Short communications

Variations in exhaled nitric oxide concentration after three types of dive

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Key words
Diving, hyperbaric, hyperoxia, expired nitric oxide, F_ENO

Abstract
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Introduction: An increase in exhaled nitric oxide concentration (F_ENO) occurs during an exacerbation of chronic obstructive lung disease or other inflammatory processes of the airway. Raised F_ENO levels are also observed during normobaric, mild hyperoxic exposures, whereas after hyperbaric hyperoxic exposure the F_ENO level is reduced. This study investigated the variations of F_ENO after three different types of dive.
Methods: Military divers participated in either a closed circuit rebreather dive (CCR, n = 17, pO_2 = 130 kPa), semi-closed circuit rebreather dive (S-CCR, n = 12, pO_2 = 180 kPa) or a compressed air dive (scuba, n = 17, pO_2 = 126 or attendant, n = 12, pO_2 = 118 kPa). Before and after each dive, the F_ENO was measured using a hand-held electrochemical analyser (Niox Mino®).
Results: All values for F_ENO fell within the normal range (5–25 ppb). A small decrease in F_ENO level was found after all dives. After CCR dives, F_ENO fell from 16.4 (± 8.0) pre-dive to 13.6 (± 7.5) ppb, after S-CCR from 16.2 (± 7.2) to 13.6 (± 6.3) ppb, scuba from 17.1 (± 5.6) to 16.1 (± 5.2) ppb and attendants from 17.7 (± 9.8) to 17.3 (± 9.1) ppb. Only after a CCR or S-CCR dive was this decrease statistically significant ($P < 0.05$).
Conclusion: In our divers, hyperbaric hyperoxia up to 180 kPa led to a small decrease in F_ENO in the conductive compartment of the lungs, the biological importance of which is unknown.

Introduction

Nitric oxide (NO) is a small molecule with the qualities of a free radical. In the human body, NO is made under the influence of NO-synthase (NOS) from the oxidation of L-arginine by NOS to L-citrulline with the release of NO.[1] Three forms of NOS are described: neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS); nNOS and eNOS are often termed constitutional NOS (cNOS).[1,2] While cNOS is constantly available and produces low quantities of NO, iNOS will produce NO in large quantities under more extreme circumstances, such as an inflammatory process.[2] In the lung, cNOS is found in the epithelium, endothelium and neurons, while iNOS is found in the epithelium and macrophages. Despite its radical qualities, the half-life of NO in air can be tens of seconds, which allows us to measure NO in the exhaled breath.[1]

NO, in reaction with oxygen, can be metabolized to nitrite (NO_2^-), nitrate (NO_3^-), and peroxynitrite ($ONOO^-$). Peroxynitrite has cell- and tissue-damaging activity which causes inflammation. This kind of inflammation plays an important role in the exacerbation of asthma or chronic obstructive pulmonary disease (COPD).[1] In 1899, Lorrain-Smith demonstrated that breathing oxygen at a partial pressure (pO_2) higher than 50 kPa can cause pulmonary damage, leading to pulmonary oedema and airway inflammation.[3] In view of this inflammation, one would expect an increase in the exhaled nitric oxide concentration (F_ENO) after exposure to a breathing gas with a pO_2 of more than 50 kPa. However, earlier studies showed inconsistent results. Raised F_ENO levels were observed during normobaric, mild hyperoxic exposures,[4,5] whereas after hyperbaric hyperoxic exposure the F_ENO level was reduced.[2,6] The aim of this study was to investigate the effect on F_ENO of three different types of dive, each using a different breathing gas with a pO_2 of more than 100 kPa.

Methods

STUDY POPULATION

All participating divers and attendants (n = 58) were professional military divers and fit to dive. All dives used to measure F_ENO were made as part of their daily routine or as part of their training. Each diver or attendant participated in only one type of dive. All participants were male and were informed about the aims of the study during a general meeting. It was explained that they could withdraw from the study at any time and the F_ENO results would not be put in their personal medical file. Before F_ENO measurement, they were asked again if they had any questions or objections regarding the study. Table 1 presents demographic data on the divers and attendants.

Table 1
Demographic data, mean (SD) for the participating divers and attendants (*n* = 58)

	CCR divers (*n* = 17)	S-CCR divers (*n* = 12)	Scuba divers (*n* = 17)	Attendants (*n* = 12)
Height (cm)	181.4 (6.2)	184.8 (5.4)	183.8 (5.9)	181.5 (7.9)
Weight (kg)	85.6 (8.7)	91.7 (10.8)	89.5 (11.6)	88.3 (13.5)
Age (years)	24.5 (2.6)*	34.3 (8.5)*	40.2 (6.5)†	43.3 (4.8)†
Smoking (pack-years)	0	0	0.9 (2.6)	0

* − $P < 0.05$ between all groups; † − $P < 0.05$ between all groups except between scuba divers and attendants

THE DIVES

Closed circuit rebreather (CCR) dive: this was a wet dive where the divers (*n* = 17) used a CCR (LAR VII, Draeger®) and breathed 100% oxygen. Maximal pressure at depth was 130 kPa (3 msw, pO_2 approaching 130 kPa) and the dive time was 60 min.

Semi-closed circuit rebreather (S-CCR) dive: this was a wet chamber dive where the divers (*n* = 12) used a S-CCR (SIVA 55, Carleton®) and breathed 60% oxygen and 40% nitrogen. Maximal pressure at depth was 300 kPa (20 msw, pO_2 approaching 180 kPa) and bottom time was 47 min. Decompression was done according to the Canadian diving tables (DCIEM Table 1: 21 msw/50 min). Total dive time was 58 min.

Scuba dive: this was a partial wet, partial dry dive where the divers (*n* = 17) used scuba (Mk 25/S550, Scubapro®) while breathing compressed air. Maximal pressure at depth was 600 kPa (50 msw, pO_2 approaching 126 kPa) with a bottom time of 14 min. Diving was done in the wet compartment of our pressure chamber; for both S-CCR and scuba dives, the water temperature in the wet chamber was 13–15°C. Decompression according to an adapted DCIEM Table 1 (51 msw/25 min) was done in the dry compartment where the divers breathed chamber air. The total dive time was 71 min. During this study, we also measured F_ENO of the attendants (*n* = 12) who stayed inside the dry compartment during this whole dive. Their maximal pressure at depth was 560 kPa (46 msw, pO_2 approaching 118 kPa).

MEASUREMENT OF F_ENO

Before and directly after every dive, the F_ENO was measured using an electrochemical hand-held NO analyser (Niox Mino®, Aerocrine AB, Sweden). Compared to on-line NO analysers, the measured F_ENO values using the Niox Mino® are statistically the same.[7] All measurements were done according to the ATS/ERS guidelines with an expiratory flow rate of 50 ± 5 $ml \cdot s^{-1}$.[8] The divers and attendants were not allowed to drink coffee, eat or smoke within 1 h before any measurement.

STATISTICAL ANALYSES

The results are presented as mean and standard deviation (SD). Regarding the F_ENO data, the Shapiro-Wilk (S-W) test (STATA Manual Reference G-M; 2003. p. 231) showed a non-normal distribution for one of the groups (chamber attendants). Therefore, we log transformed the F_ENO data after which the S-W test showed a normal distribution for all groups. Differences between the log transformed pre- and post-dive F_ENO values were analysed using the paired Student's t-test. A *P*-value < 0.05 was considered statistically significant. Analyses were performed using Stata SE software (StataCorp, version 9.2).

RESULTS

All F_ENO measurements fell within the normal range (5–25 ppb).We found no differences between the three dive groups regarding height, weight and smoking history (i.e., pack-years). There was a significant difference in age between the groups, except between scuba divers and attendants. The CCR divers were the youngest divers and the attendants the oldest (Table 1).

All dives produced a small decrease in F_ENO, with the greatest decrease after a CCR dive (-2.8 ppb) and the smallest

Table 2
F_ENO (ppb) pre- and post-diving, mean (SD) shown;
CCR – closed circuit rebreather; S-CCR – semi-closed circuit rebreather; * *P* < 0.01

	CCR divers (*n* = 17)		S-CCR divers (*n* = 12)		Scuba divers (*n* = 17)		Attendants (*n* = 12)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
F_ENO	16.4 (8.0)	13.6(7.5)*	16.2 (7.2)	13.6(6.3)*	17.1 (5.6)	16.1 (5.2)	17.7 (9.8)	17.3 (9.1)

in the attendants (-0.4 ppb). Only after a CCR or S-CCR dive did this decrease in F_ENO become statistically significant at the $P < 0.05$ level (Table 2).

Discussion

The results of the CCR and S-CCR groups, with a small decrease in F_ENO, are in line with results from earlier studies.[2,6] As the CCR and S-CCR divers were the only ones who performed a totally wet dive, submersion could play a role in this reduction. During submersion there is a central pooling of blood within the thoracic cavity. This thoracic pooling results in an increased pulmonary blood flow which leads to an increase in pulmonary artery pressure.[9] It is known that pulmonary arterial hypertension results in decreased F_ENO, so it is conceivable that increased pulmonary artery pressure could cause a drop in F_ENO.[10] Secondly thoracic pooling leads to an improved ventilation-perfusion relationship.[9] This improvement could result in a higher level of NO diffusion and, therefore, to a lower net F_ENO. Eventually, both mechanisms could cause a more pronounced decrease in F_ENO in a wet dive compared to a dry hyperbaric chamber dive.

However, compared to earlier studies, in which only dry chamber dives were performed, our findings showed a smaller decrease in F_ENO.[2,6] Therefore, submersion alone cannot fully explain the decrease in F_ENO we found. More plausible explanations for this decrease were given by Puthucheary et al who stated that hyperbaric oxygen inhibits iNOS which leads to a decrease in F_ENO.[2] Also, the presence of reactive oxygen species could scavenge NO, resulting in decreased F_ENO.[2]

As F_ENO is a biological marker, normal deviations play a role and must be taken into account. A difference of up to 2 ppb between two measurements of F_ENO can be found, so a difference of more than 4 ppb is considered to be of biological significance.[11] Regarding the Niox Mino®, which has an accuracy of +/- 2.5 ppb, a difference of more than 5 ppb is regarded as biologically significant.[7] In view of this, we conclude that, although we found a statistically significant reduction of F_ENO in the CCR and S-CCR groups, these minor changes are not bio-medically relevant.

Finally a limitation of the present study should be mentioned. We measured the F_ENO with an expiratory flow rate of 50 ± 5 $ml \cdot sec^{-1}$, according to the ATS/ERS guidelines.[8] At these flow rates, one measures the F_ENO from the bronchus down to the alveolus, but not the alveolus itself.[12] To measure the alveolar compartment F_ENO, the subject should exhale in a controlled fashion over 8–10 sec at a flow rate of at least 250 $ml \cdot sec^{-1}$, and sidestream sampling (of alveolar gases) occurs during the final part of exhalation.[10,13] As we used a flow rate of 50 $ml \cdot sec^{-1}$, we measured changes in the conductive compartment of the lungs only and not in the alveolar compartment (see Appendix). Earlier studies used flow rates of up to 100 $ml \cdot sec^{-1}$, implying that they also only measured changes of F_ENO in the conductive compartment.[2,4–6] To differentiate between the alveolar and conductive compartments, the multiple exhalation flow technique (MEFT) should be used.[14] Since hyperbaric hyperoxia produces alveolar damage, one should use expiratory flow rates of at least 250 $ml \cdot sec^{-1}$, and we strongly recommend that future studies use the MEFT to differentiate between F_ENO changes in these two compartments after exposure to an increased level of pO_2.

Appendix

The F_ENO pathway is often visualized as a two-compartment model with a conductive (airway to generation 17) and alveolar compartment (generation 18 to alveolus). F_ENO is a net result of flux and diffusion of NO in these compartments.[13] Based on this idea it is possible to calculate the F_ENO using the formula of George et al.[13]

$$F_ENO = C_{aw,no} + (C_{alv,no} - C_{aw,no}) * \exp(-D_{aw,no}/V)$$

where $C_{aw,no}$ is the airway wall concentration of NO, $C_{alv,no}$ the steady-state alveolar concentration of NO, $D_{aw,no}$ diffusing capacity of NO, and V is the exhalation flow rate.

- Healthy persons have a F_ENO between 5 and 25 ppb.[7,11,12]
- Values above 50 ppb can be found in exacerbation of asthma and chronic obstructive pulmonary disease or an acute eosinophilic airway inflammation.[12]
- Values below 5 ppb can be found in smokers or after strenuous efforts.[8]

F_ENO is not influenced by age, day-to-day or within-day variations but is reduced in females.[8,11]

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