Optimal oxygen pressure and time for reduced bubble formation in the N₂-saturated decompressed prawn

O. Ertracht, R. Arieli, Y. Arieli, R. Ron, Z. Erlichman, and Y. Adir. Optimal oxygen pressure and time for reduced bubble formation in the N₂-saturated decompressed prawn. J Appl Physiol 98: 1309–1313, 2005. First published December 3, 2004; doi:10.1152/japplphysiol.01051.2004.—Bubbles that grow during decompression are believed to originate from preexisting gas micronuclei. They showed that pretreatment of prawns with 203 kPa oxygen before nitrogen loading reduced the number of bubbles that evolved on decompression, presumably owing to the alteration or elimination of gas micronuclei (Arieli Y, Arieli R, and Marx A. J Appl Physiol 92: 2596–2599, 2002). The present study examines the optimal pretreatment for this assumed crushing of gas micronuclei. Transparent prawns were subjected to various exposure times (0, 5, 10, 15, and 20 min) at an oxygen pressure of 203 kPa and to 5 min at different oxygen pressures (P₂O values of 101, 151, 203, 405, 608, and 810 kPa), before nitrogen loading at 203 kPa followed by explosive decompression. After the decompression, bubble density and total gas volume were measured with a light microscope equipped with a video camera. Five minutes at a P₂O of 405 kPa yielded maximal reduction of bubble density and total gas volume by 52 and 71%, respectively. It has been reported that 2–3 h of hyperbaric oxygen at bottom pressure was required to protect saturation divers decompressed on oxygen against decompression sickness. If there is a shorter pretreatment that is applicable to humans, this will be of great advantage in diving and escape from submarines.

decompression; bubbles; diving

THE EVOLUTION OF BUBBLES DURING the ascent from a deep dive is the cause of decompression sickness (DCS). Bubbles that grow during decompression are believed to originate from preexisting gas micronuclei (7). A number of studies have confirmed the existence of gas micronuclei by using procedures designed to eliminate them and then counting the bubbles that evolved after decompression (2, 3, 8, 12). In some studies, high pressure was used to increase the partial pressure of the inert gas in the micronuclei, resulting in their dissolution and disappearance (2, 3, 12). Extreme pressures (2,000–3,000 kPa) were required to achieve partial protection against decompression sickness in rats (8), making this procedure for crushing gas micronuclei impractical for human use.

Denitrogenation is a common procedure employed to reduce the risk of DCS before high-altitude exposure (5, 9–11). Latson et al. (4) used oxygen to denitrogenize humans after a simulated air saturation dive, in a study of safe escape from a disabled submarine. They showed that prolonged decompression with oxygen reduced the incidence of DCS only when the subjects breathed oxygen at the saturation pressure of 250–280 kPa for a period of 2–3 h beforehand. The authors explained this reduction in the incidence of DCS as being due to the elimination of nitrogen from the body tissues and the possible elimination of gas micronuclei. Prolonged exposure of this kind to hyperbaric oxygen carries a potential risk of oxygen toxicity. We have previously shown that exposing prawns to 203 kPa oxygen for 10 min before loading them with nitrogen reduced the number of bubbles that evolved on decompression (1). We suggested that oxygen replaced the resident gas in the micronuclei; when the oxygen pressure was reduced, the oxygen was consumed and the micronuclei were either eliminated or reduced to a volume that is ineffective for bubble production. These two effects are further termed crushing.

The purpose of the present study was to determine the optimal oxygen pretreatment strategy to minimize bubble production after decompression from saturation in the prawn. We submitted small transparent prawns to various combinations of exposure time and oxygen pressure (P₂O) before they were loaded with nitrogen at 203 kPa and subjected to explosive decompression. We then evaluated the number of bubbles that evolved and their volume.

METHODS

Animals

Forty prawns (Palaeomon elegans, ~20 mm long) were collected in the shallow water along the rocky shores of the Mediterranean Sea. Their transparent shell makes it possible to conduct noninvasive microscopic observation of the animal’s tissues. The prawns were kept at a temperature of 20–25°C in a 100-liter aquarium of aerated seawater.

Determination of Inert Gas Loading

We had previously determined the time constant for inert gas (nitrogen) loading 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 400 kPa/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 at high pressure in all the prawns. We considered both the jar filled with gas-perfused seawater and the prawn whose organs are washed by its heart in an open system (with no blood vessels), as well-mixed compartments. The calculated rate constant (k) was 0.32 min⁻¹, and the following equation was derived:

\[ Pt_{N_2} = P_{N_2} - (P_{N_2} - P_{N_20})e^{-0.32t} \]  

(1)

where \( Pt_{N_2} \) is the partial pressure of inert gas in the tissue (essentially both nitrogen and argon) at exposure time \( t \), and \( P_{N_20} \) and \( P_{N_2} \) are the tissue nitrogen tension at the start of the exposure and the ambient nitrogen tension, respectively. This basic equation was used to calcu-
late nitrogen loading and unloading in the prawn’s tissues and the percent saturation when the ambient pressure was constant. All pressure changes were carried out on air, linearly with time ($P_{t_{\text{N}_2}} = P_{\text{N}_2,0} + a \times t$, where $a$ is the slope in kPa nitrogen/min). Loading of nitrogen (and unloading) during the linear change of pressure was calculated by the following expression:

$$P_{t_{\text{N}_2}} = P_{\text{N}_2,0} + a \times t - a/0.32$$

$$+ (P_{\text{N}_2,0} - P_{\text{N}_2,0} + a/0.32) \times e^{-0.32t}$$

(see APPENDIX). In all the exposures before the final explosive decompression, the nitrogen loading was 98% of complete saturation in air aerated water at 203 kPa ($P_{t_{\text{N}_2}}/P_{\text{N}_2,0} = 0.98$, Eq. 1).

### Experimental System and Procedure

The experimental procedure consisted of hyperbaric exposure followed by video-microscopic evaluation of the bubbles. The five phases (Fig. 1) of the hyperbaric exposure were: 1) compression to bottom pressure, which was carried out with bubbling of air or oxygen through the prawn’s jar in separate runs; 2) a hyperoxic pretreatment phase, which we believe crushes gas micronuclei; 3) decompression to 203 kPa; 4) remaining for a time at 203 kPa (during phases 3 and 4, air was bubbled through the jar for nitrogen loading); and 5) explosive decompression.

A prawn was placed in a glass jar 15 cm high and 8 cm in diameter that was half filled with sea water (0.5 liters) and bubbled profusely with gas. The jar was placed inside a temperature-controlled (25°C), 150-liter hyperbaric chamber (T.C.A.H.O., La Spezia, Italy). The pressure was increased linearly at 100 kPa/min, with either air or oxygen, to a predetermined bottom pressure (compression, Fig. 1). The pressure remained for a predetermined time at the bottom pressure while pure oxygen was bubbled through the water (bottom hyperoxia, Fig. 1). The flow of oxygen was then switched to air, and the pressure was changed at a rate of 100 kPa/min to 203 kPa (pressure change, Fig. 1). The prawn remained at 203 kPa for a time calculated to result in 98% nitrogen saturation ($N_2$ loading, Fig. 1) and was then subjected to explosive decompression at 300 kPa/min. Only the time and oxygen pressure for the bottom hyperoxia were changed in the different experimental protocols.

An example of the calculated nitrogen tension is shown in Fig. 1, bottom. In this example, bottom pressure is reached after 3-min compression. Bottom hyperoxia at 405 kPa lasting for 5 min is followed by 2 min of linear decompression to 203 kPa. Nitrogen loading at 203 kPa continues until 98% saturation is reached. This was calculated to occur after 7.7 min in the air-compressed prawn and after 8.6 min in the oxygen-compressed prawn.

Immediately after decompression, the prawn was transferred to a small transparent cuvette continuously supplied with fresh seawater. The cuvette was examined under the objective $(\times 40)$ of a light microscope (model CH40, Olympus, Tokyo, Japan) equipped with a video camera (model SSC-C350P, Sony, Toyohashi, Japan). Measurements were taken 15 min after final decompression, because we previously found that this is the time interval for maximal gas evolution (1). Body length and the number of stable bubbles in the body of the prawn were recorded (mobile bubbles are those that adhere to the outer shell). On the assumption that the bubbles were ellipsoids, the two diameters for each bubble were measured by use of image-analysis software (AnalySIS 3.0, SIS, Reutlingen, Germany).

### Experimental Protocol

**Series A**. Series A examined the effect of hyperoxic exposure time (bottom hyperoxia, Fig. 1) on the evolution of bubbles after decompression. Each of the 20 prawns was assigned to 10 experimental exposures consisting of two components: the bubbling gas (air or oxygen) during compression combined with each of five hyperoxic exposure times (0, 5, 10, 15, or 20 min) at a bottom pressure of 203 kPa.

**Series B**. Series B examined the effect of oxygen pressure (bottom pressure, Fig. 1) at the effective hyperoxic exposure time. This was the minimum time required to achieve the maximum reduction in bubble density, which was found in series A. Each of the 20 prawns was assigned to 12 experimental exposures consisting of two components: the bubbling gas (air or oxygen) during compression and six oxygen bottom pressures (101, 151, 203, 405, 608, and 810 kPa) for a period of 5 min (determined in series A).

To revert back to the control state with regard to the renewed presence of the assumed bubble micronuclei, an interval of at least 48 h separated each set of experiments for the same prawn (1, 2). In preliminary tests, we found that there was no difference in the number of bubbles and their volumes on repeated exposure of a group of prawns to the same profile, if the two exposures were separated by at least 48 h. Our laboratory reported previously (1) that bubble density and volume did not differ for two identical exposures to hyperbaric oxygen followed by nitrogen loading and explosive decompression. The protocols were conducted in random order with respect to the different exposure times and oxygen pressures.

### Data Analysis and Statistics

Because the prawns were not all the same size, we normalized the measured parameters to prawn volume (V). We measured the weight...
of 26 prawns and their body length \( L \). Assuming the prawn to have a density of 1 g/ml and a power of three relationship, the calculated volume (using regression) is \( V = 0.0341 \times L \left( \text{mm}^3 \right) + 0.0135 \). Bubble density and the ratio of the total gas volume (summatated bubble volume) in the prawn were normalized to its volume and expressed as the number of bubbles per microliter of prawn and microliter gas per microliter prawn, respectively. Bubble volume was calculated on the assumption that the bubbles were ellipsoids with a circular cross section.

For the different combinations of the bubbling gas during compression and exposure time or oxygen pressure at bottom hyperoxia, three parameters (bubble density, total gas volume, and mean bubble volume) were compared by two-way ANOVA using SAS 6.03 (SAS Institute, Cary, NC). Whenever the ANOVA was significant, the Duncan post hoc test was conducted for multiple comparisons of either exposure times or oxygen pressures. When the interaction was significant, we used a \( t \)-test to compare the results for the two compression gases and a specific hyperoxic time or bottom pressure. Statistical significance was set at \( P < 0.05 \).

**RESULTS**

**Series A**

Bubble density was the only parameter that showed significant dependency on the gas used during compression (Table 1; Fig. 2, bottom). The group of prawns compressed with oxygen had a lower bubble density compared with the group compressed with air \( (P < 0.05) \). Bubble density was also affected by bottom hyperoxic exposure time \( (P < 0.001) \). When no bottom hyperoxic time was given \( (0 \text{ min in the figure}) \), bubble density was highest \( (\sim 0.017 \text{ bubbles/\mu l}) \). Increasing the bottom hyperoxic exposure time to 5, 10, 15, or 20 min almost halved bubble density \( (\sim 0.01 \text{ bubble/\mu l}) \). Bubble density for all bottom times \( >0 \) (excluding 15 min, compression with air) was significantly lower than the values obtained with no bottom time. Bubble density for a hyperoxic bottom time of 15 min in the air-compressed prawns was similar to control values. Mean bubble volume (Fig. 2, middle) was not significantly affected by the compression gas or bottom hyperoxic exposure time (Table 1). The average bubble volume was \( 0.077 \pm 0.048 \mu \text{l} \). However, their interaction was found to have a significant effect \( (P < 0.05) \), implying a different response to the hyperoxic bottom time in the air- and oxygen-compressed groups. There was a tendency for bubble volume to decrease with increasing bottom hyperoxic time in the air-compressed prawns and to increase in the oxygen-compressed group. However, there was no specific bottom hyperoxic time for which the difference due to the compression gas reached statistical significance \( (t\text{-test}) \).

Total gas volume (Fig. 2, top) was not significantly affected by the compression gas, the hyperoxic bottom time, or their interaction (Table 1).

**Series B**

Bubble density (Fig. 3, bottom) was not affected by the compression gas but was significantly affected by the bottom oxygen pressure \( (P < 0.05, \text{Table 1}) \); their interaction was not significant. The overall response was a decrease in bubble density as the \( \text{PO}_2 \) increased from 21 kPa to 101 and 405 kPa. Bubble density after a bottom pressure of 405 kPa was significantly lower than after bottom pressures of 21, 101, and 203

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### Table 1. Statistical results for the different parameters

<table>
<thead>
<tr>
<th></th>
<th>Bubble Density</th>
<th>Bubble Volume</th>
<th>Total Gas Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Series A</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bottom time</td>
<td>0.001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Compression gas</td>
<td>0.05</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Interaction</td>
<td>NS</td>
<td>0.05</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Series B</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bottom pressure</td>
<td>0.05</td>
<td>0.001</td>
<td>0.05</td>
</tr>
<tr>
<td>Compression gas</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Interaction</td>
<td>NS</td>
<td>0.001</td>
<td>NS</td>
</tr>
</tbody>
</table>

A significant effect is indicated by the value that \( P \) is less than. A nonsignificant effect is indicated by NS.
kPa. There was no significant change in density at PO2 values above 405 kPa.

Mean bubble volume (Fig. 3, middle) was not significantly affected by the compression gas, but it was affected by the bottom PO2, and there was a significant effect of their interaction (Table 1). For the lower pressures of 101–608 kPa, the mean bubble volume was rather low (<0.003 μl) compared with control prawns that were not given oxygen. There may be a tendency for bubble volume to increase in the oxygen-compressed prawns that had been at a pressure of 810 kPa (a significant difference compared with all the other hyperoxic exposures, \( P < 0.05 \), Duncan’s test). However, there was no specific pressure for which the difference due to the compression gas reached statistical significance (t-test).

Total gas volume (Fig. 3, top) was not affected by the compression gas, but it was affected by the bottom oxygen pressure, with no effect of their interaction (Table 1). For a bottom oxygen pressure of 101 kPa, the gas volume was lower than in the controls (\( P < 0.05 \)) (prawns not exposed to oxygen) and showed a further significant decrease as bottom PO2 increased to 151 kPa (\( P < 0.05 \)). Thereafter, the volume remained low for all the PO2 values above 151 kPa.

**DISCUSSION**

We assumed that bubble density is a measure of the number of gas micronuclei that evolved into bubbles. Five minutes were sufficient for a maximal reduction in density of 31% (Fig. 2, bottom). Because of the risk of oxygen toxicity, the shortest possible exposure would be preferable for further evaluation using mammals. An oxygen pressure of 405 kPa yielded a maximal reduction in bubble density of 51% (Fig. 3, bottom). Therefore, the most effective pretreatment with oxygen in the prawn is compression with oxygen followed by 5 min at 405 kPa oxygen. The evolution of gas micronuclei into bubbles depends on a transition from the stable to the unstable phase (6). Thus we believe that a reduction in the number of bubbles formed represents crushing of gas micronuclei.

The total gas volume represents the part of the supersaturated gas that changed phase during decompression. Total gas volume decreased when the oxygen pressure increased from the control state (21 kPa) to 150 kPa (Fig. 3, top). Any further increase in bottom PO2 failed to affect the gas volume. Although we found no significant effect of hyperoxic bottom time on gas volume (Fig. 2, top), it seems that any exposure at all to oxygen reduced the volume compared with air. Because of the reduction in the number of gas micronuclei having the potential to grow into bubbles, more of the excess gas will be unloaded in soluble form. For the same level of supersaturation, a reduced volume of gas should reduce the risk of DCS in mammals.

Bubble size is of great significance in the risk of DCS, because small bubbles that do not block blood vessels or compress nervous tissue are “silent bubbles” that do not cause clinical DCS. Almost all of the hyperoxic pretreatments reduced the size of the bubbles (Figs. 2, middle, and 3, middle). Bubble volume increased from these low values in the oxygen-compressed prawns with longer hyperoxic bottom times (15 and 20 min) and at the highest pressure (810 kPa). With fewer nucleation sites, each nucleus may gain gas from the larger surrounding volume and therefore produce larger bubbles. If this were indeed the case, whenever density is reduced bubble volume should increase. However, for most of the combinations of hyperoxic exposure time and pressure that reduced the density, bubble volume was low.

Although there was a general reduction in the three measured parameters as bottom time and pressure increased, in some conditions the opposite effect was seen. There was an increase in bubble density for air compression and 15-min bottom hyperoxia (Fig. 2, bottom) and for high bottom pressures of 607 and 810 kPa (Fig. 3, bottom). There was an increase in bubble volume for oxygen compression and bottom hyperoxia of 810 kPa (Fig. 3, top). This may be related to two effects that counteract micronuclei crushing. Excess oxygen not removed by being consumed by the prawn or by diffusion...
after high oxygen pressures and long bottom times could contribute to the increase in bubble volume. The effect of a prolonged stay at a high bottom pressure may appear on decompression, causing hidden micronuclei to pass the threshold from the stable to the unstable state (6) and thus increase the number of potential micronuclei for bubble formation. Therefore, the preferred hyperoxic pretreatment of 5 min at 405 kPa will also result in minimal bubble volume and density.

Daniels et al. (2) tested different decomposition ratios in the shrimp and showed increased bubble density as the ratio increased from 1.5:1 to 12:1. In the present study, we tested only the ratio of 2:1. Because a higher decomposition ratio may result in more gas micronuclei forming bubbles (6), we are presently studying the hyperoxic elimination of gas micronuclei for different decomposition ratios.

The findings of the present study may have implications for diving practice. The abortion of a saturation dive or escape from a sunken submarine carries a high risk of DCS. In experiments designed to reduce this risk of DCS by simulating saturation in a sunken submarine, a high decompression risk was found after prolonged decompression (4–10 h) while breathing oxygen (4). The risk was eliminated only when the subjects breathed hyperbaric oxygen for 2–3 h at the bottom pressure. This kind of prolonged hyperoxic exposure involves a risk of oxygen toxicity and requires a large supply of oxygen. If a shorter hyperoxic pretreatment could be found that is applicable to mammals, this would be of great advantage in technical terms and reduce the risk of oxygen toxicity. A short, controlled hyperoxic exposure in the dry escape trunk of a submarine or in a diving bell can be safely performed, if it is proved advantageous in protecting against the risk of DCS.

APPENDIX

Abbreviations

\[ \text{PtN}_2 \] Tissue nitrogen tension
\[ \text{PtN}_{2,0} \] Time of linear pressure change
\[ \text{PamN}_2 \] Tissue nitrogen tension at the start of a linear pressure change
\[ a \] Slope of nitrogen pressure change with time
\[ \text{PamN}_{2,0} \] Ambient nitrogen pressure
\[ k = 0.32 \] Rate constant
\[ \text{PamN}_{2,0} \] Initial ambient nitrogen pressure

Procedure

\[
\frac{d\text{PtN}_2}{d\text{t}} = k(\text{PamN}_2 - \text{PtN}_2) \quad (1)
\]

differential change in tissue nitrogen tension
\[
\text{PamN}_2 = \text{PamN}_{2,0} + a \times t \quad (2)
\]

linear pressure change
\[
\frac{d\text{PtN}_2}{d\text{t}} = k(\text{PamN}_{2,0} + a \times t - \text{PtN}_2) \quad (3)
\]

incorporation of Eq. 1 into Eq. 2
\[
\frac{d\text{PtN}_2}{d\text{t}} + k \times \text{PtN}_2 = k \times a \times t + k \times \text{PamN}_{2,0} \quad (4)
\]

1st order linear differential equation that is solved in the following steps:

\[
\frac{d(\text{PtN}_2)}{d\text{t}} + k \times \text{PtN}_2 = k \times a \times t + k \times \text{PamN}_{2,0} \quad (5)
\]

\[
d(\text{PtN}_2) \times e^{kt} = (k \times a \times t + k \times \text{PamN}_{2,0}) \times e^{kt} \quad (6)
\]

\[
\text{PtN}_2 \times e^{kt} = \int (k \times a \times t + k \times \text{PamN}_{2,0}) \times e^{kt} \quad (7)
\]

\[
\text{PtN}_2 \times e^{kt} = \left( \text{PamN}_{2,0} - \frac{a(1/t - t)}{1 - ek} \right) e^{kt} + \frac{1}{1 - ek} \quad (8)
\]

\[
\frac{\text{PtN}_{2,0}}{1 - ek} = \frac{\text{PamN}_{2,0}}{1 - ek} + \frac{a}{1 - ek} \quad (9)
\]

where \( C \) is the integration constant

\[
\text{PtN}_2 = \left( \text{PamN}_{2,0} - \frac{a(1/t - t)}{1 - ek} \right) e^{kt} + \frac{1}{1 - ek} \quad (10)
\]

Substituting the limit conditions \( t = 0 \) and \( \text{PtN}_2 = \text{PtN}_{2,0} \) gives

\[
\text{PtN}_{2,0} = \frac{\text{PamN}_{2,0}}{1 - ek} = \text{PamN}_{2,0} + a \times t \quad (11)
\]

\[
\text{PtN}_2 = \left( \text{PamN}_{2,0} - \frac{a(1/t - t)}{1 - ek} \right) e^{kt} + \frac{1}{1 - ek} \quad (12)
\]

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REFERENCES