Influence of heliox, oxygen, and N_2O-O_2 breathing on N_2 bubbles in adipose tissue

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Hyldegaard O, Madsen J. Influence of heliox, oxygen, and N_2O-O_2 breathing on N_2 bubbles in adipose tissue. Undersea Biomed Res 1989; 16(3):185–193.—Bubbles in rat adipose tissue were studied at 1 bar after decompression from an exposure to air at 3.3 bars (absolute) for 4 h. During air breathing the bubbles grew throughout the observation period. During heliox (80:20) breathing they shrank and eventually disappeared from view. If the breathing gas was changed from heliox back to air or to N_2O-O_2 (80:20) while the bubbles still had an appreciable size, they started growing again. If the change to N_2O was done after or a few minutes before a bubble disappeared from view, it did not reappear. During breathing of 100% O_2, most bubbles containing N_2 initially grew and then maintained their size for a while before diminishing. However, some bubbles did not start shrinking during the 2–3-h observation period. The relevance of the findings to heliox treatment of CNS decompression sickness after air dives is discussed.

adipose tissue
heliox
rat
decompression sickness
bubbles
nitrous oxide
treatment
oxygen

The use of heliox breathing during recompression in serious cases of decompression sickness after air dives has been recommended by James (1). The beneficial effect of heliox breathing on N_2 bubbles in a lipid tissue such as the white substance of the spinal cord was ascribed by Hills (2) and James (1) to an outward flux of N_2 from bubbles exceeding the inward flux of He, whether the exchange of gasses in the tissue is limited by perfusion or diffusion. In the case of perfusion limitation this is because the solubility of He in blood is less than that of N_2; in the case of diffusion limitation it is because the product of diffusion coefficient and solubility in lipid is greater for N_2 than for He.

James (1) also pointed out that at equal partial pressure differences, the flux of oxygen in fat is twice that of nitrogen and 4 times that of helium, which might cause growth of N_2 bubbles during O_2 breathing and be responsible for the occasional worsening seen when oxygen is used in the treatment of decompression sickness.

In these experiments we studied the behavior of N_2 bubbles in adipose tissue during the breathing of air, 80:20% heliox, 80:20% N_2O-O_2, and 100% O_2. Breathing of N_2O-O_2 after bubbles had disappeared during heliox breathing was done to reveal possible submicroscopic bubbles that would be expected to grow and become visible under this regimen (*see* Discussion).

METHODS

Female rats weighing 250–350 g were anesthetized with sodium thiomebumal i.p. A cannula was inserted in the trachea and (with a few exceptions) a catheter was placed in a carotid artery for blood pressure registration. To keep the cannula open, saline was continuously infused at a rate of 1 ml/h. Body temperature was continuously measured in the vagina. The rat was exposed to air in a pressure chamber at 3.27 bars absolute for 4 h. The chamber temperature was set to 25–28°C, maintaining the vaginal temperature of the animal about 37°C. After 4 h the animal was decompressed in three stages over 20 min. The animal was removed from the chamber and placed under a heating lamp controlled by the vaginal thermometer, to maintain constant body temperature. The abdomen was opened in the midline, and the abdominal fat covered with a 12.5-μm-thick polyethylene membrane to prevent evaporation. The adipose tissue was illuminated with two flexible fiberoptics and studied through a Wild M-8 stereomicroscope (maximum ×80) fitted with photographic equipment. In the adipose tissue a bubble partly covered with adipocytes was selected for study. The tissue in question was covered with a piece of gas-impermeable mylar film. In some experiments a metal rod 200 μm in diameter was placed under the film as a measuring reference. In other experiments a thermoprobe was placed superficially under the film to check the temperature at the surface of the exposed tissue. After selecting a bubble, one of the following types of experiment was performed, all at 1 bar.

1. Air breathing for up to 150 min after decompression.
2. Breathing of heliox (80:20%) starting 30–60 min after decompression (Fig. 1 *left, right, first points*), followed by air breathing either from shortly before the bubble was expected to disappear or from 20 min after the bubble actually disappeared.

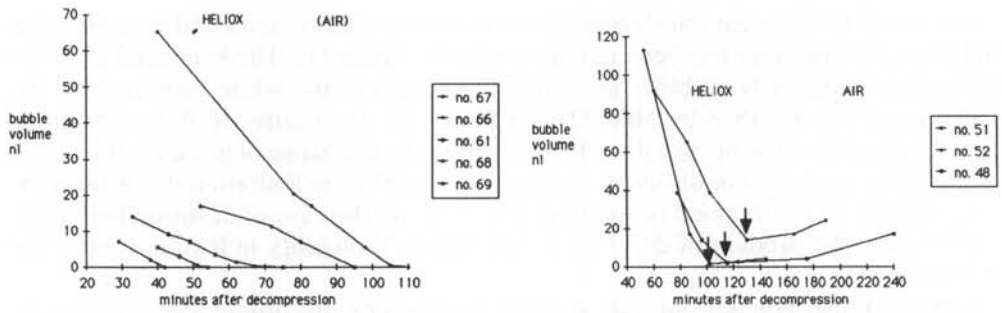

Fig. 1. Effect of heliox breathing on bubble volume. Heliox breathing started at the first point of each curve. *Left*, 20 min after disappearance of a bubble, the rat breathed air for about 1 h. *Right*, at the *arrows* the breathing medium was shifted to air.

3. Heliox (80:20%) breathing starting 40–90 min after decompression (Fig. 2 *left, right, first points*), followed after various time intervals by breathing of N_2O-O_2 (80:20%) (Fig. 2).

4. Breathing of O_2 starting 30–80 min after decompression (Fig. 3 *left, right, first points*).

At intervals the field was photographed, always at ×40. Bubble diameters were measured on the photographs and converted to absolute values by comparison with the standard rod. From the diameters, bubble volumes were calculated.

RESULTS

Development of decompression sickness

All animals treated as described developed decompression sickness as indicated by the presence of bubbles in adipose tissue. Ten of 56 animals died during the

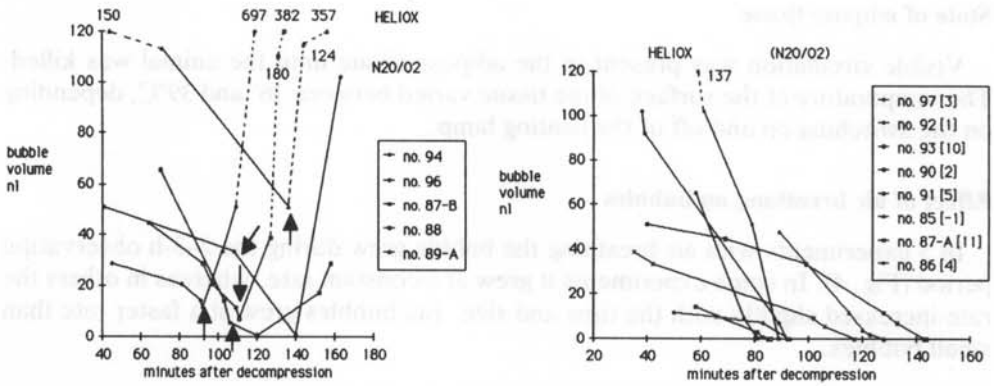

Fig. 2. Effect of N_2O breathing on bubble volume after heliox breathing. Heliox breathing started at the first point of each curve. *Left,* at the *arrows* the breathing medium was shifted from heliox to N_2O-O_2. *Broken lines* connect with points outside the scale. *Right,* in these experiments heliox was shifted to N_2O-O_2 a few minutes before or immediately after the bubble had disappeared from view. *Numbers in brackets* show minutes between the shift to N_2O and the disappearance of the bubble. If the number is negative, the shift was done after the bubble had disappeared.

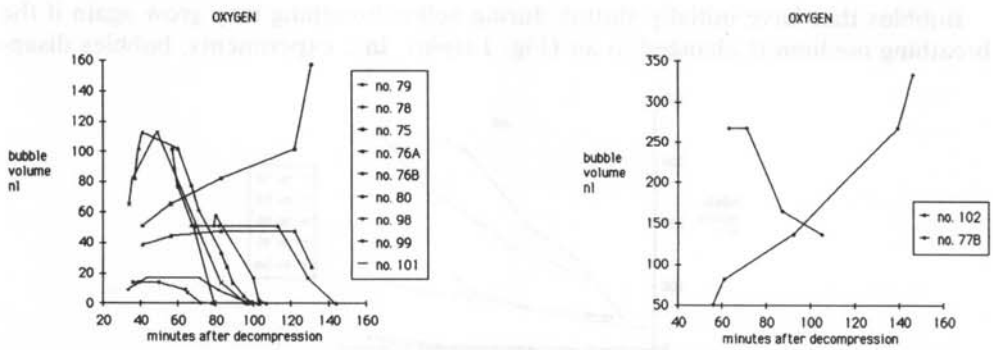

Fig. 3. Effect of oxygen breathing on bubble volume. Oxygen breathing was started at the first point of each curve. Drawn in two diagrams because of different sizes of bubbles. Experiment 77 had to be discontinued 110 min after decompression for technical reasons.

observation. Before death, bubbles were always seen in the adipose venous circulation. The rest of the animals remained in apparently stable condition until they were killed 2.5–4 h after decompression. Animals that died "spontaneously" are not considered below.

Mean arterial blood pressures

These followed a typical pattern. During the 4 h air breathing under pressure it was in the range of 160–180 mmHg. During decompression it fell to a level between 80 and 140 mmHg (usually between 100 and 120), in which range it remained with a decreasing tendency during air or heliox breathing. If the breathing medium was shifted to oxygen, blood pressure increased about 20 mmHg and remained at this new level. If the breathing medium was changed to N_2O, a transient increase of about 15 mmHg was seen.

State of adipose tissue

Visible circulation was present in the adipose tissue until the animal was killed. The temperature at the surface of the tissue varied between 36° and 39°C, depending on the switching on and off of the heating lamp.

Effect of air breathing on bubbles

In 5 experiments with air breathing the bubble grew during the 2–3-h observation period (Fig. 4). In some experiments it grew at a constant rate, whereas in others the rate increased slightly with the time and size. Big bubbles grew at a faster rate than small bubbles.

Effect of heliox breathing on bubbles

During heliox breathing, bubbles consistently diminished (Figs. 1 *left, right,* and 2 *left, right*).

Effect of air after heliox breathing

Bubbles that have initially shrunk during heliox breathing may grow again if the breathing medium is changed to air (Fig. 1 *right*). In 2 experiments, bubbles disap-

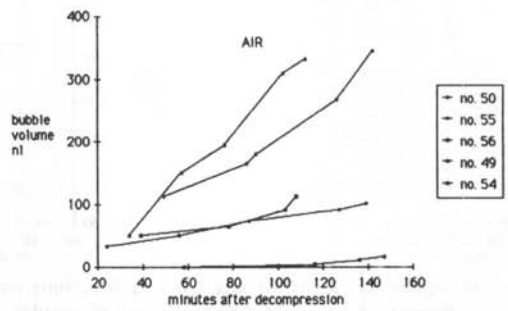

Fig. 4. Changes in bubble volume during breathing of air.

peared a few minutes after the change to air (not shown). If a bubble disappeared during heliox breathing it did not reappear during subsequent air breathing (Fig. 1 *left*).

Effect of N_2O-O_2 after heliox breathing

Bubbles that have initially shrunk during heliox breathing will grow rapidly if the breathing medium is switched to N_2O-O_2 while the bubble is still of a certain size (Fig. 2 *left*). In 7 experiments the bubble disappeared, although the shift was done 1–10 min before its estimated disappearance. The bubble did not reappear in an experiment in which the shift was done 1 min after the disappearance of the bubble (Fig. 2 *right*).

Effect of O_2 breathing on bubbles

This is illustrated in Fig. 3 *left, right*. The pattern varied from animal to animal. Eight out of 9 bubbles initially grew or remained stable for a considerable period of O_2 breathing. Two of the bubbles were in the same animal (rat 76) and close to each other, and apparently the smaller dumped its gas content into the other, which would explain the sudden volume increase in experiment 76-A. When bubbles began to diminish, this occurred at a considerable rate.

DISCUSSION

Effect of heliox and oxygen breathing on N_2 bubbles

In clinical practice, heliox treatment is combined with recompression, whereas our observations were carried out at 1 bar. However, the main effect of pressurization on the gas exchange between blood and bubbles is to increase the pressure gradients for N_2 as well as for He, and thus to speed up the changes observed.

We found that the rate at which bubbles shrink (or grow) varies considerably, probably depending on the vascularization of the surrounding tissue. There is a tendency for big bubbles to change volume at a greater rate than small bubbles, which would be expected if the surface area of the bubble is important for its gas exchange with the surroundings.

Variability is particularly conspicuous during oxygen breathing. The general pattern here is that bubbles initially grow, have an interval at constant size, and then shrink. Differences among tissues in the ratio between rate of oxygen consumption and blood perfusion must contribute to the variation between bubbles. Bubbles that initially grow fastest seem to shrink fastest too, perhaps because tissue with abundant vascularization is also tissue with high O_2 consumption.

It seems that most bubbles are eliminated in about the same time during heliox and oxygen breathing. However, two bubbles (76A and 102) were still growing 131 and 146 min, respectively, after decompression, in spite of O_2 breathing.

The observation that all N_2 bubbles diminished during heliox breathing at a time when they would grow during breathing of air is consistent with the clinical observation of an amelioration of spinal decompression sickness during treatment with

heliox. But can abdominal adipose tissue be used as a model for spinal white matter? The adipose tissue we studied contains about 85% lipid material (3), whereas CNS white matter contains only 18% (4, 5). However, in myelin sheets, where gas phase separation seems to occur according to some reports [*see* discussion by Sykes and Yaffe (6)], the lipid concentration is 35–45% of the wet weight [several references in (7)]. If gas exchange between bubble and blood is limited by the perfusion rate, a N_2 bubble should shrink during heliox breathing whether it is situated in lipid or aqueous surroundings. If, however, the exchange is diffusion-limited, a bubble should shrink in lipid but grow in aqueous surroundings (Table 1). However, the chemical composition of an inhomogeneous tissue such as white matter says little about the nature of the actual diffusion path for gas molecules, and whether the diffusion resistance is of a lipid or aqueous nature, or both. Thus the present experiments do not allow, with certainty, the prediction that the effect of heliox breathing will be the same in the CNS as in adipose tissue. However, the fact that bubbles during decompression from saturation are most likely to be formed in tissues with a high lipid content and a poor perfusion makes it probable.

Some recent papers have failed to demonstrate a beneficial effect of heliox breathing on decompression sickness after experimental air dives of dogs (8) and guinea pigs (9). However, in both these studies decompression sickness seemed to be of the pulmonary "choke" type with no indication of spinal involvement, so an ameliorating effect was not to be expected.

Possibility of stable bubble size during exchange of N_2 with He

In the introduction we explained that nitrogen bubbles in a lipid tissue would be expected to shrink during heliox breathing, whether the exchange of gasses is limited

TABLE 1
PHYSICAL PROPERTIES OF INERT GASSES

	Ostwald Solubility Coefficients at 37°C, ml gas 37°C × ml Fluid^{-1}				Diffusion Coefficients, × 10^{-6} cm^2 × sec^{-1}		Solubility Coefficient × Diffusion Coefficients, × 10^{-6} cm^2 × sec^{-1}	
	Oil[a]	Water[a]	85% Lipid[b]	Blood[a]	Oil	Water	Oil	Water
N_2	0.0745	0.0143	0.066	0.0148	7.04[c]	30.1[c]	0.525	0.430
He	0.0168	0.0098	0.016	0.0094	18.6[d]	63.2[d]	0.312	0.619
O_2	0.133	0.0271	0.117	0.0261	6.59[d]	28.2[d]	0.876	0.764
N_2O	1.458[e]	0.456	1.359[f]	0.453	5.62[d]	24.01[d]	8.194	10.949

[a]Averages from values cited by (14). A very low value for the solubility of N_2 in oil obtained with outdated technique has been omitted. [b]Calculated as weighted averages of oil and water values, disregarding possible salt effects. [c]From ref (15). [d]Calculated from N_2 data by Grahams law. [e]Calculated from oil-water partition coefficient (3.0) in ref (16). [f]Calculated from fat-blood partition coefficient (3.2) in ref (16).

by perfusion or by extravascular diffusion (1, 2). However, *in a perfusion-limited situation* it is conceivable that nitrogen bubbles in a lipid tissue will only shrink to a certain semistable volume, and that very small nitrogen bubbles will grow to the same size during heliox breathing.

The reasoning is that during its passage through the tissue the blood will lose helium and gain nitrogen. Helium and nitrogen are not only exchanged between blood and bubbles but with the tissue as well. The solubility of helium in adipose tissue is one fourth that of nitrogen (Table 1). Consequently, the fraction of helium lost that is dissolved in the tissue will be smaller than the fraction of nitrogen gained that comes from the tissue. Correspondingly, the fraction of helium lost that enters the bubbles will be greater than the fraction of nitrogen gained that comes from the bubbles. The differences between these fractions will be inversely related to the ratio between bubble volume and tissue volume (bubble:tissue volume ratio = BTR). If this ratio is large the difference between the tissue solubilities of the two gasses is unimportant, and the bubbles will shrink, as adduced by James (1) and Hills (2). However, the smaller the volume of gas phase, compared to the volume of tissue in the region considered, the more important the difference in tissue solubilities should be. Below a certain BTR the rate of helium entering the bubbles could be greater than the rate of nitrogen leaving them: the bubbles would grow. At an intermediate BTR the bubbles might be expected to maintain their volume during the exchange of nitrogen and helium. If these considerations are valid, larger bubbles should shrink to this equilibrium size, from which they would only disappear slowly under influence of the "inherent unsaturation" or "oxygen window" (10).

In the N_2-He experiments we never observed small bubbles grow or larger bubbles shrink to a stable "equilibrium volume." One reason for this might be that the hypothetical equilibrium size is below the resolving power of our microscope (bubble diameter $\approx$ 10–20 μm). The N_2O experiments were done to reveal such submicroscopic bubbles.

Nitrous oxide can be used as an amplifier of N_2 and He bubbles (11), because it dissolves 30 and 45 times better in blood than N_2 and He, respectively, and diffuses much faster through a lipid tissue into a bubble than either N_2 or He can diffuse the opposite way given similar partial pressure differences (Table 1).

The fact that no bubbles reappeared when the breathing medium was switched to N_2O-O_2 after or in some cases a few minutes before their disappearance does not support the hypothesis that bubbles persist at a submicroscopic size. Apparently they disappear from existence when they disappear from view. The most likely reason for this is that the exchange of gasses between blood and bubbles is not determined by the perfusion rate alone, and that diffusion resistance in the tissue is a factor of significance. This corresponds to the findings of Piiper et al. (12) in their study of absorption of inert gasses from subcutaneous gas pockets in rats. This explanation is supported by our finding that N_2 bubbles continued to grow for several hours during air breathing (Figs. 1 *right* and 4). Obviously, diffusion equilibrium between bubble and surrounding tissue did not occur during the experiment.

It should be noted that no firm conclusion can be drawn from our N_2O experiments, because a detailed analysis has not been performed concerning the exchange of N_2O with N_2 and He in the bubbles taking into consideration the distribution of gasses between tissue and gas phase, depending on the BTRs and the varying ratio between N_2 and He partial pressures in the bubbles.

In two experiments, 13 (no. 87) and 31 (no. 94) min elapsed before a bubble began to grow after switching the breathing medium from He to N_2O. In both experiments it was noted that the local circulation was sluggish before the shift. Several small bubbles disappeared, although the breathing medium was shifted to N_2O a few minutes before their disappearance (Fig. 2 *right*). These observations indicate that it takes a certain time to establish the concentration gradients in the tissue that are necessary to initiate bubble growth. This is remarkable considering the high diffusivity of N_2O in lipid (Table 1).

In contrast, the elimination of dissolved xenon from rabbit adipose tissue is known to be perfusion limited, because calculations of adipose tissue blood flow based on this assumption give the same results as direct measurements over a wide range of flows (13). The diffusivity of xenon in lipid is about 75% that of N_2O and about 10 and 20 times those of N_2 and He. A contributing cause for the disappearance of N_2 bubbles shrinking during heliox breathing could be that the surface tension in very small bubbles may create a pressure in the bubble sufficient to overcome the pressure gradients driving gas into the bubble.

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