addition, we did not conduct lung functions test before and after RETs, so we do not know if the lung function was reduced after RETs.

When the subjects measured $\text{F}_{\text{E}_{\text{NO}}}$ values outside the climatic chamber they always conducted the online measurement first followed by offline measurement. Anyway, there was a good correlation between these methods ($r=0.9$). We pointed that a total exhaling was needed before inhalation of NO free air to make sure of a valid $\text{F}_{\text{E}_{\text{NO}}}$ measurement but some subjects may not have followed our guidelines and this can be a confounding factor that may have affected the $\text{F}_{\text{E}_{\text{NO}}}$ values.

It should be emphasized that the V_{NO} reported in this master thesis is a product of V_{E} measured immediately and $\text{F}_{\text{E}_{\text{NO}}}$ values 5 min after test. Furthermore, we did not measure $\text{F}_{\text{E}_{\text{NO}}}$ during the RET`s. Because it is too difficult to implement an offline exhaled NO measurement immediately after high intensity exercise we had to wait 3 and 5 minutes. The V_{E} was monitored by collecting exhaled air by Douglas bags immediately post exercise and V_{NO} was calculated after these measurements, which make the results of V_{NO} less valid. Finally, we did not measure $\text{F}_{\text{E}_{\text{NO}}}$ values inside the climatic chamber before the RET`s so we do not know if there was any change in $\text{F}_{\text{E}_{\text{NO}}}$ just related to the climate at rest.

7 CONCLUSIONS

$F_{E_{NO}}$ was decreased after exercise both in the normobaric and the cold climate in healthy adults. $F_{E_{NO}}$ was reduced in the cold climate after warm up and 20, 30 and 60 min post exercise, compared to the corresponding exercise test in the normobaric climate.

The subjects returned to baseline $F_{E_{NO}}$ approximately 10 and 15 min after RET in the cold and normobaric climate, respectively, but a reduction in $F_{E_{NO}}$ was seen after 20 min and a further reduced tendency was seen 30 and 60 min post exercise in the cold climate.

Increased $F_{E_{NO}}$ from baseline (online measure) was seen 24 hours post exercise in the normobaric climate. The subjects did not reach resting $F_{E_{NO}}$ values 60 min after pretest.

The baseline $F_{E_{NO}}$ value was in average 17.2 ± 7.2 ppb, with a 95th percentile of 31.6 ppb. There was no difference between the $F_{E_{NO}}$ values baseline between the three tests.

A gender difference in $F_{E_{NO}}$ was seen, with higher values among the males both at baseline and post exercise.

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