HIGHLIGHTED TOPIC | The Physiology and Pathophysiology of the Hyperbaric and Diving Environments

Impairment of the autonomic nervous function during decompression sickness in swine

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Impairment of the autonomic nervous function during decompression sickness in swine. J Appl Physiol 106: 1004–1009, 2009. First published January 8, 2009; doi:10.1152/japplphysiol.91246.2008.—Dysautonomia has been observed in many cardiac diseases; however, its effect in decompression sickness (DCS) has not been well examined largely due to the difficulty in obtaining experimental data in human or animal subjects. In this study, we examine how DCS affects the autonomic nervous system’s (ANS) dynamics in swine. Baseline and post-DCS electrocardiograms were obtained via telemetry recordings and compared. These data were analyzed using both the power spectrum method and our recently developed principal dynamic mode (PDM) analysis. PDM is able to separate the dynamic tones of the sympathetic and parasympathetic nervous systems. Both methods demonstrated a statistically significant decrease (>55%; P < 0.05) in the dynamics of both branches of the autonomic nervous system in the swine with DCS compared with the control condition. In cardiac diseases such as myocardial infarction, ANS imbalance is often associated with a significant increase in sympathetic tone, which may or may not be counterbalanced by parasympathetic nervous activity. However, the effect of DCS is such that both branches of the ANS are depressed >55% compared with the control condition, suggesting impairment, but not imbalance, of the ANS.

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Bai Y, Mahon RT, White JC, Brink PR, Chon KH. Impairment of the autonomic nervous function during decompression sickness in swine. J Appl Physiol 106: 1004–1009, 2009. First published January 8, 2009; doi:10.1152/japplphysiol.91246.2008.—Dysautonomia has been observed in many cardiac diseases; however, its effect in decompression sickness (DCS) has not been well examined largely due to the difficulty in obtaining experimental data in human or animal subjects. In this study, we examine how DCS affects the autonomic nervous system’s (ANS) dynamics in swine. Baseline and post-DCS electrocardiograms were obtained via telemetry recordings and compared. These data were analyzed using both the power spectrum method and our recently developed principal dynamic mode (PDM) analysis. PDM is able to separate the dynamic tones of the sympathetic and parasympathetic nervous systems. Both methods demonstrated a statistically significant decrease (>55%; P < 0.05) in the dynamics of both branches of the autonomic nervous system in the swine with DCS compared with the control condition. In cardiac diseases such as myocardial infarction, ANS imbalance is often associated with a significant increase in sympathetic tone, which may or may not be counterbalanced by parasympathetic nervous activity. However, the effect of DCS is such that both branches of the ANS are depressed >55% compared with the control condition, suggesting impairment, but not imbalance, of the ANS.

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method provides the most sensitive discriminating statistics between pre- and post-DCS conditions. The prevailing hypothesis is that there will be significant autonomic imbalance in response to DCS. The question to resolve is: What happens to the dynamics of the sympathetic and parasympathetic nervous tones in DCS compared with the normobaric condition?

**MATERIALS AND METHODS**

The methods reported were conducted according to the principles set forth in the “Guide for the Care and Use of Laboratory Animals,” Institute of Laboratory Animal Resources, National Research Council, National Academy Press, 1996. Before commencing, the Naval Medical Research Center’s (NMRC) Institutional Animal Care and Use Committee reviewed and approved all aspects of this protocol. All experiments were performed at the NMRC, and the animal care facility is fully AAALAC accredited.

Animals. As part of a larger study focusing on DCS and spinal cord injury, 13 male Yorkshire swine (Biotechnical Industries, Dunsborough, PA) were housed in free running cages at an animal care facility. Animals were acclimated onsite for 5 days before any procedures. At the housing facility, food (2–2.5% of body weight twice daily; Lab Diet Mini-Pig Grower, Quality Lab Products, Elkridge, MA) and full access to water were provided.

Predive preparation. Since gait disturbance is one of the signs used to determine the presence of DCS, the animals were trained daily to walk on a treadmill over the 3 days before the dive. Each session lasted between 15 and 20 min, with a successful training session defined as the swine’s ability to sustain a speed of 1.5 miles/h for 15 min.

Electrocardiogram. Electrodes were positioned on the surface of the swine’s body (three differential leads). An internally crafted cotton vest was then placed over the electrodes and tied in place. The electrocardiogram data transmitter (Data Sciences Incorporated’s jacketed external telemetry [JET]) was connected to the electrocardiogram (ECG) cables, and after verification of the transmission test data the unit was removed, and the animal recovered for 1 day.

Hyperbaric exposure. On the day of hyperbaric exposure, the ECG telemetry system was re-connected, and baseline signals were acquired. Animals were placed in a standard dog kennel and transported to the hyperbaric laboratory. In the kennel, animals were placed within the Navy’s multiple large animal chamber (MLAC). The MLAC is a steel hulled hyperbaric chamber with 450 ft.³ of floodable volume and pressure tested to 1,000 ft. of seawater (fsw).

The MLAC was pressurized with air to 200 fsw at a rate of 30 ft³/min. Animals were monitored via closed-circuit television for signs of distress related to middle ear barotraumas (head shaking, nystagmus). Any evidence of middle ear barotraumas resulted in a decrease in hyperbaric pressure and descent at a slower rate. Chamber atmosphere was monitored with a Geotech Anagas Dive Air Analyzer (Geotech, Denver, CO). Air composition was maintained at 21 ± 2% oxygen and <0.05% CO₂ surface equivalent. Temperature (80 ± 2°F) and humidity (50 ± 5%) were controlled via an environmental control system piped to the MLAC.

After reaching 200 fsw, the animals remained at depth for a total time (from leaving surface to leaving bottom) of 24–31 min [referred to as bottom time (BT)]. Since the purpose of the parent study was to develop a spinal cord injury model, BT was adjusted throughout the study (24-min BT, n = 6; 31-min BT, n = 6). After the specified BT was reached, the chamber was decompressed at a rate of 60 fsw/min. These are the standard dive procedures used in practice to induce the spinal cord injury (5).

Post-decompression procedures. After reaching the surface, the MLAC door was opened, and the transport kennel was removed. Animals were removed from the kennel, placed in a Panepinto sling, and given diazepam (0.125 mg/kg iv). ECG signals were collected again wirelessly via the JET system into proprietary data collection software. Additional doses of diazepam (0.125 mg/kg) were permitted every 5 min (to a total dose of 2 mg/kg) if warranted.

Surface observations. Animals were observed continuously for the first hour after reaching surface pressure. Animal comfort based on respiratory distress, any vocalizations (Reyes scale), and signs of DCS were recorded. DCS was noted with the onset of cutis marmorata (skin bends) or death. In this group, all 13 animals had cutis marmorata, and 4 of the animals died. After 1 h, the animals were assessed for comfort and returned to their holding pen. Animals unable to be immediately returned to their holding pen remained in the Panepinto sling under observation until able to return to the holding pen.

Data analysis. ECG measurements were collected with a sampling rate of 750 Hz to allow accurate detection and identification of QRS complexes in the ECG (7). The QRS complexes were used to identify beat locations, and, once beat timing was determined, an instantaneous heart rate (HR) signal was created at a sampling rate of 4 Hz using the technique described (3). HR signals were down sampled to 2 Hz, and mean and trends were removed. Signal segments containing 600 data points, which corresponds to 5 min, were used for both the PSD and PDM analyses.

Analysis of HR using PDMs. The PDM is a method based on extracting only the principal dynamic components of the signal via eigen decomposition. The PDMs are calculated using Volterra-Wiener kernels based on expansion of Laguerre polynomials (17). Among all possible choices of expansion bases, some require the minimum number of basis functions to achieve a given mean-square approximation of the system output. This minimum set of basis functions is termed the principal dynamic modes of the nonlinear system. PDM specifically accounts for the inherent nonlinear dynamics of HR control, which the current PSD method is unable to do. A minimum set of basis functions is determined using a method widely known as principal component analysis, in which the dominant eigenvectors and eigenvalues are retained as they relate more closely to the true characteristics of the signal, and non-dominant eigenvectors and eigenvalues represent noise or nonessential characteristics. Thus principal component analysis separates only the essential dynamic characteristics from a signal that is corrupted by noise. We have modified the PDM technique to be used with even a single output signal of HRV data, whereas the original PDM required both input and output data. A detailed summary of the procedure has been presented in Ref. 24, and comparison to the PSD has shown that PDM is more accurate.

Although the PDMs are in time-domain representation, we convert them to the frequency domain via the fast fourier transform (FFT) to facilitate validation of the two ANS activities, since they are usually illustrated in the frequency domain. Therefore, hereafter, we will describe the PDMs’ dynamic characteristics in the frequency domain. For this study, we used six Laguerre functions with a memory length of 60. The detailed steps involved in the calculation of PDMs as well as determining the Laguerre functions and the memory lengths have been previously described (24).

Power spectral density. PSD of HR data were calculated using the method of the Welch periodogram (Matlab 6.5, Natick, MA). A 128-point (frequency resolution of 0.0078 Hz) FFT was applied, using the Hanning window and nonoverlapping segments. The high-frequency (HF) and low-frequency (LF) bands were denoted as 0.04–0.2 and 0.2–0.6 Hz, respectively, and are in agreement with previous studies (9, 22). In addition, our choice of these LF and HF bands was based on the calculated power spectra in which there was clear separation between the LF and HF bands.

Time-domain parameters. The mean HR, root-mean square of the successive difference (RMSSD) of R-R intervals, variance of HR, and approximate entropy (ApEn) values were calculated. ApEn were calculated based on the recommended embedding dimension and the threshold value of m = 2 and r = 0.15, respectively (21).

Statistical analysis. The paired Wilcoxon rank signed test (for non-normally distributed data) and paired t-test (for normally distributed data) were performed to test the significance of the PDM and
PSD in quantifying the autonomic balance. A $P$ value of $<0.05$ was considered significant.

RESULTS

A representative sample of ECG tracings at baseline and post-DCS are shown in Fig. 1, top. Note that there is no discernable difference between the two conditions in the ECG waveforms. Figure 1, bottom, shows R-R interval variability at baseline and post-DCS. Unlike the ECG signals, the R-R variability illustrates a greater difference between the baseline and DCS conditions.

The quantification of HR variability (the inverse of the R-R intervals) was performed in both time and frequency domains. The time-domain parameters (mean HR, variance of HR, RMSSD of R-R, and ApEn) all showed a decreasing trend with DCS (Table 1). However, none of these parameters is significantly different between the control and DCS conditions.

Representative parasympathetic and sympathetic dynamics via the PDM at baseline and post-DCS are shown in Fig. 2. The PDM corresponding to the sympathetic tone shows a dominant magnitude in the LF range (0.04–0.2 Hz). The PDM corresponding to the parasympathetic dynamics shows dominant magnitudes at both LF (0.04–0.2 Hz) and HF (0.2–0.6 Hz). The group average showing the significant decrease in the magnitude of these tracings with DCS is provided in Fig. 3, top and bottom, for the PSD and PDM methods, respectively. Both methods show a significant decrease in the average spectral power post-DCS compared with baseline. The spectral power in both LF and HF, and sympathetic and parasympathetic dynamics obtained by the PDM, all show significant decreases with post-DCS. The observed decrease is on the order of 55%.

Table 1. Comparison of time-domain parameters between baseline and DCS conditions

<table>
<thead>
<tr>
<th></th>
<th>HR, beats/min</th>
<th>Variance of HR</th>
<th>RMSSD</th>
<th>ApEn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prediving</td>
<td>123.63±18.72</td>
<td>6.90±1.72</td>
<td>21.38±17.83</td>
<td>1.17±0.09</td>
</tr>
<tr>
<td>Postdiving</td>
<td>109.14±39.49</td>
<td>5.13±1.96</td>
<td>21.87±17.80</td>
<td>1.00±0.24</td>
</tr>
</tbody>
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Values are means ± SE. DCS, decompression sickness; HR, heart rate; RMSSD, root-mean square of the successive difference; ApEn, approximate entropy. None of the parameters show significant difference between the two conditions.

Fig. 1. Top: a comparison of the electrocardiographic (ECG) signal during baseline and post-decompression sickness (DCS). Bottom: a comparison of the R-R intervals during baseline and post-DCS. Note the discernable difference in the R-R interval variability, whereas ECG signals do not show such contrast.

Fig. 2. Averaged principal dynamic modes pertaining to the parasympathetic (top) and sympathetic (bottom) tones during baseline (blue) and post-DCS (red) conditions. Solid and broken lines represent averages and their standard errors.
for the sympathetic and 58% for the parasympathetic dynamics post-DCS using the PDM method. The decrease in the LF and HF powers post-DCS are greater than the PDM with reductions of 65 and 69%, respectively. Variability in the results is less with the PDM since its level of significance is higher (sympathetic, \( P = 0.001 \) vs. \( P = 0.003 \); parasympathetic, \( P = 0.002 \) vs. 0.04) than the PSD.

**DISCUSSION**

The primary finding from this work is the significant reduction in both the sympathetic and parasympathetic tones post-DCS compared with baseline. The two techniques utilized to obtain the noninvasive assessment of the ANS (the PSD and PDM) concurred in revealing more than a 55% reduction in the two branches of the ANS when the swine exhibited characteristics of DCS.

Although the effect on the ANS with cardiovascular diseases has been well documented (13), the effect of hyperbaric conditions is rather scant in the literature. A notable exception is the study by Hirayanagi et al. in which they investigated the relationship between the autonomic nervous activity and stress hormones induced by hyperbaric conditions in human subjects (8).

They found elevated plasma epinephrine and higher LF power, indicating enhanced sympathetic nerve activity, and depressed parasympathetic nervous activity 3 h after a dive that was at a depth as great as 4.1 MPa (~1,340 fsw) (8). Another study found that there were some noticeable changes in the morphology of the ECG waveforms and ANS disturbances with DCS (16). Our results are in general agreement with these two studies in that we also revealed significant changes in the autonomic tones. However, the enhanced sympathetic tone reported by Hirayanagi et al. (8) does not support our observation of a decrease. This may be attributed to the fact that their divers did not experience DCS, whereas the pigs in our study did. For the human divers in Hirayanagi’s study (8), deep hyperbaric saturation diving did affect the normal function of the ANS. However, the ANS’s regulation was intact because depression of the vagal tone was counterbalanced by the elevation of the sympathetic tone. In DCS, it appears the ANS’s regulatory efficiency has degraded to the point that the compensatory effect of the sympathetic tone to a depressed vagal tone does not function, thereby leading to depression or impairment of both regulatory systems. Considering this, we speculate that the inflection point at which the sympathetic tone changes from an elevated to a depressed level may provide an early warning of impending DCS. Thus the PDM method, by tracking the dynamics of the ANS, has the potential to be used as a noninvasive diagnostic measure for DCS. Modalities to prevent DCS have been sought and the results are promising, based on short oxygen prebreathing and administration of perfluorocarbon (6). Although the PDM is not a method to prevent DCS, it could be used in conjunction with such therapy to examine the proper dosage of the perfluorocarbon and the proper duration of oxygen prebreathing by tracking the dynamics of the ANS from DCS to eventual normal condition.

The effect of diazepam (0.125 mg/kg) on the dynamics of the ANS is unsettled in the literature. A study investigating the relationship between the autonomic function and diazepam (5 mg iv) on human subjects showed that there were no significant changes in the sympathetic and parasympathetic nervous tones as assessed by the power spectral method, but it did reduce the systolic and mean blood pressure and burst rate of muscle sympathetic nerve activity (12). In another study, oral injection (0.2 mg/kg) increased sympathetic nerve activity with no significant change in the parasympathetic activity (10), whereas another study using midazolam (0.08 mg/kg) showed a decrease in both LF and HF powers in human subjects (18). A comprehensive recent study has found no change in the LF and an ~24% decrease in the HF power 15 min after treatment with diazepam (0.13 mg/kg) in human subjects; there was no change in the HF power by 30 min after administration of diazepam, however (1). In the same study, they also found no change in the LF but an ~5% decrease in the HF power after 15 min but no change 30 min posttreatment with midazolam (0.08 mg/kg) (1). Based on these studies, it can be concluded that the LF power is relatively unaffected, whereas the HF power decreases with diazepam or midazolam. The depression of the parasympathetic tone can be partly attributed to the
usage of diazepam but it does not account for all of its 55% decrease in our study. Thus the combined decrease of the sympathetic tone, which is not affected by diazepam, and the parasympathetic tone are most likely due to impairment of the ANS during DCS condition.

In our data, there were two dive time exposures. One group of swine had an exposure time of 31 min (n = 6) and the other group for 25 min (n = 6). We compared the sympathetic and parasympathetic tones between these two groups and found that there were no significant differences between them. Although one might think the time of exposure is critical, we found that the 6-min time difference between the two groups did not have a significant effect. The depth of the dive is also important as greater depths stress the ANS tones, thereby leading to increased sympathetic tone and either a concurrent decrease or no change in the parasympathetic dynamics (8, 14). Thus we expect the intensity of the ANS impairment will be directly proportional to the stresses of increasing dive times and depths.

It should be noted that four of the analyzed data sets represented the swine that died after surfacing. The sample size is too small to make a statistical comparison, but we did observe far lower HRV parameters for those swine that died than those that did not. Even when we excluded these four swine for comparison between pre- and post-DCS, our findings of significant ANS depression post-DCS were not altered.

The evidence of spinal cord injury was verified in the deceased animals by H & E staining. The spinal cord injury was diffuse in nature and consisted of hemorrhage and axonal degeneration. In addition, based on random sampling of the four surviving animals, we found all had pathological evidence of spinal cord injury. Our results are in agreement with a previous study that also found spinal cord injury with DCS (5). Also, the clinical observation of Cutis Marmarota (CM), which is often used as one of the acknowledged signs of DCS, was observed in all swine. CM is often associated with vascular congestion, vasculitis, neutrophilic infiltration, and reactive changes in endothelial cells. These changes are most evident in the capillaries and venules. In other studies, the time of onset of CM has correlated with the severity of DCS (4).

Although the mechanisms of DCS-induced ANS impairment are not fully known, recent reports suggest that injury to the spinal cord is most likely the culprit in the impairment of the ANS seen in our swine with DCS. In a study of Indian lobster divers who have experienced severe neurological decompression sickness (2), they found the majority of the severe injuries were localized to the thoracolumbar spinal cord. In a case study involving a male diver who died from severe DCS after a rapid ascent from 120 m, extensive gas embolism in cerebral and spinal arteries as well as cerebrospinal fluid spaces using postmortem CT and MRI were found (20). This case study has been confirmed in another recent study, which also found cerebrospinal dysfunction in two divers (11). Thus, given this evidence and the fact that the heart receives the ANS inputs from the spinal cord, we speculate that the injury to the spinal cord is most likely the culprit in the impairment of the ANS seen in our swine with DCS.

None of the time domain-based parameters shown in Table 1 provides statistically significant results, although there is a decreasing trend post-DCS compared with the control condition. One of these parameters is the complexity measure known as approximate entropy, which has been widely utilized in biomedicine to discern differences in information complexity between normal and diseased conditions (21). For example, ApEn has been successfully utilized to discern differences in information complexity between healthy and diseased conditions, with the prevailing evidence indicating significant decrease with disease. In our study, ApEn did show a trend of decrease, but the results were not significant.

Separation of the sympathetic and parasympathetic components has long been the objective of mathematical analyses of HRV. Historically, the ratio of the LF (0.04–0.15 Hz) to HF (0.15–0.4 Hz) power obtained from linear spectral analysis has been used as a marker of the sympathetic-parasympathetic balance in assessing HRV for human subjects. However, linear spectral analysis of HRV has not gained wide acceptance within the cardiovascular literature because it is a linear approximation of the nonlinear properties of the ANS and, therefore, does not accurately reflect the balance of the two nervous influences. Furthermore, the LF-to-HF ratio assumes that the LF is mediated only by the sympathetic nervous system, whereas the prevailing understanding is that the LF reflects both the sympathetic and parasympathetic nervous systems (7). Other recent nonlinear techniques such as symbolic dynamics (15), including ApEn (21), have been utilized because they can provide nonlinear properties of the ANS. However, these methods are also limited since they are based on determining the overall complexity of the data and are not designed to separate the two dynamics of the ANS.

The PDM, however, is a nonlinear method designed to capture and separate the two dynamics of the ANS (17). Its efficacy was demonstrated by using pharmacological agents on human subjects, and it was more accurate than the PSD method (23). In this study, both the PDM and PSD methods resulted in significant depression of both branches of the ANS. Although the PDM is a more sensitive measure than PSD, the fact that both methods provided the same results can be attributed to the fact that DCS is such a severe condition that both methods provide consistent results. Furthermore, both methods provide consistent results, supporting our finding of significant depression in both sympathetic and parasympathetic nervous system tones.

We used spectral and PDM powers in the LF in the range of 0.04–0.2 Hz and in the HF of 0.2–0.6 Hz. These LF and HF ranges concur with previously defined frequency ranges for the swine model (17). These LF and HF ranges extend beyond those found in humans (LF: 0.04–0.15 Hz; HF: 0.15–0.4 Hz), presumably because swine have higher HRs and higher spectral power (>0.4 Hz). The PDM, unlike the PSD, is able to separate the two branches of the ANS; thus we were able to show dynamics pertaining to the parasympathetic and the sympathetic nervous system (Fig. 2). Note that the parasympathetic dynamics via the PDM show significant magnitudes in both LF (0.04–0.2 Hz) and HF (0.2–0.6 Hz), whereas the sympathetic dynamics from the PDM only show significant power in the LF (0.04–0.2 Hz) range. Clearly, this is the advantage of the PDM over the PSD method since separate dynamics of both branches of the ANS can be resolved. This advantage has also been demonstrated in human subjects in our previous study using pharmacological blockades (23).
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Limitations

The current study did not involve data collection at depth, which would have yielded valuable information on the effect of pressure on the ANS without DCS. However, the literature regarding the effect of depth on the ANS has shown that this increased stress especially affects sympathetic tone (8, 14). Thus we would expect a similar increase in the sympathetic tone in our animals had we measured the data at the experimental depth. Such experiments are currently in progress.

In conclusion, this is one of the first studies to examine the effect of DCS on the dynamics of the ANS in swine. It was found that one of the detrimental consequences is the significant depression of both branches of the ANS. The often reported gait disturbances with DCS can be explained in part by the impairment of the ANS. Early detection of DCS using the PDM method is currently under study, with swine ECG data being continuously collected from a baseline state, through DCS, and finally post-DCS in the laboratory at the NMRC.

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