Time course of the reduction in nitric oxide concentration in exhaled gas after exposure to hyperbaric hyperoxia

Ida Kjelkenes and Einar Thorsen

Key words
Hyperbaric oxygen therapy, inflammation, lung function, oxygen toxicity

Abstract

(Kjelkenes I, Thorsen E. Time course of the reduction in nitric oxide concentration in exhaled gas after exposure to hyperbaric hyperoxia. *Diving and Hyperbaric Medicine*. 2009;39(2):77-80.)
Exposure to hyperoxia is associated with oxidative stress and is known to cause inflammation in the lung and the airways. Exhaled nitric oxide concentration (FE_{NO}) is a marker of some inflammatory processes in the lung and airways, and is reduced immediately after a single session of hyperbaric oxygen (HBO) exposure. The purpose of this study was to characterize the time course of this decrease in FE_{NO}. Ten patients who had HBO treatment were included. The daily HBO exposures were at a pressure of 240 kPa for 90 min. FE_{NO} was measured before a single HBO treatment session and immediately after and 30, 60, 120, and 240 minutes after. Thirteen healthy controls had FE_{NO} measured at the same time intervals as the patients without hyperoxic or hyperbaric exposure. FE_{NO} was sigificantly reduced by 30.0 (SD 22.3) % (P = 0.009) immediately after HBO treatment. It remained reduced by 27.3 (SD 19.6) % (P = 0.013) at 120 min, and had not recovered completely by 240 min. There were no changes in FE_{NO} in the control group. The results confirm the finding of a decrease in FE_{NO} immediately after exposure to hyperbaric hyperoxia. The reduction in FE_{NO} persists for up to 240 min.

Introduction

Exposure to hyperbaric oxygen (HBO) is associated with an increased production of reactive oxygen species (ROS) and may induce an acute toxic effect on the lung.[1] The ROS reacts non-specifically with DNA, fatty acids and proteins causing intracellular and membrane dysfunction. There is a dose dependent reduction in vital capacity when the oxygen pressure is over 50 kPa.[2] Forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), maximal expiratory flow rates, and transfer factor for carbon monoxide (Tl_{CO}) are all reduced, and the exposure to hyperoxia contributes to the acute effects of a saturation dive on lung function.[3] Reductions in maximal expiratory flow rates and Tl_{CO} have also been demonstrated in patients having HBO therapy.[4–6] These studies describe a variable reduction in lung function, but they all conclude that the effects on lung function of a standard HBO treatment series of 20 – 30 HBO exposures are of minor clinical importance. However, residual effects of repeated treatment series may accumulate to a long-term effect, and more sensitive indicators of oxygen toxicity than the traditional lung function variables may be needed to detect such effects.

Nitric oxide (NO) concentration in exhaled gas (FE_{NO}) is a biochemical marker of some airway inflammation.[7] It is consistently increased with the eosinophilic airway inflammation of asthma, and may be used to monitor disease severity and response to treatment.[8] With other lung disease, including chronic obstructive lung disease and exacerbations thereof which are associated with neutrophilic inflammatory responses, the FE_{NO} may be unchanged or increased.[9] FE_{NO} is consistently decreased in current smokers.[10] Because of the non-invasive technique and simple procedure, FE_{NO} measurements are ideal for monitoring inflammatory responses that are associated with changes in the NO production in the lungs and airways.

A reduction in FE_{NO} immediately after a single session of HBO exposure in patients having HBO therapy has recently been demonstrated in two studies.[11,12] In the study of Puthucheary et al, baseline FE_{NO} was larger than in the control group.[11] In the study of Taraldsøy et al, baseline FE_{NO} was not different from the control group, and it remained unchanged during the course of a four-week HBO treatment series (20 HBO exposures), with all FE_{NO} measurements taken before HBO treatment.[12] The decrease in FE_{NO} immediately after a single HBO treatment session was the same and approximately 30% on both the first and ninteenth day of treatment, indicating complete recovery within 24 hrs.

The aim of this study was to characterize the time course of the reduction in FE_{NO} after a single exposure to hyperbaric hyperoxia. The time course has to be known before further studies of the mechanisms of these changes are initiated, and the time course itself may give some indication of which mechanisms are operative.

Methods

SUBJECTS

Ten non-smoking patients (seven women), aged 43–73 years, with normal lung function and receiving HBO treatment for radiation-induced tissue injury were studied. Participants

Table 1
Subject demographics; FVC – forced vital capacity; FEV_1 – forced expiratory volume in 1 sec

	HBO exposed (n = 10)		**Controls** (n = 13)	
Age (yrs, range)	57	(43–73)	48	(24–62)
Height (m, range)	1.69	(1.55–1.79)	1.73	(1.60–1.86)
FVC (L) (SD)	3.83	(0.88)	4.71	(0.91)
FVC (% predicted) (SD)	100.3	(15.2)	106.0	(10.2)
FEV_1 (L) (SD)	2.82	(0.74)	3.67	(0.74)
FEV_1 (% predicted) (SD)	90.7	(12.9)	100.5	(12.3)

were selected from consecutive patients referred for HBO treatment. Exclusion criteria were respiratory symptoms, known heart or lung disease, diabetes mellitus and atopy. The lungs and trachea had not been included in the radiation field. Thirteen healthy subjects, all never smokers, and mainly recruited from the hospital staff served as controls. There were eight men and five women, aged 24–62 years. Subjects' characteristics, including lung function by spirometry, are given in Table 1. The study was approved by the Regional Ethics Review Committee and all subjects gave written informed consent.

PROTOCOL

The HBO exposure was at a pressure of 240 kPa for 90 min, five days a week and scheduled for 2–6 weeks. All HBO treatments were given in a monoplace hyperbaric chamber, and oxygen exposure was in three cycles of 30 minutes interrupted by five-minute breaks breathing air.

All measurements took place between 0900 h and 1600 h on one of the HBO treatment days during the second week. FE_{NO} was measured within 30 minutes before HBO treatment and was repeated as soon as practically possible after treatment, usually within 10 min. Thereafter measurements of FE_{NO} were taken 30, 60, 120 and 240 min after treatment. The baseline measurement was at least one hour after breakfast and drinking water only was allowed until the measurement at 60 min after treatment was accomplished. A meal was allowed between the measurements 60 and 120 min after HBO exposure. Measurements of FE_{NO} were not done before the start on the first day of HBO treatment. The control group followed the same time interval and food and drink restrictions as the patients.

SPIROMETRY

Forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) were measured using a wedge spirometer (Vitalograph Ltd., Buchingham, England). Spirometric testing was performed in the sitting position with a nose clip. The highest result of at least three satisfactory tests was recorded for each subject.

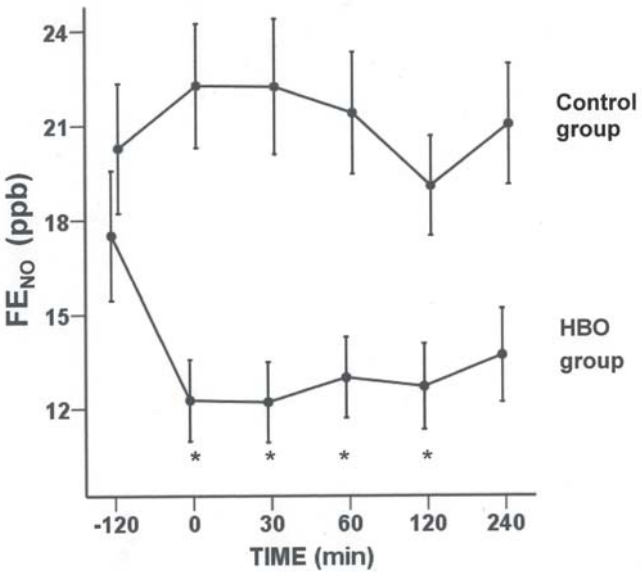
CONCENTRATION OF NITRIC OXIDE IN EXHALED GAS (FE_{NO})

Exhaled nitric oxide concentration (FE_{NO}) was measured by an online chemiluminiscence analyser (Niox, Aerocrine AB, Stockholm, Sweden). It was done with controlled expiration of 50 ml per second from total lung capacity after inhalation from functional residual capacity as recommended by the American Thoracic Society and European Respiratory Society.[13] The mean value of three tests not differing more than 10% or 2 ppb was used in the analyses.

STATISTICS

All data are given as mean (SD). Changes in FE_{NO} from baseline were tested by repeated measures ANOVA. A *P*-value < 0.05 was considered statistically significant.

Figure 1
Nitric oxide concentration (FE_{NO}), mean and SEM, in exhaled gas in the control group and HBO-exposed group; exposure took place between -120 and 0 min. * *P* < 0.05 compared with control group



Results

The results of the measurements of FE_{NO} are shown in Figure 1. FE_{NO} was significantly reduced by 30.0 (SD 22.3)% ($P = 0.009$) immediately after HBO treatment, and FE_{NO} was still significantly reduced 120 min after HBO treatment ($P = 0.013$). FE_{NO} was not statistically different from baseline after 240 min, the reduction being 21.8 (SD 15.0)% ($P = 0.083$). There were no changes in FE_{NO} in the control group.

Discussion

This study demonstrated that the reduction in FE_{NO} persists for up to four hours after exposure to hyperbaric hyperoxia. A free radical chain reaction involving NO and O_2 can propagate and scavenge NO in the gas phase of the bronchi and alveoli and within the tissue at the end of HBO therapy. In the gas phase, this process is likely to be reversed after complete washout of the gas in the lung, which takes less than ten minutes in subjects with normal lung function.[14] However, in the airway and alveolar walls more complex chemical reactions scavenging NO may still propagate.

Inducible nitric oxide synthase (iNOS) is the major airway epithelial isoform of the nitric oxide synthases.[15] iNOS activity is regulated by oxygen concentration in normobaric hyperoxic, normoxic and hypoxic conditions,[16] but there are no studies of iNOS regulation in hyperbaric hyperoxic conditions. iNOS is generally not expressed, but it is activated by inflammatory cytokines as for example peroxynitrite.[17,18] It could be expected that NO levels would rise after exposure to toxic HBO concentrations. However, the findings of this study and the study by Puthucheary et al showed the opposite. FE_{NO} was decreased after hyperbaric hyperoxia.[11] Inhibition of iNOS could be a mechanism of the reduction of FE_{NO} seen with HBO therapy.[19,20] The patients studied by Puthucheary et al had a higher baseline FE_{NO} compared with the controls.[11] This could support an upregulation of iNOS. Nevertheless, FE_{NO} was decreased after hyperoxic exposure. Taraldsøy et al did not demonstrate higher baseline levels of FE_{NO} in the HBO treated subjects.[12] Cucchiaro et al observed an upregulation of iNOS expression in rat lungs after exposure to 85% oxygen without increase in FE_{NO}.[20] This could be explained by a concomitant inhibition of iNOS activity, but biochemical mechanisms for this are not known. Another explanation could be that iNOS is induced, but the produced NO is not measurable in exhaled gas because it is scavenged in the interstitium.

Studies of rabbits have shown that NO detected in expired gas is derived primarily from the lung epithelial cells, and not by diffusion from the interstitium and blood circulation.[21] If so, it can be assumed that the reduction in FE_{NO} after HBO therapy is due to processes involving the production or scavenging of NO in the airway epithelium.

NO is involved in the regulation of vasomotor tone, and hyperoxia induces vasoconstriction. Studies of NO in coronary arteries in pigs provide evidence that hyperoxia reduces the basal release of NO leading to endothelium-dependent vasoconstriction.[22] It is not known how the bronchial circulation responds to changes in NO.

Patients having HBO treatment for some underlying disorder do not constitute an ideal group for studying these effects. The patients in Putucheary's and our studies could have ongoing inflammatory processes influencing the NO turnover, and patients having had radiation for head-and-neck cancers could have processes in the mouth with a bacterial flora influencing the FE_{NO}. Even though the patients as well as the controls had normal dynamic lung volumes, the phenomenon ought to be confirmed in studies of healthy subjects.

Conclusion

FE_{NO} is reduced for at least four hours after a single hyperbaric hyperoxic exposure to 240 kPa. The time course of the changes indicates more complex mechanisms than simple scavenging of NO by O_2 in alveolar and bronchial gas.

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Ida Kjelkenes is a student at the Medical School, University of Bergen, holding a research grant at the Institute of Medicine, University of Bergen.
Einar Thorsen, MD, PhD, is Professor of Hyperbaric Medicine, Institute of Medicine, University of Bergen and Consultant in Hyperbaric Medicine at the Department of Hyperbaric Medicine, Haukeland University Hospital, Bergen, Norway.

Address for correspondence:
Einar Thorsen, Institute of Medicine, University of Bergen, Haukeland University Hospital, N-5021 Bergen, Norway
Phone: *+47-(0)55-973973*
Fax: *+47-(0)55-975137*
E-mail: *<einar.thorsen@helse-bergen.no>*

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