Decompression-Induced Ocular Tear Film Bubbles Reflect the Process of Denitrogenation

Polona Jaki Mekjavic¹ and Igor B. Mekjavic²

PURPOSE. To confirm that the tear film bubbles observed after decompression from hyperbaric exposure are due to denitrogenation and to assess the time course of denitrogenation based on the number of ocular tear film bubbles.

METHODS. The study comprised two parts. In the first, subjects (n = 8) were compressed to a pressure of 2.0 ATA (atmospheres absolute; depth of 10 meters of sea water [msw]) for 60 minutes in a hyperbaric chamber on two separate occasions. On one occasion they breathed air, whereas on the second occasion they inspired pure oxygen. Before and within 30 minutes after each dive, the subjects' tear film was examined with a slit lamp microscope and the average number of bubbles recorded. Ultrasound reflectivity of the lens–vitreous humor compartments was also examined. In the second part of the study, subjects (n = 8) participated in two simulated dives in the hyperbaric chamber: 4.0 ATA (30 msw) for 15 minutes and 2.5 ATA (15 msw) for 180 minutes. The former was a no-stop decompression dive, whereas the latter required a 43-minute decompression stop at 3 msw. Ocular tear film examinations were conducted before the dive, as well as 30 minutes and 1 day, 2 days, and 3 days after the dives.

RESULTS. The number of tear film bubbles increased significantly (P < 0.05) after the air dives to 2.0 ATA for 60 minutes, whereas there was no significant postdecompression increase in tear film when oxygen was inspired by the subjects during the dive. Posterior lens–vitreous humor reflectivity increased significantly after decompression from 2 ATA, when air was the breathing mixture, whereas no change in reflectivity was observed when oxygen was inspired during the dive. In the second part of the study, there was a significant elevation in tear film bubbles for 2 days after the two dives (P < 0.05). There was no significant difference in the number of ocular tear film bubbles between the two dives.

CONCLUSIONS. After a hyperbaric air exposure, tear film bubbles reflect the process of denitrogenation, which may persist for up to 2 days after the decompression. (Invest Ophthalmol Vis Sci. 2007;48:3756–3760) DOI:10.1167/iovs.06-1144

From the ¹University Eye Clinic, Clinical Centre, Ljubljana, Slovenia; and the ²Department of Automation, Biocybernetics and Robotics, Jozef Stefan Institute, Ljubljana, Slovenia.

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Corresponding author: Polona Jaki Mekjavic, Eye Clinic, University Clinical Centre, SI-1000 Ljubljana, Slovenia; polona.jaki@guest.arnes.si.

During a hyperbaric exposure, the inert gas component of a diver's breathing mixture is dissolved in all tissues. Return to normobaria should follow strict decompression schedules, to allow the dissolved inert gas to be released through the lungs. An inappropriate rate of ascent, or decompression sickness, will cause the dissolved inert gas to come out of solution and create gas bubbles, which can result in decompression sickness. Decompression sickness is probably associated with the physical and inflammatory consequences of bubbles in the tissues, either through distortion of tissue structures, interruption of microvascular flow, or the stimulus of the inflammatory responses. Symptoms and signs reflect the location and size of any bubbles and can range from subclinical aches and pains to paralysis and death. Objective methods capable of detecting the formation, or presence of gas bubbles in the blood and tissues could therefore assist in the prevention and treatment of decompression sickness.

Detection of circulating bubbles with ultrasound has become the method of choice,¹ ² but has until recently required trained personnel to provide subjective analysis of the audio records. With new ultrasonic imaging techniques,³ circulating bubbles can now be graded objectively, though the methods have certain limitations in field studies.

Since Boyle's observation⁴ in a viper of "a conspicuous bubble moving to and fro in the waterish humor of one of its eyes" after rapid decompression, the eye has been largely ignored as a site for monitoring the process of postdecompression denitrogenation. Our previous study demonstrated a dose-dependent response between bubbles forming in the tear film of subjects and the depth and duration of hyperbaric exposure.⁵ The method was compared to the Doppler ultrasound method of monitoring bubbles and was found to be more sensitive, especially for dives well within the no-stop decompression limit.

Ocular tear film bubbles observed after decompression in divers wearing contact lenses have been attributed to the liberation of inert gas from the supersaturated tear film and subsequent expansion of this gas trapped between the cornea and contact lens.⁶ ¹¹ Postdecompression tear film bubbles have also been observed after asymptomatic recreational dive profiles in divers not wearing contact lenses¹²,¹³ and appear to reflect the severity of decompression much better than precordial Doppler ultrasonic monitoring.⁵ The purpose of the present study was to extend our preliminary findings that tear film bubbles observed after a dive when breathing air reflect the process of denitrogenation,¹⁴ and to evaluate the temporal change in tear film bubble formation after a dive.¹⁵

METHODS

The study comprised two parts: one tested the hypothesis that postdive tear film bubbles are an index of the process of denitrogenation and the other the hypothesis that any postdive residual nitrogen is eliminated in 24 hours, as assumed by most dive decompression tables. Of the 13 subjects (8 women, 5 men) participating in the study, only 3 (1 woman, 2 men) participated in both parts of the study, whereas the remaining 10 subjects (7 women, 5 men) were equally assigned to the two parts. The two parts of the study were conducted 1 month

3756

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apart, during which the three divers participating in both dive series abstained from diving.

Subjects participating in the study were all certified divers, and their participation in the study was subject to physician’s approval. They were all novice divers, had not participated in any dives 1 month before the study, and abstained from diving during the period of the study. The experimental protocol was approved by the Ministry of Health National Committee for Medical Ethics (Republic of Slovenia) and adhered to the tenets of the Declaration of Helsinki.

Ocular Tear Film Bubbles after Decompression

Subjects ($n = 8$; four men, four women) were compressed to a pressure of 2.0 ATA (atmospheres absolute; depth, 10 meters of sea water [msw]) for 60 minutes in a multiplace hyperbaric chamber (Marine Dynamics, San Diego, CA) on two separate occasions separated by a week. All simulated dives were conducted in accordance with the Canadian Forces Air Diving Tables and Procedures. On one occasion, the divers breathed air (AIR trial), and on the second occasion they wore a standard oxygen hood and inspired 100% oxygen (OXYGEN trial). The order of the trials was randomized among the subjects. Before, and within 30 minutes after each dive, the subjects’ tear film was examined with a slit lamp microscope (30 SL-M; Opton, Berlin, Germany). Subjects were instructed to close their eyelids for 5 seconds. Once they opened their eyes, the surface of the cornea and the conjunctival gutter, formed by the globe and eyelid, were scanned with the slit lamp microscope in a medial-to-lateral direction, against the direction of tear flow. The average number of bubbles for three scans was used for the analysis.

All simulated dives were concluded with a 1-hour observational period, during which subjects were monitored for any signs and symptoms of decompression sickness.

Ultrasound Reflectivity

Ultrasound scans were also made of the eyes before and after each dive in the AIR and OXYGEN trials. Ultrasonic examinations were conducted with the subjects supine. A B-mode (time–brightness method) ultrasonic system (model B3000; Sonomed, Lake Success, NY) was used to obtain two-dimensional sections of the eye structures, specifically to provide the anatomic position of the reflecting surfaces. An 8-MHz probe was placed on the eyelid and oriented along the visual axis from the vortex of the cornea toward the fovea on the retina. Measurements of the amplitudes of individual signals were achieved by an A-mode (amplitude modulation) ultrasonic system (model A2500; Sonomed) with logarithmic amplification. A-scan is a one-dimensional acoustic display in which echoes are represented as vertical spikes from baseline. The height of the displayed spikes indicates the strength (i.e., amplitude) of the echoes. The greater the difference in sound velocity of the media that created the acoustic interface, the stronger the echo. Thus, with the B-mode ultrasonic scans providing structural orientation, the A-mode was used to quantify changes in the reflectivity of the posterior surface of the lens before and after the simulated dives. Results of the A- and B-mode ultrasound scans were printed on thermal paper (Video Copy Processor P67UA; Mitsubishi, Tokyo, Japan) and analyzed for any decompression-induced alterations in reflectivity. For each subject, the postdecompression measurements of the lens–vitreous humor reflectivity were expressed relative to the predive reflectivity.

Persistence of Tear Film Bubbles

In the second part of the study, subjects ($n = 8$; five women, three men) participated in two dives: 4.0 ATA (30 msw) for 15 minutes and 2.5 ATA (15 msw) for 180 minutes. The former was a no-stop decompression dive, whereas the latter required a 45-minute decompression stop at 3 msw, according to the Canadian Forces Air Diving Tables and Procedures. The partial pressure of inspired nitrogen ($P_{N_2}$) in the 4-ATA and 2.5-ATA dives was 3.16 ATA and 1.98 ATA, respectively. As in the first part of the study, the order of the dives was randomized among the subjects.

Ocular tear film examinations were conducted with a biomicroscope, as described previously, before the dive, and 30 minutes and 1, 2, and 3 days after the dives.

Tear Film Examination

All subjects participating in both parts of the study, were tested for normal quantity and quality of tear film using the Shirmer test and break-up time (BUT) test before the dives.

Analysis

In the first part of the study, the number of pre- and postdive ocular tear film bubbles and acoustic reflectivity were compared by paired $t$-test. In the second part of the study, a two-way repeated measures analysis of variance (ANOVA) was used, with time and dive profile as the main factors, to compare the number of ocular tear film bubbles observed over a 3-day period after the two dive profiles. The post hoc analysis was conducted with the Fisher protected least significant difference test. The significance level was set at 5%.

RESULTS

The dives in part 1 of the study were uneventful. All subjects had some bubbles in the tear film before the hyperbaric exposure, and the mean (SD) predive number of bubbles were similar for the AIR (3.1 [2.6]) and OXYGEN (3.0 [2.1]) dives. When the divers were decompressed after having inspired 100% oxygen for the entire duration of the hyperbaric exposure, there was no significant difference between the average number of predive (3.1 [2.1]) and postdive (3.9 [2.4]) tear film bubbles. As shown in Figure 1, the number of tear film bubbles increased significantly (8.3 [5.1]) after decompression in the AIR trial, whereas there was no significant increase in the number of bubbles after decompression in the OXYGEN trial.

In the AIR trial, postdive posterior lens–vitreous humor reflectivity increased significantly (115.5% ± 12.8% of predive levels). In contrast, there was no postdive change in the average postdive posterior lens–vitreous humor reflectivity in the OXYGEN trial (Table 1).

In the second part of the study, we observed a statistically significant elevation in tear film bubbles from predive levels, for 2 days after decompression from both dives (Fig. 2). On day 3, the number of ocular tear film bubbles was not significantly
The dive to 2.5 ATA for 180 minutes simulated an average profile used during hyperbaric oxygen treatments. During such treatments, patients breathe pure oxygen at pressures ranging from 2 ATA (10 msw) to 3 ATA (20 msw). The duration of such treatments is normally 1.5 hours, although treatments of decompression sickness may last 4 hours and 45 minutes, when U.S. Navy Treatment Table 6 is used. Although patients breathe pure oxygen during such treatments, interspersed with intervals of air breathing to minimize the risk of oxygen toxicity, the personnel attending patients during such therapy, breathe air. We therefore wanted to evaluate whether ocular tear film bubble formation could be used to monitor the decompression stress of personnel attending patients during hyperbaric oxygen therapy. We recently demonstrated that there was no difference in the results of postdive ocular tear film examinations performed by two investigators,15 which suggests that tear film bubble formation is an objective method, and may assist in the development of an index that could be used by nonspecialists to monitor personnel in a hyperbaric facility on a regular basis.

**Figure 2.** Ocular tear film bubbles observed before (predive) and 30 minutes (postdive), 1, 2, and 3 days after a simulated dive to 4.0 ATA (30 msw) for 15 minutes and 2.5 ATA (15 msw) for 180 minutes. The former was a no-stop decompression dive and the latter required a 45-minute decompression stop at 1.3 ATA (3 msw). Error bars, SD.

**Table 1.** Reflectivity of the Posterior Lens-Vitreous Humor Interface after Decompression from Dives to a Simulated Depth of 10 msw (2.0 ATA) for 60 Minutes in a Hyperbaric Chamber

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Oxygen</th>
<th>Air</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>109.9</td>
<td>103.0</td>
</tr>
<tr>
<td>2</td>
<td>100.0</td>
<td>130.0</td>
</tr>
<tr>
<td>3</td>
<td>110.7</td>
<td>108.3</td>
</tr>
<tr>
<td>4</td>
<td>105.9</td>
<td>118.8</td>
</tr>
<tr>
<td>5</td>
<td>88.9</td>
<td>100.0</td>
</tr>
<tr>
<td>6</td>
<td>66.7</td>
<td>124.1</td>
</tr>
<tr>
<td>7</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>8</td>
<td>84.6</td>
<td>123.3</td>
</tr>
<tr>
<td>9</td>
<td>156.36</td>
<td>131.8</td>
</tr>
<tr>
<td>Mean</td>
<td>100.5</td>
<td>115.5*</td>
</tr>
<tr>
<td>SD</td>
<td>19.5</td>
<td>12.8</td>
</tr>
</tbody>
</table>

Data were calculated as postdecompression posterior lens-vitreous humor reflectivity (% predive reflectivity). Divers (n = 8; four men, four women) breathed either pure oxygen or air.

* P < 0.05.
Although the most accurate method of monitoring circulating bubbles is echocardiography, it is not the circulating bubbles that cause decompression sickness, but rather stationary bubbles in tissues and small blood vessels. These are difficult to detect. It is for this reason that various methods have been developed to monitor inert gas elimination after decompression, and the results used to establish indices of decompression severity. The occurrence of circulating bubbles is normally assigned a grade based on magnitude and frequency. Although increasing grades of Doppler bubble counts are correlated with an increasing probability of decompression sickness, a high Doppler bubble grade does not equate to clinical decompression sickness.

The increased acoustical reflectivity of the posterior lens after decompression in the AIR trial confirms our previous observation. The occurrence of this increased reflectivity is most likely attributable to the inert gas dissolved in the vitreous humor, since there was no change in reflectivity after decompression from the dive during which subjects breathed pure oxygen. We have speculated that nitrogen bubbles forming and accumulating in this region after decompression, may cause such changes in reflectivity. This effect may be due to a greater barrier to the diffusion of inert gas created by the lens, impairing the postdecompression diffusion of gas from the posterior chamber, a slow-diffusion tissue region with an elevated PN₂, to the fast-diffusion tissue region of the anterior chamber, with a lower PN₂. An alternate explanation might also be that the sound propagation velocity in the vitreous body changes, as a consequence of the dissolved inert gas. It is well established that factors such as temperature and age affect the mean sound wave velocity in the vitreous body. Whether elevated PN₂ and PO₂ of the vitreous body are also factors that affect sound propagation velocity remains unresolved. The reason that increased reflectivity was not observed after decompression in the OXYGEN trial, may be attributable to the uptake of the dissolved oxygen by the tissues, which would rapidly reduce the partial pressure of dissolved oxygen in the vitreous body to predive levels.

The finding that bubbles observed in ocular tear film persist for several days after hyperbaric exposure and that peak levels are achieved 2 days after the exposure may explain some of the long latencies reported in the development of signs and symptoms of decompression sickness. Furthermore, these findings do not support the concept adopted by many decompression tables that residual nitrogen returns to predive levels after a surface interval of 24 hours.

The reason for conducting the two dive profiles, was to establish a postdive situation in which most of the fast, but not the slow, tissues were fairly saturated (4 ATA for 15 minutes), and a situation where also the slow tissues were close to saturation (2.5 ATA for 180 minutes). Thus, although the inspired PN₂ was greater during the 4.0-ATA dive, PN₂ in the slow tissues most likely attained higher levels in the dive to 2.5 ATA for 180 minutes, due to the longer duration of the dive.

This finding was reflected in the tendency, albeit not significant, for greater ocular tear film bubbles after decompression from the 2.5-ATA dive compared with the 4.0-ATA dive.

Finally, the observations conducted on the diver that experienced decompression sickness are noteworthy. We obtained tear film bubble measurements before and immediately after the recompression therapy. Although the diver did not have tear film bubble scores that were much higher that those of the remaining group after the dive, his tear film bubbles decreased to predive levels immediately after the treatment.

References


Table 2. Number of Ocular Tear Film Bubbles in One Subject before and after Dives to 30 msw

<table>
<thead>
<tr>
<th>Dive Profile (Depth/Duration)</th>
<th>Predive</th>
<th>Postdive</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 msw/15 min</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>15 msw/180 min</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

The dive to 30 msw for 15 minutes was asymptomatic. The subject experienced knee pain after the dive to 15 msw for 180 minutes, but reported it only before the ophthalmic examination on day 2 after the dive. The bubble numbers for day 2 were observed immediately after the recompression therapy.