Exogenous Nitric Oxide and Bubble Formation in Divers

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ABSTRACT

DUJIĆ, Ž., I. PALADA, Z. VALIC, D. DUPLANČIĆ, A. OBAD, U. WISLØFF, and A. O. BRUBAKK. Exogenous Nitric Oxide and Bubble Formation in Divers. *Med. Sci. Sports Exerc.*, Vol. 38, No. 8, pp. 1432–1435, 2006. **Purpose:** Prevention of bubble formation is a central goal in standard decompression procedures. Previously we have shown that exercise 20–24 h prior to a dive reduces bubble formation and increases survival in rats exposed to a simulated dive. Furthermore, we have demonstrated that nitric oxide (NO) may be involved in this protection; blocking the production of NO increases bubble formation while giving rats a long-lasting NO donor 20 h and immediately prior to a dive reduces bubble formation. This study determined whether a short-lasting NO donor, nitroglycerine, reduced bubble formation after standard dives and decompression in man. **Methods:** A total of 16 experienced divers were randomly assigned into two groups. One group performed two dives to 30 m of seawater (msw) for 30 min breathing air, and performed exercise at an intensity corresponding to 30% of maximal oxygen uptake during the bottom time. The second group performed two simulated dives to 18 msw for 80 min breathing air in a hyperbaric chamber, and remained sedentary during the bottom period. The first dive for each diver served as the control dive, whereas the divers received 0.4 mg of nitroglycerine by oral spray 30 min before the second dive. Following the dive, gas bubbles in the pulmonary artery were recorded using ultrasound. **Results:** The open-water dive resulted in significantly more gas bubbles than the dry dive (0.87 ± 1.3 vs 0.12 ± 0.23 bubbles per square centimeter). Nitroglycerine reduced bubble formation significantly in both dives from 0.87 ± 1.3 to 0.32 ± 0.7 in the in-water dive and from 0.12 ± 0.23 to 0.03 ± 0.03 bubbles per square centimeter in the chamber dive. **Conclusion:** The present study demonstrates that intake of a short-lasting NO donor reduces bubble formation following decompression after different dives.
Key Words: DECOMPRESSION SICKNESS, NO, DIVING, MAN, HYPERBARIC CHAMBER

Gas will be taken up in solution by the tissues of the body during a dive proportional to the depth, and the uptake is exponentially related to the time spent under pressure. Upon returning to the surface, this excess gas must be eliminated. Gas elimination follows an exponential curve, with time constants determined by blood flow to the different tissues. If pressure is reduced faster than gas can be eliminated, the partial pressure of gas in the tissue will be higher than the environmental pressure. This supersaturation can lead to gas coming out of solution, forming bubbles. Such bubbles grow from so-called bubble nuclei that are attached to the vessel wall. These bubbles are considered the cause of clinical symptoms, decompression sickness (DCS). Such symptoms can include pain in muscles or joints or more severe symptoms from the cardiovascular and the central nervous system.

Decompression procedures have been developed to reduce the risk of injury following a dive. These procedures have the aim of reducing bubble formation, which most authors regard as the initiating mechanism for tissue injury (6). The volume of gas in bubbles is proportional to the risk for DCS (21), and the number of bubbles detected by ultrasound in the pulmonary artery is proportional to the number of vascular bubbles in the periphery (1).

Previous studies have demonstrated that a single bout of high-intensity exercise 20–24 h before the dive significantly reduces the number of bubbles in the pulmonary artery both in rats (18) and in man (3). Exercise performed immediately before a dive will not have this effect. Exercise will increase nitric oxide (NO) production, and our hypothesis is that exercise changes the surface properties of the endothelium, which will enable elimination of the precursors of the bubbles. Administration of a long-lasting NO donor had the same effect as exercise in rats, even when it was given immediately before the dive (20). Nitroglycerine, a short-acting NO donor, had similar effects when given before decompression was started in pigs (Mollerlokken et al., personal communication, 2005). The present study determines whether a short-lasting NO donor reduces bubble formation in man performing standard dives and decompression procedures.

METHODS

Study population. This study consisted of two parts. The first part was carried out in 10 experienced divers aged

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34.4 ± 4.2 (mean ± standard deviation, SD) yr, height 1.83 ± 0.1 m, weight 87.1 ± 9.6 kg, and BMI (body mass index) 25.9 ± 2.0 $kg \cdot m^{-2}$. They performed two open-water dives to 400 kPa (30 m of seawater, msw) for 30 min breathing air. The subjects were all experienced divers with considerable diving experience (both air and oxygen diving). Two of the divers were mild smokers (5 and 10 cigarettes a day). The second part of the study was performed in an additional group of six experienced divers, aged 32.2 ± 5.2 yr, height 1.84 ± 0.1 m, weight 89.0 ± 7.6 kg, and BMI 26.5 ± 2.4 $kg \cdot m^{-2}$, who performed two dry dives to 280 kPa (18 msw) in a pressure chamber for 80 min breathing air.

At the time of the study, all had a valid medical certificate for diving and were clear of all symptoms of acute illness. All experimental procedures were conducted in accordance with the Declaration of Helsinki and were approved by ethics committee of the University of Split School of Medicine. Each method and its potential risks were explained to the participants in detail, and they gave written informed consent before the experiment.

Maximal oxygen uptake. Measurement of the maximal oxygen uptake ($\dot{V}O_{2max}$) was determined in all divers 2 wk prior to the experiments using an incremental protocol on a cycle ergometer (Marquette Hellige Medical Systems 900 ERG, Milwaukee, WI). The subjects were exposed to an initial work rate of 50 W at a pace of 60 cycles per minute. They were told to sustain a constant frequency, and work rate was increased each minute by 25 W up to exhaustion, which occurred within 9–12 min in all subjects. During the entire test, oxygen uptake and pulmonary ventilation were measured with a cardiopulmonary exercise testing unit (Quark b^2, Cosmed, Rome, Italy), and heart rate (HR) was registered continuously with Polar S810i HR monitor (Polar Vantage, Finland). Criteria for assessment of $\dot{V}O_{2max}$ were a respiratory exchange ratio (RER) $\geq$ 1.1 and plateau ($\leq$ 150 mL increase) in $\dot{V}O_2$ despite increased workload. The mean $\dot{V}O_{2max}$ and the maximum HR (HR_{max}) at $\dot{V}O_{2max}$ were 41.8 ± 6.4 $mL \cdot kg^{-1} \cdot min^{-1}$ and 177 ± 9 bpm in the first group (field dives) and 47.3 ± 5.3 $mL \cdot kg^{-1} \cdot min^{-1}$ and 182 ± 11 bpm in the second group (chamber dives), respectively.

Location and duration of the field study. The present study was performed at the military base of the Croatian Navy Forces over a 2-wk period. The dive site was located in the vicinity of the base, where the divers were transported by powerboat during a 10-min ride. The site was chosen because it allowed us to perform dives of the suitable depth and duration. Sea temperature at bottom and at the decompression stop was 15°C for all dives, and outside temperature varied between 10 and 17°C.

Field diving protocol. Divers did not participate in any diving activities 7 d before this protocol. Divers equipped with wet suits in accordance with the Croatian Navy and the U.S. Navy diving manuals performed all dives. Depth of the dive was set to 30 msw, and each pair of divers was supplied with a dive computer (Mosquito, Suunto, Finland) interfaced with a personal computer for later verification of the dive profile. Descent to depth was performed in 3 min. During the bottom time, the divers swam at a low level of exertion and were told to swim on the bottom for a distance of 500 m; this was controlled by the personnel on the powerboat, and the exercise intensity corresponded to 30% $\dot{V}O_{2max}$. During the decompression period, divers were told not to perform any exercise. This protocol was chosen because, in our experience, it reliably produces significant amount of venous bubbles even if proper decompression procedures are followed. HR was continuously monitored in all divers during diving with Polar S810i HR monitor. Ascent was performed at a rate of 10 $msw \cdot min^{-1}$ with a 3-min safety stop at 3 msw.

Chamber diving protocol. The divers were compressed to 280 kPa in a hyperbaric chamber (Brodosplit, Split, Croatia) at a rate of 100 $kPa \cdot min^{-1}$ breathing air, remaining at pressure for 75 min. Then they were decompressed at a rate of 90 $kPa \cdot min^{-1}$ to 130 kPa, where they remained for 7 min before they were decompressed to the surface pressure (100 kPa) at the same rate (U.S. Navy air decompression procedure, 1996). We have shown previously that this protocol produced a significant amount of venous bubbles (16).

Pharmacological intervention. The two field and chamber dives performed by each diver were selected first as a control dive and second as a NO dive. The control and the NO dives for field dives were 2–13 d apart (for eight divers more then 6 d, and for two divers 2 d) and for chamber dives were 2–10 d apart (for five divers more then 6 d, and for one diver 2 d). Thirty minutes prior to the NO dive, the divers took 0.4 mg of nitroglycerine by oral spray (Nitrolingual (glyceriltrinitrat) manufactured by G. Pohl-Boskamp).

Predive monitoring and bubble analysis. After completion of the diving protocol, divers were transported to the facility where they took a brief shower before further monitoring. Following the shower, subjects were placed in the supine position, and an echocardiographic investigation with a phase array probe (1.5–3.3 MHz) using a Vivid 3 Expert ultrasonic scanner (GE, Milwaukee, WI) was conducted. The same experienced cardiologist performed all echocardiographic investigations. Monitoring was performed in supine position at 30 and 60 min after reaching surface pressure. Gas bubbles were seen as high-intensity echoes in the right heart and the pulmonary artery. The cardiac images were recorded on a S-VHS videotape (Sony VCR) for 60 s at rest and after two coughs. The coughs were used because we have noticed that such maneuver significantly increases the number of bubbles detected. The bubbles were graded using the method described by Eftedal and Brubakk (5). This grading system has been used extensively in several animal species as well as in man. This grading system is highly nonlinear and was converted into a linear scale (bubbles per square centimeter) using the conversion table previously described (13). The number of bubbles was determined at each of the measurement points and then integrated to give an average bubble number for the whole observation period. High-quality images were obtained in all subjects. The investigator performing the grading did not know the status of the diver. The data were

saved on a tape and digitalized on a personal computer (ATI Multimedia Center, ATI Technologies). The grading was controlled for accuracy by one of the authors (AOB) who had developed the method. The presence of patent foramen ovale (PFO) in our subjects was checked by observing whether the bubbles were crossing the atrial septum after two coughs.

Statistics. The data are presented as mean ± standard deviation (SD). The comparison between the dry and wet dive groups was done using Student's *t*-test for unpaired samples, and the comparison between bubble formation in the control dives and the dives using nitroglycerine was done using Student's *t*-test for paired samples. All analyses were done using Statistica 7.0 software (Statsoft, Inc., Tulsa, OK).

RESULTS

Following the control in-water dive, the mean bubble numbers observed over the whole decompression period was 0.87 ± 1.3 bubbles per square centimeter. This was reduced to 0.32 ± 0.7 bubbles per square centimeter when administrating nitroglycerine prior to the dive ($P = 0.04$). Figure 1 shows a representative echocardiographic image of the heart of field diver 9. Panel A clearly reveals the presence of a significant number of venous gas bubbles (grade 4) within the right chambers of the heart after the dive without NO donor, and panel B with a reduced amount of venous gas bubbles (grade 2) after the dive with NO donor.

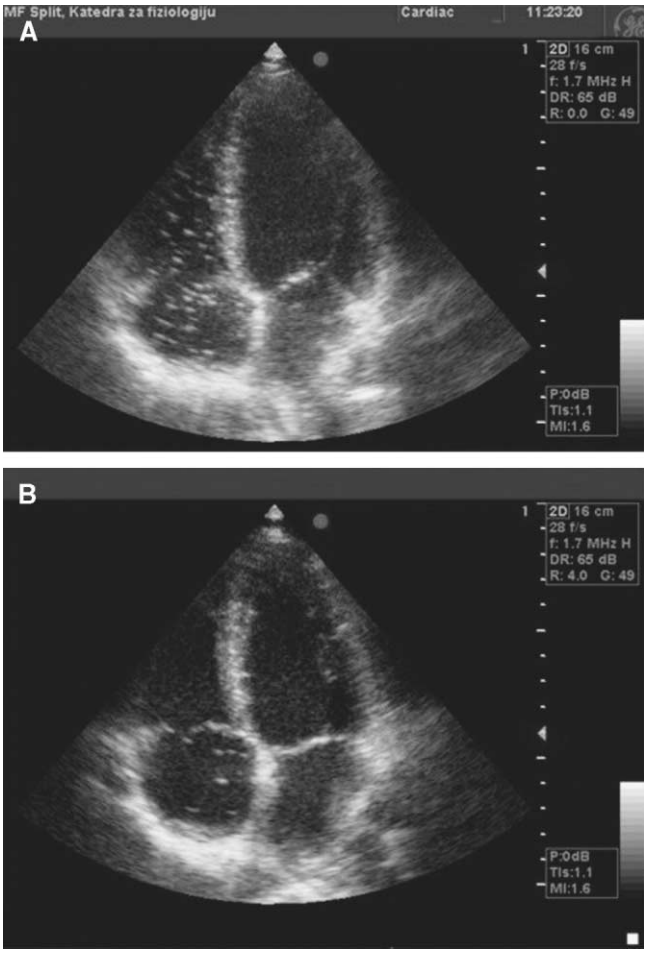

FIGURE 1—Echocardiographic apical four-chamber view of the heart in field diver 9. The image was obtained 20 min after reaching surface pressure, in the control dive (A) and after the dive with NO donor (B). The reduction of venous gas bubbles within right heart is clearly visible in panel B.

Following the dry dive, the mean bubble grade observed over the same period as above was 0.12 ± 0.23 bubbles per square centimeter, which was reduced to 0.03 ± 0.03 bubbles per square centimeter after administration of nitroglycerine prior to the dive ($P = 0.05$).

The dry chamber dive produced significantly less bubbles than the in-water dive ($P = 0.02$). On average, bubble formation was reduced by 63% in the in-water dive and 79% in the simulated dry dive.

DISCUSSION

Bubbles are probably formed from nuclei, small gas-filled bubbles that adhere to the vascular wall (8,21). If such nuclei are not present, no bubbles will be found at the supersaturations observed in the vascular system. Even slight supersaturations are adequate for venous bubble formation in man (4). The present study demonstrated that intake of a short-lasting NO donor 30 min prior to a dive significantly reduced bubble formation following decompression after dives of different duration and to different depths in man. We have previously reported that administration of a long-lasting NO donor several days or immediately prior to the dive similarly reduced bubble formation and increased survival significantly in rats (20). However, the NO donor used in that study was long lasting and was active during the whole dive and several hours after the decompression procedure. In the present study, we used a short-lasting NO donor that is active only for 15 min (10), and its effects disappear prior to starting the dive in our divers. This would suggest that nitroglycerine only triggers a protective effect on bubble formation that lasts during the dive. The beneficial effect was similar in both the dry chamber dive and the in-water dive. Together with our previous studies, it now appears well documented that NO will prevent bubble formation in different mammalian species (rats, pigs, and humans) and following different dive exposures.

The mechanisms responsible for the reduction in bubble formation by NO are not clear. We have previously speculated that NO may lead to changes in the vascular endothelium, making it less hydrophobic, and thus reduce the adherent forces that are needed for keeping the nuclei attached (18,19). Endothelial NO synthase (eNOS) has been localized to caveolae, small invaginations of the cell membrane rich in caveolin, cholesterol, and sphingolipids (15). Recently, Linder et al. (11) have shown that molecules in the NO-signaling pathway such as soluble guanylyl cyclase, cAMP-dependent protein kinase, and cGMP-dependent protein kinase are also colocalized in caveolae. Because exogenous NO reduces bubble formation, it is possible that bubble nuclei are also colocalized in the caveolae with eNOS and NO-related

molecules; this hypothesis should be investigated in the future using eNOS-knockout mice (14). However, other mechanisms may also be involved, as exercise will prevent bubble formation in seden tary but not exercised rats following blockade of NO production (19).

Nitroglycerine will reduce peripheral vascular resistance and reduce venous tone, leading to a slight reduction in cardiac output with little change in blood pressure (7). This may influence uptake of inert gas. However, as similar reductions in gas bubble number were seen with nitroglycerine in both wet and dry dives, differences in gas uptake probably do not play a major role.

Significantly fewer bubbles are produced in the dry chamber dive than in the in-water dive. According to Hennessy and Hempelman (9), dive stress can be estimated by $p\sqrt{t}$, where p is pressure in bar and t is time at bottom in minutes. Using this index, the dry dive had a $p\sqrt{t}$ of 25, and the in-water dive had a $p\sqrt{t}$ of 22 or about 14% less. In spite of this, the in-water dive produced significantly more bubbles over the observation time. Both immersion and exercise will increase cardiac output, and one would therefore expect that this would increase gas uptake in the in-water dive. The chamber dive had a considerably longer bottom time (75 vs 30 min), which would lead to a higher gas uptake in the chamber dive. However, because previous studies have shown that there can be significant differences in bubble formation between individuals (2), this may also have influenced the results. The simulated dive in this study produced 0.12 bubbles per square centimeter, which is between the values of 0.98 and 0.0125 bubbles per square centimeter seen on similar dives in two previous studies (2,3).

The number of bubbles observed in the pulmonary artery is a measure of decompression stress and is related to the risk of DCS (13). Furthermore, a number of studies have shown that there is an increased risk of central nervous symptoms in divers with a persistent foramen ovale (PFO) allowing for right to left shunts (12,17). Recently, we have shown a reduction in arterial endothelial function after a simulated single air dive (2), which we believe is a combined effect of hyperoxia and bubble formation.

This and our previous studies have demonstrated that exercise or drugs can reduce vascular bubble formation significantly. Because there is a statistical relationship between the occurrence of many vascular bubbles and DCS, this effect may be used to improve diving safety (13).

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