Comparison of recompression treatment table efficacy in a swine (sus scrofa) model of neurological decompression sickness

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Introduction / Background: Significant reductions in ambient pressure increase individual risk for decompression illness (DCI), with incidence up to 35 per 10,000 dives. In severe cases the central nervous system is often compromised (>80%), making DCI among the most morbid of diving-related injuries. While hyperbaric specialists suggest initiating recompression therapy with either a Treatment Table 6 (TT6) or 6A (TT6A), the optimal recompression treatment for severe DCI is unknown.

Methods and Materials: Swine were exposed to an insult dive at 7.06 ATA for 24 minutes and decompressed at a rate of 1.82 ATA/minute. Swine that developed neurologic DCI within one hour of surfacing were block randomized to one of four United States Navy Treatment Tables (USN TT): TT6, TT6A-air (21% oxygen, 79% nitrogen), TT6A-nitrox (50% oxygen, 50% nitrogen), and TT6A-heliox (50% oxygen, 50% helium). The primary outcome was the mean area of spinal cord lesions. Secondary outcomes included spinal cord lesion incidence and gross neurologic outcomes. We compared outcomes among these four groups and between the two treatment profiles (i.e., TT6 and TT6A).

Results: One-hundred and forty-two swine underwent the insult dive, and 61 met inclusion criteria (43%). We found no differences in baseline characteristics among groups or between profiles. We found no significant differences in functional neurologic outcomes (p=0.77 and 0.33), spinal cord lesion incidence (p=0.09 and 0.07), or spinal cord lesion area (p=0.51 and 0.17) among the four treatment groups or between treatment profiles, respectively. Animals treated with TT6 had the lowest rates of functional deficits and the fewest spinal cord lesions. Across all animals, functional neurologic deficit strongly correlated with lesion area (logistic regression, p < 0.01).

Summary / Conclusion: TT6 performed as well as the other treatment tables and is the least resource-intensive. TT6 is the most appropriate initial treatment for neurologic DCI among the tables we compared.

Mechanical damage from a growing gas bubble in the spinal cord ex-vivo

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Introduction / Background: Spinal cord decompression sickness (SC-DCS) is caused by the growth of inert gas bubbles in the microcapillaries of the spinal cord. It is widely assumed that the pressure associated with these bubbles leads to a local reduction in blood flow and thus an infarction of the spinal cord tissue. We present evidence for an alternative injury mechanism, in which the growing bubble mechanically tears the surrounding spinal cord tissue irreversibly.

Materials and Methods: High-resolution magnetic resonance imaging (MRI) is used to observe the formation and shrinkage of decompression gas bubbles in ex-vivo bovine spinal cords upon decompression and recompression inside a specially constructed MRI-compatible pressure cell. The sample is compressed at 7 ATA to saturation, then rapidly decompressed to 3 ATA and allowed to reach equilibrium. The sample is imaged in multiple directions to obtain high-resolution images of the decompression bubbles for volumetric quantification. The sample then undergoes multiple cycles of rapid decompression to 1 ATA and recompression to 3 ATA. It is scanned at the end of each cycle to follow the evolution of the bubbles. The decompression and recompression process is so fast that no substantial gas accretion from the surrounding tissue into the bubble can occur and reflects only the mechanical response of the tissue.
Results: We observe that the decompression bubbles grow substantially with each decompression / recompression cycle and do not return to their previous size. We also note that bubble formation is overall quite rare, and most likely to occur near the edges of the sample.

Summary / Conclusion: We conclude that decompression bubble formation in the spinal cord is not a reversible elastic process, but involves an irreversible tearing of the surrounding tissue. We further speculate that bubbles form preferentially in already weakened spots in the tissue. Our observations suggest that SC-DCS may be understood in some cases as a form of traumatic spinal cord injury.

Carbon dioxide retention and carotid body chemosensitivity in cold-water diving
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Introduction / Background: Cold stress and increased arterial oxygen content reduces carotid body (CB) tonic activity, which may reduce the ventilatory response to hypercapnia. We tested the hypotheses that cold water diving would lower CB chemosensitivity and augment CO2 retention more than thermoneutral diving.

Methods and Materials: Thirteen subjects completed two dives (depth: 6.1 msw; bottom time: 202 ± 11 minutes) in either cold (15°C) or thermoneutral (30°C) water. CB chemosensitivity was assessed using brief hypercapnic ventilatory response (CBCO2) tests pre-dive, 80, and 160 minutes into the dive. CBCO2 consisted of inhaling 13% CO2, 66% N2, 21% O2 for one breath repeated four times, with two minutes between hypoxic exposures. CBCO2 was calculated as the slope of the linear regression line of the peak minute ventilation (VE) in three consecutive breaths vs. the peak end-tidal CO2 tension (PETCO2) following the hypercapnic exposures. Data are reported as a change from pre-dive (mean ± SD).

Results: PETCO2 increased during the dive (cold: +1.1 ± 2.4 mmHg; thermoneutral: +2.0 ± 3.3 mmHg; time main effect; p < 0.01), but was not different between conditions. VE increased during the cold condition from pre-dive (D80: +12 ± 7 and D160: +22 ± 13 L·min⁻¹; both p < 0.01) and was greater than thermoneutral condition at both time points during the dive (D80: +5 ± 5 and D160: +3 ± 7 L·min⁻¹; both p < 0.01). CBCO2 was unchanged during the dive (p = 0.28) and was not different between conditions (p = 0.58).

Summary / Conclusion: These data indicate that CBCO2 is not attenuated during cold or thermoneutral diving and likely does not contribute to CO2 retention or changes in ventilation.

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Plasma gelsolin decreases with pressure/decompression and supplementation has anti-inflammatory effects
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Introduction / Background: There are no reliable biomarkers for decompression sickness (DCS). In our studies of proinflammatory microparticles (MPs) we have found a relationship between provocative decompression and some MPs subtypes as well as the amount of filamentous actin (F-actin) on the MPs surface. Plasma gelsolin (pGSN) is an abundant circulating protein that binds and degrades F-actin, and is depleted by inflammation. Levels of pGLN drop in a variety of human and experimental injuries, and supplementation abrogates injuries in over 20 different animal models. We hypothesized that pGLN levels would decrease in association with high-pressure exposures and supplementation may inhibit DCS in a murine model by inhibiting MPs production.

Materials and Methods: Plasma pGLN, interleukin (IL)-1β and MPs were assayed in six human volunteers before, during and two hours after exposure to 30 meters of seawater (msw) for 35 minutes and in mice subjected to 100 psi air pressure for two hours. MPs production by human or mouse neutrophils (PMN, 5.5x10⁵) was assessed ex vivo after incubation at 100 psi air for 30 minutes.
Results: In humans, MPs number rose at pressure by 2.5-fold and IL-1β by 4.6-fold (both p < 0.05), and gelsolin changed insignificantly (87 ± 3 µg/ml before and 73 ± 3 at pressure). After decompression MPs remained elevated by 2.0-fold and IL-1β by 6.0-fold (both p < 0.05), and plasma gelsolin decreased by 90% to 8.9 ± 2.3 µg/ml (p < 0.05). Mice exposed to 100 psi for two hours then decompressed exhibited similar changes. However, mice injection prophylactically with 27 mg/kg human recombinant pGLN did not demonstrate post-decompression elevations of MPs and IL-1β or a decrease in pGLN. In ex vivo studies with isolated mouse and human PMN, pGLN inhibited pressure-induced MPs production, NLRP3 inflammasome formation and IL-1β synthesis.

Summary / Conclusion: Plasma GLN may be a reliable index of exposure to provocative pressure/decompression and supplementation may have therapeutic potential for DCS treatment.

A 5 Passive acoustic mapping of decompression bubbles under ultrasound sonication
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Introduction / Background: Ultrasound at higher acoustic energies can cause tissue damage due to cavitation, but this threshold is decreased in the presence of pre-existing microbubbles. In diving research, decompression-induced venous gas emboli (VGE) are often assessed with echocardiography post-dive. With increasing portability of newer ultrasound systems and development of automated VGE analysis software, continuous monitoring is attractive to better evaluate each diver’s VGE appearance and evolution. Nevertheless, further studies regarding the potential bioeffects of acoustic cavitation of decompression bubbles is warranted. Here we investigate the cavitation energy generated by ultrasound-stimulated decompression bubbles using passive acoustic mapping (PAM), a recent technique allowing real-time, non-invasive imaging of cavitation activity.

Materials and Methods: Decompression bubbles were created by introducing air-saturated pressurized water (4 atm) to an open water-filled column through a jet nozzle. Bubbles were insonified for five seconds at a time at 30 fps at a mechanical index of 1.2 with a 1-megahertz focused transducer excited with amplified sinusoidal signals from an arbitrary function generator. A separate L12-5 linear array transducer connected to a Verasonics programmable ultrasound platform on which we implemented PAM algorithms was placed orthogonally for concurrent passive acoustic detection over the same region. PAM for both stable and unstable cavitation energy levels were generated after subtracting baseline (column sonified before starting the decompression) signals, then normalized to the highest intensity in the bubble data.

Results: Cavitation significantly increased in the presence of decompression bubbles. PAMs show higher inertial cavitation compared to stable cavitation, and higher bubble densities led to increased cavitation activity.

Summary / Conclusion: We have developed a laboratory setup to map stable and inertial cavitation levels in insonified decompression bubbles. Next, we will convert normalized maps into energy values after calibration to evaluate the potential for bioeffects over a range of sonication conditions, while investigating the effect of bubble density and size.

A 6 Gene expression in decompression sickness secondary to underwater compressed-gas diving
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Introduction / Background: Gene expression analysis and biomarker discovery on decompression sickness (DCS) is a research niche. Previous research on rats with DCS has demonstrated significant differential gene and protein expression. DCS diagnosis may be challenging, particularly since it mimics other common medical presentations. Investigating gene expression in humans with DCS compared to closely matched controls can shed light on susceptibility and biomarker profiles. Such information might benefit divers wishing to tailor their diving practices, and physicians involved in the management of divers and fitness for diving consultations. Moreover, this may lead to discovery of biomarkers to facilitate timely DCS diagnosis.
**Materials and Methods:** Subjects diagnosed with DCS presenting within eight hours of symptom onset were recruited. Controls were divers who surfaced symptom-free from ≥ 25 meters of seawater maximum depth. Whole blood was sampled at presentation and at 40-44 hours after surfacing on PAXgene® Blood RNA Tubes (PreAnalytiX, Hombrechtikon, Switzerland). RNA extraction was performed using PAXgene Blood RNA kit (Qiagen, Venlo, Netherlands). Two-step quality control (QC) followed using spectrophotometry (Nanodrop) and bioanalyzer (Agilent 2100) for RIN (RNA Integrity Number). Samples were shipped to BGI Copenhagen, where QC was repeated and RNA-SEQ performed.

**Results:** Paired blood samples were collected from nine cases and 17 controls. RNA-extraction was performed on seven random cases and seven random controls. Following two-step QC, 13 case samples and 11 control samples met required criteria. QC confirmed RIN >8.1 in all samples and RNA concentrations of >20ng/µL in all but one. RNA-SEQ was run on 24 samples. Shifts in the patterns of transcription of leukocytes were noted. Upregulation of genes associated with apoptosis, inflammation and innate immune responses were observed in both groups. Further bioinformatics are expected shortly. Abstract will be updated accordingly.

**Summary / Conclusion:** This area of research remains understudied, with data restricted mainly to animal studies. Future genome and proteome expression studies in human DCS are required.

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**Visualizing decompression stress post dive by transabdominal ultrasound**

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**Introduction / Background:** For decades pre-calculated dive tables such as the DCIEM/DRDC decompression tables have been validated in closely regulated settings on divers, prior to release to the general public. In order to check if the decompression stress after such an experimental dive was acceptable, repetitive Doppler analysis of the heart and subclavian veins or cardiac ultrasound (CUS) investigation are used to determine the amount of inert gas bubbles in the venous bloodstream. Although Doppler and CUS are considered the golden standard, it has several major disadvantages.

In order to quantify reliably bubble sound obtained by Doppler studies or acquire high quality CUS images for grading, experienced and trained personnel are required.

Our aim is to detect and assess venous bubbles after a dive, thus quantifying decompression stress, by transabdominal ultrasound imaging of the inferior vena cava (IVC).

**Materials and Methods:** An ultrasound with abdominal probe was used to image the IVC after different heliox dives, ranging from 69 to 81 meters of seawater (msw), and bottom times of 15 or 20 minutes. The table used was the DCIEM/DRDC experimental heliox-surface oxygen table. The bubble load on the IVC images were compared to apical four-chamber CUS images and the Kisman-Masurel scores for Doppler.

**Results:** In comparison to CUS the IVC images were clearer and showed more gas bubbles. Compared to Doppler scores we also observed more matching results with the IVC images as opposed to CUS, especially in the lower range of the Kisman-Masurel scale.

**Summary / Conclusion:** Imaging intravascular IVC bubbles by transabdominal ultrasound is a promising procedure to assess the presence of venous bubbles and could complement (or supersede) current methods to quantify decompression stress. In addition, due to lower training requirements of the person operating the ultrasound, it could lower the threshold for experimental hyperbaric research and possibly allow on-site adjustment of diving profiles.

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**Venous gas emboli time-series analysis from three controlled pool diving exposures at 24-hour intervals**

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**Introduction / Background:** No current methods to quantify decompression stress. In addition, due to lower training requirements of the person operating the ultrasound, it could lower the threshold for experimental hyperbaric research and possibly allow on-site adjustment of diving profiles.
Introduction / Background: The amount of venous gas emboli (VGE) after diving, assessed via echocardiography, but also their evolution over time, vary significantly between people, even when they complete identically controlled pool dives. Information, however, is scarce concerning the same subjects undergoing repeated dives. We have previously shown that, in three consecutive day dives observing VGE post-dive on a given day, as a binary outcome was dependent on whether these were observed on prior days (Denoble, et al., UHMS 2018 [NOT A FULL CITATION]). Here we re-analyze that experimental data to investigate possible changes in VGE quantity and evolution.

Materials and Methods: VGE counts were analyzed using a custom-built graphical user interface for all post-dive echocardiography recordings acquired at rest from four subjects who showed VGE on all three consecutive dives (n=84). Time-series consisting of VGE counts over the 135-minute post-dive measurement period (sampled every 20 minutes) were analyzed and their area under the curve (AUC) calculated as a measure of integrated VGE observed. To evaluate the effect of consecutive dives on VGE evolution we performed a repeated measures two-way analysis of variance with respect to dive day and time since surfacing from each dive. At each time point the mean VGE of each dive was compared with the mean of the first dive via Dunnett’s multiple comparisons testing (adjusted p-values reported).

Results: AUC and duration over which VGE were seen both decreased with each consecutive dive for all subjects. Mean VGE over all timepoints tended to decrease with consecutive dives (dive 1 vs. 3 p=0.07).

Summary / Conclusion: There is a trend for decreasing VGE amounts with each repeated dive 24 hours apart, although results will be updated once data from all subjects are analyzed. The physiological basis for this apparent acclimation remains to be explained.

Decompression illness caused by freediving in a Florida swimming hole
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Introduction / Background: This case report highlights the potential dangers of freediving that are not usually appreciated by freedivers.

Materials and Methods: We present the case of a 27-year-old man who was freediving with a buddy (30-60 feet of freshwater) at a well-known diving hole. However, on the eighth dive the diver became seriously injured. During this dive the diver interjected a stop at 30 ftw to explore a cavelike structure with air pockets. He inhaled several lungs full of this compressed gas before continuing downward. The diver began his return to the surface. During this ascent (10 ftw), he described a “pop in my chest.” He surfaced with right-sided hemiparesis, wheezing and shortness of breath. He swam to the dock, finning furiously with his left leg. He reported that he had attempted to exhale the “air” in his lungs, “But I knew I was in trouble.” The diver’s clinical course was complicated by right-sided hemiparesis and paresthesias, decompression sickness, pneumomediastinum, bilateral pneumothoracies, a swollen left thigh, suspected left thigh compartment syndrome, and elevated troponin and creatine kinase. The extent of the decompression sickness noted in this patient, was noted on MRI as ischemic regions in the left sensory and motor cortex near the vertex. The patient was transferred first to the Navy Dive Center in Pensacola, Florida and treated with a USN TT6. He was then transferred to community hospital, and finally to the WJMC in Marrero, Louisiana.

Results: Following hyperbaric oxygen therapy at WJMC, the right upper extremity and the left thigh were significantly improved. Currently the patient is applying for a medical waiver to continue his training with the USAF.

Summary / Conclusion: Freedivers need to be educated on the particular dangers of breathing compressed gas underwater when freediving. Arterial gas embolism and decompression sickness may occur, with significant injuries.

Persistent gas bubbles on radiologic imaging after recompression treatment for decompression sickness: A case report
Introduction / Background: Decompression sickness (DCS) may arise when dissolved inert gas in tissue forms extravascular and/or intravascular bubbles during depressurization. The primary treatment is 100% oxygen breathing during recompression, which increases the gas gradient and partial pressure of oxygen in the tissue. This accelerates inert gas washout, and protocols like the U.S. Navy Treatment Table 6 (TT6) are widely assumed to resolve bubbles in the course of a single treatment. We present a case with persistent bubbles apparent on computed tomography (CT) imaging after two TT6 recompression treatments.

Materials and Methods: A 37-year-old male developed multifocal upper body pain and swelling after six repetitive dives to a maximum depth of 145 feet of sea water while breathing 29% nitrox with inadequate decompression stops. He was diagnosed with musculoskeletal and lymphatic DCS and was recompressed (TT6) 25 hours after his last dive, resulting in minimal improvement. A thoracic CT scan was performed, revealing bubbles in the sternomanubrial joint and near the right glenohumeral joint. A second TT6 was provided, again with minimal symptom improvement. The diver elected to transfer to another hospital, where he underwent another CT scan 14 hours after the second TT6. This showed persisting gas in the manubriosternal joint, another gas focus near the left sternoclavicular joint, and resolution of the gas near the right glenohumeral joint. A single U.S. Navy Treatment Table 9 was provided, with partial resolution of his aches.

Results: Due to non-medical and unrelated issues the patient was lost to follow-up. He returned to spearfishing seven weeks after the incident.

Summary / Conclusion: Contrary to prevalent belief large extravascular bubbles may persist after repetitive definitive recompression treatment for DCS.

A 11

Hyperbaric oxygen treatment sensitizes gastric cancer cells to 4-amino-2-trifluoromethyl-phenyl retinate-inhibit migration

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Introduction / Background: The recurrence and metastasis of malignant tumors which may be less sensitive to some chemotherapy drugs as well as some tumor cells' susceptibility to drug resistance are important reasons for the failure of tumor treatment and the low five-year survival rate. The proliferation, migration and drug resistance of tumor cells are closely related to their hypoxic microenvironment. Gastric cancer is one of the most common gastrointestinal malignancies in the world; it is hard to diagnose in early stages and difficult to treat in late stages. Drug resistance to chemotherapy makes the efficacy of chemotherapy drugs poor. 4-amino-2-trifluoromethyl-phenyl retinate (ATPR) can effectively induce the differentiation and apoptosis of gastric cancer cells and inhibit their migration. We asked whether hyperbaric oxygen (HBO2) therapy could increase the effect of ATPR on gastric cancer cells.

Materials and Methods: First, we treated samples of the gastric cancer cell line SGC7901 with HBO2 and analyzed the change in oxygen partial pressure. The cell grouping was as follows: cell, ATPR, HBO2, ATPR+HBO2. MTT assay and wound healing experiment were used to test the viability and migration ability of SGC7901, respectively. Finally, Western blot assay detected the related proteins and preliminarily analyzed the possible molecular mechanism.

Results: The results showed that HBO2 could significantly increase the oxygen partial pressure in the cell medium. Compared with other groups, ATPR combined with HBO2 significantly inhibited the activity and migration of SGC7901. Moreover, Western blot results showed that MMP9 and GRP78 were significantly reduced after ATPR combined with HBO2.

Summary / Conclusion: HBO2 significantly enhanced the effect of ATPR on inhibiting the cell activity and migration ability of SGC7901, which may be related to decrease the expression of MMP9 and GRP78.

End Session A