

# Venous Bubble Count Declines During Strenuous Exercise After an Open Sea Dive to 30 m

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**Introduction:** The effect of post-dive exercise on bubble formation remains controversial, although the current practice of divers and aviators is to avoid strenuous exercise after diving. Previously, we have shown that exercising 24 h before a dive, or during a decompression stop, significantly reduces bubble formation in man. The objective of this study was to determine whether a short period of strenuous post-dive exercise promotes venous bubble formation. **Methods:** Seven male military divers performed an open-sea field dive to a maximum depth of 30 m for 30 min. At maximum depth, subjects performed mild underwater fin swimming, followed by standard decompression. Diving was followed by a post-dive exercise session consisting of short, strenuous incremental upright cycle ergometry, up to 85% of maximal oxygen uptake, for about 10 min. Subjects were monitored for venous gas bubbles in the right heart with an echo-imaging system starting 20 min post-dive while in the supine position, during cycle ergometry in the seated upright position, and immediately after exercise in a supine position. **Results:** The average number of bubbles was  $1.5 \pm 1.4$  bubbles  $\cdot$   $\text{cm}^{-2}$  20 min after diving. Changes in posture from supine to seated upright resulted in significant reduction of bubbles to  $0.6 \pm 1.3$  bubbles  $\cdot$   $\text{cm}^{-2}$  ( $p = 0.043$ ), with further reduction to  $0.2 \pm 0.3$  bubbles  $\cdot$   $\text{cm}^{-2}$  at the end of exercise ( $p = 0.02$ ). No cases of DCS or intra-pulmonary shunt were observed during or following post-dive exercise. **Discussion:** These results suggest that post-dive strenuous exercise after a single field dive reduces post-dive gas bubble formation in well-trained military divers. Additional findings are needed for normal sports divers.

**Keywords:** decompression sickness, physical activity, ultrasound, open-sea diving, gas bubbles.

GENERALLY, IT IS accepted that inert gas bubbles in the vascular and/or extravascular space are the cause of decompression sickness (DCS), and that the occurrence of many bubbles is linked to a higher risk of developing DCS (22). Micronuclei were proposed as intermediates in venous gas bubble formation after diving, since the supersaturations encountered are well below what is necessary for de novo nucleation (15) and the half-life of micronuclei is around 1 h (6). Once the number of micronuclei is reduced it takes about 10–100 h to regenerate them (31). Gas bubbles are frequently observed by echocardiography after diving, and in most cases they are asymptomatic.

Exercise after diving, shortly before, or after decompression to altitude, can promote bubble formation (6). Webb et al. (27) recently failed to show that moderate dynamic exercise (around 50% of maximal oxygen uptake) after altitude exposure delayed onset of DCS or reduced recurring DCS. The translation of data taken at

high-altitude to open-sea diving is not straightforward, since altitude DCS involves decompression from the saturation condition, whereas sub-saturation decompressions are associated with diving. Thus, it is thought that gas bubble formation is greater with high-altitude exposure and that cerebral symptoms are more frequent in high-altitude DCS than in diving (14).

We recently reported that interval, high-intensity exercise 24 h before a dive and mild exercise performed during decompression stops both significantly reduce bubble formation and, therefore, diminish the risk for DCS (9,10). This finding was confirmed recently for exercise 2 h before a dive (1). Nevertheless, the controversy on this topic in recent years has not been solved (4), and further larger studies are needed in order to quantify amount and timing of exercise, as well as to investigate potential mechanisms. Micronuclei are not stable in the blood and two stabilizing factors were proposed: the presence of intercellular hydrophobic crevices on the endothelial surface where gas nuclei are trapped (15,16) and the concept of surface-acting molecules like proteins, platelets, and surfactant (31). Potential biochemical processes involved in the protective effect of strenuous exercise on bubble formation include exercise-induced increase in vascular endothelial nitric oxide (NO) bioavailability and heat shock protein 70 expression (30). Although NO blockade increases bubble production in sedentary rats, exercise still has protective effects, suggesting NO-dependent and NO-independent mechanisms (29). Huang et al. (17) have recently shown that heat shock pretreatment attenuates air bubble-induced acute lung injury and suggested this novel mechanism of diving acclimatization.

Strenuous exercise in the post-dive period may not be associated with DCS after field diving (11), although the main emphasis of that study was to demonstrate whether intra-pulmonary shunts are recruited with ex-

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ercise after dive, and the left side of the heart was preferentially investigated. Therefore, the purpose of this study was to investigate the effect of a short period of strenuous post-dive exercise on venous bubble formation in the right heart and pulmonary artery. The objective of this study was to determine whether post-dive exercise after field diving promotes venous bubble formation.

## METHODS

### *Study Population*

The study subjects were seven military divers between 30 and 39 yr old. The subjects were all experienced non-smoking divers with considerable diving experience (both air and oxygen diving). Diver mean weight was  $85.9 \pm 8.5$  kg, height  $182.1 \pm 4.9$  cm, and body mass index  $25.9 \pm 2.0$   $\text{kg} \cdot \text{m}^{-2}$ . At the time of the study, all had a valid medical certificate for diving and were free of any symptoms of acute illness. All experimental procedures were conducted in accordance with the Declaration of Helsinki, and were approved by the Ethics Committee of the University of Split School of Medicine. Methods were explained in details to the divers, and written informed consent obtained from each participant.

### *Maximal Oxygen Uptake*

Maximal oxygen uptake ( $\dot{V}_{\text{O}_{2\text{max}}}$ ) and maximum heart rate were determined in all divers 1 wk prior to the experiments using an incremental protocol on the 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  $\cdot$  min<sup>-1</sup>. They were instructed to sustain a constant frequency while the work rate was increased each subsequent minute by 25 W up to exhaustion. Exhaustion occurred after 9–13 min in all subjects. During the entire test, oxygen uptake and lung ventilation were measured with a spirometry testing unit (Quark b<sup>2</sup>, Cosmed, Rome, Italy) and heart rate (HR) was registered continuously with a Polar S810i HR monitor (Polar Vantage, Kempele, Finland). Criteria for assessment of  $\dot{V}_{\text{O}_{2\text{max}}}$  included a respiratory exchange ratio  $\geq 1.1$  and a plateau ( $\leq 150$  ml increase) in  $\dot{V}_{\text{O}_2}$  with an increase in workload. The mean  $\dot{V}_{\text{O}_{2\text{max}}}$  was  $41.5 \pm 9.6$  ml  $\cdot$  kg<sup>-1</sup>  $\cdot$  min<sup>-1</sup> (mean  $\pm$  SD) and the maximum HR was 178.5  $\pm$  9.9 bpm.

### *Location and Duration of the Study*

The study was conducted at the military base of the Croatian Navy Forces during a 1-wk period. Divers were transported by powerboat to a location within 10 min of the base's vicinity. The exact location was chosen based on its suitability to perform dives of the desired depth and duration. Bottom sea temperature and at the decompression stop depth was 15°C for all dives, while ambient temperature varied between 15–17°C.

### *Diving Protocol*

All dives were performed by divers equipped with wet suits in accordance with the Croatian Navy and

U.S. Navy diving manual (25). Maximum depth was set to 30 m. Divers were supplied with a diving computer (Mosquito, Suunto, Vantaa, Finland) interfaced with a personal computer for later verification of the diving profile. Divers were instructed to swim a distance of 500 m once they reached the maximum depth that was controlled by the personnel on the powerboat, mimicking 30% of their  $\dot{V}_{\text{O}_{2\text{max}}}$ . During the decompression period, divers were instructed not to perform any exercise, since we have shown previously that mild exercise during decompression reduces post-dive bubble formation (10). Ascent rate was set at 9 m  $\cdot$  min<sup>-1</sup> with a decompression stop at 3 m for 3 min. This protocol was chosen because, from our experience, one can reliably induce a significant amount of venous bubbles by exercising, even if the proper decompression procedures are followed. HR was continuously monitored in all divers during their dives with the Polar S810i HR monitor.

### *Post-Dive Monitoring and Bubble Analysis*

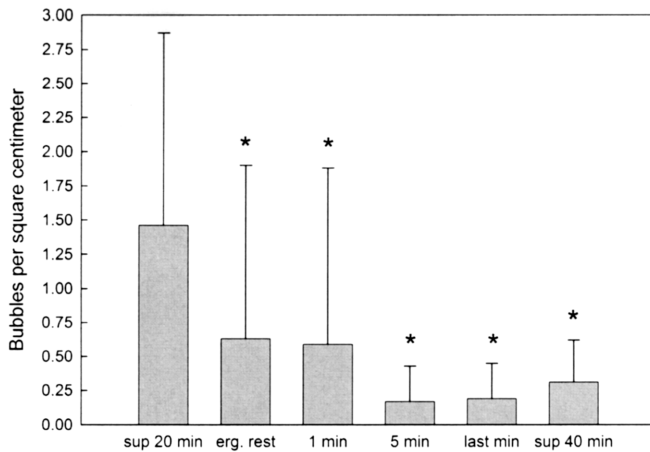
After completion of the diving protocol, divers were transported to a facility where they took a brief shower before further monitoring. Following the shower, subjects were placed in the supine position and an echocardiographic investigation with a phase array probe (1.5–3.3 MHz) using a Vivid 3 Expert ultrasonic scanner (GE, Milwaukee, WI) was conducted. All echocardiographic investigations were performed by the same experienced cardiologist. High quality images were obtained in all subjects and stored on S-VHS tapes for later evaluation and analysis. Monitoring was performed in the supine position within 20 min of reaching surface pressure. Next, subjects were transferred to a cycle ergometer, and bubbles were monitored in an upright, seated and resting position, and then during the incremental protocol up to 85% of  $\dot{V}_{\text{O}_{2\text{max}}}$ . This exercise protocol lasted between 30–40 min after diving. We selected this time period because we have previously shown that bubble formation is greatest at this time after diving (9). Each diver started cycling at 50 W for 1 min. Exercise intensity was increased by 25 W each minute until they reached 250 W. During cycling, high-quality images of both the right and left atria and ventricles were obtained in all subjects in order to determine if there was a transfer of venous bubbles through the pulmonary circulation. Images were graded as previously described (12). Detailed information about this technique is presented elsewhere (9).

### *Statistical Analysis*

All data are presented as mean  $\pm$  SD. Due to the small sample size ( $n = 7$ ), the non-parametric Wilcoxon matched pairs test was used to examine changes in venous bubble formation post-dive at the different time-points. Statistical significance was set at  $p < 0.05$ . All analyses were done using Statistica 7.0 software (Statsoft, Inc., Tulsa, OK).

## RESULTS

All seven divers successfully completed the designed protocol and none developed any signs/symptoms of



**Fig. 1.** The average venous gas bubble number per cm<sup>2</sup> for all seven divers. It is clearly visible that the shift from supine position to upright posture on the cycle ergometer immediately reduces the number of detected bubbles. Sup 20 min – number of bubbles 20 min after reaching the surface in supine position; erg. rest – number of bubbles during rest on the cycle ergometer; 1 min, 5 min, last min – number of bubbles during first, fifth, and last minute of exercise on cycle ergometer; sup 40 min – number of bubbles 40 min after reaching surface in supine position. Values are mean ± SD. \* p < 0.05 compared with sup 20 min.

DCS. Twenty minutes post-dive the average number of bubbles was  $1.46 \pm 1.41 \cdot \text{cm}^{-2}$ . Postural change from supine to the seated upright position resulted in a significant reduction of bubbles to  $0.63 \pm 1.27$  bubbles  $\cdot \text{cm}^{-2}$  ( $p = 0.043$ ), while at the end of exercise a further reduction to  $0.19 \pm 0.26$  bubbles  $\cdot \text{cm}^{-2}$  was found ( $p = 0.02$ , from baseline, **Fig. 1**). During exercise, bubble grade did not change significantly ( $0.59$  vs.  $0.19$  bubbles  $\cdot \text{cm}^{-2}$ ,  $p = 0.11$ ). Resumption of the supine position after exercise did not cause further changes. Duration of exercise on the cycle ergometer lasted approximately 10 min. No cases of intra-pulmonary shunt were observed during or following post-dive exercise.

## DISCUSSION

Exercise, which is an inherent part of professional military and commercial diving, is considered a risk factor for DCS (5). Open-sea diving is associated with a moderate level of exercise depending on sea currents, temperature, and stress, among other things. Intensive exercise immediately before diving may produce microscopic muscular injuries, which may promote bubble formation (26). We recently found that a single bout of interval exercise 24 h before a dive significantly reduces the number of bubbles in the right side of divers' hearts (9). These results are in concordance with previous studies performed in rats (28). The uptake and elimination of inert gas is dependent on blood flow. Exercise at the maximum depth of a dive will increase blood flow and consequently nitrogen uptake because of active hyperemia in skeletal muscles (13). With more nitrogen in the body the time for elimination of this additional nitrogen should be longer. Dick et al. (7) have shown that more nitrogen is indeed eliminated after a dive with exercise on the bottom phase, although Rademacher et al. (24) did not confirm this finding. Muth et al. (21) have reported that moderate exercise

(100 W cycling for 30 min) starting 10 min after a shallow non-decompression simulated dive to 20 msw for 20 min causes increased nitrogen elimination lasting up to 2 h after the dive. They have speculated that the exercise-induced increase in nitrogen elimination could lead to an increased chance of bubble formation, although they have previously shown that even with simulated dives to 50 msw for 30 min no intravascular bubbles were detected with precordial Doppler (24). Mild exercise during a decompression stop increases gas elimination via increased alveolar ventilation, and it reduces the number of venous bubbles detected after diving (10,18). The positive effects on venous bubble formation of a rather short 3-min exercise period during a decompression stop suggest that the nitrogen pool for bubble formation is coming from the "faster" tissues (10).

Webb et al. (27) recently reported that moderate dynamic exercise (three times 15-min dual cycle ergometry) after high-altitude exposure does not result in delayed onset of DCS or recurrent DCS. Their study was prompted by anecdotal reports of DCS presumably arising from post-exposure exercise that had prompted U.S. Air Force personnel to avoid exercise soon after altitude exposure. Previously, Pollard et al. (23) have shown that post-dive exercise after a simulated dive to 80 msw for 2 h in the rat is significantly associated with more DCS. However, this decompression stress is much larger than the diving profile used in the current study (dive to 30 msw for 30 min), with only one decompression stop at 3 msw for 3 min. We have shown that post-dive exercise of the same intensity and duration as that used in the present study does not increase the risk for developing DCS (11).

This study is the first to investigate the relationship between post-dive exercise and venous bubble formation. Our subjects exercised at  $\sim 85\% \dot{V}O_{2\text{max}}$  for about 10 min (from 30 to 40 min) after diving because we previously found the highest bubble grade forming at that time (9). In this study, we have found that post-dive strenuous exercise is associated with an 8-fold reduction in bubble formation, in contrast to our hypothesis that it would promote venous bubble formation based on the previous reports of increased nitrogen elimination with post-dive exercise after simulated dives to 20 msw (21). This finding was unexpected and the mechanism is presently unknown, although simulated and field diving cannot be easily compared when the additional stressors present in a real diving situation are factored in. The other possible difference could be the rather high intensity of exercise used in this study vs. moderate exercise in the other report (21). The most likely cause of reduced bubble formation during post-dive exercise is the increased blood flow that occurs during exercise, which may cause a "wash-out" of bubble nuclei at the surface of endothelial cells. Bubble nuclei are probably needed for bubble formation, and in the vasculature, it is most likely that crevice-based bubbles (e.g., at the endothelial cell junctions) produce the bubbles that result in emboli. If these bubbles were in fact "washed away," the crevice-stabilized nuclei would most likely still be present and able to regenerate

more bubbles after the cessation of exercise, but the time-course of this is not certain. Thus, the true cause of this reduction remains elusive and very poorly understood, and other effects of exercise must be considered as well in future studies (e.g., NO, heat shock proteins, etc.).

Despite the high intensity of the exercise in this study, no cases of DCS were seen, although the sample size was too small to make any conclusions. The change in post-dive posture from supine to upright and seated resulted in a significant reduction of bubble formation from 1.5 to 0.6 bubbles · cm<sup>-2</sup>. This finding was unexpected, and we do not know the mechanism. Possibly this finding is due to a posture-induced decrease in cardiac output (of about 20%) (20), or the reduced post-dive cardiac output of a similar degree (8). Both effects should decrease the return of peripheral gas bubbles toward the central vascular pool, where the bubbles were monitored.

An association between adiposity, aerobic fitness, and bubble formation has been recently suggested. Carturan et al. (3) have shown that aerobically fitter divers produced fewer bubbles after field dives than older, fatter, or poorly physically fit recreational divers. Our military divers were in excellent physical condition based on the ability to withstand a workload in the cycle ergometer between 275 and 375 W, but their measured maximal oxygen uptake was in the moderate range (around 42 ml · kg<sup>-1</sup> · min<sup>-1</sup>). In our previous study with 13 recreational divers, bubble formation was unrelated to the diver's age, body mass index, or maximal oxygen uptake (9), although this finding was limited because of the small sample size, while Carturan et al. (3) investigated this issue in 50 recreational divers. This fact is even more accentuated in the current study with only seven divers.

#### Study Limitations

This study was performed on a limited number of divers and, therefore, results must be viewed cautiously before giving divers recommendations to exercise freely immediately post-diving, especially in view of recently reported post-dive reductions in cardiac output and gas exchange (8), right and left heart systolic function (19), and arterial endothelial function (2). Transportation of divers in a powerboat from the diving site could have potentially reduced the number of bubbles seen at first observation. Moreover, our results may be considered limited due to the use of only one type and intensity of exercise after diving.

#### Conclusions

No signs/symptoms of decompression sickness were found with short periods of strenuous exercise after a single field open-sea dive in this study. These results indicate that post-dive upright cycle exercise reduces gas bubble formation in well-trained military divers. Additional findings are needed for normal sports divers.

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