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ORIGINAL ARTICLE

Is a 12-h Nitrox dive hazardous for pulmonary function?

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Abstract

Purpose Prolonged exposure to a high partial pressure of oxygen leads to inflammation of pulmonary tissue [pulmonary oxygen toxicity (POT)], which is associated with tracheobronchial irritation, retrosternal pain and coughing, and decreases in vital capacity (V_C). The nitric oxide (NO) concentration in exhaled gas (FeNO) has been used as an indicator of POT, but the effect of SCUBA diving on FeNO has rarely been studied. The study presented here aimed to assess alterations to pulmonary function and FeNO following a 12-h dive using breathing apparatus with a relatively high partial pressure of oxygen.

Methods Six healthy, male, non-smoking military SCUBA divers were recruited (age 31.8 ± 2.7 years, height 179 ± 0.09 cm, and body weight 84.6 ± 14 kg). Each diver completed a 12-h dive using a demand-controlled semi-closed-circuit rebreather. During the 12 h of immersion, divers were subjected to 672 oxygen toxicity units (OTU).

A complete pulmonary function test (PFT) was completed the day before and immediately after immersion. FeNO was measured using a Nobreath™ Quark (COSMED™, Rome, Italy), three times for each diver. The first datapoint was collected before the dive to establish the "basal state", a second was collected immediately after divers emerged from the water, and the final measurement was taken 24 h after the dive.

Result Despite prolonged inhalation of a hyperoxic hyperbaric gas mixture, no clinical pulmonary symptoms were observed, and no major changes in pulmonary function were detected. However, a major decrease in FeNO values was observed immediately after emersion [0–12 ppb (median, 3.8 ppb)], with a return to baseline [2–60 ppb (median, 26 ppb) 24 h later (3–73 ppb (median, 24.7 ppb)].

Conclusion These results suggest that if the OTU remain below the recommended limit values, but does alter FeNO, this type of dive does not persistently impair lung function.

Keywords Hyperoxia · Diving · Pulmonary oxygen toxicity · Fractional concentration of exhaled nitric oxide · FeNO

Abbreviations

ER*V*	Expiratory reserve volume
FEF 25–75%	Forced expiratory flow 25–75%
FeNO	Concentration in exhaled nitric oxide
FEV_1	Forced expiratory volume in 1 s
FV_C	Forced vital capacity
IR*V*	Inspiratory reserve volume
msw	Meter sea water (is a unit of pressure used in underwater diving)
NO	Nitric oxide
OTU	Oxygen toxicity units (OTU)
PEF	Peak expiratory flow
PFT	Pulmonary function test
POT	Pulmonary oxygen toxicity
ppb	Parts per billion
R*V*	Residual volume
SD	Standard deviation
TLC	Total lung capacity
TL_{CO}	Transfer factor for carbon monoxide
UPTD	Unit pulmonary toxic dose

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V_C Vital capacity
V_t Tidal volume

Introduction

For operational reasons, military divers must sometimes dive for more than 12 h consecutively. To minimize the risk of decompression sickness, prolonged dives are only done at shallow depth, i.e., less than 20 meter sea water (msw). However, because closed-circuit rebreathers are used, divers may nevertheless be exposed to a high partial pressure of oxygen (PO_2) for several hours.

Prolonged exposure to a high PO_2 leads to inflammation of the lung tissue, a phenomenon which has been called pulmonary oxygen toxicity (POT) (van Ooij et al. 2013). This is the toxic effect of oxygen on the lungs first described by Dr. James Lorrain Smith in 1899 (Smith 1899). Clinical symptoms may appear after just 2 h when PO_2 exceeds 0.5 ATA (Clark and Lambertsen 1971a, b). The severity of POT has been shown to be directly related to the total dose of oxygen inhaled. Early symptoms of POT in humans include tracheobronchial irritation with retrosternal pain and coughing (Klein 1990).

To determine the maximum acceptable daily dose of oxygen for a subject, the concept of Unit Pulmonary Toxic Dose (UPTD) has been proposed (Clark 1988). One UPTD corresponds to the effect produced by pure O_2, a PO_2 of 1 ATA, breathed for 1 min. In the diving community, the UPTD is more commonly referred to as the oxygen toxicity unit (OTU), which was first described by Hamilton et al. (1988) following observation of repetitive excursions, surfacing techniques, and oxygen procedures for habitat diving (REPEX). Hamilton used these data to write procedures for the National Oceanic and Atmospheric Administration, establishing oxygen exposure limits for REPEX.

Whatever it is called, oxygen toxicity is indexed on a decrease in pulmonary vital capacity (V_C). To minimize the risk of POT, the US Navy allows divers diving with oxygen or mixed gas to receive a maximum of 615 units during any single ordinary operational dive. The upper limit for a single exposure has been set at 1425 UPTD (Wright 1972; Arieli et al. 2002). These loads lead to a median decrease in V_C of 2% and 10%, respectively.

POT not only reduces V_C, but also forced expiratory volume in 1 s (FEV_1) and maximal expiratory flow rates (FEF 25%, FEF 50%, FEF 75%). All these values decrease after exposure to oxygen (Thorsen et al. 1993; Clark et al. 1995). An altered transfer factor for carbon monoxide (TL_{CO}) has also been described in patients undergoing HBO therapy (Thorsen et al. 1998).

In the field of respiratory medicine, exhaled breath is increasingly being subjected to molecular analyses. Thus, the fraction of exhaled nitric oxide (FeNO), which is easy to measure, rapid and convenient is used a biochemical marker of airway inflammation (Kharitonov and Barnes 2001). FeNO is currently used clinically to assess the severity of asthma, to detect exacerbation of COPD disease, and to determine a patient's response to corticosteroid therapy (Kharitonov and Barnes 2000). Since the technique to measure FeNO is non-invasive and simple to use, measurement of this parameter has been adopted as the preferred technique to monitor inflammatory responses associated with changes in the production of NO at the level of the airways (Maziak et al. 1998). Since an increase in FeNO is associated with inflammation of the airways, it has been used to assess the inflammation associated with POT. However, studies measuring FeNO after exposure to hyperbaric oxygen gave paradoxical results (Schmetterer et al. 1997; Lemaitre et al. 2002; Puthucheary et al. 2006; Taraldsoy et al. 2007; Kjelkenes and Thorsen 2009). Whereas in animal studies (male Sprague–Dawley rats), hyperoxia was clearly demonstrated to increase the FeNO (Cucchiaro et al. 1999), in humans contradictory results have been reported. Some authors reported an increase (Lemaitre et al. 2002), others a decrease (Taraldsoy et al. 2007; Kjelkenes and Thorsen 2009; Caspersen et al. 2013) and still others found no change (Schmetterer et al. 1997; Lemaitre et al. 2002) in FeNO values following hyperoxia. Further analysis of the phenomenon revealed that low-intensity hyperoxic normobaric air leads to an increase in FeNO values, whereas after hyperbaric hyperoxic exposure, FeNO is reduced (Puthucheary et al. 2006; Taraldsoy et al. 2007; Kjelkenes and Thorsen 2009).

Surprisingly, only one study so far measured FeNO after hyperbaric oxygen exposure during immersion (van Ooij et al. 2010). Although the authors reported a significant decrease in FeNO following immersion, it fell within the limits of biological variation. In addition, the diving conditions used, with relatively deep (46–51 msw), short-duration dives (41 and 71 min), are not relevant in an occupational context for military divers who may have to spend long periods (up to 12 h) underwater at shallow depth breathing Nitrox or oxygen (to increase autonomy, reduce the risk of decompression sickness and maintain operational discretion).

In sum, little is known about the negative effects of hyperoxia on lung tissue after a very long wet dive using a rebreather. To compensate for this lack of information, the study described here assessed modifications to pulmonary function and FeNO in a group of young healthy, well-trained divers following a 12-h dive at 7–20 msw using a breathing apparatus with a relatively high partial pressure of oxygen (121–170 kPa). Data were collected before, immediately after and 24 h after performing the dive.

Materials and methods

Subjects

Six male military SCUBA divers were recruited. The characteristics of the study population were as follows (mean ± SD): age 31.8 ± 2.7 years, height 179 ± 0.09 cm, and body weight 84.6 ± 14 kg. All volunteers were healthy non-smokers with no history of cardiovascular or pulmonary disease. The methods and potential risks related to the study were explained to participants in detail before beginning the experiments, and all subjects gave written informed consent for their participation. All experimental procedures were conducted in line with the Declaration of Helsinki, and the study protocol was approved by the local Ethics Committee (Comité de Protection des Personnes-CPP Sud Méditerranée V, ref 16.077).

Diving conditions

Each diver completed 12-h dives in water at 18 °C, wearing their own, well-fitted protective neoprene wetsuit. Divers used a demand-controlled semi-closed-circuit rebreather (Oxymixgers™ Aqualung ™, Carros, France). For 10 h, they remained static at 20 msw, breathing a Nitrox 50 gas mixture (50% oxygen, 50% nitrogen, $PO_2 = 121$ kPa). For the remaining 2 h, they performed fin exercises at 7 msw, breathing oxygen ($PO_2 = 170$ kPa). Divers were subjected to a total OTU of 672 during the 12 h of immersion (Fig. 1).

Data collection

Before and immediately after each immersion, divers were meticulously examined by a physician to detect pulmonary impairment. All data were collected by a physician trained in the use of the systems implemented, and the measurements thus showed good reproducibility.

Assessing pulmonary function

A complete pulmonary function test (PFT) was performed the day before and immediately after immersion using a Quark Pulmonary Function Testing™ system (COSMED™, Rome, Italy). All measurements were performed in accordance with guidelines, statements and technical standards published by the European Respiratory Society (ERS).

Spirometry included measurements of peak expiratory flow (PEF), forced vital capacity (FV_C), forced expiratory volume in 1 s (FEV_1), FEV_1/FV_C ratio, and forced expiratory flow 25–75% (FEF 25–75%.) To test lung volumes, tidal volume, inspiratory reserve volume, expiratory reserve volume (ERV), residual volume (RV), lung capacity (TLC), inspiratory capacity, functional residual capacity, and V_C were measured. Single-breath diffusing capacity for carbon monoxide (TL_{CO}) was also monitored.

FeNO measurement

Participants were asked to refrain from drinking coffee or eating for 1 h before the dive. FeNO was measured three times for each diver, using a Nobreath™ Quark system (COSMED™, Rome, Italy) (Fig. 2). The first measurement was performed before the dive to determine the "basal state", a second one was taken immediately after emerging from the water ("after

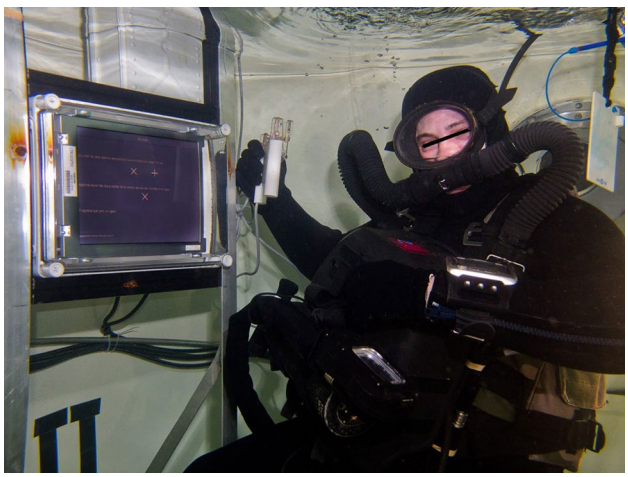

Fig. 1 A military diver during the 12-h immersion. Written informed consent was obtained from the individual for the publication of this image

Fig. 2 Assessing FeNO immediately after the dive. Written informed consent was obtained from the individual for the publication of this image

emersion"), and the final measurement was taken 4 days after the experimental dive. The measurement system is based on the principle of detection by an electrochemical sensor, and meets the standards set out for the measurement of nitric oxide at an expiratory flow rate of 50 ml s^{-1} in the recommendations published in 2005 by the American Thoracic Society and the European Respiratory Society. The system is compatible with non-invasive, simple and rapid measurements; results are immediately available. Particulate matter is also measured in parts per billion (ppb); this parameter provides information at the level of the bronchial conductive pathways rather than the alveolar level, which requires a specific apparatus and protocol.

Statistical analysis

The data are presented as mean and standard deviation (SD) or median and interquartile variance. Statistical analyses were performed using SIGMASTAT™ 3.0 software (SPSS Inc., Chicago, Illinois). Due to the small size of our series, non-parametric tests were selected. A Wilcoxon test was used to compare data related to pulmonary function. A one-way analysis of variance (ANOVA) with repeated-measures and the post hoc Holm–Sidak test was used to compare FeNO values. The threshold for significance was set to $p < 0.05$.

Results

No clinical symptoms of POT (coughing, dyspnea, lung pain) or other complications were observed in subjects following the experimental dives.

Before immersion, all PFT values were normal. After immersion, only FEV_1 was increased (+2%, $p = 0.031$) compared to baseline values (Table 1). It is important to note that neither FV_C nor TL_{CO} values were affected by the 12-h hyperoxic dive.

The baseline FeNO values ranged from 2 to 60 ppb (median, 26 ppb). Immediately after emersion, the values ranged from 0 to 12 ppb (median, 3.8 ppb). One day (24 h) after diving, values had returned to a range from 3 to 73 ppb (median, 24.7 ppb) (Table 2, Fig. 3).

These differences between FeNO values immediately after immersion and values recorded before and 24 h after immersion were statistically significant (repeated one-way ANOVA; $p = 0.0081$). No statistical differences were observed before and the day after immersion.

Discussion

Despite prolonged (12 h) inhalation of a hyperoxic hyperbaric gas mixture (OTU = 672), no clinical indication of pulmonary toxicity was observed in the divers studied here,

Table 1 Pulmonary function data recorded before and immediately after immersion for 12 h

	Before	After	Delta %	*p*
FV_C (L)	5.28 ± 0.3	5.35 ± 0.25	1.3	0.063
TLC (L)	7.09 ± 0.9	6.9 ± 0.6	− 2.7	0.438
RV/TLC (%)	21.32 ± 3.8	19.94 ± 7.95	− 6.5	0.438
PEF (L s^{-1})	9.21 ± 2.5	9.4 ± 2	2	0.688
FEV_1 (L)	4.3 ± 0.5	4.4 ± 0.4	2.3	0.031
FEV_1/FV_C (%)	79.3 ± 9	79.1 ± 7	− 0.3	1
FEF 25/75 (L s^{-1})	3.61 ± 0.8	3.96 ± 1.5	9.6	0.438
TL_{CO}/AV, (mmol min^{-1} kPa^{-1} L^{-1})	1.5 ± 0.1	1.51 ± 0.25	0.6	0.438

FV_C forced vital capacity, *TLC* total lung capacity, *RV* residual volume, *PEF* peak expiratory flow, *FEV_1* forced expiratory volume in 1 s, *FEF 25–75%* forced expiratory flow 25–75%, *TL_{CO}* single-breath carbon monoxide transfer factor, *AV* alveolar volume

[a]Statistical comparisons were based on a Wilcoxon test comparing data before and immediately after the dive

and no major changes in pulmonary function were detected

Table 2 Concentration in exhaled nitric oxide (FeNO) before, immediately after and 1 day after immersion

Subjects	Before	Immediately after		1 day after	
	a	b	c	d	
	FENO (ppb)	FENO (ppb)	b/a (%)	FENO (ppb)	d/a (%)
1	8	1	− 88%	6	− 25%
2	60	4	− 93%	73	22%
3	14	4	− 71%	22	57%
4	2	0	− 100%	3	50%
5	59	12	− 80%	32	− 46%
6	13	2	− 85%	12	− 8%
Median	26	3.8		24.7	
Interquartile variance	51	3		26	

FeNO is expressed as ppb. Variation of FeNO data after immersion is expressed as percentage compared to data observed before immersion

during the PFTs. In contrast, a large and significant decrease in FeNO values was observed immediately after emersion, with a return to the basal state 24 h after surfacing.

These results suggest that these diving conditions do not induce POT.

Cellular mechanisms of POT

The cellular mechanisms resulting in POT are thought to be due to the impairment of DNA and cell membranes as a

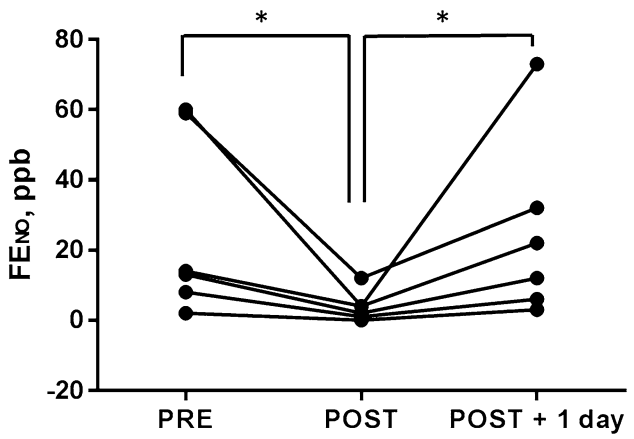

Fig. 3 FeNO data recorded before and immediately after the 12-h immersion and 24 h after the dive. Statistical comparisons were performed based on one-way analysis of variance (ANOVA) with repeated-measures and a post hoc Holm–Sidak test. $*p < 0.05$ significant difference between time-points. *FeNO* fractional concentration of exhaled nitric oxide

result of significant oxidative stress. This stress is thought to activate the biochemical cascades of the inflammatory reaction, with recruitment of neutrophils, and fibrinogen (Thom 2011). The extent of damage to pulmonary tissue is determined by the duration of exposure and can be split into two successive phases. First, a local reversible exudative inflammation of the pulmonary parenchyma occurs, with capillary and endothelial edema, leading to a decrease in type I alveolar cells, and an influx of inflammatory cells (Bryan and Jenkinson 1988; Allen et al. 2009) which cause tracheobronchial irritation and substernal pain (Bryan and Jenkinson 1988). These changes are reversible, and the lung returns to its normal state when the partial pressure of the inhaled oxygen drops below 0.5 ATA. However, prolonged exposure to high doses of oxygen can result in dose-dependent cellular alteration that may lead to pulmonary fibrosis. Thus, if exposure to oxygen continues at a high partial pressure, proliferative phase fibroblasts and type II alveolar cells infiltrate the inflamed endothelia and true pulmonary fibrosis ensues (Robinson et al. 1974; Montgomery et al. 1989). With continued oxygen inhalation, the air–blood membrane increases in thickness four- to fivefold, leading to loss of diffusion capacity (Kapanci et al. 1972; Robinson et al. 1974). The rate at which these changes occur is directly related to the PO_2 of the gas inhaled and can occur after just 3 h at a PO_2 of 3 ATA during a dry dive (Klein 1990).

POT and clinical symptoms

Clinical symptoms of POT include bronchial irritation, retrosternal pain and coughing (Klein 1990). The extent of these clinical symptoms is very variable depending on the subject, with significant individual variations in sensitivity to POT. However, in general, exposure to a PO_2 greater than 0.5 ATA is considered potentially damaging for the lungs. Exposure to even a relatively low PO_2 for a long period can damage the tracheal mucosa and result in impaired mucus clearance (Sackner et al. 1975).

Although symptoms of POT (coughing, chest pain when breathing, and dyspnea) precede changes in pulmonary function and are thus likely to be a more sensitive indicator of POT (Klein 1990; Shykoff 2005), the occurrence of these symptoms is so variable between individuals that it is considered a poor index of O_2 tolerance (Klein 1990). For this reason, lung function parameters are preferred as they provide a more objective monitoring index. Many lung function parameters have been studied to identify the most useful for identifying POT. Of all the parameters tested, the most frequently studied is the V_C, even though it is not the most affected by inhaled oxygen and can also be very variable from one individual to the next.

Since POT is directly related to the total dose of oxygen inhaled, a unit of measure has been developed to estimate the maximum amount of oxygen that can be inhaled without risk. Thus, in 1971, Clark and Lambertsen (1971a, b) developed the concept of a UPTD. Based on pulmonary tolerance curves, they produced a mathematical model where one UPTD is the degree of pulmonary toxicity induced by breathing 100% oxygen continuously at 101 kPa for 1 min. The total amount of UPTD for continuous oxygen exposure at constant pressure can then be calculated using the following equation:

$$\mathrm{UPTD} = t \cdot \sqrt[-1,2]{\frac{0.5}{PO_2 - 0.5}}.$$

Once the UPTD value has been calculated, it can be compared to values in a reference table proposed by the University of Pennsylvania (Wright 1972). This table relates the oxygen dose to the predicted percentage of decrease in V_C. Thus, the UPTD is indexed on the extent of the expected decrease in V_C. As indicated in "Introduction", the maximum UPTD recommended for ordinary professional diving, 615, represents a median decrease in V_C of 2%.

In the diving community, the UPTD is more commonly referred to as the OUT (Hamilton et al. 1988). Like the UPTD, one OTU is the degree of POT produced by breathing 100% O_2 continuously at a pressure of 1 atmosphere absolute (ATA) for 1 min. For these authors, if the OTU over 24 h does not exceed 850, no clinical symptoms of POT are expected to appear.

In our study, to minimize decompression sickness, dive depth was between 7 and 20 msw. At these depths, the partial pressure of oxygen was relatively low (121–171 kPa)

even when using a hyperoxic gas mixture. Consequently, the OTU calculated after 12 h immersion was around 672. This value is below the REPEX threshold (850 OTU), but higher than the threshold originally proposed by Lambertsen (615 OTU). Our results confirm that compliance with the OTU thresholds proposed by Hamilton avoids the occurrence of clinical symptoms of POT.

Lung volumes and airflow

According to Wright's publication (1972), the median decrease in V_C based on total units of pulmonary dose is expected to be about 2%. Our results showed a 1.3% increase for FV_C, and a 2.7% decrease for TLC after the 12-h dive. Based on these findings, we can reasonably affirm that the dive profile used by military divers in this study causes no changes to lung function detectable based on a PFT.

The pathophysiological cause of an oxygen-induced decrease in V_C has not yet been fully elucidated, but purely pathological changes at the alveolar-capillary level could be involved. Interstitial edema is another of the pathological features of POT that could lead to a decrease in V_C (Kapanci et al. 1972).

All previously described effects of hyperoxia (normo- or hyperbaric) on pulmonary function were observed in dry conditions, without immersion. Immersion also causes a decrease in V_C, mainly due to the drop in ERV (Prefaut et al. 1976). It could be, therefore, hypothesized that in SCUBA diving, the effects of immersion could play an additional role in the impact hyperoxia has on lung function.

Transfer factor for CO

According to Thorsen et al. (1993) "Diffusion capacity of the lung for carbon monoxide … seems to be a more sensitive indicator of oxygen toxicity than the classic vital capacity". As discussed above, the damage to pulmonary tissue associated with POT is mainly localized in the alveolocapillary membrane. Since the diffusing capacity is altered by any deviation at this anatomical level, it is obvious why measuring diffusing capacity could be useful in this context. This utility is supported by recent studies performed by van Ooij et al. (2011, 2014), presenting data suggesting that measuring V_C is not sufficient to assess and monitor POT in divers breathing oxygen, and that changes in diffusing capacity for either carbon monoxide (Dl_{co}) or nitrogen monoxide (Dl_{no}) may be more informative.

Although a drop in TL_{CO} has been described as part of POT (Thorsen et al. 1998), our results showed no variations, and normal values were maintained in divers following the 12-h dive. This result can probably explained, once again, by the cumulative doses of O_2 to which the divers were exposed (672 OTU). This exposure is not sufficient to induce symptoms of oxygen toxicity.

A temporary decrease in the lung's capacity to take up carbon monoxide (TL_{CO}) has been described after a wet dive (Dujic et al. 1993), with the maximum decrease recorded at 20-min post-immersion. This effect was significantly correlated with venous gas microbubbles detected by Doppler ultrasound. A control condition, where subjects breathed pure oxygen during decompression resulted in no microbubbles or significant changes to TL_{CO}, suggesting that venous gas microbubbles may account for the change in TL_{CO} observed after diving. The reduction in carbon monoxide diffusion capacity was significantly greater in subjects who had venous gas microbubbles compared to subjects without (Catron et al. 1986). In our study, no venous gas microbubbles were detected in any diver following the dive, and TL_{CO} values were unchanged compared to baseline values.

FeNO

FeNO measurement was fairly recently adopted as a routine clinical tool to measure pulmonary inflammation. Nitric oxide (NO) is an oxidation product of L-arginine and is synthesized throughout the respiratory tract by nitric oxide synthases (Paraskakis et al. 2006).

As FeNO is associated with inflammation of the lower airways, it is possible that inflammation linked to POT will also result in changes to exhaled nitrogen monoxide concentrations. Although studies measuring FeNO after hyperbaric oxygen exposure reported inconsistent results (Schmetterer et al. 1997; Lemaitre et al. 2002; Puthucheary et al. 2006; Taraldsoy et al. 2007; Kjelkenes and Thorsen 2009), only one so far measured FeNO after hyperbaric oxygen exposure during immersion (van Ooij et al. 2010). Although these authors reported a significant decrease in FeNO, the value itself fell within the limits of biological variation—which range from 15 parts per billion (ppb) to 50 ppb (Dweik, Boggs et al. 2011).

FeNO and dry dives

As FeNO is associated with inflammation of the lower airways, NO levels might be expected to increase after exposure to HBO, but as indicated in the introduction, in humans conflicting results have been reported (Lemaitre et al. 2002; Taraldsoy et al. 2007; Kjelkenes and Thorsen 2009; van Ooij et al. 2010; Caspersen et al. 2013). Some authors have attempted to explain these contradictory results, and two hypotheses have emerged. First, endogenous NO could be scavenged by proteins and free radicals (Cucchiaro et al. 1999; Caspersen et al. 2013). Thus, according to Kjelkenes and Thorsen (2009), the reduced FeNO observed following HBO therapy (Pedoto et al. 2003) could be caused

by inhibition of inducible nitric oxide synthase (iNOS). Indeed, iNOS activity is regulated by oxygen concentration in normobaric hyperoxic, normoxic and hypoxic conditions (Dweik et al. 1998), however, no studies have yet investigated iNOS regulation in hyperbaric, hyperoxic conditions. Alternatively, the recommended exhalation flow rate of 50 ml s^{-1} could be too low to accurately determine FeNO values in these conditions (van Ooij et al. 2013).

FeNO and wet dives

An increase in FeNO occurs upon exacerbation of chronic obstructive pulmonary disease and with other inflammatory processes of the airway and raised FeNO levels have also been reported during normobaric, mild hyperoxic exposures. In contrast, after hyperbaric hyperoxic exposure, the FeNO level was reduced. To the best of our knowledge, only one study investigated variations in FeNO after immersion dives (van Ooij et al. 2010). In this study, wet dives with hyperbaric hyperoxia up to 180 kPa resulted in a small, but significant, decrease in FeNO (from around from 17 ppb pre-dive to 13 ppb, after). According to the authors of this study, submersion could play a role in the reduction observed.

Immersion is associated with peripheral vascular translocation towards the central pulmonary circulation (Castagna et al. 2017). This pulmonary vascular congestion leads to an increase in pressure in the pulmonary artery (Pendergast et al. 2015). Previous work by Geigel et al. (Geigel et al. 1999) indicated that this increase in pressure was associated with a drop in FeNO. It is, therefore, conceivable that the increase in pressure in the pulmonary artery induced by the "blood shift" associated with immersion could cause a drop in FeNO. This hypothesis should be considered with caution as Berg's study shows that in blood-perfused lungs or live animals, FeNO cannot be taken as an accurate reflection of lung NO production, and changes in FeNO must be interpreted cautiously in the context of blood or hemoglobin-containing lung perfusates (Berg et al. 2000).

It has also been demonstrated that the immersion-induced blood shift leads to an improved ventilation–perfusion relationship (Pendergast and Lundgren 2009). Taking these data into account, Van Ooij et al. (2010) suggested that the decreased FeNO observed after wet dives could been explained by a higher level of NO diffusion in the blood due to this improved ventilation–perfusion relationship. Our data—a decrease in the values of FeNO just after a dive, with a return to normal values 24 h post-dive—support this hypothesis, and this mechanism could thus lead to a more pronounced decrease in FeNO following a wet dive compared to a simulated dive in a dry hyperbaric chamber.

The almost total disappearance of FeNO after our 12-h dive is not in line with a pulmonary inflammatory process induced by hyperoxic exposure in divers. As mentioned previously, the hyperoxic exposure level here, 672 OTU, does not seem sufficient to trigger pulmonary inflammation. Nevertheless, although some subjects showed no variation in FeNO values, others exhibited a considerable decrease, reflecting individual variability in FeNO values after wet dives. Consequently, submersion itself may be an unlikely source of protection against POT.

Conclusion

In our study, even after 12-h immersion breathing Nitrox, no clinical pulmonary impairment, or alteration of PFTs were observed, despite the massive decrease in some subjects FeNO. One of the limitations of this study is the study population. All divers were young, fit, athletic, non-smokers without pulmonary pathology. They were very well trained and frequently completed long-duration dives. These divers were, therefore, regularly exposed to long-lasting hyperoxia. It is possible that their bodies have developed antioxidant defense mechanisms to counteract POT. Moreover, during their training at the diving school, divers were selected to eliminate individuals susceptible to hyperoxia. As a result, none of the subjects involved in the study were expected to show any adverse effects of hyperoxia. Furthermore, it is important to remember that results can be highly variable between divers, especially with regard to clinical pulmonary symptoms, decrease in PFT values and FeNO values (Klein 1990; Shykoff 2005).

The results presented here show that if the OTU remains below the recommended limit values, long-duration dives while breathing an oxygen-enriched mixture appear not to impair lung function. Indeed, no clinical pulmonary symptoms were observed, and pulmonary function, as assessed by complete PFT, was normal. Our study is the very first to confirm the results presented by van Ooij et al. (2010), even after prolonged immersion dives. However, because of a the considerable inter-individual variability of the risk of developing POT, more sensitive and earlier lung-toxicity markers must be developed for use in routine clinical practice, such as measurement of exhaled volatile organic compounds.

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Author contributions OC, CB and JEB conceptualized and designed the study, performed the experiments, analyzed the data, interpreted the results of experiments, and prepared the figures. OC and JEB drafted, edited, and revised the manuscript. OC, CB and JEB approved the final version of the manuscript.

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Compliance with ethical standards

Conflict of interest The authors have no competing interests to disclose in relation to this study. The results of the present study do not constitute endorsement by the European JAP, and are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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