Increased oxygen before and during decompression reduces bubble formation in rats

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Skogland S, Stuhr LEB, Sundland H, Marstein S, Hope A, Increased oxygen before and during decompression reduces bubble formation in rats. Undersea and Hyperb Med 2003; 30(1): 36-45 - The aim of this study was to test the hypothesis that increased oxygen partial pressure shortly before and during decompression from hyperbaric pressures would decrease venous gas bubble formation. Bubbles were detected by an ultrasound Doppler technique in conscious, freely moving rats. All rats were exposed twice to 6 bar for 2 hours. In exposure A, the breathing gas mixture was 1 bar O_2 and 5 bar N_2. In exposure B, the breathing gas was changed to 2 bar O_2 and 4 bar N_2, 5 min prior to decompression. The decompression rate was 0.1 bar $\cdot$ s^{-1} in both groups. Significantly fewer bubbles were detected after decompression in exposure B compared to A. The angiotensin converting enzyme (ACE) concentration in serum was measured as an indicator of possible damage to the pulmonary endothelium induced by bubbles. However, no correlation between ACE and bubble amount was found. In conclusion, this study in conscious rats indicates that safer decompression may be obtained by increasing the oxygen partial pressure before and during decompression.

ultrasound, Doppler, bubbles, hyperbaric exposure, angiotensin converting enzyme

INTRODUCTION

The main cause for decompression sickness (DCS) is the development of bubbles both in the blood and/or in the tissue (1,2). Gas bubbles produced by decompression have been reported in several animal studies (3-6) and in humans (7,8). Asymptomatic "silent" venous bubbles (not inducing DCS) have been observed (8-10), and may have both biological and clinical relevance (10,11). Since silent bubbles occur in dives, it is of interest to evaluate the factors that may influence such bubble formation. In this way, one may be able to improve existing decompression procedures and thereby avoid DCS.

Previous studies have concluded that surface oriented working dives in the range of 30-50 msw (4-6 bar) may result in a higher incidence of DCS than shallower dives (12). These

observations prompted a Norwegian research program to develop safer and more effective diving tables in this depth range. The present study is one of the activities in this program. However, experimental decompression studies on humans where the risk of DCS is high should be limited and animal studies and mathematical models pursued first.

Gas bubbles produced by decompression have been reported in rats (3,13). Since we had relevant experimental experience with this animal (6,14-16), we established a method for detecting circulating gas bubbles in freely moving rats using the ultrasound Doppler technique (3,6). This method has the advantage of detecting differences in the decompression stress and bubble load at sub-DCS level, with a reduced risk of paralysis or death of the animal. Additionally, fewer animals were required and repeated measurements on the same animal were possible. Although the method used does not detect all the bubbles produced in the body, the vena cava probe measures bubbles originating from the hind part of the body. This may include intravascular bubble formation in tissues such as muscle, skin, subcutaneous fat, bones etc.

The main objective of this study was to evaluate differences in bubble formation after breathing 1 bar O_2 or 2 bar O_2 5 min prior to decompression, and with concomitant oxygen percentages of 16.7 or 33.3% during linear decompression. Since it has been observed that "silent" bubbles may release angiotensin converting enzyme (ACE) from pulmonary endothelial cells in humans (9), we also wanted to determine whether serum ACE and bubble amount correlated in our rat model.

METHODS

Animals

Eight male albino Wistar rats of the same age and weight (approximately 250 g) were used for the bubble measurements. Additionally, 6 rats were sham-operated and 4 rats were normal controls for angiotensin converting enzyme (ACE) experiments. The rats were kept 4 and 4 together in cages in 12/12 hours day/night cycles. The animals had free access to standard rat food and water. The experimental procedures were approved by the Norwegian Experimental Animal Board and the Animal Use Committee at the University of Bergen.

Pressure chamber

A pressure chamber of 130 l, with an internal diameter of 50 cm was used. For inspection and video supervision, there were two windows in the chamber wall. Penetrators for gas in-/outlet, and electrical signals/power support, were run through the chamber rear end. A pressure transducer (Tronic line 891.13.500, 0-10 bar, Wika Alexander Wiegand, Klingenberg, Germany) was placed outside the chamber. This transducer had a resolution of 0.05 bar in the pressure range from 1 to11 bar. Temperature readings were recorded by a chamber thermistor (EUS-U-V5-0, Grant Instruments, Cambridge, England). Heating of the chamber was obtained by circulating hot water in a copper tube coiled around the chamber. A water heater, with a reservoir and a circulating pump (Hetofrig CB7 and 03PF623 Heto Lab. Equipment, Denmark), delivered the hot water. The oxygen partial pressure inside the chamber was measured with an oxygen cell (C3, Middlesborough, UK). The chamber atmosphere was mixed using a 12 V fan (Sprofona SJ-80Y12A, Seiko Electronics, Japan). CO_2 content was kept low by use of a scrubber material (Sodasorb; Molecular Products United Drug Co Ltd, Essex, UK).

Doppler measurements and bubble scoring

Detection of gas bubbles in the blood vessels using the ultrasound Doppler technique has been described previously (3,4,6,17). An ultrasound Doppler flow meter (EME Pioneer TC 2020, Eden Mediziniche Elektronik, Uberlingen, Germany) was used in the 20 MHz pulsed mode, designed for invasive extravascular, high quality, Doppler registration of blood flow velocity. A 20 MHz ultrasound probe (DBF120A-CP-4.0 Crystal Biotech, Hopkinton, MA) was used with a 1 mm piezoelectric crystal placed in a C-formed silastic cuff. The distance between probe and ultrasound unit was kept as short as possible by placing the pre-amplifier inside the chamber approximately 40 cm from the probe.

The ultrasound Doppler system uses a FFT (Fast Fourier Transform) analysis of the flow signal. A high quality signal is therefore vital to evaluate an experiment. The threshold (optional from 1-64 dB) had to be fitted individually in each experiment, depending of the signal intensity of the actual flow signal. Each sudden increase in signal intensity was automatically saved and numbered as an event. Simultaneously the audible bubbles were recorded manually. The observation time was from 10 minutes before decompression to 1 hour after decompression. Bubbles were quantified as bubble grade (Tables 1 and 2) or as the number of bubbles per 30 sec (Table 2, Fig. 2). In experiments with high bubble grades, the single detections could cover more than one bubble and scores should be considered as conservative estimates of actual bubble number. In low bubble grade experiments the estimate will reflect the correct amount. All ultrasound Doppler signals were recorded on a DAT recorder (Sony DTC-55 ES) for post experimental bubble scores.

Table 1: Grades of bubbles as a measure of decompression stress.

Grade	Bubble amount
0	No bubbles
1	<1 bubble·s^{-1}; sporadic occurrence
2	<1 bubble·s^{-1}; continuous occurrence
3	1-5 bubbles·s^{-1}; continuous occurrence
4	>5 bubbles·s^{-1}; continuous occurrence in periods (bubble showers)
5	Recompression necessary; (V. cava blood flow decreased markedly)

Modified from Spencer and Clarke (8)

Anesthesia and surgery

The rats were anesthetized with Hypnorm-Dormicum in a solution of sterile water, given sc in a dose of 0.25 ml $\cdot 100^{-1}$ g. The ultrasound Doppler probe was implanted on the posterior vena cava, caudal to the renal veins, through a 1.5 cm right flank incision. Two stitches were placed in the soft silastic cuff to hold the Doppler probe in place on the blood vessel. The probe wires were connected to a contact (ERA 00250 CTL, Lemo, Ecublens, Switzerland) mounted in a silicone plate (approximately 2 cm in diameter), which was placed subcutaneous in the neck region. The Doppler flow signal was checked to be satisfactory prior to closing the incision by stitching up the muscle layer. Thereafter the wire was coiled up under the skin before closing the skin wound. The rats were then given 0.2 ml Temgesic, buprenorphinum 0.3 mg $\cdot$ ml^{-1} (Reckitt & Colman), for analgesia.

The habitat

A special small habitat was constructed to obtain a rapid change in the breathing gas composition from 1 bar to 2 bar O_2 and to minimize fire risk. The habitat was an acrylic cylinder, 25 cm in diameter and 42 cm in height. When this cylinder was closed, there was no direct connection between the atmosphere inside the habitat and chamber atmosphere. The two gas lines - one used for air or nitrogen injection, the other for oxygen - were connected to the habitat. During pressurization, the habitat was pressurized but an equalizing valve maintained equal pressures inside habitat and chamber. When the predetermined pressure was reached, the valve closed automatically and there was no further gas exchange. Inside the habitat there was a separate fan (Sprofona SJ-80Y12A, Seiko Electronics, Japan) and CO_2 scrubber (Sodasorb; Molecular Products United Drug Co Ltd, Essex, UK). Temperature and O_2 recordings were also made from the habitat in the same way as from the pressure chamber.

During decompression, the exhaust valve in the pressure chamber was opened. When the pressure inside the habitat exceeded the chamber pressure, the equalizing valve opened and the pressure was equalized.

During O_2 injection, the total pressure in the system was compensated through the exhaust valve. Shifting from 1 to 2 bar O_2 could be done in about two minutes. When the O_2 pressure was lowered from 2 to 1 bar, the system was flushed with air. To lower the risk of fire, no bedding was used in the bottom of the cage during the 2 bar O_2 experiments.

Experimental procedure and pressure profiles

A flexible cable was connected to the implanted contact in the rat and to a swivel (Lehigh Valley Electronics, USA), which enabled the rat to move freely. The other end of the swivel was connected to the ultrasound equipment via a penetrator through the chamber wall. The pressure chamber was then closed. Each rat was exposed twice (exposure A and B) 2 days apart in random order, and all rats were pressurized to 6 bar. The rat was kept at this pressure for 2 hours. In exposure A, nitrogen was injected instead of air when the oxygen pressure reached 1 bar to obtain a breathing gas mixture of 1 bar O_2 and 5 bar N_2. In exposure B, O_2 was injected 5 min before decompression in order to obtain a nitrox breathing gas mixture consisting of 2 bar O_2 and 4 bar N_2 (Fig. 1). The decompression rate was 0.1 bar $\cdot$ s^{-1}. A 3-min stop was performed at 1.5 bar. The gas was switched back to air immediately after decompression to 1 bar. The pressure and gas profiles are shown in Figure 1.

ACE measurements

Anesthesia (Hypnorm-Dormicum) was administered subcutaneously following ultrasound measurements in the last experiment. A 2.5-ml blood sample was drawn from the femoral artery via a P-50 catheter, and centrifuged at 3000 rpm for 15 min. 1 ml serum was used for the ACE measurements with 3-(2-furylaryloyl)-L-phenylalanyl-glycylglycine (FAPGG) as substrate. A detailed description is reported by Johansen et al. (18).

Statistics

The Wilcoxon signed Rank test and a t-test were used for paired comparison of exposure A and B. A significance level of 95% ($p<0.05$) was considered statistically significant. Values are presented as means ± standard deviation.

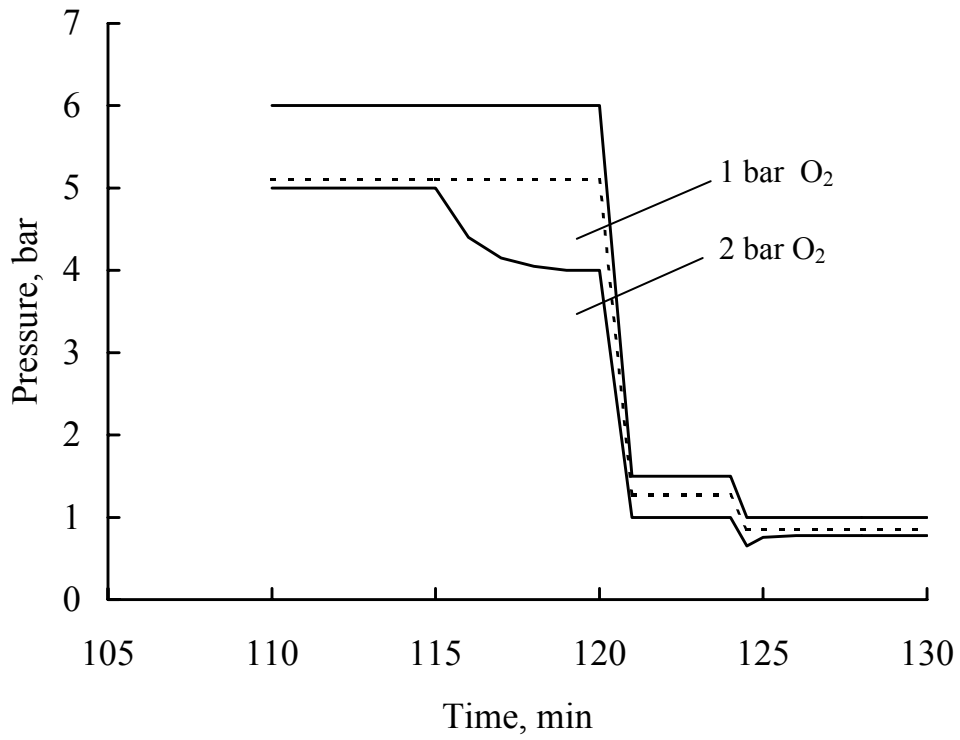

Figure 1. Ambient pressure (upper solid line), and partial pressure of nitrogen during 1 bar O_2 (dashed line) and 2 bar O_2 (lower solid line) before, during and after decompression.

RESULTS

Bubble production showed great scatter among the animals. Of a total of 16 exposures, 12 produced bubbles (Table 2). Three of the four experiments without bubbles were in the 2 bar O_2 group. Four rats had significantly fewer bubbles after the 2 bar O_2 exposure, and two rats had approximately similar amount of bubbles in both exposures. Two rats in exposure A (1 bar O_2) had bubble grade 5 (Table 2) and were recompressed 22 and 24 minutes after decompression. The maximal bubble amount of every 30 s period is shown in Fig. 2. At 40 min post decompression cumulative bubble detection was 5087 ± 6088 and 852 ± 1894 bubbles, (average

± SD) in exposure A and B respectively ($p<0.05$, Wilcoxon signed ranks test, Table 2). The difference in bubble grade was not statistically significant (2.9 ± 2.0 and 1.3 ± 1.5, Table 2). Bubbles occurred within 2.5 min post decompression in 50% of the animals with bubbles. After 13.5 min all bubble positive animals bubbled (Table 2).

Table 2: Maximum bubble count in 30 s periods, cumulative bubble amount at 40 min, and bubble grade following decompression from 6 to 1 bar in the two different nitrox mixtures.

Animal no.	1 bar O_2 ("Air")			2 bar O_2 ("Nitrox")		
	Maximum	Cumulative	Bubble grade	Maximum	Cumulative	Bubble grade
1	249	7046	4	383	5427	4
2	487	16241	4	6	112	1
3	354	10964	5	82	1205	3
4	4	25	1	0	0	0
5	5	18	1	4	49	1
6	61	714	3	0	0	0
7	0	0	0	0	0	0
8	350	5691	5	4	25	1
Average	189	5087	2.9	60[1)]	852[2)]	1.3[1)]
SD	195	6088	2.0	134	1894	1.5

Wilcoxon signed ranks test: [1)] NS vs 1 bar O_2 [2)] $p<0.05$ vs 1 bar O_2

Animals with bubbles (group A and B) had an average ACE concentration of 159.6 ± 20.6 $mU \cdot ml^{-1}$ compared to 222 ± 34.1 $mU \cdot ml^{-1}$ in control rats ($p<0.05$). In sham-operated rats an insignificantly lower ACE concentration was observed (168.5 ± 32.5 $mU \cdot ml^{-1}$).

DISCUSSION

The present study indicated that rats rapidly decompressed from 6 bar produced fewer bubbles when the oxygen tension was doubled immediately prior to and during decompression.

The time period from surgery to the last experiment never exceeded 5 days. By exceeding this period of time, we have previously observed local inflammation around the silicon plate in the neck region. In addition, the Doppler signal may be lost due to adipose and connective tissue growth in the area between the vessel wall and the crystal in the probe.

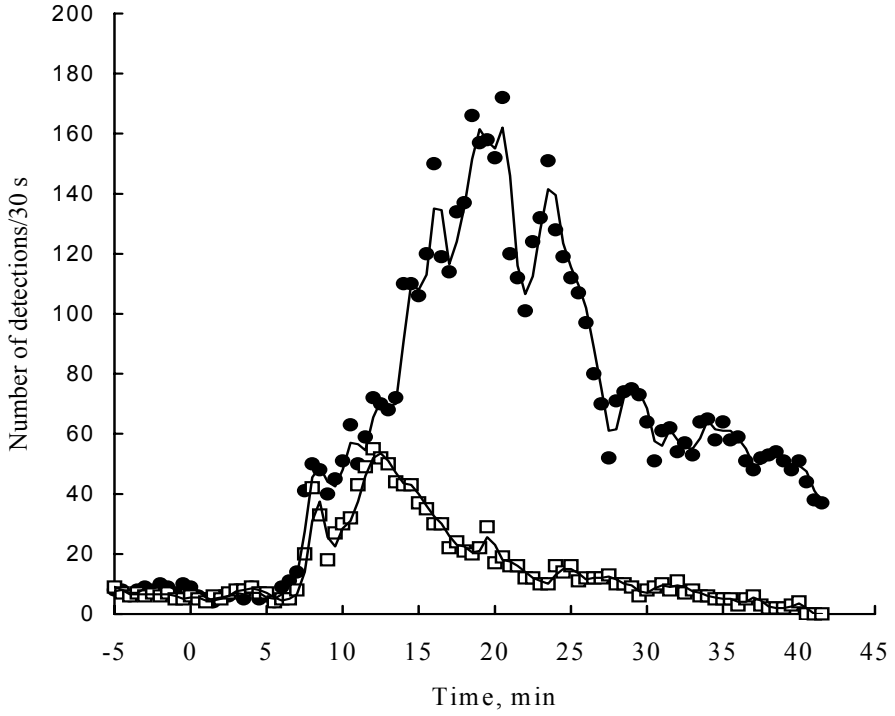

Figure 2. Average number of bubbles, determined as detections per 30 sec, after decompression from 6 bar with 1 bar O_2 (closed symbols) or 2 bar O_2 (open symbols). The lines are trend lines.

The possibility of repeated exposures of a single rat by this method has great advantages. The rat was its own control and the number of animals used may thereby be reduced to a minimum. Acclimatization to decompression stress may occur according to Eckenhoff and Hughes (19). This has been attributed to crushing of bubble nuclei by large pressure increases before the actual experimental pressure reduction. However, in two rat studies (3, 16) no difference in bubble threshold between 1st and 2nd exposures was observed. To eliminate possible acclimatization effects in our study, 4 rats were first exposed to 1 bar O_2 (exposure A) whereas the other 4 rats started with exposure B.

In the present rat study, we could not copy human tables because the smaller the animal, the greater the pressure-reduction required for gas bubbles and DCS symptoms (3). This is due mainly to differences in physiological parameters like heart rate, body weight and respiratory exchange (7). Both absolute pressure reduction and the decompression rate in the present study were therefore significantly greater than that normally producing bubbles and DCS in man. For bubbles to form during rapid decompression, an approximately 4 to 1 ratio in pressure reduction can be found comparing rat and man (2,3,7). According to Berghage et al. (7), there is a close

correlation between heart rate and body weight with respect to maximum saturation pressure from which an organism can be decompressed abruptly to surface. These two variables also correlate highly with $\Delta P_{ED_{50}}$, the pressure reduction from saturation necessary to produce DCS in 50% of the subjects/organisms. Thus, a $\Delta P_{ED_{50}}$ of 6 bar in the rat corresponds to 1.7 bar in man (7), confirming the 4 to 1 ratio. This relationship further suggests that the 5 min O_2 breathing period before decompression in our rat study would correspond to a 20 min O_2 breathing period in humans. The 3 min stop in rats at 1.5 bars would indicate a 12 min stop at the same depth in humans. Even fewer bubbles would be expected if the period was extended for O_2 enriched breathing. However, possible negative effects of prolonged elevated pO_2 should be kept in mind.

In operational diving, the recommendation to use high O_2 in combination with surface decompression tables would probably be limited to the decompression period. To obtain the same effect on bubble formation in humans, the total period of elevated O_2 would have to be much longer, and probably far from what would be suggested in human tables. The small, but statistically significant reduction in bubble formation detected in the 2 bar vs. 1 bar O_2 exposure in rats would probably not be detected in a human study using an identical O_2 breathing period.

Oxygen breathing has been shown to be an effective method of enhancing inert gas elimination from the tissues. The most probable reason for why fewer bubbles are observed at increased pO_2 is the concomitant reduced tissue nitrogen tension prior to decompression. Another important factor, influencing gas elimination, is the perfusion rate in the tissues. Different studies have shown that oxygen breathing reduces the cardiac output and induces vasoconstriction in different organs (20,21). Such vasoconstriction would tend to decrease nitrogen elimination. However, during the Doppler monitoring period the rats in both groups were breathing air and vasoactivity should not influence the results.

Berghage (7) reported that the time to onset of DCS symptoms post decompression was approximately 6 min in 50 % of rats exposed to a heliox gas mixture. In previous heliox experiments we observed that initial bubble appearance in 50% of the animals occurred within 1 min post decompression (16). The present findings in nitrogen-oxygen showing that 50% of the animals developed bubbles within 2.5 min post decompression would therefore indicate a delayed onset of DCS symptoms in air compared to that of heliox (7).

Changes in ACE have been postulated to be an index of damage to the pulmonary endothelium in both animals and humans (22,23). Since the present dive profile did induce gas bubbles, these bubbles could have damaged the lung endothelium. However, the results showed a significantly lower serum ACE content in animals with bubbles compared to normal control rats. This is in contrast to observations in a recent human study by Marabotti et al (9).

The present study concludes that increasing the pO_2/pN_2 ratio 5 minutes before and during decompression reduced the number of intravascular bubbles in freely moving rats.

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