Effect of heliox, oxygen and air breathing on helium bubbles after heliox diving.

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Hyldegaard O, Jensen T. Effect of heliox, oxygen and air breathing on helium bubbles after heliox diving. Undersea Hyperb Med 2007;34(2):107-122. In helium saturated rat abdominal adipose tissue, helium bubbles were studied at 101.3 kPa during breathing of either heliox(80:20), 100% oxygen or air after decompression from an exposure to heliox at 405 kPa for one hour. While breathing heliox bubbles initially grew for 15-115 minutes then shrank slowly; three out of 10 bubbles disappeared in the observation period. During oxygen breathing all bubbles initially grew for 10-80 minutes then shrank until they disappeared from view; in the growing phase, oxygen caused faster growth than heliox breathing, but bubbles disappeared sooner with oxygen breathing than with heliox or air breathing. In the shrinking phase, shrinkage is faster with heliox and oxygen breathing than with air breathing. Air breathing caused consistent growth of all bubbles. With heliox and oxygen breathing, most animals survived during the observation period but with air breathing, most animals died of decompression sickness regardless of whether the surrounding atmosphere was helium or air. If recompression beyond the maximum treatment pressure of oxygen is required, these results indicate that a breathing mixture of heliox may be better than air during the treatment of decompression sickness following heliox diving.

INTRODUCTION

Decompression sickness (DCS) following heliox dives, where the bubbles consists of helium (He), has in several cases shown dramatic deterioration during recompression treatment using air as the breathing gas (1-4). In keeping with this, divers who develop DCS after air diving, where the bubbles mainly consists of nitrogen (N_2), have been treated successfully with recompression breathing heliox, in some cases with a rapid recovery (1, 5-8). The beneficial effect of heliox breathing on N_2 bubbles in a lipid tissue such as the white substance of the spinal cord (9-11) was ascribed by Hills (12) and James (1) to an outward flux of N_2 from the bubbles exceeding the concomitant inward flux of helium. This should be expected whether the exchange of gases in the tissue is limited by perfusion or diffusion. In the case of perfusion limitation, because the solubility of He in blood is less than that of N_2 (13,14); in the case of diffusion limitation because the permeability, i.e. the product of diffusion coefficient and solubility coefficient, in lipid is greater for N_2 than for helium (13-15).

The standard treatment of DCS following air diving is recompression breathing oxygen (O_2). Since O_2 is consumed metabolically and substituted by the highly soluble CO_2, one should not expect bubbles to grow during O_2 breathing. However, at equal partial pressure differences, the greater solubility of O_2 in blood compared to both N_2 and He, as well as a greater permeability in lipid than both N_2 and He, might cause growth of nitrogen bubbles during O_2 breathing and account for the occasional worsening seen when O_2 is used

in the treatment of DCS after air diving (1,16-18). According to the reasoning above and our previous reports as described below, it is conceivable that O_2 breathing could also cause growth of He bubbles.

In previous reports (19,20) we found that decompression-induced nitrogen bubbles in rat adipose tissue will initially grow, then shrink and disappear during O_2 breathing at sea level (19). Further, both heliox and O_2 breathing at sea level has a preventive effect on the development of spinal DCS in rats monitored by spinal evoked potentials, when compared with air breathing; the effect of heliox seems to be superior to that of oxygen (21). Since conventional treatment of DCS involves recompression, we have also studied the combined effect of recompression and O_2 breathing on bubbles injected into lipid and aqueous tissues of rats decompressed from a prolonged air exposure (22). We found that O_2 breathing will cause bubbles to disappear faster than during breathing of air. However, O_2 breathing caused a short lasting initial increase in bubble size. The surprising observations of air (- or N_2) bubbles growing during O_2 breathing raised the question whether He bubbles in a helium-saturated tissue would behave in the same manner?

In offshore deep diving, heliox is used as a breathing gas (23). In case of DCS the bubble will consist mainly of helium. The treatment of DCS following heliox bounce or saturation diving is recompression using either pure O_2, an O_2-enriched breathing mix with N_2 or a heliox breathing mixture (16). Oxygen is 7 times more soluble in lipid tissue than helium (13,14) and twice as soluble as nitrogen. In blood, O_2 forms a chemical compound with haemoglobin - oxyhaemoglobin - increasing the oxygen transport capacity of blood. Further, O_2 is dissolved physically better than both $N_2$and He in blood (13,14) and since the solubility of He in both blood and lipid as well as its permeability in lipid are less than those of N_2 (12), He bubbles in adipose tissue after a heliox dive could be expected to grow even faster during breathing of O_2 than do N_2 bubbles. If this is the case, then recompression using heliox might have advantages in cases of DCS after heliox diving compared to the standard recompression using oxygen.

We further studied whether subsequent air breathing would cause He bubbles to grow causing deterioration of DCS (1-4). Such bubble growth would be in favour of breathing helium (i.e. heliox) over nitrogen (i.e. nitrox) in the treatment of DCS after heliox diving.

METHODS

Pressurization system

Compression and decompression was performed in a specially designed pressure chamber (chamber volume 0.02m^3) with a horizontal viewing port in the lid - see Figure 1. A small fan (ventilator), placed in the bottom of the chamber mixed the chamber atmosphere at frequent intervals. Penetrations were made for a chamber atmosphere heating system. The breathing mixture was supplied continuously via silicone tubing at a pressure slightly above chamber pressure with a T-connection for the rat's tracheal cannula. The tube was connected to the exhaust outlet via a specially designed overboard dump valve.

The chamber was compressed on He as chamber gas only. The overboard dump valve was adjusted to maintain a maximum positive pressure corresponding to 1.2 - 1.6 cm H_2O above the chamber pressure. The rat's tracheal cannula was connected via the T-connection to the respiratory gas circuit, through which the breathing gas free-flowed at 1500-2000 ml/minute, as verified by a flow meter. All rats remained unaffected with respect to blood pressure and ventilation when they were connected to the overboard dump valve system.

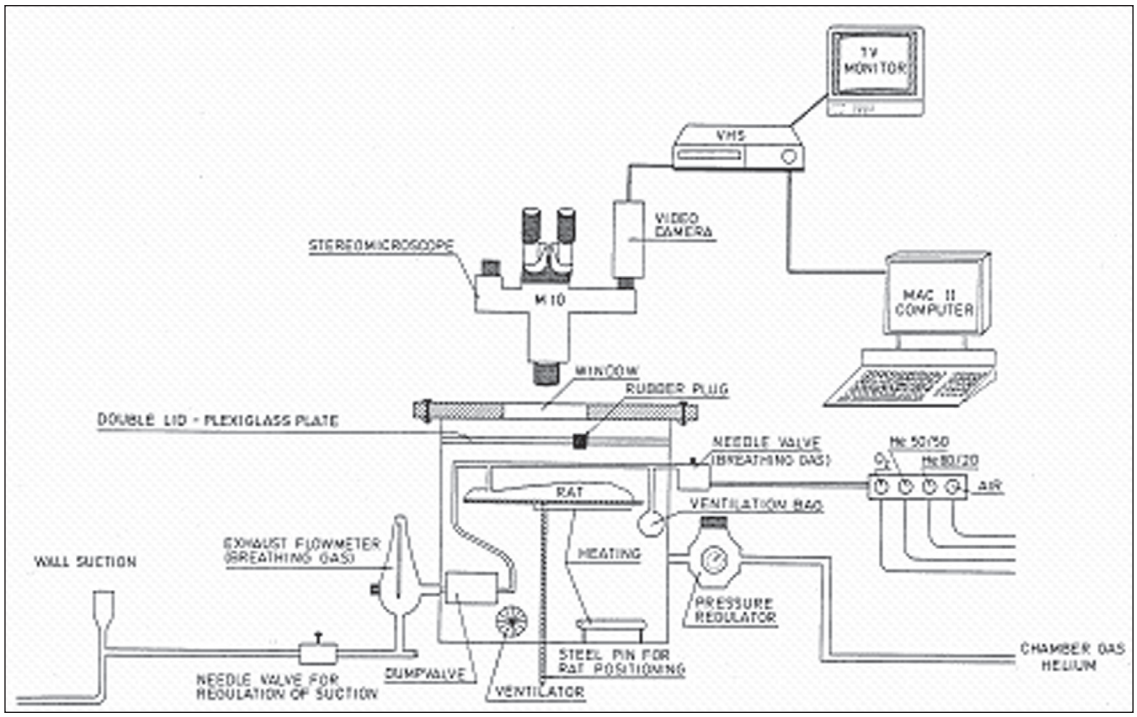

Fig.1. Experimental set-up during heliox saturation experiments. The square around the animal is the pressure resistant walls of the chamber.

Animal preparation and experimental protocol

Fat female wistar rats weighing 250-350 gram were anaesthetised with sodium thiomebumal (0.1 g/kg) intraperitoneally. Before putting the rat in the pressure chamber the rat was placed supine on the operating table and a cannula was inserted in the trachea (Polythylene tubing - ID 1.5 mm). A catheter was placed in the left carotid artery for continuous blood pressure registration. The latter was kept patent by a continuous infusion of non-heparinized saline by means of a syringe pump at a rate of 1 ml per hour. Mean arterial blood pressure (MAP) was measured throughout the experiment by means of a Statham AA pressure transducer as was body temperature by means of a thermocouple placed in the vagina. The vaginal thermometer was connected to a thermostat pre-set at 37°centigrade. In some experiments a thermo probe was placed superficially on to the tissue to check the temperature at the surface of the exposed tissue. A continuous record of temperature and mean arterial blood pressure was obtained on a recorder.

The abdomen was opened in the midline and the abdominal adipose tissue exposed. The exposed tissue was covered with gas impermeable mylar membrane and a polyethylene membrane to prevent evaporation. Once inside the chamber the rat was connected to the respiratory gas circuit. The pressure chamber was modified with a double lid, the lower consisting of a translucent Plexiglas plate, in order to contain the He atmosphere in the pressure chamber during the bubble injection phase - see Figure 1. The anaesthetized and surgically prepared animal with the abdominal adipose tissue exposed was placed in the pressure chamber and the connections made as outlined above. From hereon the rat was given heliox (i.e. 20% O_2 in 80% He) to breathe. Subsequently, the top steel lid of the pressure chamber was mounted. The chamber atmosphere was then changed from air to

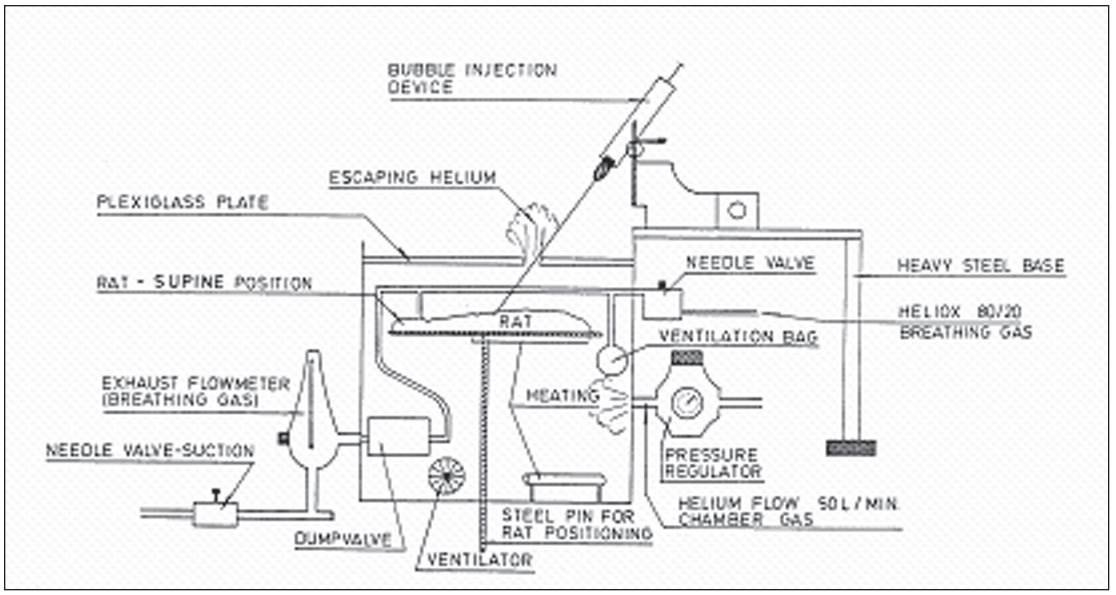

Fig.2. Experimental set-up during bubble injection phase. Note that the top steel lid is removed and that the transparent Plexiglas plate holds back the helium atmosphere surrounding the rat. The square around the rat is the pressure resistant wall of the chamber.

100% He by slowly flushing the chamber with approximately 250 litres of He over 15 minutes. The He atmosphere was continuously measured. The chamber temperature was set to 32-34.5 °C.

After two hours of heliox (80:20) breathing at 101.3 kPa with He as the chamber atmosphere, the upper steel lid was removed from the chamber. The rubber plug in the Plexiglas cover was removed, as was the polyethylene and mylar membrane from the exposed tissue. With the micromanipulator attached to a stand adjacent to the chamber, the micropipette was guided to the adipose tissue and two to four bubbles, widely separated and each consisting of 1-1.5 µl of He (i.e. the chamber atmosphere), were injected into the adipose tissue – see Figure 2.

The principle of the injection technique has been described in a previous report (20). The He atmosphere was controlled by flushing He at a high flow rate (approximately 30-50 litres/minute) while continuously measuring the He content in the chamber - see Figure 2. Subsequently, the mylar- and polyethylene membrane was repositioned over all of the exposed tissue, the Plexiglas plate opening sealed and the pressure chamber closed. The injection phase lasted from 15-45 minutes during which time the rat continued breathing heliox (80:20). After bubble injection and closing the chamber the animal was compressed to 405 kPa for 1 hour breathing heliox (80:20) and with He as the chamber gas. After 1 hour at 405 kPa the animal was decompressed over 7.5 minutes in three stages.

After decompression one to three spherical bubbles were selected for study. From then on, the pressure was maintained at 101.3 kPa and the animal continued breathing heliox (80:20) or switched to either oxygen or air breathing at 4-38 minutes post decompression. Bubble dimensions were recorded periodically for up to 255 minutes post decompression or until the bubbles disappeared from view. The rat was removed from the pressure chamber and placed under the operating binoculars at which time the thorax and abdomen were opened for a wider microscopic scan for intra- or extra vascular gas formation before exanguination.

The experiments were conducted in agreement with the Declaration of Helsinki II and approved by the Danish Animal Ethical Committee.

Bubble monitoring system

Bubbles were observed through the viewing port at x40 magnification by means of a Wild M10 stereo microscope with a long focal length objective as described in a previous report (22). A colour video camera was fitted on the microscope, with the field displayed on a TV-screen and recorded on VHS-videotape. Real-time images could be transmitted to a Macintosh IISi computer - see also Figure 1. The bubbles were then analysed on the computer using the NIH Image version 1.61 program (24) by measuring the bubble diameter and calculating the volume or by measuring visible bubble area (air breathing experiments).

Data analysis and statistics

Heliox, oxygen and air breathing experiments: Bubble *shrinking rate* was calculated as nanolitres per minute ($nL \bullet min^{-1}$ (slope of a line from the first point of observation until disappearance of the bubble or last point of observation)). If the last measured bubble size was greater than the first measured bubble size the shrinking rate was given a negative value indicating over all growth. Similarly, the bubble *growth rate* was calculated as $nL \bullet min^{-1}$ (slope of a line from first point of observation until maximal measured bubble size). Average values of bubble shrinking- or growing rates are given +/- SD. The calculated bubble shrinking rates as well as growth rate were analysed by means of one-way ANOVA to see if the calculated group mean values were different from zero. The difference between mean values of the various treatment groups were then analysed by use of the Student Newman-Keuls procedure for multiple comparison of means between groups. When several bubbles were studied in one animal, their mean value was used in the statistical comparison.

Treatment groups were also compared by means of 4-fold χ^2-test dividing the experiments into "bubbles disappeared" or "bubbles not disappeared". We further compared the effect of the heliox pressure exposure on survival or death from DCS in animals breathing heliox, O_2 or air by means of 4-fold χ^2-test dividing the experiments into "dead from DCS" or "not dead from DCS".

Comparability of treatment groups: Statistical analysis by means of ANOVA was performed between groups with respect to possible differences in the size of injected bubbles, time used during the bubble injection phase and time from decompression to first observation. For all comparisons $P < 0.05$ is regarded the limit for significance.

Furthermore, the bubble growth ratio for the O_2 breathing animals was compared to our previous results on bubble growth ratio during O_2 breathing after air exposures (19) by means of 4-fold χ^2-tests (25,26). The bubble growth ratio was calculated from first observation point to the point of maximal observed bubbles size in the observation period and $P<0.05$ is regarded as the limit for significance. The experiments were divided into "bubble growth ratio ≥1.23" or "bubble growth ratio ≤ 1.23", where 1.23 is the smallest observed growth in the O_2 treatment group after heliox diving.

RESULTS

General condition of animals and development of decompression sickness

In all 41 animals were used. Animals that died spontaneously are not considered below. Five animals were discarded from the study for technical reasons. After the 1 hour heliox exposure to 405 kPa pressure 27 of the remaining 36 animals developed visible gas formation in

the blood vessels of the exposed bowel. Twenty of those animals died in the observation period. Three animals died immediately and within 10 minutes after decompression during either heliox (80:20) (one animal) or O_2 breathing (two animals) and before bubble measurements could be performed. Seventeen animals died during air breathing—dividing the animals into two groups with 8 animals in the bubble observation group (see Figure 5, page 115) and 9 animals died during air breathing while surrounded by room air. Animals died during air breathing between 15 and 182 minutes after decompression.

Before death, many bubbles were always seen in the veins. Of the remaining 16 living animals, many bubbles were seen in the vessels of the bowel in 9 animals in the beginning of the observation period. These animals remained unaffected with respect to MAP and ventilation as those 7 animals in which no intravascular gas formation was observed. Of the 16 animals that lived through the entire observation period, no bubbles were observed intra- or extra-vascularly in animals breathing either heliox (N=5), O_2 (N=7) or air (N=4), when examined under the microscope. During decompression from the 405 kPa pressure exposure the rat's ventilation rate increased until a few minutes after decompression was ended. The vaginal temperature remained constant at 37°C throughout the experiment.

State of adipose tissue

Except for the animals breathing air perfusion were clearly visible in the small vessels with a diameter of approximately 10-15µm and seemed unaffected throughout the experiment. During the experiments the temperature at the surface of the tissue varied between 35.5-36.5°C.

Mean arterial blood pressure - MAP

This followed a typical pattern. In the beginning of the experiment before the chamber atmosphere was changed from air to He the MAP was in the range of 120-140 mmHg. When the chamber atmosphere was changed to He over 15 minutes the MAP fell to a range of 75-125 mmHg, the interval between 100-110 mmHg being most frequent. During the one hour pressure exposure the blood pressure was in the range of 80-130 mmHg, the interval between 100-110 mmHg as the most frequent. During decompression the pressure fell transiently to about 80 mmHg. In heliox(80:20) breathing rats the MAP was in the range of 80-100 mmHg in the post decompression observation period. When O_2 breathing was started the MAP increased about 10-20 mmHg and remained at this new level with the MAP about 100-110 mmHg in the observation period. During breathing of air the MAP was in the range of 80-125 mmHg with a consistently decreasing tendency towards the end of the observation period and dropped towards zero before death.

Comparability of heliox, oxygen and air breathing experiments

By means of one-way ANOVA test we found that the three experimental groups did not differ significantly from each other with respect to the size of injected bubbles or with time used during bubble injection, nor with the time from decompression to first observation. There were no differences between the groups with respect to the distribution of animals with bubbles or no bubbles. All comparisons were at $P>0.1$.

Effect of heliox (80:20) breathing on helium bubbles

During heliox breathing, see Figure 3, all bubbles (N=10) initially grew for a period of 15-115 minutes where after they started to shrink slowly. Three bubbles disappeared within the observation period. Larger bubbles

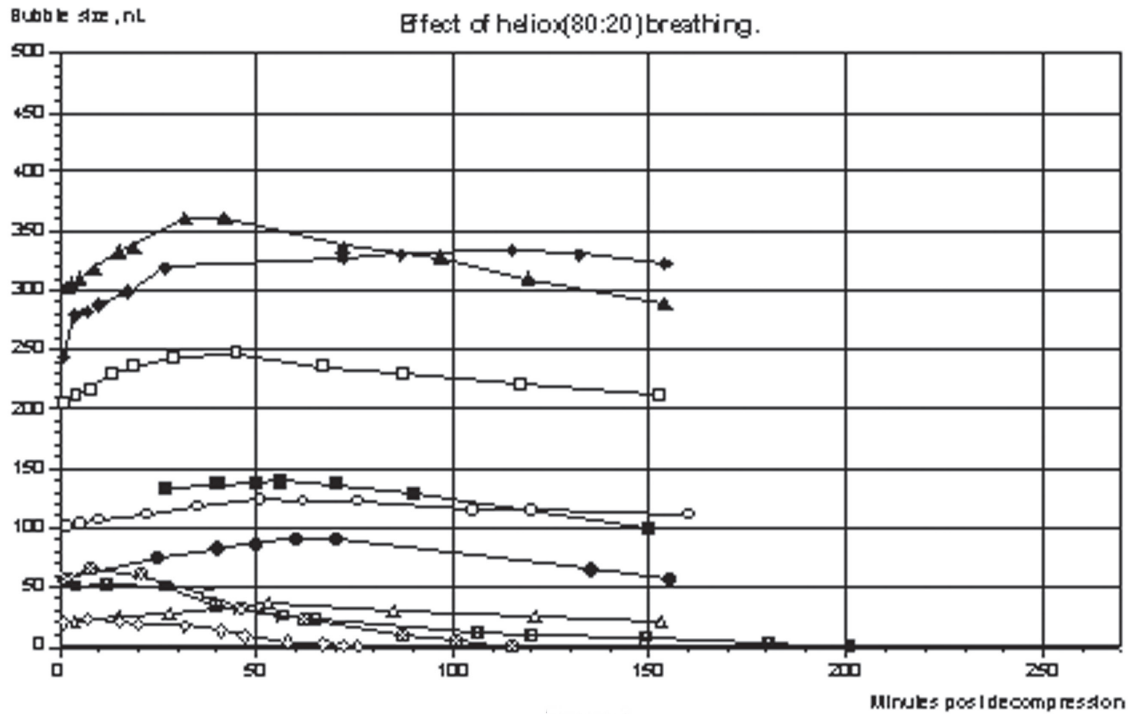

Fig.3. Effect of heliox (80:20) breathing on helium bubbles in rat adipose tissue at 101.3 kPa. Heliox breathing from first point on the curves.

appeared to grow more and for a longer period than smaller bubbles. The shrinkage rate during heliox (80:20) breathing (N=5) was 0.09 nL•min^{-1} (SD: +/- 0.21 nL•min^{-1}). When calculating the shrinkage rate, 5 out of 10 bubbles were larger at the end of the observation period than at the first point of observation. The bubble growth rate (N=5) was on average 0.76 nL•min^{-1} (SD: +/- 0.67 nL•min^{-1}). One rat died immediately after decompression while breathing heliox.

Effect of oxygen breathing on helium bubbles

During O_2 breathing (see Figure 4, page 114) all bubbles (N=9) initially grew. Most bubbles grew for 10-15 minutes. However, one bubble grew for 80 minutes. One bubble increased volume 6 fold. After the initial growth the bubbles would start to shrink at a considerable rate until they disappeared from view. The average bubble shrinking rate during O_2 breathing (N=7) was 1.10 nL•min^{-1} (SD: +/- 0.78 nL•min^{-1}. The growth rate (N=7) was 4.51 nL•min^{-1} (SD: +/- 2.77 nL•min^{-1}). Two rats died immediately after decompression and with in a few minutes after the switch to O_2 breathing. The bubble growth ratio was > 1.23 in all 9 bubbles studied. In our previous results (19), the bubble growth ratio was > 1.23 in 4 out of 9 bubbles when DCS-induced N_2 bubbles were studied during O_2 breathing after air diving.

Effect of air breathing on helium bubbles

During air breathing (see Figure 5) 8 out of 9 bubbles grew consistently throughout the observation period. Bubble growth continued until death of the animal apparently due to many bubbles present in their veins. Of the 9 animals exposed to air breathing during subsequent bubble visualisation 8 animals died during the observation period with bubbles

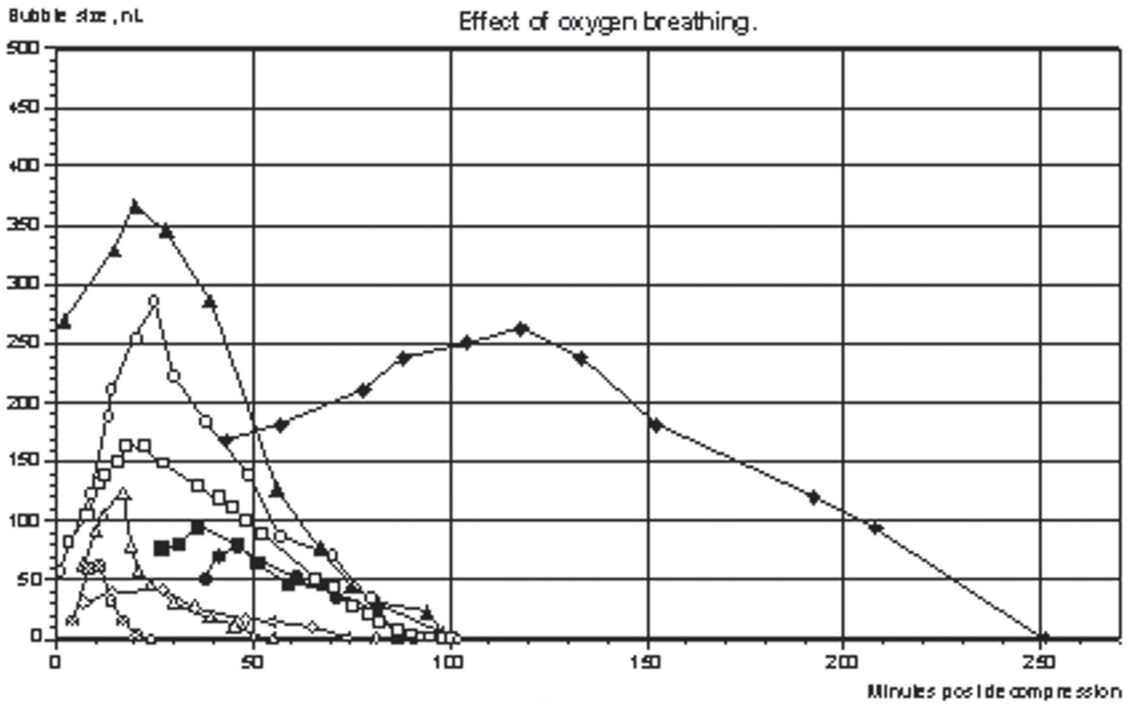

Fig.4. Effect of oxygen breathing on helium bubbles in rat adipose tissue at 101.3 kPa. Oxygen breathing from first point on the curves.

appearing in the venous vessels – see Figure 6. During growth, injected bubbles became irregular in shape – see Figure 6, page 119. Accordingly, their size were initially measured as visible surface area and subsequently recalculated to the volume of a sphere. Bubble growth rate during air breathing was 2.39 $nL \bullet min^{-1}$ (SD: +/- 0.82 $nL \bullet min^{-1}$).

Another 12 animals underwent the same heliox saturation and pressure profile but without exposure of the abdominal adipose tissue (see discussion). The chamber atmosphere was changed from He to air immediately after surfacing from the heliox pressure exposure. Of these 12 animals 9 died with many bubbles present in their veins. Animals died between 15 and 182 minutes after decompression. In the three surviving animals no bubbles were seen. In all 17 of 21 air-breathing animals died in the observation period due to massive venous gas formation.

Comparison of bubble shrinking and growth rates

ANOVA analysis showed that the mean shrinking rate during O_2 breathing (N=7) was significantly faster as compared to heliox breathing (N=5) ($P<0.05$) – see Table 1. The bubble shrinking rate during air breathing (N=9) were significantly slower than during either heliox (N=5) or O_2 (N=7) breathing ($P<0.001$). The rate of growth was significantly faster during breathing of O_2 as compared to heliox ($P<0.05$). The growth rate during air breathing was not different from that of heliox or O_2 breathing – see Table 1.

Comparison of bubble disappearance and death by DCS

During heliox breathing bubbles disappeared from view in 1 out of 5 animals while during O_2 all bubbles disappeared from view (N=7). No bubbles (N=9) disappeared

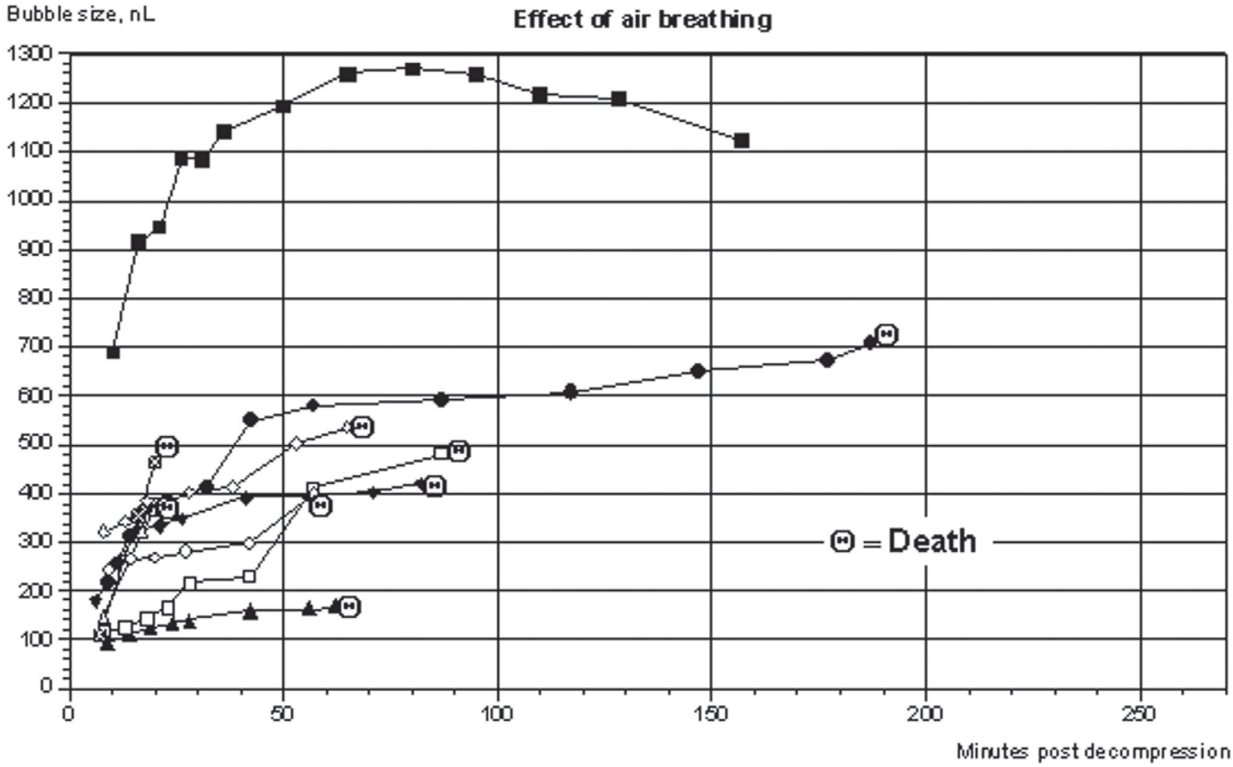

Fig.5. Effect of air breathing on helium bubbles in rat adipose tissue at 101.3 kPa. Air breathing from first point on the curves.

from view during air breathing. 4-fold χ^2-test show that bubbles disappear significantly faster from view during O_2 breathing as compared to heliox and air breathing ($P<0.001$) – see Table 1.

Table 1. Effect of heliox(80:20), oxygen and air breathing on bubble Evolution after heliox diving.

Breathing gas (N=number of animals)	Bubble shrinking rate nL x min^{-1} ± SD	Bubble growth rate nL x min^{-1} ± SD	Bubbles disappeared	Bubbles not disappeared
Heliox (N=5)	0.09 ± 0.21 [+)] (N=5)	0.76 ± 0.67 (N=5)	1	4
Oxygen (N=7)	1.10 ± 0.78 [+)] (N=7)	4.51 ± 2.77 [++)] (N=7)	7[¤)]	0
Air (N=9)	- 2.39 ± 0.82 (N=9)	2.39 ± 0.82 (N=9)	0	9

[+)] Heliox and oxygen bubble shrinking rate is different from air breathing ($P<0.001$). Oxygen bubble shrinking rate is different from heliox breathing ($P<0.05$).

[++)] Oxygen bubble growth rate is different from heliox breathing ($P<0.05$).

[¤)] Bubble disappearance during oxygen breathing is different from heliox breathing ($P<0.05$) and air breathing ($P<0.001$).

Of the 35 animals exposed to the He pressure profile 1 animal died during breathing of heliox, while during breathing of O_2 2 of 9 animals died. Seventeen of 21 animals exposed to air breathing after the heliox dive, died. Four-fold χ^2-test show that air breathing caused a significantly higher death rate compared to heliox ($P<0.02$) and O_2 breathing ($P<0.01$).

DISCUSSION

Effect of heliox (80:20) breathing

In the present experiments the animals were exposed to 3.5 hours of heliox (80:20) breathing before bubble observations were started. Since the tissue/blood partition coefficient λ for He is smaller than for N_2 in lipid (13,19), the tissue will saturate faster with He than N_2 will desaturate when the

breathing mixture is changed from air to heliox (80:20) (27). Giving a N_2 tissue half time of about 30 minutes (28,29) it seems justified to assume that the abdominal adipose tissue is almost completely desaturated (i.e. 3.5 hours corresponds to 7 halftimes) of its N_2 and at the same time completely saturated with helium. In previous reports we found that air breathing after decompression will cause N_2 bubbles in adipose tissue (-or spinal white matter) to grow for hours in the observation period (19,20). During breathing of heliox they will consistently shrink until they disappear from view. If the breathing medium is changed from air to pure O_2 they will initially increase in size, and sometimes after a time interval at constant size, shrink and disappear in about the same time as during breathing of heliox. The short growth and beginning shrinking of the He bubbles (see also Figure 3) as compared to the continuing growth of N_2 bubbles may be explained by the fact that He is less soluble in fat compared to N_2 making less gas available for bubble formation. Further, in the present heliox experiments the He will desaturate the tissue faster than would N_2, since the lipid tissue/blood partition coefficient (λ) is smaller for helium than for nitrogen. Opposing the effects above may be the presence of a counter-current mechanism (see discussion later) that would tend to maintain He in bubble and tissue counteracting shrinking of the He bubbles.

Obviously, several mechanisms influence the effect of a breathing gas shift on bubble volume. During steady breathing the tissue inert gas partial pressure will come in equilibration with the partial pressure of the alveolar gas and arterial blood. The time necessary for this saturation depends on the blood flow rate of the tissue and the tissue/blood partition coefficient (λ). Following a breathing gas shift from one inert gas (breathing gas A) to the other (breathing gas B) the following mechanisms may be important:

1) if the blood solubility for the initial breathing gas A is higher than for breathing gas B then this should, everything else being equal, lead to more gas A being carried away from the bubble by blood than what goes into the bubble of gas B and should make the bubble shrink.

2) if the partition coefficient of gas A (λ_A) is greater than gas B (λ_B) then the breathing gas with the greater partition coefficient (λ = Solubility of gas in lipid / solubility of gas in blood) will desaturate at a rate slower than gas B will saturate the tissue. This will, all else equal, lead to a state of transient super saturation of the tissue and will cause bubble growth (27,30). However, if the situation is reversed (i.e. gas B the initial breathing gas and of which the bubbles consist), then switching to a gas with a slower rate of tissue saturation should cause the bubble to shrink.

3) Counter-current - or shunting of gas by diffusion - is a mechanism whereby gas is shunted between arteries and veins that lie in close contact. The existence of counter-current gas exchange has been demonstrated in skeletal muscle and brain tissue by Sejrsen et al (31), Piiper et al in muscle (32) and discussed by Homer et al (33) and Van Liew (34). Whether a counter-current mechanism is at work in adipose tissue is uncertain. However, in rat adipose tissue we have observed that the arteries entering the adipose tissue lie in intimate contact with the major veins removing blood from the tissues. The possible counter-current effect on bubble evolution will depend on the permeability and the contact area of the involved gases, i.e. on their molecular weight and their solubility in the medium separating arterial and venous blood and the contact area.

4) if the gas exchange is limited primarily by extra vascular diffusion of gases, then the permeability of gases in tissue will be important (1,12). If the permeability of the initial breathing gas A is greater than the permeability of breathing gas B, the bubble

should grow after a breathing gas shift from gas A to gas B. If a breathing gas shift from gas B to gas A was done, then the bubble should shrink.

5) Blood perfusion rate. A rise in the blood perfusion rate will, every thing else being equal, lead to a faster wash-in as well as washout of gases decreasing the time for complete bubble resolution independently of the breathing gas.

6) Metabolic consumption. Due to the metabolic conversion of O_2 into CO_2 in the tissue cells the oxygen tension will drop from approximately 104 mmHg in the alveolar gas phase to 40 mmHg in the venous effluent. Concomitantly, because of its high solubility the carbon dioxide tension will rise from 40 mmHg in arterial blood to only 46 mm Hg in venous blood. If the sum of alveolar partial pressures is 760 mmHg, the total gas tension in venous blood is 701 mmHg. Thus, there is an inherent unsaturation at a difference of 59 mmHg, which is called the oxygen window (35,36). This oxygen window effect will cause bubbles to shrink regardless of the gas they may consist of all though, as predicted by Van Liew HD et al. (37), the magnitude of the oxygen window effect may change with ambient pressure.

7) When bubbles get small enough surface tension will increasingly tend to collapse them.

Which of the 7 factors dominates gas exchange will depend on many factors such as blood perfusion rate, the tissue in question, gas load from the previous exposure, ambient pressure, bubble size and the physical properties of the breathing gases involved as well as in which sequence they are being breathed.

Effect of oxygen breathing on helium bubbles

When a bubble is generated by decompression or injected into a tissue, its oxygen partial pressure must be low as determined by the partial pressure in the surrounding tissue (38,39). After a breathing gas shift to 100% O_2 the initial bubble growth is caused by a flux of O_2 into the bubble, in the beginning favoured by the high partial pressure of dissolved O_2 when the arterial partial pressure of O_2 is greater than 100mmHg, subsequently by the great O_2 carrying capacity of haemoglobin. At equal partial pressure differences blood will carry more dissolved O_2 to the tissue than it can concomitantly remove inert gas because the solubility of O_2 in blood is greater compared with both helium and nitrogen. Further, the permeability in fat is greater for O_2 than for either nitrogen or helium (13,14). At the same time, inert gas from the tissue may be dumped into the bubble when the inert gas partial pressure of the bubble is reduced by dilution with oxygen. This will happen to a greater extent when the surrounding adipose tissue is saturated with N_2 than with He because of the slower desaturation of the former.

The effect of O_2 breathing (see Figure 4) is similar to our previous results when N_2 bubbles are studied in the post decompression period during O_2 breathing (19). Compared to the present heliox breathing experiments (see Figure 3) the effect of O_2 on bubble size corresponds to the difference in the initial growth rate of the bubbles, which was significant ($P<0.05$). This must partly be caused by the much higher transport capacity of arterial blood for O_2 than for He as outlined before; partly by dumping of dissolved He from the supersaturated tissue into the bubble where the partial pressure of He is being reduced by dilution with oxygen. Further, the permeability of O_2 into fatty tissue is four times that of helium (1,12). Again, the effect of a diffusive counter-current shunt would tend to preserve the He in bubble and tissue furthering growth of the bubble during O_2 breathing. Counter- current would at the same time impede O_2 transport to the bubble, but O_2 would be less affected by

this mechanism than helium. When the growth ratio during O_2 breathing on He bubbles in our present experiments is compared to the growth ratio during O_2 breathing on N_2 bubbles from our previous report (19) the greater increase in bubble size during O_2 breathing on He bubbles is significant ($P<0.05$). An explanation of the apparently larger O_2 effect on He than on N_2 bubbles may be sought in the following mechanisms:

a) if gas exchange is limited by perfusion then the lower solubility of He in blood should promote bubble growth since He will be removed from bubble and tissue at a rate slower than nitrogen.

b) in case of a diffusive shunt, i.e. counter-current, this will retain the more diffusible He gas in the tissue and bubble more than nitrogen. Since fat desaturates 2.45 times faster with He than with N_2 the bigger growth of He than of N_2 bubbles during O_2 breathing suggest that gas exchange between bubble and blood is more important for bubble size than exchange between bubble and tissue.

Apparently, the growth rate decrease with time from decompression as the tissue is desaturated - see Figure 4. The fast disappearance of the bubbles must mainly be due to metabolic elimination of O_2, once He has been replaced.

In both heliox and O_2 breathing animals, larger bubbles seem to grow more and for a longer period than do smaller bubbles - see also Figures 3 and 4. This would be expected if the surface area of the bubble is important for its gas exchange with the surroundings. Similar observations are seen when bubbles form in-situ in rat abdominal adipose tissue after air diving (19). In this report bubbles were injected and not created by decompression. If the sizes of bubbles formed in-situ (19) are compared to the size of injected bubbles studied during O_2 breathing in this report, they are not different from each other ($P>0.2$).

Effect of air breathing on helium bubbles

During air breathing, bubbles would grow irregular in shape depending on their initial size and the tissue elasticity – see also Figure 6 showing a typical oval to near ellipsoid bubble shape in an air breathing animal. Accordingly, their size were initially measured as visible surface area and subsequently recalculated to the volume of a sphere. The fact that bubble area was recalculated to volume adds an uncertainty to our results. However, an increase in bubble area should only imply an even greater increase in volume. Further, since air breathing caused prolonged and consistent growth of all bubbles as well as increased rate of death caused by systemic venous gas formation, the present results indicate not only a quantitative difference from heliox or O_2 breathing but also a qualitative difference as well. Considering a breathing gas shift from heliox to air (i.e. N_2), as in the present experiments, we can predict the following based on the assumptions given above (points 1-7 previously in discussion):

1. Blood solubility of He is less than that of N_2 by a factor of approx. 1.5. This should, everything else being equal, lead to less He being carried away from the bubble than N_2 enters and should make the bubbles *grow*.

2. $\lambda N_2 > \lambda He$ in lipid tissues by a factor of approximately 2.5. This means that heliox desaturates the tissue faster than N_2 saturates it thus making bubbles *shrink*.

3. Counter-current, where the highly diffusible gas He will diffuse from veins to arteries better than N_2 diffuses from arteries to veins, will tend to reduce the amount of He that leaves the bubble and therefore conserve He in the bubble. Since He transport away from the bubble is affected more by the mechanism than N_2 transport to the bubble, bubble growth is promoted. This is the case if the gas exchange in the artery/vein interface is dominated by

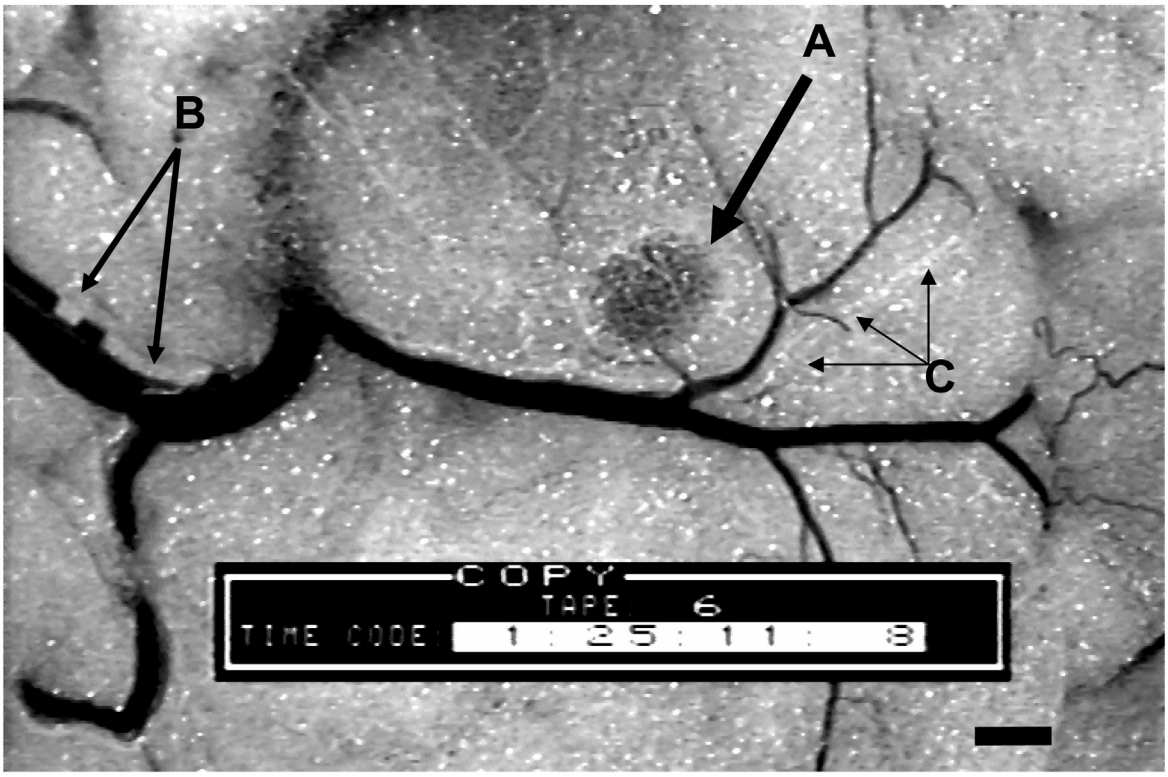

Fig.6. Picture of injected helium bubble during air breathing (arrow A). Note intravascular venous gas formation (arrows B and C). Time reference shown in lower middle of picture – 1 hour and 25 minutes after decompression from heliox dive. Lower right corner, bar = 200μm.

diffusion though an aqueous medium. If this diffusion barrier is lipid the effect should be reversed.

4. The permeability of N_2 is greater than for He in a lipid tissue. Thus, if gas exchange is limited mainly by extra vascular diffusion, more N_2 would enter the bubble and tissue than He leaves.

5-7. A rise in blood perfusion rate, metabolic O_2 consumption and bubble surface tension will affect the size of bubbles in the same manner regardless of the breathing gas and will make the bubble shrink.

Judging from our previous reports and the present experiments our results suggest that factors 1, 3 and 4 are of greatest importance in determining the behaviour of bubbles in adipose tissue under these conditions. The experiments where air breathing animals were exposed to a surrounding atmosphere consisting of room air instead of He was done in order to exclude a possible bubble growth promoting effect of inwards diffusion of He, a mechanism coined by Lambertsen et al. as superficial isobaric counter diffusion (40). The results indicate that air breathing following heliox bounce diving will promote bubble growth regardless of the surrounding atmosphere and that superficial isobaric counter diffusion was not a mechanism of significant importance in these experiments.

The growth of He bubbles during O_2 breathing at 101.3 kPa following a heliox dive may be important during transport of the diver to a recompression facility. DCS symptoms after heliox saturation dives are usually of the bend-type (i.e. pain in and around major joints) (16) and thus probably originate in aqueous rather than in lipid tissues. In a previous study, where air bubbles were injected into three non-lipid tissues (41) we found that O_2 breathing caused

bubbles in muscle to shrink and disappear faster than during breathing of air. However, in both tendon and the eye O_2 breathing caused a short lasting increase in bubble size followed by shrinking and disappearance of the bubbles (41). This transient increase in bubble size during O_2 breathing may be more pronounced if O_2 is breathed after a heliox exposure where the bubbles consist of helium. Partly because the solubility of He in blood is less than that of N_2 leading to less He being carried away by the blood from a He bubble than N_2 from a N_2 bubble during O_2 breathing (1). Partly, because counter-current mechanisms (31,33,34,42) may be more effective maintaining He, rather than N_2 in a bubble.

However, in aqueous tissues the permeability of He is greater than that of N_2 and almost the same as for oxygen (13, 14). Therefore, if gas exchange were limited by extra-vascular diffusion in these tissues bubble growth during O_2 or air breathing should not be expected. However, in our previous report (41), heliox (80:20) breathing promoted bubble shrinking and resolution compared with air breathing suggesting that further experiments are warranted in order to test whether and to what degree He bubbles in aqueous surroundings will grow during O_2 or air breathing.

In recent years a new kind of recreational diving has emerged, so called "technical diving", using different breathing mixtures such as tri-mix (i.e. N_2, He and O_2) or heliox, thus avoiding negative consequences of breathing air at great depths (i.e. N_2 narcosis (43) and O_2 toxicity (44). The standard treatment of a diver with DCS from a heliox bounce dive would be O_2 breathing at a maximal pressure of 284 kPa (45). However, if this is not sufficient to treat the symptoms of the diver then recompression to higher pressure using inert gases may be necessary. Most treatment facilities will be able to raise the pressure by having the diver breathing air. However, air recompression after heliox diving, especially where the diver has been breathing the heliox mixture all the way to the surface (or during accidental blow-up to the surface), may dramatically worsen the symptoms (1,2,4). The present results indicate that a breathing mixture of heliox is preferable to air for the treatment of DCS following heliox diving. However, further experiments on the combined effect of recompression and heliox, O_2 or air breathing on He bubbles seem warranted.

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