J Appl Physiol 103: 757–762, 2007.
First published June 28, 2007; doi:10.1152/japplphysiol.00155.2007.

Effect of hypobaric air, oxygen, heliox (50:50), or heliox (80:20) breathing on air bubbles in adipose tissue

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Submitted 6 February 2007; accepted in final form 21 June 2007

Hyldegaard O, Madsen J. Effect of hypobaric air, oxygen, heliox (50:50), or heliox (80:20) breathing on air bubbles in adipose tissue. *J Appl Physiol* 103: 757–762, 2007. First published June 28, 2007; doi:10.1152/japplphysiol.00155.2007.—The fate of bubbles formed in tissues during decompression to altitude after diving or due to accidental loss of cabin pressure during flight has only been indirectly inferred from theoretical modeling and clinical observations with noninvasive bubble-measuring techniques of intravascular bubbles. In this report we visually followed the in vivo resolution of micro-air bubbles injected into adipose tissue of anesthetized rats decompressed from 101.3 kPa to and held at 71 kPa corresponding to ~2.750 m above sea level, while the rats breathed air, oxygen, heliox (50:50), or heliox (80:20). During air breathing, bubbles initially grew for 30–80 min, after which they remained stable or began to shrink slowly. Oxygen breathing caused an initial growth of all bubbles for 15–85 min, after which they shrank until they disappeared from view. Bubble growth was significantly greater during breathing of oxygen compared with air and heliox breathing mixtures. During heliox (50:50) breathing, bubbles initially grew for 5–30 min, from which point they shrank until they disappeared from view. After a shift to heliox (80:20) breathing, some bubbles grew slightly for 20–30 min, then shrank until they disappeared from view. Bubble disappearance was significantly faster during breathing of oxygen and heliox mixtures compared with air. In conclusion, the present results show that oxygen breathing at 71 kPa promotes bubble growth in lipid tissue, and it is possible that breathing of heliox may be beneficial in treating decompression sickness during flight.

altitude; decompression sickness; gas exchange

IN RATS decompressed to sea level after hyperbaric exposure, we previously found that decompression-induced nitrogen bubbles in adipose tissue (10) and air bubbles injected into the white matter of the spinal cord will initially grow, then shrink and disappear during oxygen breathing (11, 12). In combination with recompression, oxygen breathing causes a smaller increase in bubble volume of much shorter duration than that seen during oxygen breathing at sea level (8). Since the initial growth of bubbles during oxygen breathing at hyperbaric conditions is smaller and of shorter duration than that observed at sea level, it seems possible that oxygen breathing at hypobaric conditions will cause greater growth of air bubbles than during normobaric conditions. Bubble kinetic studies suggest that metabolic gases, i.e., oxygen, carbon dioxide, and water vapor, are important contributors to bubble volume and may be of greater importance during hypobaric conditions (29) than in the normo- or hyperbaric situation (7). Further, animal studies as well as theoretical models have shown that oxygen may contribute significantly to the evolution of bubbles during decompression sickness (DCS) (5, 6, 18, 22, 26).

Flying after diving may induce DCS during flight despite correct decompression procedures since most pressurized aircrafts normally maintain the cabin pressure equivalent to ~2,500 m above sea level corresponding to a barometric pressure of ~75 kPa (21). Several cases of DCS have occurred in this situation (20, 27, 31). Accordingly, the purpose of the present experiments was to study the behavior of nitrogen bubbles during oxygen breathing at subatmospheric pressure (like flying after diving) and to study if heliox mixtures have advantages in the treatment of such bubbles.

METHODS

Animal preparation and experimental protocol. Female Wistar rats weighing 250–350 g were anesthetized with sodium thiomebumal (0.1 g/kg) intraperitoneally. A cannula was inserted in the trachea (Polyethylene tubing, ID 1.5 mm). A catheter was placed in the left carotid artery for blood pressure registration. It was kept patent by a continuous infusion of saline by means of a syringe pump (SAGE Instruments, model 341) at a rate of 1 ml/h. Mean arterial blood pressure (MAP) was measured throughout the experiment by means of a Statham AA pressure transducer placed inside the pressure chamber. In some experiments, a thermoprobe (Ellab) was placed on the surface of the exposed tissue to check its temperature. A continuous record of temperature and MAP was obtained on a Goerz RE 520 and a Servogor recorder. The abdomen was opened in the midline, and the abdominal adipose tissue was exposed. After the adipose tissue was exposed, a micropipette was guided to the tissue, and two to three air bubbles usually in the volume range of 10–80 nl (with a few exceptions between 140 and 400 nl) were injected superficially into the adipose tissue. The injection technique has been described previously (12). The tissue with the bubbles in question was covered with gas-impermeable mylar. A polyethylene membrane was placed over all exposed tissue to prevent evaporation. The animal was then transferred to the pressure chamber, lying supine and fixed to the operating and heating platform. The tracheal cannula was connected to the T-shaped tube in the chamber breathing system. Once the animal was connected to the breathing system, its position was adjusted so that the tissue studied would be 2 cm below the window of the chamber. The stereomicroscope was positioned, and the videotape recorder started recording the microscopic picture. Spherical bubbles were selected for study. The predecompression bubble dimensions were obtained. The vacuum pump was started, and the chamber was evacuated to 71 kPa (i.e., 0.7 bar) absolute in 2 min. The

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second bubble observation, reflecting bulk decompression, was then obtained. From then on, the pressure was maintained at 71 kPa. After 30–35 min of air breathing, the breathing gas was changed isobarically to either breathing of oxygen, heliox (50:50), or heliox (80:20) with control animals breathing air throughout the observation period. The bubbles were observed for up to 220 min or until they disappeared from view. The animal was then recompressed to normobaric pressure. The rat was removed from the pressure chamber. With the rat still attached to the operating and heating platform, the thorax and abdomen were opened, and the animal was examined under the microscope for intra- or extravascular gas formation before exsanguination. All bubble observations were done at 71 kPa. The experiments and use of anesthetized animals were approved by a Government-granted license from the Danish Animals Ethical Committee under the Department of Justice.

Experimental set-up and pressurization system. Compression and decompression were performed in a specially designed pressure chamber with a horizontal viewing port 16 cm in diameter (see Fig. 1 in Ref. 8). The anesthetized animal was placed supine on a circular plate which could be removed from the pressure chamber and serve as an operating platform. This platform also contained a built-in heating system, which was controlled by a vaginal thermometer maintaining body temperature at 37°C (see Fig. 1 in Ref. 8). In the bottom of the chamber penetrations were made for a chamber atmosphere heating system consisting of an electrical heater. A small fan, placed in the bottom of the chamber, mixed the chamber atmosphere.

The breathing mixture was supplied continuously at a pressure slightly above chamber pressure and flowed inside the chamber through an 8-mm-ID silicone tube with a small latex rubber breathing bag and a T-connection for the rat's tracheal cannula. The tube was connected to the exhaust outlet via a specially designed overboard dump valve from Ottestad Breathing Systems. The overboard dump valve was adjusted to maintain a 1.5–2.0 cm ethanol positive pressure (corresponding to 1.2–1.6 cmH_2O) above the chamber pressure with a breathing gas flow rate at 2,000 ml/min (room pressure), as verified by a Puritan-Bennett flowmeter, attached outside the chamber to the outlet from the overboard dump valve. To have the overboard dump valve work properly, an extra Plexiglas chamber of ~2 liters in volume was constructed and connected to the exhaust outlet and to the vacuum pump. Once the vacuum pump (Brooke Crompton, Parkinson Motors) was started, the smaller chamber would reach its preset vacuum pressure at 61 kPa absolute within 10–15 s, thus ensuring a constant suction and the necessary pressure drop to the outside from the dump valve placed inside the main chamber.

Bubble observations and video recordings were done as described in our previous report (8). Using the NIH Image version 1.61 program (25), the volume of the spherical bubbles was calculated from the diameter. The computer program was calibrated by comparison with a metal rod of known diameter, 200 μm in diameter, placed on top of the adipose tissue in the observed field.

Data analysis and statistics. Bubble "net disappearance rate" was expressed as the mean net disappearance rate (in nl/min), i.e., the slope of a line from the measured bubbles size at the time of the gas shift (at 30–35 min after decompression) from air to either oxygen, heliox (50:50), or heliox (80:20) breathing to disappearance of the bubble. In the air experiments, the first observation after 30 min was used as starting point. If a bubble did not disappear, the average net disappearance rate was calculated as the slope of the line connecting the first observation after gas shift (30–35 min) with the last observation. If a bubble did not shrink but grew after the breathing gas shift, it was given a negative value, indicating growth. Average values of bubble net disappearance rates are given ± SD. To examine whether the difference between two mean values of calculated bubble net disappearance rates was different from zero, test for normality by means of Shapiro-Wilk followed by ANOVA was performed on the difference between mean values in the different treatment groups (1, 2). The difference between mean values in the treatment groups was then analyzed by use of the Student-Newman-Keuls procedure for multiple comparison of means between groups (1, 2, 28).

The bubbles were also analyzed with respect to growth rate from the time of breathing gas shift (30–35 min) until maximal bubble size was measured. In the air-breathing animals, where no gas shift was done, the growth rate was also measured from the 30-min observation point to maximal observed bubble size. If a bubble did not grow but shrank after the breathing gas shift, it was given a negative value, indicating shrinkage. Bubble growth rates were analyzed by means of ANOVA and the Student-Newman-Keuls procedure for multiple comparisons of means between groups (1, 2, 28).

In addition, bubbles were analyzed with respect to their growth ratio. Bubble growth ratio is calculated as maximal measured bubble size in the observation period divided by observed bubble size at 30 min postdecompression (i.e., time of breathing gas shift). Four-fold χ^2 test was used to analyze bubble growth ratio, dividing the experiments into "bubbles growth $\geq$ 1.34 ratio" or "bubble growth $\leq$ 1.34 ratio," where 1.34 ratio is the smallest observed bubble growth ratio in the oxygen treatment group (1, 2). Further, the bubble growth ratio for the hypobaric (71 kPa) oxygen breathing animals was compared with our previous results (10) at 101.3 kPa by means of fourfold χ^2-tests (1, 2) (see DISCUSSION). Bubbles were also compared with respect to "bubbles disappeared" or "bubbles not disappeared" by means of fourfold χ^2-tests (1, 2).

Statistical analysis by means of ANOVA was performed between groups with respect to possible differences in the size of injected bubbles, time from decompression to first observation, bubble growth caused by immediate decompression, and bubble growth until breathing gas shifts. When several bubbles were studied in one animal, their mean value was used in the statistical comparison. For all comparisons, $P < 0.05$ is regarded the limit for significance.

RESULTS

General condition of animals. All animals seemed unaffected with respect to blood pressure measurements when decompression was initiated and the rat was breathing spontaneously while connected to the overboard dump valve system at 71 kPa pressure. Twenty-six animals were used, but data from five had to be discarded for different technical reasons: one rat died because of an accidental air inflation through the arterial carotis catheter, causing massive gas embolism; there was one case of accidental overheating and one case of failure in the breathing gas supply system. Two air experiments had to be discontinued as the arterial carotis catheter accidentally disconnected within the chamber. In no case were bubbles observed intra- or extravascularly when the animals were examined under the microscope after the hypobaric exposure before being killed by exanguination. The rat's vaginal temperature remained constant at 37°C during the observation period.

State of adipose tissue. During the observation period, perfusion in the smaller vessels with a diameter of approximately 10–15 μm was clearly visible and seemed unaffected throughout the experiment. During the experiments the temperature at the surface of the tissue varied between 36 and 37°C.

MAP. Before decompression to 71-kPa atmospheric pressure, the MAP was in the range of 100–160 mmHg. During air breathing at 71 kPa, the MAP was within the range of 80–100 mmHg, with a slowly decreasing tendency through the observation period. When oxygen breathing was started, an increase in the MAP of about 20–30 mmHg was seen, and the MAP remained at this new level throughout the experiment. When heliox (50:50) breathing was started ($n = 4$), the MAP in-

Table 1. *Breathing gas effects on bubble growth and net disappearance rates in rat adipose tissue at 71-kPa ambient pressure*

	Air ($n = 6$)	Oxygen ($n = 7$)	Heliox (50:50) ($n = 4$)	Heliox (80:20) ($n = 4$)
Bubble growth rate, nl/min	0.56±0.50	6.43±4.53*	0.14±0.93	−0.18±0.91
Bubble net disappearance rate, nl/min	−0.14±0.27†	1.66±1.16	1.46±0.24	1.05±0.37

Values are means ± SD; n = no. of animals. *Bubble growth rate during oxygen breathing different from air, heliox (50:50), and heliox (80:20) breathing: $P < 0.01$. †Bubble net disappearance rate during air breathing different from oxygen, heliox (50:50), and heliox (80:20) breathing: $P < 0.05$.

creased 20–30 mmHg and remained at this new level in the observation period. During heliox (80:20) breathing at 71 kPa, the MAP was in the range of 80–120 mmHg, with 80–100 mmHg as the most frequent interval.

Comparability of the experimental groups. Since no animals were exposed to any pressure changes before bubble injection and observation, all animals in the different treatment groups presented are comparable with respect to the degree of tissue supersaturation during observations. ANOVA analysis showed no significant differences with respect to the size of the injected bubbles in the different treatment groups or with time from decompression to first bubble observation, bubble growth caused by the effect of immediate decompression, or bubble growth during air breathing before gas shift ($P > 0.1$).

Effect of breathing gases on bubbles at 71 kPa. The calculated net disappearance and growing rates during air, oxygen, heliox (50:50), and heliox (80:20) breathing are shown in Table 1.

During air breathing ($n = 6$ animals), all bubbles ($n = 14$) initially grew for about 30–80 min, after which they remained stable or began to shrink slowly (see Fig. 1). No bubbles disappeared in the observation period. Only 5 bubbles of 14 shrank to a size smaller than the size observed at the 30-min point. The bubble growth ratio was smaller than 1.34 in four of six animals.

During oxygen breathing ($n = 7$ animals), all bubbles ($n = 13$) initially grew for a period of 15–85 min (see Fig. 2). Subsequently, they shrank at a considerable rate, and 12 of 13 bubbles disappeared in the observation period. One bubble grew for 60 min and another grew for 85 min, both in the same animal. The bubble that grew for 85 min did not disappear in the observation period. Most bubbles increased their volume two-to fourfold. One bubble however, increased its volume sevenfold before it began shrinking and disappeared from view.[1] The bubble growth ratio was equal to or larger than 1.34 in all animals.

During heliox (50:50) breathing ($n = 4$ animals), 7 of 11 bubbles ($n = 11$) initially grew for a period of 5–30 min. From then on, bubbles shrank at a considerable rate until they disappeared from view (see Fig. 3). Two bubbles maintained a constant volume for 10–15 min and then started to shrink until they disappeared from view. Two bubbles started shrinking immediately upon gas shift (see Fig. 3). The bubble growth ratio was smaller than 1.34 in three of four animals.

After shift to heliox (80:20) breathing ($n = 4$ animals), 6 bubbles of 10 ($n = 10$) grew slightly or remained stable for a period of 20–30 min, from which point they began shrinking until they disappeared from view. Four bubbles immediately started to shrink when heliox (80:20) breathing was started. All bubbles disappeared in the observation period (see Fig. 4). Bubble growth ratio was smaller than 1.34 in all animals.

Comparison of bubble growth and net disappearance rates. ANOVA followed by multiple comparisons among the groups showed that the growth rate of bubbles during oxygen breathing was significantly greater than during air, heliox (50:50), and heliox (80:20) ($P < 0.01$). There were no significant differences between the bubble growth rates of breathing air, heliox (50:50), or heliox (80:20). The net disappearance rates during oxygen, heliox (50:50), and heliox (80:20) were significantly faster compared with air breathing ($P < 0.05$). There was no significant difference between the net disappearance rates of bubbles during oxygen, heliox (50:50), or heliox (80:20) breathing ($P > 0.1$).

Comparison of bubble growth ratio. Fourfold χ^2-test showed that oxygen breathing caused a significantly greater bubble growth ratio compared with air and heliox (50:50) breathing ($P < 0.05$), as well as with heliox (80:20) breathing ($P < 0.01$). There were no difference in bubble growth ratio between air and heliox (50:50) and heliox (80:20)-breathing animals.

Comparison of bubbles disappeared with bubbles not disappeared. Fourfold χ^2-test showed that the number of animals in which all bubbles disappeared in the observation period during air breathing (0 of 6) was significantly different from oxygen (6 of 7), heliox (50:50) (4 of 4), and heliox (80:20) (4 of 4) breathing ($P < 0.05$). There was no significant difference between the effect of heliox (50:50), heliox (80:20), or oxygen breathing ($P > 0.2$).

DISCUSSION

Bubbles initially increased in volume with a factor 101.3/71 = 1.43, corresponding to the immediate effect of decompression from 101.3 kPa to 71 kPa absolute pressure, which lasted 2 min. For the next 28 min, all bubbles grew because of the tissue supersaturation (see below). From then on, their fate depended on the breathing mixture as outlined below.

Air breathing. From 30 min after decompression, the bubble size was largely stable, indicating equilibrium between the effects of oxygen window and overpressure in the bubble caused by surface tension and tissue elasticity and the effect of the modest tissue supersaturation, which was initially 30 kPa, declining with a N_2 half-time of 29 min since the tissue perfusion is 0.105 ml blood·g^{-1}·min^{-1} (19) and the partition coefficient (λ) for nitrogen between 85% lipid and blood is 0.066/0.0148 for rat abdominal adipose tissue (10).

Oxygen breathing. In previous papers (10, 12), we have shown that bubbles in rat adipose tissue created by decompression or by microinjection of air in spinal white matter during breathing of oxygen will grow for a period of 10 to more than

[1] It should be noted that this bubble changed its initial spherical shape during the observation phase such that the volume of the bubble at its maximal size was estimated as a combination of a sphere and an ellipsoid.

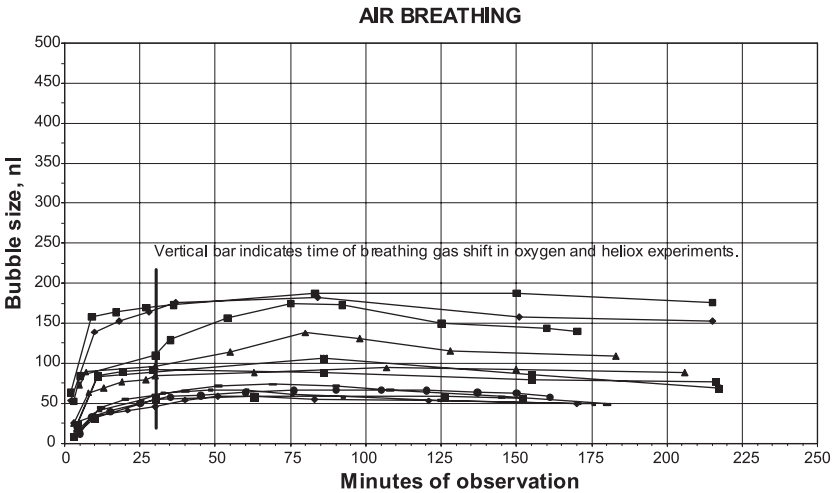

Fig. 1. Effect of air breathing at 71 kPa on air bubbles in rat adipose tissue. Air breathing from first point on the curves. The initial volume increase, i.e., between the first two points of the curves, is mainly the effect of decompression.

100 min, after which they disappear at a fast rate. This transient growth can be explained *1*) by the greater capacity of blood for oxygen than for nitrogen transport caused mainly by hemoglobin when the Po_2 in the tissue is 12–13 kPa; at higher Po_2, by the difference in solubility coefficients (0.022 ml gas·ml blood^{-1}·atm^{-1} for oxygen, 0.014 for nitrogen), i.e., at equal partial pressure differences, blood will carry more dissolved oxygen to the tissue than it can concomitantly remove inert gas; and *2*) by the increasing gradient for diffusion of nitrogen from the surrounding tissue to the bubble as the nitrogen in the bubble is diluted by oxygen. Accordingly, the effect is furthered by the increasing fraction of oxygen in the bubble but wanes as the tissue is desaturated for nitrogen. As the oxygen partial pressure in the bubble increases, the oxygen diffusion gradient from blood to bubble decreases, while the oxygen gradient from bubble to tissue increases. From a certain point, the total loss of oxygen and nitrogen from the bubble will exceed the gain of oxygen and nitrogen, and the bubble will start shrinking as oxygen is used by the surrounding tissue. *3*) The greater permeability (i.e., solubility coefficient × diffusion coefficient) (10, 32) of oxygen than of nitrogen in lipids may also be of importance.

In the present experiments at 71 kPa, air bubbles expanded more during oxygen breathing than did decompression-induced bubbles in previous observations at 101.3 kPa (10, 12) (mean growth ratio for normobaric experiments = 1.73 and mean growth ratio for hypobaric experiments = 2.35, $P < 0.01$ by 4-fold χ^2-test). This difference is the more remarkable as the nitrogen supersaturation in the present experiment were considerably less than in the observation at 101.3 kPa, where the animals had been exposed to a 4-h 327 kPa dive before the observation (10). Several mechanisms may explain the enhanced oxygen effect at hypobaric conditions. *1*) Since Po_2 in the region below 12–13 kPa will be reached sooner in the hypobaric than in the normobaric observations, oxygen transport by hemoglobin (and the Bohr effect) must be of quantitatively greater importance in the hypobaric experiments. *2*) A certain amount of oxygen molecules (mol) brought by the arterial blood will have a greater volume at 71 kPa than at 101.3 kPa. Accordingly, the delivered oxygen will expand a bubble more the lower the ambient pressure. *3*) It is also conceivable that the vasoconstrictor effect of oxygen is more pronounced at 101.3 kPa than at 71 kPa, which would favor the microcirculation in the latter case.

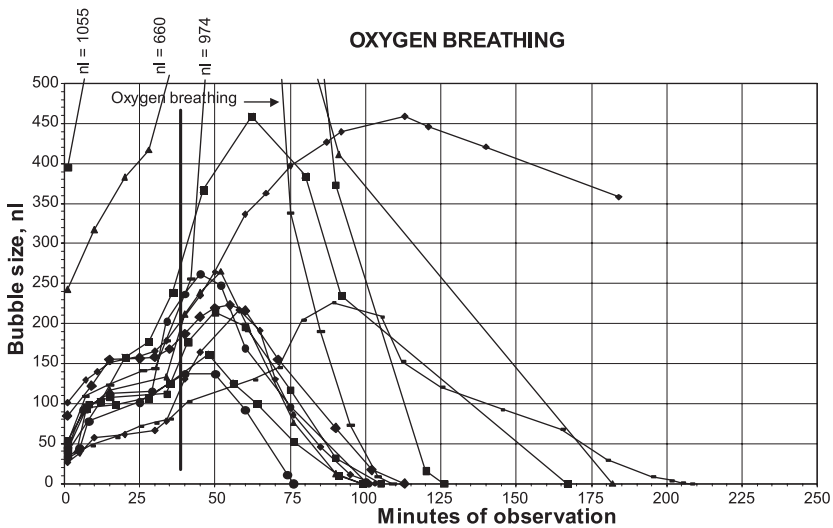

Fig. 2. Effect of oxygen breathing at 71 kPa on air bubbles in rat adipose tissue. Oxygen breathing starts from the black vertical bar 30–35 min postdecompression. Note that maximal observed bubble size is indicated for 3 bubbles that were outside the frame.

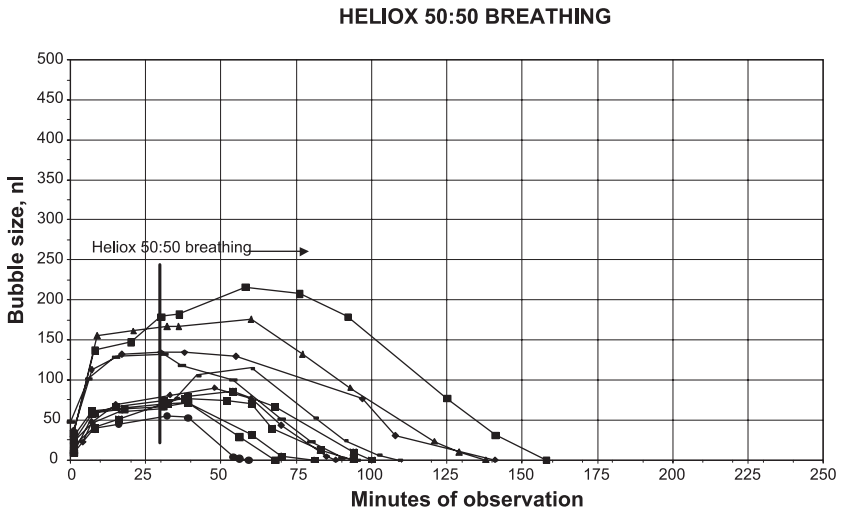

Fig. 3. Effect of heliox (50:50) breathing at 71 kPa on air bubbles in rat adipose tissue. Heliox (50:50) breathing starts from the black vertical bar at 30–35 min postdecompression.

The above considerations would also apply to our previous observations of a greater increase in bubble size during oxygen breathing at normobaric pressure (10) than during recompression (284 kPa) (8).

Heliox breathing. The faster disappearance of bubbles in lipid tissues during breathing of heliox compared with air breathing has been discussed in our previous papers (8–12) and explained by a greater solubility of nitrogen in blood than helium and possibly by a countercurrent diffusion mechanism (8, 12–14). During breathing of heliox (50:50), the picture was intermediate between oxygen and heliox (80:20) breathing as could be expected since part of the transient volume increase is caused by the raised oxygen partial pressure adding to the growth of bubbles as described above.

Breathing of heliox (80:20) at 71 kPa caused all bubbles to disappear in about the same time as during oxygen breathing. In contrast to our previous reports where bubbles were observed at 101.3 kPa (10) and 284 kPa (8), there was a certain time delay before the bubbles began to shrink, and a few increased marginally (Fig. 4) in size as during air breathing (Fig. 1) before shrinking. A reason for this difference may be the smaller partial pressure gradient for both helium and nitrogen under the present conditions. However, the observed effect of helium on bubble growth is marginal and will have only little effect on bubble radius.

The present results may be of importance for divers who develop DCS during flying after diving. DCS at altitude may develop in divers who have dived less than 12–48 h before flying (20, 27, 30). This problem is of interest not only during flying after diving. Extravehicular activities (EVA or space walking) during space flight involve decompression of the space shuttle's cabin pressure with the risk of the formation of venous gas embolism (16, 23) and DCS (17). Although pilots in fighter aircrafts and astronauts use denitrogenation by prebreathing of oxygen before exposure to hypobaric conditions (33, 34), the risk of DCS is not completely eliminated even after hours of oxygen breathing (24). Accidental pressure drop in the cabin pressure of air, as well as spacecraft's, may also occur (16, 24). Unless a portable recompression chamber is available, recompression is not directly accessible during spaceflight, and oxygen breathing together with intravenous fluid administration is the only possible treatment. The effect of oxygen prebreathing in diving may have limited effect on the incidence of postdive DCS (4).

Previous results (9–12) suggest that heliox (80:20) breathing may be beneficial during transportation of a diver suffering

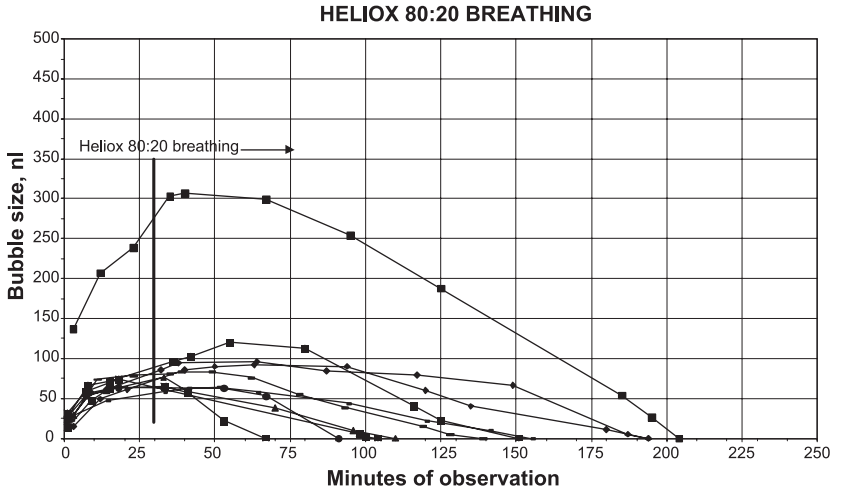

Fig. 4. Effect of heliox (80:20) breathing at 71 kPa on air bubbles in rat adipose tissue. Heliox (80:20) breathing starts from the black vertical bar at 30–35 min postdecompression.

from DCS at sea level since nitrogen bubbles disappear about as fast during heliox(80:20) breathing as during oxygen breathing without the initial bubble growth seen during oxygen breathing. Further, the variability of bubble behavior in these and previous experiments (8, 10, 12) is particularly conspicuous during oxygen breathing and seems unpredictable. In the present study, we found that air bubbles in a lipid tissue will grow more during oxygen breathing at hypobaric conditions than at normobaric or hyperbaric conditions. Growth of bubbles during oxygen breathing, although transient, is obviously undesirable in a clinical situation. However, it is difficult to make clinical recommendations from bubble studies in adipose tissue although previous experiments have demonstrated a similar behavior of bubbles in rat spinal cord (11, 12). In addition to undesirable effects (bubble growth and vasoconstriction), oxygen has of course many positive effects, being vital and antiedematous, and may reduce the tendency of leukocytes to block microvessels after exposure to bubbles (3, 11, 15, 35). The optimal treatment pressure of oxygen must depend on the balance between these effects.

It is concluded that breathing of heliox is preferable over air breathing. Since oxygen breathing at higher altitudes may promote bubble growth in lipid tissues more than at sea level, it seems likely that breathing of heliox may be beneficial in treating DCS during flight. However, the optimal oxygen partial pressure in the heliox mixture remains to be established. Our results also suggest that experiments testing the effect of oxygen and of an oxygen-enriched helium breathing mixture at higher altitudes are warranted.

ACKNOWLEDGMENTS

Engineer Kurt Sørensen's and engineer Sven Lohman's help in the construction of the pressure and vacuum chamber is gratefully appreciated as is the assistance of laboratory technician Ian Godfrey for help in the manufacture of the glass micropipettes. A special thanks is given to senior Hyperbaric Supervisor Michael Bering Sifakis in assisting us with chamber support and maintenance. This project would have been impossible without it.

GRANTS

The present work was supported by AGA AB Medical Research Fund, The Laerdal Foundation for Acute Medicine, Idrættens Forskningsråd, The Novo Nordisk Fund, Fonden til Lægevidenskabens Fremme and The Divers Alert Network-DAN Europe Foundation.

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