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Combined effect of denucleation and denitrogenation on the risk of decompression sickness in rats

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Decompression sickness in the rat following a dive on trimix: recompression therapy with oxygen vs. heliox and oxygen

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Arieli R, Svidovsky P, Abramovich A. Decompression sickness in the rat following a dive on trimix: recompression therapy with oxygen vs. heliox and oxygen. *J Appl Physiol* 102: 1324–1328, 2007. First published December 28, 2006; doi:10.1152/jappphysiol.01195.2006.—Trimix (a mixture of helium, nitrogen, and oxygen) has been used in deep diving to reduce the risk of high-pressure nervous syndrome during compression and the time required for decompression at the end of the dive. There is no specific recompression treatment for decompression sickness (DCS) resulting from trimix diving. Our purpose was to validate a rat model of DCS on decompression from a trimix dive and to compare recompression treatment with oxygen and heliox (helium-oxygen). Rats were exposed to trimix in a hyperbaric chamber and tested for DCS while walking in a rotating wheel. We first established the experimental model, and then studied the effect of hyperbaric treatment on DCS: either hyperbaric oxygen (HBO) (1 h, 280 kPa oxygen) or heliox-HBO (0.5 h, 405 kPa heliox 50%-50% followed by 0.5 h, 280 kPa oxygen). Exposure to trimix was conducted at 1,110 kPa for 30 min, with a decompression rate of 100 kPa/min. Death and most DCS symptoms occurred during the 30-min period of walking. In contrast to humans, no permanent disability was found in the rats. Rats with a body mass of 100–150 g suffered no DCS. The risk of DCS in rats weighing 200–350 g increased linearly with body mass. Twenty-four hours after decompression, death rate was 40% in the control animals and zero in those treated immediately with HBO. When treatment was delayed by 5 min, death rate was 25 and 20% with HBO and heliox, respectively.

technical diving; helium; hyperbaric chamber

TECHNICAL DIVING USING TRIMIX (a mixture of helium, nitrogen, and oxygen) has become more widespread over the past two decades. Trimix has been used to reduce the time required for decompression on short, deep dives. Workman (20) stressed the reduced decompression time in trimix compared with air and nitrox diving, and Keller and Buhlmann (11) emphasized the advantage of decompression with heavy gases after short, deep dives. Berghage et al. (4) have shown that in short dives (15 min), decompression sickness (DCS) risk in rats is lower when a helium-nitrogen-oxygen mixture is used rather than a combination of oxygen and either one of the inert gases. Van Liew and Burkard (18) found in a theoretical study that peak bubble volume is lower in trimix than it is with helium or nitrogen alone. Trimix was adopted by the French Navy for short dives (the time from leaving the surface to starting the ascent must not exceed 20 min) to a depth between 45 and 80 m (1), and it has been used for short excursions to 80 m from nitrox saturation dives (22). Trimix has also been used to reduce the risk of high-pressure nervous syndrome, nitrogen narcosis, and oxygen toxicity in very deep dives (2, 3, 8, 17).

DCS is not common in trimix diving (5, 21). There are no general validated decompression procedures (7) and no specific recompression treatment for DCS resulting from trimix diving. There has been no investigation of hyperbaric recompression in rats with DCS after trimix diving. Because the load of two inert gases is involved, the efficacy of recompression treatment could vary depending on whether oxygen or heliox is used. The purpose of the present study was to validate a rat model for DCS following decompression from a trimix dive and to compare recompression treatment using oxygen or heliox.

METHODS

Animals

White male Sprague-Dawley rats were used. The experimental procedure was approved by the Israel Ministry of Defense Animal Care Committee, and the rats were handled in accordance with internationally accepted humane standards.

Exposure Cage and Experimental System and Procedure

Exposure cage. The exposure cage was a metal, double-walled cage (25 × 11 × 12 cm). One wall for observation of the animal and the top cover, which could be opened, were made of Plexiglas. Thermoregulated water was pumped through the double wall to control the ambient temperature. The incoming gas flowed through a metal container attached to the cage wall for temperature equilibration before entering the cage. A thermistor (Telethermometer YSI 400A, Yellow Springs Instrument, Yellow Springs, OH) was inserted through the top of the cage.

Experimental system. The exposure cage was placed in a 150-liter hyperbaric chamber (Roberto Galeazzi, La Spezia, Italy). The flow of trimix, oxygen, or heliox through the cage was controlled by a needle valve and by observation of a flowmeter situated inside the hyperbaric chamber. The outgoing gas exited via a bypass tube into the atmosphere of the hyperbaric chamber. A small portion of the outgoing gas was directed out of the hyperbaric chamber (this was controlled by another needle valve), passed through a flowmeter, and sampled for oxygen concentrations by an oxygen analyzer (Servomex, Sussex, UK). Water hoses were connected to ports in the hyperbaric chamber and to the ports in the exposure cage for recirculation of the thermoregulated water (C/H Temperature Controller Bath and Circulator 2067, Forma Scientific, Marietta, OH). The temperature inside the exposure cage was kept at 27°C.

A pneumatic rotating cylindrical cage was constructed from two commercial running wheels (diameter 21 cm, width 21 cm). A door was cut in each running wheel to enable the rat to be placed inside. The engine of a pneumatic drill was adjusted with transmission wheels and a rubber band to run the cylindrical cage, while the rotation rate was controlled using a pressure gauge. The cage was designed to operate both in normobaric and hyperbaric conditions.

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However, because of a limited supply of trimix, it was not used inside the hyperbaric chamber.

Experimental procedure. The rat was placed in the exposure cage inside the hyperbaric chamber. The animal was unrestrained, and it could move about freely inside the cage. The pressure in the chamber was raised at a rate of 180 kPa/min. During this procedure, until the desired pressure was reached, the gas mixture flowing through the cage was trimix. For each bottom pressure, the trimix yielded a nitrogen pressure of 405 kPa and an oxygen pressure of 142 kPa, with the balance helium. The rat was observed through a window in the hyperbaric chamber for any signs of distress. After decompression to atmospheric pressure, the experimental rats were given hyperbaric treatment, whereas control rats were left untreated. The rat was freed from the exposure cage and was placed inside the cylindrical cage rotating at a perimeter speed of ~3 m/min for 0.5 h. This was established to be a sufficient length of time for almost all cases of DCS to become evident (12). The rat was observed for signs of decompression sickness while walking for 0.5 h, as well as at 2 and 24 h after decompression (using the rotating cylindrical cage for a short period). When obvious difficulties were observed while walking in the rotating wheel, the rat was removed from the wheel for further observation. Signs of DCS according to Lillo and Parker (13) consisted of walking difficulties, abnormal breathing patterns, forelimb and/or hindlimb paralysis, rolling in the rotating wheel, convulsions, and death. Outcome was divided into three categories: "No DCS"; "DCS," DCS excluding death; and "Death," when signs of DCS culminated in death.

Experimental Protocol

Series A. In *series A*, we established the experimental model. We determined the exposure protocol, and we tried to find a combination of exposure time, depth, and decompression rate that would yield the desired percentage of DCS. The initial exposures were calculated using the algorithm suggested by Lillo and Parker (13) to result in 85% DCS. Later on, we changed the parameters of depth, exposure time, and decompression rate to achieve an ~50% risk of DCS. We also tested the effect of body mass and repeated exposure on the risk of DCS. There were four phases in this series. 1) In the initial exposures ($n = 17$), the body mass range was 150–400 g, bottom pressure 910–1,320 kPa, and decompression rate 100–10 kPa/min. 2) We carried out 78 exposures of 18 rats weighing 250–350 g to pressures from 1,110 to 1,320 kPa, with a decompression rate of 25–100 kPa/min. Rats were used once a week, provided they had no signs of DCS before the next exposure. From the results, we selected a pressure of 1,110 kPa, 30-min bottom time, and a decompression rate of 100 kPa/min as our model for the investigation to follow. 3) The effect of body mass on the risk of DCS was tested in 72 rats by a 30-min exposure to 1,110 kPa and decompression at a rate of 100 kPa/min. 4) The effect of repeated weekly exposures was further studied on 44 rats weighing 250–350 g exposed to 1,110 kPa for 30 min and decompressed at a rate of 100 kPa/min. A rat that suffered DCS was not used for any further exposures.

Series B. In *series B*, we studied the effect of hyperbaric hyperoxic treatment on DCS. Each protocol in *series B* was a consequence of the results obtained in previous protocols. In this series, we used rats in the weight range 250–350 g. The hyperbaric exposure was at 1,110 kPa (100 m of seawater) for 30 min, and decompression was carried out at a rate of 100 kPa/min. Hyperbaric treatment after decompression was either hyperbaric oxygen (HBO) at 280 kPa for 1 h (similar to US Navy Table 6, Ref. 16) or heliox-HBO, 0.5 h at 405 kPa on heliox (He-O₂ mixture, 50%-50%) followed by 0.5 h at 280 kPa on oxygen (similar to Table Cx 30, Ref. 10). There were five protocols. 1) Twenty rats were used for a control exposure, in which there was no treatment after decompression. *Protocols 2 and 3* tested the effect of HBO treatment immediately after a trimix dive or on the following trimix exposure, respectively. 2) Twenty rats were treated by HBO immediately after decompression from the hyperbaric exposure. Rats that survived without any residual signs of DCS were exposed to trimix again after being given a week to recover. 3) The rats that survived the control exposure (*protocol 1*) without signs of DCS were left for a week before a further trimix exposure followed by immediate HBO treatment. Because immediate HBO treatment had a 100% success rate, in *protocols 4 and 5* the treatment was given after a 5-min delay for the purpose of comparing HBO and heliox. 4) Twenty rats were treated by HBO 5 min after decompression to the surface from the trimix exposure. 5) Twenty rats were treated by heliox-HBO 5 min after decompression to the surface from the trimix exposure.

Statistical Analysis

A logistic model used to compare the three treatments and the control group in *series B* proved to be unsolvable. The Fisher exact test was therefore used to compare pairs of groups for the frequency of "no DCS" and "DSC+Death." Differences between the groups were examined for statistical significance at 2 and 24 h postdecompression.

RESULTS

Time Course of DCS in Rats

The course of the development of DCS is summarized in Table 1. The data are from 40 rats that had at least 1 symptom of DCS. Death occurred during the final stage of decompression in six rats. Peak incidence of death was during the 30-min period of walking (12 animals), mostly in the early stages. Four animals died in the interval between the end of the initial 30-min observation period and the examination conducted at 2 h. Only three animals were found dead 24 h after decompression. All of the remaining symptoms were more frequent during the initial 30-min observation period. The prevalence of all symptoms decreased markedly 2 h after decompression, and none was seen at 24 h. In no case was the first appearance of a symptom later than the 30-min period of walking.

Table 1. Prevalence of symptoms of DCS in 40 rats weighing 250–350 g exposed to 1,110 kPa for 30 min and decompressed at a rate of 100 kPa/min that suffered any symptom of DCS

Symptom	Immediately After Decompression	During 30 min Walking	2 h After Decompression	24 h After Decompression
Death	15	45	55	63
Hindlimb paralysis	10	45	3	
Walking difficulties		23	5	
Abnormal breathing patterns	3	25		
Rolling in the rotating wheel		18	3	
Convulsions	5	20		

Values are prevalence (in %) given for 4 stages of observation. Each number expresses the percentage of the whole group of 40 rats; only death is a cumulative percentage. DCS, decompression sickness.

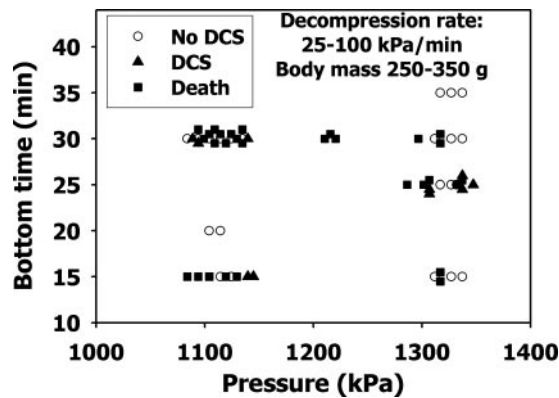


Fig. 1. Results from different trimix (a mixture of helium, nitrogen, and oxygen) exposures (bottom time, pressure, and decompression rate). Symbols are clustered around their pressure-time point to be discernible. DCS stands for any symptom of decompression sickness, excluding death.

Series A

1) In our initial efforts to find a trimix exposure that would yield 85% decompression sickness, we used Lillo and Parker's algorithm (13). However, we were unable to produce DCS using the exposure time calculated for 910 and 1,110 kPa. From these initial trials, it became clear that rats weighing <200 g are highly resistant to DCS, whereas heavy rats, ~400 g in weight, are very sensitive. We also noted that a slow decompression rate protected rats from DCS, and that almost no DCS occurred after decompression from 910 kPa. 2) We therefore exposed rats having a body mass in the range 250–350 g to pressures >1,100 kPa, followed by decompression at >25 kPa/min. The results are shown in Fig. 1. All three DCS categories are represented in the pressure and time ranges tested. Exposure for 30 min to 1,110 kPa with a decompression rate of 100 kPa/min gave ~50% DCS+Death, and it was selected for further assessment. 3) The percentage of DCS+Death in 72 rats with varying body mass is shown in Fig. 2. Rats with a body mass of 100–150 g had no DCS at all. The risk of DCS in rats having a body mass of 200–350 g increased linearly with body mass ($P < 0.0001$). In the DCS-sensitive body mass range, the risk increased by 8% for each 100-g increase in body mass. The effect of repeated exposures is shown in Fig. 3. Some of the rats were given HBO treatment after the first exposure, and are therefore excluded from the

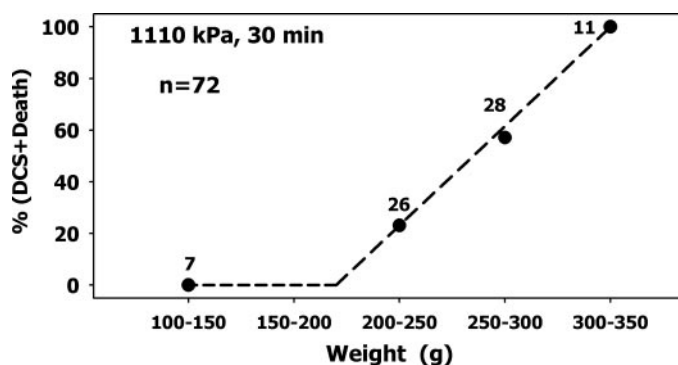


Fig. 2. Percentage of DCS+Death as a function of body mass. Exposure condition is shown, and the number of rats in each body mass group is given above the symbol.

calculation of risk for this exposure. The trend noted for a reduced risk of DCS with an increasing number of exposures was not significant (Fisher exact test). The probability of DCS+Death was 53% in the first exposure, declining to 38% in the third exposure and 25% in the fourth (only 4 rats). Each case of DCS+Death relates to a different rat.

Series B

In the control group (*protocol 1*), by the end of the 30-min period of walking, eight rats had no DCS, four had signs of DCS, and eight had died. At 24 h, all four animals with signs of DCS had recovered. No DCS was recorded in 20 rats given HBO treatment immediately after decompression (*protocol 2*). When these rats were used a week later for a further trimix exposure, nine were dead at 2 h postdecompression. Of the 20 rats exposed to trimix as a control group (*protocol 1*), by 2 h, 8 had died and 3 had DCS. A week later, none of the 12 surviving animals had any discernible signs of DCS, and after exposure to trimix followed by immediate HBO treatment, they continued to exhibit no signs of DCS (*protocol 3*). When the 20 rats treated by HBO after the first exposure were compared with the 20 control rats, a beneficial effect of immediate HBO treatment was found at 2 h ($P < 0.0001$) and at 24 h ($P < 0.003$).

When 5 min separated decompression to the surface and HBO treatment (*protocol 4*), four rats died and six developed DCS within those 5 min. After 1 h, one of the six rats with DCS had died, two remained with DCS, and three had recovered. At 24 h, the last two animals with DCS had also recovered. Twenty-four hours after decompression, 40% of animals from the control trimix exposure had died (*protocol 1*, Fig. 4), compared with only 25% when HBO treatment was given after a 5-min delay (*protocol 4*, Fig. 4). The reduced number of deaths when treatment was given after a 5-min delay was not significantly different from the control group at 2 h or at 24 h. The results for the 30-min postdecompression period following *protocol 1* (the control exposure) are also shown in Fig. 4. It can be seen that by 24 h, the four rats with DCS had recovered.

The outcome of treatment given after a 5-min delay (HBO, *protocol 4*; heliox, *protocol 5*) is shown in Fig. 5 for three points in time at which we observed the animals: 5 min after decompression, 95 min after decompression at the end of

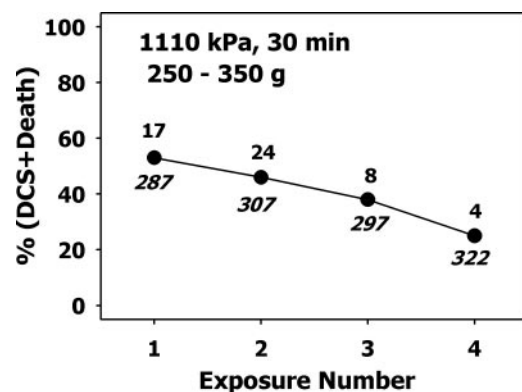


Fig. 3. Percentage of DCS+Death as a function of exposure number. Exposure condition and weight range are shown. The number of rats in each exposure is given above the symbol, and the mean weight in italics below the symbol.

wheel walking, and at 24 h postdecompression. The outcome of the two treatments was similar (not significantly different), and it may confer a slight, nonsignificant advantage over the control state. The percentage of animals that did not have DCS was 80% in the heliox-treated rats (heliox compared with control, $P = 0.07$), and it was 75 and 60% in the HBO-treated and control rats, respectively. Immediate HBO treatment had a large advantage over the control state ($P < 0.003$) and over HBO or heliox treatment given with a 5-min delay ($P < 0.05$).

DISCUSSION

In our search of the literature, we were unable to find a description of the time course of DCS in the rat. Spiess et al. (15) exposed rats to 689 kPa air for 30 min and noted that when death occurred, it did so most often within minutes of decompression. This is in agreement with our data. Our findings also agree with those of Lillo et al. (12), who noted that all cases of DCS appeared within the first 30 min of observation. The present description of the time course of DCS in the rat can be used for further studies. In contrast to humans, no permanent disability was found in rats, and evidently in this respect their response to DCS is different.

In the discussion of our experimental model, we stressed that body mass is an important factor in sensitivity to DCS in the rat. Sensitivity was very low at a body mass below 150 g, and increased linearly between 200 and 350 g (over a range of 150 g). The slope shown in Fig. 2 is greater than that derived for trimix in rats over a range of 50 g body mass [Lillo et al. (12)]. Weight-related sensitivity may be explained by increased fat content, which enables the storage of more inert gas, and by a reduction in the specific metabolic rate and tissue perfusion, which leads to slower gas clearing, in heavy rats.

There may have been an acclimation response on repeated exposure (Fig. 3), when the risk of DCS tended to decrease with the number of hyperbaric exposures. When trimix followed HBO treatment as the second exposure, the number of animals without DCS was slightly greater (11 as opposed to 9), and this may also be related to acclimation. There is a similarity between our findings here and the accepted process of

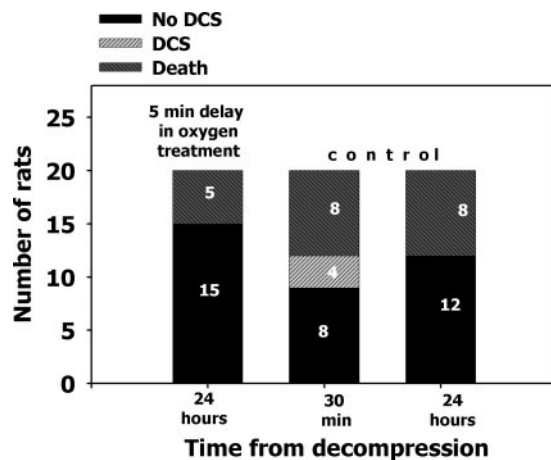


Fig. 4. Occurrence of DCS and death after decompression from 30 min at 1,110 kPa trimix when hyperbaric oxygen treatment was given 5 min after decompression (left), and the control exposure (middle and right). Time from decompression appears below the bars. The number of rats in each category appears in the appropriate segment of the bar.

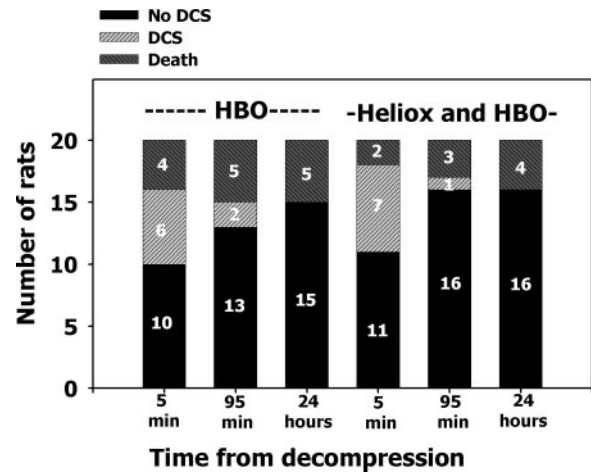


Fig. 5. Occurrence of DCS and death after decompression from 30 min at 1,110 kPa trimix, when hyperbaric oxygen or heliox treatment was given 5 min after decompression. Bars represent observation at three points in time: 5 min after decompression, at the end of the 30 min period of walking that followed a 1-h hyperbaric treatment, and at 24 h postdecompression. The number of rats in each category appears in the appropriate segment of the bar.

acclimation to diving. Walder (19) suggested seven daily dives as the half-time for acclimation to diving. Eckenhoff and Hughes (6) showed that 12 daily dives had no effect on venous bubbles, but itching was reduced. The rats in the present study were exposed to hyperbaric conditions at intervals of 1 wk, to find out whether a week is sufficient to eliminate any effect of a previous exposure on the following one. The acclimation effect should be considered when designing any study of DCS that involves repeated exposures.

In a number of previous studies, oxygen treatment was commenced immediately after decompression. The ability of rats to reach maximal exercise was reduced at 45 min, and more so at 60 min, after decompression from a 120-min exposure to air at 612 kPa (14). Immediate HBO treatment (45 min at 284 kPa) after decompression relieved the decompression stress with regard to the ability to exercise in a rotating cage and leukocyte β_2 -integrin-mediated adherence in brain blood vessels (14). In anesthetized rats decompressed from a 1-h exposure to air at 385 kPa, immediate treatment with either normobaric oxygen or normobaric heliox (80/20) reduced the incidence of death (over a 3-h observation period) from 48% to 14% and 19%, respectively (9). The adverse effect on the elimination of spinal evoked potentials was reduced from 75% to 42% and 8%, respectively. The authors concluded that heliox treatment seemed to be superior to oxygen. It was impossible in the present study to compare immediate treatment by HBO with heliox, because of the 100% recovery rate after immediate HBO treatment following trimix exposure. We therefore commenced the treatment after a 5-min delay. No superiority of heliox and HBO over HBO alone was found in the present study.

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DISCLOSURES

The opinions and assertions contained herein are the private ones of the authors, and are not to be construed as official or as reflecting the views of the Israel Naval Medical Institute.

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