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ORIGINAL ARTICLE

Oxygen pretreatment as protection against decompression sickness in rats: pressure and time necessary for hypothesized denucleation and renucleation

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Abstract Pretreatment with HBO at 300–500 kPa for 20 min reduced the incidence of decompression sickness (DCS) in a rat model. We investigated whether this procedure would be effective with lower oxygen pressures and shorter exposure, and tried to determine how long the pretreatment would remain effective. Rats were pretreated with oxygen at 101 or 203 kPa for 20 min and 304 kPa for 5 or 10 min. After pretreatment, the animals were exposed to air at 1,013 kPa for 33 min followed by fast decompression. Pretreatment at 101 or 203 kPa for 20 min and 304 kPa for 10 min significantly reduced the number of rats with DCS to 45%, compared with 65% in the control group. However, after pretreatment at 304 kPa for 5 min, 65% of rats suffered DCS. When pretreatment at 304 kPa for 20 min was followed by 2 h in normobaric air before compression and decompression, the outcome was worse, with 70–90% of the animals suffering DCS. This is probably due to the activation of "dormant" micronuclei. The risk of DCS remained lower (43%) when pretreatment with 100% O_2 at normobaric pressure for 20 min was followed by a 2 h interval in normobaric air (but not 6 or 24 h) before the hyperbaric exposure. The loss of effectiveness after a 6 or 24 h interval in normobaric air is related to micronuclei rejuvenation. Although pretreatment with hyperbaric O_2 may have an advantage over normobaric hyperoxia, decompression should not intervene between pretreatment and the dive.

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Introduction

A sudden or rapid reduction in ambient atmospheric pressure may occur during escape from a disabled submarine, the ascent from an aborted dive, or high altitude flight, and involves a serious risk of decompression sickness (DCS). It is widely accepted that DCS is caused by the formation of bubbles in gas-supersaturated tissues during decompression, and it is also accepted that decompression bubbles grow from pre-existing micronuclei in the tissues (Evans and Walder 1969; Tikuisis and Gerth 2003; Vann et al. 1980).

A novel mechanism was suggested by Arieli et al. (2002) for replacement of the resident gas in the micronuclei by pretreatment with oxygen, before the exposure to hyperbaric air and subsequent decompression. The rationale behind the theoretical use of hyperbaric or normobaric O_2 as pretreatment has been discussed previously, and was demonstrated by the emerging bubbles in the transparent prawn (Arieli et al. 2002, 2007b; Ertracht et al. 2005), by decompression sickness in the rat (Arieli et al. 2009; Katsenelson et al. 2007, 2009) and by precordial Doppler ultrasound in humans (Castagna et al. 2009; Landolfi et al. 2006). In short, the animal is exposed at the start of the dive to hyperbaric oxygen (HBO), at which stage it is suggested the resident gas in the hypothetical micronuclei will be replaced by oxygen. When this exposure is terminated and the oxygen is switched to air, the oxygen pressure in the animal's tissues will decrease. During this time, the oxygen from the micronuclei inside the tissues could be consumed by the animal and the micronuclei should shrink. Due to the

faster diffusion rate of oxygen and the proximity of the mitochondria, the oxygen will probably be consumed faster than nitrogen is loaded into the micronuclei. Thus, on decompression, these hypothetical micronuclei (though probably not all of them, due to their variability) could be below the threshold for bubble growth, as shown in the analysis of Tikuisis (1986). Therefore, fewer bubbles will be likely to emerge upon decompression, which should reduce the risk of DCS.

A quantitative description of this model is shown in Fig. 1, in which the parameters for the rate of gas exchange from a spherical microbubble having unit volume (volume = 1, $r = [3/(4\pi)]^{1/3}$) at a pressure of 101 kPa (1 atmosphere absolute, ATA) were calculated. When the O_2 pressure is increased from that of oxygen in air (21 kPa) to normobaric oxygen (101 kPa), the surface area of the microbubble does not change. With further elevation of the oxygen pressure, the microbubble is compressed and its surface area becomes smaller. The gas pressure gradient from the resident gas in the microbubble to the tissue, which is flushed with oxygen, increases linearly with the inspired oxygen pressure. The rate of gas exchange between microbubble and tissue is linearly related to the product of the surface area and the gas pressure gradient. Thus, as the oxygen pretreatment pressure increases, there will be faster elimination of the resident gas from the microbubble, mainly on the transfer from air to 101 kPa oxygen.

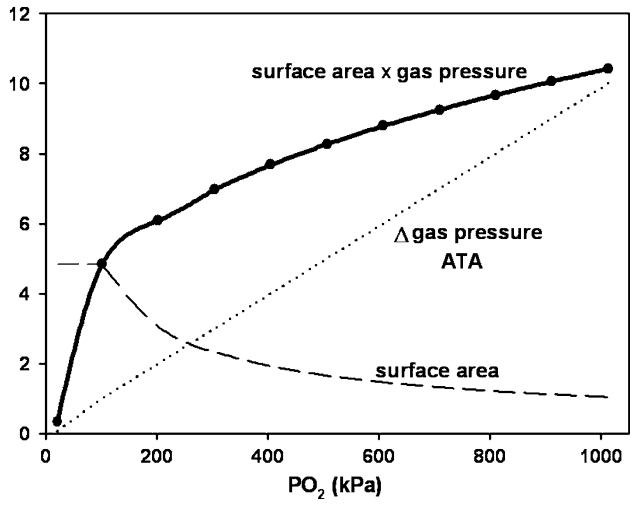


Fig. 1 Product of surface area and gas pressure (*solid line*; linearly related to the rate of gas exchange) for a spherical microbubble starting with unit volume (volume = 1) at 1 atmosphere absolute (ATA), as a function of inspired oxygen pressure. Volume and surface area (*dashed line*) decrease as total pressure increases. Inert gas pressure difference (bubble minus inspired) increases linearly (*dotted line*) with the increase in total pressure. For appropriate scaling, surface area units are related to initial volume (arbitrary unit = 1 and therefore initial surface area is 4.84), pressure units are in ATA, and the product of these two parameters gives units of surface area × gas pressure difference

In a previous study (Katsenelson et al. 2007), rats were given oxygen pretreatment at a pressure of 300–500 kPa for 20 min. Pretreatment with HBO was chosen because in a previous study in prawn (Arieli et al. 2002), normobaric oxygen had proven less effective. However, because prolonged exposure to high pressures of oxygen carries a risk of oxygen toxicity in mammals, and because in certain situations the availability of oxygen and high-pressure facilities may be limiting factors, it would be of value to investigate whether shorter exposure to a lower PO_2 is effective in reducing the risk of DCS. The simple model in Fig. 1 predicts that the main effect may also be obtained by using normobaric oxygen. From the experience that has accumulated in our laboratory (Arieli and Hershko 1994; Arieli et al. 2001), no central nervous system oxygen toxicity was expected in any of the HBO exposures employed in our previous study (Katsenelson et al. 2007).

The present study was designed to examine the effectiveness of oxygen pretreatment at different pressures and duration and also to examine how long before the dive the oxygen pretreatment is effective.

Methods

DCS in rats was assessed using the rotating wheel method, which is based on previously reported studies (Arieli et al. 2007a; Katsenelson et al. 2007; Kayar et al. 1998; Lillo and Parker 2000). The effect of oxygen pretreatment pressures below 300 kPa was studied in *Series A*, the duration of oxygen pretreatment below 20 min in *Series B*, and renucleation time in *Series C*.

Animals

Eighty male Sprague–Dawley rats weighing 274 ± 11 g were used in *Series A* and *B*. In *Series C*, 88 rats were used in *Procedure A*, and 122 rats in *Procedure B*; these animals weighed 296 ± 13 g. The experimental procedure was approved by the Israel Ministry of Defense Animal Care Committee, and the rats were handled in accordance with the principles of laboratory animal care under the surveillance of a veterinary surgeon.

Experimental system

Exposures were conducted in a double-walled, thermoregulated metal cage which enables continuous observation of the animal. The ambient temperature was kept in the range 25–28°C. The exposure cage was placed in a 150-l hyper-

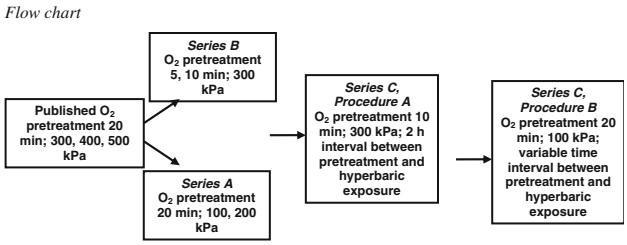
baric chamber (Roberto Galeazzi, La Spezia, Italy). A pneumatically operated cylindrical cage, which could be rotated at a speed of 3 m/min (Kayar et al. 1998), was used to diagnose DCS by observing the animals' gait and behavior following the exposure. The experimental system has been described in detail in a previous study (Katsenelson et al. 2007).

Experimental procedure

Before any exposure, the animal was placed in the rotating cage to ensure a normal motion pattern. Two animals at a time were placed in the exposure cage, which was placed in the hyperbaric chamber.

For normobaric hyperoxic pretreatment, the oxygen concentration inside the cage was increased until it reached 100% at atmospheric pressure. For hyperbaric hyperoxic pretreatment, the pressure was raised linearly to the desired level at a rate of 101 kPa/min using oxygen. In *Series A* and *B*, the oxygen was switched to air after the hyperoxic pretreatment and the pressure was increased linearly at 101 kPa/min to 1,013 kPa. Rats remained at this pressure breathing air for 33 min to achieve the inert gas loading which had resulted in decompression sickness in 65% of the control rats (Katsenelson et al. 2007). This exposure resulted in almost complete saturation of rats with nitrogen (time constant of −0.068 yields at 32 min 89% saturation, Lillo and Parker 2000), which ruled out any lasting denitrogenation effect. In *Series C*, after the hyperoxic pretreatment the rats stayed at normobaric level breathing air for a predetermined period, and was then exposed to 1,013 kPa for 32 min. This 1-min difference in exposure time brought the rat to the same calculated nitrogen load as in *Series A* and *B*. At the end of the hyperbaric exposure, animals were subjected to rapid decompression at 202 kPa/min. Following decompression, the rats were immediately placed inside the cylindrical cage rotating at ∼3 m/min for 30 min. The animal's motion pattern in the cage enabled the observer to make an early diagnosis of DCS according to the following symptoms: walking difficulties, abnormal breathing patterns, forelimb and/or hind limb paralysis, rolling around in the cage, convulsions, and death. The rats were checked again after 2 and 24 h (Lillo and Parker 2000). For the purpose of data analysis, the decompression results were scored as "No DCS" when there were no signs of DCS, "DCSrec" when the above-mentioned symptoms apart from death were observed but the animal always recovered, or "Died" when DCS symptoms culminated in death. The results were compared with those of the control group, which was exposed to hyperbaric air without hyperoxic pretreatment.

Experimental protocol



Series A and B: pressure and duration of oxygen pretreatment Twenty rats were used for each of four pretreatment protocols, in which a constant exposure time was combined with two different oxygen pressures, or a constant oxygen pressure with two different exposure times. In *Series A*, the rats were pretreated with HBO at 203 or 101 kPa for 20 min. In *Series B*, the rats were pretreated with HBO at 304 kPa for 10 or 5 min. In the Control protocol reported earlier (Katsenelson et al. 2007), the rats were compressed to 1,013 kPa on air for 32 min without HBO pretreatment and then decompressed. Three more experimental groups to be presented for the purpose of comparison later in this study were also taken from our previous investigation (Katsenelson et al. 2007). In these groups, the rats were pretreated with HBO at 304, 405, or 507 kPa for 20 min.

Series C: How long is denucleation effective? To determine how long the beneficial effect of oxygen pretreatment might last, it would first have to be determined whether hyperbaric or normobaric oxygen is the appropriate oxygen pretreatment, and a time interval would then have to be left between oxygen pretreatment and the hyperbaric air exposure. It can be seen from the model in Fig. 1 and the combined results from *Series A* and Ref. 17, that the effectiveness of denucleation increases to a certain extent when an elevated oxygen pressure is employed and when air pressurization starts immediately after the oxygen pretreatment. Decompression for the purpose of an interval in normobaric air may increase the potential for bubble production. When two identical dives were performed after a 2 h interval in normobaric air, with or without oxygen prebreathing, the bubble score as measured by precordial Doppler ultrasound was higher in the second dive (Castagna et al. 2009). Thus two opposing hypothetical processes would appear to be at work: denucleation by means of oxygen pretreatment and the transformation of "dormant" into effective nuclei (renucleation) by decompression. These two opposing features may operate if hyperbaric oxygen is used for pretreatment. We therefore investigated in *Procedure A* whether HBO would be appropriate for the study of renucleation.

Series C, Procedure A Because 1 year had elapsed between *Series A* and *B* and *Series C*, a further two groups

of 20 animals were tested in control conditions (without oxygen pretreatment) and with oxygen pretreatment at 304 kPa for 10 min. To evaluate the effect of hyperbaric oxygen, four groups (10–14 rats) were given oxygen pretreatment at 304 kPa for 10 min, followed by a 2 h interval in normobaric air before the hyperbaric air exposure. Whenever it appeared that the procedure was worsening DCS outcome, we discontinued that part of the experiment without continuing to the total of 20 animals. To rule out any possibility that the pretreatment might be inappropriate, we varied the final stage of the HBO pretreatment protocol in the four groups: (1) decompression on air; (2) decompression on oxygen; (3) the addition of 5 min on air at 304 kPa before decompression, (4) the addition of 20 min on air at 304 kPa before decompression. The last two profiles were chosen to have low oxygen periods at the end of the pretreatment for the consumption of oxygen from the hypothesized nuclei.

Series C, Procedure B In *Procedure A*, DCS outcome worsened in comparison with both control and the baseline oxygen pretreatment ("Results"; Table 2). Normobaric oxygen for 20 min was therefore chosen as the oxygen pretreatment. In *Procedure B*, after pretreatment with normobaric oxygen for 20 min, groups of 30 rats spent 0, 2, 6, or 24 h in normobaric normoxia, after which they were exposed to air at 1,013 kPa for 32 min.

Data analysis and statistics

Fisher's exact test was used to determine the relationship between the different pretreatment protocols and DCS outcome. The analysis includes the results of the present report, and a comparison with those obtained in a previous study (Katsenelson et al. 2007).

Results

All cases of DCS occurred within 30 min of decompression. When obvious signs of DCS appeared, the rat was immediately removed from the rotating cage. Most of the symptomatic rats which survived the 30-min observation period had recovered completely after 2 h and resumed normal walking, except for three animals which were symptomatic and died after 45 min, 2, and 4 h. Although animals were not specifically monitored for oxygen toxicity by recording electrical discharges in the EEG, no obvious symptoms of oxygen toxicity, which usually follow the first electrical discharge in the rat, were observed in any of the animals exposed to HBO.

Series A and B Table 1 shows the three outcome categories: No DCS, asymptomatic; DCSrec, symptomatic with later recovery; Died, symptomatic animals that died of DCS, for the four different hyperoxic pretreatment protocols in the present study (*Series A and B*), and for the control group and three additional hyperoxic pretreatment protocols taken from our previous investigation (Katsenelson et al. 2007) for comparison.

The trend toward a reduced percentage of symptomatic rats with increasing oxygen pressure, seen from normobaric hyperoxia at 101 kPa to hyperbaric oxygen at 507 kPa (Table 1, lines 4–8, left column), was not significant. Pretreatment at 101 or 203 kPa for 20 min in the present study reduced the number of symptomatic rats to 9, compared with 13 in the control group. These protocols reduced the mortality rate to 2 and 5, respectively, compared with 9 in the control group. There was no significant difference between these two hyperoxia-pretreated groups and the other HBO-pretreated groups (Katsenelson et al. 2007). Therefore the five hyperoxia-pretreated groups were combined into one, and compared with the control group. HBO pretreatment significantly reduced the number of symptomatic rats for the two categories asymptomatic and symptomatic ($P < 0.05$), and for the three categories No DCS, DCSrec, and Died ($P < 0.025$). Mortality from DCS was significantly lower in the hyperoxia-pretreated rats than in the control group ($P < 0.005$).

It can be seen from Table 1 that HBO pretreatment at 304 kPa for 5 min was less effective than the other oxygen pretreatment protocols, resulting in less asymptomatic rats

Table 1 Effect of hyperoxic pretreatment protocols on symptoms of DCS in rats, Series A and B

Experimental groups	No DCS (*n*)	DCSrec (*n*)	Died (*n*)	DCSrec + Died (% affected rats)
Control[a]	7	4	9	65
5 min oxygen at 304 kPa	7	7	6	65
10 min oxygen at 304 kPa	11	6	3	45
20 min oxygen at 101 kPa	11	7	2	45
20 min oxygen at 203 kPa	11	4	5	45
20 min oxygen at 304 kPa[a]	12	5	3	40
20 min oxygen at 405 kPa[a]	12	5	3	40
20 min oxygen at 507 kPa[a]	13	4	3	35

n, Number of rats; *No DCS*, DCS symptoms were not observed; *DCSrec*, DCS symptoms were observed, and the animals later recovered; *Died*, animals which died from DCS

[a] Results from Katsenelson et al. (2007)

Table 2 Effect of hyperoxic pretreatment at 304 kPa for 10 min on symptoms of DCS in rats, with a 2 h interval between pretreatment and the hyperbaric air exposure (Procedure A in Series C)

Experimental groups	No DCS (*n*)	DCSrec (*n*)	Died (*n*)	DCSrec + Died (% affected rats)
Control, no HBO pretreatment	8	4	8	60
Baseline HBO pretreatment: hyperbaric air compression follows directly from the HBO pretreatment	12	2	6	40
2 h at normobaric pressure between HBO and hyperbaric air exposure				
Decompression from pretreatment with air	4	0	10	71
Decompression from pretreatment with oxygen	4	0	10	71
5 min air at 304 kPa before decompression	1	1	8	90
20 min air at 304 kPa before decompression	3	1	6	70

n, number of rats; *No DCS*, DCS symptoms were not observed; *DCSrec*, DCS symptoms were observed, and the animals later recovered; *Died*, animals which died from DCS

Table 3 Effect of pretreatment with normobaric hyperoxia for 20 min, when a period of normobaric normoxia separated the oxygen pretreatment and hyperbaric air exposure (Procedure B in Series C)

	No DCS (*n*)	DCS rec (*n*)	Died (*n*)	DCSrec + Died (% affected rats)	Died (% affected rats)
Control[a]	15	8	19	64	45
Normobaric air interval after oxygen pretreatment, h					
0[b]	19	7	4	37	13
2	17	0	13	43	43
6	7	1	22	76	73
24	8	2	20	73	67

n, number of rats; *No DCS*, DCS symptoms were not observed; *DCSrec*, DCS symptoms were observed, and the animals later recovered; *Died*, animals which died from DCS

[a] Rats from Tables 1 and 2

[b] Rats from Table 1

and more dead rats. Oxygen pretreatment at 304 kPa for 10 or 20 min resulted in similar numbers of symptomatic rats. When the 10- and 20-min HBO pretreatment groups were combined into one and compared with the control group, it was found that HBO pretreatment significantly reduced the number of symptomatic and dead rats due to DCS for the three categories ($P < 0.05$). Mortality from DCS in the rats pretreated with HBO for 10 or 20 min (three of the total rats in each group) was significantly lower than the nine dead rats in the control group ($P < 0.025$).

Series C The results from *Procedure A* are given in Table 2. The outcome in the control group and for oxygen pretreatment at 304 kPa for 10 min was similar to the data in Table 1 (a difference of 1 between symptomatic and asymptomatic rats). The percentage of DCS for all four variations of the HBO pretreatment was higher than for the control and baseline oxygen pretreatments. There was no difference in DCS outcome for the four modes of decompression from HBO pretreatment when followed by a 2 h delay before hyperbaric air exposure, and these four groups were therefore combined into one for the statistical evaluation. The difference between control, baseline HBO treatment, and HBO pretreatment with a 2 h delay was significant for the three categories No DCS, DCSrec, and Died ($P < 0.0036$), for the two categories No DCS and DCSrec + Died ($P < 0.016$). DCS risk after a 2 h delay was even greater than in the control rats. This led us to conclude that HBO pretreatment followed by an interval at normobaric pressure is not beneficial.

The results from *Procedure B* are shown in Table 3. When normobaric oxygen pretreatment was followed immediately by hyperbaric air exposure, the percentage of DCSrec + Died was significantly reduced from 64% in the control group to 37% ($P < 0.05$), and the percentage of Died from 45 to 13% ($P < 0.019$). A trend toward a reduction in the percentage of symptomatic rats (DCSrec + Died) was still evident when a 2 h delay separated oxygen pretreatment and the hyperbaric air exposure ($P = 0.064$). However, there was no difference in the Died category between control and a 2 h delay, with a significant difference ($P < 0.010$) between a 2 h delay and immediate exposure after pretreatment. No beneficial effect was preserved when a 6 or 24 h delay separated oxygen pretreatment and the hyperbaric air exposure. A trend may be

seen toward an increased risk of DCS in these last two groups compared with control. The difference between these groups was not significant for symptomatic rats. However, there were more deaths (Died category) after a 6 h delay ($P < 0.016$) with a trend toward this after a 24 h delay ($P = 0.059$).

Discussion

A limitation dictated by the methods was the impossibility of determining any further subdivisions for the severity of DCS within the framework of the study protocol. A rat which showed signs of DCS on the rotating wheel was immediately removed. If the rat had been left on the wheel, its DCS would have become more severe. It was therefore impossible to compare rats which completed the run on the wheel with those which did not. Because all of the rats which suffered DCS and survived 2 h after decompression recovered with no residual effects after 24 h, there was no "permanent damage" category. For that reason, only three categories were chosen: No-DCS, DCSrec and Died.

This study supports the notion that pretreatment with HBO prior to inert gas exposure reduces the incidence of DCS after fast decompression in rats, and determines the most effective pressure and time for such HBO pretreatment. As will be seen from the comparison with previous results (Katsenelson et al. 2007), hyperoxic pretreatment at pressures ranging from 101 to 507 kPa appears to be effective in reducing the incidence of DCS in rats, and seems to gain in effectiveness with elevation of the oxygen pressure. However, the initial step in the progression to higher pressure, from control conditions to normobaric hyperoxia, provided the highest level of protection. In addition, to be effective, the pretreatment period should be longer than 5 min. Thus for the rat, the most effective pretreatment would perhaps be at 101 kPa for 10 min, with minimal use of oxygen to lower the risk of oxygen toxicity.

Pretreatment with hyperbaric oxygen was shown to reduce the severity of DCS in rats by reduction of β-integrin and white cell adhesion (Martin and Thom 2002). However, the present results of DCS occurring mainly at the end decompression point to gas phase effect rather than inflammatory one. If the beneficial effect on cell adhesion was the main effect, no worsening would be expected when decompression followed HBO. It therefore seems more reasonable to relate the effects of oxygen pretreatment to denucleation–renucleation mechanisms. Because rats attain more than 94% saturation during 9 min of compression plus 33 min at depth (Lillo and Parker 2000), the effect of denitrogenation should be negligible.

Pretreatment at 101 kPa in the prawn had almost no protective effect compared with 203 kPa (5), which differs from the present findings in the rat. This can be related to the vascular system in mammals, which transports the inspired oxygen to the vicinity of the microbubble, as opposed to the non-vascular system of the prawn.

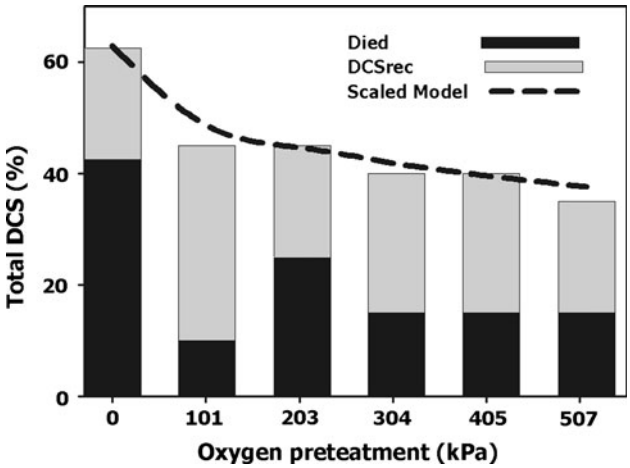


Fig. 2 Total incidence of DCS (symptomatic rats) among the control and hyperoxia-pretreated rats. The curve representing the rate of gas exchange from hypothetical micronuclei is scaled—3.2 × [20−(Pgas × surface)]—from surface area × gas pressure in Fig. 1, to fit one point of the data (pretreatment at 203 kPa oxygen) on the total incidence columns

If there is a hyperoxic pretreatment time which will completely eliminate the available effective population of gas nuclei, continuing pretreatment beyond that limit would not bring about any further reduction in the risk of DCS. It was assumed that shortening the HBO pretreatment period to less than 20 min would reveal this turning point. After HBO pretreatment at 304 kPa for 20 min (Katsenelson et al. 2007) and 10 min, the number of asymptomatic animals in a group of 20 rats was 12 and 11, respectively. After HBO pretreatment at 304 kPa for 5 min, the number of asymptomatic rats dropped to 7 as in the control group. It would therefore appear that the critical division between the periods for maximal effect and almost no effect in the rat is between 5 and 10 min.

In Fig. 2, the total incidence of DCS (symptomatic rats) is plotted for the control and hyperoxia-pretreated rats. It can be seen that normobaric oxygen pretreatment considerably reduces the incidence of DCS in rats compared with the control group, and that increasing the oxygen pressure results in a further reduction. When the theoretical rate of gas exchange was scaled from the model (surface area × gas pressure, Fig. 1) to one of the data points in Fig. 2, a good match was found between the theoretical line and the experimental results. This may be seen as additional support for the hypothesis on denucleation. Although a trend was noted toward a reduced risk of DCS with the increase in oxygen pretreatment pressure, this did not prove to be statistically significant. It is quite probable that

increasing the number of animals in each group would emphasize the differences. However, it was chosen not to increase the number of animals solely for that purpose. Bosco et al. (2010) have shown in divers that in-water oxygen prebreathing at a depth of 6 msw or 12 msw yielded lower bubble scores than prebreathing oxygen at the surface. Their findings agree with the evidence for increased protection against DCS as the prebreathe oxygen pressure increases.

The HBO pretreatment procedure for denucleation presented here may be a major step towards reducing the risk of DCS in humans. Three studies on humans pretreated with pure oxygen, at normobaric pressure for 30 min, 1.6 ATA for 45 min, and 1.0, 1.6, or 2.2 ATA for 20 min, are encouraging (Bosco et al. 2010; Castagna et al. 2009; Landolfi et al. 2006).

There may be an acclimation response on repeated exposure to high pressure, when the risk of DCS tends to decrease with the number of hyperbaric exposures. Walder (1967) suggested seven daily dives as the half-time for acclimation to diving. Eckenhoff and Hughes (1984) showed that 12 daily dives had no effect on venous bubbles, but itching was reduced. The risk of DCS tended to drop with repeated weekly hyperbaric exposures in rats (Arieli et al. 2007a). This acclimation may be related to a reduction of effective gas micronuclei. Daniels et al. (1984) calculated the half-time for gas micronuclei regeneration in the shrimp as 8–10 h. Castagna et al. (2009) showed in humans that oxygen pretreatment is effective in reducing the bubble count 100 min after treatment. A prolonged effect of denucleation may be of advantage in diving. Our experimental set-up was used to study renucleation time by selecting various intervals between oxygen pretreatment and the hyperbaric air exposure.

Although the efficacy of pretreatment may increase with elevation of the oxygen pressure (Table 1; Fig. 2), it may be to the diver's disadvantage if a period at surface pressure intervenes between pretreatment and the dive. In the present study, the severity of DCS increased beyond that observed in control conditions (no oxygen pretreatment) when there was an interval in normobaric air between HBO pretreatment and the hyperbaric air exposure (Table 2). Decompression from HBO pretreatment may cause ineffective gas nuclei to become effective, a process which can override denucleation to yield an eventual disadvantage. In repetitive diving, it might be expected that gas nuclei which grew into bubbles and filtered out by the lungs in the first dive will be the cause of a reduction in available nuclei for forthcoming dives. However, the opposite was the case; bubble scores were seen to increase in a sequence of dives (Castagna et al. 2009; Dunford et al. 2002; Hahn 1995). This may be explained by the proposed mechanism of expansion and activation of "dormant" gas nuclei, which in turn can explain why better protection was obtained in divers after normobaric oxygen pretreatment (Castagna et al. 2009) than after HBO at 1.6 ATA with a normobaric air interval before the dive (Landolfi et al. 2006). It is known that the risk of DCS increases with repeated dives, and that this may not be predicted by dive models (Douglas and Milne 1991; Dunford et al. 2002; Hahn 1995; Marroni 1995; Meliet 1995). Bubble score in the second dive on the same day is greater than in the first dive, but decreases with diving days (Dunford et al. 2002). All of this can be explained by the recruitment of "dormant" nuclei during decompression from the first dive and a consequent increase in the risk of DCS on the next dive, whereas with continued diving on the following days the store of "dormant" nuclei is depleted. This manifests as adaptation, with a reduced risk of DCS. Evidently, the findings of the present study suggest that HBO pretreatment is possible only if it is followed by exposure to increased pressure, but not by decompression. Normobaric oxygen pretreatment would therefore be preferable as a means of decreasing the risk of DCS following hyperbaric exposure (diving) or before high altitude flight.

We propose a number of mechanisms that may underlie oxygen pretreatment and influence the effectiveness of gas micronuclei (the potential for bubble growth). (1) Denucleation: oxygen replaces the resident gas and is later consumed, making the gas micronuclei ineffective. (2) Renucleation: due to decompression, which increases the volume of "dormant" micronuclei to render them effective. (3) Renucleation: during oxygen breathing, the inflow of oxygen into the micronuclei should be faster than the outflow of the nitrogen. This is because arterial oxygen delivery is higher than venous carrying capacity for nitrogen and blood oxygen permeability being 70% greater than nitrogen. Therefore, the volume of the micronuclei temporarily increases, thus rendering "dormant" micronuclei effective. (4) Renucleation: the accepted hypothesis of prevalence of gas micronuclei points to some unknown mechanism for their production. After disappearance of some effective gas micronuclei, this unknown natural mechanism may reproduce the basic level of the gas micronuclei. The effective oxygen pretreatment is the combination of oxygen pressure and time at which denucleation overrides the renucleation mechanisms.

It is preferable to have almost no interim normobaric air period between oxygen breathing and the hyperbaric air exposure (Table 3). The percentage of symptomatic rats as related to the normobaric air interval between oxygen breathing and the hyperbaric air exposure is shown in Fig. 3. When a 2 h normobaric air interval separated oxygen pretreatment and the hyperbaric air exposure, the percentage of symptomatic rats was similar to that obtained when there was no normobaric air interval and lower than

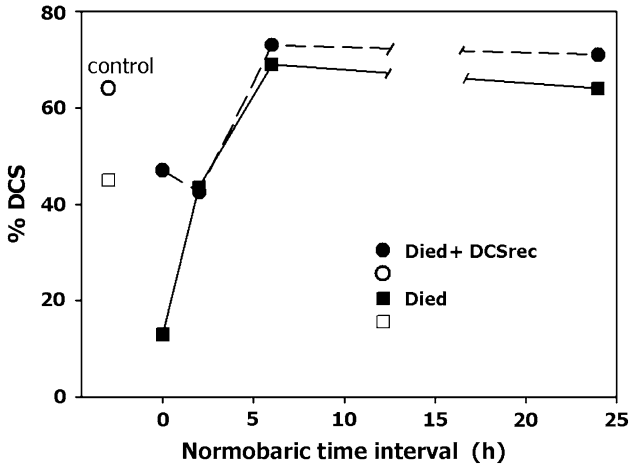


Fig. 3 Percentage of symptomatic rats (DCSrec + Died) and Died among the control (*open symbols*) and hyperoxia-pretreated animals (*full symbols*), as a function of the normobaric air interval between oxygen breathing and the hyperbaric air exposure

in the control rats. After 6 or 24 h, oxygen pretreatment had no beneficial effect and may even have increased the risk of DCS. However, the risk of the most severe outcome, death, was higher with prolonged normobaric air intervals of 6 and 24 h. An interval of 6 h probably resulted in regeneration of the effective gas micronuclei, as proposed in mechanisms 3 and 4. These findings are not far removed from the calculated half-time of 8–10 h for gas micronuclei regeneration in the shrimp (Daniels et al. 1984), and agree with effective oxygen pretreatment in divers, which lasted at least 1.7 h (Castagna et al. 2009). It therefore appears that diving immediately after oxygen breathing is most effective in reducing the risk of DCS.

In summary of the present results with reported studies on divers: breathing pure normobaric oxygen just before the dive could reduce the risk of DCS, and hyperbaric oxygen below toxic levels could add to the beneficial effect. A period of 20–30 min may be sufficient. However, no decompression should intervene between oxygen prebreathing and the dive.

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