Effect of oxygen and heliox breathing on air bubbles in adipose tissue during 25-kPa altitude exposures

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Randsøe T, Kvist TM, Hyldegaard O. Effect of oxygen and heliox breathing on air bubbles in adipose tissue during 25-kPa altitude exposures. *J Appl Physiol* 105: 1492–1497, 2008. First published August 28, 2008; doi:10.1152/japplphysiol.90840.2008.—At altitude, bubbles are known to form and grow in blood and tissues causing altitude decompression sickness. Previous reports indicate that treatment of decompression sickness by means of oxygen breathing at altitude may cause unwanted bubble growth. In this report we visually followed the in vivo changes of micro air bubbles injected into adipose tissue of anesthetized rats at 101.3 kPa (sea level) after which they were decompressed from 101.3 kPa to and held at 25 kPa (10,350 m), during breathing of oxygen or a heliox(34:66) mixture (34% helium and 66% oxygen). Furthermore, bubbles were studied during oxygen breathing preceded by a 3-h period of preoxygenation to eliminate tissue nitrogen before decompression. During oxygen breathing, bubbles grew from 11 to 198 min (mean: 121 min, ±SD 53.4) after which they remained stable or began to shrink slowly. During heliox breathing bubbles grew from 30 to 130 min (mean: 67 min, ±SD 31.0) from which point they stabilized or shrank slowly. No bubbles disappeared during either oxygen or heliox breathing. Preoxygenation followed by continuous oxygen breathing at altitude caused most bubbles to grow from 19 to 179 min (mean: 51 min, ±SD 47.7) after which they started shrinking or remained stable throughout the observation period. Bubble growth time was significantly longer during oxygen breathing compared with heliox breathing and preoxygenated animals. Significantly more bubbles disappeared in preoxygenated animals compared with oxygen and heliox breathing. Preoxygenation enhanced bubble disappearance compared with oxygen and heliox breathing but did not prevent bubble growth. The results indicate that oxygen breathing at 25 kPa promotes air bubble growth in adipose tissue regardless of the tissue nitrogen pressure.

decompression sickness; preoxygenation

FLYING AFTER DIVING may induce decompression sickness (DCS) during flight despite standard decompression procedures, since most pressurized aircrafts normally maintain the cabin pressure equivalent to ~2,500 meters above sea level corresponding to a barometric pressure of ~75 kPa (20). Several cases of DCS have occurred in this situation (18, 25, 28). Loss of cabin pressure in commercial and military aircrafts or in the spacecraft and during extravehicular activities (EVA procedures) could also result in DCS (6). In the first case, DCS would result from a near normobaric nitrogen saturation exposure. Oxygen breathing combined with fast decent from altitude, followed by ground level oxygen breathing or recompression with hyperbaric oxygen is the standard treatment for altitude DCS (19). For astronauts and fighter pilots, DCS is prevented by a period of oxygen breathing to reduce tissue nitrogen. Animal studies as well as theoretical models have shown that oxygen may contribute significantly to the evolution of bubbles during DCS (4, 5, 10, 16, 21, 24). In previous reports we found that nitrogen bubbles in adipose tissue in rats decompressed to sea level after a hyperbaric exposure (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). When air bubbles injected into lipid and aqueous tissues are exposed to the combined effects of oxygen breathing and recompression, a smaller increase in bubble volume and of much shorter duration than that seen during oxygen breathing at sea level is observed (13). In a recent report (14) we followed the in vivo volume changes of air bubbles injected into adipose tissue of rats decompressed from 101.3 kPa and held at 71 kPa, corresponding to an altitude of ~2,750 m above sea level or equivalent to a maximum allowable cabin pressure in commercial aircraft. We found that oxygen breathing at 71 kPa will cause increased growth of air bubbles compared with oxygen breathing at sea level (14). Under similar conditions, bubble growth during breathing of heliox(50:50) or heliox(80:20) mixtures were significantly less pronounced compared with oxygen breathing (14). Since oxygen breathing at 71 kPa caused increased bubble growth compared with oxygen breathing at sea level or during hyperbaric conditions, it seems possible that oxygen breathing at higher altitudes will promote bubble growth even more. 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 relatively greater importance during hypobaric conditions (27) than in the normo- or hyperbaric situation (7).

Accordingly, the purpose of the present experiments was to study the behavior of nitrogen bubbles during oxygen breathing at 25 kPa, corresponding to ~10,350 m above sea level and by means of preoxygenation, eliminating tissue nitrogen, to study the net contribution of tissue nitrogen to the injected air bubble evolution while breathing oxygen at this altitude. Furthermore, we investigate whether a heliox breathing mixture would be advantageous in the treatment of such bubbles.

METHODS

Animal preparation and experimental protocol. As in previous reports (9, 10, 13), non-pregnant female Wistar rats weighing 250–350 g with free access to food and water were chosen because of their abundant and transparent, abdominal adipose tissue into which bubbles can be injected and clearly viewed through the microscope. The

rats 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 denitrogenated saline by means of a syringe pump (SAGE Instruments model 341) at a rate of 1 ml/h. To avoid any bubble formation during saline infusion at altitude, denitrogenated saline was prepared by means of boiling and subsequent storing in sterile gas-tight syringes with Luer lock. Mean arterial blood pressure (MAP) was measured throughout the experiment by means of a Statham AA pressure transducer placed inside the pressure chamber. A continuous record of temperature and mean arterial blood pressure was obtained on Picolog data collection software installed on a personal computer. The abdomen was opened in the midline and the abdominal adipose tissue was exposed. Part of the bowel including cecum was gently lifted aside, wrapped in saline-soaked gauze, and covered with polyethylene membrane to avoid evaporation. With a 2.0-mm ID cannulla, the cecum was perforated and the cannulla left in situ to function as drainage for any expanding bowel gases during decompression. After exposing the adipose tissue, a glass micropipette mounted on a 5 μl Hamilton syringe was guided to the tissue, and two to three air bubbles, usually in the volume range of 2–500 nl, were injected superficially into the adipose tissue using a UMP2 ultra precision pump from WPI. The principle of the injection technique was described previously (12). During decompression, bubbles will grow irregular in shape caused by the expanding air explained by the effect of Boyle-Mariotte and the tissue elasticity of the adipose tissue allowing the bubble expansion. Accordingly, their size was measured as visible surface area, a method that adds an uncertainty to our results. However, an increase in bubble area should only imply an even greater increase in volume. 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 a 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. The breathing gas, i.e., oxygen or heliox(34:66) (i.e., 34% helium and 66% oxygen), was connected, and in the preoxygenation experiments, oxygen breathing continued without interruption. The predecompression bubble dimensions were obtained. The vacuum pump was started, and the chamber was evacuated to 25 kPa (i.e., 0.25 Bar) absolute in 36 min with two stops. The second bubble observation, reflecting bulk decompression, was then obtained. From then on the pressure was maintained at 25 kPa during which period the rats continued breathing either oxygen or heliox(34:66). At 25 kPa, this heliox breathing mixture is marginally hypoxic [i.e., hypoxia defined as $P_{arterial}O_2$ less than 7.3 kPa or 55 mmHg (22)] with an inspiratory oxygen partial pressure ~13 kPa (or 95 mmHg), an arterial oxygen hemoglobin saturation just below 90% with an $P_{alveolar}O_2$ of ~7.4 kPa (or 55.5 mmHg). The heliox mixture was verified using a Teledyne helium/oxygen ANALYZER by Vandagraph with a measuring accuracy of 1–2%.

With the rat inside the chamber at 25 kPa, the bubbles were observed for up to 220 min or until they disappeared from view. All bubble observations and comparisons were done at 25 kPa. At the end of the observation period, the animal was recompressed from 25 kPa to normobaric pressure at 101.3 kPa (sea level). 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. The animal experiments 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. The anesthetized animal was placed supine on a circular plate that 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 an average of 37°C [see Fig. 1 in (13)]. 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. The breathing and pressurization system has been described in a previous report (14).

Bubbles were observed through the chamber window at ×40 magnification by means of a Wild M10 stereomicroscope with a long focal-length objective. The bubble field was illuminated by two flexible fiber optic light guides attached to a Volpo Intralux 5,000 lamp. A Kappa CF 15/2 color video camera was fitted to the microscope, and the field was both displayed on a TV screen and recorded on VHS videotape [see Fig. 1 in (13)]. With a frame grabber board, real-time images could be grabbed to a Macintosh IISi computer. With the use of the NIH Image version 1.61 program (23), the visible surface area of the bubbles were calculated by means of automated planimetry. 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. Bubbles were examined with respect to bubble growth time defined as time of observed bubble growth from first observation at 25 kPa until the time of maximal bubbles size was measured. Bubble growth time is expressed in minutes and mean values of bubble growth time are given ±SD. The bubbles were also analyzed with respect to mean growth rate (mm²/min⁻¹) from the time of first observation at 25 kPa (at 36–40 min) until maximal bubble size was measured. If a bubble did not grow but shrank while at 25 kPa, it was given a negative value indicating shrinkage. Mean values of bubble growth rates are given ±SD.

Bubble "net disappearance rate" was expressed as the mean net disappearance rate in square millimeters per minute, i.e., the slope of a line from the measured bubbles size at the time of first bubble observation at 25 kPa to disappearance of the bubble. If a bubble did not disappear, the mean net disappearance rate was calculated as the slope of the line connecting the first observation at 25 kPa (at 36–40 min) with the last observation. If a bubble did not shrink but grew it was given a negative value indicating growth. Mean values of bubble net disappearance rates are given ±SD. To examine whether the difference between two mean values of calculated bubble growth time, bubble growth, or net disappearance rates, were different from zero, test for normality by means of Kolmogorov and Smirnov (KS) test followed by ANOVA was performed on the difference between mean values in the different treatment groups (1, 2, 26). 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, 26). When several bubbles were studied in one animal, their mean value was used in the statistical comparison.

Furthermore, bubbles were analyzed with respect to their mean growth ratio. Bubble growth ratio is calculated as maximal measured bubble size in the observation period divided with the first observed bubble size at 25 kPa immediately after decompression. Four-fold χ^2 test was used to analyze bubble growth ratio, dividing the experiments into: "bubble growth ≥ 1.20 ratio" or "bubble growth ≤ 1.20 ratio," where 1.20 ratio is the smallest observed bubble growth ratio in the

oxygen treatment group (i.e., all bubbles in the oxygen treatment group grew as a minimum 1.2-fold; Refs. 1, 2). 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 at 25 kPa, and observed bubble size caused by the immediate effect of decompression to 25 kPa. For all comparisons, $P < 0.05$ is regarded the criteria for significance.

RESULTS

General condition of animals. All animals seemed unaffected with respect to blood pressure measurements when decompression was initiated and the rats were breathing spontaneously while connected to the overboard dump valve system at 25-kPa pressure. Thirty-six animals were used of which three animals died from DCS and seven had to be discarded for different technical reasons; two animals died caused by overheating and one experiment was stopped because of failure in the Picolog data-collection software. One experiment was stopped because of failure in the video recording and one animal died due to sudden exsanguination caused by accidental disconnection of the a. carotis catheter. Two preoxygenated rats died during the decompression phase at 40- and at 25-kPa pressures without any apparent bubble formation when the animals were examined under the microscope after the hypobaric exposure. In some of the heliox(34:66) experiments, a transient period of hyperventilation was noted in which one of the animals died of DCS.

The rat's vaginal temperature remained at 37°C but with fluctuations within an interval of 35.5–38.5°C during the decompression phase cooling the pressure chamber and the subsequent reheating of the chamber. During decompressions, some bowel expansion was observed. Peristaltic movements were lively and visible throughout the experiment. During the observation period, adipose tissue perfusion in the smaller vessels with a diameter of ~10–15 μm was clearly visible and seemed unaffected throughout the experiment.

Mean arterial blood pressures. With no differences among the treatment groups, the mean arterial blood pressure (MAP) showed a similar pattern during the experiment. Before decompression to 25-kPa atmospheric pressure, the MAP was in the range of 100–160 mmHg in all groups. While at 25 kPa the MAP was within the range of 80–150 mmHg with 100–120 mmHg as the most frequent interval. A slowly decreasing tendency in MAP throughout the observation period, but within the above interval, was observed for all groups.

Comparability of the experimental groups. ANOVA showed no significant differences with respect to the size of the injected

bubbles in the different treatment groups, time from decompression to first bubble observation, or bubble growth caused by the effect of immediate decompression as well as first observed bubble size at 36–40 min at 25 kPa ($P > 0.1$).

Effect of breathing gases on bubbles at 25 kPa. The calculated bubble growth time as well as the growth and net disappearance rates during oxygen, heliox(34:66), and oxygen breathing preceded by 3 h of preoxygenation are shown in Table 1.

During oxygen breathing ($n = 8$ animals), all bubbles ($n = 18$) initially grew for a period of 11–198 min (mean 121 min, ±SD 53.4; see Fig. 1). No bubbles disappeared in the observation period. Most bubbles increased their visible surface area from two to four times and one rat died of decompression sickness during the observation period. Bubble growth rate was 0.012 mm²·min⁻¹ (±SD = 0.007) and net disappearance rate −0.0045 mm²·min⁻¹ (±SD = 0.0041). The bubble growth ratio was equal to or larger than 1.20 in all observed bubbles.

During heliox(34:66) breathing ($n = 8$ animals), 17 of 18 bubbles initially grew for a period of 30–132 min (mean 67 min, ±SD 31.0; see Fig. 2). Bubble growth rate was 0.015 mm²·min⁻¹ (±SD = 0.018) and net disappearance rate was −0.0045 mm²·min⁻¹ (±SD = 0.0063). No bubbles disappeared in the observation period. Two rats died of decompression sickness during the observation period. The bubble growth ratio was smaller than 1.20 in 4 of 18 bubbles.

When oxygen breathing at altitude was preceded by a 3-h period of normobaric oxygen breathing ($n = 10$ animals), 17 bubbles of 20 grew or remained stable for a period of 19–179 min (mean 51 min, ±SD 47.7 min) from which point they remained constant or began to shrink slowly. Bubble growth rate was 0.007 mm²·min⁻¹ (±SD = 0.008) and net disappearance rate was 0.0022 mm²·min⁻¹ (±SD = 0.0068). Three bubbles shrank consistently and eight bubbles disappeared in the observation period (see Fig. 3). Bubble growth ratio was smaller than 1.20 in 12 of 20 bubbles.

Comparison of bubble growth time, growth, and net disappearance rates. ANOVA followed by multiple comparisons among the groups showed that the growth time was significantly longer during oxygen breathing compared with heliox(34:66) ($P < 0.05$) and when oxygen breathing was preceded by preoxygenation ($P < 0.01$). The faster bubble net disappearance rate observed in preoxygeneated animals were not quite significant compared with non-preoxygenated animals breathing either oxygen or heliox(34:66) ($P = 0.07$). Bubble growth or net disappearance rates during oxygen or heliox breathing were not different ($P > 0.1$). See Table 1.

Comparison of bubble growth ratio. A fourfold χ^2 test showed that when oxygen breathing at altitude was preceded

Table 1. *Gas effects on bubble growth time, growth rate, and net disappearance rates in rat adipose tissue at 25-kPa ambient pressure*

	Oxygen ($n = 8$)	Heliox(34:66) ($n = 8$)	Preoxygenation ($n = 10$)
Bubble growth time, min	121±53.4	67±31.0*	51±47.7*
Bubble growth rate, mm²·min⁻¹	0.012±0.007	0.015±0.018	0.007±0.008
Bubble net disappearance rate, mm²·min⁻¹	−0.0045±0.0041	−0.0045±0.0063	0.0022±0.0068†

Values are means ± SD. *Bubble growth time in animals exposed to 3 h of preoxygenation followed by oxygen breathing at altitude is different from oxygen ($P < 0.01$) and heliox(34:66) breathing ($P < 0.05$) without preoxygenation. Heliox(34:66) breathing is different from oxygen breathing ($P < 0.05$). †$P = 0.07$ compared with non-preoxygenated animals breathing either oxygen or heliox(34:66).
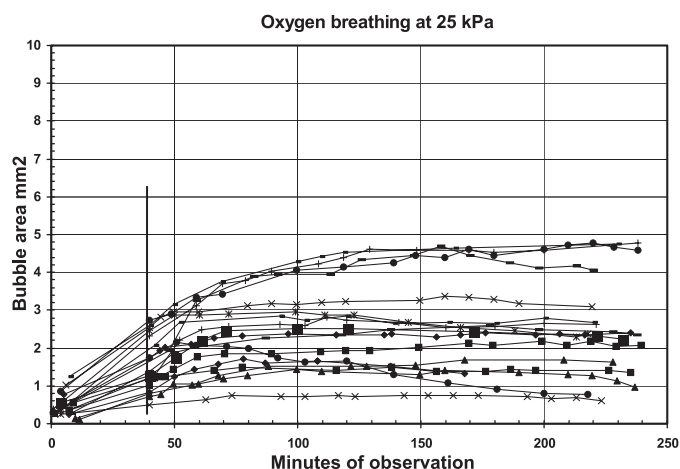
Fig. 1. Effect of oxygen breathing at 25 kPa on air bubbles in rat adipose tissue. Oxygen breathing from first point on the curves. The initial volume increase, i.e., between the first 2 points of the curves, is mainly the effect of decompression. The vertical bar indicates the time of arrival at 25 kPa during decompression from 101.3 kPa. Each curve represents 1 bubble.

Fig. 3. Effect of oxygen breathing at 25 kPa on air bubbles in rat adipose tissue when preceded by preoxygenation. Oxygen breathing from first point on the curves. The initial volume increase, i.e., between the first 2 points of the curves, is mainly the effect of decompression. The vertical bar indicates the time of arrival at 25 kPa during decompression from 101.3 kPa. Each curve represents 1 bubble.

by 3 h of preoxygenation bubble growth ratio was significantly smaller than during both oxygen ($P < 0.001$) and heliox(34:66) breathing ($P < 0.05$). There were no differences between oxygen- and heliox-breathing animals ($P > 0.1$).

Comparison of bubbles disappeared with bubbles not disappeared. A fourfold χ^2 test showed that the number of bubbles that disappeared in the observation period when oxygen breathing at altitude was preceded by preoxygenation (8 of 20) was significantly different from both oxygen (0 of 18) and heliox(34:66) (0 of 18) breathing ($P < 0.01$).

DISCUSSION

Bubbles initially increased in volume with a factor 101.3/25 = 4.05 corresponding to the immediate effect of decompression from 101.3- to 25-kPa absolute pressure, which lasted 36 min. When decompression to a subatmospheric pressure of 25 kPa was reached, bubbles initially grew fast then stabilized
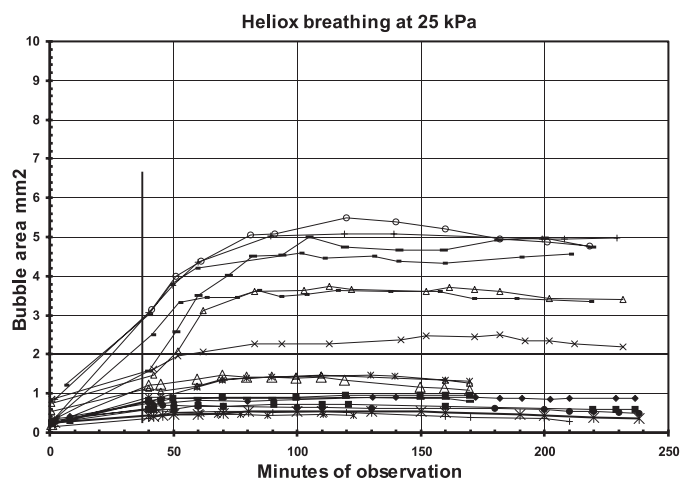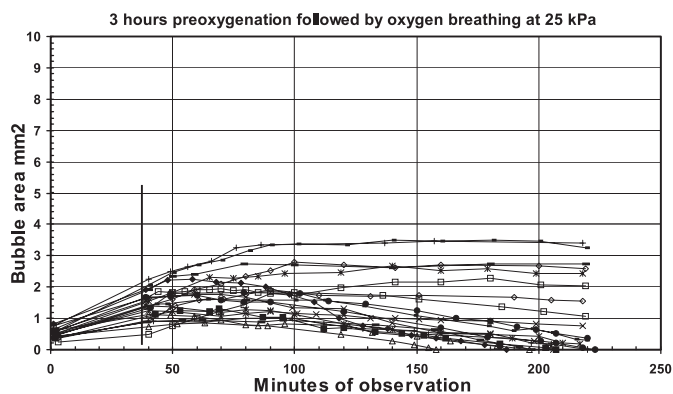

Fig. 2. Effect of heliox(34:66) breathing at 25 kPa on air bubbles in rat adipose tissue. Heliox breathing from first point on the curves. The initial volume increase, i.e., between the first 2 points of the curves, is mainly the effect of decompression. The vertical bar indicates the time of arrival at 25 kPa during decompression from 101.3 kPa. Each curve represents 1 bubble.

or started to shrink slowly depending on the breathing mixture and tissue nitrogen super saturation. The initial faster bubble growth seen in the oxygen and heliox(34:66) experiments may partly be explained by the effect of the nitrogen tissue super saturation, which would be ~60 kPa disregarding the effect of oxygen breathing during the decompression phase, which lasted 36 min. The effect of oxygen or heliox breathing during decompression will further reduce the nitrogen tissue super saturation to ~30 kPa and subsequently decline with a tissue N_2 $T1/2 = 29$ min since the tissue perfusion is 0.105 ml $\times$ g^{-1} $\times$ min^{-1} blood (17) and the partition coefficient (λ) for nitrogen between 85% lipid and blood is 0.066/0.0148 for rat abdominal adipose tissue (10). When the level of decompression to 25 kPa was reached their fate depended on the breathing mixture as outlined below.

Preoxygenation. Three hours of preoxygenation (mean time: 217 min) did not prevent bubble growth during subsequent oxygen breathing despite almost complete tissue nitrogen elimination (see Fig. 3). At 217 min oxygen prebreathing, more than seven tissue half-times has elapsed because the tissue nitrogen half-time in rat adipose tissue is ~29 min (17). This justifies the assumption that all nitrogen has been washed out of the adipose tissue at the time of bubble injection and decompression. When the bubble is injected into the preoxygenated adipose tissue, it will initially consist of 79% nitrogen. During continued oxygen breathing at 25 kPa the arterial oxygen partial pressure $P_{arterial}O_2$ is:

$$P_{arterial}O_2 = P_{alveolar}O_2 = 25.0 \text{ kPa} - 6.3 \text{ kPa } (P_{H_2O})$$
$$- 5.3 \text{ kPa } (P_{alveolar}CO_2) = 13.4 \text{ kPa}.$$

After decompression some nitrogen is still present in the bubble. Disregarding the effect of surface tension and assuming a $P_{bubble}CO_2$ of 6.1 kPa, $P_{bubble}O_2$ is:

$$P_{bubble}O_2 = 25.0 \text{ kPa} - 6.3 \text{ kPa } (P_{H_2O}) - 6.1 \text{ kPa } (P_{bubble}CO_2)$$
$$- P_{bubble}N_2$$
$$= 12.6 \text{ kPa} - P_{bubble}N_2 \text{ kPa}.$$

Consequently, an oxygen partial pressure difference between arterial blood and bubble of 0.8 (=13.4–12.6) +

$P_{bubble}N_2$ kPa exists, explaining bubble growth. As nitrogen is lost by diffusion from the bubble, $P_{bubble}O_2$ rises, and at certain level oxygen begins to diffuse from bubble to the surrounding tissue. At the same time the oxygen partial pressure difference is reduced toward 0.8 kPa, and most bubbles start to shrink, since less oxygen is now received from the blood than is given off to the surrounding tissue. Some bubbles disappear during the observation period, whereas others apparently enter a state of equilibrium, with a near constant bubble size during the observation period. This difference between bubbles must depend on local conditions such as vascularization around the bubble, the metabolic activity of the surrounding tissue, and to a lesser extent, the bubble surface area. If surface tension has any significant effect, it will be to further bubble shrinking tendency; the smaller the bubble is, the greater the effect surface tension will have.

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 100 min after which they disappear at a fast rate. When air bubbles in adipose tissue are studied at 71 kPa (14) during oxygen breathing, all bubbles will grow transiently and most bubbles shrink and disappear at a rate significantly faster than during air breathing ($P < 0.01$). In the present experiments air bubbles were found to grow, then stabilize or shrink only very slowly and remain visible throughout the observation period during oxygen breathing. The fact that no bubbles disappeared but most bubbles grew consistently during oxygen breathing at 25 kPa are at variance with our previously reported observations (14). However, several mechanisms may explain the enhanced oxygen effect on air bubbles at 25 kPa.

In oxygen breathing animals that have not been preoxygenated, the tissue surrounding the bubble will be supersaturated with nitrogen, $P_{tissue}N_2$ being ~40 kPa at the beginning of the observation period, since the time for decompression corresponds to about one halftime for nitrogen in adipose tissue (17). Consequently, nitrogen from the tissue will contribute to bubble growth and dilute oxygen in the bubble, furthering diffusion of oxygen from arterial blood to bubble. Thus a more pronounced and protracted growth of bubbles is to be expected than in the preoxygenated animals. Bubble growth must be supported by the higher solubility of oxygen in blood (both physical and chemical) than of nitrogen in blood. Concomitantly, nitrogen diffuses from bubble to blood at a decreasing rate as $P_{bubble}N_2$ decreases. When nitrogen is finally cleared from tissue [$T_{1/2} \approx 29$ min (17)] and bubble, the situation is similar to that in the preoxygenated animals. As seen in Fig. 1 most of the bubbles maintain their size in the latter part of the observation period indicating equilibrium between oxygen gained from the blood and oxygen lost to the surrounding tissue. It is also conceivable that the vasoconstrictor effect of oxygen is more pronounced at normobaric conditions and at 71 kPa than at 25 kPa, which would favor the microcirculation and thus increase oxygen delivery even more in the latter case.

The above considerations would also apply to our previous observations of a greater increase in bubble size during oxygen breathing at 71 kPa (14) than seen at normobaric pressure (10) and during recompression (284 kPa) (13). Growth of bubbles during oxygen breathing, although transient, is obviously undesirable in a clinical situation. In addition to undesirable effects (bubble growth and vasoconstriction) oxygen has of course many positive effects, being vital, anti-edematous, and reducing the tendency of leukocytes to block microvessels after exposure to bubbles (3, 11, 15, 29). The optimal treatment pressure of oxygen must depend on the balance between these partly opposing effects.

Heliox breathing. During heliox(34:66) breathing in rats without preoxygenation the air bubble will grow in the first part of the observation period because of in-diffusion of nitrogen from the surrounding supersaturated tissue as well as in-diffusion of oxygen and helium from the blood. At the same time, nitrogen is removed from the bubble by the perfusing blood. In the later part of the observation period nitrogen has been eliminated from tissue and bubble and diffusion equilibrium for helium exist among blood, tissue, and bubble. As in the other experiments, the balance between oxygen uptake from the blood and oxygen diffusion to the surrounding tissue now determines bubble size. Comparison of Figs. 1 and 2 reveal no obvious difference in bubble evolution, although bubble growth time was shorter in the heliox experiments ($P < 0.01$).

In some heliox experiments a transient period of hyperventilation was observed likely caused by minor fluctuations in the oxygen content of the heliox mixture used and the fact that 66% oxygen content at 25 kPa is at the limit of a hypoxic mixture, which will be compensated by hyperventilation, thus lowering the alveolar partial pressure of CO_2. Previous results (8, 10, 12) suggest that heliox(80:20) breathing may be beneficial during transportation of a diver suffering 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. However, as ambient pressure decreases, an increase in inspiratory oxygen fraction becomes necessary, thus reducing the maximal allowable helium fraction in the breathing gas. Since the effects of 34% helium in these experiments were only marginal, i.e., reducing bubble growth time but not preventing bubble growth nor increase bubble net disappearance rate, the results do not support the recommendation for the use of a heliox breathing mixture at this altitude.

The present results differ from our previous observations of bubbles in adipose tissue during oxygen breathing at 284 (13), 101.3 (10), or 71 kPa (14). In these experiments the bubbles would disappear after an initial volume increase, the transient bubble growth and bubble disappearance time tending to increase with lower pressure. In the present experiments the bubbles tend to grow and reach a stable size. However, in our earlier experiments the bubbles have all been spherical. After decompression to 25 kPa they assume various shapes, differing from either round or ellipsoid, determined by the tissue. For this reason we have had to asses bubble size by the visible bubble area instead of volume. For the same reason the role of surface tension in bubble disappearance is uncertain. Compared with our previous reports (10, 14), as bubbles grow more than fourfold during the decompression phase from 101.3 to 25 kPa, surface tension must, everything else being equal, be significantly reduced in the present experiments. The degree of nitrogen super saturation at the beginning of the observation period also varies between the different experiments. The fast disappearance of bubbles during oxygen breathing in previous reports was mainly ascribed to the oxygen consumption of the

surrounding tissue. Since this is hardly lower in the present experiments, it seems likely that the fast disappearance was in great part caused by the surface tension of the spherical bubbles. The same considerations apply to the heliox experiments. While the lower solubility in blood of helium compared with nitrogen must be important for the shrinkage of nitrogen bubbles during heliox breathing (10), surface tension must be instrumental in their disappearance.

It is concluded that oxygen breathing at 25 kPa promotes bubble growth in lipid tissues more than at sea level or at 71 kPa (14) and that oxygen breathing at 25 kPa will not enhance bubble disappearance under these experimental 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). On the basis of the present results and our previous reports, it is conceivable that between the altitude exposures of 25 and 71 kPa, bubbles should eventually start to shrink and disappear during oxygen breathing when descending from 25 kPa toward sea level pressure. Our results suggest that experiments testing the effect of oxygen and of an oxygen-enriched helium breathing mixture at altitudes lower than 25 kPa are warranted.

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