Analysis of clinical outcomes of linear vs. deep stop decompression from 3.5 to 6 atmospheres absolute (350 – 600 kpa) in awake rats

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ABSTRACT

Recreational divers are introducing “deep stops” at half the depth (HD-DS) to reduce the risk of spinal DCS with only Doppler evidence to support it. Therefore this research was designed to show the effect of an HD-DS on spinal DCS manifestations by evaluating whether: (1) air diving-induced spinal DCS could be produced in awake, freely moving rats at 3.5-6.0 atm abs (350-600 kPa); and (2) whether the introduction of an HD-DS reduced spinal DCS in such a model. Fifty-one female, Wistar rats (221 to 450 g) underwent one-hour compression at 350 to 600 kPa with seven minutes of decompression with / without a five-minute DS (HD-DS / No-DS). Animals were observed for three hours. Outcomes were classified as: (1) asymptomatic; (2) breathing difficulties; (3) paralysis/weakness; (4) immobility; or (5) death. Eight animals, exposed to 385 kPa air breathing for 60 minutes followed by a three-staged decompression of 7.5 minutes, remained asymptomatic. The profile is known to produce spinal DCS in anesthetized rats. Eleven animals were then used to determine the threshold for DCS: 500 kPa. A total of 14 animals were compressed to 550 kPa (Group 1). Group 1-A (n=8) No-DS; Group 1-B (n=6) HD-DS; 18 were compressed to 600 kPa (Group 2). Group 2-A (n=8) No-DS; Group 2-B (n=10) HD-DS.

Results: (1) 385 kPa protocol did not produce visible DCS manifestations in awake rats. The binomial probability of no DCS in this sample size is 0.002818 for the proportion expected from a published report. The binomial probability of no fatalities is 0.005346. (2) No animals developed spinal DCS when assessed by visible paralysis / weakness or immobility, so no difference could be shown. Group 1-A: two deaths; two breathing abnormalities; four asymptomatic. Group 1-B: all asymptomatic. Difference recorded for breathing difficulties (p=0.0483); none for fatalities (p=0.2024). Group 2 mortality was 55% (n=10). Group 2-A and 2-B: no difference for death (p=0.6063) or breathing problems (p=0.2084).

Conclusions: This model could not evaluate HD-DS for the prevention of spinal DCS in rats.

INTRODUCTION

Decompression sickness (DCS) in scuba divers remains an important clinical problem, with current decompression models being unable to prevent it completely [1]. Neurological DCS is of greatest concern, particularly spinal DCS [2-3]. To reduce the incidence of spinal DCS, some recreational divers are now introducing empirical “deep stops” at half the depth (HD-DS) during decompression, with only limited scientific evidence of a reduction in Doppler bubble scores to support this practice [4-7]. No evidence exists to support the belief that overt signs and symptoms of spinal decompression sickness may be reduced. This research was designed, therefore, to determine the effect of the deep stop on spinal DCS manifestations in awake rats.

Various animal models have been used to study spinal DCS [8]. In general, larger awake animals develop spinal DCS similar to the clinical and pathological findings in humans [9]. Most experiments using smaller awake animals, on the other hand, produce either no effect or rapidly fatal DCS with severe cardiovascular symptoms [10]. Typically, high pressures and rapid decompression
are employed that do not compare favorably to human exposures of interest [11-13]. Gross assessment of outcomes show neurological findings, but the cause is rarely verified. Some studies have used indirect peripheral nerve conductivity to assess possible spinal DCS, but this is not as reliable as direct measurement of spinal cord functionality or histopathological findings confirming spinal DCS [11].

Previous work by Hyldegaard et al. [14], using anesthetized and ventilated rats, showed a high incidence of spinal DCS (52%; n=13) following one-hour air compression to 3.8 atm abs (385 kPa) with a 7.5-minute decompression. Spinal DCS was determined by a reduction (n=3) or loss (n=9) of somatosensory evoked potentials (SSEPs) by measurements of direct ascending primary neuron fibers within the spinal cord. Spinal cord DCS was further verified by histopathological lesions primarily in the white matter of the cord, and comparable to lesions found in larger animals [15] and in humans [16]. Following this experience, the authors planned a series of titrated decompressions using awake, female Wistar rats. The objective was to provide a simple, economical way to produce a high numbers of animals with spinal DCS manifestations in order to compare the difference in clinical DCS outcomes following linear (No-DS) vs. HD-DS decompression.

Accordingly, this study tested two hypotheses:

(1) Does clinical spinal DCS occur in awake animals after compression to 385 kPa in 60 minutes with a decompression of 7.5 minutes? And, if so,

(2) Does an HD-DS reduce the incidence of clinical spinal DCS in an awake rat model?

The study was approved by the Ethics Committee of the Centre for Experimental Animal Research at the University of Stellenbosch, Faculty of Medicine. The study was funded by Divers Alert Network (DAN) Europe and DAN Southern Africa.

**MATERIALS & METHODS**

Unless otherwise specified, all pressures are shown in kPa absolute. Gauge pressure values were used in the
three figures to illustrate decompression schedules ending at zero gauge pressure.

**Pressure exposures**
Fifty-one female Wistar rats (weight: 221-450 g) underwent one-hour compression in an experimental animal recompression chamber. Different groups of animals followed different decompression schedules: Eight followed a protocol of air breathing at 385 kPa (285 kPa gauge pressure) in 60 minutes with a decompression of 7.5 minutes in three stages, *i.e.*, the Hyldegaard *et al.* profile [14] (*facing page*).

When no manifestations were found at this pressure, 11 animals were exposed to pressures between 420 and 600 kPa to determine the threshold for manifestations of DCS. It was found to be 500 kPa. Then, 32 rats were used to compare the clinical outcomes at 550 (Group 1; *n* = 14) and 600 kPa (Group 2; *n* = 18) respectively with either No-DS (Subgroup A) or decompression with a deep stop (Subgroup B, Table 1, above).

Of the 14 animals compressed to 550 kPa (Group 1), eight did No-DS (Group 1-A), and six did an HD-DS (Group 1-B), (*Figure 2, below*). All groups were matched for weight due to the known association with susceptibility to DCS [17].

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**TABLE 1**

<table>
<thead>
<tr>
<th>GROUP 1 (<em>n</em>=14)</th>
<th>GROUP 2 (<em>n</em>=18)</th>
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<tbody>
<tr>
<td>Exposure to 550 kPa</td>
<td>Exposure to 600 kPa</td>
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<tr>
<td>Subgroup 1A</td>
<td>Subgroup B</td>
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<tr>
<td>No-DS</td>
<td>HD-DS</td>
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<tr>
<td>(<em>n</em>=8)</td>
<td>(<em>n</em>=6)</td>
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**FIGURE 2**

FIGURE 2 – Decompression of Group 1: after 60 minutes at 550 kPa (450 kPa gauge pressure)
When the incidence of DCS manifestations at 550 kPa appeared lower than found by Hyldegaard in ventilated animals, another 18 animals were compressed to 600 kPa (Group 2, above). Again, eight did No-DS (Group 2-A) and 10 did HD-DS (Group 2-B).

**Experimental protocol**

At the beginning of each experimental procedure, the animals were transferred from the holding cages in the experimental animal facility at Tygerberg Centre for Experimental Animal Research (CEAR) to an experimental cage and then transferred to the hyperbaric facility. The animals were allowed to acclimatize for a period of three hours before being transferred to the recompression chamber.

The animals had unlimited access to food and water throughout. They were then compressed in batches of no more than three, with each animal in a separate cage. This was done to avoid carbon dioxide accumulation or breathing restrictions, as the animals tended to nest and often grouped together in ways that could restrict breathing or cause significant rebreathing of exhaled air.

CO₂ levels were not measured directly. However, previous experience and calculations of estimated CO₂ production, duration of the exposure, the large internal volume of the chamber and ventilation at 30 minutes offered assurance that CO₂ accumulation was not a confounding factor.

After 30 minutes at pressure, the 80L (7.21-cubic-foot) recompression chamber was flushed with fresh compressed air at a rate of 200 Lpm for five minutes without changing pressure. The animals stayed at the target pressure for a period of one hour, after which they were decompressed. The temperature in the chamber was stable within five minutes of compression to pressure (22°C±1).

Pressure was monitored by means of an analogue Bourdon-tube gauge (scale 0 to 1 MPa) and a digital pressure gauge, both calibrated within the preceding three months to National Standards. Dual gauges were employed to allow for visual determination of any inaccuracies; the analogue gauge provided a clear indication of scale, while the digital gauge provided for direct electronic data recording.
Upon surfacing, the cages were removed from the chamber and the animals were observed in their cages for visible signs of DCS for a total of three hours or until death occurred.

**Evaluation of DCS symptoms**

Manifestations were classified as follows:

1. asymptomatic – no visible signs of discomfort or any abnormalities;
2. breathing difficulties – tachypnea or dyspnea, both transient and persistent;
3. paralysis or weakness – weakness or loss of mobility in a limb;
4. immobility – unable to move upon handling;
5. death.

After decompression the animals were actively monitored at regular intervals if they were not moving spontaneously. The animal care committee did not approve of a three-hour assessment of mobility and function using a continuous treadmill. Accordingly, animals were assessed by tilting the cages every 10 minutes so that the animals were required to walk. Inability to support their own weight, or lack of movement of a limb was recorded as weakness or paralysis respectively. Breathing abnormalities were easily visible. At the end of three hours, the highest-grade manifestations were recorded. Survivors were euthanized with halothane; death occurred within one minute.

The experimental protocol also made provision for perfusion fixation of the spinal cords of symptomatic animals to verify the presence of histopathological changes using the protocol described by Hyldegaard [14]. As no animals qualified for the procedure, it is not detailed here.

**Statistical procedures**

The assessment of DCS symptoms is based on non-parametric observations of which we cannot assume that data to have a normal distribution. For this reason non-parametric statistical analysis was performed by means of the Mann-Whitney test. The data was captured in a STATA database and analyzed using the Stata/IC 10.0 software (StataCorp Inc.). The Wilcoxon Rank Sum (Mann-Whitney) test was used to assess the differences in outcomes between subgroups. The comparability of the mean weights of the two groups was assessed with the two-sample T-test with equal variances.

**RESULTS**

Group 1 animals exposed to 550 kPa – subgroups A and B: Both groups were matched for weight. Difference in weight (grams) was -9.3; CI: -21.4 – 2.9; \( p=0.1225 \).

Group 2 animals exposed to 606 kPa – subgroups A and B: Both groups were matched for weight, but a wider weight range of animals was selected in order to determine whether this would produce greater DCS susceptibility in this model given the primary objective of eliciting spinal DCS. Differences in mean weight in grams was -40.2; 95%CI: -113.5 – 33.1; \( p=0.262 \).

**HYPOTHESIS 1**

Of the eight animals compressed to 385 kPa, with decompression over 7.5 minutes according to the Hyldegaard protocol, all remained asymptomatic without any visible manifestations of DCS throughout the three hours of observation. The weight of the animals was similar to those used by Hyldegaard, i.e., 300 – 350 g [14]. For the proportion expected from the Hyldegaard study, the binomial probability that no DCS would be observed in eight animals in our study is 0.002818, and the probability of observing no fatalities in these animals is 0.005346.

**HYPOTHESIS 2**

Reducing spinal DCS

As no animals developed clinical features suggestive of spinal DCS, this hypothesis could not be tested. Accordingly, histopathological assessment was not undertaken to verify spinal DCS had indeed occurred. The results are summarized in Table 2 (Page 46). No animals developed spinal DCS, so no difference could be shown using No-DS vs. DS.

In Group 1 (n=14), eight underwent No-DS decompression (Group 1-A); six completed an HD-DS at 325 kPa (Group 1-B). In Group 1-A (n=8) there were two deaths; two had breathing abnormalities and four remained asymptomatic. Group 1-B (n=6) all remained asymptomatic. The difference recorded for breathing difficulties between the groups was significant (\( p=0.0483 \)) and favored the HD-DS group. There was no difference for fatalities (\( p=0.2024 \)).

In Group 2 (n=18), eight did No-DS (Group 2-A), and 10 did an HD-DS at 350 kPa (Group 2-B). In Group 2-A (n=8) there were five deaths, two breathing abnormalities and one remained asymptomatic. In Group 2-B (n=10) there were five deaths, two breathing abnormalities and three were asymptomatic. Mortality in Group 2 was 55% (n =10), with no difference between
Group 2-A and 2-B for death ($p=0.6063$) or breathing problems ($p=0.2084$). The power to detect difference was low (46.5%). However, as no spinal DCS had been observed in any of the animals, with the mortality now reaching 55% ($n=10$), the investigators concluded that the model would not support the primary hypotheses and decided to terminate the study at this point.

**DISCUSSION**

Rat models have been used extensively in the study of DCS [8]. Recent research by Lillo *et al.* has suggested that experimental data may be transferable to human exposures [17]. However, their model was based on varying inert gases and high pressure exposures from 485 kPa to 945 kPa (with ultrarapid decompression of less than 10 seconds, producing largely intravascular gas bubbles with cardiovascular symptoms in awake animals. Air exposures were applied to only 20 rats and included a 10-minute stop at 485 kPa. DCS evaluation was based on visible symptoms such as breathing problems, paralysis or death. The study did not include an examination of the causes for walking abnormalities or paralysis where these were observed. Our protocol made provision for histopathological verification of the nature of walking difficulties. However, as none emerged, this component could not be addressed. Also, the Lillo profiles were not comparable to the ones used by Hyldegaard, and therefore probability predictions using this model were not undertaken. In Group 2, the weight range also exceeded the parameters of the Lillo model, making it inappropriate to extrapolate their findings to ours.

It is evident that the awake rat is quite resistant to DCS, especially if its body weight is within the lower range of 150 -250 grams [18]. To be applicable to human exposures, however, the comparison of HD-DS vs. No-DS required the use of longer decompressions from a moderate pressure.

The threshold for visible manifestations of DCS in the awake rats was 500 kPa. At this pressure a statistical difference was found (albeit at low power) for breathing problems and fatalities. This is in general agreement with the previous observations by Lillo and co-workers. However, this did not support the primary hypothesis relating to spinal DCS. Further pressure and decompression combinations could have been attempted, but the authors believed that this fell outside the scope and intent of the original study. Also, they were not aware of any other awake rat model that had an exposure and decompression schedule comparable to typical human behavior.

Hyldegaard *et al.* were able to achieve a reliable incidence of spinal DCS using a pressure of 385 kPa for 60 minutes with a 7.5 minute decompression. Their rats were anesthetized and ventilated and showed evidence
of somatosensory evoked potential (SSEP) changes as well as the development of histopathological lesions comparable to those seen in humans that verified the appearance of spinal DCS. Encouraged by these possibilities, this study attempted to confirm the findings by Hyldegaard in the awake freely moving animals and then to extend the application towards evaluating the protective effects of an HD-DS in reducing spinal DCS. Unfortunately, we were unable to replicated this in awake animals.

The authors therefore concluded that awake rats may have reduced vulnerability to DCS in general and for spinal DCS in particular. The combination of general anesthesia and positive pressure ventilation may affect DCS risk. Hypothetically, positive intrathoracic pressures may reduce offgassing from the spinal cord and increase susceptibility to spinal DCS [19-20]. Unpublished findings by Hyldegaard have shown that the effects are not limited to ventilated rats, however. Accordingly, it does seem that it is the immobility and / or the anesthesia that are predisposing factors. Whether this is due to hemodynamic effects, increased ventilation during movement and exercise with greater elimination of inert gas, or due to intrathoracic and intrathecal pressure changes is not known.

There may be merit in continuing the study to assess the value of HD-DS in reducing breathing abnormalities and death at 500 kPa or testing the effect of HD-DS by means of the anesthetized rat model producing verifiable SSEP changes and histological lesions according to the protocol by Hyldegaard et al. [14]. Even though the rats would be anesthetized, the lesions remain comparable to those seen in humans and occur in a pressure range more comparable to human exposure. Accordingly, further research on HD-DS vs. N-DS using anesthetized animals may have merit.

CONCLUSION
Using pressures comparable to human exposures in an awake, freely moving rat model, no clinical signs of DCS occurred that could be attributed to spinal DCS. Accordingly, the benefit of deep stops could not be evaluated with this model.

REFERENCES