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Exhaled nitric oxide and lung function after moderate normobaric hyperoxic exposure

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ABSTRACT

Introduction: Pulmonary oxygen toxicity is associated with inflammatory responses in the airways and alveoli. The purpose of this study was to investigate whether the changes in exhaled nitric oxide (FE_{NO}) after exposure to normobaric hyperoxia (NBO), 100% oxygen (O_2) at 1 atmosphere absolute (atm abs) for 90 minutes, are associated with changes in lung function.

Methods: Eighteen healthy non-smoking subjects were exposed to NBO breathing 100% oxygen and to breathing ambient air, both for 90 minutes on separate days and in random order. Dynamic and static lung volumes, maximal expiratory flow rates, distribution of ventilation including closing volume and slope of phase III of the nitrogen washout curve (ΔN_2), diffusion capacity (D_LCO) and FE_{NO} were measured before and after the exposures.

Results: The mean reduction in FE_{NO} was 20% (SD=20) after the NBO exposure ($p<0.001$). Static and dynamic lung volumes, maximal expiratory flow rates, D_LCO and distribution of ventilation were unchanged. No association was found between the changes in the lung function variables and the change in FE_{NO}.

Discussion: Unchanged indices of distribution of ventilation and maximal expiratory flow rates indicate no small airways' dysfunction, and unchanged D_LCO suggests preserved gas transfer in the lung despite a significant reduction in FE_{NO}. FE_{NO} might be an index of oxygen exposure, but further studies over a wide range of oxygen exposures are necessary to establish the role of FE_{NO} as a marker of pulmonary oxygen toxicity.

INTRODUCTION

Pulmonary oxygen toxicity is associated with inflammatory responses in the airways and alveoli [1,2]. There is a dose-response relationship between oxygen exposure measured as unit pulmonary toxic dose (UPTD) and the decrease in vital capacity (VC) [3]. However, reductions in maximal expiratory flow rates and diffusion capacity of the lung for carbon monoxide (D_LCO) have been demonstrated after exposures to hyperoxia where changes in VC were neither found nor predicted [4, 5, 6], and symptoms like chest tightness often precede a reduction in VC.

Nitric oxide (NO) in exhaled gas is a marker of some inflammatory processes in the lung. An increased fraction of nitric oxide in exhaled gas (FE_{NO}) is associated with the eosinophilic inflammation characteristic of atopic asthma and with some viral infections [7]. FE_{NO} is not generally increased in chronic obstructive pulmonary disease (COPD), which is associated with a neutrophilic inflammation, but may be increased during exacerbations [8]. FE_{NO} is generally lower in smokers compared with non-smokers [9]. Exposures to normobaric and hyperbaric hyperoxia have been shown to reduce the FE_{NO} by ~20-70% [10,11,12] in an apparently dose-dependent manner. Partitioning FE_{NO} into its flow-independent components indicate no change in the alveolar nitric oxide concentration, but a reduction in the bronchial nitric oxide flux [11,13]. The reduction in FE_{NO} in these studies was not accompanied by reductions in forced vital capacity (FVC) or forced expired volume in one second (FEV_1). It is not known whether it is associated with changes in other lung function variables, in particular indices of small airways function and gas transfer.

The production of NO is influenced by the partial pressure of oxygen (pO_2). NO production is decreased in hypoxia, and lower NO concentration contributes to pulmonary hypoxic vasoconstriction and high altitude pulmonary edema [14]. An increase in NO production

TABLE 1 – Subjects' characteristics

	Female (*n* = 10)	Male (*n* = 8)	Total (*n* = 18)
Age *(years)*	24 (1)	24 (2)	24 (2)
Weight *(kg)*	59 (7)	78 (6)	67 (11)
Height *(cm)*	170 (5)	184 (6)	176 (9)
FVC *(% predicted)*	96 (9)	99 (10)	97 (10)
FEV$_1$ *(% predicted)*	98 (12)	93 (10)	96 (11)

Table 1: Descriptive data and lung function measured by forced vital capacity (FVC) and forced expired volume in one second (FEV$_1$) in the subjects (mean (SD).

has not been demonstrated in hyperoxia, but there is an upregulation of inducible nitric oxide synthase (iNOS) [15]. NO reacting with superoxide-anion forming peroxynitrite is thought to contribute to the toxic effects of hyperoxia [16]. Neurogenic NO-dependent mechanisms appear to be involved in development of severe pulmonary oxygen toxicity at partial pressures of oxygen higher than 250 kPa, with pulmonary edema and respiratory failure [17].

The VC is apparently not sufficiently sensitive to identify the early development of pulmonary oxygen toxicity as far as other lung function variables may be reduced without an accompanying reduction in VC. The purpose of this study was to investigate whether a hyperoxic exposure known to result in a 20-25% reduction in FE$_{NO}$ [13] is associated with changes in established lung function variables, including D$_L$CO and indices of distribution of ventilation.

METHODS
Subjects
Eighteen healthy subjects (eight males) exposed to normobaric hyperoxia, 100% O$_2$ at normal atmospheric pressure (NBO) for 90 minutes were included. Their mean age was 24 years (range 22-28). All subjects were non-smokers and functioned as their own control, breathing ambient air. The subjects' descriptive data are given in Table 1. The study was a randomized crossover design approved by the Regional Committee for Medical Research Ethics, and written informed consent was given by all subjects.

Protocol
The subjects sat passively with a nose clip breathing 100% oxygen for 90 minutes at normal atmospheric pressure through a low-resistance two-way non-rebreathing T-shape^{TM} valve (Hans Rudolph, Inc. Kansas City,

USA). The oxygen was provided from a compressed source. In the control exposure for 90 minutes the subjects were breathing air directly from the atmosphere. NBO exposure and control exposure were on different days and in random order. The pre-exposure measurements started at least one hour after the last meal between 3 and 6 p.m. and drinking water only was allowed until all tests were finished. The lung function measurements were done in the same sequence each time starting with FE$_{NO}$, and then the single-breath oxygen test, D$_L$CO, the multiple-breath nitrogen washout test and, finally, dynamic lung volumes. The lung function tests were conducted in this order to prevent – as much as possible – each test from affecting one another. It is known that a forced expiratory maneuver may influence FE$_{NO}$, which is why measurement of dynamic lung volumes was the last test. During the single-breath oxygen test and intra-breath D$_L$CO test the subjects had a three- to four-minute break between each maneuver. The measurements were done within 30 minutes before the exposure and starting with FE$_{NO}$ measurements 15 minutes after exposure. A pre-test was performed for practice purposes one week before, and subjects with FE$_{NO}$ >50 ppb (parts per billion) or atopic diseases were not included in the study.

Exhaled nitric oxide
FE$_{NO}$ was measured at an expiratory flow rate of 50 mL·s^{-1} with an online chemiluminescence analyzer (Eco Medics AG, Duernten, Switzerland). The mean of three measurements one minute apart with a variation of less than 10% was accepted. All measurements were performed before and after exposure according to recommendations specified by the European Respiratory Society and the American Thoracic Society [18].

Lung function measurements
All lung function tests were performed on a Vmax Encore 229 (Viasys Healthcare Inc., Yorba Linda, Calif. USA), which measures flow using a mass flow meter and integrates flow to get volume. The single-breath oxygen test measures closing volume (CV) and the slope of phase III of the nitrogen washout curve (Δ N$_2$), which are indices of distribution of ventilation and are related to small airways function. The intra-breath method was

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used to measure D_LCO as a measure of pulmonary gas transfer function and pulmonary blood flow. The multi-breath nitrogen washout was used for measurement of static lung volumes, and additional information related to distribution of ventilation can be derived from the nitrogen washout curve. All lung function tests were performed with a nose clip in a sitting position. All indices were calculated with the algorithms supplied with the software from the manufacturer. Volume and test gas calibrations were done before each test.

Distribution of ventilation – single-breath oxygen test

CV was measured with the single-breath oxygen test after a single inhalation of 100% oxygen from residual volume (RV) to total lung capacity (TLC). ΔN_2 was calculated over the mid-expiratory range.

Diffusion capacity for carbon monoxide (D_LCO)

D_LCO was measured with the intra-breath method [19]. The subjects inhaled the test gas with 0.3% CO, 0.3% methane, 0.3% acetylene and 21% oxygen in nitrogen from RV to TLC. Without any breath-hold at TLC, exhalation was at a rate of 0.5 $L \cdot s^{-1}$ and the concentration of the test gases were measured during the exhalation. D_LCO was not corrected for hemoglobin concentration. Effective alveolar volume (V_A) was calculated based on the dilution and washout of methane, and D_LCO and pulmonary blood flow (Q_{pulm}) based on the rate of change of CO and acetylene concentration. The diffusion coefficient for carbon monoxide (Kco) was calculated as $D_LCO \cdot V_A^{-1}$.

Static lung volumes

The multiple-breath nitrogen washout method was used to measure functional residual capacity (FRC). When a stable end-expiratory volume was established, the subjects switched to breathing 100% oxygen until the end-tidal nitrogen concentration was less than 2%. After that the subjects did a full inspiration to TLC followed by a full expiration to residual volume (RV). TLC was calculated as the sum of FRC and inspiratory capacity and RV as the difference between FRC and expiratory reserve volume. The nitrogen washout time (NWT) was the time taken to bring end-tidal nitrogen concentration below 2%, and the lung clearance index (LCI) was the number of turnovers of FRC to bring it below 2%. NWT and LCI were considered as indices of distribution of ventilation. Poorly ventilated lung regions will need longer time for washing out the nitrogen and a larger volume must be ventilated to bring the end-tidal nitrogen concentration below 2%.

Dynamic lung volumes and maximal expiratory flow rates

Forced vital capacity (FVC), forced expired volume in one second (FEV_1) and peak expiratory flow rate (PEF) were obtained from the highest values of at least three satisfactory forced expiratory maneuvers from TLC. Maximal expiratory flow rates at 25-, 50- and 75% of FVC expired ($FEF_{25\%}$, $FEF_{50\%}$, $FEF_{75\%}$) and mean mid-expiratory flow rate ($FEF_{25-75\%}$) were taken as the highest values from flow-volume loops where FVC was not less than 95% of the highest FVC [20].

Statistical analysis

Repeated measures ANOVA for group differences were used for comparison of FE_{NO} and the lung function variables pre- and post-exposure. The difference between pre-exposure values on different days was included in the analysis. Any relationships between the changes in FE_{NO} and changes in the lung function variables were analyzed by linear regression analysis. A p-value <0.05 was considered significant. Data are expressed as mean (SD =).

RESULTS

There was a significant reduction in FE_{NO} from 19.5 (SD = 7.4) to 15.2 (SD = 5.8) ppb ($p < 0.001$) after exposure to NBO, and a smaller reduction from 21.6 (SD = 8.8) to 20.1 (SD = 8.2) ppb ($p < 0.05$) after the control when breathing ambient air. There were no changes in static and dynamic lung volumes, maximal expiratory flow rates, D_LCO or distribution of ventilation *(Table 2)*. CV was not detectable in three subjects at any time of investigation. In four subjects who had a detectable CV before NBO exposure, it was not detectable after the exposure. In the 11 subjects with detectable CV before and after exposure there was no significant change. The subjects did not report any symptoms including coughing or chest tightness during or after exposure.

DISCUSSION

The reduction in FE_{NO} of 20% was consistent with the findings of previous studies with a comparable oxygen exposure [12,13]. The reduction in FE_{NO} after exposure to hyperoxia has been shown to be predominantly an airway effect with a reduction in bronchial nitric oxide flux without changes in alveolar NO concentration [11,13]. In this study the reduction in FE_{NO} was not associated with changes in maximal expiratory flow rates or indices of distribution of ventilation indicative of physiological small airways dysfunction. A reduction

TABLE 2 - Lung volumes, diffusion capacity and indices of distribution of ventilation before and after exposure to normobaric hyperoxia (NBO) and when breathing ambient air for 90 minutes, mean (SD)

	NBO ($n = 18$) PRE	POST	AMBIENT AIR ($n = 18$) PRE	POST
DYNAMIC LUNG VOLUMES, MAXIMAL EXPIRATORY FLOW RATES				
FEV_1 (L)	4.01 (0.81)	4.09 (1.01)	4.01 (0.83)	4.06 (0.89)
FVC (L)	4.82 (1.14)	4.74 (1.12)	4.83 (1.20)	4.79 (1.12)
PEF (L $\cdot$ s^{-1})	9.52 (2.17)	9.86 (2.38)	10.1 (2.86)	10.0 (2.73)
$FEF_{25-75\%}$ (L $\cdot$ s^{-1})	4.20 (0.95)	4.23 (0.89)	4.1 (0.88)	4.20 (0.84)
$FEF_{25\%}$ (L $\cdot$ s^{-1})	8.27 (1.70)	8.50 (1.83)	8.72 (2.23)	8.73 (2.10)
$FEF_{50\%}$ (L $\cdot$ s^{-1})	5.24 (1.23)	5.28 (1.17)	4.92 (1.14)	5.15 (1.00)
$FEF_{75\%}$ (L $\cdot$ s^{-1})	2.24 (0.69)	2.26 (0.70)	2.23 (0.70)	2.25 (0.61)
STATIC LUNG VOLUMES				
VC (L)	4.84 (1.13)	4.78 (1.11)	4.76 (1.22)	4.71 (1.19)
TLC (L)	6.47 (1.19)	6.25 (1.19)	6.35 (1.31)	6.33 (1.21)
RV (L)	1.63 (0.39)	1.4 (0.36)	1.59 (0.39)	1.62 (0.42)
FRC (L)	3.36 (0.63)	3.12 (0.69)	3.31 (0.76)	3.28 (0.64)
INTRA-BREATH DLCO				
D_LCO (mmol $\cdot$ m^{-1} $\cdot$ kPa^{-1})	9.1 (2.8)	8.9 (2.4)	8.9 (2.5)	9.0 (2.5)
Q_{PULM} (L $\cdot$ m^{-1})	4.7 (1.2)	4.1 (0.8)	4.5 (1.2)	4.2 (1.0)
V_A (L)	5.74 (1.41)	5.66 (1.37)	5.69 (1.36)	5.65 (1.34)
DISTRIBUTION INDICES				
LCI	5.0 (0.5)	5.0 (0.4)	5.1 (0.4)	5.2 (0.4)
NWT (min)	2.3 (0.7)	2.3 (0.8)	2.2 (0.6)	2.4 (1.0)
ΔN_2 (%/L)	1.84 (0.73)	1.91 (0.74)	1.86 (0.77)	1.98 (0.83)
CV (%VC)	9.3 (2.6)	9.9 (2.6)	8.8 (2.0)	8.9 (2.3)

Note: Forced expired volume in one second (FEV_1), forced vital capacity (FVC), peak expiratory flow rate (PEF), mean mid-expiratory flow rate ($FEF_{25-75\%}$), maximal expiratory flow rates at 25-, 50- and 75% of FVC expired ($FEF_{25\%}$, $FEF_{50\%}$, $FEF_{75\%}$), vital capacity (VC), total lung capacity (TLC), residual volume (RV), functional residual capacity (FRC), diffusion capacity for carbon monoxide (DLCO), pulmonary blood flow (Q_{pulm}), alveolar volume (VA), lung clearance index (LCI),) nitrogen washout time (NWT), delta N_2 (ΔN_2), closing volume (CV). There were no significant differences between measurements.

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in maximal expiratory flow rates has been demonstrated after repeated hyperbaric oxygen treatment sessions at a pO_2 of 240 kPa for 90 minutes [12] and 300 kPa for 3.5 hours [6] without accompanying changes in VC or FEV_1.

The method chosen for measuring static lung volumes was the multiple-breath nitrogen washout since it gives additional information on distribution of ventilation. During the oxygen exposure nitrogen being dissolved in tissues has been washed out and lower nitrogen content in the body would result in lower static lung volumes measured after NBO by this technique. The overestimation of FRC is normally ~200 mL compared with the helium dilution method and will be less after breathing oxygen for 90 minutes. There was a 30- to 45-minute period breathing air with nitrogen wash-in before the post-exposure measurement uploading the nitrogen stores. This could explain a trend for a lower FRC of 240 mL after the NBO exposure. However, there were no changes in LCI and NWT, which would be expected to be lower if lower body stores of nitrogen contributed to a reduction in FRC; and the change was not statistically significant.

During NBO exposure the oxygen was provided from a compressed source, whereas in the control exposure the subjects were breathing air directly from the atmosphere and not compressed air through the low-resistance Hans-Rudolph T-valve. Breathing resistance and humidity have been different between the exposures, but with resting ventilation only, the added work of breathing and respiratory water loss have been low. These effects have probably not influenced the static lung volumes significantly; and they were not significantly different.

In the present study, no changes in D_LCO, Q_{pulm} or effective alveolar volume were found after exposure to NBO for 90 minutes. D_LCO is a compound measure influenced by lung volume accessible for gas exchange, diffusion of gas over the alveolar-capillary membrane, pulmonary capillary blood flow and hemoglobin concentration. Partitioning FE_{NO} into its flow-independent components has shown unchanged alveolar NO concentration after a similar hyperoxic exposure [11,13]. Unchanged D_LCO supports the conclusion that alveolar integrity and gas exchange is preserved after a hyperoxic exposure of this magnitude. However, the results of D_LCO after exposure to hyperoxia are ambiguous. Subjects exposed to higher oxygen pressures ranging from 203-304 kPa for two-12 hours demonstrated similar results [21,22], whereas other studies have shown that D_LCO was decreased by 8-20% after prolonged exposures to a pO_2 higher than 100 kPa for 3.5-13 hours [4,23].

Fothergill and Gertner [11] measured FE_{NO} after six- and eight-hour exposures to a pO_2 of 203 kPa and found a reduction in FE_{NO} of 60-70%. There were no statistically significant group mean changes in forced inspiratory VC and D_LCO adjusted for hemoglobin content, but there was a trend for a reduction in D_LCO the day after the exposure when FE_{NO} had normalized. This may indicate different time courses for changes in different lung function variables after exposure to hyperoxia and may explain the different results in different studies. In that study subjects with the lowest FE_{NO} after the exposure were the first to develop symptoms of pulmonary oxygen toxicity. Further, subjects with elevated baseline FE_{NO} were less disposed to pulmonary oxygen toxicity compared to subjects with low baseline FE_{NO}. Thus, FE_{NO} might be a useful marker of individual susceptibility to pulmonary oxygen toxicity. The results from the present study found no association between the 20% reduction in FE_{NO} and the lung function variables, but the hyperoxic exposure was low and no change in VC was predicted. Therefore, FE_{NO} might be its own index of oxygen exposure rather than a direct marker of pulmonary oxygen toxicity. VC is still probably the best marker of pulmonary oxygen toxicity, but it may have limitations since FE_{NO}, D_LCO and maximal expiratory flow rates can be reduced before any reduction in VC occurs [5]. A limitation of this study was that only one oxygen exposure was performed. Extended studies with different oxygen exposures over a wide range may give more information about lung function variables and the possible relationship to FE_{NO}.

In the respiratory tract, NO is derived from various cellular sources such as neutrophils, endothelial and epithelial cells and airway and vascular smooth muscle cells [24]. Three distinct isoforms of NO synthase (NOS) have been identified: inducible NOS (iNOS), endothelial NOS (eNOS) and neuronal NOS (nNOS). The production of NO may aggravate or attenuate the toxic effects of oxygen [25]. Preserved endothelial function and endothelial NO production may be protective, but neurogenic NO-mediated mechanisms appears to be involved in the development of overt pulmonary edema at a pO_2 higher than 250 kPa [17]. Cucchiaro et al. [15] found that hyperoxia upregulates iNOS expression in the lungs of rats without increasing FE_{NO}. These findings suggest that NO may either be chemically scavenged in a hyperoxic environment or its synthesis may be inhibited. When tetrahydrobiopterin is oxidized to dihydrobiopterin it is no longer functional as a co-factor in the NO synthesis. It may then be speculated that exposure to high oxygen

pressures results in reduced NO production in all three isoforms of NOS influencing both vascular and neurogenic activity.

In conclusion, the reduction in FE_{NO} after a 90-minute exposure to 100 % oxygen at normal atmospheric pressure was not associated with changes in lung function variables. FE_{NO} might be its own index of oxygen exposure, but further studies over a wide range of oxygen exposures are necessary to establish the role of FE_{NO} as a possible marker of pulmonary oxygen toxicity.

Disclosures
The authors have no conflicts of interest or financial ties to disclose.

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