

# Effect of in-water oxygen prebreathing at different depths on decompression-induced bubble formation and platelet activation

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**Bosco G, Yang Z, Di Tano G, Camporesi EM, Faralli F, Savini F, Landolfi A, Doria C, Fanò G.** Effect of in-water oxygen prebreathing at different depths on decompression-induced bubble formation and platelet activation. *J Appl Physiol* 108: 1077–1083, 2010. First published February 25, 2010; doi:10.1152/jappphysiol.01058.2009.—Effect of in-water oxygen prebreathing at different depths on decompression-induced bubble formation and platelet activation in scuba divers was evaluated. Six volunteers participated in four diving protocols, with 2 wk of recovery between dives. On *dive 1*, before diving, all divers breathed normally for 20 min at the surface of the sea (Air). On *dive 2*, before diving, all divers breathed 100% oxygen for 20 min at the surface of the sea [normobaric oxygenation (NBO)]. On *dive 3*, before diving, all divers breathed 100% O<sub>2</sub> for 20 min at 6 m of seawater [msw; hyperbaric oxygenation (HBO) 1.6 atmospheres absolute (ATA)]. On *dive 4*, before diving, all divers breathed 100% O<sub>2</sub> for 20 min at 12 msw (HBO 2.2 ATA). Then they dove to 30 msw (4 ATA) for 20 min breathing air from scuba. After each dive, blood samples were collected as soon as the divers surfaced. Bubbles were measured at 20 and 50 min after decompression and converted to bubble count estimate (BCE) and numeric bubble grade (NBG). BCE and NBG were significantly lower in NBO than in Air [ $0.142 \pm 0.034$  vs.  $0.191 \pm 0.066$  ( $P < 0.05$ ) and  $1.61 \pm 0.25$  vs.  $1.89 \pm 0.31$  ( $P < 0.05$ ), respectively] at 20 min, but not at 50 min. HBO at 1.6 ATA and 2.2 ATA has a similar significant effect of reducing BCE and NBG. BCE was  $0.067 \pm 0.026$  and  $0.040 \pm 0.018$  at 20 min and  $0.030 \pm 0.022$  and  $0.020 \pm 0.020$  at 50 min. NBG was  $1.11 \pm 0.17$  and  $0.92 \pm 0.16$  at 20 min and  $0.83 \pm 0.18$  and  $0.75 \pm 0.16$  at 50 min. Prebreathing NBO and HBO significantly alleviated decompression-induced platelet activation. Activation of CD62p was  $3.0 \pm 0.4$ ,  $13.5 \pm 1.3$ ,  $10.7 \pm 0.9$ ,  $4.5 \pm 0.7$ , and  $7.6 \pm 0.8\%$  for baseline, Air, NBO, HBO at 1.6 ATA, and HBO at 2.2 ATA, respectively. The data show that prebreathing oxygen, more effective with HBO than NBO, decreases air bubbles and platelet activation and, therefore, may be beneficial in reducing the development of decompression sickness.

decompression sickness; bubble formation; platelet activation; oxygen prebreathing; open sea dive

IT HAS LONG BEEN KNOWN that a rapid decrease in hydrostatic pressure promotes bubble formation in blood and tissues, an essential factor contributing to the development of decompression sickness (DCS) (11, 43). Manifestation of DCS can range from minimal symptoms to neurological consequences, including cognitive impairment and motor-sensory dysfunction, to death. Postdive DCS can be effectively treated by hyperbaric oxygenation (HBO) (33); however, HBO service is not always

available immediately. It would be beneficial if protective measures could be applied to reduce or prevent the development of DCS. Decompression tables that account for the depth and length of the dive enhance the safety of decompression (43). Nevertheless, following the advice of decompression tables is no guarantee that DCS will be avoided, because the risk of developing DCS is not only determined by the depth and length of the dive, but also by other factors, including platelet activation (24, 37). It has been reported that microbubbles could induce platelet activation (3, 42). Platelet activation may be involved in prothrombotic and proinflammatory processes (25) and, consequently, may be involved in the development of DCS.

As suggested by an early study, micronuclei exist within living tissue under normal conditions (19). During decompression, these gas micronuclei may expand and grow into bubbles and cause DCS (4, 9). It is reasonable to think that reduction or elimination of these micronuclei may reduce decompression-induced bubble formation and, consequently, the risk of DCS. Theoretically, this may be achieved by breathing pure oxygen under normobaric or hyperbaric conditions, which leads to a higher oxygen content in the tissues. This increased oxygen content rapidly diffuses into the micronuclei in exchange for nitrogen. Nitrogen then rapidly diffuses out of the micronuclei and is eliminated from the body. The oxygen then is absorbed by the surrounding tissue to cause rapid decay of the micronuclei. These processes have been defined as denucleation and denitrogenation. This theory has been supported by studies that demonstrate significantly reduced decompression-induced bubble formation in HBO-pretreated animals, believed to be due to the elimination of bubble nuclei (1, 2, 13, 48). HBO has been observed to eliminate most of the gas nuclei in decompressed prawns, therefore reducing the number and size of bubbles during decompression (1). Our previous study showed that HBO prebreathing significantly reduced decompression-induced bubble formation and platelet activation in simulated dives in an HBO chamber (29). A recent study demonstrated that prebreathing normobaric oxygen (NBO) also decreased venous gas emboli formation, with a prolonged protective effect (10, 21). It has been reported that more air bubbles were detected in divers when the dives were performed in the open sea than in the hyperbaric chambers (20). Both dry and wet dives are associated with hyperoxia, increased density of breathing gas, and decompression stress, with possible formation of venous bubbles. However, open sea dives are also associated with immersion, the mechanical load of the breathing apparatus, a high level of physical activity, and exposure to

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cold environment. Immersion in cold seawater results in breathing colder and denser gas and may also, by inducing peripheral cutaneous vasoconstriction in conjunction with the immersion effect, potentiate greater central pooling of blood than in dry dives. Water immersion-induced changes in hemodynamic, neuroendocrine, and autonomic activities have been well reviewed by Pendergast and Lundren (36). The effect of prebreathing HBO on bubble formation and platelet activation in open sea divers has not been investigated. The present study was designed to compare the effect of prebreathing NBO or HBO on bubble formation and platelet activation in open sea divers.

## METHODS

**Study subjects.** Six healthy, well-trained, male recreational divers voluntarily participated in a four-dive protocol in the Tremiti Islands, Italy (Table 1). All experimental procedures were approved by the Human Ethics Committee of the University of Chieti and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from each volunteer subject. All divers declared “no” to questions about 1) consuming medications and 2) diving or flying 48 h prior to the study.

**Experimental protocol.** The experiment was conducted in open sea off the Tremiti Islands, with a water temperature of  $20 \pm 5^\circ\text{C}$ . The depth of the dive was set at 30 m seawater [msw; 4 atmospheres absolute (ATA)] with 20 min of bottom time. All divers breathed compressed air ( $\text{N}_2\text{O}_2$ ) from self-contained underwater breathing apparatus (scuba) gear during their bottom time.

The diving protocol is depicted in Fig. 1. The study protocol included four diving exposures, with 2 wk of recovery prior to each successive dive. On *dive 1*, before diving, all subjects were asked to breathe air for 20 min on the surface of the sea (Air). On *dive 2*, before diving, all divers were asked to breathe 100% oxygen for 20 min on the surface of the sea (NBO). On *dive 3*, before diving, all divers were asked to breathe 100% oxygen for 20 min while submerged 6 m below the surface of the sea (HBO 1.6 ATA). On *dive 4*, before diving, all divers were asked to breathe 100% oxygen for 20 min while submerged 12 m below the surface of the sea (HBO 2.2 ATA). Oxygen was provided via a prefilled tank. After the pretreatment, all divers dove to 30 m, where they stayed for 20 min breathing from scuba gear. The ascent rate was set at 10 m/min, with a decompression stop at 5 m for 3 min, according to the US Navy Manual Diving Table. All subjects were asked to perform the same mild workload at the bottom on an underwater bicycle (OKEO, GE-Italy) at a pedaling rate of 25 rpm to ensure no difference of ventilation and gas exchange in all dives, guided by the Borg category ratio 0–10 scale at an intensity level of 3 (6). On each dive, as soon as the divers surfaced, blood samples were collected. The bubbles were measured at 20 and 50 min after decompression at rest and after exercise (3 flexions of the legs).

**Bubble monitoring and analysis.** Doppler signals were obtained using a Hadeco SonoMate 300G 2-MHz probe and dual-ear headphones. Precordial Doppler signals were recorded on a digital appa-

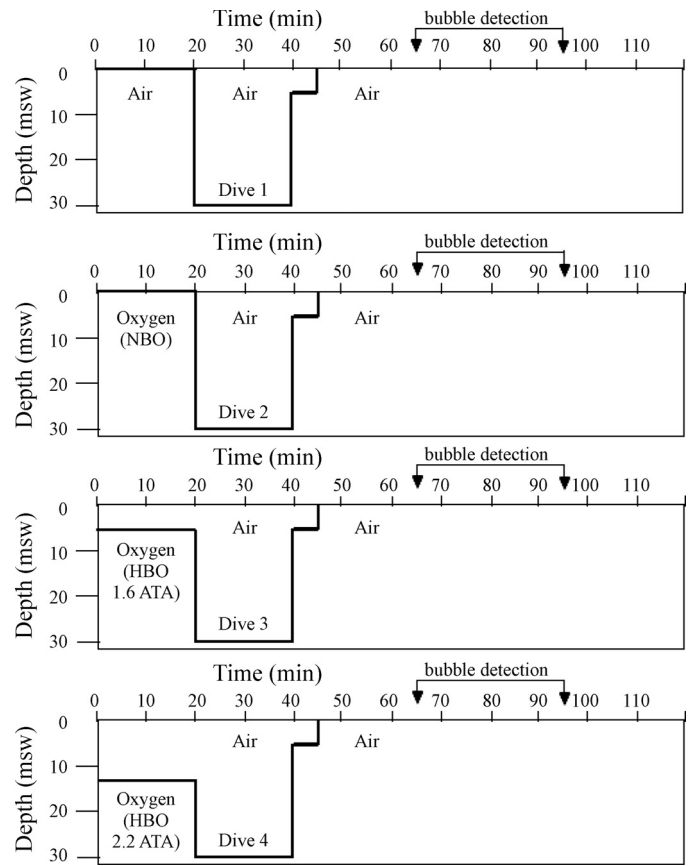


Fig. 1. Study protocol for *dives 1–4*. Divers prebreathed oxygen at different depths of the sea for 20 min before diving. Then they dived to 30 m seawater [msw [4 atmospheres absolute (ATA)]] for 20 min with air. Blood samples were collected immediately after the divers surfaced. Bubble detection was performed at 20 and 50 min after the divers surfaced. HBO, hyperbaric oxygenation; NBO, normobaric oxygenation.

ratus (Panasonic IC Recorder RR-US360) to achieve high-quality echo and stored with USB exit for personal computer connection. Prior to diving, 1-min precordial Doppler signals were recorded for each subject as examples of bubble-free heart sound. These signals served as the baseline for comparisons during subsequent signal grading after completion of the dive. The Doppler probe was placed at the left sternal border and manipulated until the flow sounds were strong, with valve sounds audible in the background. Bubble sounds were analyzed using Wave-Purity Professional software (Berlin, Germany), which allows graphic representation of the Doppler signal.

The auditory output from the bubble detector was categorized using the Kisman-Masurel (KM) code (27). The three-digit KM code (each digit ranging from 0 to 4) represents the frequency of bubbles (number of bubbles per cardiac cycle), the duration (percentage of cardiac

Table 1. *Physiological characteristics of divers*

Subject No.	Age, yr	Body Weight, kg	Height, cm	Blood Pressure, mmHg	Body Fat, %	BMI
S1	41	69.3	175	135/85	17.8	22.63
S2	48	86.3	176	140/85	23.5	27.86
S3	21	74.6	188	135/70	11.6	21.11
S5	33	71.2	171	115/85	17.9	24.35
S6	51	106.7	176	140/90	33.1	34.45
S7	35	101.8	177	125/80	25.6	32.49
Mean $\pm$ SD	38.2 $\pm$ 11.0	85.0 $\pm$ 16.1	177 $\pm$ 6		21.6 $\pm$ 7.5	27.1 $\pm$ 5.4

BMI, body mass index.

Table 2. Conversion of KM codes to KM bubble grades

fpA	BG	fpA	BG	fpA	BG	fpA	BG
111	I-	211	I-	311	I	411	II-
112	I	212	I	312	II-	412	II
113	I	213	I+	313	II	413	II+
114	I	214	II-	314	II	414	III-
121	I+	221	II-	321	II	421	III-
122	II	222	II	322	II+	422	III
123	II	223	II+	323	III-	423	III
124	II	224	II+	324	III	424	III+
131	II	231	II	331	III-	431	III
132	II	232	III-	332	III	432	III+
133	III-	233	III	333	III	433	IV-
134	III-	234	III	334	III+	434	IV
141	II	241	III-	341	III	441	III+
142	III-	242	III	342	III+	442	IV
143	III	243	III	343	III+	443	IV
144	III	244	III+	344	IV-	444	IV

KM, Kisman-Masurel; BG, bubble grade [according to Nishi et al. (35)]; fpA, 3 digits of KM code (frequency, percentage, amplitude).

cycles with bubbles at rest or the number of cardiac cycles with elevated bubble sound after the specified movement), and the amplitude of the bubble signal relative to normal cardiac background sounds. The KM codes were subsequently converted to a bubble grade (BG) from 0 to IV based on a 12-point ordinal scale (I-, I, I+, II-, . . . , IV-, IV) according to Nishi et al. (35) (Table 2). BG for the precordium at rest and during movement was converted to bubble count estimates (BCE, in bubbles/cm<sup>2</sup>) using a scale and a numeric bubble grade developed by Eftedal et al. (17).

**Blood sampling and platelet preparation.** Immediately after the divers surfaced, blood samples were carefully drawn from an antecubital vein through a 20-gauge needle by specially trained staff. The samples were injected into Vacutainer tubes (Becton Dickinson) containing 3.2% trisodium citrate and centrifuged at room temperature for 10 min at 200 g. Platelet-rich plasma was obtained with a Pasteur pipette and then fixed by addition of an equal volume of 2% (vol/vol) paraformaldehyde in PBS. The samples were washed twice with 1 ml of PBS.

The fixed platelets were placed in polypropylene tubes containing 10  $\mu$ l of anti-human CD41, 10  $\mu$ l of anti-human CD61-phycoerythrin (PE), and 10  $\mu$ l of anti-human CD62p-PE. As a negative control, 10  $\mu$ l of mouse IgG1-PE were added in place of CD61p-PE and anti-CD62p-PE, and 10  $\mu$ l of mouse IgG2a-FITC were added in place of anti-CD41a-FITC. All samples were immediately placed on ice for 30 min in darkness. The stained cells were washed twice, and the pellet was resuspended in 500  $\mu$ l of PBS and analyzed by flow cytometry.

**Flow cytometry.** Flow cytometric analysis was performed with a flow cytometer (Coulter Elite, Coulter Electronics, Miami, FL) equipped with a 488-nm helium-neon laser. The flow cytometer was calibrated with microbeads (Coulter) to verify the light scatter and the fluorescence signal reproducibility. Analysis was performed by evaluation of  $\geq 10,000$  cells for each sample. Each region of interest (platelet population) was programmed into the computer to analyze

each platelet sample with the same parameters. The platelet population was identified by gating the CD41a-positive cells. The negative and positive delineator was determined by gating 2% background staining on the isotype control fluorescence. The percentage of PE (CD61 and CD62p)-positive events in this population was then determined. A separate linear gate was used to determine mean PE fluorescence in arbitrary units on a logarithmic scale.

**Statistical analysis.** Values are means  $\pm$  SD. The platelet data were analyzed with a two-way analysis of variance with repeated measures and Tukey's post hoc test to determine the difference between experimental conditions and each dive. BCE and numeric bubble grade were compared using Wilcoxon's test for paired values.  $P < 0.05$  was considered statistically significant.

## RESULTS

All participants completed the experiment without signs or symptoms typical of mild or severe DCS.

As shown in Tables 3 and 4, NBO prebreathing significantly reduced air bubble numbers at 20 min, but not at 50 min. HBO prebreathing significantly reduced air bubble numbers at 20 and 50 min. The effect of HBO was similar at 1.6 and 2.2 ATA.

As shown in Table 5, diving significantly increased percent activation of CD41a, CD61, and CD62p. Pretreatment with NBO or HBO significantly attenuated the decompression-induced increase in activation of CD41a, CD61, and CD62p.

## DISCUSSION

The main findings of the present study are as follows: 1) prebreathing NBO for 20 min immediately before an open seawater dive while breathing air from scuba significantly reduced decompres-

Table 3. Bubble count estimate at 20 and 50 min after diving

	20 min		50 min	
	Rest	After Leg Flexions	Rest	After Leg Flexions
Nonpreoxygenation	0.191 $\pm$ 0.066	0.271 $\pm$ 0.068	0.125 $\pm$ 0.060	0.158 $\pm$ 0.040 <sup>f</sup>
NBO	0.142 $\pm$ 0.034 <sup>a</sup>	0.175 $\pm$ 0.042	0.125 $\pm$ 0.061	0.150 $\pm$ 0.045
HBO				
1.6 ATA	0.067 $\pm$ 0.026 <sup>b,c</sup>	0.092 $\pm$ 0.020 <sup>b,d</sup>	0.030 $\pm$ 0.022 <sup>b,c</sup>	0.067 $\pm$ 0.025 <sup>b,c,g</sup>
2.2 ATA	0.040 $\pm$ 0.018 <sup>a,c,e</sup>	0.075 $\pm$ 0.028 <sup>a,c,e,f</sup>	0.020 $\pm$ 0.020 <sup>a,c</sup>	0.063 $\pm$ 0.025 <sup>a,c,e,g</sup>

Values are means  $\pm$  SD, expressed as bubbles/cm. NBO, normobaric oxygenation; HBO, hyperbaric oxygenation; ATA, atmospheres absolute. <sup>a</sup> $P < 0.05$ ; <sup>b</sup> $P < 0.01$  vs. nonpreoxygenation. <sup>c</sup> $P < 0.05$ ; <sup>d</sup> $P < 0.01$  vs. NBO. <sup>e</sup> $P < 0.05$  vs. HBO (1.6 ATA). <sup>f</sup> $P < 0.05$ ; <sup>g</sup> $P < 0.01$  vs. Rest.



Table 4. Numeric bubble grade at 20 and 50 min after diving

	20 min		50 min	
	Rest	After Leg Flexions	Rest	After Leg Flexion
Nonpreoxygenation	1.89 ± 0.31	2.00 ± 0.29 <sup>f</sup>	1.50 ± 0.37	1.72 ± 0.23 <sup>f</sup>
NBO	1.61 ± 0.25 <sup>a</sup>	1.83 ± 0.28 <sup>f</sup>	1.50 ± 0.41	1.67 ± 0.30
HBO				
1.6 ATA	1.11 ± 0.17 <sup>b,c</sup>	1.28 ± 0.13 <sup>b,c</sup>	0.83 ± 0.18 <sup>b,c</sup>	1.11 ± 0.17 <sup>b,c,g</sup>
2.2 ATA	0.92 ± 0.16 <sup>a,c,e</sup>	1.17 ± 0.19 <sup>a,c,f</sup>	0.75 ± 0.16 <sup>a,c</sup>	1.08 ± 0.16 <sup>a,c,f</sup>

Values are means ± SD. <sup>a</sup>*P* < 0.05; <sup>b</sup>*P* < 0.01 vs. nonpreoxygenation. <sup>c</sup>*P* < 0.05 vs. NBO. <sup>e</sup>*P* < 0.05 vs. HBO (1.6 ATA). <sup>f</sup>*P* < 0.05; <sup>g</sup>*P* < 0.01 vs. Rest.

sion-induced bubbles detected at 20 min, but not at 50 min, postdive; 2) prebreathing HBO significantly reduced detected air bubbles at 20 and 50 min postdive; 3) decompression-induced air bubbles were more effectively reduced by prebreathing HBO than by prebreathing NBO; and 4) decompression-induced platelet activation was more effectively alleviated by prebreathing HBO than by prebreathing NBO. It has been reported that repetitive multiday diving significantly decreases the high bubble grade incidence in recreational divers (16). To avoid the possible effect of diving and HBO on bubble formation from previous dives, for all divers, 2 wk of recovery time separated subsequent dives.

Denucleation and denitrogenation have been hypothesized to be an essential mechanism underlying the beneficial effect of prebreathing oxygen on decompression-induced bubble formation (2). Denucleation is based on the fact that micronuclei exist within living tissue under normal conditions (19). These micronuclei expand and grow into bubbles during decompression (4, 31). Reduction of the number and size of these preexisting micronuclei would decrease decompression-induced bubble formation and, consequently, the risk of DCS. Theoretically, prebreathing oxygen would significantly increase tissue content of oxygen, which rapidly diffuses into the micronuclei in exchange for nitrogen, which rapidly diffuses out. When prebreathing oxygen under pressure, the size of preexisting micronuclei decreases in inverse proportion to the pressure. The oxygen is then absorbed by the surrounding tissue to cause rapid decay of the micronuclei. This process has been described as denucleation (2). This theory has been supported by the finding of Arieli et al. (1) that when decompressed prawns are pretreated with HBO, most of the micronuclei are eliminated, thereby reducing the number and size of bubbles formed. In a recent study, Katsenelson et al. (26) observed that when rats decompressed from 1,013 kPa were pretreated with HBO, the incidence of DCS was significantly reduced. We previously reported that prebreathing HBO reduced decompression-induced bubble formation and platelet activation in divers subjected to decompression in an HBO chamber (29). Blatteau and Pontier (5) examined the effect of in-water recompression to 6 msw

(1.6 ATA) vs. NBO breathing on bubble formation in divers during surfacing. They observed that bubble count was significantly lower for postdive NBO than for control divers breathing air. In-water recompression with oxygen dramatically suppressed circulating bubble formation, with a bubble count significantly lower than for NBO or controls (5). The present study shows the beneficial effect of prebreathing oxygen in water. The data of Blatteau and Pontier and our results suggest that pre- and postdive in-water oxygen breathing may be beneficial in reducing decompression-induced bubble formation. Whether combined pre- and postdive in-water oxygen breathing have an added beneficial effect in further reducing bubble formation warrants additional investigation.

Denitrogenation occurs when the tissue nitrogen resulting from an air exposure is washed out by breathing oxygen before decompression. During the scuba dive, increased hydrostatic pressure generates an increase in oxygen and nitrogen partial pressure. According to Henry's law, a proportional relationship exists between the solubility of a gas in a liquid and the partial pressure of that gas above the liquid. Therefore, body tissues saturate with nitrogen during a dive. During surfacing, the sum of the gas tension in the tissues may exceed 1 ATA to create a state of supersaturation. If decompression is sufficiently rapid and extensive, the excess nitrogen may create bubbles from preexisting gas micronuclei (47). Breathing pure oxygen significantly reduces the alveolar nitrogen partial pressure, creating a large difference of partial pressure between pulmonary alveoli and surrounding tissues, therefore speeding nitrogen washout. Breathing pure oxygen, combined with an increase in ambient pressure to 2.2 ATA, dissolves oxygen in the blood plasma and in all body cells, tissues, and fluids at >10 times normal concentration (from 20% to 100% oxygen is a 5-fold increase, from 1 ATA to 2 ATA can double this again to a 10-fold, or 1,000%, increase). This greatly increased oxygen content and hydrostatic pressure would more effectively wash out nitrogen from micronuclei. The better effect of HBO than NBO pretreatment in reducing bubble formation is probably

Table 5. Platelet activation

Glycoprotein	Baseline	Air	NBO	HBO	
				1.6 ATA	2.2 ATA
CD41a	20.0 ± 8.5	21.7 ± 8.7*	17.7 ± 5.7†	20.5 ± 9.1†	16.3 ± 6.4
CD61	26.7 ± 7.2	34.0 ± 6.7*	30.9 ± 6.3*†	25.2 ± 7.2†	29.2 ± 7.8*†
CD62p	3.0 ± 0.4	13.5 ± 1.3*	10.7 ± 0.9*†	4.5 ± 0.7*†	7.6 ± 0.8*†

Values are means ± SD, expressed as percentage. Baseline, before compression; Air, after protocol described for *dive 1*; NBO, after protocol described for *dive 2* with 100% NBO; HBO (1.6 ATA), after protocol described for *dive 3* with HBO at 1.6 ATA and 100% oxygen; HBO (2.2 ATA), after protocol described for *dive 4* with HBO at 2.2 ATA and 100% oxygen. \**P* < 0.05 vs. Baseline. †*P* < 0.05 vs. Air.

due to its ability to provide significantly more oxygen to the entire body to replace nitrogen.

The denitrogenation effect of oxygen can be mathematically estimated, as described in detail in our previous study (29). To simplify the computation, the Bhulmann ZHL-12 algorithm was used to explain how denitrogenation can reduce decompression-induced bubble formation. The detailed computation is shown in the APPENDIX. The mathematical data show that 1) prebreathing HBO is more effective in removing nitrogen from so-called fast and very fast compartments; 2) the effectiveness of denitrogenation improves as the oxygen partial pressure rises until it reaches >2.0 ATA, at which the effectiveness no longer improves; and 3) at >2.4 ATA, oxygen behaves as an inert gas in terms of decompression. These mathematical data also show that there is no significant difference in nitrogen tensions in these four compartments at the end of the bottom time of the 4 ATA/20 ft dive under different oxygenation protocols. This is consistent with the report of Castagna and colleagues (10) that the role of denitrogenation alone may not be predominant in the effectiveness of oxygen prebreathing in the removal of the decompression-induced venous gas bubble.

According to these hypotheses, prebreathing NBO would also be beneficial in reducing decompression-induced bubble formation. This has been validated by recent studies. In a simulated submarine escape study, Gennser and Blogg (21) suggested that prebreathing NBO for a period as short as 15 min may be efficacious in helping reduce the initial bubble load in their goat model. Castagna et al. (10) recently reported that 30 min of NBO prebreathing ending 15 min prior to an open-water dive decreased decompression-induced air bubbles. The present study shows that prebreathing NBO for 30 min immediately before diving significantly reduces air bubbles detected at 20 min, but not at 50 min. This is consistent with the findings of Gennser and Blogg that prebreathing NBO for 15 min before submarine escape lowers the total amount of bubbles detected postescape, particularly in the 30 min immediately after surfacing. These initially detected bubbles are believed to be originally from the central nervous system and other fast tissues (21). The study of Gennser and Blogg and our findings suggest that prebreathing NBO may produce partial denitrogenation and denucleation, mainly in the fast tissues.

Drastic pressure changes during decompression not only induce air bubble formation, but also initiate a biochemical cascade with serious indirect effects, including leukocyte adhesion, platelet aggregation, and complement activation (14, 18, 22). Additionally, microbubble-induced endothelial damage causes tissue factor expression and subsequent platelet activation and thrombus generation (49). A key role of platelets in the pathogenesis of DCS has been suggested (7, 29, 32). Platelet accumulation around air bubbles in the blood is due to a cellular reaction, as well as the physicochemical flotation process, as demonstrated by Ritz-Timme et al. (40). Adherence and aggregation of platelets to the bubble surface have been observed (38), and these activated platelets may be involved in the formation of microthrombi in lung vessels after decompression in a rat model of DCS (37). The data from a recent study with a rat model of DCS suggest that thrombus generation and platelet activation likely participate in bubble-induced platelet aggregation, which could play a key role in the pathogenesis of DCS (39). Our previous study suggested that even

mental and physical stress during open-water dives may enhance platelet activation (7). Numerous markers, including CD41 (GPIIb), CD61 (GPIIIa), and CD62p (P-selectin), can be assayed to detect platelet activation (12). CD41 and CD61 are predictive of platelet activation markers for bleeding disorders due to platelet aggregation and adhesion abnormalities (12). Increased levels of CD62p have been reported as potential markers of life-threatening thrombotic diseases, such as stroke, thrombosis, and coronary artery disease (30). These activated platelets may participate in the formation of thrombi in experimental DCS (28).

The reduction of nitrogen partial pressure reduces platelet activation; this hypothesis is supported by the fact that breathing nitrox, rather than air, reduces the level of decompression-induced platelet activation (3). An animal study showed that acute exposure to HBO at 2.4 ATA for 90 min significantly reduces the maximal rate of ADP- and collagen-induced platelet aggregation (15). We previously reported that HBO, when administered immediately before simulated diving, significantly reduces decompression-induced platelet activation (29). The present study further shows that HBO prebreathing alleviates decompression-induced platelet activation in open-sea divers. Furthermore, our study shows that NBO prebreathing may also alleviate platelet activation. The platelets are mainly circulating in the fast tissue. NBO significantly increases oxygen content in the fast tissue, reduces decompression-induced air bubble formation, and, therefore, alleviates decompression-induced platelet activation.

In the present study, decompression from 4 ATA significantly increased activation of CD41, CD61, and CD62p, potentially increasing the risk for divers with underlying medical problems. It is unclear whether decompression-induced platelet activation poses any danger to healthy divers. However, enhanced platelet activation has been linked to the development of ischemic stroke (41). Platelet activation is also observed in patients with atherothrombotic lesions, arrhythmias (15), and renal insufficiency (23). Considering that platelet activation is also associated with microbubble formation during decompression (3, 44, 45, 46), the reduction of platelet activation may be beneficial to reduce the risk of DCS.

In summary, prebreathing NBO or HBO immediately before diving may be beneficial in open-sea scuba divers. Prebreathing HBO seems more effective than NBO in reducing decompression-induced bubble formation and platelet activation. The present study suggests that prebreathing oxygen at depth of the water may be beneficial in reducing the development of DCS.

#### APPENDIX

To simplify the computation, the Bhulmann ZHL-12 algorithm was used to explain how denitrogenation can reduce decompression-induced bubble formation using following formula

$$P_t = P_0 + (P_1 - P_0) \times [1 - e^{-0.69315 \times t/T_{(1/2)}}]$$

where  $P_0$  is nitrogen tension at the surface of the sea (1 ATA  $\times$  0.79 = 0.79 ATA),  $P_1$  is nitrogen tension at 30 m below the sea (4 ATA  $\times$  0.79 = 3.16 ATA), and  $P_t$  is nitrogen tension in the compartment at time  $t$ . Here, only a very fast, a fast, a slow, and a very slow compartment [ $T_{(1/2)} = 2.65, 26.5, 114, \text{ and } 635 \text{ min}$ ] are taken into consideration. Inspired fraction of  $O_2$  is considered theoretically to be 100% during NBO and HBO prebreathing, and nitrogen fraction is considered to be 79% during air breathing.

On *dive 1*, after diving to 30 m below the sea for 20 min on air

$$P_{t(\text{very fast compartment})} = 0.79 + (3.16 - 0.79) \times (1 - e^{-0.69315 \times 202.65}) = 3.14 \text{ (ATA)}$$

$$P_{t(\text{fast compartment})} = 0.79 + (3.16 - 0.79) \times (1 - e^{-0.69315 \times 202.65}) = 1.75 \text{ (ATA)}$$

$$P_{t(\text{slow compartment})} = 0.79 + (3.16 - 0.79) \times (1 - e^{-0.69315 \times 20114}) = 1.06 \text{ (ATA)}$$

$$P_{t(\text{very slow compartment})} = 0.79 + (3.16 - 0.79) \times (1 - e^{-0.69315 \times 20365}) = 0.84 \text{ (ATA)}$$

On *dive 2*, after 20 min of prebreathing oxygen at the surface of the sea

$$P_{t(\text{very fast compartment})} = 0.79 + (0 - 0.79) \times (1 - e^{-0.69315 \times 202.65}) = 0.004 \text{ (ATA)}$$

$$P_{t(\text{fast compartment})} = 0.79 + (0 - 0.79) \times (1 - e^{-0.69315 \times 202.65}) = 0.46 \text{ (ATA)}$$

$$P_{t(\text{slow compartment})} = 0.79 + (0 - 0.79) \times (1 - e^{-0.69315 \times 20114}) = 0.69 \text{ (ATA)}$$

$$P_{t(\text{very slow compartment})} = 0.79 + (0 - 0.79) \times (1 - e^{-0.69315 \times 20365}) = 0.77 \text{ (ATA)}$$

After 20 min diving to 30 m below the sea on air

$$P_{t(\text{very fast compartment})} = 0.004 + (3.16 - 0.004) \times (1 - e^{-0.69315 \times 202.65}) = 3.14 \text{ (ATA)}$$

$$P_{t(\text{fast compartment})} = 0.46 + (3.16 - 0.46) \times (1 - e^{-0.69315 \times 202.65}) = 1.56 \text{ (ATA)}$$

$$P_{t(\text{slow compartment})} = 0.69 + (3.16 - 0.69) \times (1 - e^{-0.69315 \times 20114}) = 0.98 \text{ (ATA)}$$

$$P_{t(\text{very slow compartment})} = 0.77 + (3.16 - 0.77) \times (1 - e^{-0.69315 \times 20365}) = 0.82 \text{ (ATA)}$$

On *dive 3*, after 20 min of prebreathing oxygen at 6 m below the sea (1.6 ATA)

$$P_{t(\text{very fast compartment})} = 0.79 + (-0.6 - 0.79) \times (1 - e^{-0.69315 \times 202.65}) = 0.00 \text{ (ATA)}$$

$$P_{t(\text{fast compartment})} = 0.79 + (-0.6 - 0.79) \times (1 - e^{-0.69315 \times 202.65}) = 0.22 \text{ (ATA)}$$

$$P_{t(\text{slow compartment})} = 0.79 + (-0.6 - 0.79) \times (1 - e^{-0.69315 \times 20114}) = 0.63 \text{ (ATA)}$$

$$P_{t(\text{very slow compartment})} = 0.79 + (-0.6 - 0.79) \times (1 - e^{-0.69315 \times 20365}) = 0.75 \text{ (ATA)}$$

After 20 min diving to 30 m below the sea on air

$$P_{t(\text{very fast compartment})} = 0.00 + (3.16 - 0.00) \times (1 - e^{-0.69315 \times 202.65}) = 3.14 \text{ (ATA)}$$

$$P_{t(\text{fast compartment})} = 0.22 + (3.16 - 0.22) \times (1 - e^{-0.69315 \times 202.65}) = 1.41 \text{ (ATA)}$$

$$P_{t(\text{slow compartment})} = 0.63 + (3.16 - 0.63) \times (1 - e^{-0.69315 \times 20114}) = 0.92 \text{ (ATA)}$$

$$P_{t(\text{very slow compartment})} = 0.75 + (3.16 - 0.75) \times (1 - e^{-0.69315 \times 20365}) = 0.81 \text{ (ATA)}$$

On *dive 4*, after 20 min of prebreathing oxygen at 12 m below the sea (2.2 ATA)

$$P_{t(\text{very fast compartment})} = 0.79 + (-1.2 - 0.79) \times (1 - e^{-0.69315 \times 202.65}) = 0.00 \text{ (ATA)}$$

$$P_{t(\text{fast compartment})} = 0.79 + (-1.2 - 0.79) \times (1 - e^{-0.69315 \times 202.65}) = 0.00 \text{ (ATA)}$$

$$P_{t(\text{slow compartment})} = 0.79 + (-1.2 - 0.79) \times (1 - e^{-0.69315 \times 20114}) = 0.56 \text{ (ATA)}$$

$$P_{t(\text{very slow compartment})} = 0.79 + (-1.2 - 0.79) \times (1 - e^{-0.69315 \times 20365}) = 0.74 \text{ (ATA)}$$

After 20 min diving to 30 m below the sea on air

$$P_{t(\text{very fast compartment})} = 0.00 + (3.16 - 0.00) \times (1 - e^{-0.69315 \times 202.65}) = 3.14 \text{ (ATA)}$$

$$P_{t(\text{fast compartment})} = 0.00 + (3.16 - 0.00) \times (1 - e^{-0.69315 \times 202.65}) = 1.28 \text{ (ATA)}$$

$$P_{t(\text{slow compartment})} = 0.56 + (3.16 - 0.56) \times (1 - e^{-0.69315 \times 20114}) = 0.85 \text{ (ATA)}$$

$$P_{t(\text{very slow compartment})} = 0.74 + (3.16 - 0.74) \times (1 - e^{-0.69315 \times 20365}) = 0.79 \text{ (ATA)}$$

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#### DISCLOSURES

No conflicts of interest are declared by the authors.

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