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Exhaled Nitric Oxide Concentration in the period of 60 minutes after Submaximal Exercise in the Cold

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Running head: Exhaled nitric oxide and exercise in the cold

Keywords: exhaled nitric oxide, exercise, cold environment, temperate environment

Abstract

Background: Fractional expired nitric oxide (FE_{NO}) is decreased after exercise. The effect of exercise in the cold upon FE_{NO} is unknown.

Purpose: To examine changes in FE_{NO} after a short, high intensive exercise test in a cold and in a temperate environment.

Methods: Twenty healthy well trained subjects (8 females) aged 18-28 years performed an 8 minutes exercise test at 18°C (SD=1.0) and -10°C (SD=1.2) ambient temperature. The tests were performed in a climate chamber in random order. The workload corresponded to 90 - 95% of peak heart rate (HR_{peak}) during the last four minutes. FE_{NO} was measured offline. Exhaled gas was sampled in Mylar® bags using a collector kit with a flow restrictor and analysed within two hours. FE_{NO} was measured before exercise and repeatedly during the first hour after. ANOVA for repeated measures was used to compare differences in FE_{NO} after exercise between environments.

Results: There was no difference in baseline FE_{NO}. A significant difference in FE_{NO} between environments was found after warm-up and from 20 – 30 minutes after exercise, with FE_{NO} being lower after exercise in the cold ($p < 0.05$). The maximal reduction in FE_{NO} was seen 5 min after exercise and was not different between environments.

Conclusion: Recovery of FE_{NO} was slower after exercising in -10°C compared with 18°C.

Introduction

The effect of exercise on the concentration of nitric oxide in exhaled gas (eNO), or the fraction of nitric oxide in exhaled gas (FE_{NO}), is extensively investigated. In most studies, there is a reduction in eNO and an increase in the production rate of NO (V_{NO}) during and in the first 15-20 minutes after exercise (Chirpaz-Oddou *et al.*, 1997;Maroun *et al.*, 1995;Persson *et al.*, 1993a;Trolin *et al.*, 1994). However, some studies report no changes (Bauer *et al.*, 1994;Iwamoto *et al.*, 1994;Shin *et al.*, 2003). In one study there was an increased eNO 2-3 hours after a marathon, and it was suggested that an increased eNO could modulate exercise-associated inflammatory responses (Bonsignore *et al.*, 2001). Eosinophilic airway inflammation is associated with an increased eNO, but exercise induced bronchoconstriction (EIB) and sports asthma are not typically associated with eosinophilic inflammation in non-atopic subjects (Bougault *et al.*, 2009). The prevalence of sports asthma is high in cross-country skiers who exercise in a cold and dry environment (Heir & Oseid, 1994;Larsson *et al.*, 1993).

The physiological mechanisms of reduced eNO after different types of exercise in different environmental conditions are still not fully understood. Respiratory heat and water losses are increased in a cold and dry environment. To our knowledge, only one group has examined eNO in healthy subjects exercising in cold air (Therminarias *et al.*, 1998). During exercise in cold air, eNO was lower than in temperate air up to a load of approximately 75% of maximal oxygen uptake, but there was no difference thereafter including a short 10 min recovery period (Therminarias *et al.*, 1998).

3

Bronchial obstruction is associated with a lower bronchial surface area and a lower NO flux from the bronchial surface to the bronchial lumen (Verbanck *et al.*, 2012;Pendergast *et al.*, 1999). Mechanical stress failure during high ventilatory demands in the cold may cause inflammatory responses (Bougault *et al.*, 2009) which may have effects on eNO later in the recovery phase.

The aim of the present study was to assess eNO measured as the fraction of NO in exhaled gas (FE_{NO}) before and repeatedly up to 60 minutes after a short, high intensive exercise bout in a cold and in a temperate environment in healthy, well trained males and females.

We hypothesized that FE_{NO} is lower after exercise in a cold compared with a temperate environment, and that the time course for recovery after exercising in the cold is different.

Materials and methods

Design

The present study had an open, randomized crossover design. A pre-test was followed by two 8 minutes exercise tests performed in two different climatic environments, one test in a temperate environment at 18˚C (1.0) [mean ± one standard deviation (SD)] with an actual humidity of 6.12 $g·m^{-3}$ (0.5) and one test in a cold environment at -10˚C (1.2) with an actual humidity of 0.89 $g·m^{-3}$ (0.09) in randomized order. All tests were performed at the same time of the day and were completed within 14 days with a minimum of 48 hours between each test.

The present study was part of a larger study aiming to assess the effect of different environments including altitude (Stang *et al.*, 2014) upon $FE_{NO.}$

Subjects

Twenty healthy, non-smoking, non-snuffing subjects (8 females) were included in the study. Subject's characteristics are given in Table 1. Inclusion criteria were a maximal oxygen uptake (VO_{2max}) >40 ml·kg^{-1}·min^{-1} for females and >50 ml·kg^{-1}·min^{-1} for males measured in a pre-test. Exclusion criteria were FE_{NO} >30 parts per billion (ppb), a positive exercise induced bronchoconstriction (EIB) test defined as a reduction in forced expiratory volume the first second (FEV_1) of ≥10% from baseline, and symptoms of any other respiratory diseases such as viral or bacterial infections.

Exercise and intake of food or drink containing nitrite or nitrate was prohibited on the day of experiments and the subjects could only drink water the last hour before the exercise tests according to the American Thoracic Society and the European Respiratory Society recommendations (2005). The subjects were told to eat and drink approximately the same 24 hours before and 24 hours after each test to avoid the effect of for example high fat meals and caffeine upon FE_{NO}. All subjects signed a written informed consent before the pre-test. The study was approved by the Regional Medical Ethics Committee in Oslo, Norway, and performed according to the principles stated in the Declarations of Helsinki.

Test protocols

The workload used in the 8 minutes exercise test was individually estimated based on the pre-test. The pre-test consisted of an incremental VO_{2max} test on a treadmill (Woodway, ELG 2, Germany) with increasing running velocity every minute until exhaustion according to Åstrand and Rodahl et al.(Aastrand *et al.*, 2003). The subjects were breathing through a mouthpiece (2700 Series: Hans Rudolph Inc, USA)

and expired air was sampled continuously and analysed for O_2 and carbon dioxide (CO_2) using an Oxycon Pro analyser (Erich Jaeger GmbH, Hoechberg, Germany). The mean value of the two highest successive measures of VO_2 recorded over 60 seconds was defined as VO_{2max}. Heart rate (HR) was assessed every minute with a Polar Electro OY (Kempele, Finland) and the highest value was defined as HR_{peak}. Spirometry was performed approximately 5 minutes before and 6 - 10 minutes after exercise to exclude subjects with EIB. FE_{NO} was measured before spirometry.

The main exercise tests were carried out in a climatic chamber (Norwegian Sub Diving Techniques A/S, Haugesund, Norway). The subjects performed two exercise tests, one in a temperate and one in a cold environment. The exercise test was an eight minutes maximal running bout on a treadmill (Bodyguard Cardionics 2313, Cardionics AB, Sweden) after 15 minutes warm-up at 75-80% of HR_{peak}. Running velocity was adjusted during the first four minutes to achieve a work load corresponding to 90-95% of HR_{peak} for the last four minutes (Carlsen *et al.*, 2000;Stensrud & Carlsen, 2008). Heart rate was monitored electronically with a Polar Electro OY, (Kempele, Finland) and registered every minute. Minute ventilation (V_E) was measured 5 min before exercise, after warm up and 0, 10 and 20 minutes after exercise. FE_{NO} was measured 5 min before exercise, after warm up, and then 5, 10, 15, 20, 30 and 60 minutes after the exercise test. The subjects remained in the climatic chamber for 20 minutes post exercise which means that measurements after warm up and after 5, 10, 15 and 20 minutes were performed inside the chamber in a cold or temperate environment, respectively. The FE_{NO} measurements at baseline, 30 and 60 minutes after exercise tests in both climates were thus performed in a temperate environment outside the chamber.

Exhaled nitric oxide

FE_{NO} (ppb) was measured both online and offline with a chemiluminescence analyser (EcoMedics AG, Duerten, Switzerland) according to the current recommendations from the ATS/ERS (2005). Only offline samples could be collected in the cold environment and only offline results are used in the data analyzes and presented in the result chapter. However, online measurements were performed in parallel with the offline sampling when the subjects were outside the climatic chamber as a control of the validity of the offline measurements. There was a strong correlation, (R^2=0.88) (p<0.001) between FE_{NO} measured online and offline.

Subjects inhaled NO-free air to total lung capacity and then exhaled for approximately 10 seconds filling gas tight metal foil Mylar® bags (EcoMedics – Offline Collection Kit, Duerten, Switzerland). Measurements were performed in triplicates according to the guidelines (ATS/ERS, 2005), and the mean of three measurements were used in the analyses. A visual feedback system helped to maintain a positive pressure of 10-12 cmH$_2$0 to ensure closure of the soft palate and keeping the expiratory flow rate over the resistor at 50 ml·s^{-1}. The Mylar® bags were analysed within two hours after sampling. Ambient NO concentration was registered every day and before baseline measurements.

Minute ventilation

Exhaled air was collected in Douglas-bags (Prieur *et al.*, 1998) at baseline, after warm-up, and 0, 10 and 20 minutes after the exercise test. The subjects wore a nose clip and breathed for 60 seconds through a three-way valve (2700 series; Hans Rudolph Inc, USA). The volume of the expired gas was measured within one hour after sampling (Ventilation measuring system, model S-430, KL-Engineering,

Northridge, California, USA) and corrected to body temperature pressure saturated condition (BTPS).

Spirometry

Spirometry was performed only at pretest to exclude subjects with EIB. Forced expiratory volume in one second (FEV_1), forced vital capacity (FVC) and forced expiratory flow at 50% of FVC (FEF_{50}) were measured by maximum expiratory flow volume loops (Masterlab, Erich Jaeger®, Würztburg, Germany). All measurements were within the general acceptable criteria of the ERS (1997) and predicted values were according to Quanjer et al. (Quanjer *et al.*, 1993). Spirometry was always performed after the measurement of FE_{NO}.

Statistical analysis

ANOVA for repeated measurements (mixed model) was used to analyse changes in FE_{NO} after the exercise tests with time and environmental condition as independent variables. Possible associations between FE_{NO} and V_E, as well as online and offline measurements of FE_{NO} were analysed by Pearson's correlation coefficient. All statistical analyses were performed with Statistical Package of Social Science (SPSS) version 15.0. Demographic data are given as mean ± one standard deviation (SD). Results are given as mean with 95% confidence intervals (CI), unless otherwise stated. The study was initially powered to detect a 15% change in FE_{NO} and a sample size of 20 subjects was found to be required. Level of significance was set to $p < 0.05$.

Results

There was no significant difference in baseline FE_{NO} before exercise in the two climates.

A significantly lower FE_{NO} was found after warm-up and at 20 minutes (p<0.01) and 30

minutes (p<0.05) after exercise in the cold.

The maximum reduction in FE_{NO} was found 5 minutes after exercise in both

environments. FE_{NO} was reduced by 29% (12, 38) [mean with 95% CI] (p<0.001) and

11% (1, 21) (p<0.01) 5 and10 minutes after exercise in the temperate environment, and

by 33% (24, 42) (p<0.001), 16% (4, 28) (p<0.01) and 11% (-2, 24) (p<0.01) 5, 10 and

15 minutes after exercise in the cold (Fig.1B and Table 3b.).

Mean running speed was significantly lower in the cold compared with the temperate

environment (p<0.01). However, no difference in exercise intensity measured as HR_{peak}

or V_{Epeak} was observed (Table 2).

Discussion

The maximal reduction in FE_{NO} was seen immediately after exercise and was not

different between exercise in a cold and in a temperate environment. However, a

reduction in FE_{NO} was apparent after exercise in the cold and the recovery to baseline

was slower.

The time course of the reduction in FE_{NO} is consistent with the studies of Therminarias et

al. (Therminarias *et al.*, 1998) and Pendergast et al.(Pendergast *et al.*, 1999). In the first

study the subjects exercised in a temperate and cold environment at 22 and -10°C, and

the largest differences in eNO were seen after submaximal workloads. In the other study

the subjects performed exercise immersed in water of 20, 30 and 35°C, and the largest

9

differences in eNO were seen at lower workloads. The ambient conditions had an effect on baseline eNO in these studies. We did not measure FE_{NO} after acclimatization to the cold, all baseline measurements were performed in a temperate environment and the first measurement in the cold was performed immediately after warm-up (Fig.1).

The measurement method of FE_{NO} used in the present study is a relatively new expression which refers to the fraction of nitric oxide measured at a constant flow rate, and we will use that notation when referring to own results. Other studies have used eNO and some with other measurement techniques (Chirpaz-Oddou *et al.*, 1997;Iwamoto *et al.*, 1994;Maroun *et al.*, 1995;Trolin *et al.*, 1994;Bauer *et al.*, 1994;Shin *et al.*, 2003;Therminarias *et al.*, 1998;Phillips *et al.*, 1996). We have therefore chosen to refer to what is reported in the respective studies.

Therminarias et al. (1998) reported a significant decrease in eNO by increasing exercise intensities, approximately until 50% of VO_{2peak}, in the cold environment (-10°C) as compared to control (22°C). Thereafter no differences in eNO between the two climates were seen. However eNO decreased from baseline by increasing intensities in both environments (Therminarias *et al.*, 1998). Our results showed a significant reduction in FE_{NO} after warm up in the cold climate as compared to the temperate climate. Pendergast et al. (1999) found a significant decrease in eNO in ten male subjects who cycled immersed in water of 20, 30 and 35˚C. They reported significantly lower eNO in 20˚C compared to 30 and 35˚C at workloads of 50 and 100W respectively, and suggested that a possible explanation was a reflex reduction of NO release in the lung due to bronchial obstruction and vasoconstriction occurring

10

in cold climates. Further, an enhanced alveolar clearance of NO could be due to immersion and increased pulmonary blood volume. (Pendergast *et al.*, 1999).The baseline values of eNO were significantly different between the climates in these studies probably because the baseline values were measured in different ambient conditions (Therminarias *et al.*, 1998;Pendergast *et al.*, 1999).

Therminarias et al. (1998) found a decrease in FEV_1 of 5% in healthy well-trained males exercising in the cold (Therminarias *et al.*, 1998). The initial decrease in eNO could be caused by the effect of cold air on the vasomotor tone of the bronchial muscles. They also emphasized that since endogenous NO-production is involved in bronchial dilation, it cannot be excluded that this lack of production may favor the appearance of airway obstruction (Therminarias *et al.*, 1998). However, airway obstruction, by itself, causes a lower FE_{NO} because the bronchial surface area is reduced and thereby the flux of NO into the bronchial lumen (Verbanck *et al.*, 2012)

The literature does not agree on the mechanisms of the changes in eNO after exercise. It has been related to ventilation and increased airflow rates during exercise (Persson *et al.*, 1993b;St Croix *et al.*, 1999). Vascular NO production has been linked to increased cardiac output (Q) during exercise through shear stress on the capillary wall and axial backdiffusion to the blood (Hemmingsson & Linnarsson, 2009). However, NO has a short half-life (Gaston *et al.*, 1994) and combined with a high affinity to haemoglobin (Ricciardolo, 2003), it has been argued that changes in systemic and/or pulmonary vascular formation of NO are not necessarily detectable in exhaled breath (St Croix *et al.*, 1999).

In the present study, the mean running velocity was significantly higher in the temperate climate compared to the cold. Since no significant differences in HR_{peak} and VE_{peak} were seen between the two climates, we may assume that an equal amount of stress was applied to the pulmonary system during exercise. This is in agreement with Terada et al. (Terada *et al.*, 2001), but not Phillips et al. (Phillips *et al.*, 1996). However, high intensive exercise may produce discrepancies in eNO between athletes and sedate subjects (Maroun *et al.*, 1995). Maroun et al (1995) found a smaller decrease in eNO among elite athletes as compared to trained and sedate subjects during constant workload exercise at VO_2 of 1, 2 and 4 $l \cdot min^{-1}$ (Maroun *et al.*, 1995). They suggest that increased vascular and/or epithelial production of NO may be the main cause and that enhanced vascular NO production may be the result of increased shear stress and upregulation of endothelial NO synthase gene expression (Maroun *et al.*, 1995). To ensure a homogenous group relative to aerobic capacity and to be comparable with athletes, all subjects in the present study performed a VO_{2max} test before inclusion.

Bonsignore et al (2001) suggest that NO may modulate inflammatory processes in the airways induced by high intensive exercise (Bonsignore *et al.*, 2001). They found significantly higher FE_{NO} 2-3 hours after a marathon in amateur runners and this was associated with a low expression of L- selectin and CD11b/CD18 in induced sputum (Bonsignore *et al.*, 2001). Because FE_{NO} was measured only until 60 minutes after exercise in the present study, a later rebound effect of the changes in FE_{NO} cannot be excluded. Further investigation assessing FE_{NO} regularly longer than 60 minutes after exercise is needed to confirm the results in the present study as well as the results reported by Bonsignore et al (2001).

The main limitations of the present study are related to mechanisms explaining the changes in FE_{NO} after exercise in the cold environment. Measurements of physiological variables during exercise were not done and inflammatory markers from blood or airways were not obtained.

Conclusion

Exercise in a cold environment has an acute effect on FE_{NO} and recovery is slower as compared with exercise in a temperate environment. The initial acute reduction in FE_{NO} could be related to bronchial constriction and vasoconstriction related to exposure to the cold.

Conflict of interest statement

Trine Stensrud, Julie Stang, Einar Thorsen and Veslemøy Bråthen have none competing interests to declare.

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FIGURE LEGEND

Figure 1. Changes in the fraction of exhaled nitric oxide (FE$_{NO}$) and % changes in FE$_{NO}$ from baseline (pre) to after warm up (post w. u.) and 5-60 minutes after an 8 minutes exercise test, in a cold ($\triangledown$)and temperate ($\blacksquare$) environment. Results are given as mean with 95% confidence intervals.
&=significant different between climates ($p<0.05$), *= significant different from baseline ($p<0.05$) (n=20)