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EFFECT OF OXYGEN BREATHING AND PERFLUOROCARBON EMULSION TREATMENT ON AIR BUBBLES IN ADIPOSE TISSUE DURING DECOMPRESSION SICKNESS

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Running head: Treatment of DCS with perflurocarbon

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ABSTRACT:

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 extra vascular 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 extra vascular bubble growth upon 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 anaesthetized rats decompressed from a 60 minutes 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 when compared to air breathing. Combined oxygen breathing and PFC infusion caused faster bubble disappearance when compared to oxygen breathing. The combined effect of oxygen breathing and PFC infusion did neither prevent nor increase transient bubble growth time, rate- or growth ratio when compared to oxygen breathing alone. We conclude, that oxygen breathing in combination with PFC infusion cause 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

Introduction:

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 intravascular administrated perfluorocarbon emulsions (20, 26, 30). Several reports describe a beneficial effect of combined oxygen breathing and perflurocarbon (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 extra vascular bubbles upon 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 due to 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 extra vascular 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 extent 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 extra vascular micro air bubbles injected into the adipose tissue of rats, decompressed from a one hour hyperbaric air exposure at 385 kPa (14) during continuous monitoring of local adipose tissue oxygen partial pressure (P_tO_2).

Methods:

Animal Preparation and Experimental Protocol:

Fat female wistar rats weighing 250-350 gram were anaesthetized with intraperitoneal sodium thiomebumal (0.1 g/Kg) and subcutaneous buphrenorphine (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 way for administering PFC. It was kept patent by a continuous infusion of non-heparinized 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 pre-set at 37° centigrade. A continuous real time 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 micro-catheter and a Licox® thermo probe were placed inside the adipose tissue for continuous measuring of P_tO_2. P_tO_2 values were registered every 10-15 minutes 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 non-traumatic 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 P_tO_2 Licox® catheters were made. The P_tO_2 catheter was then auto calibrated 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 minutes 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 one hour at 385 kPa the animals were decompressed over 7.5 minutes 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 8-15 minutes. 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 as positioned, and the videotape recorder started recording the microscopic picture. Bubble dimensions were measured periodically for up to 200 minutes 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 extra vascular gas formation before exsanguination. The experimental use of anaesthetized 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 was performed in a specially designed pressure chamber with a horizontal viewing port 16 cm in diameter. The anaesthetized 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 figure 1. in (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 x40 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 figure 1. in (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 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 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 ($\mu m^2 \bullet min^{-1}$),* 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 $\mu m^2 \bullet min^{-1}$, *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 were 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. Fishers 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 as a minimum 1.034-fold). Bubbles were also compared with respect to *"bubbles disappeared"* or *"bubbles not disappeared"* by means of Fishers Exact test.

The mean values of the post decompression P_tO_2 (*mmHg*) measurements for each animal were calculated. To examine whether the difference between two mean values of P_tO_2 measurements were different from zero, test for normality by means of Kolmogorov and Smirnov (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 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 the limit for significance.

Results:

General conditions of animals and development of Decompression sickness:

In all 43 animals were used. Twenty animals were used in the air-breathing group and 4 of them died of DCS during the observation period of which 3 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 three animals had to be discharged from the study due to failure in the injection pump. 10 animals had implanted a P_tO_2 Licox catheter. All of the 10 oxygen-breathing animals and all of the 13 animals breathing combined oxygen with PCF infusion survived the observation period and within both groups all had a P_tO_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 post decompression. 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 extra vascular bubble could be seen in the adipose tissue.

Mean arterial blood pressure-MAP:

Before and during the one-hour hyperbaric pressure exposure, the MAP was stabile 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 P_tO_2:

For all treatment groups, mean bubble growth time, growth rate and mean net disappearance rate's, 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 figure 1).The mean net disappearance rate (See Table 1) during air breathing was negative indicating an overall bubble growth. In air breathing animals, P_tO_2 mean value was 51 mmHg (SD: +/- 18 mmHg). Four animals died during the observation period of which the 3 animals that died from within 20-32 minutes after decompression had ample bubbles present in their veins when examined immediately post mortem. No visible bubbles were found in the animal that died 170 minutes 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 figure 2). In oxygen breathing animals, P_tO_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 observation period; four bubbles shrank consistently without initial growth (See figure 3 and Table 1). During combined oxygen breathing and PFC infusion, P_tO_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 post decompression. 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 when compared to 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 where compared to 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 were not different from oxygen breathing or when oxygen breathing was compared to 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 when compared to air breathing ($P < 0.05$). There was no significant difference when oxygen breathing was compared to either combined oxygen breathing with PFC infusion or air breathing ($P > 0.1$)

Comparison of bubbles disappeared with bubbles not disappeared:

Fishers 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 P_tO_2 values after decompression:

There was a significant difference in mean P_tO_2 values in air breathing animals when compared to oxygen and combined oxygen breathing and PFC treated animals ($P < 0.05$). The greater P_tO_2 during combined PFC infusion and oxygen breathing as compared to oxygen breathing alone was not quite significant ($0.1 > P > 0.05$).

Discussion:

Effect of Air breathing: From 27-152 minutes after decompression bubbles grew during breathing of air. This bubble growth can be explained by the effect of the tissue N_2 super saturation. After decompression from 385 kPa to 101.3 kPa breathing air (i.e. 79% nitrogen), the N_2 super saturation was approximately 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 hours after decompression most bubbles became stable reflecting equilibrium between growth provoking influences (i.e. tissue super saturation) 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 minutes, after which they will disappear at a fast rate. In these experiments, oxygen breathing caused a similar transient bubble growth lasting from 16 – 126 minutes 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 PO_2 in the tissue is 12-13 kPa; at higher PO_2 by the difference in solubility coefficients (0.022 ml gas x ml

318 blood^{-1} x atm^{-1} for oxygen, 0.0148 ml gas x ml blood^{-1} for N_2), i.e. at equal partial pressure differences, blood will
319 carry more dissolved oxygen to the tissue than it can concomitantly remove inert gas; and 2) by the greater
320 oxygen permeability (i.e., solubility coefficient x diffusion coefficient) in the lipid tissue than of N_2 (18, 38).
321 Further, 3) bubble growth is initially favored by the increasing gradient for diffusion of N_2 from the supersaturated
322 surrounding tissue into the bubble as the N_2 in the bubble is diluted by oxygen. This effect is furthered by the
323 increasing fraction of oxygen in the bubble but wanes as the tissue is desaturated for N_2. Obviously, bubble
324 growth must also be influenced by 4) the blood flow rate and thereby 5) degree of oxygen induced
325 vasoconstriction as well as 6) the rate of oxygen consumption by the tissue, because a high ratio between
326 oxygen delivery and oxygen consumption would favor bubble growth. As the oxygen partial pressure in the
327 bubble increases, the oxygen diffusion gradient from blood to bubble decreases, while the oxygen gradient from
328 bubble to tissue increases. From a certain point, the total loss of oxygen and N_2 from the bubble will exceed the
329 gain of oxygen and N_2, and the bubble will shrink as oxygen diffuses to the surrounding metabolizing tissue and
330 N_2 will be exhaled because of the increased oxygen window (36). In the present experiment, four bubbles did not
331 disappear within the observation period but maintained their augmented size (See figure 2). This variable and
332 unpredictable bubble behavior is particularly conspicuous during oxygen breathing and corresponds to our
333 previous observations (13, 15, 17).

334 Effect of combined oxygen breathing and PFC infusion: Perfluorochemicals are synthetic
335 straight chain or cyclic hydrophobic hydrocarbons first developed for the industry but later made miscible with
336 water through combination with organic emulsification agents and thereby adapted for biological use. Their most
337 important property of relevance to biology is as vascular gas transporters due to their high capacity for dissolving
338 respiratory gases (19, 30, 31). This ability has made the PFC emulsions obvious candidates for treating DCS and
339 several reports describe a beneficial effect of treating experimental animals suffering from severe DCS. This
340 include improved survival rate of rodents treated with combined PFC infusion and oxygen breathing as well as
341 PFC infusion and air breathing upon the reach of surface after rapid decompression (21, 22, 32). Combined PFC
342 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 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 (approximately 1.0 ml) and thereby improved peripheral micro circulation, although the volume of the PFC infusion in these experiments represents less than 5% of the total accumulated saline infusion (5-6 ml) during the experiment and estimated total rat blood volume of 16-23 ml giving a rat weight of 250-350 gram (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-volume % at 101.3 kPa at 37°C. The carrying capacity of PFC emulsions for N_2 may approach up to 50-volume % at 101.3 kPa at 37°C, which stands in contrast to N_2's poor solubility in whole blood or plasma (N_2 solubility in

368 plasma is 0.015 volume % at 37°C) (18, 19, 32, 38). Furthermore, these emulsions have an average particle size
369 of less than 0.2 μm in diameter (red blood cells is 7 μm in diameter) which gives them a large surface area for
370 gas transfer and possibly an ability to improve oxygen delivery in hypoxic tissue peripheral to vascular
371 obstruction, by passing through plasma gaps. This should make PFC emulsions ideal for eliminating N_2 and
372 improving oxygen delivery and thereby reducing the risk of DCS (19, 31, 32). The study by Novotny et al. (26)
373 demonstrated that PFC infusion were able to increase tissue off-gassing of xenon from muscle by some 33%.
374 The bubble growth observed is obviously undesirable, but despite the significantly faster growth
375 rate seen during combined oxygen breathing and PFC infusion when compared to air-breathing animals, bubble
376 growth ratio was significantly smaller during combined oxygen breathing and PFC infusion when compared to air
377 breathing. Further, there were no differences in growth rate, growth time or growth ratio during combined oxygen
378 breathing and PFC infusion when compared to the oxygen-breathing group. Accordingly, under the present
379 experimental conditions we do not find any reason for reservations with respect to the use of PFC infusion when
380 compared to oxygen breathing alone, since the combination of the two treatment modalities did not enhance
381 transient bubble growth. Treatment of DCS after air diving usually combines recompression and pure oxygen
382 breathing due to oxygen's many positive effects such as, improving oxygenation, enhance elimination of
383 dissolved inert gases, ability of being anti-edematous and reducing the tendency of leukocytes to block micro
384 vessels after exposure to bubbles and decrease platelet aggregation (39). However, oxygen breathing during
385 recompression therapy may also result in some undesirable effects. These involve oxygen induced
386 vasoconstriction (3, 4) and limitations in maximal therapeutic partial pressure and duration due to oxygen-toxicity
387 (7). If victims of DCS undergo recompression it is possible that cerebral oxygen toxicity (seizures) might result as
388 it was demonstrated by Mahon et al. (24) and others (8, 29). An effect ascribed to the hyperbaric hyperoxia
389 caused by the PFC infusion. From previous studies (8, 24) it has been demonstrated that treatment with PFC at
390 depth was not as effective as PFC administered at surface. However, whether the treatment of DCS with a
391 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 cytokines 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 as compared to oxygen breathing alone. The present experiments indicates, 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.

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Legends:

Figure 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.

Figure 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.

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

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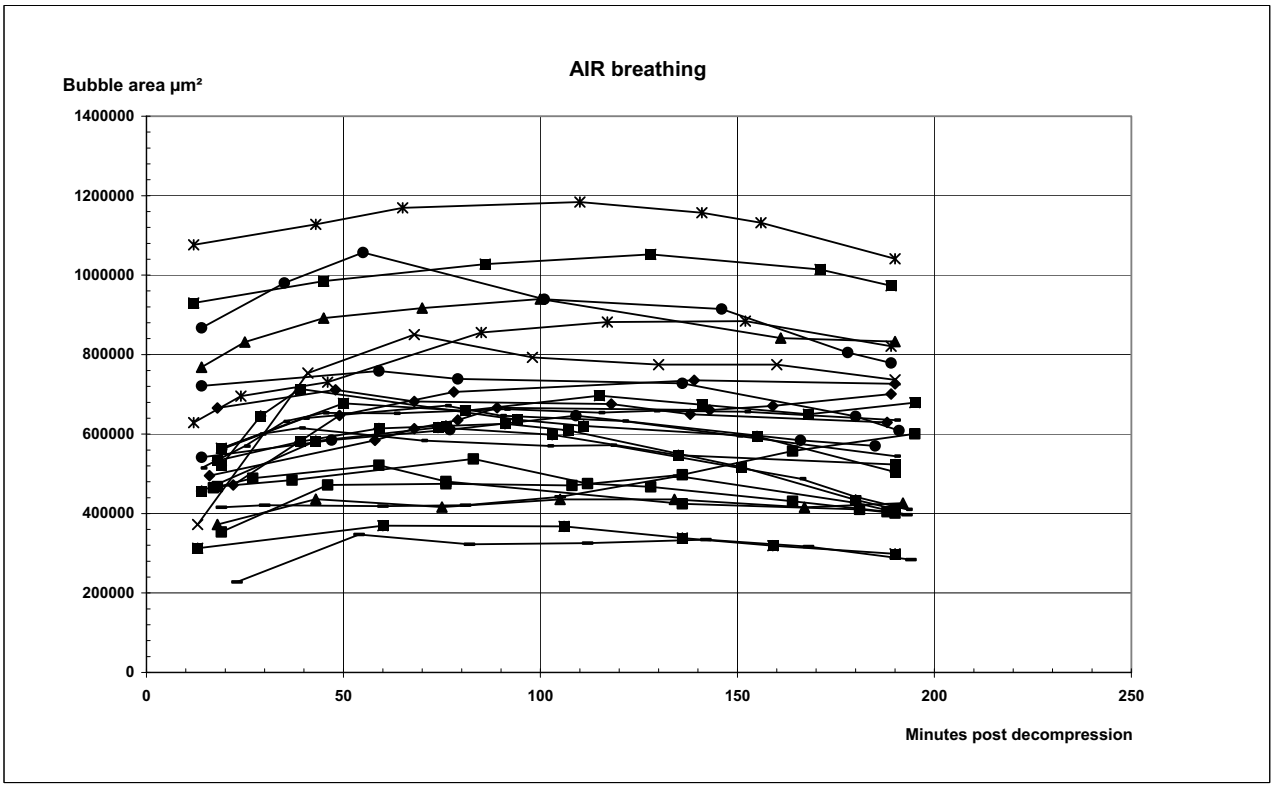

Figure 1.

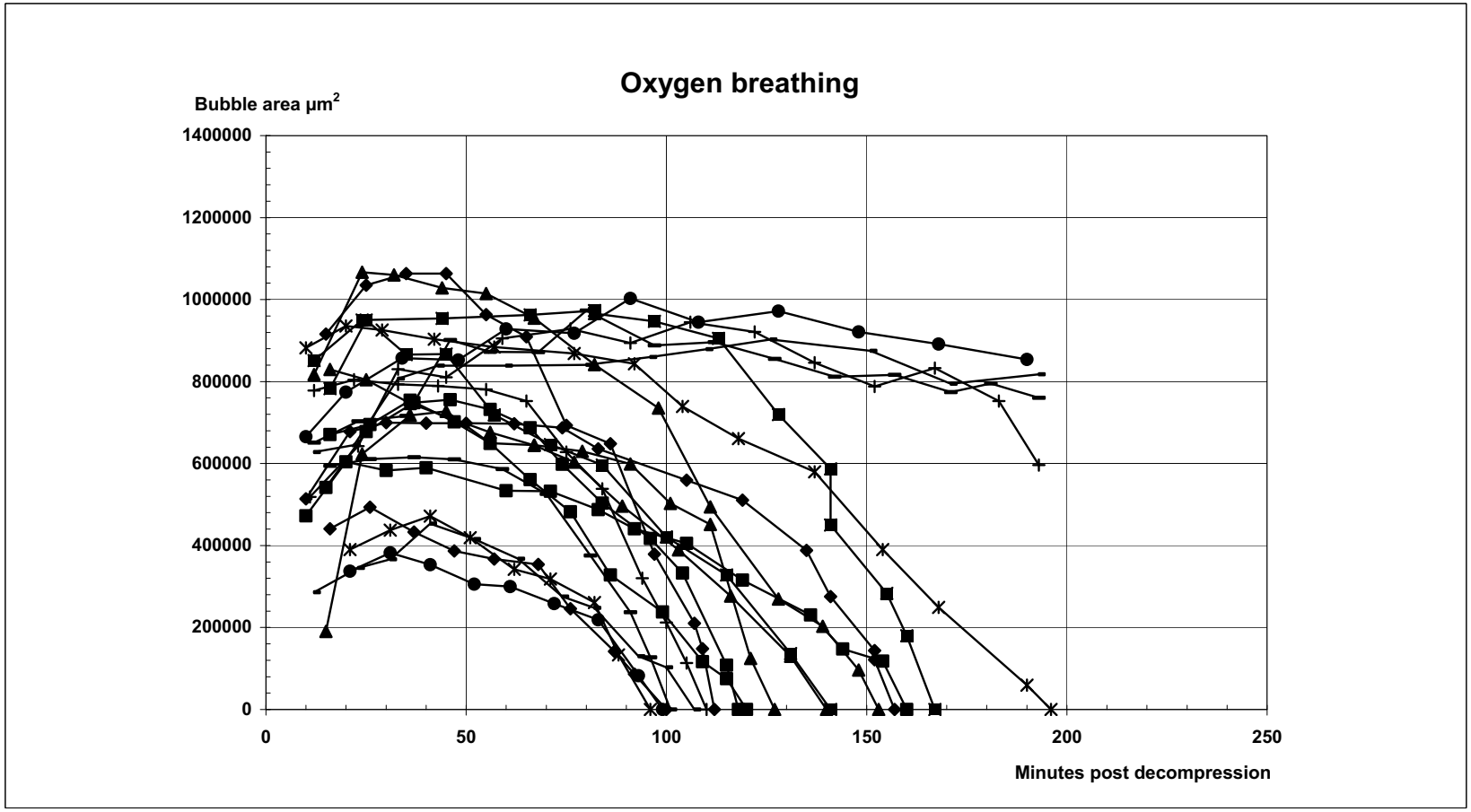

Figure 2.

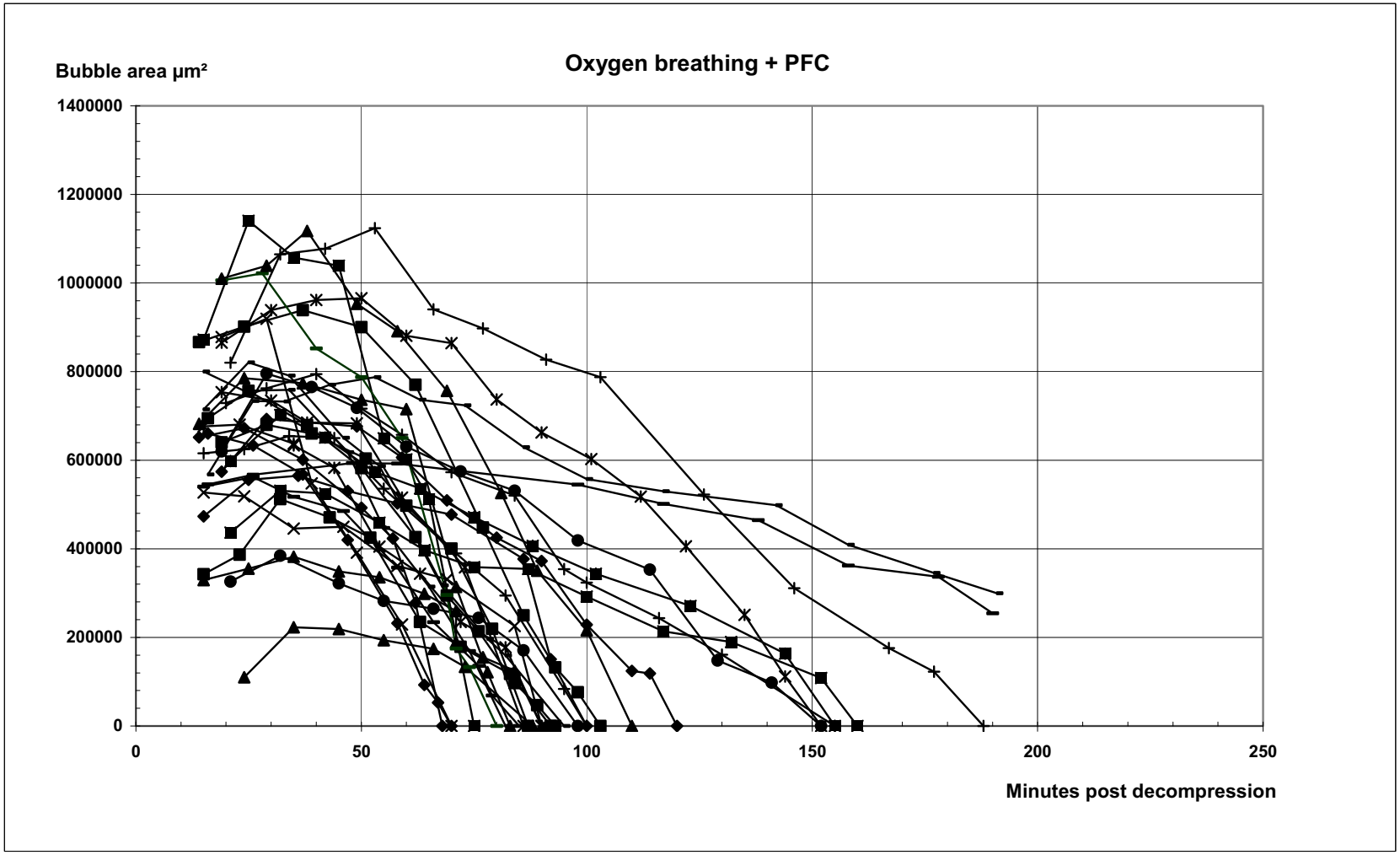

Figure 3.

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

	Growth time minutes	Growth rate μm^2/min	Net disappearance rate μm^2/min	Bubbles disappeared	Bubble growth ratio > 1,034
Air (N = 13)	86.0 (+/- SD 26.8[(1)])	2544 (+/- SD 1460[(2)])	-420 (+/- SD 806[(3)])	0 of 25[(5)]	25 of 25[(6)]
Oxygen (N = 9)	46.3 (+/- SD 24.2)	5501 (+/- SD 3346)	3844 (+/- SD 3571[(4)])	17 of 21	20 of 21
Oxygen + PFC (N = 11)	31.0 (+/- SD 9.0)	8271 (+/- SD 7449)	7479 (+/- SD 4286)	29 of 31	24 of 31

Values are means +/- SD; N = number of animals

(1) Bubble growth time during air breathing different from oxygen- and combined oxygen breathing with PFC infusion: $P < 0.05$

(2) Bubble growth rate during air breathing different from combined oxygen breathing with PFC infusion: $P < 0.05$

(3) Bubble net disappearance rate during air breathing different from oxygen breathing ($P < 0.01$) and combined oxygen breathing with PFC infusion ($P < 0.0001$)

(4) Bubble net disappearance rate during oxygen breathing different from combined oxygen breathing with PFC infusion ($P < 0.05$)

(5) Bubble disappearance during air breathing different from oxygen- and oxygen breathing with PFC infusion ($P < 0.0001$)

(6) Bubble growth ratio during air breathing different from combined oxygen breathing with PFC infusion ($P < 0.05$)

TABLE 1.