Surface tension and bubble formation after decompression in the pig

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Abstract

Following decompression, there is a considerable difference in detected venous gas emboli (VGE) between individuals; this study is an exploration of the role surface tension may play in the differences. We measured serum surface tension in 26 anesthetized pigs before (*predive*) and after a dive (*postdive*) to 300 kPa for 3 hours. Gas bubbles in the pulmonary artery were monitored continuously from the beginning of decompression and continued throughout 120 minutes after the dive. Maximum bubble levels were reached about 30 minutes after surfacing. *Predive* surface tension was significantly higher than *postdive* values (66.8 ± 1.0 dynes/cm, n=26) vs. (66.4 ± 1.0 dynes/cm, n=26). We found a significant negative correlation between *predive* surface tension and the number of bubbles that were generated as a result of the dive. A significant negative correlation was also observed between the generated vascular bubbles and *postdive* surface tension. We conclude that small surface tension differences between individuals may influence vascular bubble formation, and that formation of VGE may itself lower surface tension.

Introduction

Intravascular gas bubbles are formed during most decompressions [1]. However, at any level of exposure, there is a considerable individual variation in the number of vascular bubbles formed [2]. At present, the reason for this is unknown.

The formation of bubbles in the circulation is growth of gas bubbles from micronuclei [3]. These micronuclei are either stable nuclei on surfaces in the body or they are established *de novo* by cavitation. Cavitation is brought on by disruption of the molecular cohesive forces in the liquid, this cohesion work is directly proportional to the surface tension of the fluid [4]. Thus a low surface tension would favor the formation of cavitation nuclei. Decompression bubbles (venous gas emboli) are probably not formed *de novo*, but require nuclei from which to grow [5-7]. One hypothesis is that these nuclei are small (1-2 μm) gas-filled bubbles [3]. Theoretically, the formation and stability of a gas bubble is affected by the surface tension of the fluid; a low surface tension favors bubble formation.

Walder [8] demonstrated in a large number of human individuals that there was an inverse relationship between the level of surface tension in serum and the susceptibility to altitude decompression sickness (DCS). In two separate experiments with a total of 97 individuals Walder showed a 5% lower surface tension in the susceptible group compared to the non-susceptible group. Subjects completing a test without showing any signs or symptoms of decompression sickness were classified as non-susceptible. He could furthermore show in three DCS susceptible individuals that, by drinking 1.5 liters of isotonic saline (surface tension; 72 dynes/cm at 25°C) in one hour, the surface tension in serum increased about 6%. In subsequent altitude exposures these divers did not have any signs of decompression sickness.

Bubble stability is affected by the surface tension, which is the force that draws the bubble to its smallest size. This is evidenced by the Laplace equation for a gas bubble:

$$\Delta P = P_{int} - P_{abs} = 2\,\gamma/r \qquad (1)$$

where γ is the surface tension and r is the bubble radius, P_{int} is the internal gas pressure of the bubble and P_{abs} is the external absolute gas pressure. The internal pressure in the bubble is proportional to r^{-3}. Thus it is the surface tension that determines the overpressure inside the bubble at one particular bubble radius. The smaller the bubbles, the greater is the inner pressure caused by

surface tension [9]. The surface tension of the blood will determine the ΔP required to initiate expansion of the bubbles when supersaturation is present [8]. If gas tensions are higher inside than outside the bubble, the pressure difference, ΔP, will result in an outward diffusion of gas and the bubble will shrink and finally dissolve. The dimension of surface tension is force per unit length (dynes/cm; i.e. 0.001 N/m).

A surfactant will reduce the surface tension by locating on the bubble surface and thus reducing the driving pressure, ΔP, needed for the bubble to collapse; stabilizing the bubble. Furthermore, the presence of an organic skin on the bubble surface may decrease outward diffusion of gas, further stabilizing the bubble [10,11]. In sea-water, this effect will stabilize bubbles of a size between 0.07 and 1 μm [12]. Furthermore, once bubbles have formed, if the surface tension is low, less energy will be required to make them grow [13].

A previous study at our laboratory demonstrated that the surface tension in serum varied with 1% between individuals and with 1% in each individual over a period of 6 months [14].

The present study was initiated to determine if variations in surface tension among animal subjects would affect the formation of gas bubbles in decompression and thus be a factor in determining the variation in bubble formation observed. To our knowledge there is no previously published data on this approach.

Methods

Twenty-six (11 female and 15 male) domestic farm pigs, Norsk Landsvin, (weight between 18.3 - 25.1 kg) were used in this study. All experiments were performed in accordance with the principles for the care and use of animals. The experimental protocol was approved by the Norwegian Council for Animal Experimentation and was monitored by the Head of the Animal Research Facility at the Medical Faculty, Norwegian University of Science and Technology.

Anaesthesia

Fifteeen to twenty minutes before induction of anaesthesia, the animals received pre-medication consisting of 7-9 mg/kg of azaperonum (Stresnils, Jansen) intramuscularly followed by 1 mg atropinsulphate (Atropin, Hydro Pharma). Anaesthesia was induced by 5 mg/kg thiopental sodium (Thiopenthon Natrium, Nycomed Pharma) and 20 mg/kg ketamine (Ketalar, Parke Davis). A tracheostomy was performed, allowing the animal to breathe spontaneously in the supine position. Anaesthesia was maintained by a continuous i.v. infusion of ketamine in 0.9% NaCl (30 mg kg^{-1} h^{-1}). A bolus dose of α-chloralose was injected to regulate the respiratory pattern (10-15 mg/kg, Sigma St. Louis). The level of anaesthesia was checked by observation and by control of blood gases. Body temperature was regulated to 37.5 - 38.0 °C by use of a feed-back system regulating the temperature in the chamber. Catheters were introduced into the jugular vein and moved into the pulmonary artery for measurement of pressure and blood sampling. A catheter was placed into the femoral artery and blood was taken for surface tension measurements before the dive (*predive*) and 30 minutes after the dive (*postdive*).

Dive profile

After placement in a 300 l pressure chamber, the pigs were compressed to 300 kPa in 2 minutes and stayed there for 180 minutes, after which they were decompressed to the surface at a rate of 100 kPa/minute. They were divided into 5 groups according to the gas mixture breathed in the second hour at the bottom. All the pigs were breathing 21 kPa O_2 in N_2 during the first and third hour at the bottom. In the second hour the gas was switched to a mixture of 21 (n=7), 50 (n=4), 100 (n=6) and 200 (n=5) kPa oxygen in helium or 100 (n=4) kPa oxygen in nitrogen. This was part of a study to determine the effect of oxygen tension on inert gas washout [15].

Bubble detection

All pigs were monitored for gas bubbles using ultrasonic scanning during the dive and up to 120 minutes *postdive*. A transesophageal echocardiographic transducer (TEE-probe, 7.5 MHz, CFM 750, Vingmed A/S, Horten, Norway) was inserted into the esophagus providing a 2D image of the right ventricle, the main pulmonary artery and aorta. Air bubbles were observed as bright spots in the blood. The ultrasonic images were transmitted to a computer (Macintosh II) for automatic quantification of the number of gas bubbles in the pulmonary artery [16]. Bubble counting was started at the beginning of decompression and continued for 120 minutes *postdive*. The bubbles are presented as bubbles/cm^2.

Nitrogen detection

To evaluate the nitrogen level in the pigs' venous blood, 1 ml sample was taken through a catheter placed into the pulmonary artery. The samples were drawn into gas-tight syringes and were analysed for nitrogen content using

the gas extraction technique described elsewhere [17]. The variability of this technique is found to be around 5% of the mean.

Surface tension measurements

Serum was used for the measurements. All measurements were done between 21 - 23 °C. The surface tension of serum was measured with a drop volume method by using a surfactometer (AmnioTECH, Vitafaktum AB, Høllviken, Sweden). This instrument was originally developed to measure the surface tension of amniotic fluids and has previously been described in detail by Åberg and Gislen [18]. This device has also been employed in determining surface tension in human individuals at our laboratory [14]. The drop volume method proved to be very accurate; the mean coefficient of variation based on 100 observations with 6 parallel measurements was less than 0.5% [14]. The serum density was set at 1.028 g/cm^3 [19]. Each serum sample was measured 6 times, and the value is given as mean ± SD.

Statistical analysis

The data were subjected to analysis using Mann-Whitney U and Wilcoxon signed rank tests for unpaired and paired data as appropriate. Spearman's rank correlation was used to test any significance of correlation between parameters of interest. Kruskal-Wallis test was used to compare groups breathing different gas mixes. The results were expressed as mean and standard deviation. The level of significance was set at $P \leq 0.05$.

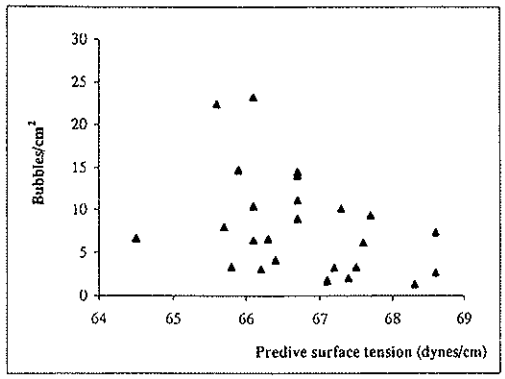

Figure1. Relationship between the number of gas bubbles detected in the pulmonary artery 30 minutes *postdive* and surface tension *predive*, presented individually for each pig (n=26).

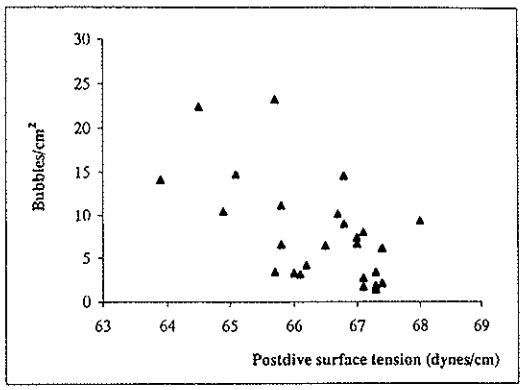

Figure 2. Relationship between the number of gas bubbles detected in the pulmonary artery 30 minutes *postdive* and surface tension *postdive*, presented individually for each pig (n=26).

Results

Intravascular bubbles were detected in all 26 pigs. The mean of the maximum number of bubbles observed was 8.0 ± 6.0 bubbles/cm^2 (range 1.3 - 23.2 bubbles/cm^2). This maximum value was reached approximately 30 minutes after surfacing. Three pigs died *postdive* due to massive bubbling, all experienced arterial bubbles, no arterial bubbles were detected in the remaining pigs.

There were no significant differences in nitrogen concentration in the pulmonary artery at the end of the bottom phase [15] or in *predive* surface tension between the groups, thus results from all pigs have been treated together.

There was a significant negative correlation ($r = 0.4$, $P = 0.024$) between *predive* surface tension and the number of vascular bubbles (Fig.1). The pig with the lowest bubble number (1.35 bubbles/cm^2) had a surface tension of 68.3 dynes/cm as compared to 65.6 dynes/cm for the pig with the highest bubble number (22.39 bubbles/cm^2). A significant negative correlation was observed between the number of vascular bubbles generated from the dive and *postdive* surface tension as seen in Fig.2 ($r = 0.6$, $P = 0.002$).

Individual surface tensions, *pre* and *postdive* values, from all pigs are presented in Fig.1 and Fig.2, respectively. The interindividual difference in *predive* surface tension was 1.5% (n=26). The mean surface tension of *predive* and *postdive* values was 66.8 ± 1.0 dynes/cm (range 64.5 - 68.6 dynes/cm, n=26) and 66.4 ± 1.0 dynes/cm (range 63.9 -68.0 dynes/cm, n = 26), respectively. The *predive* surface tension was significantly higher than the *postdive* values ($P = 0.034$).

There was no significant difference in *predive* surface tension between male and female pigs. There was a significant positive correlation between bodyweight and the number of vascular bubbles formed upon decompression ($r = 0.6$, $P = 0.002$), but no correlation was found between surface tension and bodyweight.

Discussion

This study has shown that there is a significant correlation between the level of surface tension in serum before the dive and the number of bubbles formed after decompression. Our results confirm the theory presented in the introduction that a low surface tension favors bubble formation. Further, these results also support Walder's observations that susceptibility to DCS is due to the level of surface tension [8]. Although Walder had no information about the number of gas bubbles formed in his divers, there is a general agreement that DCS symptoms are caused by gas bubbles [20] and a correlation exists between the number of gas bubbles observed in the pulmonary artery and DCS [21]. The above results indicate that lowering surface tension may increase bubble formation.

Even if the pigs in this study breathed different gas mixtures during the second hour of exposure to pressure, there was no difference in nitrogen levels in the pulmonary artery immediately prior to decompression. This indicates that the level of inert gas supersaturation was similar in all animals.

However, Malette et al. demonstrated in rats pretreated with a surface tension reducing agent (*Antifoam A*), a reduced mortality after decompression [22]. These rats were exposed to a lethal dose of gas emboli caused by a rapid decompression. In their study a lower surface tension *predive* was protective against the presence of the massive gas emboli formed after decompression. They explained the protective effect of *Antifoam A* with a lower resistance to flow by allowing the gas emboli to move from a vital area to a benign location. The actual mechanism of obstruction to normal blood flow has been shown to be the high surface tension and the inability to achieve sufficient pressure to drive the bubbles through the pulmonary capillary bed [23]. When vascular obstruction occurs as a result of gas embolism, the driving pressure required to push the bubble through the vessel and restore blood flow will decrease as the surface tension is lowered [24]. In the present study, the diving profile caused a lethal dose of gas emboli in only 3 animals. We may thus speculate that the lowering of surface tension in the rat study by Malette and colleagues was not sufficient to increase the number of bubbles to a level that would influence mortality. The main effect of the lowering of surface tension would then indeed be an increased bubble transport through the lungs.

The present study demonstrated that the presence of gas bubbles reduced the level of surface tension *postdive* and that this reduction is related to the number of bubbles (Fig. 1). Our observation is supported by Hills and Butler [24] who claimed that surfactant molecules can migrate to the surfaces of trapped pulmonary bubbles and cause a decrease in surface tension. Further, Hills reported reduced surface tension by released bubbles after decompression in spinal tissue immersed in saline [25]. Walder observed in 16 divers performing 44 altitude exposures, a significant reduction in *postdive* surface tension [8].

However, the presence of surfactant on the bubble surface may increase the time for dissolution. Yount et al. [26] postulated that the presence of surfactant on the bubble surface will increase bubble lifetime by reducing diffusivity. There is some experimental evidence that this is actually the case [12]. Thus, the presence of surfactant may tend to stabilize gas bubbles. Furthermore, the rate of increase in the radius of a bubble when supersaturation is present is inversely proportional to the surface tension of the surrounding fluid [27]. One explanation for the reduction in surface tension *postdive* may be the adsorption of an organic skin on the surface of the bubbles. However, reduction of surface tension by exposure to a hyperbaric environment can not be excluded, as several authors have shown that surface tension can be reduced by the introduction of various inert gases [28,29].

How surface tension-reducing agents affect air embolism has previously been studied by several authors [23,30,31]. Eiseman et al. showed that a reduction of surface tension of 11%, reduced the mortality from coronary air embolism in dogs from 95 to 77% when *antifoam* was administered intravenously with the emboli [23]. In another study, a protective effect from venous air embolism was afforded by prior reducing surface tension by 13% in dogs [30]. Another study in pigs showed that reduction of surface tension by approximately 15% prior to air infusion gave a rise in the pulmonary vascular resistance [31], indicating a further penetration of gas into the vascular bed. Both the air embolism studies and the decompression study cited above, indicate that reducing surface tension prior to massive gas embolization reduces mortality. Our study demonstrates that a low *predive* surface tension increases the number of vascular bubbles formed. This must mean that if a large number of gas bubbles are present, any additional bubble load will have a negligible effect on mortality. However, after less stressful decompressions, a high surface tension *predive* may be beneficial by reducing the number of bubbles formed.

A positive correlation was found between bodyweight and number of vascular bubbles. It has previously been reported that bodyweight is an important factor in

determining susceptibility to decompression illness in rats [32].

No significant difference in surface tension was found between genders, this in contrast to our study in humans [14]. We have found no data in the literature on surface tension related to gender in animals.

The interindividual surface tensions observed in these pigs was only slightly higher (1.5% vs. 1%) compared to those we found in 25 human individuals prior to this study [14]. It is surprising that these small differences in surface tension values will influence bubble formation in the pigs. Thus, it is tempting to speculate that the great variability of response to decompression in divers' tendency to form gas bubbles, may in part be explained by surface tension differences. The interindividual difference in surface tension found by others varies from 0.6% in 24 human individuals [33] to 6% in 71 human individuals [34]. Walder observed in one experiment, including 46 altitude divers, an interindividual difference in surface tension of 1.6% [8]. Among these divers some experienced DCS while others showed no signs or symptoms of DCS. However, in Walder's study no data about bubble counts were given.

In the human study at our laboratory we concluded that surface tension is not a static parameter but varies about 1% within each individual over time [14]. This may explain the intraindividual difference in generating bubbles after being exposed to identical pressure profiles at different times. It may also be worth considering to increase surface tension prior to a dive, as observations in the present and other studies imply that even small changes in surface tension may influence bubble formation. This may be achieved by increasing surface tension by drinking water, which has a surface tension of 72.8 dynes/cm at 20 °C [35]. Alcohol should be avoided, as ethanol (34%) in water has a surface tension of 33.2 dynes/cm at 20 °C [35]. Similarly, meals rich in fat should be avoided, as available data indicate that the diet influences serum levels of triglycerides and cholesterol [36]. In the previous human study we concluded that a high lipid level in serum caused a lower surface tension compared to normal serum [14].

In conclusion, our data indicate that small surface tension differences observed between individuals may have an important effect on the individual's variability in vascular gas bubble formation upon decompression. A positive relation was found between bodyweight and number of vascular bubbles. However, a negative correlation was observed between vascular bubbles and *post-dive* surface tension, suggesting migration of surface active molecules to the bubble surface. More work is needed to determine to what extent these results are applicable to human decompression bubbles.

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