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Exhaled nitric oxide after high intensity exercise at 2800 m altitude

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ABSTRACT

Background: Nitric oxide (NO) concentration in exhaled gas is a marker of some inflammatory processes in the lung and endogenous NO plays a role in the physiological responses to exercise and altitude. The aim of this study was to compare changes in exhaled NO concentration 5-60 min after high intensity exercise at 2800 m and at 180 m altitude.

Methods: Twenty trained healthy volunteers (12 males), aged 19-28 years, were included in this open, cross-over study. Subjects performed two exercise tests at different altitudes; 2800 m and 180 m, in a randomized order. The fraction of NO in exhaled gas (FE_{NO}) was measured 5 min before and 5-60 min after 8 min of running on a treadmill at a heart rate (HR) of 90% of peak HR. Peak HR was assessed during a pre-test at 180 m. Ambient temperature was 20.1 ^{o}C (SD=1.2), and relative humidity 40.2% (SD=3.2). FE_{NO} measurements were corrected for altitude gas density effects and converted to partial pressure of NO (PE_{NOcorr}).

Results: PE_{NOcorr} was reduced from 1.47 (1.21, 1.73) milliPascal (mPa) at baseline to 1.11 (0.87, 1.34) mPa 5 min after exercise at 2800 m and from 1.54 (1.24, 1.84) to 1.04 (0.87, 1.22) mPa 5 min after exercise at 180 m. There was no difference in PE_{NOcorr} between exercise at 2800 m and 180 m, and PE_{NOcorr} was normalized within 20 min.

Conclusions: Exercise at 2800 m induces a similar acute reduction in exhaled nitric oxide concentration as compared with 180 m in healthy subjects.

Key words: airways inflammation; altitude; athletes; endurance sports; exercise; ventilation

INTRODUCTION

Endogenous nitric oxide (NO) is a vasodilator involved the physiological response to exercise in healthy humans, i.e. pulmonary gas exchange. Shortly after exercise, the fraction of NO in exhaled gas (FE_{NO}) is decreased (Maroun *et al.* 1995; Persson *et al.* 1993; Phillips *et al.* 1996). However, NO output increases during and shortly after exercise due to increased minute ventilation (V_E) (Phillips *et al.* 1996; Verges *et al.* 2005), and an increased FE_{NO} has been reported in both healthy and asthmatic subjects from 20 minutes to 2 hours after exercise (Bonsignore *et al.* 2001; De Gouw *et al.* 2001).

FE_{NO} is shown to depend on the partial pressure of oxygen (PO_2) in inspired air (Schmetterer *et al.* 1997), and correlate with arterial oxygen pressure (P_aO_2) in healthy humans (Tsuchiya *et al.* 2000). When sampling at altitude it is recommended to express FE_{NO} as partial pressure of NO (PE_{NO}) to account for differences in ambient pressure (Hemmingsson *et al.* 2009). Brown and co-workers (Brown *et al.* 2006) measured PE_{NO} in 47 subjects at sea level, 2800 m and 4200 m altitude, and found that average PE_{NO} decreased with increasing altitude. Exposure to acute hypoxia initializes a number of adjustments in the cardiopulmonary system to maintain adequate O_2 delivery to the tissues (Gale *et al.* 1985). Endogenous NO present in the lungs and airways has been proposed to be an important factor regulating hypoxia-induced pulmonary vasoconstriction (Phillips *et al.* 1996). Altitude training programs in elite endurance sports, e.g. cross country skiing, are often located at low to moderate altitudes between 1000 and 3000 m. Although these altitudes is believed to pose little to no direct health risks(Bergeron *et al.* 2012), ambient air at altitude is often cold and dry which means that exercise at high intensities at altitude may provoke inflammatory processes in the respiratory system in athletes (Carlsen 2013).

Exercise-induced changes in FE_{NO} during hypoxic exposure have been assessed in previous studies showing no differences after exercise at normal atmospheric pressure breathing normoxic and hypoxic air (St Croix *et al.* 1999; Verges *et al.* 2005). However, the partial pressure of NO in exhaled gas (PE_{NO}) is reduced in hypobaric hypoxia compared to normobaric hypoxia at rest (Hemmingsson & Linnarsson 2009) and whether the same applies after exercise is yet to be determined. The main aim of the present study was to assess the acute changes in PE_{NO} after exercise at 2800 m and 180 m altitude. We hypothesized a greater reduction in PE_{NO} after heavy exercise at 2800 m compared with 180 m.

MATERIALS AND METHODS

Subjects and design

The present study had an open cross-over design with subject acting as their own controls. A pre-test was followed by two exercise tests carried out in random order at two different altitudes; 180 m and 2800 m. Data collection was carried out from September to January 2009/2010 and occurred between 0800 am and 1600 pm. All testing were completed within 14 days, with a minimum of 24 hours between each test day to allow complete NO recovery (Verges *et al.* 2006). Twenty healthy, non-smoking subjects (12 male) volunteered to participate in the study. Subject characteristics are given in Table 1. Inclusion criteria were a maximal oxygen uptake (VO_{2max}) >40 $ml \cdot kg^{-1} \cdot min^{-1}$ for females and >50 $ml \cdot kg^{-1} min^{-1}$ for males, FE_{NO} <30 parts per billion (ppb) and a negative exercise induced bronchoconstriction (EIB) test defined as a reduction in forced expiratory volume in the first second (FEV_1) less than 10% from baseline. Subjects showing symptoms of any respiratory diseases, such as viral infections during the test period, were excluded from the study. Exercise and intake of food or drink containing nitrite or nitrate was prohibited on the day of experiments(2005). One

hour before testing the subjects could only consume water. All subjects signed a written informed consent. The study was approved by the Regional Medical Ethics Committee in Oslo, Norway, and performed according to the principles stated in the Declaration of Helsinki.

Pre-test

The pre-test was carried out to exclude subjects with low physical fitness levels, elevated FE_{NO} or EIB, and to establish peak heart rate (HR_{peak}). Subjects performed a running test on a treadmill (Woodway GmbH, Weil am Rhein**,** Germany) with running velocity increasing by 1 $km \cdot hour^{-1}$ every minute until exhaustion, as described by Hermansen (1973). Subjects breathed through a mouthpiece (2700 Series: Hans Rudolph Inc., USA) and expired gas was sampled continuously from a mixing chamber. Expired gas was analysed for O_2 and carbon dioxide (CO_2) content (Oxycon Pro, Erich Jaeger GmbH, Hoechberg, Germany). FE_{NO} measurements and spirometry were done approximately 5 minutes before the exercise test and spirometry, measured by maximal expiratory manoeuvres (Masterlab, Erich Jaeger®, Germany), was repeated 6 minutes after exercise.

Exercise test

The exercise test was performed on a treadmill (Bodyguard Cardionics 2313, Cardionics AB, Sweden), and we used a 8-minute exercise-induced asthma (EIA) provocation protocol as described in the European Respiratory Society (ERS) guidelines for clinical exercise testing (1997). Running velocity was increased the first four minutes of the test to achieve a work load of ≥90% of HR_{peak}. The exercise tests were carried out in a combined altitude and climate chamber (Norsk Undervanns Teknikk A/S, Haugesund, Norway) and the subjects remained in the altitude chamber for 20 minutes after the exercise test. In the control experiment at 180 m mean barometric pressure (P_B) was 98.7 kPa (SD=1.1) (740.3 mmHg), mean ambient temperature was 20.2 ˚C (SD=1.1), and relative humidity was 40.0% (SD=3.3). The partial pressure of oxygen was 20.9 kPa (SD=0.1). During hypobaric exposure ambient pressure was reduced to 72.8 kPa (SD=0.2) (546.0 mmHg), corresponding to an altitude of 2800 m. The ambient temperature was 20.1 ^{o}C (SD=1.2) and relative humidity was 40.2% (SD=3.1). Before the running test, subjects warmed up for 15 minutes at an intensity of 75% of HR_{peak}. HR was monitored electronically (Polar Electro OY, Kempele, Finland) and registered every minute. Minute ventilation (V_E) was assessed by subjects breathing through a mouthpiece (2700 series; Hans Rudolph Inc, USA) connected to Douglas bags for 1 minute. Measurements were done during the first minute after warm-up and after exercise test, in addition 10 and 20 minutes after exercise. Within 30 minutes after sampling the Douglas bags were analysed for gas volume which was corrected to BTPS conditions. The complete test protocol is illustrated in Figure 1.

Measurement of exhaled nitric oxide

FE_{NO} was measured using a chemiluminescence analyser (EcoMedics AG, Duerten, Switzerland) according to current recommendations(2005). Both online and offline measurements were performed, however only offline samples could be collected in the altitude chamber. 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). A visual feedback system helped to maintain a positive pressure between 10-12 cmH_20 to ensure closure of the soft palate and a constant expiratory flow rate. Mylar bags were analysed after recompression within 1 hour after sampling. Online measurements were performed with a similar procedure except with subjects exhaling through a mouthpiece and a gas sample was continuously drawn into the chemiluminescence analyser. All measurements were performed in triplicates. Mean values

with a difference of less than 10% were used in the analysis. Online and offline measurements correlated significantly ($p<0.001$, $R^2=0.82$).

Partial pressure of exhaled nitric oxide

FE_{NO} was converted to PE_{NO} (mPa) to express the molar concentration of NO ($PE_{NO}=FE_{NO}\cdot(P_B-P_{H2O})$). Furthermore, PE_{NO} was corrected for differences in expiratory flow rate between 180 and 2800 m (PE_{NOcorr}) due to differences in gas density as described by Caspersen et al. (2012).

Statistical analysis

ANOVA for repetitive measurements (mixed models) was used to compare changes in PE_{NOcorr} after the exercise tests. Possible associations between PE_{NOcorr} and HR or V_E, as well as comparisons of online and offline measurements, were assessed by Pearson's correlation coefficient. All statistical analyses were performed with Statistical Package of Social Science (SPSS) version 18.0. Results are given as means with 95% confidence intervals (CI), unless otherwise stated. The study was powered to detect a 15% change in PE_{NOcorr} with a power of 80%. The level of significance was set at 0.05.

RESULTS

All subjects completed testing without difficulty. During the exercise tests, heart rate (HR) and running velocity was significantly higher at 180 m compared to 2800 m ($p<0.004$) (Table 2). We observed no difference in V_E between the tests (Table 3). After exercise, there was no difference in HR or V_E between 2800 m and 180 m (Table 3).

Partial pressure of exhaled nitric oxide

PE_{NOcorr} was lower at 2800 m after warm up compared to at 180 m ($p=0.002$) , but no difference was seen after the exercise tests. Peak decrease in PE_{NOcorr} was observed 5 min after the exercise challenge in both altitudes (Figure 2). PE_{NOcorr} decreased by 22.5% (-33.9, -11.0) ($p=0.01$), or from 1.47 mPa (1.21, 1.73) to 1.11 mPa (0.87, 1.34), after exercise at 2800 m, and by 28.5% (-37.4, -19.6), from 1.54 mPa (1.24, 1.84) to 1.04 mPa (0.87, 1.22), at 180 m ($p<0.001$) (Table 3). PE_{NOcorr} was normalized within 10 min in both altitudes (Figure 2).

Fractional exhaled nitric oxide at altitude

FE_{NO} increased by 23.1% (CI 8.8, 37.4) after warm up at 2800 m altitude, from 15.9 19.1 ppb (Table 4). After exercise there was a decrease in FE_{NO} parallel to the changes in PE_{NOcorr} (Table 4). Mean baseline FE_{NO} were 16.62 ppb (13.37-19.87) on day 1 and 15.95 ppb (13.06, 18.83) on day 2. There was no difference between baseline FE_{NO} at the two days ($p=.61$).

DISCUSSION

We hypothesized that exercise at 2800 m altitude would induce a greater decline in PE_{NO} compared to exercise at 180 m. After corrections for ambient gas density and expiratory flow rate at 2800 m (PE_{NOcorr}), no difference was found between the two tests. This indicates that there was no additional effect of acute altitude exposure upon PE_{NO} after exercise. Reduced PE_{NO} was observed 5 min after end of exercise, and sustained for less than 10 minutes after both exercise tests. This finding is in agreement with other studies (Chirpaz-Oddou *et al.* 1997), and may be linked to normalized V_E and HR after exercise.

Exercise in altitude

Our results are in agreement with those of Verges et al. (2005) who showed that FE_{NO}

decreases after exercise in both normoxic and hypoxic conditions. Verges and co-workers assessed FE_{NO} levels at a flow rate of 170 ml·s^{-1}, and reported a 16.6% (-31.2, -2) decrease in healthy subjects after cycling at 60% of maximal power output for 5 minutes. In the present study, PE_{NO} was reduced by 22.5% and 28.5% after running at 90% of HR_{peak} at 2800 m and 180 m, respectively. . The difference between these studies may be addressed the higher exercise intensity, longer duration and different types of exercise activities. Furthermore, Verges and co-workers used inhalation of hypoxic gas (FiO_2=15%), and in the present study, subjects exercised in reduced barometric pressure and our results was thus expressed as partial pressure of exhaled NO (PE_{NOcorr}) to adjust for differences in gas density.
Decreased FE_{NO} after exercise is well documented (De Gouw *et al.* 2001; Terada *et al.* 2001; Verges *et al.* 2005) and has previously been related to increased airflow rates during exercise (Persson *et al.* 1993; St Croix *et al.* 1999). Also, altered vascular NO production during exercise has been linked to increased cardiac output through shear stress on the capillary wall (Hemmingsson & Linnarsson 2009). However, NO has a short half-life (Gaston *et al.* 1994) and combined with a high affinity for haemoglobin (Ricciardolo 2003), changes in systemic and/or pulmonary vascular formation of NO is not necessarily detectable in exhaled breath (St Croix *et al.* 1999). This problem elucidates the difficulties by linking pulmonary NO to exercise related to peripheral vasodilatation.
Reduced PE_{NO} under hypoxic conditions is thought to be caused by an adaptive pulmonary vasoconstriction to redistribute blood flow to better oxygenated areas of the lung (Brown *et al.* 2006). Verges et al. (2005) found a greater NO decrease with subjects breathing hypoxic air as compared to normoxic air. Our study showed a similar decline in both hypobaric and normobaric pressures, with the exception of a reduction after warm up at 2800 m. A recent study by Faiss et al. (2013) showed a lower ventilatory response to exercise and a lower PE_{NO} in hypobaric hypoxia corresponding to 3000 m altitude compared to normobaric hypoxia (PO_2=14.7%). Higher cardiac output and higher ventilation are physiologically associated with altitude (Gale *et al.* 1985), and could affect NO levels by increasing NO scavenging by pulmonary blood and/or increased NO output through high V_E.

Methodological aspects
The exercise protocol used in the present study was originally developed to provoke EIB in patients with EIA (ERS *Task Force Guidelines*, 1997), and was chosen with the purpose to apply maximal amount of strain upon the airways and pulmonary vascular system. Lung function was measured by spirometry before and after a pre-test performed by running until exhaustion on a treadmill, to ensure that subjects showed no evidence of EIB before inclusion to the project. Strenuous exercise have shown to produce different responses in FE_{NO} between athletes and sedate subjects (Maroun *et al.* 1995). This might be due to higher levels of ventilation and/or cardiac output in athletes. To ensure a homogenous group in terms of physical fitness level, all subjects performed a maximal exercise test and had to exceed a certain physical fitness level to be included in the study to be comparable with athletes. Average O_2 saturation (S_aO_2) has been shown to decrease at altitudes similar to the one used in the present study, both during rest (Brown *et al.* 2006) and exercise (Verges *et al.* 2005). Decreased pulmonary NO may contribute to pulmonary gas exchange abnormalities in athletes, probably through its effect on V_A/Q matching (Verges *et al.* 2005). Measurements of arterial oxygen saturation (SaO_2) were not assessed during exercise in the present study.
In the present study, mean HR and running velocity were statistical significantly lower during exercise at 2800 m compared to 180 m (Table 3). To standardize the exercise intensity in order to compare the two exercise tests performed at different barometric pressures is complicated and may be considered the main limitation of the present study protocol. Measurements of HR were used to control for the exercise intensity of ≥ 90% of HR_{peak},

whereas HR_{peak} was assessed at a pretest at 180 m. To compare exercise at different altitudes is challenging. It is known that exposure to moderate and high altitudes increases HR at rest and during sub-maximal exercise (Gale *et al.* 1985) and to use HR as a measure of exercise intensity can be misleading. This makes it difficult to explain the discrepancies between the two exercise tests, although the clinical relevance of these differences may not be crucial. No differences were observed in V_E during the two tests (Table 2), suggesting that elimination of NO in expired gas was similar. However, NO output could not be estimated as the Douglas bags were not analysed for NO content.

Measuring exhaled NO at different altitudes

Expiratory flow rate must be standardized during measurements of FE_{NO}, so that exhaled gas passes through the airways at the same rate and NO is transferred from the airway wall to the airway lumen for the same period of time (Brown *et al.* 2006). Reduced gas density causes an increase in expired flow rate for the same driving pressure with the offline sampling equipment used in this study. Hemmingsson et al. (2009) studied the altitude effect upon two NO analysers and suggests that to avoid methodological errors, the flow regulator should be readjusted to give a volume flow of 50 $ml \cdot s^{-1}$ at the altitude of interest. In the present study, FE_{NO} was increased during exposure to 2800 m (Table 3), but no difference was observed in PE_{NO} after corrections. This finding confirms that exhaled NO should be expressed as PE_{NO} when comparing measurements assessed at different altitudes(Hemmingsson *et al.* 2009).

Clinical applications

A problem with the use of exhaled NO as an inflammatory marker is the large intra-individual (Olivieri *et al.* 2006), and day-to-day variability. Different measurement procedures and equipment may account for some of the variation. A difference in FE_{NO} of 20% or 4 ppb is considered significant. The reduction in FE_{NO} or PE_{NO} was 22-28% after exercise which is greater than the expected random variability. The results from the present study show that effects of altitude and physical activity must be taken into account, when evaluating the results of FE_{NO} or PE_{NO} for monitoring asthmatic subjects.

Conclusion

The results from the present study show that high intensity exercise for 8 minutes at 2800 m and 180 m induces a similar decrease in PE_{NOcorr} in healthy subjects. Methodological considerations and correction for effects of altitude on PE_{NO} must be performed when measuring exhaled NO concentration at altitude. More research is needed to establish any relationship between exhaled NO concentration and gas exchange during and after exercise at sea level and different altitudes.

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Conflicts of interest

The authors have nothing to declare.

References

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

ATS/ERS Recommendations for Standardized Procedures for the Online and Offline Measurement of Exhaled Lower Respiratory Nitric Oxide and Nasal Nitric Oxide, 2005. *American Journal of Respiratory and Critical Care Medicine*, (2005); **171:** 912-930.

Bergeron M. F., Bahr R., Bärtsch P., Bourdon L., Calbet J. A., Carlsen K.-H., Castagna O., González-Alonso J., Lundby C., Maughan R. J., Millet G., Mountjoy M., Racinais S., Rasmussen P., Singh D. G., Subudhi A. W., Young A. J., Solligard T. & Engebretsen L. International Olympic Comitee consensus statement on thermoregulatory and altitude challenges for high-level athletes. *Br J Sports Med*, (2012); **46:** 770-779.

Bonsignore M. R., Morici G., Riccobono L., Insalaco G., Bonanno A., Profita M., Paternò A., Vasalle C., Mirabella A. & Vignola A. M. Airway inflammation in noonasthmatic amateur runners. *Am J Physiol Lung Cell Mol Physiol*, (2001); **281:** L668-676.

Brown D. E., Beall C. M., Strohl K. P. & Mills P. S. Exhaled nitric oxide decreases upon acute exposure to high-altitude hypoxia. *Am J Hum Biol*, (2006); **18:** 196-202.

Carlsen K.-H. Sports in extreme conditions: The impact of exercise in cold temperatures on asthma and bronchial hyper-responsiveness in athletes. *Br J Sports Med*, (2013); **46:** 796-799.

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

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

Gale G. E., Torre-Bueno J. R., Moon R. E., Saltzman H. A. & Wagner P. D. Ventilation-perfusion inequality in normal humans during exercise at sea level and simulated altitude. *Journal of Applied Physiology*, (1985); **58:** 978-988.

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

Hemmingsson T., Horn A. & Linnarsson D. Measuring exhaled nitric oxide at high altitude. *Respir Physiol Neurobiol*, (2009); **167:** 292-298.

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

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

Olivieri M., Talamini G., Corradi M., Perbellini L., Mutti A., Tantucci C. & Malerba M. Reference values for exhaled nitric oxide (reveno) study. *Respir Res*, (2006); **7:** 94-100.

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

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

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

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

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

Terada A., Fujisawa T., Togashi K., Miyazaki T., Katsumata H., Atsuta J., Iquchi K., Kamiya H. & Togari H. Exhaled nitric oxide decreases during exercise-induced bronchoconstriction in children with asthma. *Am J Respir Crit Care Med*, (2001); **164:** 1879-1884.

Tsuchiya M., Tokai H., Takehara Y., Haraguchi Y., Asada A., Utsumi K. & Inoue M. Interrelation between oxygen tension and nitric oxide in the respiratory system. *Am J Respir Crit Care Med*, (2000); **162:** 1257-1261.

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

Verges S., Tonini J., Flore P., Favre-Juvin A., Levy P. & Wuyam B. Exhaled nitric oxide in single and repetitive prolonged exercise. *J Sports Sci*, (2006); **24:** 1157-1163.

Table 1 Subject characteristics. Results are listed as medians (with range). *p<0.05.

	Male (n=12)	Female (n=8)	Total
Age (yrs)	23 (19-27)	23 (19-28)	23 (19-28)
Height (cm)	177 (168-188)	165 (158-192)*	173 (158-188)
Weight (kg)	74 (59-83)	57 (46-68)*	66 (46-83)
VO_{2max} ($ml \cdot kg^{-1} min^{-1}$)	62 (52-67)	53 (47-65)*	60 (47-67)
VE_{peak} ($l \cdot min^{-1}$)	160 (108-204)	111 (89-128)*	157 (89-204)
HF_{peak} (beats$\cdot$ min^{-1})	187 (173-197)	190 (178-201)	189 (173-201)
FEV_1 (% of predicted)	105 (86-115)	96 (82-109)*	101 (82-115)
FE_{NO} baseline (ppb)	21 (7-30)	14 (7-22)*	20 (7-30)

Table 2 Average running velocity, ventilation the first minute after exercise (V_{E1}) and peak heart rate (HR_{peak}) during an 8 min exercise test at 2800 m and at 180 m. Results are listed as means with standard deviations (sd). Level of significance (p) is set as 0.05.

	2800 m	150 m	p
Velocity ($km \cdot h^{-1}$)	11.3 (±0.9)	12.7 (±1.1)	0.0000004*
V_{E1} ($l \cdot min^{-1}$)	86 (±29.1)	84.5 (±27.1)	0.8
HR_{peak} (%)	89 (±5.5)	91 (±5.5)	0.004*

Table 3 Absolute values of partial pressure of nitric oxide sampled offline at baseline, after warm up and 5-60 min after high intensity exercise. Measurements made at 2800 m were corrected for an increase in expiratory flow rate (PE_{NOcorr}). Results are given as means with 95% confidence intervals.

PE_{NOcorr} (mPa)	2800 m	180 m
Baseline	1.47 (1.21, 1.73)	1.54 (1.24, 1.84)
After warm-up	1.27 (1.03, 1.15)	1.47 (1.24, 1.84)
5 min	1.11 (0.87, 1.34)	1.04 (0.87, 1.34)
10 min	1.24 (0.99, 1.48)	1.29 (1.05, 1.52)
15 min	1.38 (1.12, 1.63)	1.41 (1.18, 1.64)
20 min	1.47 (1.22, 1.73)	1.58 (1.30, 1.85)
30 min	1.49 (1.23, 1.74)	1.62 (1.33, 1.92)
60 min	1.49 (1.17, 1.74)	1.55 (1.26, 1.83)

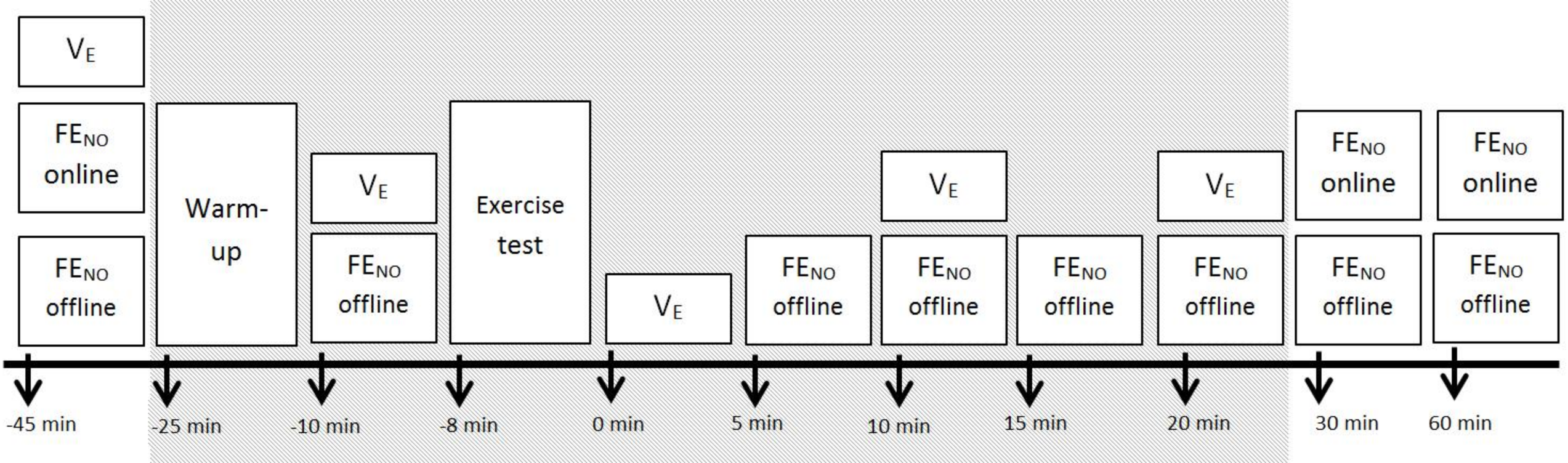
* Different from baseline, †different from 180 m. P<0.005

Table 4 Absolute values of fractional exhaled nitric oxide (FE_{NO}) measured offline and online at baseline, after warm up and 5-60 min after high intensity exercise. Samples collected at 2800 m altitude are corrected for an increase in expiratory flow rate (FE_{NOcorr}). Results are given as means with 95% confidence intervals.

	2800 m		180 m	
FE_{NO} (ppb)	**Offline**	**Online**	**Offline**	**Online**
Baseline	15.9 (13.1, 18.8)	17.7 (14.1, 21.3)	16.6 (13.4, 19.9)	18.4 (14.6, 22.0)
After warm-up	19.1 (15.5. 22.8)†	-	15.9 (13.0, 18.8)	-
5 min	16.7 (13.2, 20.2)†	-	11.3 (9.4, 13.2)*	-
10 min	18.6 (15.0, 22.2)†	-	13.9 (11.4, 16.5)*	-
15 min	20.7 (16.8, 24.5)*†	-	15.3 (12.8, 17.7)	-
20 min	22.1 (18.3, 25.9)*†	-	17.1 (14.1, 20.0)	-
30 min	16.1 (13.3, 18.8)	18.5 (14.7, 22.2)	16.7 (13.6, 17.8)	19.5 (15.4, 23.6)
60 min	15.7 (12.7, 18.8)	18.4 (14.6, 22.2)	16.7 (13.6, 17.8)	19.3 (15.0, 23.5)

* Different from baseline, †different from 180 m. P<0.005

Figure 1 Timeline of test procedure with the gray area illustrating duration of hypoxic exposure of 2800 m altitude. Measurements of fractional exhaled nitric oxide (FE_{NO}) were performed at baseline, after warm-up and 5 – 60 min after exercise. Minute ventilation (V_E) was assessed at baseline, after warm up, immediately after exercise and 10 and 20 min after exercise.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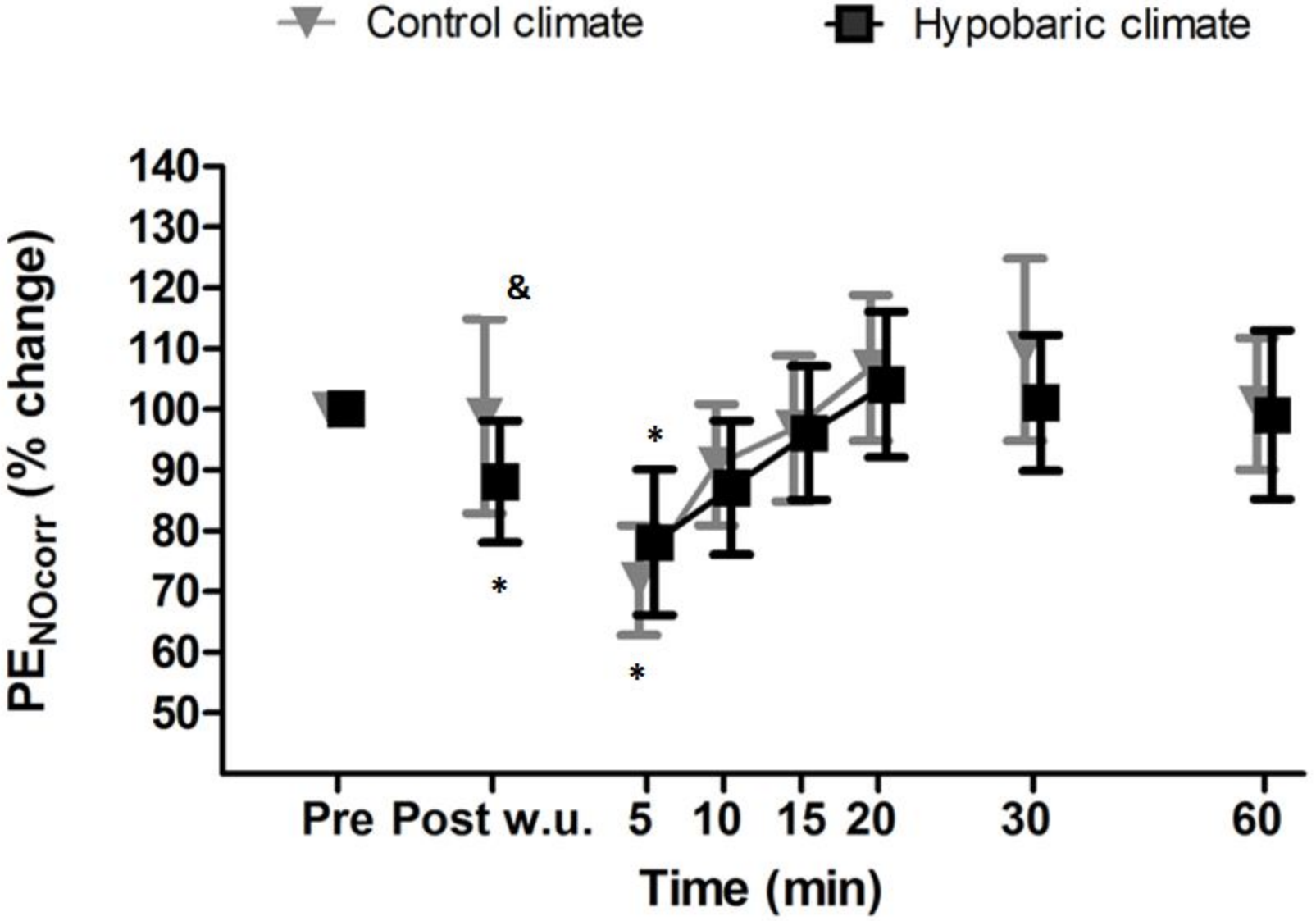
FE_{NO} offline
FE_{NO} online
FE_{NO} offline
FE_{NO} online
FE_{NO} offline
-45 min
-25 min
-10 min
-8 min
0 min
5 min
10 min
15 min
20 min
30 min
60 min

Figure 2 Change (%) in partial pressure of nitric oxide corrected for gas density at 2800 m and 180 m (PE_{NOcorr}) from baseline (pre) to after warm up (post w. u.) and 5-60 minutes after to 8 min exercise tests, one at 2800 m and one at 180 m. Results are given as means with 95% confidence intervals.

& Significantly different between tests ($p<0.05$).

* Significantly different from baseline ($p<0.05$).


Control climate
Hypobaric climate
PE_NOcorr (% change)
140
130
120
110
100
90
80
70
60
50
&
*
*
*
Pre
Post
w.u.
5
10
15
20
30
60
Time (min)