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Bronchial nitric oxide flux and alveolar nitric oxide concentration after exposure to hyperoxia.

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ABSTRACT

Background: The fraction of nitric oxide in exhaled gas ($F_{E_{NO}}$) is reduced with 30-70% after exposure to partial pressures of oxygen (PO_2) of 200-240 kPa for 90 min. The purpose of this study was to partition $F_{E_{NO}}$ into its flow independent alveolar and bronchial components. A reduced bronchial NO flux ($J_{aw}NO$) is associated with induced bronchoconstriction, while increased alveolar NO concentration (C_ANO) is associated with increased alveolar dead space. ***Methods:*** Twelve patients undergoing hyperbaric oxygen (HBO_2) therapy for 90 min at a PO_2 240 kPa and twenty healthy subjects exposed to normobaric hyperoxia (NBO_2) breathing 100% oxygen for 90 min were compared to a control group of 6 subjects breathing ambient air. $F_{E_{NO}}$ was measured at flow rates from 30 to 250 $mL{\cdot}s^{-1}$ before and after the exposures and the Högman Märilainen algorithm was used to calculate $J_{aw}NO$ and C_ANO. ***Results:*** $F_{E_{NO}}$ at an expiratory flow rate of 50 $mL{\cdot}s^{-1}$ was reduced from 17.6 ± 8.3 to 12.3 ± 6.3 ppb after HBO_2 exposure and from 17.8 ± 6.2 to 13.3 ± 5.2 ppb after NBO_2 exposure. There was a significant reduction in $J_{aw}NO$, but unchanged C_ANO. There were no changes in the control experiment. ***Discussion:*** The reduction in $F_{E_{NO}}$ after exposure to normobaric and hyperbaric hyperoxia appears to be predominantly an airway effect. An unchanged and low C_ANO indicate preserved integrity of the gas exchange units without increased alveolar dead space at rest.

Key words: Diving; Hyperbaric oxygen therapy; Lung function; Oxygen toxicity

INTRODUCTION

Nitric oxide (NO) is produced in the upper and lower respiratory tract by a large variety of cell types, such as vascular endothelial cells, neuronal cells, alveolar macrophages, and bronchial and alveolar epithelial cells (4). The endothelial and neuronal nitric oxide synthases are constitutive and produce NO in picomol and fentomol concentrations, contributing minimally to the concentration of NO in exhaled gas. The NO in the airway gas is synthesized predominantly in bronchial epithelial cells by inducible NO synthase (iNOS) in nanomol concentrations. An increase in $F_{E_{NO}}$ is seen with some inflammatory processes in the airways. It is consistently increased in atopic asthma (14), in association with some viral infections and exacerbations of chronic obstructive lung disease (17). It is decreased in smokers and in association with some bacterial infections, cystic fibrosis and ciliary dyskinesia (16). The functional significance of an increased or reduced $F_{E_{NO}}$ in these conditions is not known.

The fraction of nitric oxide in exhaled gas ($F_{E_{NO}}$) is reduced with 30-70% after exposure to hyperbaric hyperoxia for 90 min at partial pressures of oxygen (PO_2) of 200-240 kPa in patients having hyperbaric oxygen (HBO_2) therapy (22;27). The reduction in $F_{E_{NO}}$ persists for more than 4 hrs, but is apparently normalised within 24 hrs (15). A reduction in $F_{E_{NO}}$ of 55-63% has been demonstrated in healthy divers exposed to a PO_2 of 203 kPa for 6-8 hrs in a hyperbaric chamber (10). There are conflicting results in humans exposed to normobaric hyperoxia (NBO_2). Puthucheary et al. (22) found no changes after breathing 100% or 40% O_2 for 90 min in healthy subjects serving as a control group in their study of patients having HBO_2 therapy. Tsuchiya et al. (30) showed that $F_{E_{NO}}$ was reduced after breathing 100% O_2 for 50 min, but unchanged after breathing 40% O_2 for 50 min in subjects that were mechanically ventilated during anaesthesia. Schmetterer et al. (24) found that $F_{E_{NO}}$ was increased with 25% when breathing 100% O_2.

Alveolar nitric oxide concentration (C_ANO) and bronchial NO flux ($J_{aw}NO$) contribute to the concentration of NO in exhaled gas. By measuring $F_{E_{NO}}$ at different expiratory flow rates, the alveolar and bronchial contributions to $F_{E_{NO}}$ can be estimated. C_ANO and $J_{aw}NO$ can be estimated by several models based on analysis of the relationship between the inverse of expiratory flow rate and $F_{E_{NO}}$. There are small differences between models, but the nonlinear regression model by Högman and Märilainen may be more accurate than linear models (13). C_ANO is normally low and close to zero because of its fast reaction rate with haemoglobin. It may be increased due to increased alveolar dead space and with increased axial diffusion of NO into the alveoli due to bronchoconstriction (31). A reduced $F_{E_{NO}}$ and C_ANO are associated with primary pulmonary hypertension (12). Fothergill and Gertner (10) showed that $J_{aw}NO$ was reduced after the exposure to a PO_2 of 203 kPa for 6 hrs in healthy divers without changes in C_ANO.

The purpose of this study was to partition $F_{E_{NO}}$ into its flow independent alveolar and bronchial components in patients exposed to HBO_2 therapy and in healthy subjects exposed to NBO_2 breathing 100% oxygen. It was hypothesised that any derangements of alveolar structure and function due to oxygen toxicity might result in a change in the alveolar component despite an overall reduction in $F_{E_{NO}}$.

METHODS

Subjects

Twelve patients (7 men) undergoing HBO_2 therapy, twenty healthy subjects (10 men) exposed to normobaric hyperoxia breathing 100% oxygen (NBO_2), and six healthy subjects (3 men) serving as control group breathing ambient air (AA) were included. The patients received HBO_2 therapy for chronic radiation-induced injury in the pelvic or head and neck regions, but had not radiation to the thoracic region. Two had treatment for cardiovascular disease with β-blockers. Seven patients were

previous smokers (5 men), having stopped smoking more than 6 months before HBO_2 treatment. Their mean age was 50 years (range 35-63). The healthy subjects constituting the NBO_2 and control groups had a mean age of 27 and 28 yrs (range 20-39) and were non-smokers. The subjects' characteristics are given in Table 1. The study was approved by the Regional Committee for Medical Research Ethics, and written informed consent was given by all subjects.

[Table 1 here]

Protocol

The patients were exposed to HBO_2 daily for four weeks for 90 min in a monoplace hyperbaric chamber. The chamber was compressed to a pressure of 240 kPa within 10-15 min. The oxygen exposure was in three intervals of 30 min interrupted by 5 min breaks inhaling air from an oronasal mask. Then they were decompressed for 7-10 min to normal ambient pressure. The treatment took place between 9 and 11 am. All patients had breakfast at 7 am and drinking water only was allowed until the measurements were finished.

The healthy subjects sat passively for 90 min with a nose-clip inhaling 100% oxygen or ambient air at normal atmospheric pressure through a two-way non-rebreathing T-shape^{TM} valve (Hans Rudolph, inc. Kansas City, USA). The exposure took place between 3 and 6 pm and 2 hrs after the last meal. Drinking water only was allowed until the measurements were finished.

F_{ENO} was measured at flow rates of 30, 50, 100 and 250 $mL \cdot s^{-1}$ ($F_{ENO,\,30}$, $F_{ENO,\,50}$, $F_{ENO,\,100}$, $F_{ENO,\,250}$), with an on-line chemiluminescence analyser (Eco Medics AG, Duernten, Switzerland) 10-30 min before and 10-20 min after the exposures. For the normobaric hyperoxic exposure $F_{ENO,\,50}$ were measured during the exposure as well at 30, 60 and 90 min. The subject removed the mouthpiece and nose-clip, exhaled slowly to residual volume before inhaling to total lung capacity and then exhaled

directly into the NO analyzer. The mean of three measurements 1 min apart with a variation of less than 10% was accepted. The Högman Meriläinen algorithm (13) was used to estimate C_ANO and J_{aw}NO. All measurements were performed according to the recommendations specified by European Respiratory Society and American Thoracic Society (1).

Forced vital capacity (FVC) and forced expired volume in one second (FEV_1) were measured before and after the exposures on a wedge spirometer (Vitalograph Ltd., Buckingham, England). The highest value obtained from three satisfactory forced expiratory manoeuvres was reported. Spirometry was done after the F_{ENO} measurements.

Statistical analyses

Paired-samples t-test was used for comparison of F_{ENO} before and after the exposures to hyperbaric and normobaric hyperoxia and ambient air. A p value <0.05 was considered significant. Data are expressed as mean ± SD.

RESULTS

Hyperbaric oxygen exposure

There was a 30 ± 9% reduction in $F_{ENO,\ 50}$ from 17.6 ± 8.3 to 12.3 ± 6.3 ppb ($t_{(11)}$ = 6.8, p<0.001) 15-20 min after the HBO_2 exposure. F_{ENO} at the other expiratory flow rates was significantly reduced as well (Fig. 1A). There was a significant reduction in J_{aw}NO ($t_{(11)}$ = 4.2, p<0.001), but no change in C_ANO ($t_{(11)}$ = 0.74, p = 0.47) (Table II). FVC and FEV_1 were unchanged.

[Table II here]

Normobaric oxygen exposure

There was a 25 ± 9% reduction in $F_{ENO,\ 50}$ from 17.8 ± 6.2 to 13.3 ± 5.2 ppb ($t_{(19)}$ = 9.6, p<0.001) 15-20 min after the NBO_2 exposure, and there was a significant reduction in F_{ENO} at the other expiratory flow rates (Fig. 1B). There was a significant reduction in $J_{aw}NO$ ($t_{(19)}$ = 4.9, p<0.001), but no change in C_ANO ($t_{(19)}$ = 0.8, p = 0.44) (Table II). $F_{ENO,\ 50}$ was not different from baseline to 30 min into the NBO_2 exposure. Thereafter there was a gradual decrease at 60 and 90 min approaching the 15 min post-exposure value (Fig. 2). There was no change in FEV_1, ($t_{(19)}$ = 1.7, p = 0.10) but a small and statistically significant ($t_{(19)}$ = 2.3, p = 0.03) reduction in FVC of 0.05L after the NBO_2 exposure. All respiratory parameters remained unchanged in the control group (Fig. 1C).

[Fig. IA, B and C here]

[Fig. 2 here]

DISCUSSION

The results suggest that $F_{ENO,\ 50}$ is reduced with ~30% after a single 90 min HBO_2 treatment session, which is consistent with previous studies (15;22;27). $F_{ENO,\ 50}$ was reduced with 25% after breathing 100% O_2 for 90 min. There was a gradual decrease in $F_{ENO,\ 50}$ during the NBO_2 exposure indicating a dose-dependant response (Fig. 2). The larger reduction in $F_{ENO,\ 50}$ after 6 and 8 hrs exposures to a PO_2 of 203 kPa in the study of Fothergill and Gertner (10) than after 90 min to 240 and 100 kPa in this study also indicate a dose-dependant response. However, Schmetterer et al. (24) found that F_{ENO} increased with 25% when breathing 100% O_2 for 10 min. There was a trend of an increase in F_{ENO} after 30 min exposure to NBO_2 (Fig. 2), but thereafter there was a gradual decrease. No measurements were done immediately after the start of oxygen breathing. If there is an initial response with an increase in $F_{ENO,\ 50}$ this could explain the difference between the study of Schmetterer et al. (24) with a very short exposure time and the others.

The reduction in F_{ENO} after exposure to hyperoxia found in the present study appears to be predominantly an airway effect. At any axial position in the airways the flux of NO into the airway lumen depends on several variables in addition to the concentration of NO in the airway such as tissue thickness, airway diameter and thereby surface area, and endogenous production and consumption rates (29). Induced bronchoconstriction has been shown to reduce F_{ENO}. One mechanism that probably plays an important role in the effect of airway constriction or dilation on F_{ENO} is backdiffusion of NO from the bronchial compartment towards the alveolar zone (31). Reduced small airways conduction is an early sign of development of pulmonary oxygen toxicity. This is consistent with reduced F_{ENO} in the bronchial compartment.

In the present study, no change in C_ANO was found. This is in agreement with the study by Fothergill and Gertner (10), which showed that C_ANO was unchanged after exposure to a PO_2 of 203 kPa for 6 hrs. Nitric oxide on the alveolar level is important for regulation of blood flow in the alveoli, which may influence the distribution of the ventilation-perfusion ratio (V_A/Q) and gas exchange. Tsuchiya et al. (30) showed that there is an association between reduced F_{ENO} and increased alveolar-to-arterial oxygen difference. A reduced F_{ENO} is associated with pulmonary hypertension (12) and induced bronchoconstriction (31), while an increase in C_ANO is associated with increased alveolar dead space. This could imply that the bronchial component caused the reduction in F_{ENO} and the pulmonary blood supply was unchanged. NO will not be transferred to pulmonary capillary blood if the ventilated regions are poorly perfused, and will go back to the conductive airways when exhaled.

The risk of developing pulmonary oxygen toxicity is present during professional diving or long term HBO_2 therapy. Exposure to a PO_2 higher than 40-50 kPa results in a toxic effect on the lung with reduced vital capacity (5;8), maximal expiratory flow rates (6) and diffusion capacity (5;7) depending

on exposure time. Oxygen toxicity is associated with inflammatory responses in the airways and in the alveoli causing alveolar epithelial and endothelial dysfunction and eventually pulmonary edema (9;23). Exposure to hyperoxia contributes to a reduction in maximal expiratory flow rates in patients having HBO_2 therapy (18;28) and to the long term effects of diving on the lung (26;28). The results of the present study showed a significantly lower FVC after the exposure to hyperoxia in the group of healthy subjects exposed to NBO_2. This is not in agreement with other studies and with the predictions based on oxygen dose of unit pulmonary toxic doses (UPTD) that found no difference (7), and is not considered to be of any clinical importance. Whether a reduction in F_{ENO} is related to the development of oxygen toxicity is not known.

In previous studies, patients having HBO_2 therapy have been compared with matched control groups with respect to age, gender and smoking habits (15;27). No significant change in F_{ENO} over a period of 4 hrs was demonstrated in these control groups. If anything, there was a small increase that is consistent with the demonstration of a diurnal variation with an increase in F_{ENO} of ~15% from the morning into the afternoon (21;25). However, Kharitonov et al. (14) found no diurnal or day to day variation in F_{ENO}, and a reproducibility of ~10%. The reduction in F_{ENO} in the patients having HBO_2 therapy and the healthy subjects exposed to NBO_2 was 25-30%, and larger than the expected random variation. There was a larger interindividual variation in the response in the patients than in the healthy subjects, which constituted a more homogenous group. Baseline F_{ENO} and the response to HBO_2 treatment was not different in the two patients treated with β-blockers compared with the other patients.

The patients exposed to HBO_2 were significantly older than the healthy subjects exposed to NBO_2 and ambient air. There was a difference in height and weight between males and females in all groups and males exposed to HBO_2 had lower FVC and FEV_1 compared to females. There are conflicting results in the literature whether F_{ENO} is associated with age and height among adults (11). Baraldi et al. (2) found

no correlation, while Buchvald et al. (3) reported an obvious age dependency of F_{ENO} in healthy children. In a random population sample, F_{ENO} was associated with height but not gender (20). Olin et al. (19) suggested that the upper normal values of F_{ENO} in never-smoking adults range from 24 to 53 ppb, depending on age and height. The median F_{ENO} was 37 ppb among subjects >60 yrs and 19 ppb among subjects <30 yrs. The variability in baseline F_{ENO} was larger in the patients. The somewhat larger reduction in F_{ENO} after HBO_2 exposure in patients could be due to age differences and heterogeneity of the group. The hyperoxic exposure was larger in this group. Baseline C_ANO was also larger in the patients exposed to HBO_2. Previous smoking history, ageing and radiation therapy might have caused an increased alveolar dead space in this group, which is associated with an increase in C_ANO.

In conclusion, the reduction in F_{ENO} after exposure to normobaric and hyperbaric hyperoxia appears to be predominantly an airway effect. An unchanged and low C_ANO indicate preserved integrity of the gas exchange units without increased alveolar dead space at rest.

ENDNOTES

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TABLES

TABLE I. DEMOGRAPHICS AND LUNG FUNCTION MEASURED BY FORCED VITAL CAPACITY (FVC) AND FORCED EXPIRED VOLUME IN ONE SECOND (FEV_1) IN PATIENTS EXPOSED TO HYPERBARIC OXYGEN (HBO_2) THERAPY, HEALTHY SUBJECTS' EXPOSED TO NORMOBARIC OXYGEN (NBO_2) AND THE CONTROL GROUP BREATHING AMBIENT AIR (AA) (MEAN ± SD).

	HBO_2	**NBO_2**	**AA**
	Female/Male (n = 5/7)	**Female/Male (n = 10/10)**	**Female/Male (n = 3/3)**
Age (years)	50 ± 11*	27± 4	28 ± 4
Weight (kg)	78 ± 16*	67 ± 10	71 ± 9
Height (cm)	174 ± 8	172 ± 9	172 ± 10
FVC (% predicted)	95 ± 15	102 ± 13	104 ± 7
FEV_1 (% predicted)	90 ± 17	99 ± 11	97 ± 9

* = $p<0.05$ comparing HBO_2 therapy group with the other groups.

TABLE II. ALVEOLAR NO CONCENTRATION (C_ANO), BRONCHIAL NO FLUX ($J_{aw}NO$), FORCED VITAL CAPACITY (FVC) AND FORCED EXPIRED VOLUME IN ONE SECOND (FEV_1) MEASURED BEFORE AND AFTER EXPOSURE TO HYPERBARIC HYPEROXIA (HBO_2), NORMOBARIC HYPEROXIA (NBO_2) AND IN A CONTROL GROUP BREATHING AMBIENT AIR (AA) (MEAN ± SD).

	HBO_2 (n = 12)		**NBO_2 (n = 20)**		**AA (n = 6)**	
	Before exposure	**After exposure**	**Before Exposure**	**After exposure**	**Before exposure**	**After exposure**
C_ANO (ppb)	1.6 ± 1.5	1.4 ± 0.6	0.8 ± 1.3	0.6 ± 0.9	1.2 ± 0.3	0.9 ± 0.4
$J_{aw}NO$ ($pL \cdot s^{-1}$)	1045 ± 545	741 ± 407**	1201 ± 624	825 ± 417**	987 ± 343	1072 ± 464
FVC (l)	4.26 ± 0.73	4.24 ± 0.79	4.82 ± 1.06	4.77 ± 1.03*	4.97 ± 1.34	4.95 ± 1.37
FEV_1 (l)	3.28 ± 0.54	3.25 ± 0.62	3.97 ± 0.79	3.93 ± 0.81	3.91 ± 1.11	3.93 ± 1.17

* = $p<0.05$, ** = $p<0.01$.

CAPTIONS FOR FIGURES

Fig. 1: Expired nitric oxide ($F_{E_{NO}}$) at different flow rates before and after exposure to; A) hyperbaric hyperoxia (HBO_2) therapy in twelve patients, B) normobaric hyperoxia (NBO_2) breathing 100% O_2 in twenty healthy subjects and C) in a control group of six subjects breathing ambient air (AA), all for 90 min (Mean ± SD). ** = $p<0.001$.

Fig. 2: Expired nitric oxide ($F_{E_{NO}}$) at a flow rate of 50 $mL \cdot s^{-1}$ ($FE_{NO,\,50}$) 10 min before, during at 30, 60 and 90 min, and 15 min after exposure to normobaric hyperoxia breathing 100% oxygen (NBO_2) and in a control group breathing ambient air (AA) for 90 min (Mean ± SD). * = $p<0.05$, ** = $p<0.001$.