

Decompression from Saturation Using Oxygen: Its Effect on DCS and RNA in Large Swine

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Introduction: The use of hyperbaric oxygen (HBO) to expedite decompression from saturation has not been proven and may increase risk of toxicity to the pulmonary system. To evaluate any benefit of HBO during decompression, we used a 70-kg swine model of saturation and examined lung tissue by microarray analysis for evidence of RNA regulation.

Methods: Unrestrained, non-sedated swine were compressed to 132 fsw (5 ATA) for 22 h to achieve saturation. Animals then underwent decompression on air (AirD) or HBO (HBOD) starting at 45 fsw (2.36 ATA). Animals were evaluated for Type I and Type II decompression sickness (DCS) for 24 h. Control (SHAM) animals were placed in the chamber for the same duration, but were not compressed. Animals were sacrificed 24 h after exposure and total RNA was isolated from lung samples for microarray hybridizations on the Affymetrix platform. **Results:** There was no evidence of Type I DCS or severe cardiopulmonary DCS in any of the animals; abnormal gaits were noted only in the HBOD group (4/9). Three genes (nidogen 2, calcitonin-like receptor, and pentaxin-related gene) were significantly up-regulated in both the AirD and HBOD groups compared to controls. Three other genes (TN3, platelet basic protein, and cytochrome P450) were significantly down-regulated in both groups. **Conclusions:** HBO during decompression from saturation did not reduce the incidence of DCS. Gene regulation was apparent and similar in both the AirD and HBOD groups, particularly in genes related to immune function and cell signaling.

Keywords: accelerated decompression, oxygen pre-breathe, decompression sickness, decompression illness, DCS, oxygen toxicity.

EXPOSURE TO HYPERBARIC conditions for a prolonged period results in all tissues becoming saturated with inert gas. Once saturation is reached the individual may remain at depth without incurring further decompression obligation. However, decompression from a saturation state is generally lengthy; for example, decompression from saturation at 132 fsw (5 ATA) is more than 57 h (1).

Although there is no definitive etiology for decompression sickness (DCS), bubble formation from gas exiting tissues as ambient pressure decreases is thought to initiate a cascade of events leading to clinical manifestations that can range from mild joint pain to death (6). When environments are well controlled, decompression follows established schedules, allowing the time needed to off-gas slowly, minimizing bubble formation (4). This is achieved either by controlled ascent or by recompression in a hyperbaric chamber immediately upon surfacing.

In emergency situations such as a disabled submarine or commercial diving mishap, proper execution of an optimally timed decompression may be compromised (19). It may also be important to reduce time at depth to

minimize physical stress imposed by the dive. One theoretical method of accelerating decompression is the use of hyperbaric oxygen (HBO) breathing. The benefits of breathing HBO during decompression are thought to largely result from elimination of accumulated N₂ in tissue (3).

However, oxygen at increased partial pressures may lead to toxicity that involves the pulmonary system and/or the central nervous system (CNS). There is evidence that 100% oxygen in hyperbaric conditions leads to decreased vital capacity (8) and increased lung permeability (10). Thus it is possible that while the use of oxygen may beneficially decrease decompression time from saturation, it may concomitantly increase oxygen toxicity risk. We used a 70-kg swine saturation model to examine and compare the use of HBO during decompression from 132 fsw (5 ATA) to an identical air only decompression profile. We then harvested the lungs to examine the effects of staged decompression with HBO or air on high-throughput measurement of RNA regulation.

METHODS

The experiments 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, our Institutional Animal Care and Use Committee reviewed and approved all aspects of this protocol. The institutional animal care facility is fully AAALAC accredited and staff members are familiar with our swine saturation model. Neutered male Yorkshire swine ($N = 26$; 68.2 ± 0.6 kg; Biotechnical Industries, Dunsborough, PA) were housed in free running

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cages at the onsite animal care facility for 5 d prior to any procedures. Food was administered twice daily (2–2.5% bodyweight; Lab Diet Mini-Pig Grower, Quality Lab Products, Elkridge, MD) with free access to water.

Surgical Preparation

On the day prior to the experiment, animals underwent external jugular vein catheterization. Anesthesia induction was performed with ketamine (Ketaject 100 mg · ml⁻¹; Phoenix Pharmaceutical, St. Joseph, MO, 20 mg · kg⁻¹) and xylazine (Xyla-Ject 100 mg · ml⁻¹; Phoenix Pharmaceutical, 2 mg · kg⁻¹), intramuscularly. After induction, animals were maintained on Isoflurane inhalant anesthesia (Halocarbon Products, Rover Edge, NJ, 2–5%) via mask. The external jugular vein was catheterized with a 14-Ga., 30-cm single lumen catheter (Central Venous Catheterization Set; Arrow International, Inc., Reading, PA) via the modified Seldinger technique (2). The catheter was advanced to 8–10 cm from the skin incision site, sutured in place, and taped to the skin. Using connector tubing (Edwards Life Science, Irvine, CA) the catheter was brought through a vest (designed and crafted in house) with an exit site on the dorsal thorax of the swine. Full ambulation after recovery was verified prior to return to the holding pen, where the animal remained overnight.

On the day of the hyperbaric exposure animals were transported to the hyperbaric lab and placed into a Plexiglas box (30" × 42" × 38" manufactured in house) within our Multiple Large Animal Chamber (MLAC). The MLAC is a steel-hulled hyperbaric chamber of 450 cu ft floodable volume and pressure tested to 1000 fsw (31.3 ATA) (Bay Tank Inc, Panama City, FL). The Plexiglas boxes allow for animal isolation and control of the breathing gas mixture while maintaining full range of movement. The external jugular vein catheter was connected to a sterile line, fed through Tygon tubing with a swivel top, and out of the Plexiglas box. The sterile line then passed through a hull penetrator port of the MLAC and was connected to a high-pressure positive displacement infusion pump (Mini pump; Milton Roy, Ivyland, PA). This system allowed for delivery of medication at depth if there was evidence of animal distress. Water was available ad libitum throughout the hyperbaric exposure via a drinking valve (Hog Nipple; Edstrom Industries, Waterford, WI); food was not provided during exposure.

Hyperbaric Protocol

The MLAC was pressurized with air to 132 fsw (5 ATA) at a rate of 30 ft · min⁻¹. Animals were monitored

via close circuit television for any signs of distress related to middle ear barotrauma. The animals remained at 132 fsw (5 ATA) for 22 h to achieve saturation (12). During this time the animals were unrestrained and able to move at will within the Plexiglas boxes. The chamber and Plexiglas box atmospheres were monitored with a Geotech Anagas Dive Air Analyzer (Geotech, Denver, CO). Plexiglas box atmosphere O₂ was maintained at 21 ± 2%; CO₂ was maintained at < 0.05% surface equivalent. Temperature was held at 80 ± 2°F and humidity at 50 ± 5% via an environmental control system piped to the MLAC.

After the 22-h saturation period, paired animals were designated AirD (air decompression) or HBOD (hyperbaric oxygen decompression). All animals ascended at a rate of 5 fsw · min⁻¹ (1.15 ATA · min⁻¹) to 85 fsw (3.57 ATA). Ascent was paused for specific times at the depths indicated in Table I. The gas mixture remained air for all stops until 45 fsw (2.36 ATA), at which time Group HBOD animals were switched to 100% O₂ breathing, while Group AirD animals continued air breathing (Table I).

Breaks from breathing O₂ were given every 60 min for 15 min. These air breaks were considered 'dead time' and did not reduce the time required at the listed depth. At a depth of 5 fsw (1.15 ATA), both groups breathed air until the completion of that decompression stop and then ascended to surface pressure. Travel between stops occurred at a rate of 5 fsw · min⁻¹ (1.15 ATA · min⁻¹). The total duration of the compression, hyperbaric exposure at 132 fsw (5 ATA), and decompression was 58.75 h.

A third group (SHAM) underwent the same pre-dive procedures as the experimental groups and was placed in the chamber, which was then manually sealed and where they remained (not compressed) for the same duration as the AirD and HBOD groups (58.75 h). All chamber conditions (temperature, humidity, light) in the SHAM group were replicated as in the experimental groups except for noise associated with compression/decompression procedures.

Upon decompression the MLAC chamber door was opened and individual observers for each animal entered. Animals were fitted with a tail pulse oxymeter (VetOx 4404, Heska Corp, Loveland, CO); pulse and oxygen saturation were recorded every 5 min as well as any signs of cutis marmorata (skin bends) or distress. After an initial 2-h observation period the animals walked out of the MLAC. Animals were evaluated and graded for limb dysfunction by a blinded observer based on a modified Tarlov Scale (0 = paraplegia; 1 = minimal movement; 2 = stands with assistance; 3 = stands alone; 4 = weak walk; 5 = normal gait) (17,18). Afterwards the

TABLE I. DECOMPRESSION PROFILE FOR AIRD AND HBOD GROUPS.

Depth (fsw)	85	80	75	70	65	60	55	50	45	40	35	30	25	20	15	10	5
Time at Depth (min)	305	115	125	130	135	145	150	165	60	65	70	75	80	90	100	180	215
Cumulative (h:min)	5:05	7:00	9:05	11:15	13:30	15:55	18:25	21:10	22:10	23:15	24:25	25:40	27:00	28:30	30:10	33:10	36:25

Animals in both AirD and HBOD groups decompressed on identical schedules as shown by time (min) spent at specific depths (fsw). Numbers in bold and italic indicate times when HBOD animals breathed 100% O₂, while AirD animals remained on air.

animal was placed in a holding pen to move about freely. After a final examination 24 h after surfacing the animal was sacrificed.

Decompression Sickness Definitions

DCS was clinically divided into three broad categories: 1) cutis marmorata; 2) pain-only DCS; and 3) severe DCS. Cutis marmorata is a skin manifestation that appears as hyperemia, progressing to dark, violet patches. Pain-only DCS includes limb lifting, foot curling, vocalization, and generalized agitation such as pacing and "head butting." Cutis and pain-only are considered Type I DCS. Type II DCS includes severe and life-threatening illness of two varieties, neurological and cardiopulmonary. In the present study, neurological DCS was defined as motor deficit (limb weakness, repeated inability to stand after being righted by the investigator) and paralysis (limb flaccidity, areflexia, hypotonia). This was further defined by the modified Tarlov scale performed prior to hyperbaric exposure, immediately following, and 24 h after decompression. Cardiopulmonary DCS was defined as clinical evidence of severely compromised oxygenation and/or hemodynamic instability; more specifically, a hemoglobin saturation < 80%, mean heart rate $\geq$ 150% baseline, and mean observed respiratory rate $\geq$ 200% baseline, all sustained for $\geq$ 1 min.

The absence of cutis marmorata and cardiopulmonary DCS limited statistical analysis of DCS. A simple Fisher's Exact Test was used to analyze the dependence of treatment groups on gait. All individuals with a Tarlov score of < 5 at any point after decompression were categorized as "weak walk."

Pulmonary Tissue Collection, RNA Isolation, and Microarray Analysis

After final observations were recorded 24 h post-surfacing, the animals were euthanized with an intravenous injection of Euthosol (1.5 cc/10 lb bodyweight). At the time of necropsy the thoracic cavity was opened and a small sample was collected from the right lower lobe and placed in RNAlater (Ambion, Austin, TX). Necropsy lung tissues were collected from six AirD animals, eight HBOD, and four SHAM controls and preserved in RNAlater (Ambion). Isolation of RNA and microarray analysis were performed by Cogenics, Inc. (Durham, NC). Briefly, RNA was isolated from the lung samples using RNeasy Mini columns (Qiagen, Valencia, CA) according to the manufacturer's protocol. The quantity and purity (A260/280 ratios 2.0-2.1) of extracted RNA was evaluated using a Nanodrop ND-100 spectrophotometer (NanoDrop Technologies, Wilmington, DE) and its integrity measured using an Agilent Bioanalyzer (Agilent Technologies, Wilmington, DE).

All phases of the microarray analysis, through preparation, synthesis, purification, hybridization, scanning, and quality checks, were performed using the Affymetrix GeneChip protocol (Affymetrix, Inc., San Jose, CA). In brief, 1 μ g of total RNA from each individual lung sample, along with poly A spikes (labeling control), were converted to double-stranded cDNA using the GeneChip

One-Cycle cDNA Synthesis Kit. After second-strand synthesis, cDNA was purified with the GeneChip Sample Cleanup Module. The resulting double-stranded DNA was then used to generate multiple copies of biotinylated cRNA by in vitro transcription with the GeneChip 3'-Amplification Reagent Kit for IVT Labeling. The A260/280 ratio and yield of each of the cRNAs were 2.0-2.1. For each sample, 10 μ g of biotinylated cRNA spiked with bioB, bioC, bioD, and cre (hybridization control) was hybridized to arrays using the GeneChip® (900624) Porcine Genome Array for 17 h at 45°C. Following hybridization, all arrays were washed and stained in an Affymetrix GeneChip Fluidics Station and scanned with a GeneChip® Scanner 3000. Quality checks and data analyses were carried out using GeneChip Operating Software. Differentially expressed transcripts were assessed for the enrichment of possible biological processes using High-Throughput GoMiner (<http://discover.nci.nih.gov/gominer/hi-thruput-defs.jsp>) to leverage the gene ontology.

Analysis of the Gene Expression Measured by Microarray

To assess the reproducibility of the gene expression measurements from biological replicates, a Pearson correlation coefficient was calculated based on the intensity measurements for all features present on the microarray for each pair-wise combination of biological replicates at the 0-h time point. A 1.0 value indicates a perfect correlation while a 0 value indicates no correlation. The average correlation within each group was 0.9834 (SHAM), 0.9835 (AirD), and 0.9845 (HBOD). These values indicate that the intensity data was well correlated between different individuals at the 0 time point. The similarity of the values indicates that the normal variability associated with individual animals was consistent within each of the treatment groups. To identify differentially expressed transcripts, an average result for the animals within each treatment and SHAM control groups was first calculated. Then ratios were calculated, comparing a treatment group to the SHAM group. The following criteria were used to identify differentially expressed transcripts: log₁₀ intensity $\geq$ -0.8, absolute fold-change $\geq$ 1.3, log ratio *P* value $\leq$ 0.001.

RESULTS

Decompression Illness

There was no evidence of cutis marmorata, pain, cardiopulmonary DCS, or overt neurologic injury in any of the animals. The Tarlov score for each individual prior to decompression was 5 (maximum possible). At the two observation points after decompression (2 h, 24 h), abnormal Tarlov scores were noted only in the HBOD group. Two animals (22%) scored 4 immediately after the decompression; one of those recovered and scored 5 at 24 h. Two animals had a normal Tarlov upon surfacing, but scored 4 at the 24-h observation.

A Tarlov score of < 5 at any point after decompression was categorized as "weak walk" (Table II). Using Fisher's

TABLE II. HIND LIMB GAIT ANALYSIS OF EXPERIMENTAL ANIMALS.

Group	Normal Gait	Weak Walk
AirD	9	0
HBOD*	5	4
SHAM	8	0

Animals were scored for hind limb gait by the modified Tarlov scale. Data represent the number of animals with either normal gait (Tarlov score = 5) or weak walk (Tarlov scores ≤ 4); grading was performed immediately upon reaching surface and 24 h after decompression. * Statistically significant difference between AirD and HBOD ($P = 0.041$ Fisher's Exact).

exact test, no difference was detected between the AirD group and the SHAM group. However, there was a statistical difference between the AirD group and the HBOD group ($P = 0.041$) and a difference approaching significance between the SHAM group and the HBOD group ($P = 0.053$).

Gene Expression

At 24 h, both the AirD and HBOD groups exhibited statistically significant alterations affecting 37 genes in

the lungs compared to the SHAM group. Overall, there were 20 up- and 17 down-regulated genes that were significantly altered in most or all of the animals in the AirD (Table III) and HBOD (Table IV) groups. The differentially expressed genes were classified into eight categories based on their general biological function. The AirD group animals exhibited a broad range of up- and down-regulated gene alterations (Fig. 1).

Most of the up- and down-regulated alterations (35% and 52%, respectively) were noted in genes associated with immune function. Animals in the HBOD group exhibited a similar pattern of up-regulated genes; changes were primarily related to similar functional gene families: cell signaling (30%), molecular pathways (25%), structural (20%), and immune function (25%) (Fig. 1). As was observed in the AirD group, the majority of down-regulated genes in the HBOD group were associated with immune function (52%). Other genes families that were down-regulated included molecular pathways (12%), metabolism (12%), structural (6%), and cell signaling function (6%).

TABLE III. PROFILE OF UP- AND DOWN-REGULATED GENES FROM LUNGS OF THE AIRD GROUP.

Gene Description	Code	N
Up-Regulated		
caspase 7 isoform delta; ICE-like apoptotic protease 3	BX923579	3
cytoskeletal beta actin	BI118288	3
Chemokine	BF192019	3
heterogeneous nuclear ribonucleoprotein	BQ601998	3
nidogen 2 (predicted)	CN156760	2
WAP disulfide domain 1 precursor; prostate stromal protein ps20	CF363052	2
WAP disulfide core domain 1 precursor; homolog (mouse)	CF361753	2
calcitonin receptor-like receptor	NM_214095	2
pentaxin-related gene, rapidly induced by IL-1 beta	CN153343	2
haloacid dehalogenase-like hydrolase domain containing 2	CF794576	2
Clone rfcc27b_h3.y1.abd, mRNA sequence	CF365719	2
TCN2	CO956727	2
placenta-specific 8	BI404223	2
optimedin form A	CD572353	2
IRRE like 3	CF368148	2
Growth arrest, DNA-damage-inducible protein GADD45 beta	CF175279	2
cytochrome P450 2B22	NM_214413	2
Growth arrest, DNA-damage-inducible protein GADD45 beta-like	BF708594	2
Myotubularin-related protein 9; myotubularin related protein 8	BF080890	2
RNA binding, signal transduction associated phosphotyrosine protein	CK459782	2
Down-Regulated		
similar to immunoglobulin kappa light chain variable region O11	AF334741.1	4
platelet basic protein	NM_213862	4
TN3	NM_214147	3
immunoglobulin VDJ region mRNA	U38212.1	3
cytochrome P450 3A26	CO990609	3
immunoglobulin VDJ region mRNA	U38217.1	3
immunoglobulin kappa light chain VJ region	CF180359	3
eosinophil chemotactic cytokine; ortholog of mouse chitinase,	CF368668	3
interleukin 2 receptor gamma	NM_214083	3
MHC class II antigen (SLA-DRB) mRNA for SLA-DR1 beta1	AB016750.1	3
RING finger protein 13 isoform 1	BI184073	3
immunoglobulin kappa light chain VC region	AF334739.1	3
immunoglobulin kappa light chain VJ region	BI336606	3
Nucleolin	AJ657247	3
bA30415.1 (novel lipase)	CB474923	3
Nucleolin	BP157354	3
secreted phosphoprotein-I	NM_214023	3

Genes significantly up- or down-regulated in the lungs of the AirD animals compared to the SHAM group. N = number of animals that demonstrated significant changes.

TABLE IV. PROFILE OF UP- AND DOWN-REGULATED GENES FROM LUNGS OF HBOD GROUP.

Gene Description	Code	N
Up-Regulated		
nidogen 2	CN156760	8
prostate stromal protein ps20	CF363052	8
prostate stromal protein ps20	CF361753	8
scr.msk.p1.Contig2, mRNA sequence	CF364971	8
Chimerin 1 (GTPase-activating protein, rho, 2)	BQ597587	8
reticulin 1 isoform A; neuroendocrine-specific protein	CK457674	8
disintegrin/metalloproteinase w thrombospondin motif 9	CO989594	8
alpha 1 type XVIII collagen isoform 1 precursor; endostatin	CN155194	8
calcitonin receptor-like receptor	NM_214095	7
pentaxin-related gene, rapidly induced by IL-1 beta	CN153343	7
melanoma cell adhesion molecule	BQ605024	7
alpha 2 type IV collagen preproprotein; canstatin	CO950243	7
Sus scrofa cDNA 5, mRNA sequence.	BG609032	7
scr.msk.p1.Contig1, mRNA sequence	BQ601005	7
chimerin 1	CD572334	7
scr.msk.p1.Contig1, mRNA sequence	BF710490	7
similar to collagen alpha 1(IV) chain precursor	BI183311	7
matrix metalloproteinase 1 (type I collagenase)	CA779388	7
heat shock protein 47	CN160329	7
enabled homolog	AJ653947	7
Down-Regulated		
immunoglobulin kappa light chain variable region O11	AF334741.1	8
TN3	NM_214147	8
Immunoglobulin VDJ region	U38212.1	8
Scinderin	CO989361	8
alveolar macrophage-derived chemotactic factor-I	NM_213867	8
chemokine (C-X-C motif) ligand 13 (B-cell chemoattractant)	CF787657	8
immunoglobulin mu heavy chain constant region	S42881.1	8
platelet basic protein	NM_213862	7
cytochrome P450 3A26	CO990609	7
Immunoglobulin VDJ region	U38217.1	7
Clone G502 immunoglobulin kappa light chain VJ region	CF180359	7
porcine inhibitor of carbonic anhydrase	NM_213847	7
tetranectin (plasminogen binding protein)	BI186214	7
similar to RIKEN cDNA 2010001M09	BX916934	7
371,602 MARC	BI343446	7
Ig gamma 2b chain constant region	M81771.1	7
uncoupling protein 2	NM_214289	7

Genes significantly up- or down-regulated in the lungs of the HBOD animals as compared to controls. *N* = number of animals demonstrating significant changes.

Microarray analysis of mRNA isolated from lungs following the dive revealed that six specific genes were up-regulated (three genes) or down-regulated (three genes) in the majority of pigs in both the AirD and HBOD groups (Tables III and IV). The genes that were significantly up-regulated in both groups were nidogen 2, calcitonin receptor-like receptor, and pentaxin-related gene. The genes that were down-regulated in both treatment groups were TN3, platelet basic protein, and cytochrome P450 3A26. Three of these altered genes (calcitonin receptor-like receptor, pentaxin-related gene, and platelet basic protein) are related to immune function.

DISCUSSION

In the present study we demonstrated that this staged decompression schedule from 132 fsw (5 ATA), with or without HBO, did not result in overt DCS manifestations of skin bends, pain, or cardiopulmonary dysfunction. However, the HBO decompression profile was associated with an increase in abnormal gait which was mild in nature and not associated with overt neurologic

defects. It is well established that HBO increases nitrogen elimination and decreases bubble size (3). While HBO use as part of a decompression schedule has been correlated with decreased decompression requirements from non-saturated states, there is surprisingly scant literature on the use of HBO during decompression from saturation.

Human testing of HBO in decompression from saturation has not demonstrated clear benefit. The Navy Experimental Diving Unit (NEDU, Panama City, FL) tested an accelerated decompression schedule with HBO in humans from saturation at 50 fsw (2.52 ATA). There was no decrease in the incidence of DCS (15). The protocol tested was similar to the profile presented here, where HBO was initiated after decompression from saturation began. This led to testing HBO use prior to the start of decompression (oxygen pre-breathe), which safely decreased decompression time (15).

In the NEDU human study, several reasons were considered to explain the lack of benefit seen from HBO. These included the potential vasoconstrictive effects

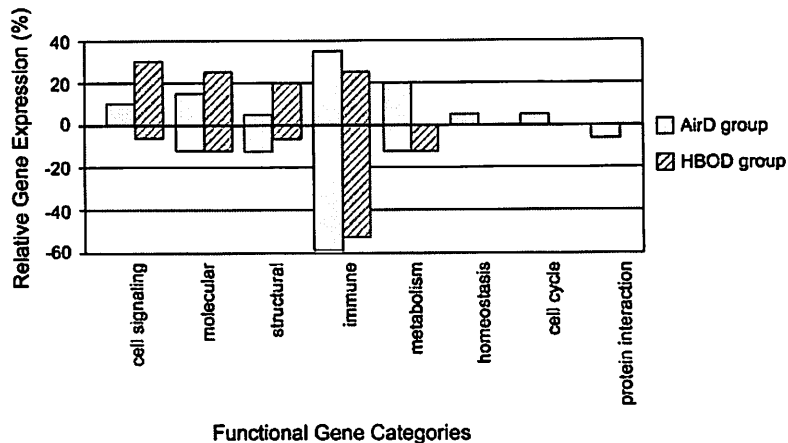


Fig. 1. Categorization of genes exhibiting significantly altered expression in AirD and HBOD groups compared to controls (SHAM). Data is presented by functionally categorized genes in animals from AirD (gray bars) and HBOD (hatched bars) groups with > 1.5-fold changes compared to sham-treated controls.

from HBO, the oxygen contribution to bubble formation, and the potential for oxygen toxicity. One of the main concerns with saturation is the tissue beds that take up (and release) inert gas slowly (i.e., slow compartments). In these tissue beds small decrements of inert gas elimination may have a greater clinical impact. Also, given the air break requirements (to prevent oxygen toxicity), slow compartments are more difficult to purge of inert gas. Though these concerns are theoretical, the lack of HBO benefit in both animal and human decompression schedules from saturation could simplify operations by eliminating its consideration altogether.

If HBO is not beneficial to decompression from saturation, concerns of toxicity are important and warranted, given that the level of oxygen exposure may be greater than that considered safe for humans. Clark (8,9) described the unit pulmonary toxic dose (UPTD) to formulate oxygen exposures that would result in a 5% decrease in vital capacity in 50% of human subjects. If we consider the decompression from saturation and oxygen exposure of the HBO decompression schedule, a calculated UPTD of > 3800 is reached; it is generally accepted that a UPTD of < 1380 is safe. Though these values were contrived for humans, it gives us an opportunity to compare oxygen exposure levels. Additional insight into the effects of HBO may be gained from gene expression studies.

Compression/decompression with hyperbaric air leads to alterations in the levels of inflammatory mediators, including cytokines, complement and adhesion molecules, and the accumulation of neutrophils (7,21). The present work supports this, describing a strong down-regulation of immune genes that occurs regardless of the oxygen treatment. Furthermore, in addition to immune functions, we observed an up-regulation of genes related to cell signaling, molecular pathways, and structural functions in lungs from swine treated with O₂. Perhaps O₂ saturation during decompression stimulated essential biological processes to facilitate the capacity of swine to maintain normal homeostasis. Of interest, in the air-treated swine, the same gene groups were up-

regulated in addition to metabolic, homeostasis, and cell cycle groups, albeit at lower levels. We observed specific genes that were significantly altered in almost all animals, regardless of the treatment during decompression. Of note, three of these altered genes were related to immune function (calcitonin receptor-like receptor, pentaxin-related gene, and platelet basic protein). Calcitonin receptors are novel G protein-coupled for the calcitonin gene-related peptide (CGRP) family of peptides, which play important roles in the mediation of effects such as ischemic preconditioning, antigen presentation within the immune system, apoptosis, and other stress-induced effects (20,22). Pentraxin 3 (PTX3) is a tumor necrosis factor and interleukin-1 β -stimulated gene is an important component of the innate immune response (11). Platelet basic protein is a chemokine family member known to have a broad range of functions as signaling molecules and cationic antimicrobial peptides. It is released upon platelet activation and leads to increased plasma levels of β -thromboglobulin and neutrophil-activating peptide-2 (13,14), which are involved in inflammatory responses.

Interestingly, expression of the first two genes was up-regulated in both AirD and HBOD groups, while the platelet basic protein gene was down-regulated in both groups. This suggests that responses may be more linked with aspects of the dive profile that both groups had in common (e.g., pressure exposure, saturation, elevated partial pressures of gases comprising air, initial decompression) rather than the differential exposures to oxygen during the final phase of decompression. Nonetheless, the identification of common factors or pathways involving these genes may lead to novel therapies, including inflammatory cytokine receptor antagonists or specific antibodies.

In addition, this observation suggests an immune component in DCS as postulated in previous findings that described an increase of inflammatory markers (IL-6, TNF α , IFN γ) in rat brain, lung, muscle, and serum 6 h after decompression that were decreased at 24 h (5).

The authors speculated that nitrogen bubbles in the blood, extracellular and intracellular, may stimulate an inflammatory reaction. Elevation of P-selectin was also observed in rat lungs that may have been related to DCS (5). A separate swine study demonstrated a strong correlation between increased incidence of neurologic DCS and acute lung and liver inflammation as characterized by leukocyte aggregation and activation (16). Future studies should identify the cells responsible for these inflammatory responses and examine additional tissues and organs that may be affected by immunomodulation after DCS.

Though the lungs studied here were collected 24 h after decompression, it is evident that compression/decompression influences a variety of pathways. The single time point (24 h) for tissue collection is not sufficient to permit speculation on the role played by regulation of the identified genes. However, we hope that this study encourages further work into the molecular effects of decompression. During this decompression schedule from saturation at 132 fsw (5 ATA), the use of HBO may have led to a worse clinical outcome compared to the same decompression schedule using air. Demonstrating the numerous genes regulated should provide insights and justification for further research regarding the molecular mechanisms observed during decompression and HBO exposure.

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