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Hyperbaric oxygen may reduce gas bubbles in decompressed prawns by eliminating gas nuclei

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Arieli, Yehuda, Ran Arieli, and Amit Marx. Hyperbaric oxygen may reduce gas bubbles in decompressed prawns by eliminating gas nuclei. *J Appl Physiol* 92: 2596–2599, 2002. First published February 22, 2002; 10.1152/japplphysiol.01059.2001.—It is accepted that gas bubbles grow from preexisting gas nuclei in tissue. The possibility of eliminating gas nuclei may be of benefit in preventing decompression sickness. In the present study, we examined the hypothesis that hyperbaric oxygen may replace the resident gas in the nuclei with oxygen and, because of its metabolic role, eliminate the nuclei themselves. After pretreatment with oxygen, prawns were 98% saturated with nitrogen before explosive decompression at 30 m/min. Ten transparent prawns were exposed to four experimental profiles in a crossover design: *1*) 10-min compression to 203 kPa with air; *2*) 10-min compression with oxygen; *3*) 10-min compression with oxygen to 203 kPa followed by 12 min air at 203 kPa; and *4*) 10 min in normobaric oxygen followed by compression to 203 kPa with air. Bubbles were measured after explosive decompression. We found that pretreatment with hyperbaric oxygen (*profile C*) significantly reduces the number of bubbles and bubble volume. We suggest that hyperbaric oxygen eliminates bubble nuclei in the prawn.

decompression sickness

DECOMPRESSION SICKNESS IS the main hazard in underwater activity such as diving and rescue from a disabled submarine. At high pressure, inert gas is loaded in the tissues. During decompression, this supersaturated gas is released in the form of bubbles. The pathological effect of these bubbles is decompression sickness (2). Various protocols may be used to reduce the risk of decompression sickness. These include elimination of the inert gas by breathing oxygen before escape from a disabled submarine, as investigated in goats, rats, and humans (14, 19). In the above-mentioned protocols, the purpose of the oxygen is either to reduce the partial pressure of the inert gas in the breathing mixture, as with the use of oxygen-enriched gas mixtures (nitrox, a nitrogen-oxygen mixture) in diving, or to wash out the resident inert gas. However, there are conditions where high risk is unavoidable, such as during escape from a disabled submarine (4, 14) and an aborted dive.

The hypothesis that gas bubbles are formed by the expansion and growth of bubble nuclei present in tissue is widely accepted (5, 9, 10, 15, 18, 20). A number of hypothetical models have been suggested for these gas nuclei: gas nuclei trapped in crevices on the surface of hydrophobic particles, gas nuclei composed of nonsoluble gas, and gas nuclei coated by surface-active molecules or by an elastic skin (8–10, 17). The growth of a nucleus into a bubble is retarded by the action of surface tension (11). Exposure of the animal to a very high pressure for a short period of time considerably reduced the evolution of bubbles and decompression sickness (3, 18) This was related to the elimination of bubble nuclei. Tikuisis (15), using the model of a gas nucleus in a hydrophobic conical or elliptical crevice, suggested that high pressure caused a reduction in the volume of the nucleus so that on subsequent decompression it did not expand over the critical radius to form a bubble. Thus the elimination of gas nuclei may be beneficial in reducing the risk of decompression sickness. The elimination of gas nuclei by prepressurization, as employed in rats and crabs (13, 18), carries too great a risk for humans.

However, the resident gas in the nucleus can be exchanged with a gas in the tissue by diffusion. If the tissue is perfused with pure oxygen, oxygen may replace the resident gas in the nucleus. When the oxygen tension is reduced, the oxygen might then be consumed from the bubble nucleus, thus eliminating it completely. It is also possible that in hyperbaric conditions the elimination of gas nuclei will be accelerated. Elevated pressure will increase the partial pressure difference between the resident gas in the nucleus and the oxygen in the tissue. Thus the elimination of a nucleus will consist of two phases: *phase I*, replacement of the resident gas by oxygen; and *phase II,* consumption of the oxygen from the nucleus. Reduction of tissue oxygen pressure by replacing the oxygen with air could enhance the consumption of oxygen from the nucleus. Using our established prawn model (1), we tested the hypothesis that pretreatment of a prawn with hyperbaric oxygen before an air-saturation dive would reduce the formation of bubbles on explosive decompression. Although the prawn is an invertebrate with gill respiration, which has no real vascular system, no internal skeleton, and lacks hemoglobin to

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carry oxygen or carbon dioxide, its tissues do not differ basically from those of a vertebrate where the mechanisms of bubble formation are concerned (3, 12, 13).

METHODS

Animals. The small (~50 mm) prawn *Palaemon elegans* is very common in the shallow water along the rocky shores of the Mediterranean Sea. Its transparent shell makes it possible to conduct noninvasive microscopic observation of the animal's tissues. Ten prawns were kept at a temperature of 20–25°C in a 100-liter aquarium of aerated seawater.

Determination of nitrogen loading. Our laboratory previously determined the time for gas (nitrogen) saturation by exposing prawns to hyperbaric conditions (203 kPa) for periods of 1, 2, 4, 8, and 10 min, followed by decompression at a rate of 40 m/min (1). The volume of gas in the bubbles was measured when it peaked 15 min after decompression. It was found that the volume reached a plateau after ~8 min of compression in all the prawns. The calculated rate constant (K) was 0.32 min^{-1}, and the time constant ($1/K$) was 3.13 min, in a nonlinear regression of the following equation: $P_{N_2} = P_{N_2\,S} - (P_{N_2\,S} - P_{N_2\,0})e^{-Kt}$, where P_{N_2} is the nitrogen partial pressure in the tissue (essentially both nitrogen and argon) at hyperbaric exposure time t, and $P_{N_2\,0}$ and $P_{N_2\,S}$ are the P_{N_2} values at the start of the hyperbaric exposure and when saturation is reached, respectively. Using the equation for gas saturation, we calculated the resident nitrogen in the control prawn and the residual nitrogen in the experimental prawn after oxygen exposure and loading of nitrogen at high air pressure. We found that after 10 min of hyperbaric exposure at 203 kPa the calculated loading of nitrogen in the control prawn was 154.2 kPa. After 10 min of oxygen followed by 12 min of air in the experimental prawn, the calculated P_{N_2} was also 154.2 kPa. At saturation, P_{N_2} is the pressure minus water vapor pressure multiplied by the fraction of both nitrogen and argon. At 203 kPa, $P_{N_2} = (202.6 - 3.2) \times 0.79 = 157.5$ kPa, and thus exposing the control group to 10 min of hyperbaric air and the experimental group to 12 min will yield the same nitrogen loading of 98% saturation.

Experimental system and procedure. A prawn (mean length 47.6 ± 5 mm) was placed in a small receptacle (15 × 12 × 12 cm) filled with continuously gas-bubbled seawater inside a temperature-controlled (25°C) 150-liter experimental hyperbaric chamber (102 × 52 cm in diameter; T.C.A.H.O., La Spezia, Italy). A period of exposure to bubbling gas, which yielded 98% saturation at 203 kPa, was followed by decompression at a rate of 30 m/min. Immediately thereafter, the prawn was transferred into a small transparent cuvette continuously supplied with fresh seawater and examined under the objective (×40) of a light microscope (model CH40, Olympus, Tokyo, Japan) equipped with a video camera (model SSC-C350P, Sony, Toyohashi, Japan). Prawns were examined 15 min after decompression. Only stable bubbles in the body of the prawn were counted (mobile bubbles are those that adhere to the outer shell). On the assumption that the bubbles were ellipsoids, which have a circular cross section, image-analysis software (AnalySIS 3.0, SIS, Reutlingen, Germany) was used to measure two diameters for each bubble and to calculate their volume of the bubbles.

Experimental protocol. All hyperbaric exposures were conducted at 203 kPa. Preliminary trials of explosive decompression from 304 kPa proved to be lethal for these prawns (4). In a crossover design, the 10 prawns were assigned to the following six experimental exposures in sequence, each followed by explosive decompression at 30 m/min: *1*) *profile A,* 10 min of hyperbaric exposure with aerated water (control); *2*) *profile B*, 10 min of hyperbaric exposure with oxygenated water; *3) profile C,* 10 min of hyperbaric exposure with oxygenated water followed by 12 min with aerated water; *4*) *profile D,* 10 min of oxygenated water in normobaric conditions followed by 12 min of hyperbaric exposure with aerated water; *5*) *profile C*, 10 min of hyperbaric exposure with oxygenated water followed by 12 min with aerated water; and *6*) *profile A*, 10 min of hyperbaric exposure with aerated water. *Exposures 5* and *6* were conducted 2 wk after *exposure 4*. Before each exposure session, prawns were examined under the microscope to confirm the absence of gas bubbles in their body tissues. An interval of at least 48 h separated each set of experiments for the same prawn because Daniels et al. (3) found complete regeneration of gas nuclei within 2 days. Repetition of the exposures to *profiles A* and *C* in reverse order as *exposures 5* and *6* was designed to rule out a possible effect of experimental *exposures 1* and *2* on the number of bubbles in *exposures 3* and *4*.

Data analysis and statistics. Three parameters were compared in the six exposures (for the same 10 prawns): the number of bubbles, the volume of a single bubble, and the percentage of prawns with bubbles. Differences between the six exposures were analyzed by Excel software (Microsoft, Redmond, WA), using one-way ANOVA for the first three parameters and X^2 for the fourth. Because we could not identify all the prawns individually, we could not use repeated-measures ANOVA. When there was a significant difference between the various protocols, the means were compared by using Duncan's multiple-range test. Differences between means were considered significant for $P < 0.05$.

RESULTS

In most cases, bubbles were observed proximate to the border between segments, from the head to the abdomen. No bubbles were observed inside the gut or in the leg joints. An example of three bubbles observed in an air-exposed prawn after explosive decompression is shown in the micrograph in Fig. 1. Figure 2 shows the number of bubbles in a prawn for the six exposures. Statistical analysis yields significant differences in the number of bubbles (ANOVA, $P < 0.002$). Duncan's multiple-range test applied to the data in Fig. 2 divides it into two distinct subgroups. One group includes all

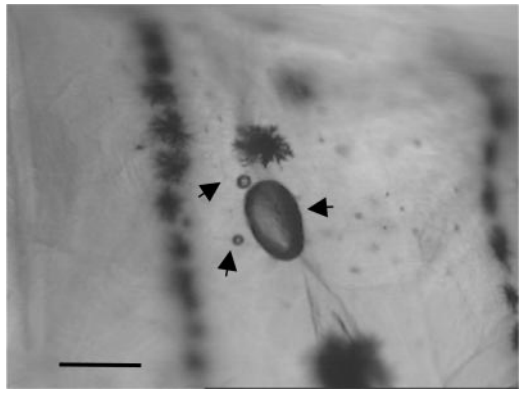

Fig. 1. Micrograph of 3 bubbles (marked by arrows) in the body of a prawn 15 min after explosive decompression. This prawn was previously subjected to hyperbaric air (203 kPa) for 10 min. It is possible that the fusion of a number of smaller bubbles led to the formation of the large bubble in the figure. Bar = 200 μm.

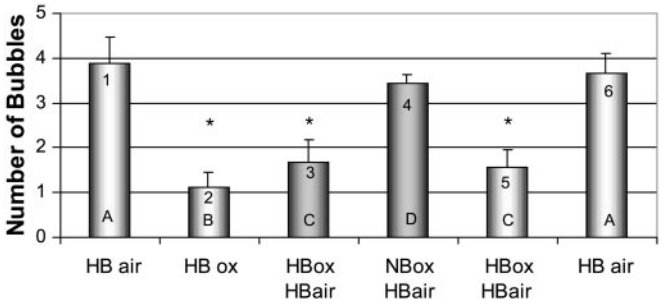

Fig. 2. Number of bubbles in a prawn after explosive decompression for the 4 experimental profiles (*A–D*) and the 6 exposures: *1*) control, 10 min in hyperbaric aerated water, (*profile A*, HB air); *2*) 10 min in hyperbaric oxygenated water (*profile B*, HB ox); *3*) 10 min in hyperbaric oxygenated water followed by 12 min in hyperbaric aerated water (*profile C*, HB ox-HB air); and *4*) 10 min in normobaric oxygenated water followed by 12 min in hyperbaric aerated water (*profile D*, NB ox-HB air). *Profiles C* and *A* were employed in *exposures 5* and *6*, respectively. Values are means ± SE. The letter on each column indicates the experimental profile, and the number is the exposure number. *Significant difference from control, $P < 0.03$.

prawns treated with hyperbaric oxygen (*profiles B* and *C*), whereas the other profiles (*A* and *D*) comprise the second group. Exposure to 203 kPa for 10 min in oxygen (*profile B*) and for 10 min in hyperbaric oxygen before 12 min in hyperbaric air (*profile C*) significantly decreased bubble formation. Figure 3 shows the mean bubble volume in the prawns for the six exposures. The differences between the six exposures were significant (ANOVA, $P < 0.04$). Duncan's multiple-range test applied to the data in Fig. 3 divides the experimental groups into two subgroups. All prawns treated with oxygen (*exposures 2–5*) fell into one group. Those prawns exposed to hyperbaric air (*exposures 1* and *6*) fell into the other group. Figure 4 shows the percentage of "bubbled" prawns in each exposure. Exposure to hyperbaric oxygen reduced the percentage of prawns in which bubbles were observed to 60% on *profile B* ($P < 0.04$). The trend seen for a reduction to 80% on *profile C* was not significant.

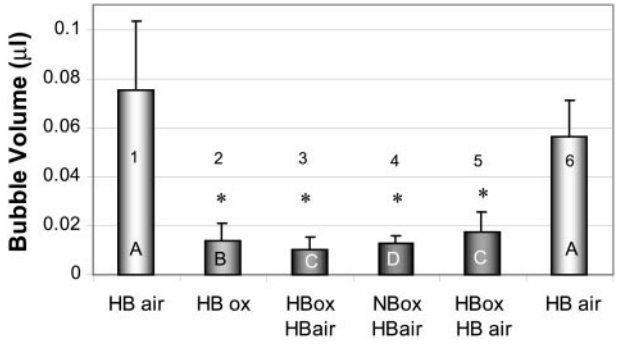

Fig. 3. Mean bubble volume in the prawns after explosive decompression for the 4 different profiles and 6 exposures. See Fig. 2 legend for definitions and further explanation. Values are means ± SE. Differences between the 6 exposures were significant (ANOVA, $P < 0.04$). Duncan's multiple-range test divides the experimental groups into 2 subgroups. Prawns treated with hyperbaric oxygen fell into 1 group (marked with an asterisk). Prawns exposed to hyperbaric air are included in the other.

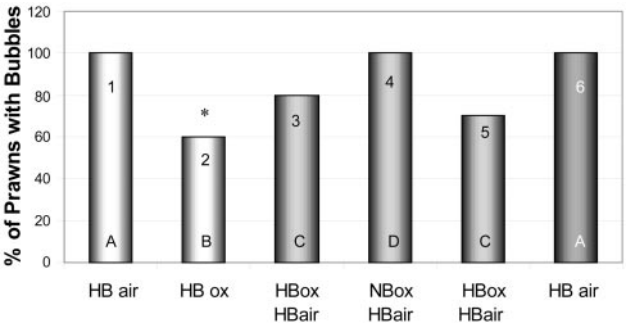

Fig. 4. Percentage of prawns in which bubbles were observed 15 min after explosive decompression. See Fig. 2 legend for definitions and further explanation. *Significant difference from control, $P < 0.05$.

DISCUSSION

Our purpose in this investigation was to test the hypothesis that saturation with pure oxygen may eliminate gas nuclei in the intact prawn. The transparent shell of this prawn makes it possible to carry out direct observation and measurement of gas bubble dynamics in the intact animal. Our main findings in this investigation were that saturation with pure hyperbaric oxygen significantly reduces the number of bubbles and the mean volume of a bubble in the nitrogen-loaded prawn. These results were obtained on exposure to oxygen and under combined exposure to oxygen followed by air but not after preexposure to normobaric oxygenation. The results from the repeated exposures, *exposures 5* and *6*, proved that an interval of 48 h between each set of measurements is sufficient to ensure return to baseline. We suggest that regeneration of gas nuclei de novo was completed even after their partial elimination by pure hyperbaric oxygen. This is also substantiated by our results from *exposure 4* (*profile D*), in which the number of bubbles was not different from *exposure 1*, and by a previous study (3), which demonstrated complete recovery of the number of bubbles 48 h after hyperbaric pretreatment.

It is widely accepted that gas bubbles either originate in tissue at a point of friction between solid structures immersed in an aqueous solution, i.e., tribonucleation (12, 13), or are formed from preexisting gas nuclei (3, 5, 10, 15, 18, 20). The existence of bubble nuclei is not completely understood because, in a bubble with a very small radius, elevation of the internal hydrostatic pressure will cause outward diffusion and collapse of the bubble (8, 12). The mechanisms that cause gas nucleus stabilization in tissue for a prolonged period of time are also as yet unclear. Tikuisis (11) elaborated a model in which gas nuclei would be formed in preexisting conical or elliptical hydrophobic crevices at the liquid-gas interface. Our results are in agreement with the gas nucleus hypothesis, according to which it may be predicted that exposure to hyperbaric oxygen will result in replacement of the resident gas in the crevice or in the stabilized bubble in the first stage. In the second hypothesis, metabolic processes will eliminate the oxygen. Liquid will then replace the consumed oxygen, abolishing the conditions required to form a

gas nucleus. It is not likely that the dominant cause for bubble generation in the present study was friction resulting from movement of the prawns (12, 13). We could not observe any differences in the pattern of movement of the prawns in any of the exposure profiles, and nitrogen loading and decompression were the same in *profiles A* and *C*. To eliminate ~50% of the observed bubbles by what has been assumed to be crushing of micronuclei, Daniels et al. (3) exposed shrimps to very high air pressure (2 MPa) for 10 min. In the present investigation, we used pressure reduced by one order of magnitude (203 kPa) combined with pure oxygen to reach the same level of nucleus reduction. Thus we suggest that treatment with hyperbaric oxygen could be of considerable practical advantage in eliminating gas nuclei in humans, despite the differences between humans (mainly their internal skeleton and closed circulatory system) and prawns. This is also in agreement with physiological findings demonstrating that preoxygenation provides improved protection against decompression sickness (14, 19), although no distinction was made between nuclei elimination and denitrogenation.

We suggest that an exposure to oxygen at 203 kPa eliminates most of the gas nuclei in the prawn, reducing the number and size of bubbles that develop and the risk of decompression sickness. The transparent prawn *P. elegans* may be a simple and appropriate model for studying certain specific aspects of decompression sickness. The effectiveness of the mild hyperoxic exposure warrants further studies in mammals.

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