J Appl Physiol 107: 1857–1863, 2009.
First published October 22, 2009; doi:10.1152/japplphysiol.00785.2009.

Effect of oxygen breathing and perfluorocarbon emulsion treatment on air bubbles in adipose tissue during decompression sickness

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Submitted 17 July 2009; accepted in final form 15 October 2009

Randsoe T, Hyldegaard O. Effect of oxygen breathing and perfluorocarbon emulsion treatment on air bubbles in adipose tissue during decompression sickness. *J Appl Physiol* 107: 1857–1863, 2009. First published October 22, 2009; doi:10.1152/japplphysiol.00785.2009.—Decompression sickness (DCS) after air diving has been treated with success by means of combined normobaric oxygen breathing and intravascular perfluorocarbon (PFC) emulsions causing increased survival rate and faster bubble clearance from the intravascular compartment. The beneficial PFC effect has been explained by the increased transport capacity of oxygen and inert gases in blood. However, previous reports have shown that extravascular bubbles in lipid tissue of rats suffering from DCS will initially grow during oxygen breathing at normobaric conditions. We hypothesize that the combined effect of normobaric oxygen breathing and intravascular PFC infusion could lead to either enhanced extravascular bubble growth on decompression due to the increased oxygen supply, or that PFC infusion could lead to faster bubble elimination due to the increased solubility and transport capacity in blood for nitrogen causing faster nitrogen tissue desaturation. In anesthetized rats decompressed from a 60-min hyperbaric exposure breathing air at 385 kPa, we visually followed the resolution of micro-air bubbles injected into abdominal adipose tissue while the rats breathed either air, oxygen, or oxygen breathing combined with PFC infusion. All bubble observations were done at 101.3 kPa pressure. During oxygen breathing with or without combined PFC infusion, bubbles disappeared faster compared with air breathing. Combined oxygen breathing and PFC infusion caused faster bubble disappearance compared with oxygen breathing. The combined effect of oxygen breathing and PFC infusion neither prevented nor increased transient bubble growth time, rate, or growth ratio compared with oxygen breathing alone. We conclude that oxygen breathing in combination with PFC infusion causes faster bubble disappearance and does not exacerbate transient bubble growth. PFC infusion may be a valuable adjunct therapy during the first-aid treatment of DCS at normobaric conditions.

gas exchange; first aid; solubility; hyperoxia; oxygen window

CONVENTIONAL TREATMENT of decompression sickness (DCS) consists of recompression in a pressure chamber combined with hyperbaric oxygen breathing (HBO). The purpose is to reduce bubble size by compression of the gas phase, improve tissue oxygenation, and enhance elimination of dissolved inert gas. The first goal is achieved solely by recompression, whereas breathing 100% oxygen will best achieve the other two goals by reducing the uptake of inert gases and creating a larger oxygen window (11, 25, 34). Another way to improve oxygen delivery and reduce the harmful effect of inert gas bubbles is by use of intravascularly administered perfluorocarbon (PFC) emulsions (20, 26, 30). Several reports describe a beneficial effect of combined oxygen breathing and PFC treatment on survival rate and faster intravascular bubble clearance in animals suffering from experimental DCS (8, 9, 21, 22, 32).

In previous reports, Hyldegaard et al. have shown that decompression-induced nitrogen bubbles in adipose tissue (13) or micro-air bubbles injected into tendon (12) and the white substance of the spinal cord (15) will initially grow, then shrink and disappear during oxygen breathing at sea level. This undesirable initial bubble growth during oxygen breathing can be explained by a greater flux of oxygen into the bubble than the concomitant net flux of nitrogen out of the bubble, mostly due to the higher carrying capacity of oxygen than nitrogen in blood.

According to the reasoning above, it is conceivable that combined oxygen breathing and PFC infusion could promote the growth of extravascular bubbles on decompression due to the increased oxygen supply. In keeping with PFC's high capacity for dissolving nitrogen, it also seems possible that the initial bubble growth seen during oxygen breathing could be either reduced or even eliminated because of the greater solubility of nitrogen in PFC and thereby a greater transport capacity of nitrogen in blood causing faster nitrogen desaturation.

In vivo observation of extravascular bubble size changes in animals suffering from DCS and treated with oxygen breathing and PFC infusion has to our knowledge not been reported before. Therefore we decided to extend previous experiments (12, 13, 15) and at 101.3 kPa examine the effect of air, 100% oxygen breathing, and combined 100% oxygen breathing with PFC infusion on the size of extravascular micro-air bubbles injected into the adipose tissue of rats, decompressed from a 1-h hyperbaric air exposure at 385 kPa (14) during continuous monitoring of local adipose tissue oxygen partial pressure (Pt_{O_2}).

METHODS

Animal Preparation and Experimental Protocol

Fat female Wistar rats weighing 250–350 g were anesthetized with intraperitoneal sodium thiomebumal (0.1 g/kg) and subcutaneous buprenorphine (0.01–0.05 mg/kg). Before putting the rat in the pressure chamber, the rat was placed supine and fixed to an operating and heating platform on top of an aqueous insulating layer. 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 and as a way for administering PFC. It was kept patent by a continuous infusion of nonheparinized 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 pressure transducer from Edwards Life Sciences placed inside the pressure chamber, and body temperature was measured by a thermometer placed in the vagina. The vaginal thermometer was connected to a thermostat preset at 37°C. A continuous real-time

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record of temperature and MAP was obtained on a PC via a Picolog data collection software.

The abdomen was opened in the midline, and the abdominal adipose tissue was exposed. A Licox oxygen microcatheter and a Licox thermoprobe were placed inside the adipose tissue for continuous measuring of Pt_{O_2}. Pt_{O_2}values were registered every 10–15 min during the observation period. The exposed tissue was covered with gas-impermeable Mylar membrane and a polyethylene membrane to prevent evaporation. Thereafter the abdomen was closed, by pinching the skin flaps together with nontraumatic surgical clamps.

The animal was then transferred to the pressure chamber attached to the operating and heating platform. Once inside the chamber the tracheal cannula was connected to the T-shaped tube in the chamber breathing system. The connections for arterial blood pressure registration (MAP), rat vaginal thermometer, thermo- and Pt_{O_2}Licox catheters were made. The Pt_{O_2}catheter was then autocalibrated by connecting the Licox thermo- and oxygen sensor cables to the Licox CMP tissue oxygen pressure monitor by Integra NeuroSciences. The top steel lid of the pressure chamber was mounted and all the experimental groups of animals were then exposed to 60 min of hyperbaric air breathing at 385 kPa. During compression, chamber temperature was set to 32–36°C to maintain a body temperature of 37°C. After 1 h at 385 kPa the animals were decompressed over 7.5 min in three stages (14). After decompression the breathing gas was changed immediately to 100% oxygen, with control animals breathing air throughout the observation period. Subsequently, the upper steel lid was removed from the chamber, and for a predetermined designated experimental group, PFC was administered through the carotid catheter at a dose of 2.7 mg/kg.

Removing the surgical clamps reopened the abdomen, and the polyethylene and Mylar membrane was removed from the exposed tissue. With a WPI micromanipulator attached to a stand adjacent to the chamber, a glass micropipette mounted on a 5-μl Hamilton syringe was guided to the adipose tissue, and two to six bubbles in the volume range of 0.5–1.0 μl were injected widely separated into the adipose tissue near the Licox catheter using a UMP2 ultra precision pump from WPI. The principle of the injection technique has been described previously (15). Injection time lasted from 8 to 15 min. Subsequently, the Mylar and polyethylene membrane was repositioned over all of the exposed tissue, and a translucent Plexiglas plate was positioned over the pressure chamber to prevent cooling of the rat. The stereomicroscope was positioned, and the videotape recorder started recording the microscopic picture. Bubble dimensions were measured periodically for up to 200 min or until the bubbles disappeared from view. All bubble observations were done at 101.3 kPa. Finally the animal was removed from the pressure chamber and placed under the operating binoculars. With the rat still attached to the operating and heating platform, the thorax and abdomen were opened for a microscopic scan for intra- or extravascular gas formation before exsanguination. The experimental use of anesthetized animals was approved by a Government-granted license from the Danish Animal Ethical Committee at the Department of Justice and conducted in agreement with the Declaration of Helsinki II.

Pressurizing System and Bubble Monitoring 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 Ref. 16). 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 (16).

Bubbles were observed through the chamber window at ×40 magnification by means of a Wild M10 stereomicroscope with a long-focal-length objective. Two flexible fiber-optic light guides, attached to a Volpo Intralux 5000 lamp, illuminated the bubble field. 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 Ref. 16). With a frame grabber board, real-time images could be grabbed to a Macintosh IISi computer. Using the NIH Image version 1.61 program (27), the visible surface area of the bubbles was 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 101.3 kPa until the time of maximal measured bubble size. Bubble growth time is expressed in minutes, and mean values of bubble growth time are given ± SD. Bubbles were also analyzed with respect to growth rate (μm^2/min), calculated from the time of first bubble observation at 101.3 kPa until maximal measured bubble size was measured. Mean values of bubble growth rates are given ± SD.

Bubble net disappearance rate was expressed as the mean net disappearance rate (in μm^2/min), i.e., the slope of a line from the measured bubble size at the time of first bubble observation at 101.3 kPa to disappearance of the bubble. If a bubble did not disappear, the mean net disappearance rate was calculated as the slope of a line connecting the first observation at 101.3 kPa with the last point of observation. If the last measured bubble size was greater than the first measured bubble size, the net disappearance rate was given a negative value, indicating an overall bubble growth. Mean values of net disappearance rates are given ± SD.

To examine whether the difference between two mean values of calculated bubble growth time, bubble growth rate, 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, 33). The difference between mean values of the various treatment groups was then analyzed by use of the Student-Newman-Keuls procedure for multiple comparison of means between groups. When several bubbles were studied in the same animal, their mean value was used in the statistical comparison.

Bubbles were also 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 101.3 kPa immediately after bubble injection. Fisher's exact test was used to analyze bubble growth ratio, dividing the experiments into "bubble growth ≥ 1.034 ratio" or "bubble growth ≤ 1.034 ratio", where 1.034 ratio is the smallest observed bubble growth ratio in the oxygen treatment group (i.e., in the oxygen treatment group, one bubble only had a shrinking phase, 20 bubbles grew a minimum 1.034-fold). Bubbles were also compared with respect to "bubbles disappeared" or "bubbles not disappeared" by means of Fisher's exact test.

The mean values of the postdecompression Pt_{O_2}(mmHg) measurements for each animal were calculated. To examine whether the difference between two mean values of Pt_{O_2}measurements were different from zero, test for normality by means of KS test followed by one-way ANOVA was performed on the difference between mean values in the different treatment groups. The difference between mean values of the various treatment groups were then analyzed by use of

Table 1. *Effect of air, oxygen, and combined oxygen + PFC infusion on air bubbles in rat adipose tissue at 101.3 kPa during DCS*

	Growth Time, min	Growth Rate, mm^2/min	Net Disappearance Rate, μm^2/min	Bubbles Disappeared	Bubble Growth Ratio > 1.034
Air (n = 13)	86.0±26.8[a]	2,544±1,460[b]	−420±806[c]	0 of 25[e]	25 of 25[f]
Oxygen (n = 9)	46.3±24.2	5,501±3,346	3,844±3,571[d]	17 of 21	20 of 21
Oxygen + PFC (n = 11)	31.0±9.0	8,271±7,449	7,479±4,286	29 of 31	24 of 31

Values are means ± SD; n = no. of animals. PFC, perfluorocarbon; DCS, decompression sickness. [a]Bubble growth time during air breathing different from oxygen- and combined oxygen breathing with PFC infusion ($P < 0.05$). [b]Bubble growth rate during air breathing different from combined oxygen breathing with PFC infusion ($P < 0.05$). [c]Bubble net disappearance rate during air breathing different from oxygen breathing ($P < 0.01$) and combined oxygen breathing with PFC infusion ($P < 0.0001$). [d]Bubble net disappearance rate during oxygen breathing different from combined oxygen breathing with PFC infusion ($P < 0.05$). [e]Bubble disappearance during air breathing different from oxygen- and oxygen breathing with PFC infusion ($P < 0.0001$). [f]Bubble growth ratio during air breathing different from combined oxygen breathing with PFC infusion ($P < 0.05$).

the Student-Newman-Keuls procedure for multiple comparison of means between groups (1, 2, 33).

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

RESULTS

General Conditions of Animals and Development of DCS

In all, 43 animals were used. Twenty animals were used in the air-breathing group, and four of them died of DCS during the observation period of which three died within 20–32 min after decompression and before bubble injection and observation could be made. Within the 16 of the air-breathing animals surviving the observation period, 13 had measurable injected bubbles and 3 animals had to be discharged from the study due to failure in the injection pump. Ten animals had implanted a Pt_{O_2} Licox catheter. All of the 10 oxygen-breathing animals and all of the 13 animals breathing combined oxygen with PFC infusion survived the observation period, and within both groups all had a Pt_{O_2} Licox catheter inserted. Of the 10 oxygen-breathing animals, nine had injected bubbles, and of the 13 PFC-treated animals, 11 had bubble injections.

The animals were observed for up to 200 min postdecompression. When the abdominal and thoracic cavities of the 39 rats that lived through the observation period (i.e., 16 air breathing, 10 oxygen breathing, and 13 breathing oxygen combined with PFC infusion) were opened for microscopic examination before exsanguinations, no bubbles were visible in the veins. In some rats an occasional extravascular bubble could be seen in the adipose tissue.

MAP

Before and during the 1-h hyperbaric pressure exposure, the MAP was stable and in the range of 150–180 mmHg as the most frequent interval for all three groups. After the decompression period, all three groups had a slowly decreasing tendency in the MAP, with the most frequent interval at the range of 100–160 mmHg in the end of the observation period. In air-breathing animals dying during the observation period, the MAP had an abrupt decrease before death.

Temperature and State of Adipose Tissue

The rat temperature remained at 37°C while during decompression cooling the pressure chamber a slight decrease of temperature to 35.5–36°C was observed. After decompression and at 101.3 kPa ambient pressure, the rat's vaginal temperature remained stable at 36–37°C for the rest of the observation period. In all experiments, adipose tissue blood perfusion in the smaller vessels of 10–15 μm in diameter was clearly visible during the observation period.

Comparability of the Experimental Groups

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 from decompression to first bubble observation ($P > 0.1$).

Effect of Breathing Gases and PFC Infusion on Bubbles and Pt_{O_2}

For all treatment groups, mean bubble growth time, growth rate, and mean net disappearance rates, as well as bubbles disappeared and growth ratios, are given in Table 1.

Air breathing. During air breathing (n = 13 animals), all bubbles (n = 25) initially grew for about 27–152 min, after which they remained stable or began to shrink slowly; two bubbles continued growing during the entire observation period and no bubbles disappeared during the observation period (see Fig. 1).The mean net disappearance rate (see Table 1) during air breathing was negative, indicating an overall bubble growth. In air-breathing animals, Pt_{O_2} mean value was 51 mmHg (SD ±18 mmHg). Four animals died during the observation period, of which the three animals that died from within 20–32 min after decompression had ample bubbles present in their veins when examined immediately postmortem. No visible bubbles were found in the animal that died 170 min after decompression.

Oxygen breathing. During oxygen breathing (n = 9 animals), 16 bubbles (n = 21) initially grew for about 16–46 min, after which point they shrank until disappearing from view; one bubble shrank consistently without initial growth, while four bubbles were growing for up to 126 min, from which point they started shrinking slowly without disappearing during the observation period (see Fig. 2). In oxygen-breathing animals, Pt_{O_2} mean value was 77 mmHg (SD ±25 mmHg). No animals died during the observation period.

Oxygen breathing with PFC infusion. During combined oxygen breathing with PFC infusion (n = 11 animals), 27 bubbles (n = 31) grew for about 15–53 min, after which point 25 bubbles shrank until disappearing from view, while two bubbles shrank slowly without disappearing during the obser-

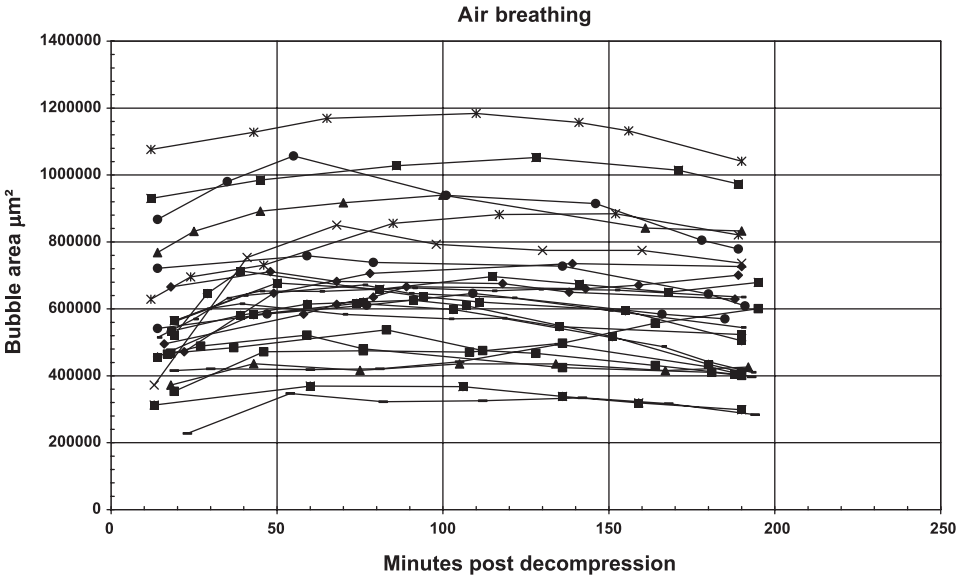

Fig. 1. Effect of air breathing on air bubbles in adipose tissue. Air breathing from first point on each curve. Individual symbols represent 1 bubble curve.

vation period; four bubbles shrank consistently without initial growth (see Fig. 3 and Table 1). During combined oxygen breathing and PFC infusion, Pt_{O_2} mean value was 99 mmHg (SD ±36 mmHg). In three of the PFC-treated animals, occasional bubbles were observed in abdominal veins within 30 min postdecompression. No animals died during the observation period.

Comparison of Bubble Growth Time, Growth Rate, and Net Disappearance Rate

ANOVA followed by multiple comparison of means between groups showed that bubble growth time was significantly longer during air breathing compared with both oxygen breathing and combined oxygen breathing with PFC infusion ($P < 0.05$). There were no differences in bubble growth time when combined oxygen breathing with PFC infusion was compared with oxygen breathing alone. Bubbles had a significantly faster growth rate during combined oxygen breathing with PFC infusion than during air breathing ($P < 0.05$). Bubble growth rate during combined oxygen breathing and PFC infusion was not different from oxygen breathing or when oxygen breathing was compared with air breathing. The net disappearance rate of bubbles during oxygen breathing was significantly faster than during air breathing ($P < 0.01$) and combined oxygen breathing with PFC infusion caused a significantly faster net disappearance rate than during both air ($P < 0.0001$) and oxygen breathing ($P < 0.05$).

Comparison of Bubble Growth Ratio

Mean bubble growth ratio was significantly smaller during combined oxygen breathing with PFC infusion compared with air breathing ($P < 0.05$). There was no significant difference when oxygen breathing was compared with either combined oxygen breathing with PFC infusion or air breathing ($P > 0.1$).

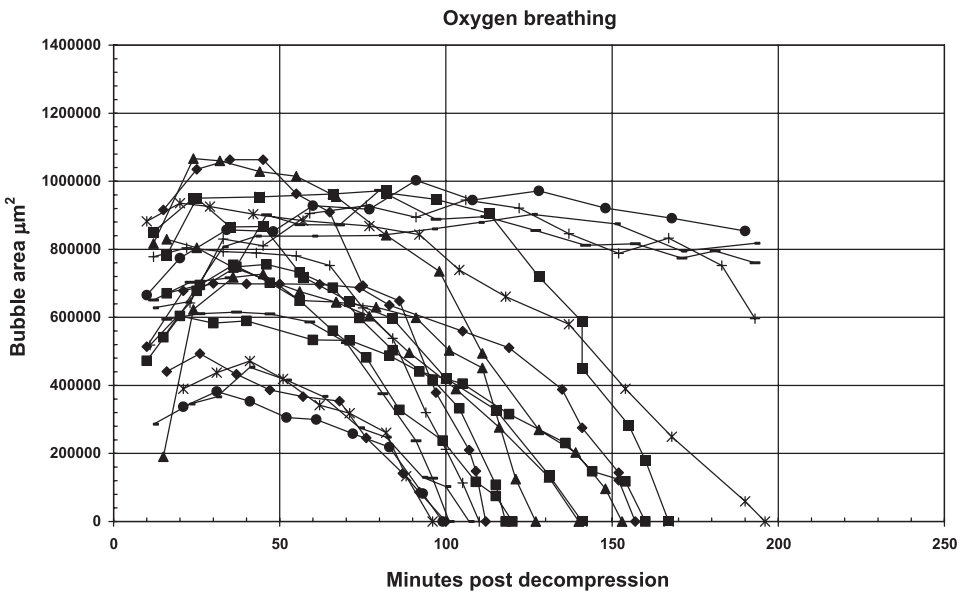

Fig. 2. Effect of oxygen breathing on air bubbles in adipose tissue. Oxygen breathing from first point on each curve. Individual symbols represent 1 bubble curve.

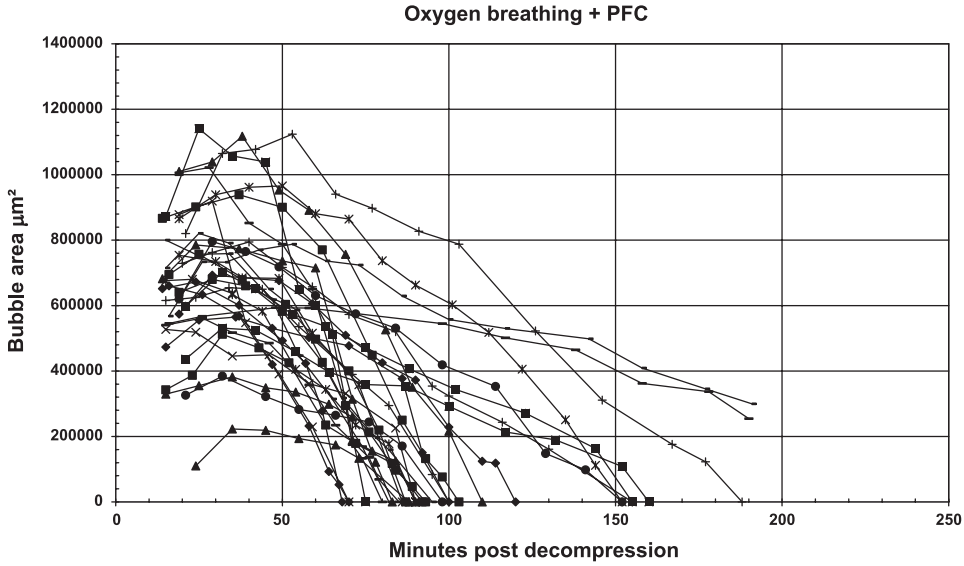

Fig. 3. Effect of combined oxygen breathing and perfluorocarbon (PFC) infusion (2.7 mg/kg) on air bubbles in adipose tissue. Oxygen and PFC breathing from first point on each curve. Individual symbols represent 1 bubble curve.

Comparison of Bubbles Disappeared With Bubbles Not Disappeared

Fisher's exact test showed that the number of bubbles that disappeared in the observation period during both oxygen breathing and combined oxygen breathing with PFC infusion was significantly different from air breathing ($P < 0.0001$). There was no significant difference in bubble disappearance between the group breathing oxygen or oxygen combined with PFC infusion.

Comparison of Pt_{O_2} Values After Decompression

There was a significant difference in mean Pt_{O_2}values in air-breathing animals compared with oxygen and combined oxygen breathing and PFC-treated animals ($P < 0.05$). The greater Pt_{O_2}during combined PFC infusion and oxygen breathing compared with oxygen breathing alone was not quite significant ($0.1 > P > 0.05$).

DISCUSSION

Effect of Air Breathing

From 27 to 152 min after decompression, bubbles grew during breathing of air. This bubble growth can be explained by the effect of the tissue N_2 supersaturation. After decompression from 385 kPa to 101.3 kPa breathing air (i.e., 79% nitrogen), the N_2 supersaturation was ~170 kPa, declining with a N_2 half-time of 29 min since the tissue perfusion is 0.105 ml blood·g^{-1}·min^{-1} (23) and the partition coefficient (λ) for N_2 between 85% lipid and blood is 0.066/0.0148 for rat abdominal adipose tissue (23, 38). From 1 to 2 h after decompression most bubbles became stable, reflecting equilibrium between growth-provoking influences (i.e., tissue supersaturation) and shrinkage-promoting factors such as the oxygen window effect (36, 37) and overpressure in the bubble caused by surface tension and tissue elasticity. After some time the N_2 tissue partial pressure will come in equilibration with the partial pressure of the alveolar gas and arterial blood and the only driving force for bubble elimination will be the oxygen window and over pressure in the bubble as outlined above.

Effect of Oxygen Breathing

In previous reports, we have shown that bubbles in rat adipose tissue created by decompression (13) or by microinjection of air in spinal white matter (15) during oxygen breathing will grow for a period of 10 to more than 100 min, after which they will disappear at a fast rate. In these experiments, oxygen breathing caused a similar transient bubble growth lasting from 16 to 126 min after decompression. This transient growth can be explained *1*) by the greater capacity of blood to carry more dissolved oxygen to the tissue than it can concomitantly remove inert gas caused mainly by hemoglobin when the P_{O_2} in the tissue is 12–13 kPa; at higher P_{O_2} by the difference in solubility coefficients (0.022 ml gas·ml $blood^{-1}$·atm^{-1} for oxygen, 0.0148 ml gas/ml blood for N_2), 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 greater oxygen permeability (i.e., solubility coefficient × diffusion coefficient) in the lipid tissue than of N_2 (18, 38). Further, *3*) bubble growth is initially favored by the increasing gradient for diffusion of N_2 from the supersaturated surrounding tissue into the bubble as the N_2 in the bubble is diluted by oxygen. This effect is furthered by the increasing fraction of oxygen in the bubble but wanes as the tissue is desaturated for N_2. Obviously, bubble growth must also be influenced by *4*) the blood flow rate and thereby *5*) degree of oxygen-induced vasoconstriction as well as *6*) the rate of oxygen consumption by the tissue, because a high ratio between oxygen delivery and oxygen consumption would favor bubble growth. 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 N_2 from the bubble will exceed the gain of oxygen and N_2, and the bubble will shrink as oxygen diffuses to the surrounding metabolizing tissue and N_2 will be exhaled because of the increased oxygen window (36). In the present experiment, four bubbles did not disappear within the observation period but maintained their augmented size (see Fig. 2). This variable and

unpredictable bubble behavior is particularly conspicuous during oxygen breathing and corresponds to our previous observations (13, 15, 17).

Effect of Combined Oxygen Breathing and PFC Infusion

Perfluorochemicals are synthetic straight-chain or cyclic hydrophobic hydrocarbons first developed for the industry but later made miscible with water through combination with organic emulsification agents and thereby adapted for biological use. Their most important property of relevance to biology is as vascular gas transporters due to their high capacity for dissolving respiratory gases (19, 30, 31). This ability has made the PFC emulsions obvious candidates for treating DCS, and several reports describe a beneficial effect of treating experimental animals suffering from severe DCS. This includes improved survival rate of rodents treated with combined PFC infusion and oxygen breathing as well as PFC infusion and air breathing on reaching the surface after rapid decompression (21, 22, 32). Combined PFC and oxygen breathing has also demonstrated a reduction in morbidity and mortality in swine saturation models (8, 9). The advantages of using a PFC emulsion for treating DCS is due to its ability of increasing oxygen delivery to stricken tissue as well as the ability to rapidly dissolve and transport N_2 from tissues to the lungs, thereby preventing the formation of harmful N_2 gas emboli. Whereas hemoglobin binds oxygen actively in a sigmoid dissociation curve, PFC liquids are passive gas transporters and both oxygen and N_2 dissolve into and come out of solution in a linear fashion based on the partial pressure gradient of the gas (19, 30, 31).

In the present experiment, combined PFC infusion and 100% oxygen breathing caused bubbles in adipose tissue to grow at a faster rate than during air breathing and shrink at a faster rate than during both air and 100% oxygen breathing. The initial bubble growth and subsequent shrinking of the bubbles seen while administering PFC can be explained by the same mechanisms that apply for oxygen breathing and as described above. However, since the rate of oxygen consumption is hardly any different within the three experimental groups, the faster growth rate seen during PFC infusion must in part be attributed to *1*) the increased oxygen supply, leading to increased oxygen flux into the bubble, thereby causing an increased gradient for diffusion of N_2 from the surrounding supersaturated tissue to the bubble, as N_2 in the bubble is diluted by oxygen. Further, the faster net disappearance rate must be influenced by *2*) a greater transport capacity of N_2 in blood caused by the intravascular PFC emulsions, thus increasing the rate of tissue N_2 desaturation and presumably *3*) a limitation in the negative effect of oxygen-induced vasoconstriction due to a possible increase in plasma volume and flow by the PFC infusion ($\sim$1.0 ml) and thereby improved peripheral microcirculation, although the volume of the PFC infusion in these experiments represents $<$5% of the total accumulated saline infusion (5–6 ml) during the experiment and estimated total rat blood volume of 16–23 ml given a rat weight of 250–350 g (5, 28). Finally, *4*) part of the initial bubble volume increase may be reduced by the fact that the animals were given oxygen and PFC immediately after decompression causing a minor reduction in N_2 tissue partial pressure before bubble observations were initiated.

While whole blood (hematocrit 41%) may carry 21% oxygen, PFC emulsions may carry up to 60 vol% at 101.3 kPa at 37°C. The carrying capacity of PFC emulsions for N_2 may approach up to 50 vol% at 101.3 kPa at 37°C, which stands in contrast to the poor solubility of N_2 in whole blood or plasma (N_2 solubility in plasma is 0.015 vol% at 37°C) (18, 19, 32, 38). Furthermore, these emulsions have an average particle size of $<$0.2 μm in diameter (red blood cell is 7 μm in diameter), which gives them a large surface area for gas transfer and possibly an ability to improve oxygen delivery in hypoxic tissue peripheral to vascular obstruction, by passing through plasma gaps. This should make PFC emulsions ideal for eliminating N_2 and improving oxygen delivery and thereby reducing the risk of DCS (19, 31, 32). The study by Novotny et al. (26) demonstrated that PFC infusion was able to increase tissue off-gassing of xenon from muscle by $\sim$33%.

The bubble growth observed is obviously undesirable, but despite the significantly faster growth rate seen during combined oxygen breathing and PFC infusion compared with air-breathing animals, bubble growth ratio was significantly smaller during combined oxygen breathing and PFC infusion compared with air breathing. Further, there were no differences in growth rate, growth time, or growth ratio during combined oxygen breathing and PFC infusion compared with the oxygen-breathing group. Accordingly, under the present experimental conditions we do not find any reason for reservations with respect to the use of PFC infusion compared with oxygen breathing alone, since the combination of the two treatment modalities did not enhance transient bubble growth. Treatment of DCS after air diving usually combines recompression and pure oxygen breathing due to oxygen's many positive effects, such as improving oxygenation, enhanced elimination of dissolved inert gases, ability of being anti-edematous and reducing the tendency of leukocytes to block microvessels after exposure to bubbles and decrease platelet aggregation (39). However, oxygen breathing during recompression therapy may also result in some undesirable effects. These involve oxygen-induced vasoconstriction (3, 4) and limitations in maximal therapeutic partial pressure and duration due to oxygen toxicity (7). If victims of DCS undergo recompression it is possible that cerebral oxygen toxicity (seizures) might result as it was demonstrated by Mahon et al. (24) and others (8, 29), an effect ascribed to the hyperbaric hyperoxia caused by the PFC infusion. From previous studies (8, 24) it has been demonstrated that treatment with PFC at depth was not as effective as PFC administered at surface. However, whether the treatment of DCS with a combination of oxygen breathing and PFC infusion at lower pressures, thus reducing or completely avoiding the risks of oxygen toxicity, may be equally effective as standard oxygen treatment pressures [i.e., 284 kPa, US Navy Treatment Table 6 (35)] remains to be investigated.

During the course of DCS treatment, a fast bubble resolution rate without transient bubble growth is essential (25, 34). In previous reports, heliox (80:20) or heliox(50:50) breathing at sea level or during recompression have been shown to enhance bubble disappearance rate in lipid and aqueous tissues, without transient bubble growth as seen during oxygen breathing (12, 13, 15, 16). Further, since the solubility of N_2 in blood and PFC is greater than that of helium (18, 19, 38), experiments testing the combined effect of a heliox breathing mixture with PFC infusion on the rate of N_2 bubble resolution in lipid and aqueous tissues seem warranted. DCS and bubbles in blood are known to activate

several different immune mechanisms such as cytokine activation (10), as well as complement and platelet activation (6). Whether PFC will have any effect on these secondary bubble effects during treatment of DCS should be further studied.

In conclusion, the present results do not support the hypothesis that combined oxygen breathing and PFC infusion enhances transient bubble growth in adipose tissue compared with oxygen breathing alone. The present experiments indicate that although transient bubble growth caused by the increased oxygen tension is not eliminated, the combined effect of oxygen breathing and PFC infusion at sea level will result in faster bubble disappearance explained by a combined effect of the increased oxygen window and the greater N_2 transport capacity in blood increasing the rate of N_2 desaturation.

ACKNOWLEDGMENTS

The assistance of laboratory technician Ian Godfrey for help in the manufacture of the glass micropipettes is greatly appreciated. Thanks are given to senior Hyperbaric Supervisor Michael Bering Sifakis in assisting us with chamber support and maintenance.

The support given from Alliance Pharmaceutical, San Diego, CA, for supplying the perfluorocarbon emulsion Oxygent is greatly appreciated. This project would have been impossible without it.

GRANTS

The present work was supported by The Danish Council for Independent Research Medical Sciences (Grant Nos. 271-05-0096 and 271-07-0016), The A. P. Møller Foundation for the Advancement of Medical Science , The Laerdal Foundation for Acute Medicine, and The DAN Europe Research Foundation.

DISCLOSURES

No conflicts of interest are declared by the authors.

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