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Exhaled nitric oxide concentration and decompression-induced bubble formation: An index of decompression severity in humans?

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ABSTRACT

Introduction: Previous studies have highlighted a decreased exhaled nitric oxide concentration (F_E NO) in divers after hyperbaric exposure in a dry chamber or following a wet dive. The underlying mechanisms of this decrease remain however unknown. The aim of this study was to quantify the separate effects of submersion, hyperbaric hyperoxia exposure and decompression-induced bubble formation on F_E NO after a wet dive.
Methods: Healthy experienced divers ($n = 31$) were assigned to either (i) a group making a scuba-air dive (Air dive), (ii) a group with a shallow oxygen dive protocol (Oxygen dive) or (iii) a group making a deep dive breathing a trimix gas mixture (deep-dive). Bubble signals were graded with the KISS score. Before and after each dive F_E NO values were measured using a hand-held electrochemical analyzer.
Results: There was no change in post-dive values of F_E NO values (expressed in ppb = parts per billion) in the Air dive group (15.1 ± 3.6 ppb vs. 14.3 ± 4.7 ppb, $n = 9$, $p = 0.32$). There was a significant decrease in post-dive values of F_E NO in the Oxygen dive group (15.6 ± 6 ppb vs. 11.7 ± 4.7 ppb, $n = 9$, $p = 0.009$). There was an even more pronounced decrease in the deep dive group (16.4 ± 6.6 ppb vs. 9.4 ± 3.5 ppb, $n = 13$, $p < 0.001$) and a significant correlation between KISS bubble score >0 ($n = 13$) and percentage decrease in post-dive F_E NO values ($r = -0.53$, $p = 0.03$).
Discussion: Submersion and hyperbaric hyperoxia exposure cannot account entirely for these results suggesting the possibility that, in combination, one effect magnifies the other. A main finding of the present study is a significant relationship between reduction in exhaled NO concentration and dive-induced bubble formation. We postulate that exhaled NO concentration could be a useful index of decompression severity in healthy human divers.

1. Introduction

In the human body, nitric oxide (NO) is produced from the oxidation of L-arginine to L-citrulline and released upon NO-synthase (NOS) activation. Three forms of NOS are classically described: neuronal NOS (nNOS), endothelial NOS [1] and inducible NOS (iNOS). In the lung eNOS and nNOS, present in the endothelium and neurons, are constantly available and produce low quantities of NO. Whereas iNOS, found in the epithelium and macrophages, produces NO in a large quantities under pathological conditions, such as an inflammatory processes [2]. NO, endogenously produced by lung tissue, has various physiological effects on both the ventilation and lung perfusion [3].

Attempts to measure NO production in biological environments are often fraught with difficulty due to both the low quantity and high metabolic rates of NO. In addition, detection of NO in biological fluids is complicated by the relative chemical instability of this radical molecule [4]. Fortunately, the lungs provide a unique opportunity to directly measure NO as a gaseous molecule in exhaled air from the airways and alveoli. When a tissue layer containing NO is brought into contact with air, NO molecules transform from soluble phase into gas phase and move across the tissue–air interface [5]. NO can be measured in exhaled air by a non-invasive approach as a direct and quantitative marker of NO production [6]. The exhaled nitric oxide (F_E NO) concentration reflects a dynamic balance between NO production rate and the

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distribution of this molecule within the conductive airway and alveolar compartment. F_E NO concentrations are not influenced by age, day-to-day or within-day variations but are lower in females [7]. Healthy humans have a F_E NO between 5 and 25 ppb (parts per billion) [8]. Values above 50 ppb can be found in exacerbation of asthma and chronic obstructive pulmonary disease or an acute eosinophilic airway inflammation. Values below 5 ppb can be found in smokers or after a strenuous exercise [9].

Studies have shown that even safe decompression can induce acute vascular/endothelial dysfunction after a single dive in a dry chamber [10] and/or after a wet dive with various breathing mixtures including air [11,12], nitrox [13] and Trimix [14]. A previous study aimed to determine if F_E NO concentration might provide a reliable and sensitive non-invasive biological marker of decompression stress in humans. Experienced divers performed varying dive protocols hypothesized to generate varying levels of decompression-induced bubble formation. The results showed a significant decrease in post-dive F_E NO values for the dive protocol with higher bubble grades and a positive linear relationship between the percent change in post-dive F_E NO values and bubble score. There was no difference in this linear relationship between dive protocols, per se. The authors concluded that expired NO concentrations do not provide a useful measure of decompression stress when dives are performed within decompression table limits [15].

The relationship between post-dive bubble scores and decompression severity itself is controversial. In 1979 Gardette examined 27 cases of decompression sickness (DCS) among 232 simulated dives by experienced commercial divers and found 30% had post-dive bubble scores at rest [16]. In 1985 Bayne found no relation between bubble scores and clinical DCS comparing 8 cases among 83 simulated chamber dives [17]. In his landmark thesis Sawatsky identified that a bubble grade of 0, 1 or 2 was associated with a very low risk of DCS, a bubble grade of 3 was associated with an approximate risk of DCS of 5% and a bubble grade of 4, the maximum, a risk of around 10% [18].

Hypothesizing that the diving activities performed in field conditions induce many environmental and physiological related responses, the aim of the present study was to investigate the respective contributions of in-water submersion, hyperbaric hyperoxia and decompression-induced bubble formation on exhaled nitric oxide (F_E NO) concentration in healthy experienced divers.

2. Methods

2.1. Study population sample

Thirty-one healthy male volunteers were recruited (age 28.2 ± 7.9 year with a body mass index ranging from 22 to 26 kg m^{-2}). All subjects were experienced military divers (Mine Clearance Divers and Combat swimmers) from the French Navy and certified physically fit to dive. None had previously experienced decompression sickness. In the present study each diver performed only one type of dive protocol. Subjects had not taken any drug affecting pulmonary function or exhaled NO concentration [19] within 14 days prior to the test. They were not exposed to pressure or to immersion for three days before the experiment. The protocol was approved by the Committee on the Ethics of Research in Human Experimentation of Marseille and was performed in accordance with the Helsinki Declaration of 1975, revised in 2000, for the conduct of human research.

2.2. Study design

The subjects were each assigned to one of three groups. In the first group ($n = 9$) they performed an open-sea scuba air dive (Air dive group). In the second group ($n = 9$) each subject performed an open-sea dive breathing oxygen and using a closed circuit rebreather (Oxygen dive group). The third group ($n = 13$) performed a deep-dive breathing a trimix gas mixture and using a semi-closed circuit rebreather (Trimix dive group). Both types of rebreather met or were well within limits for work-of-breathing defined by European Normative EN14143:2003 for rebreathers, especially at the moderate respiratory minute volumes in this study. The SCUBA sets used by the Air dive group complied with EN250:2000 Respiratory equipment – Open Circuit Self Contained Compressed Air Diving Apparatus.

2.3. Dive protocol

In the Air dive group each subject performed an open-sea scuba dive to 250 kPa (at depth 15 msw) for a total of 40 min including a linear decompression rate of 15 msw min^{-1} and no stop decompression according to the French Navy MN90 schedule. Descent was instructed to be without delay but not with discomfort and all divers were at the target depth within 1 min. The total time of in-water immersion was 40 min. In the Oxygen dive group each subject performed an Oxygen dive breathing 100% oxygen and using a closed circuit rebreather (FROGS®, Aqualung, Carros, France). Maximal pressure at depth was 170 kPa (7 msw) and total dive time was 40 min with no decompression stop. In the third group, each subject performed a deep dive using a semi-closed circuit rebreather (CRABE®, Aqualung, Carros, France). In the Trimix dive group each subject breathed a trimix gas mixture with 18% oxygen, 41% helium and 41% nitrogen. Maximal pressure at depth was 900 kPa (80 msw) and the bottom time was 10 min. The partial pressure of oxygen at depth was 1.6 ATA. According to the French Navy protocol, the decompression rate was 15 msw min^{-1} with a 3-min duration decompression stop at 12-msw depth, 3-min at 9-msw depth, 3-min at 6-msw depth and 12-min at 3-msw depth. During decompression at 6 and 3 msw each subject breathed 100% oxygen. The total time of hyperbaric exposure and immersion was 40 min.

For each protocol the total time of water immersion was 40 min. Each dive was performed in sea-water and field conditions with a sea-temperature of 17–19 °C. During the bottom phase in all protocols divers performed a moderate fining exercise at a regular frequency. All were provided with the same diving material and thermal protection equipment. They were dressed in a 5-mm neoprene wet suit with hood, boots, gloves and a thin neoprene top plus masks and fins.

2.4. Measurement of exhaled nitric oxide

F_E NO was measured with an electrochemical hand-held NO analyzer (Niox Mino, Aerocrine AB, Solna, Sweden). All measurements were performed 30 min before and after each dive. They were recorded during a controlled expiration of 50 ± 5 ml s^{-1} from total lung capacity as recommended by the American Thoracic Society and according to the ATS/ERS guidelines [20,21]. F_E NO concentration values measured using the Niox Mino are statistically the same as on-line NO analyzers [8]. The divers were not allowed to drink coffee, eat or smoke within 6 h before any measurement. The mean value of three tests not differing more than 10% or 2 ppb was used in the analysis.

2.5. Bubbles analysis

In each dive protocol the bubble measurement lasted 3 min during which time the subjects were at rest. Circulating bubble detection was performed on the precordial area by an experienced operator using a pulsed Doppler equipped with a 2 MHz probe