Approved, DCN# 43-5229-19

ONR-NAVSEA UNDERSEA MEDICINE PROGRAM REVIEW

May 14 – 16, 2019
Durham, North Carolina

2019 ONR Undersea Medicine / NAVSEA Deep Submergence Biomedical Development Program Review
May 14-16, 2019

Monday, May 13

EARLY BIRD SOCIAL – ROOF AT THE DURHAM					5:00 PM	

Tuesday, May 14

REGISTRATION					7:00 AM	
Chapman	sandra.chapman@navy.mil	Welcome/Umed NNR Program Manager Comments	ONR	INTRODUCTION	8:00 AM	15
Waters T.	edward.t.waters@navy.mil	DSBD Program Manager Comments	DSBD	INTRODUCTION	8:15 AM	15
Webb	ryan.m.webb@navy.mil	SEA00C Program Manager Comments	SEA00C	INTRODUCTION	8:30 AM	15
Waters D.	Deborah.k.waters@navy.mil	PMS391 Program Manager Comments	PMS391	INTRODUCTION	8:45 AM	15
Woodson	peter.woodson@socom.mil	NSW Force Medical Officer Comments	NSW	INTRODUCTION	9:00 AM	15
BREAK					9:15 AM	15

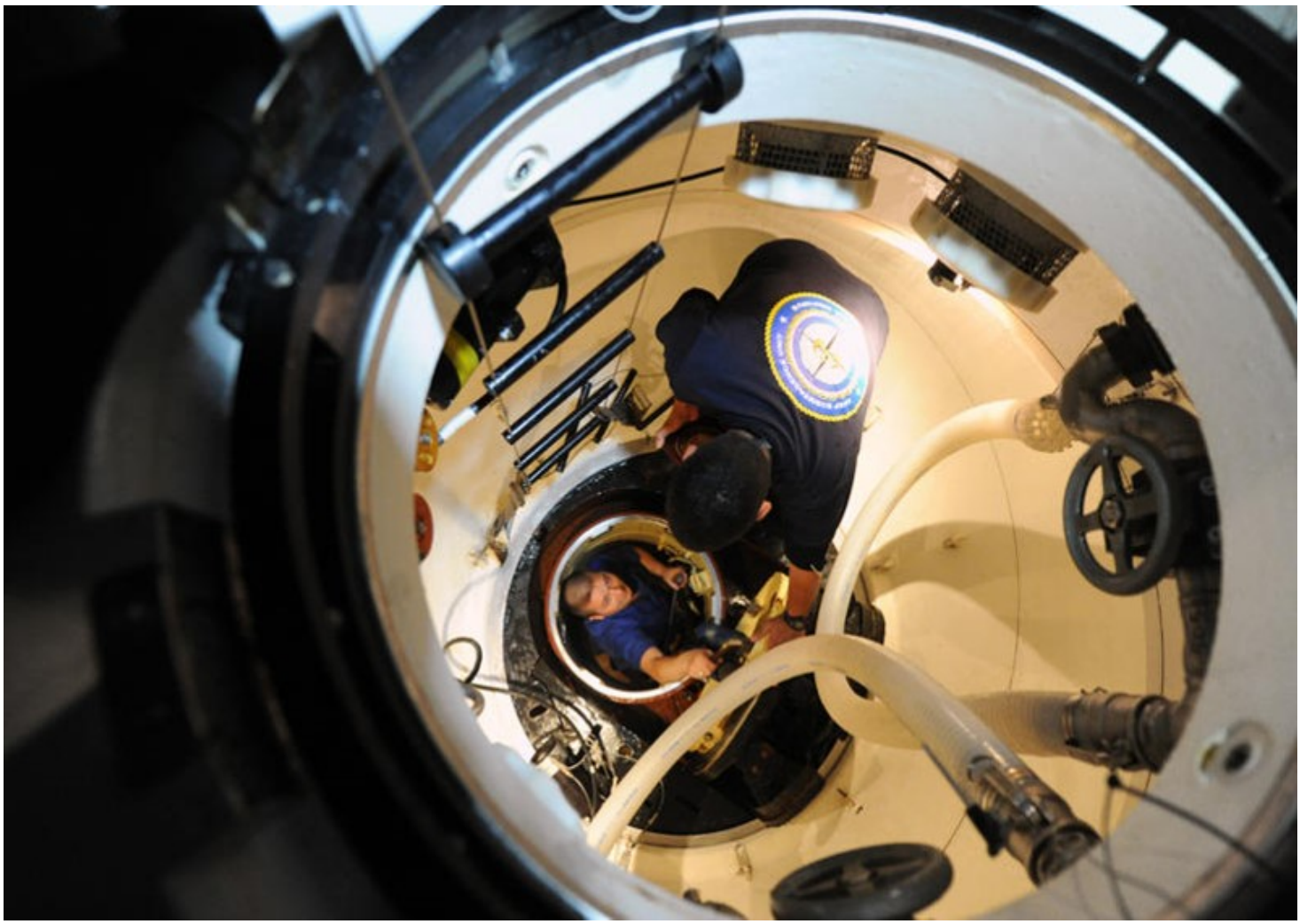
PI	Email	Title	Org.	Session	Time	Time (min)
Mcknight	jcm20@st-andrews.ac.uk	Neurobiological and Physiological Measurements From Free-Swimming Marine Mammals	ONR	GILS	9:30 AM	15
Mason	mason@chemistry.harvard.edu	Porous Metal-Organic Liquids as a New Platform for Investigating Gas-Liquid Interactions	ONR	GILS	9:45 AM	15
Ilardo	melissailardo@gmail.com	Functional Genomics Approaches To Breath-Hold Diving	ONR	GILS	10:00 AM	15
Nocera/Villagran	dnocera@fas.harvard.edu/dino@utep.edu	Studies Toward Closed-Loop Underwater Breathing Applications (SCUBA)	ONR	GILS	10:15 AM	20
QUESTIONS					10:35 AM	10
BREAK					10:45 AM	15
Boron	wfb2@case.edu	Gas Channels/ Molecular Mechanisms and Pathways for Gas Transport Across Biological Membranes	ONR	HYPERBARIC PHYSIOLOGY	11:00 AM	15
Hall	aaron.a.hall6.civ@mail.mil	Assessment of Doxycycline as an Adjunctive Therapy to Prevent Decompression Sickness in Swine	NAVSEA	DECOMPRESSION SICKNESS (DCS)	11:15 AM	15
Moon	richard.moon@duke.edu	Altitude Decompression Tables	NAVSEA	DECOMPRESSION SICKNESS (DCS)	11:30 AM	15
QUESTIONS					11:45 AM	15
LUNCH					12:00 PM	60
Murphy	francis.g.murphy1@navy.mil	Modernization of Navy Thalmann Algorithm Dive Planner	NAVSEA	DECOMPRESSION SICKNESS (DCS)	1:00 PM	15
Doolette	david.doolette@navy.mil	Navy Dive Computer & Dive Planner Algorithm for Constant 1.3 atm PO_2-in-Helium Diving to 300 fsw	NAVSEA	DECOMPRESSION SICKNESS (DCS)	1:15 PM	15
Andrew	brian.t.andrew@navy.mil	Manned Validation of VVAL 79 Air Decompression Schedules for Short Bottom Times in the 130-140 fsw Range	NAVSEA	DECOMPRESSION SICKNESS (DCS)	1:30 PM	15
Gerth	wayne.a.gerth@navy.mil	21st Century Surface Supplied Heliox Decompression Tables	NAVSEA	DECOMPRESSION SICKNESS (DCS)	1:45 PM	15
Mitchell	sj.mitchell@auckland.ac.nz	ONR-G Gas Narcosis in Hyperbaric Environments	ONR	HYPERBARIC PHYSIOLOGY	2:00 PM	15
QUESTIONS					2:15 PM	15
BREAK					2:30 PM	15
Myers	christopher.m.myers1.ctr@navy.mil	Impact of Repeated 6-hour Hyperoxic Exercise Dives at 1.35 ATA on Muscular Performance and Cardiovascular Endurance (RIP-X)	ONR/NAVSEA	DIVER PERFORMANCE & MEDICINE	2:45 PM	15
Syski	andrew.syski@navy.mil	Pulmonary Oxygen Toxicity with an Oxygen Partial Pressure of 1 atm: Possible Mitigation	NAVSEA	OXYGEN TOXICITY	3:00 PM	15
Hall	aaron.a.hall6.civ@mail.mil	Determining DISSUB Survival Rates of 70 Kg Swine Rescued Using SRDRS Standard Operating Procedures	NAVSEA	OXYGEN TOXICITY	3:15 PM	15
Hall	aaron.a.hall6.civ@mail.mil	Evaluation of Tiotropium Bromide Efficacy to Reduce Pulmonary O_2 Toxicity in Divers - FDA Procedure	NAVSEA	OXYGEN TOXICITY	3:30 PM	15
Malmstadt	malmstad@usc.edu	Connecting Lipid Oxidation To Cellular Dysfunction In Hyperbaric Oxygen Toxicity	ONR	OXYGEN TOXICITY	3:45 PM	15
Piantadosi	claude.piantadosi@duke.edu	Oxidative Tissue Damage Mitigation Using Anti-Epileptic Drugs	ONR	OXYGEN TOXICITY	4:00 PM	15

	QUESTIONS				4:15 PM	15
	ADJOURN				4:30 PM	
	MEET & GREET – TOBACCO ROAD SPORT CAFÉ				5:30 PM	
Wednesday, May 15						
	REGISTRATION				7:30 AM	
Chapman	sandra.chapman@navy.mil	Welcome/Umed NNR Program Manager Comments	ONR	INTRODUCTION	7:55 AM	5
PI	**Email**	**Title**	**Org.**	**Session**	**Time**	**Time (min)**
Howle	laurens.howle@duke.edu	Evaluation of and Advances in O_2 Toxicity Models	NAVSEA	OXYGEN TOXICITY	8:00 AM	15
Dean	jdean@health.usf.edu	Cellular Mechanisms of CNS O2 Toxicity During CO_2 Retention and Ketone Metabolic Therapy	ONR	OXYGEN TOXICITY	8:15 AM	15
Ciarlone	geoffrey.e.ciarlone.mil@mail.mil	Assessment of Ketone Ester for the Induction of Ketosis and Delay of CNS O_2 Toxicity in Swine	NAVSEA	OXYGEN TOXICITY	8:30 AM	15
Derrick	bruce.derrick@duke.edu	Ketogenic Diet for Reduction of CNS O_2 Toxicity Symptoms in Working Divers	NAVSEA	OXYGEN TOXICITY	8:45 AM	15
Chon	kchon@engr.uconn.edu	Feasibility of Electrodermal Activity for Detecting Seizures Elicited By CNS O_2 Toxicity Underwater	ONR	DIVER PERFORMANCE & MEDICINE	9:00 AM	15
D'Agostino	ddagosti@health.usf.edu	Optimizing Ketone Metabolic Therapy	ONR	DIVER PERFORMANCE & MEDICINE	9:15 AM	15
	QUESTIONS				9:30 AM	15
	BREAK				9:45 AM	15
Kernagis	dkernagis@ihmc.us	Ketones For High Intensity Mission Operations	ONR	DIVER PERFORMANCE & MEDICINE	10:00 AM	15
Johnson	blairjoh@buffalo.edu	Role of O_2 Breathing on Carotid Body Sensitivity	ONR	OXYGEN TOXICITY	10:15 AM	15
Kelly	karen.r.kelly8.civ@mail.mil	Evaluation of Thermoregulatory Differences Between Insulated Clothing Options During Cold-Water Diving	ONR	DIVER PERFORMANCE & MEDICINE	10:30 AM	15
Campbell	james.e.campbell3.ctr@navy.mil	Measurement of Regional Heat Exchange Using Direct Calorimetry in Cold Water	NAVSEA	DIVER PERFORMANCE & MEDICINE	10:45 AM	15
Maguire	brian.j.maguire12.ctr@mail.mil	Epidemiological Analyses of US Navy Diver Separation Health Assessments	NAVSEA	DIVER PERFORMANCE & MEDICINE	11:00 AM	15
Hostler	dhostler@buffalo.edu	Optimizing Performance During Topside Operations and Diving at Altitude	NAVSEA	DIVER PERFORMANCE & MEDICINE	11:15 AM	15
	QUESTIONS				11:30 AM	15
	LUNCH				11:45 AM	60
Freiberger	john.freiberger@duke.edu	Does Heart Rate Variability Predict Performance Impairment in Divers?	NAVSEA	DIVER PERFORMANCE & MEDICINE	12:45 PM	15
Beaudoin	monique.beaudoin@jhuapl.edu	Applied Systems Engineering to Improve Operational Guidance and Human Safety for Immersion in Warm Water Environments	NAVSEA	DIVER PERFORMANCE & MEDICINE	1:00 PM	15
Reimers	sreimers@pccii.com	Flexible Recompression Chamber	NAVSEA	DIVER PERFORMANCE & MEDICINE	1:15 PM	15
Lance	rachel.lance@duke.edu	Development of a Pulse Oximeter to Independently Monitor Oxygen Levels in Rebreather Divers	NAVSEA	DIVER PERFORMANCE & MEDICINE	1:30 PM	15
	QUESTIONS				1:45 PM	15
	BREAK				2:00 PM	15
McMurtrie	paul.mcmurtrie@navy.mil	Diver Augmented Vision System (DAVD)	ONR/NAVSEA	DIVER PERFORMANCE & MEDICINE	2:15 PM	15
Cunningham	blair.cunningham@codaoctopus.com	CODA Octopus 3D Sonar & DAVD GEN1 Development	ONR/NAVSEA	DIVER PERFORMANCE & MEDICINE	2:30 PM	15
Kvasic	igor.kvasic@fer.hr	Adriatic-Advanced Diver-Robot Interaction Capabilities	ONR	DIVER PERFORMANCE & MEDICINE	2:45 PM	15
Moon	richard.moon@duke.edu	Sildenafil for Prevention of Immersion Pulmonary Edema (SIPE)	NAVSEA	DIVER PERFORMANCE & MEDICINE	3:00 PM	15
Lance	rachel.lance@duke.edu	S.T.E.M.	ONR	MISCELLANEOUS	3:15 PM	15
Broderick	tbroderick@ihmc.us	Biotechnology to Improve Cold-Water Operator Performance	ONR	DIVER PERFORMANCE & MEDICINE	3:30 PM	15

	QUESTIONS				3:45 PM	15
	ADJOURN				4:00 PM	
	COCKTAIL HOUR – CAMERON ATHLETIC CENTER, DUKE UNIVERSITY				6:00 PM	60
	GALA BANQUET – CAMERON ATHLETIC CENTER, DUKE UNIVERSITY				7:00 PM	
Thursday, May 16						
	REGISTRATION				8:30 AM	
Waters T.	edward.t.waters@navy.mil	DSBD Program Manager Comments	NAVSEA	INTRODUCTION	8:55 AM	5
PI	**Email**	**Title**	**Org.**	**Session**	**Time**	**Time (min)**
Reinhart	paul.n.reinhart.ctr@mail.mil	Effects of Disabled Submarine (DISSUB) Stressors on Submariner Cognition	NAVSEA	SUBMARINE RESCUE	9:00 AM	15
Rangamani	padmini.rangamani@eng.ucsd.edu	YIP Non-Equilibrium Thermodynamics of Biological Membranes	ONR	HYPERBARIC PHYSIOLOGY	9:15 AM	15
Casper	brandon.m.casper4.civ@mail.mil	Development of an Interactive Software Application to Provide Recommendations for Human Exposure to Underwater Noise	NAVSEA	HYPERBARIC PHYSIOLOGY	9:30 AM	15
Casper	brandon.m.casper4.civ@mail.mil	Development of a Methodology for Characterization of Acoustic Technologies of Naval UUV Existing and Future Assets	NAVSEA	HYPERBARIC PHYSIOLOGY	9:45 AM	15
Eckmann	eckmann@uphs.upenn.edu	Mitochondrial Stress and Cellular Protection in Undersea Medicine	ONR	HYPERBARIC PHYSIOLOGY	10:00 AM	15
	QUESTIONS				10:15 AM	15
	BREAK				10:30 AM	15
Glandon	glandonh@uncw.edu	Lipid Composition and Nitrogen Solubility of the Spinal Cord and Brain	ONR	HYPERBARIC PHYSIOLOGY	10:45 AM	15
Johnson	blairjoh@buffalo.edu	Autonomic Activity and Water Immersion	ONR	DIVER PERFORMANCE & MEDICINE	11:00 AM	15
Whybourn	lesley.a.whybourn.fm@mail.mil	Medical Response Strategies to Optimize Survival of DISSUB Escapees	NAVSEA	SUBMARINE RESCUE	11:15 AM	15
Schlader	zjschlad@buffalo.edu	Hyperthermia and Hypohydration in Disabled PRM	NAVSEA	SUBMARINE RESCUE	11:30 AM	15
	QUESTIONS				11:45 AM	15
	LUNCH				12:00 PM	60
Sobakin	sobakin@wisc.edu	Improving Safety of Submarine Escape and Rescue from Shallow Depth	NAVSEA	SUBMARINE RESCUE	1:00 PM	15
Keegan	jkeegan@mide.com	Joints for Lightweight Atmospheric Diving Suits	ONR	NEXT GENERATION ATMOSPHERIC DIVING SYSTEM (ADS)	1:15 PM	15
Sorensen	sorensek@spawar.navy.mil	Contaminated Water Research	ONR	MISCELLANEOUS	1:30 PM	15
Howle	laurens.howle@duke.edu	Transfer of Duke University Dive Trial Data to US Navy	NAVSEA	DECOMPRESSION SICKNESS (DCS)	1:45 PM	15
Thom	sthom@smail.umaryland.edu	Micro-particles, Platelet-Neutrophil Aggregation and Decompression Sickness	ONR	DECOMPRESSION SICKNESS (DCS)	2:00 PM	15
Hibbs	stephen@quasarusa.com	Development of Diver Biometric Device (DBD) SBIR Phase II N151-078	ONR	DIVER PERFORMANCE & MEDICINE	2:15 PM	15
	QUESTIONS & CLOSING COMMENTS				2:30 PM	15
	ADJOURN				2:45 PM	
	HYPERBARIC CHAMBER TOUR, DUKE UNIVERSITY				4:00 PM	

Approved, DCN# 43-5229-19

Day 1: Tuesday, May 14, 2019



2019 ONR-NAVSEA UNDERSEA MEDICINE PROGRAM REVIEW

NEUROBIOLOGICAL AND PHYSIOLOGICAL MEASUREMENTS FROM FREE SWIMMING MARINE MAMMALS

FAHLMAN, A., MCKNIGHT, C., TYACK, P., SHORTER, K.A., BOGDAN, P., WHELAN, H.

Fundación Oceanogràfic de la Comunitat Valenciana
Carrer Eduardo Primo Yúfera, 1, 46013 València, Valencia
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Ph: +34 654 492 427

Background

Diving mammals must function effectively during extended periods without access to atmospheric oxygen. In achieving this they exhibit dramatic cardiovascular responses to diving, including intense bradycardia and major re-distribution of blood flow. Despite these circulatory adjustments, marine mammals can routinely experience extreme hypoxemia even during normal foraging dives, with arterial blood O_2 tensions as low as 3 mmHg. How obligate aerobic organs, such as the brain, tolerate regular hypoxia and avoid acute deleterious effects of low oxygen in diving mammals is currently unknown, but may be underpinned by complex hemodynamic adjustments associated with the gross circulatory changes that these animals exhibit. Oxygen management determines both dive duration and activity levels that can be maintained, and is therefore critical for determining the physiological (and thus behavioral) envelope of deep diving animals, and the consequences of anthropogenic stressors (for example ocean noise and changes in food availability) on their fitness and survival. Thus, a better understanding of these physiological changes may help better understand how these species are able to function for long periods without access to O_2.

Objectives

We will develop a archival near-infrared spectroscopy (NIRS) data logger to study cerebral and systemic changes in hemoglobin oxygenation levels in marine mammals. To enable the use of NIRS on cetaceans we propose to investigate the following scientific questions:

1) Can the propagation and transmission of light in the near IR spectrum be characterized for tissues of cetaceans? If so, can these sensors be used to measure hemodynamics of cetacean cerebral tissue?
2) How does the depth of light penetration change as a function of separation between NIRS optodes?
3) Can relationships between hemodynamics, blood oxygenation and metabolic function be derived experimentally for superficial tissues and in the brain?
4) How can measurements from physiological sensors (NIRS, heart rate, respirometry) be combined with estimates of mechanical work and power to improve our ability to predict metabolic cost in free-swimming animals?

Methods

Objective 1: Experimental verification of a NIRS system for the measurement of hemodynamics, oxygenation and metabolic function in both core (brain) and peripheral (blubber and muscle) tissues in a representative marine mammal, bottlenose dolphins.

A) **Investigate and characterize light propagation through blubber (spectrophotometry, Monte Carlo photon propagation modelling, fMRI):**
B) **Measure physiological response to breath-holding:**

Objective 2: Create and experimentally validate relationships to predict physiological responses and energetics of swimming and diving animals using data from a bio-logging tag that combines NIRS and motion sensors.

A) **Localize animal and measure swimming kinematics**
B) **Estimate center of mass, work, and power**
C) **Estimate energy expenditure using wearable bio-logging sensors:**

Objective 3: Characterize the hemodynamic, oxygenation and metabolic dynamics of free ranging dolphins using the validated system and experimentally derived models.

A) Free swimming managed animals
B) **Free ranging wild animals**

Results

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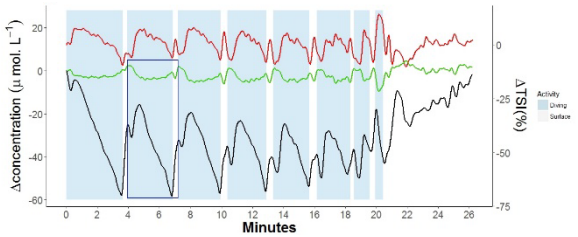
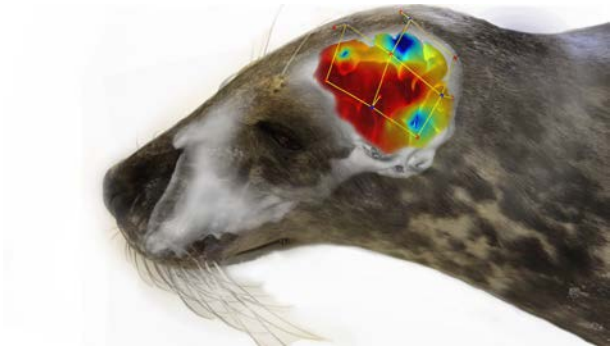


[Figure 1] Example trace of cerebral blood volume and hemoglobin oxygen dynamics during a sequence of dives from a juvenile harbor seal. Blood volume [ΔtHb], hemoglobin oxygenation [ΔHb_{diff}] and tissue saturation index (ΔTSI%) dynamics across all the dives in a trial; where the green line = blood volume, black line = hemoglobin oxygenation and red line = tissue saturation index. A single dive sequence of a 3min dive followed by a 45sec post-dive surface interval is indicated by the blue box.]

Notes:



POROUS METAL-ORGANIC LIQUIDS AS A NEW PLATFORM FOR INVESTIGATING GAS-LIQUID INTERACTIONS

Jarad A. Mason, Malia Wenny

Department of Chemistry & Chemical Biology, Harvard University
mason@chemistry.harvard.edu; 617-496-3481

Background

The development of advanced porous materials for the reversible, high-capacity adsorption of specific guest molecules is critical to the success of many emerging technologies in energy, sustainable development, and medicine. The research pursued in this project targets a fundamentally new class of porous materials—metal-organic liquids—that features both intrinsic porosity and fluidity, affording novel behaviors and previously inaccessible functionality.

Conventional liquids possess tiny, transient pores whose fleeting existence is due to thermally induced fluctuations in local density. Here, the local structure—and porosity—of liquids will be strongly influenced by directional interactions mediated by coordination bonds, leading to enhanced gas solubility and transforming conventional relationships between the microscopic structure and bulk macroscopic properties of liquids. Moreover, imparting porosity to liquids will offer access to properties that are not possible in conventional porous solids, including surface tension, tunable viscosity, motion by capillary action, surface wetting, and the ability to flow and fill a volume of any size or shape.

Our approach to the design of metal-organic liquids with intrinsic microporosity is expected to lead to the creation of a new phase space of porous materials that exhibit both fundamentally insightful and technologically important properties. Top-performing materials are expected to open new research directions within gas storage and separations and provide fundamental insights into gas-liquid interactions relevant to understanding the behavior of gases in both natural and synthetic systems.

Objectives

The objectives of this project are to: 1) synthesize metal-organic liquids with well-defined, transient micropores; 2) characterize the structure and properties of porous metal-organic liquids; and 3) investigate gas absorption and transport properties of porous metal-organic liquids.

Methods

The directionality and tunability of coordination bonds will be leveraged to create liquids with transient microporosity. Specifically, inorganic cations—either bare metal cations or metal-centered complexes—will be combined with organic ligands specifically designed to 1) bridge multiple metals through directional coordination bonds oriented to promote the formation of micropores and 2) facilitate low-temperature melting transitions through the rational manipulation of enthalpic and entropic effects. The resulting metal-organic liquids will feature transient micropores that rapidly form and collapse locally—as coordination bonds break and reform—affording a constant average porosity across the bulk liquid at equilibrium.

The strategies being pursued in our initial efforts to synthesize organic ligands for porous metal-organic liquids are directed at two overarching goals: 1) maximize the thermodynamic driving force—enthalpic and entropic—for metal-organic compounds to exist as a liquid, rather than a solid, at as low of a temperature as possible, and 2) direct the formation of transient micropores in the liquid state. Specifically, we have proposed six ligand design principles that are inspired by the well-established “crystal engineering” design rules of metal-organic frameworks to promote porosity through coordination chemistry and the “anti-crystal engineering” design rules of ionic liquids to promote low melting temperatures through rational liquid design.

Results

Our project officially began on March 11, 2019. As such, we are in the initial stages of beginning our research effort to synthesize porous liquids and do not have new results to report at this time.

Notes:

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review
Sponsor: ONR
Project Status: NEW

MOLECULAR BASIS FOR ADAPTATIONS TO DIVING

Melissa Ilardo

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melissailardo@gmail.com (808)208-4404

Background

The human response to hypoxia, or low oxygen, is a pressing issue in a variety of medical contexts. However, the genetic and physiological variation that underlies hypoxia tolerance is poorly understood. Until recently, the only natural human system in which the genetics of hypoxia tolerance had been investigated was populations adapted to high altitudes (Ilardo, Moltke et al. 2018). Importantly, these populations undergo *chronic* hypoxia due to sustained lower oxygen levels, initiating a well characterized hypoxia response. Another study system for hypoxia is a group of traditional divers called the Sea Nomads who, due to their marine-based lifestyle, regularly experience *acute* hypoxia during repeated bouts of breath hold dives (Schagatay, Lodin-Sundström et al. 2011).

It was recently discovered that a group of Sea Nomads called the Bajau have evolved enlarged spleens to enhance their diving capabilities (Ilardo, Moltke et al. 2018). In all mammals, including humans, the spleen contracts during diving to inject a supply of oxygenated red blood cells into the circulatory system (Hurford, Hochachka et al. 1996, Stewart and McKenzie 2002). Therefore, a larger spleen presumably prolongs dive time in this population. The large spleen phenotype was shown to be associated with a genetic variant in the gene *PDE10A*, believed to affect spleen size by stimulating thyroid hormone production. Mouse studies have found a drastic reduction of spleen size in *Pax8*$^{-/-}$ mice with congenital thyroid hormone (T4) deficiency (Angelin-Duclos, Domenget et al. 2005). Notably, spleen size was shown to be recoverable through an injection of T4 to artificially raise thyroid hormone levels. The difference in spleen size appeared to be attributable to T4-induced increased red blood cell proliferation in the spleen. This suggests the mode of action in the Bajau is increased spleen size as a consequence of increased thyroid hormone levels. As no studies have been conducted on the connection between thyroid hormone levels and spleen size in humans, the molecular mechanisms underlying this relationship are yet unknown.

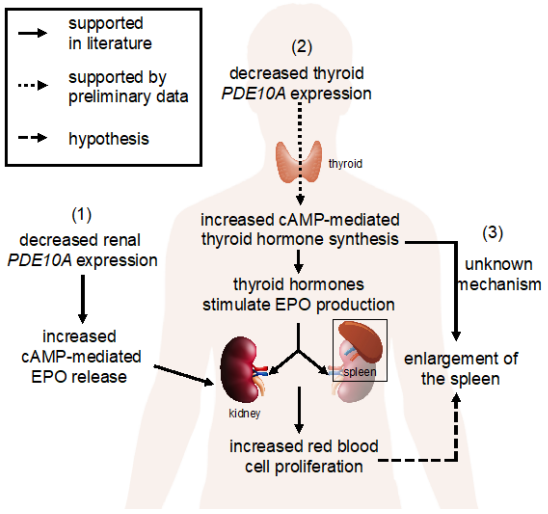

Fig.1. Three proposed pathways by which PDE10A inhibition increases spleen size.

Another possible mechanism by which the variant observed in the Bajau could increase spleen size is through stimulation of erythropoietin (EPO) production and release, which would in turn stimulate splenic erythropoiesis. EPO release in the kidney is cAMP dependent, and has been previously shown to be stimulated by phosphodiesterase inhibitors (Sherwood, Burns et al. 1987, Batmunkh, Krajewski et al. 2006). It has also been demonstrated that thyroid hormones can directly stimulate EPO production and red blood cell proliferation (Golde, Bersch et al. 1977, Popovic, Brown et al. 1977, Dainiak, Hoffman et al. 1978, Fandrey, Pagel et al. 1994, Jelkmann 2011). Therefore, the observed increase in spleen size might be a consequence of two different, simultaneous means of stimulating EPO production in response to *PDE10A* inhibition (see Figure 1).

Objectives

We propose to investigate recently discovered adaptations to hypoxia tolerance using a functional genomic approach, the results of which will help to design pharmacological applications relevant to the Navy.

Methods

Using our conditional PDE10A knockout mouse model, we will perform a global knockout of PDE10A by crossing a transgenic mouse strain line expressing Cre recombinase under the control of a promoter that directs expression specifically in the germline promoter with mice carrying a floxed-PDE10A allele. *PDE10A*$^{F/F}$ mice will be generated by Cyagen, as no such mouse currently exists. We will use the *PDE10A*$^{F/F}$ mouse line to perform physiological and biochemical experiments including: *in vivo* ultrasound spleen measurements, *ex vivo* spleen measurements (length and mass), histological analysis of thyroids, flow cytometry of splenic and bone marrow cells, T4 and TSH measurements, hematological measurements, western blot analysis of cAMP-signaling components in thyroid tissue, and mRNA measurements of EPO levels in renal tissue.

Results

We used a pharmacological approach to reduce *PDE10A* activity in a murine model. We administered the selective *PDE10A* inhibitor, MP-10, via intraperitoneal (*ip*) injection (10 mg/kg), using published protocols (Beaumont, Zhong et al. 2016, Hankir, Kranz et al. 2016) in males from age 4 weeks. After 4 and 9 weeks of drug administration, we measured spleen size using an ultrasound. We found a significant difference in spleen size between the control and treated groups at both time points (p = 0.0494, 0.0488 respectively), with the treated groups (representing the Bajau variant) having larger spleens. This was confirmed through *ex vivo* measurements of spleen length, which we also found to be significantly larger in the MP-10 treated animals (p = 0.015). We collected thyroid tissue and analyzed thyroglobulin levels through a Western blot, which confirmed that the inhibitor increased thyroid hormone production. Analysis of the erythropoietic cell composition in spleen tissue by flow cytometry demonstrated that the spleens of the treated mice contained an excess of apoptotic cells trending towards significance (Figure 2A), and a significant excess of basophilic erythroblasts (Figure 2B), a red blood cell (RBC) precursor. These data are consistent with the results from the *Pax8*$^{-/-}$ knockout mouse study (Angelin-Duclos, Domenget et al. 2005). Interestingly, we found no difference between hematological parameters such as RBC count between treated and control mice (p = 0.6956).

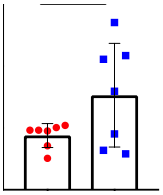

Fig. 2. Flow cytometry data from spleens of control vs MP-10 treated male mice. Chronically treated mice (10mg/kg daily for 9 weeks) demonstrated (A) a borderline significant excess of apoptotic cells (p = 0.068) and (B) a significant excess of basophilic erythroblasts (p = 0.018).

An increase RBC proliferation and splenomegaly without a corresponding increase in RBC count has also been observed in the ablation of *Nix*, an anti-apoptotic factor in erythrocytes (Diwan, Koesters et al. 2007). This has been argued to be due to increased capacity for erythrocyte sequestration in the enlarged spleen.

Combined, our preliminary results suggest that the large spleen phenotype observed in the Bajau is replicable in mice via inhibition of *PDE10A* and that the underlying mechanism involves elevated proliferation and turnover of RBCs, which could have important implications for hematological disorders. Our goals are therefore to expand our pharmacological experiments as well as to generate a genetic model of this phenotype: thyrocyte-specific *PDE10A* knockout mice, which we will use to understand the means by which *PDE10A* acts in thyroid to increased erythropoiesis and spleen enlargement.

Notes:

OXYGEN GENERATION AND EXTRACTION FROM SEAWATER

Daniel G. Nocera

Harvard University
dnocera@fas.harvard.edu, (617)495-8904

Background

The oxygen evolution reaction (OER), in which water is anodically converted to oxygen, has great value for the generation of breathable oxygen from water, especially for extended autonomous human-based undersea operations. For this application, the effects of seawater on the long-term stability of the catalyst become an important consideration as well as the selective generation of oxygen. Seawater as a potential oxygen source is challenging because the halide salts present in seawater introduce potentially undesirable products, most notably chlorine and bromine. Further complicating the situation is the fact that the thermodynamic standard potentials for these reactions are within a few hundred millivolts of that for OER. Thus, successful water oxidation catalysts operating in seawater must be capable of demonstrating exceptionally high kinetic selectivity for oxygen generation over other the possible oxidative side-reactions.

Objectives

The most basic issue of obtaining oxygen from seawater in appreciable amounts has not yet been solved. Oxygen may be produced from seawater, in principle, by water electrolysis but for underwater diving applications many basic science challenges must be addressed:

Objective 1: A key design element for oxygen evolution by electrolysis of seawater is to maximize water oxidation versus halogen oxidation. Differential electrochemical mass spectrometry instrumentation was constructed as a robust assay to assess the overpotentials for water versus halide oxidation. The assay allows a quantitative readout for determining faradaic efficiency and thus selectivity of OER catalysis from seawater versus the oxidation of other species such as organics and salts.

Objective 2: Oxidic metal catalysts of the metals of cobalt and nickel for oxygen evolution by water electrolysis were assessed. Prior work had shown that these catalysts are stable in seawater owing to their ability to self-heal themselves during catalytic operation. A comprehensive model for self-healing in seawater, necessary for long term catalytic stability is under development.

Objective 3. With instrumentation and new catalysts in hand, the ability to generate oxygen from seawater without producing harmful side products was evaluated.

Methods

Catalysts of cobalt and nickel oxides in phosphate and borate electrolytes were prepared by electrodeposition from phosphate and borate electrolytes. Differential electrochemical mass spectrometry (DEMS) was used to quantify the production of oxygen and halogen from seawater. The oxygen and side-product generation was driven electrochemically with potential vs time sweeps. The DEMS technique is particularly valuable for measuring halogen gas production as these masses are easily detected by mass spectrometry. Hypochlorite was quantified using the spectroscopic method offered by a N,N-diethyl-p-phenylenediamine assay. Seawater was collected from the Boston Harbor from the Fort Point Channel location in the harbor.

Results

Chloride is present in seawater at high concentrations (~0.5 M), and thus constitutes the greatest potential source of toxic byproducts. Using DEMS, we investigated the products of water oxidation from seawater from the Boston Harbor. Seawater solutions were flowed through the DEMS electrochemical cell and cyclic voltammetry was employed for current-time measurements because it enabled for the facile examination of potential byproducts over a wide potential range.

Figure 1 shows the results of DEMS experiments for a cobalt phosphate (CoP_i) catalyst operating in Boston Harbor seawater. No chlorine or bromine was observed over the entire potential range examined. The excellent selectivity against halogen production with these catalysts is likely a result of their ability to operate at potentials in neutral pH environments, that are positive of the production of halogen gas. This hypothesis is currently under investigation.

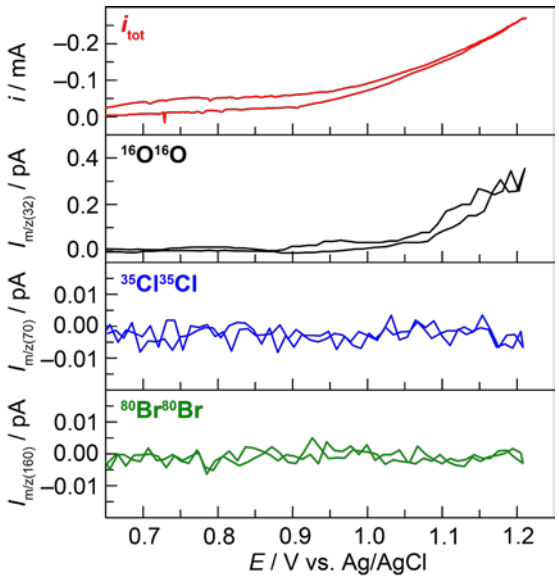

Fig. 1. DEMS experimental data for a CoB_i film operated in water collected from Boston Harbor. Cyclic voltammetry was performed at a scan rate of 5 mV/s. From top: Current response, m/z = 32, m/z = 70, and m/z = 160.

While chlorine was not detected in the reaction headspace, halogen may react with water in neutral to form hypochlorous (HClO) acid. Thus, we sought to determine whether we were forming HClO and ClO^- during electrolysis. We used a spectroscopic detection method to quantify HClO/ClO^- and found faradaic efficiencies for HClO/ClO^- production to be 1.4 ± 0.5 % for CoP_i. This amount of hypochlorous is low but nonetheless is intolerable for diving applications. By switching to a nickel borate (NiB_i) catalyst, no halogen gas was produced by DEMS and the amount of hypochlorous was reduced to 0.4 ± 0.1 %, demonstrating that an optimal catalyst may be realized with rational design.

In conclusion, we have used DEMS to demonstrate that two oxidic, self-healing OER catalysts of cobalt and nickel phosphate/borate are capable of selectively generating oxygen from seawater while simultaneously suppressing the oxidation of chloride and bromide to their respective halogen gases. We have shown that hypohalous acids are produced in miniscule amounts and may be eliminated through continued catalysis design. Work in the coming project period will begin defining the guidelines for such catalyst development. We anticipate that these results will be beneficial to applications that target the generation of oxygen using seawater as an oxygen source.

Notes:

GAS TRANSPORT THROUGH BIOLOGICAL MEMBRANES

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Background

Gases cross biological membranes via two major routes: the lipid bilayer and membrane proteins (gas channels). Other impermeant "blocking" proteins displace membrane lipids and partially shield gas molecules from access to the lipid bilayer. Thus, besides properties of the gas, membrane permeability depends on [1] membrane-lipid composition, [2] identity/abundance of blocking proteins, and [3] identity/abundance/activity of gas channels. All 3 parameters depend on cell type, and probably on physiological/pathological status and genetics/epigenetics.

Naval Relevance

Gas channels represent a paradigm shift, and understanding them could be transformative by: [1] Identifying biomarkers (e.g., polymorphisms, epigenetic changes) for enhancing warfighter performance (e.g., ↑ O_2 & CO_2 transport, especially at altitude) or decreased susceptibility to "dys-gasias"—gas pathophysiology (e.g., DCI, O_2 toxicity). [2] Developing drugs to block channels selectively or increase expression to mitigate dysgasias. [3] Creating designer channels that, embedded in gas-tight membranes, would form ultra-selective gas filters (e.g., for CO_2 scrubbing).

Objectives

(1) Understand how gases move through membrane lipids, as modulated by blocking proteins.

(2) Understand how gases move through membrane proteins: (a) Aquaporins (AQPs), (b) Rhesus (Rh) proteins, and (c) new candidates.

Long-term goal: Prepare the field for the 3 major areas of Naval relevance. For example, blocking the central pore of AQP5 in alveolar pneumocytes would reduce O_2 uptake and mitigate pulmonary O_2 toxicity; blocking the monomeric (H_2O) pores would mitigate pulmonary edema at altitude.

Methods

Our highly integrated group of PIs attack problems iteratively using several major techniques: (1) Protein expression and purification; (2) X-ray membrane-protein crystallography; (3) electron crystallography; (4) molecular dynamic (MD) simulations; (5) fabrication of synthetic membranes and reconstitution of membrane proteins in them; (6) microfluidic approaches to measure membrane permeability to gases in synthetic giant unilamellar vesicles (GUVs); (7) electrophysiology and (8) a novel bubble technique on gas-channel expressing *Xenopus* oocytes; (9) stopped-flow (SF) analysis of red blood cells; (10) whole-mouse physiology, including analysis of arterial blood gases, blood chemistries, and hematology of gene knock-outs and knock-ins; and (11) voluntary and forced exercise assays.

Results

The following are highlights of progress this year.

(1) Manuscripts: We have submitted several, including those on:

(1a) First AQP7 structure: We contrast AQP7—using X-ray crystallography, MD, and physiology—with its bacterial homolog GlpF. Major insights include the first gating of a monomeric pore by a residue undergoing conformational changes deep in the pore, blockade of the pore to water (and probably gas) transport by glycerol, and far greater structural flexibility of AQP7, which contributes to its far greater permeability.

(1b) First O_2 channels: Using SF spectroscopy of hemoglobin—backed up by proteomics, hematology, live-cell microscopy, flow cytometry, and mathematical modeling—we show that AQP1 and RhAG are O_2 channels, accounting for ~60% of total RBC permeability. Other channel(s) account for at least an additional ~30%. Plan: Use mouse knockouts to find the other channel(s).

(1c) Engineering gas-conducting and non-conducting channels by site-directed mutagenesis. As reported in a recently submitted manuscript, our structural, MD and sequence analyses of SoPIP2;1 and bovine AQP1 (bAQP1)—chosen for its high-resolution crystal structure—predict that two SoPIP2:1 residues (W79 and F207) hinder gas permeation through

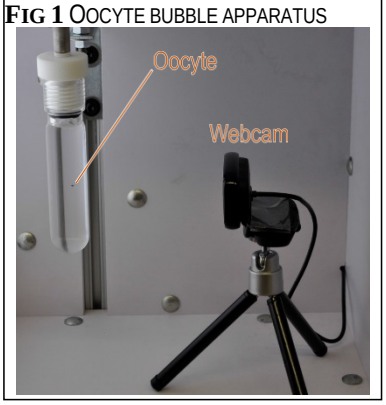
the central pore of SoPIP2;1. In bAQP1 and other gas-conducting AQPs, both aromatic residues are replaced by aliphatic residues. We find F207 to stabilize the pore-occluding conformation of W79. Computationally mutating F207 to Ala (its equivalent in bAQP1) allows O_2 to partition favorable along the pore. Conversely, computationally mutating the equivalent bAQP1 residues L56 and A179 to Trp and Phe, respectively, results in obstruction of the bAQP1 central pore. These mutants are primitive designer channels. Plan: [1] Assay designed mutant channels in oocytes, using electrophysiology and bubble approach (see point #3) for N_2 and O_2. [2] Express and purify mutant proteins for incorporation into GUVs (see point #5).

(2) First structure of a channel with gas in central pore (see Quad Chart Fig. A): The Boron Lab showed that the Rh channels bacterial AmtB and human RhAG, RhBG, and RhCG are permeable for both CO_2 and NH_3. Moreover, RhAG conducts O_2 (point 1b, above), and RhAG and AmtB conduct N_2 (point 4, below) The NH_3 moves through the 3 monomeric pores. To address the issue of how Rh proteins conduct non-polar gases, we set out to trap noble gases (Xe, Kr) in the channels of a high-resolution AmtB crystal.

We conducted standard diffraction experiments using our in-house Xenon high-pressure derivatization chamber to probe putative gas channels. Prerequisites for such experiments are highly diffracting and sturdy native crystals. At our last data-collection cycle at the Argonne National Lab APS synchrotron facility, we collected several complete anomalous data sets (at 1.45 Å wavelength) of Xe-derivatized AmtB crystals diffracting to better than 1.8 Å resolution. Two of the data sets exhibited significant anomalous signals, resulting in the localization of 12 Xe atoms with varying occupancies. The 4 Xe sites of highest occupancy are in hydrophobic pockets lining the central pore of the trimer. Plan: [1] Complement AmtB structural data with MD and physiology. [2] Attempt to obtain analogous data from Xe-derivatized crystals of AQPs. [3] Attempt to obtain structures of AQPs as blocked by inhibitors.

(3) Gas-bubble assay for N_2. A major bottleneck has been the lack of a transport assay for this gas of immense Naval importance. We now have that assay: we inject a *Xenopus laevis* oocyte (~1.3 mm diam) with N_2 gas, which now causes the cell to float. A computer-controlled pressure regulator then applies enough pressure (P_{Clamp}) to the air phase to compress the bubble sufficiently that the cell sinks to 5 cm below the air-water interface. As N_2 diffuses from bubble to cytosol, and across the plasma membrane to the outside, the bubble collapses and the cell sinks. However, guided by an image from a webcam (FIG 1), the computer lowers P_{Clamp} enough to re-expand the bubble and maintain neutral buoyancy. This assay could revolutionize the study of gas channels. Plan: [1] Complete ongoing computer modeling. [2] Extend assay to other gases (O_2, CO_2, Xe).

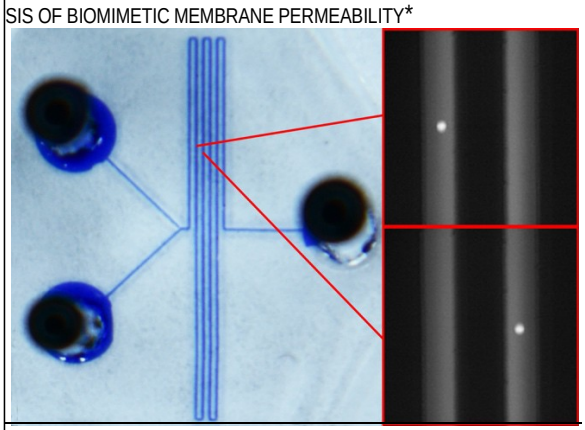
FIG 1 OOCYTE BUBBLE APPARATUS



(4) First N_2 channels (see Quad Chart Fig. B): Using the bubble assay, we can compare the time course of P_{Clamp} for control oocytes or those expressing candidate N_2 channels. We have found that Nod26 (AQP from root nodules of N_2-fixing plants), RhAG, and AmtB all conduct N_2. Plan: [1] Study central-pore mutants. [2] Examine a wide range of candidate N_2 or O_2 channels.

(5) High-throughput microfluidics for measuring CO_2 transport across biomimetic membranes. We previously showed that we can construct GUVs from both lipids and gas-impermeant block copolymers, incorporate the *Spinacia oleracea* (spinach) aquaporin SoPIP2;1 into the membranes of these GUVs, and observe CO_2 transport into the GUVs. We have now implemented a microfluidic system for high-throughput measurements of gas transport into GUVs containing a pH-responsive fluorophore that dims as $[CO_2]$ increases. At the device inlet (FIG 2), we mix a GUV-containing stream with a CO_2-rich

FIG 2 MICROFLUIDIC DEVICE FOR HIGH-THROUGHPUT ANALYSIS OF BIOMIMETIC MEMBRANE PERMEABILITY*

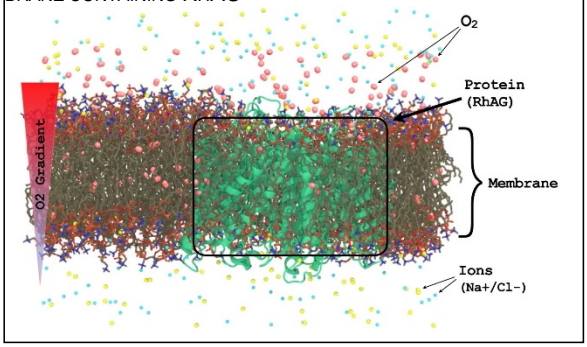


*The entire device, visualized with blue dye in the channel, is on left. Representative micrographs of GUVs flowing through the device are shown in insets with pointers indicating the approximate GUV positions. Channels are 80 μm wide.

stream. As GUVs flow along a serpentine channel, CO_2 permeates the membrane under videomicroscopic observation. By capturing GUV images at various positions along the device, we can compute the time course of CO_2 permeation. This system permits rapid collection of statistically robust permeability data for GUVs incorporating various lipid/block-copolymer compositions, gas channels, and blocking channels. Plan: Systematically examine CO_2 permeability as we: [1] Vary lipid/block-copolymer composition. [2] Introduce blocking proteins [3] Introduce purified channel proteins, wild type (WT) vs. mutants.

(6) Innovation: creation of MD gas gradients. Previous MD predictions of membrane permeability relied on extrapolations of free-energy calculations. We have now developed a novel method to generate transmembrane chemical gradients in MD simulations, mimicking closely physiological experimental conditions (e.g., osmotic gradients used in measurements of osmotic water permeability, P_f). This approach allows us to determine membrane permeability via mass-flux calculations. We used grid-steered MD (G-SMD) to overcome challenges posed by the commonly used periodic nature of MD simulations in the creation of chemical gradients. An imposed force field, confined to a region near the simulation periodic boundary, unidirectionally drives target molecules (e.g., O_2) into the next periodic cell. This approach chemically deprives one side of the membrane of the substrate (e.g., O_2), while enriching the opposite side. As a result, we do not bias molecules throughout the majority of the simulation volume as we create a self-replenishing chemical gradient.

FIG 3 MD SIMULATION SHOWING O_2 GRADIENT ACROSS MEMBRANE CONTAINING RHAG*



This method has allowed us for the first time to atomistically analyze the diffusion of molecules across membranes and determine their permeability under chemical-gradient conditions. We have applied this novel technique to determine the gas permeability of membranes containing by various proteins, such as RhAG in **FIG 3**. Plan: Systematically examine gas (O_2, CO_2, N_2) permeability for conditions that parallel those in GUV experiments: [1] Vary lipid/block-copolymer composition. [2] Introduce blocking proteins [3] Introduce purified channel protein (WT vs. mutants).

(7) Mouse performance. Preliminary data show that AQP1-knockout mice exhibit significantly less spontaneous voluntary running on activity wheels than their WT counterparts, at 21% and 11% ambient O_2. Plan: [1] Complete activity-wheel study on WT, AQP1-KO, RhAG-KO and double KO mice. [2] Complete installation of treadmill with $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ capability. [3] Determine peak $\dot{V}_{O_2}$ for 4 mouse genotypes. [4] Extend studies to KO of AQP5 (alveolar pneumocytes).

Notes:

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: NAVSEA
Project Status: COMPLETED

ASSESSMENT OF DOXYCYCLINE AS AN ADJUNCTIVE THERAPY TO PREVENT DECOMPRESSION SICKNESS IN SWINE

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Background

Decompression sickness (DCS) is an omnipresent risk in both operational diving and disabled submarine rescue (DISSUB) scenarios. While recompression therapy is the gold standard for treating DCS, adequate chamber capacity may not be available during operations with large numbers of casualties or in remote locations. Further, DISSUB rescuees may need to transfer to recompression chambers at surface pressures (surface interval), should transfer under pressure capabilities be lost or disabled. Development of an adjunct therapy which is compatible with recompression therapy would reduce DCS associated morbidity among divers and DISSUB rescuees awaiting definitive treatment. A pilot study at NMRC suggested that IV prophylaxis of doxycycline (30mg/kg) increased survival and reduced spinal cord injury in a swine model of DCS. The current study aimed to corroborate and expand on these findings with regards to dosing and operational suitability.

Objectives

1) Compare the efficacy of low dose (3mg/kg) doxycycline to that of high dose (30mg/kg) and saline control for reducing mortality following severe DCS in 20kg swine.

2) Evaluate the effect of high dose doxycycline on surface interval survival following maximum pressurized rescue module (PRM) decompression from air saturation at 70fsw in 70kg swine.

Methods

Experiment 1: Swine (20kg, n=69) received treadmill familiarization and had an ear vein catheter placed. The following day the swine were randomized to one of three treatment arms (n=23/arm): saline, low dose doxycycline (3mg/kg) or high dose doxycycline (30mg/kg). The swine received the appropriate treatment via infusion through the ear vein catheter. The swine were then subjected to a 240fsw bounce dive with a 31-minute total bottom time. Upon surfacing, swine were transferred to a Panepinto sling for a 1-hour observation period. Swine which survived the observation period were transferred to their runs for 24 hours with periodic observation. Swine which survived the 24-hour observation interval had neurological evaluation using a modified Tarlov scoring system. The swine were then humanely euthanized and transported to necropsy for perfusion/fixation and tissue collection.

Experiment 2: Swine (70kg, n=20) will be randomized into 2 groups: saline or high dose doxycycline (30mg/kg). Animals will then be sedated with ketamine/xylazine (20mg/kg and 2 mg/kg, IM respectively), anesthetized with isoflurane, and have a central jugular catheter placed. The swine will then be shaved, have electrodes attached, and be fitted with a custom jacket. The animals will be allowed to recover overnight. The next morning, two swine will be transported to their respective boxes within the multiple large animal hyperbaric chamber (MLAC). Once placed in the chamber, each animal will have the electrodes attached to the recording devices and the central catheter connected to the through-hull pump. The swine will then be compressed to a depth of 70fsw on air at a maximum rate of 60fsw/min and remain on bottom for a total bottom time of 22 hours. After 21 hours elapsed bottom time, the swine will receive their infusion of either doxycycline or saline via the jugular catheter. At the conclusion of the 22-hour bottom interval, animals will return to surface pressure using maximum decompression rate of the PRM. On surface the swine will be monitored for a 2-hour observation period within the MLAC. Following the 2-hour observation period the swine will returned to their runs for 24 hours of periodic monitoring.

Results

Phase 1: The results shown in Figure 1 indicate no survival or latency benefit from the prophylactic, 3mg/kg dose. The 30mg/kg dose (40% survival), however, demonstrated a 60% increase in survival rate when compared to saline

controls (25% survival). The median survival time for the 30mg/kg group of swine was 24.5min, a 40% increase when compared to saline controls. The high dose doxycycline group also had longer mean latencies for all types of DCS and mortality (Table 1). The study, however, was underpowered using the pilot study results, and the findings described above are not statistically significant.

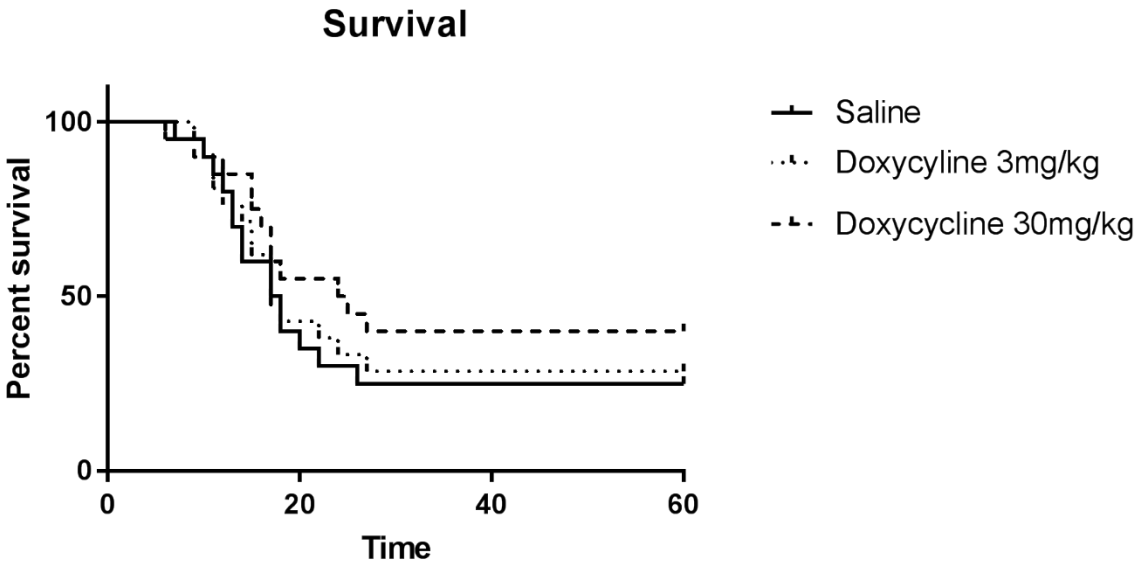

Figure 1. Swine survival rates following a 240fsw bounce dive (31min bottom time).

Latencies (min)	Saline	SEM	P Value	Doxycycline (3mg/kg)	SEM	P Value	Doxycycline (30mg/kg)	SEM	P Value
Cutis Marmorata	3.90	0.396	n/a	3.70	0.341	0.9609	4.84	0.947	0.4520
Cardiopulmonary DCS	5.78	0.688	n/a	5.88	0.561	0.9356	6.22	1.034	0.9169
Neurological DCS	8.33	1.247	n/a	11.18	1.180	0.2080	9.40	1.376	0.5573
Death	15.47	1.276	n/a	15.93	1.354	0.8159	16.75	1.810	0.7946

Table 1. DCS symptom latencies in swine treated with Saline and Doxycycline

Phase 2: The experimental execution is ongoing.

Notes:

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: NAVSEA
Project Status: ONGOING

ALTITUDE DIVING

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Background

At altitude, for a given depth-time exposure, bubble formation is more likely because of the reduced ambient pressure. Several approaches have been used to amend sea level decompression procedures for dives at altitude, largely based on the predicted value vs. ambient pressure of a constant (M) defined as maximum allowable tissue tension before any symptom of DCS occurs:

- Constant ratio translation (Cross corrections)
- Constant ratio extrapolation
- Linear extrapolation of M-values
- Model using a continuous distribution of tissue half-times
- Phase volume constraint in a correlated bubble model

While several decompression procedures have been produced, there has been very little systematic testing, and the relative safety of tables generated by most of these methods is uncertain. The Navy has promoted the use of Cross corrections, in which a virtual dive depth is calculated by multiplying the actual depth by the ratio of sea level pressure to the ambient pressure at the dive site, then implementing USN air decompression tables using the virtual depth. For example, virtual depth for a 60 fsw dive at 12,000 ft (barometric pressure=483 mmHg, 0.636 ATA) would be 60*760/483=94 fsw, for which the decompression profile should be for a dive to 100 fsw. Virtual depth of decompression stops is calculated by multiplying the stop depth by the reciprocal of the same ratio. While the Cross correction method is included in the US Navy Diving Manual, it has never been formally tested.

Other physiological changes at altitude that could affect performance or decompression safety include altitude-specific illnesses, such as acute mountain sickness (AMS). AMS consists of fatigue, disturbed sleep, headache, loss of appetite, nausea and vomiting. Incidence and severity of these conditions depend on altitude, rapidity of ascent, residence at low vs. high altitude before ascent and individual susceptibility. AMS is predicted to occur in 50% of highly active males transported rapidly to 3500 m (11,483 ft) and 70% at 4000 m (13,123 ft). AMS usually peaks 18-24 hours after arrival at altitude and dissipates spontaneously within 40-48 hours. High altitude pulmonary edema (HAPE) and high altitude cerebral edema (HACE) are less common, typically occurring in 1-5% of individuals transported rapidly to high altitude. These conditions and the hypoxia associated with altitude can also impair psychomotor and exercise performance.

Physiological changes also include changes in intravascular volume and fluid distribution among tissue compartments. Altitude exposure can result in either fluid retention or diuresis at different times. Microcirculatory blood flow derangement has been demonstrated during acute exposure to high altitude. These physiological changes could also affect susceptibility to DCS but cannot be accounted for by any current decompression models.

Loss of acclimatization and thus return of susceptibility to altitude illness usually occurs a few days after return to low altitude. It has been speculated that exposure to hyperbaric hyperoxia (i.e. during a dive at altitude) might induce rapid de-acclimatization and thus return of AMS symptoms. This phenomenon was not reported previously, however the hyperoxic exposures during previous studies were brief and may not have delivered a sufficient dose of oxygen to initiate reversal of acclimatization.

Objectives

- Test Cross corrections for no-stop air dives to 60 fsw at altitudes of 8,000 and 10,000 ft.
- Test Cross corrections for no-stop enriched O_2 (35% O_2) dives to 100 fsw at altitudes of 10,000 and 12,000 ft
- At 15,000 ft altitude after acclimatization, assess the degree to which AMS recurs after a 2 hour exposure to PO_2=1.3 ATM.

Methods

Specific dive profiles were selected in discussion with NAVSEA.

(A). Testing of Cross corrections breathing air at 8,000 (0.743 ATA) and 10,000 ft (0.688 ATA). Subjects are decompressed in a hypobaric chamber to 8000 ft (N=12) or 10,000 ft (N=16). At

8,000 ft AMS is unlikely, and the subjects remain at altitude for 12 hours before diving. At 10,000 ft, more severe symptoms are likely, thus the subjects remain there for 48 hours before commencing diving, after which AMS symptoms are likely to be minimal.

A 60 fsw no-stop dive has been tested at each altitude (2.82 ATA at sea level, 2.56 ATA at 8,000 ft, 2.51 ATA at 10,000 ft). Using Cross corrections, the virtual depth for both altitudes is 90 fsw, for which the no-stop time is 30 minutes. During the dive subjects are submersed and perform mild exercise in 28°C water. Ascent rate is 30 ft/min. Upon surfacing the divers are monitored for 12 hours for symptoms of DCS and transthoracic echocardiography (rest and leg/arm motion) at 5, 15, 30, 60 and 120 minutes after surfacing. After that, measurements are continued until no bubbles are detected. Bubbles in the right ventricle are graded according to a modified Eftedal-Brubakk scale. Subjects are returned to local atmospheric pressure 12 hours after surfacing from each dive. DCS will be treated according to standard protocols.

The more provocative dives (10,000 ft) were studied before the ones at 8,000 ft, thus allowing for any needed adjustments in bottom time to be made at the higher altitude. This will increase the confidence for the same bottom time at the lower altitude.

(B). Testing of a no-stop dive to 100 fsw at 10,000 ft and 12,000 ft breathing 35% O_2. For this series of experiments all subjects remain at altitude for 48 hours before diving. Appropriate depth-time profiles have been assessed by calculating the equivalent sea level air depth for each of these dives (PN_2 values 2.42 and 2.36 ATM, respectively), yielding equivalent air depths of 68 and 66 fsw, respectively. Cross corrected virtual depths are 99 and 104 fsw, yielding no-stop times of 25 and 20 minutes, respectively. As with the air dives described above, the more provocative dives (12,000 ft, N=28) will be completed before the ones at 10,000 ft (N=16), which will increase the confidence for the bottom time used at the lower altitude.

(C). Testing to determine whether a high PO_2 dive would reverse altitude acclimatization and re-establish susceptibility to AMS. Subjects (N=8) will ascend in the chamber to 15,000 ft in a graded fashion over 12 hours. Then, after 48 hours they will perform a hyperoxic dive by breathing 100% O_2 for 120 minutes at 1.3 ATA. Divers will then return to 15,000 ft and remain at that altitude for 24 hours to allow for AMS symptoms to recur.

For all studies Lake Louise AMS are collected every 12 hours and symptoms treated as needed. Subjects are assessed for high altitude pulmonary edema (HAPE) and high altitude cerebral edema (HACE). Occurrence of either HAPE or HACE requires that the subject be returned immediately to 1 ATA and treated appropriately. All subjects are weighed daily; 24-hour urine collections are obtained, from which samples are stored indefinitely for future analysis. Blood samples are collected every 24 hours for measurement of hemoglobin and hematocrit, and for long-term storage of plasma and DNA. This will provide a databank for future analysis, specifically pertinent for the study and prevention of altitude illness. Of the various altitude illnesses, AMS is the most common and the one most likely to interfere with Navy diving operations at altitude. This databank will be one of very few in the world and could be used to test hypotheses pertinent to AMS prevention.

Results

Institutional approval and informed consent was obtained from all volunteers. All 60 fsw air dives at 8,000 (N=12) and 10,000 ft (N=16) have been completed. A total of 4 dives (100 fsw/20 minutes) have thus far been completed at 12,000 ft (35% O_2-bal N_2).

Altitude (ft)	N	M/F	Age (Y)	BMI ($kg.m^{-2}$)	VO_2 Peak ($mL.kg^{-1}.min^{-1}$)
8,000	12	8/4	28.1 (21-28)	25.0 (22-33)	38.5 (31-45)
10,000	16	8/8	29.0 (23-38)	24.0 (17-33)	42.0 (31-57)
12,000	4	1/3	26.5 (25-29)	22.0 (20-24)	40.9 (38-44)

Altitude (ft)	Depth (fsw)	BT (min)	DCS	VGE
8,000	60	30	0/12	0/12
10,000	60	30	0/16	0/16
12,000	100	20	0/4	1/4 (up to E-B grade 3 30-120 min post dive)

*Bottom time

Due to practical considerations related to altitude-depth switchover, limitations on vacuum pumps and slowed descent due to tender-related ear equilibration compression/decompression times have been slower than standard. 'Bottom time' has therefore been defined as actual time at maximum depth. Profiles tested under this modified definition will provide for more conservative dives when applied to dives using the traditional definition of bottom time.

Notes:

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: NAVSEA
Status: ONGOING

MODERNIZATION OF NAVY THALMANN ALGORITHM DIVE PLANNER

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Background

The Thalmann Algorithm Navy Dive Planner (NDP) became necessary in order to support planning Navy Special Warfare (NSW) dives using the Navy Dive Computer (NDC). Development of the NDP commenced in 1999 yielding the first accredited version, 3.03, of the NDP being released in 2007 with a subset of its current feature set designated the NSW configuration. The functionality of the NDP was expanded with issue of version 4.03 in 2013 to encompass most of U.S. Navy Diving operations including special warfare, explosive ordnance disposal, saturation, salvage, and ships husbandry. Since the creation of the NDP five versions of the NDC have been acquired to increase U.S. Navy Diving capabilities. In 2018, the U.S. Navy Disabled Submarine Rescue Decompression Planner (DISSUB RDP) was developed based upon version 4.03 of the NDP. The DISSUB RDP is essential for planning safe and efficient decompressions of large numbers of rescuees. Modernizing the NDP is a critical step to maintaining current U.S. Navy diving capabilities.

The current NDP and DISSUB RDP were both developed using Microsoft Visual Basic 6.0 (VB6) within the Windows XP operating system. Mainstream support for VB6 was ended by Microsoft in 2005 and extended support ended in 2008. Without ongoing support for VB6, both the NDP and DISSUB RDP must be classified as legacy software to remain compliant with NAVSEA cybersecurity guidelines. Maintenance and enhancement of the NDP has become difficult as compilers for VB6 are not supported on versions of the Windows operating system issued after Windows XP. Migration of the NDP and DISSUB RDP code bases to modern software architecture and a programming language will not only improve the overall performance of these planning tools, but is essential to ensure that these tools can be supported in the future, particularly as the demand for their use becomes more widespread.

Objectives

Proposed work will complete with issue of version 5.00 of the NDP written in the Microsoft C# .Net programming language. C# .Net is a general purpose programming language with no currently foreseeable obsolescence. During the migration from VB6 to C# .Net the NDP and DISSUB RDP will be combined into a single code base. This merger will enable the enhanced Dive History Edit functionality in the DISSUB RDP to appear in the NDP as a standard feature. Combining the NDP and DISSUB RDP will also result in significant cost savings (approximately $80K) by reducing the requirements for independent verification in the Verification, Validation, and Accreditation (VV&A) process to encompass third party review of only a single software package. This project will ensure the NDP remains available and approved for use.

Methods

All software development is being conducted in the C# .Net language. Minor updates to the existing functionality will be considered as time allows, but will be carefully considered for their impact on schedule prior to being undertaken. The verification and validation documentation will be generated following SECNAV instruction 5200.40 and to be consistent with existing Navy Experimental Diving Unit (NEDU) software documentation. Independent review of the verification and validation documentation will be conducted by Sandia National laboratory.

Results

Thus far two user communities have provided feedback on their current experience using the NDP and/or RDP. Design of the data structures has been completed. A new contract software developer has been hired to assist with the project and has begun software development. The project is currently on schedule.

Notes:

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: NAVSEA
Project Status: COMPLETED

NAVY DIVE COMPUTER AND NAVY DIVE PLANNER ALGORITHM FOR CONSTANT 1.3 ATM PO2-IN-HELIUM DIVING TO 300 FSW

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Background

The diver-carried Navy Dive Computer (NDC) provides increase in decompression efficiency compared to using decompression tables, particularly for multi-level and repetitive diving. This is because the NDC calculates decompression guidance from the actual depth-time history and does not require costly depth and time round-up to select a decompression schedule from a table. The U.S. Navy does not have an NDC for diving deeper than 200 fsw and therefore cannot support NDC diving to the full 300 fsw range of the U.S. Navy 1.3 atm PO_2 He-O_2 Decompression Tables.

The 1.3 atm PO_2 He-O_2 Decompression Tables are based on the LEM-he8n25 probabilistic decompression model and all the schedules have a near-uniform probability of decompression sickness (P_{DCS}) near 2.3%. LEM-he8n25 was derived by numerical optimization to a large data base of dive profiles and decompression sickness (DCS). The schedules for depths deeper than 200 fsw in the 1.3 atm PO_2 He-O_2 Decompression Tables are calculated with an algorithm that iteratively exercises the LEM-he8n25 model to find the shortest schedule that does not exceed a target P_{DCS} of 2.3%. This algorithm is compute-intensive and is not implemented in any existing diver-worn dive computer. The schedules for depths of 200 fsw or shallower were computed with the less demanding 'deterministic' Thalmann Algorithm parameterized with XVal-He-4, and it is a variant (XVal-He-4B) which is implemented in the NSW He III 200-1.3 NDC. For dives deeper than 200 fsw, the XVal-He-4B-Thalmann Algorithm produces schedules with unacceptably high estimated P_{DCS} (see Figure 1).

Objectives

The principal objective was to develop a parameter set for the Thalmann Algorithm that closely reproduces the 1.3 atm PO_2 He-O_2 Decompression Tables across the entire 300 fsw operational depth range. This parameter set could be programmed into the current generation of NDC. Secondary objectives were to develop additional Thalmann Algorithm parameter sets for extended bottom times beyond those in the U.S. Navy Diving Manual and for decompression schedules with higher target P_{DCS} (4% and 5%), and consequently faster decompression. These parameter sets would be suitable for implementation in a future NDC and would allow selection of P_{DCS} in accord with mission requirements.

Methods

The Thalmann Algorithm controls gas supersaturation in a collection of theoretical 'tissue' compartments with different half times. Thalmann Algorithm parameter sets include a table giving the maximum permissible tissue tension (MPTT) at each decompression stop depth in each compartment. Traditionally, deterministic algorithms are developed through an iterative process of adjusting MPTTs and man-testing resulting schedules. The MPTTs in XVal-He-4 were derived by least-squares fit to a large and diverse 'standard' set of decompression schedules produced using the man-tested LEM–he8n25 model with a target 2.3% P_{DCS}. The resulting MPTTs were those that caused the Thalmann Algorithm to produces schedules as close as possible to LEM-he8n25 2.3% P_{DCS} schedules.

This fitting approach was modified to produce new parameter sets. Standard sets were produced at higher resolution of stop times than for the original method, which allow greater refinement of fitted parameter sets. An exact rather than approximate objective function was minimized in the least-squares fitting. In addition to fitting MPTTs, other algorithm parameters were varied including compartment half-times and saturation-desaturation ratios that allow slower gas washout than uptake. Choosing these latter parameters was guided by inspection of characteristic decompression stop times in exploratory decompression tables computed with LEM-he8n25 with the same target P_{DCS} values used to compute the standard sets.

Results

Three new parameter sets were developed. XVal-He-9_023 Thalmann Algorithm emulates all schedule in the 1.3 atm PO_2 He-O_2 Decompression Tables (see Figure 1) as well as LEM-he8n25 2.3% P_{DCS} schedules for much longer bottom times than in the tables. With XVal-He-9_040 and XVal-He-9_050, the Thalmann Algorithm can compute decompression schedules for undersea operations to depths up to 300 fsw and bottom times up to 4 h with P_{DCS} near 4 % or 5 %, respectively.

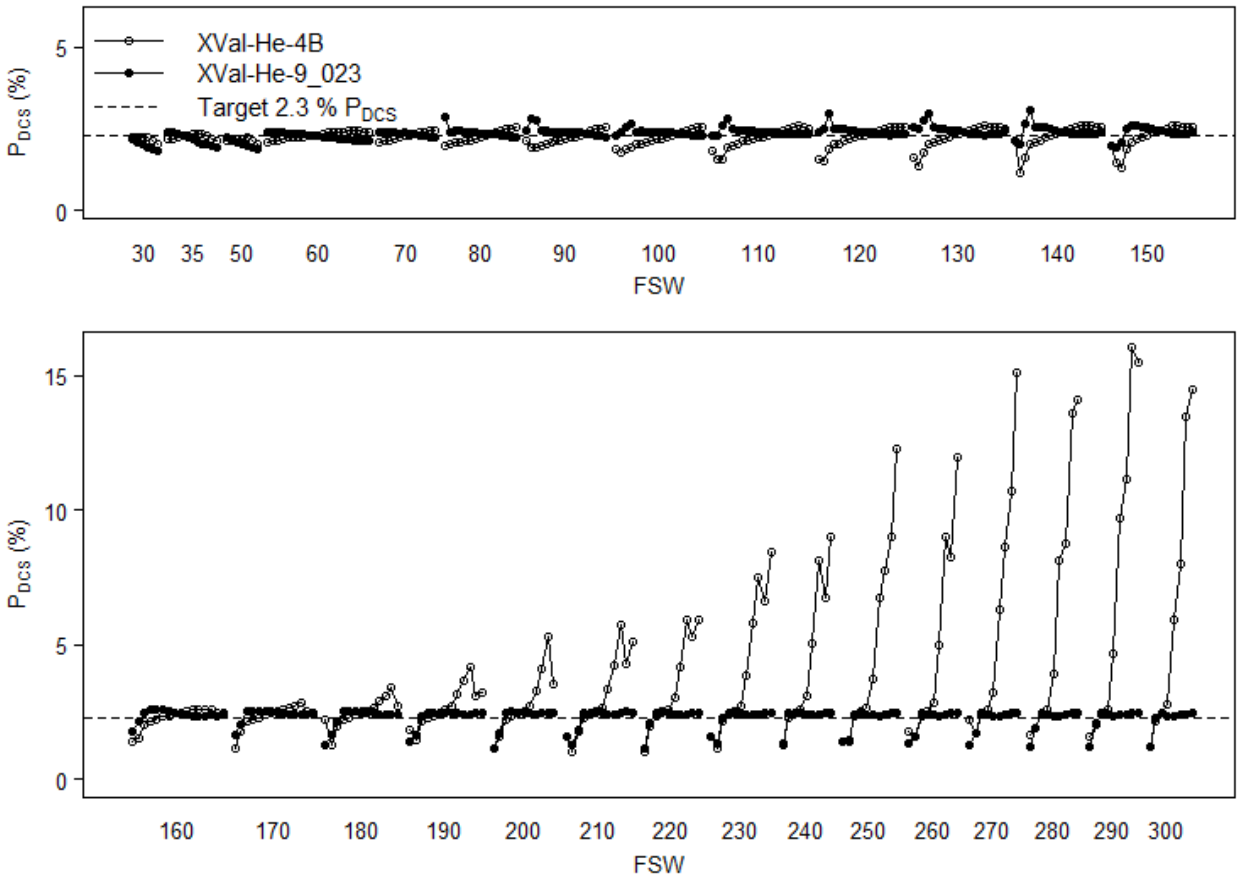

Fig.1. LEM-he8n25 estimated PDCS XVal-He-4B- and XVal-He-9_023-Thalmann Algorithm schedules. Each point is the LEM-he8n25-estimated P_{DCS} of schedule for a different depth / bottom time combination. Depth groups are indicated along the bottom axis, and within each depth group, schedules are for bottom times from 15 to 90 minutes.

Notes:

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: NAVSEA
Project Status: ONGOING

MANNED VALIDATION OF U.S. NAVY DIVING MANUAL, REVISION 7 (VVAL-79 THALMANN ALGORITHM) SCHEDULES FOR SHORT BOTTOM TIME, DEEP AIR DECOMPRESSION DIVES

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Background
The U.S. Navy Diving Manual Air Decompression Table was promulgated in 2008, and a revised version, calculated with the VVal-79 Thalmann Algorithm, was promulgated in 2016. In a departure from previous air decompression procedures, these tables replaced the 10 fsw last decompression stop with a 20 fsw last stop. The Swedish Armed Forces Diving and Naval Medicine Center (DNC) conducted a laboratory dive trial using the 2008 Air Decompression Table. 32 dives to 132 fsw (40 msw) for a 20-minute bottom time resulted in two cases of decompression sickness (DCS), and a median venous gas emboli (VGE) measurement at rest of KM grade III.

Objectives
The primary aim was to provide a relatively precise estimate of the probability of DCS of the VVal-79 Thalmann Algorithm schedule for 132 fsw for a 20-minute bottom time under dive conditions (exercise and water temperature) typical of previous U.S. Navy trials. The potential secondary aim was to compare the probability of DCS of the 132 fsw for 20-minute bottom time air decompression schedule with a 20 fsw last decompression stop to the same schedule but with a traditional 10 fsw last stop.

Methods
An air decompression schedule to 132 fsw for a 20-minute bottom time with a 9-minute stop at 20 fsw was computed with the VVal-79 Thalmann Algorithm. Dives were conducted in 85 °F (29 °C) water in the Ocean Simulation Facility at the Navy Experimental Diving Unit. Divers dressed in swimsuit and t-shirt and breathing from surface supplied air performed approximately 90 Watts cycle ergometer work while on the bottom and rested during decompression. Divers were monitored with 2-D echocardiography at 20-minute intervals for 2-hours postdive, measuring VGE at rest and with limb flexion graded with an expanded EB scale. Up to 96 man dives were planned in an adaptive sequential trial designed to stop if DCS incidence exceeded 5% at 5% significance. A stop occurring in 48 or fewer dives would result in a shift to testing a 132 fsw for 20-minute schedule computed with a 10 fsw last stop.

Results
48 man-dives have been completed on the 20 fsw last stop schedule and have resulted in no cases of DCS. The median (IQR) peak VGE grades were 3 (2–3) at rest and 3 (3–4a) with movement. VGE grades remained elevated at two hours postdive with median grade 2 (1–3) at rest and 3 (1–3) with movement.

Notes:

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: NAVSEA
Status: ONGOING

21ST CENTURY SURFACE-SUPPLIED HELIOX DECOMPRESSION TABLES

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Background
The current U.S. Navy Surface-Supplied Helium-Oxygen Decompression Table, which is an edited version of an original 1939 issue, has a limited record of success in operational dives to depths of 240 feet sea water (fsw) or shallower. Recent theoretical evaluations indicate that tabulated schedules for dives to deeper depths, depths that are increasingly within the scope of desired U.S. Navy diving operations, will incur unacceptably high risks of decompression sickness (DCS). There is consequently need for a new table of surface-supplied heliox decompression schedules recomputed from first principles with appropriately conservative ascent rules.

Our present LEM-he8n25 model for DCS incidence in heliox dives, validated for application to closed circuit constant inspired oxygen partial pressure (PO_2) dives, over-estimates DCS risk (P_{DCS}) for surface-supplied constant inspired oxygen fraction (FO_2) heliox dives. As a result, LEM-he8n25 prescribes overly conservative schedules for constant inspired FO_2 heliox dives. A new model is required to produce a new table of time-efficient surface-supplied heliox decompression schedules with acceptable P_{DCS} for deep dives.

Objectives
The present program will culminate with issue of a man-validated table of schedules for surface-supplied heliox diving based on a new model for DCS incidence in heliox dives.

Methods
The program consists of two two-year phases. The first phase, funded by the Joint Program Committee-5/Military Operational Medicine Research Program (JPC-5/MOMRP), comprises development of a new probabilistic model of DCS incidence and time of occurrence in heliox dives and generation of a new table of schedules for constant inspired FO_2 heliox diving. The second phase, to be funded by the NAVSEA Deep Submergence Biomedical Development (DSBD) Program, comprises man-validation of the new table.

Phase I (FY 2019, 2020) Milestones:
- Establish an expanded calibration data set.
- Develop a model optimization and evaluation framework.
- Implement a heliox Three-Region Unstirred Tissue – Multiple Bubble (3RUT-MB) model.
- Optimize candidate models about the expanded calibration data set.
- Down-select to the most suitable model.
- Generate a new table of surface-supplied heliox decompression schedules.
- Write protocol for man-trial validation of the new table.

Phase II (FY 2021, 2022; NAVSEA DSBD funded): Man-trials will be conducted in the Navy Experimental Diving Unit Ocean Simulation Facility to validate the new table.

Results
The program commenced with receipt of JPC-5 funding in January 2019. The DSBD Program Manager has signed a Knowledge Transfer Agreement to assume funding of the man-trial phase of this program upon completion of Phase I.
- New software development tools have been acquired and implemented:
  - C# .Net
  - Extreme Optimization
  - Oxyplot
- New model fitting architecture has been developed:
  - Models execute in parallel using symmetric multiprocessors
  - Visualizer has been built to facilitate debugging
  - Exact gain solution has been implemented for bubble models
  - A 3RUT-SB model has been implemented in both single and multi-gas versions and verified against FORTRAN mother code.
- A new HeN2_2016 data set, including seven newly-coded subsets with 1441 man-dives, has been compiled for model calibration.

The project is currently on schedule.

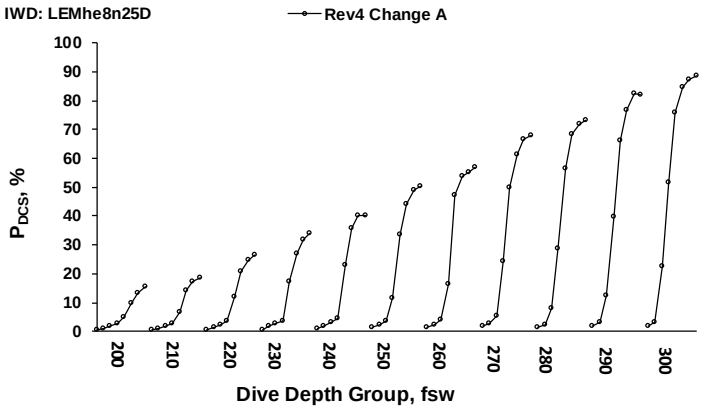

Figure 1. Model-estimated probabilities of DCS (P_{DCS}) of the normal exposure and exceptional exposure in-water decompression schedules in the current Surface Supplied Helium Oxygen Decompression Table. P_{DCS} may be overestimated by as much as a factor 4 for longer deeper dives. The model remains unsuitable for calculating a replacement surface-supplied heliox decompression table.

Notes:

GAS NARCOSIS IN HYPERBARIC ENVIRONMENTS

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Background

A Navy diver may experience narcosis induced by gases respired under pressure. The degree of narcosis is dependent on ambient pressure and the gas mixture breathed. Nitrogen is known to cause narcosis. Helium is non-narcotic, and the narcotic potential of other gases such as oxygen and carbon dioxide is poorly characterized.

The principal hazard of gas narcosis in diving is euphoria, overconfidence and loss of judgment. This may start a chain of events culminating in a serious diving accident. No early warning signal is available, and narcosis negatively influences self-assessment. Therefore, studying narcosis and how to detect it is of great importance for operational military divers undertaking hazardous missions such as mine counter measures.

The effects of gas narcosis have been studied using psychometric testing and evoked potential measurements. Neither are suitable for continuous non-distracting monitoring of gas narcosis. Attempts have been made to use the electroencephalogram (EEG) as a continuous measure. However, “traditional” spectral power analysis of EEGs is not robust in reliably distinguishing what appears to be a subtle incremental cognitive inhibition. The present studies will utilise quantitative EEG (qEEG) techniques including cortical functional connectivity, source localisation and dynamic causal modelling which may allow development of a qEEG algorithm for gas narcosis.

Objectives

There are two objectives. The first is to develop a qEEG algorithm to objectively measure the narcotic effects of inspired gases during diving. The second is to apply that algorithm in determining the relative narcotic effects of nitrogen, oxygen and carbon dioxide. This will facilitate informed decisions about acceptable gas mixes for different underwater tasks.

Ultimately, it is conceivable that a qEEG method could be used as a monitoring system that is wearable underwater in a diving helmet for real-time estimation of narcotic impairment.

Methods

The project has 4 sub-studies.

The first study explored the use of qEEG to measure subtle degrees of narcosis in a laboratory environment. Twelve subjects were exposed to 20%, 30% and 40% nitrous oxide (in oxygen) at 1 ATA. We measured EEG (ActiveTwo, Biosemi, The Netherlands) using a 32 active sintered Ag-AgCl electrode system, sampling at 2048 Hz with 24 bit resolution. An additional 2 electrodes were placed under the eyes for eye movement recording. The utility of pupillometry as a measure of gas narcosis effects was investigated. It was originally planned to develop the qEEG narcosis algorithm using data gathered in this phase, but we proceeded to the second study without doing so (see results).

In the second study 12 divers are exposed two times to pressures of 2.8 ATA and 6 ATA. In one exposure the subjects breathe air and in the other they breathe heliox: 21% oxygen in helium (a non-narcotic gas). This will allow separation of any EEG effects of the pressure exposure *per se* from the effects of nitrogen. Subjects complete a computer-based math test (Icon dive computer (Mares, Italy) with a math test based on the Psychology Experiment Building Language Battery) and critical flicker fusion frequency (CFFF) measurements at both pressures. The EEG measurements from this study will now form the basis for development of the qEEG algorithm.

With any isolated effects of pressure excluded (or at least measured) in study two, the third study will investigate any narcotic effect of oxygen by exposing 12 divers to 1.0, 1.4 and 2.8 ATA in the hyperbaric chamber, while breathing 100% oxygen. These exposures have been chosen to be operationally relevant in various settings. The EEG, math and CFFF performance will be measured at each pressure. The qEEG algorithm will be applied to look for signs of narcotic effect, and to compare the three different levels of inspired oxygen pressure. Any narcotic effects of oxygen will be benchmarked against the previously measured effects of nitrogen.

In the fourth study, 12 divers will be exposed to breathing mixtures containing carbon dioxide (CO_2) in fractions which clamp the end tidal CO_2 to normal (35-45mmHg), elevated (45-50mmHg), and (if tolerated) high levels (55-60mmHg). In the first visit subjects will breathe heliox at the surface with exposure to these three levels of end tidal CO_2. In the two subsequent visits the divers will undergo hyperbaric exposure to 6 ATA breathing air on one visit and heliox (21% oxygen) on the other. The end tidal CO_2 will be clamped at three levels as above during these exposures. EEG math performance and CFFF will be measured in all exposures. This protocol will allow evaluation of the narcotic effect of elevated CO_2 in isolation, and its synergy (if any) with nitrogen narcosis.

Results

The grant was activated 14 months ago. Logistics, compliance and subject recruitment for studies one and two have been completed. Study one is finished and study two is more than half completed (15/24 pressure exposures complete).

Study 1: All measurements (see methods) were successfully completed in 12 subjects. All subjects exhibited cognitive impairment, but preliminary evaluation of the EEG data demonstrated two principle sub-groups of subjects who behaved in different ways. Algorithm development is complicated in this setting, though it can be achieved using a multi-classifier algorithm approach. However, it seemed sensible to confirm that subjects behave the same way (or not) in the air dive exposures before proceeding down this pathway. Development of the qEEG algorithm has therefore been deferred until the end of study 2. Evaluation of pupillometry suggested it to be only marginally sensitive to the highest nitrous oxide concentration, and it is unlikely to be a suitable method for quantifying narcosis in diving.

Study 2: After logistic adaption of the necessary equipment to the hyperbaric chamber (hull penetrators, safety testing etc) study 2 has commenced using local technical diver subjects (Figure 1). Fifteen of 24 exposures have been completed and it is expected that all measurements are completed before June 2019.

Fig.1. Diver inside the hyperbaric chamber performing math test.

Other progress: Mr Vrijdag conducted a literature review on monitoring gas narcosis in divers and is conducting a systematic review on oxygen narcosis. He passed his provisional year evaluation for continuation in his PhD program in March 2019. An account of this project and the pupillometry results were presented at the Tricon Meeting (joint meeting of SPUMS, EUBS and SAUHMA) in South-Africa, September 2018.

Conclusion

After 14 months this project is well underway. The primary goal is development of a repeatable objective qEEG algorithm for evaluating narcosis that is not subject to practice effects or distractions. The program will also extend knowledge of gas narcosis physiology, in particular the narcotic potential of oxygen and carbon dioxide, and any interaction or synergism with nitrogen narcosis at elevated ambient pressures. This will increase the safety and working capability of Navy divers, by informing selection of gas mixtures suitable to the intended task and working depth.

Notes:

2019 ONR Undersea Medicine & NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: ONR/NAVSEA
Project Status: Ongoing

IMPACT OF REPEATED 6-HOUR HYPEROXIC EXERCISE DIVES AT 1.35 ATA ON MUSCULAR PERFORMANCE AND CARDIOVASCULAR ENDURANCE

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Background
Hyperoxic conditions can lead to oxygen toxicity and decrements in human performance following single and repeated long-duration 100% O_2 water immersion (WI)s. This project examines the potential links between hyperoxia and reduced cardiovascular and muscular performance.
This research addresses the needs of increasing diver safety, readiness, and mission accomplishment. This work will provide a scientific interpretation of the data to enable better dive planning for long-duration, repeated dive operations. This effort will enable the future development of countermeasures to enhance safety, human performance, and mission completion.

Objectives
The specific objectives of this project are to 1) characterize changes in physiological and neuropsychological performance after single and repeated hyperoxic exercise dives, 2) determine from our measured list of potential mediators those which are associated with the altered function, 3) examine the degree to which acute and repeated hyperoxic exercise dives alter biomarkers of oxidative stress, muscle breakdown, and microbiota, and 4) determine the extent of recovery by 72-hrs post-dive for decrements in physiological and neuropsychological function.

The broad, long-term objectives of this line of research under the Warfighter Human Performance Program are to 1) characterize performance and pulmonary, cognitive, endocrine, and overall pathophysiological function under varying dive conditions for provision of guidance to enable better dive planning, 2) determine through direct measurements and modeling the cause and the mediators of the decrements in performance, and 3) develop countermeasures to improve warfighter physiological function and performance.

Methods
Divers (n=16) will complete a dive week (5 consecutive 6-hr exercise dives with 18-hr surface intervals) and a single dive (6-hr exercise dive with an 18-hr surface interval) while breathing 100% O_2 at 1.35 ATA. Each dive day consists of pre- & post-dive testing, and 6-hr exercise dives consisting of 30-min biking and 30-min rest intervals. The target workload for the exercise interval is heart rate = 105±5 beats/min.

Endurance time on a treadmill at 85% of maximal aerobic capacity ($\dot{V}O_{2\,max}$) is measured a few days before diving (pre-WI baseline: BL), within two hours post-WI after the fifth dive (PD), and three days after the fifth dive (PD3). For the 85% $\dot{V}O_{2\,max}$ protocol, heart rate (HR) and blood lactate are measured during a walking warm-up (5 min), flat run (3 min), and every three minutes during a run at 10% grade until volitional exhaustion. Cardiac output (Q: SF_6 rebreathing, Innocor) is measured. Stroke volume (SV) is calculated as Q/HR, and the change in plasma volume (PV) from BL is calculated from hemoglobin and hematocrit (Dill-Costill equation) before exercise. Treadmill data for BL, PD, and PD3 are compared at the run time matching the last data point for the shortest of the three runs.

The pulmonary function protocol uses flow-volume loops measured at baseline, pre-WI and post-WI, and 24-hr and 72-hr after the single dive and the final dive for the DW.

Neuromuscular performance testing is completed at baseline, pre-WI and post-WI, and 24-hr and 72-hr after the single dive and the final dive for the dive week. Specific testing includes the following:

1. Evaluate neuromuscular strength and endurance contraction performance of the leg, forearm and biceps brachii using the following exercise testing protocols:
    a. Maximum voluntary isometric contraction (MVIC) knee extension and elbow flexion.
    b. Maximum handgrip (MHG) strength
    c. Maximum handgrip endurance and 50-repetition knee extension endurance fatigue test.
2. Measure neuromuscular activity with surface electromyography.

Venous blood samples are taken pre- & post-WI on WIs 1, 3, 5, 24-hr post-WI, & 72-hr post-WI. The venous samples are evaluated for serum levels of reactive oxygen species (ROS) and oxidative stress, and indicators of systematic inflammation.

Urine samples are taken BL, WI 1 & 5, and 24-hr post-WI to measure indications of intestinal permeability through changes in sucralose levels given during these time points. Fecal samples are collected at BL, WI 1 & 5, and 24-hr post-WI to measure to measure microbiota.

Results

Data collection is ongoing and is expected to be completed in October 2019. The Technical Report is due to NAVSEA in June 2020.

Notes:

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: NAVSEA
Project Status: COMPLETED

PULMONARY OXYGEN TOXICITY WITH OXYGEN PARTIAL PRESSURE 1 ATM: POSSIBLE MITIGATION

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Background

A Disabled Submarine (DISSUB) could lose its atmospheric control and become pressurized. An internal pressure of 5 atmospheres absolute (ata) of air would expose survivors to a toxic level of 1 atmosphere (atm) of O_2. Time to rescue of at least 72 hours could lead to serious symptoms/pathology and make accelerated decompression or recompression treatment difficult. There is thus an impetus to further understand the mechanisms of pulmonary oxygen toxicity and develop potential mitigations to aid in submarine rescue planning.

Pulmonary oxygen toxicity is a function of PO_2, not of oxygen fraction. Unit Pulmonary Toxicity Dose (UPTD) is a model that attempts to predict a decrement in forced vital capacity (FVC) from any combination of time and PO_2 exposure. Even though the mechanisms of pulmonary injury are known to be different at a different PO_2, it has been assumed that the mechanisms are the same at the same PO_2 with differing atmospheric pressure.

Periodic air breaks have been shown to significantly delay the onset of pulmonary oxygen toxicity in man at PO_2 of 2 atm. No studies examining air breaks have been conducted in man at PO_2 of 1 atm.

Objectives Clearly define the development of pulmonary oxygen toxicity at a PO2 of 1atm and test if normoxic intervals could delay or prevent development of pulmonary oxygen toxicity, using Pulmonary Function tests (PFTs) and symptomology as endpoints. Measure plasma levels of inflammatory cytokines before, during and after exposures, to compare between study arms as potential indicators of oxidative stress.

Methods

Subjects were healthy military members without known pulmonary disease. Medical non-rebreather face masks supplied flow of 100% oxygen through a 6-15 lpm high-flow bubble humidifier as needed to keep the non-rebreather mask reservoir bag 2/3 full during breathing (between 10 and 15 lpm flow, typically). Portable O2 cylinders used for hygiene and bathroom trips. PFTs conducted at baseline and every 8 hours. Symptoms assessed every 4 hours.

Fig.1. Physiology lab setup. Subjects watched movies while breathing high flow 100% O2 through a Hudson RCI non-rebreather facemask with reservoir.

Results

No significant reduction in forced vital capacity (FVC), forced expiratory volume (FEV1), forced expiratory flow (FEF25-75) or lung diffusion (DLCO) was observed. Twenty-six percent of subjects reported minor symptoms of airway irritation at 48 hours with reported full recovery within 24 hours of returning to ambient air breathing. Plasma levels of circulating inflammatory mediators varied widely among subjects, and only TNF-α was significantly ($P < 0.05$) reduced, falling by 15% within 24 hours of initiation of 100% oxygen breathing.

To verify subjects were able to receive 1 atm O_2, the last six subjects' masks were periodically monitored. Thirty-two spot checks with an O_2 Gas Analyzer demonstrated that the inspired oxygen fraction (FiO2) averaged 98.6 ± 2.6%. The protocol was terminated after completion of the control arm, as there was no decrement to show improvement against. Discordance with these findings and those of hyperoxic studies conducted at hyperbaric atmosphere suggest there may be a difference in the mechanism of toxicity between normobaric and hyperbaric environments at the same PO_2.

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: NAVSEA
Project Status: Ending

DETERMINING DISSUB SURVIVAL RATES OF 70 KG SWINE RESCUED USING SRDRS STANDARD OPERATING PROCEDURES

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Background

The ARA San Juan incident raised numerous concerns regarding disabled submarine (DISSUB) rescue execution. The US Submarine Rescue System Decompression Plan (SRSDP) outlines the mobilization of assets and execution of DISSUB rescue. However, many of these theoretically feasible operational scenarios have not been tested in humans or large animal surrogates. The theorized maximum survivable internal pressure for DISSUB rescue is 132fsw; however, the effects of pulmonary oxygen toxicity at this depth for the duration of rescue are unknown. Previous animal work demonstrated that swine experience significant pulmonary edema after 96 hours at 100% surface oxygen, which is equivalent in partial pressure to breathing air at 132fsw. The effects of pulmonary edema on nitrogen off-gassing and decompression sickness (DCS) are unknown. In the current study we directly tested the aforementioned knowledge gaps, focusing on the survivability of breathing air at 132fsw, timing of appearance and severity of pulmonary oxygen toxicity, and feasibility of oxygen accelerated decompression following prolonged saturation at depth.

Objectives

- Determine the mortality rate and cardiopulmonary function of 70kg swine exposed to hyperbaric air at 132fsw for 172 hours followed by the 33-hour oxygen accelerated decompression table described in the SRSDP.
- Determine the incidence and severity of decompression sickness in 70kg swine exposed to hyperbaric air at 132fsw for 172 hours followed by the 33-hour oxygen accelerated decompression table described in the SRSDP.

Methods

Swine (70kg, n=6) were acclimatized and had baseline functional neurologic evaluation (modified Tarlov). The swine were sedated for placement of recording pads and custom-fitted jacket for electrocardiography (ECG), bio-impedance, and electroencephalography (EEG). The animals were recovered and moved into a self-contained box within our multiple large animal chamber (MLAC), where baseline vital signs were collected. The swine then dove at a maximum rate of 60fsw/min to 132fsw for a planned bottom latency of 172 hours with continuous environmental (e.g. carbon dioxide, temperature, and humidity) and physiologic monitoring. Carbon dioxide levels were limited to 1.6% surface equivalent value by gas flow into the boxes. The animals had continuous, free access to water and food. Death was recorded as last heart beat on ECG. The swine were then decompressed at a rate of 60fsw/min.

Results

All swine exposed to 5ATA hyperbaric air died during the exposure. Mean latency to death was 52.2 ± 1.69hrs. Gross necropsy yielded findings of pulmonary edema and gelatinous pleural effusion consistent with cardiopulmonary compromise. Histopathology of tissue samples collected during necropsy demonstrated damage to the tracheal epithelium consistent with tracheobronchitis. Additional findings included interlobular pulmonary edema and protein-rich pleural effusions.

Conclusions

Exposure to hyperbaric air at 5ATA in the presence of elevated CO2 was not survivable beyond 55hrs. Evidence of pulmonary oxygen toxicity was present in all exposed animals.

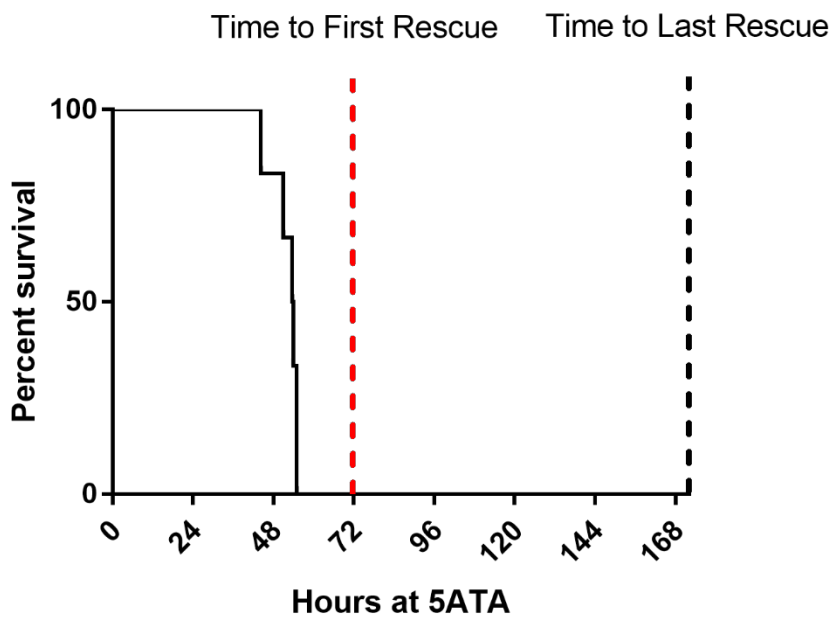

Figure 1. Kaplan Meier plot of survival latency for swine exposed to 5ATA air. Time to first rescue and Time to Last Rescue derived from SRS rescue plan are annotated on the graph.

Notes:

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: NAVSEA
Project Status: New Start

Evaluation of Tiotropium Bromide Efficacy to Reduce Tracheobronchitis and Pulmonary Function Decrements in Divers During Operationally Relevant Hyperbaric Oxygen Exposures

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Background

Military divers breathing enriched oxygen mixtures are limited in their diving duration due to the risk of lung damage termed "pulmonary oxygen toxicity (PO_2T)". The US Navy Dive Manual restricts using the MK16 MOD 1 closed circuit oxygen rebreather to 240 minutes per day and 16 hours per week to minimize PO_2T risk. These guidelines limit mission capabilities and suggest decreased diver safety if exceeded. Increasing the dive limits would allow deeper and longer dives as well as more dives per week, increasing mission capability, readiness, and diver safety by mitigating PO_2T.

Anticholinergics are a promising pharmacologic intervention to treat PO_2T, as vagally-derived acetylcholine may be implicated in the pathogenesis of airway remodeling and smooth muscle contraction. Tiotropium bromide is one such anticholinergic that is inexpensive, FDA approved, and readily available. Animal studies performed at NMRC demonstrated that tiotropium bromide may prevent PO_2T from prolonged oxygen exposure. Furthermore, there were no observed safety issues associated with tiotropium prophylaxis in swine exposed to hyperbaric oxygen. Our next step is to define the regulatory and experimental pathway required for human experimentation. This one-year study was intended to establish an investigational new drug (IND) application for use of tiotropium bromide for the prevention of PO_2T symptoms, secure a clinical partner, and provide a cost analysis for the required human studies.

Objectives

1) **Establish a partnership with a clinical research group**

2) **Prepare required documentation to support FDA IND application**

3) **Establish a plan to satisfy required regulatory and fiscal requirements**

Results

Established a collaboration with Dr. Richard Moon at Duke University. Non-provisional patent (IPT) is currently being filed which will enable contact with Boehringer Ingelheim (SPIRIVA® manufacturer).

Notes:

CONNECTING LIPID OXIDATION TO CELLULAR DYSFUNCTION IN HYPERBARIC OXYGEN TOCIXITY

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Background

Hyperbaric oxygen (HBO_2) toxicity, leading to acute pulmonary and central nervous system damage, is an ongoing risk to Navy divers using high-oxygen gas mixes. Understanding the molecular etiology of oxygen toxicity is key to improving diving protocols, identifying which divers are at risk at high oxygen partial pressures, and predicting long-term effects among those who show no acute symptoms.

The effects of HBO_2 toxicity include acute CNS symptoms such as nausea, vertigo, convulsions, and ultimately unconsciousness.[1] There has been significant work to uncover how HBO_2 alters neurophysiology on the level of cells and tissues. The foundational work in neuronal sensitivity to HBO_2 was reviewed in 2003 by Dean et al.[2] Single-cell studies show that at the cellular level, elevated oxygen partial pressure (P_{O2}) under hyperbaric conditions can increase neuronal excitability and firing rate.[3, 4] This is likely due to the production of reactive oxygen species (ROS) in HBO_2 conditions; the increase in firing rate is reduced in the presence of antioxidants and can also be stimulated by the addition of chemical oxidant species.[5]

It is likely that the site of action for ROS in altering neuronal behavior is the plasma membrane. Previous ONR-funded work from the Malmstadt lab has demonstrated that plasma membrane mechanics, permeability, and structure are radically altered by the presence of lipid oxidation products in the lipid bilayer [6-9].

In addition to altering key biophysical properties of the bilayer that are essential for physiological function, HBO_2-induced changes to lipid bilayer composition alter how lipid-influenced membrane proteins function. The Malmstadt lab has recently performed extensive studies of the lipid-dependent functionality of the human serotonin 5-HT_{1A} receptor (5-HT_{1A}R). 5-HT_{1A}R is a G protein-coupled receptor (GPCR). The synaptic receptors for many neurotransmitters—including serotonin, dopamine, GABA, glutamate, and acetylcholine—are GPCRs.

Objectives

The goal of this proposal is to achieve ***three interconnected objectives*** that will reveal how hyperbaric oxidation leads to physiological dysfunction by altering lipid bilayer properties:

1. Quantify changes to lipid composition in cells that have been treated with hyperbaric oxygen and with oxidation systems designed to reproduce the chemical effects of hyperbaric oxygen.
2. Determine how lipid bilayer mechanics and permeability change when bilayer compositions match exactly those of the plasma membranes of cells that have been treated with hyperbaric oxygen.
3. Quantify changes to the functional behavior of key neurotransmitter receptor proteins in lipid bilayers with compositions that match exactly those of the membranes of cells that have been treated with hyperbaric oxygen.

Ultimately, this work is necessary to not only understand the molecular mechanism of hyperbaric oxygen toxicity but also to develop potential medical interventions (diet, antioxidant therapy, drugs to affect neurotransmitter biology) that will minimize the risk to Navy personnel.

Methods

Objective 1

We grew U87 glioblastoma cells—a model for brain cells—in high-oxygen conditions, simulating physiological HBO_2 exposure. We monitored the viability of these cells and tracked changes in their lipid composition. These cells were used as the source of giant plasma membrane vesicles (GPMVs). GPMVs are micron-scale vesicular structures that can be induced to bud from the surfaces of cells under various conditions. In work going forward, we will extract lipids from them to perform a full lipidomic assay of changes in cell plasma membrane content under oxidation as well as to serve as protein incorporation matrices for the studies of Objective 3.

Objective 2

We extracted GPMVs from cells under oxidative stress and monitored their morphology, using this as a reporter of mechanical properties (deviation from a spherical morphology indicates increased membrane flexibility, typically a result of increased area per lipid). Going forward, we will apply techniques of micropipette aspiration to make precise measurements of lipid bilayer bending modulus and area dilation modulus.

Objective 3

We fabricated GUVs containing various concentrations of synthetic oxidized lipid and incorporated 5-HT_{1A}R. We performed activity assays on these GUVs, comparing the 5-HT_{1A}R activation rate in GUVs with varying degrees of oxidation. Going forward, we will repeat these experiments in GUVs comprised of lipids extracted from GPMVs formed from cells grown in oxidative conditions.

Results

Objective 1

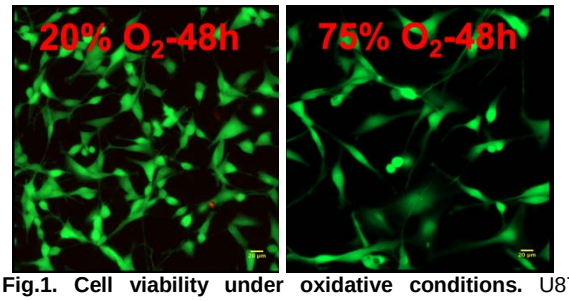

Fig.1. Cell viability under oxidative conditions. U87 glioblastoma cells were grown under either normoxic (left) or hyperoxic (right) conditions. Hyperoxic cells show a significant decrease in viability, measured at 17% of normoxic viability by an MTT assay.

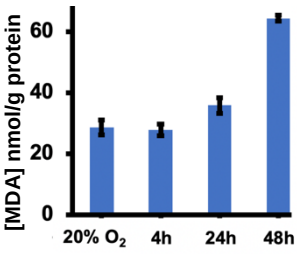

Fig.2. Cell viability is inversely correlated to the degree of oxidation in the plasma membrane. This figure shows the results of the MDA assay, which produces the colometric indicator MDA at a concentration proportional to the degree of oxidation in the cell plasma membrane. Compared to the normoxic control, there is a significant increase in chemical oxidation at 24 and 48h of hyperoxic exposure.

Cell viability was measured as cells were exposed to hyperoxic (75% O_2) conditions. **Fig. 1** shows that viability decreases significantly at 48 h hyperoxic exposure; we quantified this with an MTT assay which showed the hyperoxic cells had 17% the viability of the normoxic cells. **Fig. 2** shows that this decrease in cell viability is directly correlated to oxidation of lipids.

Objective 2

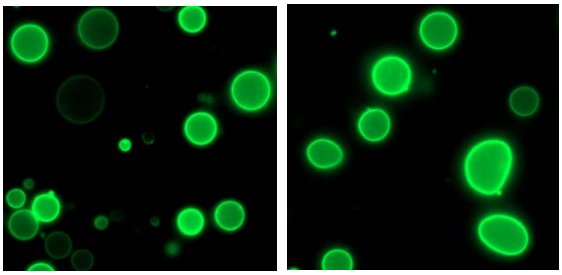

Fig.3. Morphology changes of GPMVs from oxidized cells. GPMVs were taken from cells grown in normoxic (left) and hyperoxic (75% O_2 at 48 h, right) conditions.

Fig. 3 shows how GPMV morphology changes with oxidation. Hyperoxic GPMVs show significant morphological deformation, most likely indicating excess area induced by changes in lipid structure. This indicates that the membranes of these cells have altered mechanical properties, including increased deformational flexibility.

Objective 3

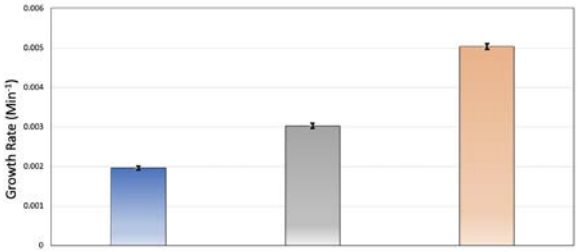

Fig.4. Activation rate of 5-HT_{1A}R as a function of lipid concentration. The y-axis shows the rate of protein activation while the three bars show lipid composition: 0% oxidized (blue), 7.5% oxidized (grey), and 17.5% oxidized (orange).

Fig. 4 shows a significant increase in the activity/excitability of the serotonin receptor as the fraction of oxidized lipids in a synthetic membrane is increased.

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Notes:

OXIDATIVE TISSUE DAMAGE AFTER HBO_2 EXPOSURE USING SELECTED FDA-APPROVED ANTI-EPILEPTIC DRUGS (AEDS)

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Background

Breathing oxygen at partial pressures ≥ 2.0 atmospheres absolute (ATA) is common in hyperbaric oxygen (HBO_2) therapy, during mixed gas diving, and in potential DISSUB situations. It can become acutely toxic to the central nervous system (CNS) culminating in tonic-clonic seizures. Convulsions in diving is a neurological emergency that we cannot now prevent, making this area critical to Navy Diving and Submarine Medicine. We previously determined that certain FDA-approved, antiepileptic drugs (AEDs) alone and in combinations prevent HBO2 seizures for up to four times the normal latent period in unrestrained mice exposed to 100% O_2 at 5 ATA. Although seizures are blocked for long periods of time, we do not yet know whether AEDs mitigate or promote biochemical oxidant damage to neurons or glia during maximal tolerable exposures. Because HBO2 seizures share electrophysiological features with idiopathic epilepsy, we have tested FDA-approved antiepileptic drugs (AEDs) for CNS O_2 toxicity prevention. We measured the anti-seizure effects of ten AEDs that act on specific molecular targets known to be involved in seizure generation and found that sodium-channel antagonists, carbamazepine (CMZ) and lamotrigine (LTG), and GABA transmission enhancers, tiagabine (TGB) and gabapentin (GBP), displayed high efficacy against HBO_2 seizures. We then tested combinations of these selected AEDs and found two combinations that prolonged the seizure latency by five- to six-fold with no change in the cerebral vascular response to HBO_2. The combination of two AEDs from different functional classes (TGB+CMZ and GBP+LTG) are the most effective against seizure activity, but combinations containing tiagabine (TGB) are the best. We are using TGB, a GABA enhancer, in combination with a sodium channel antagonist (either lamotrigine or carbamazepine) to measure to measure the impact of prolonged HBO_2 exposures (with/without seizures) on biochemical and functional variables in adult male mice (25-30g) that represent neurotransmitter depletion and/or oxidative tissue damage. For these drugs, the cerebral blood flow response to HBO_2 is normal, and brain tissue PO_2 remains elevated, which may allow increases in oxidant tissue damage during the prolonged HBO_2 exposures. The studies are accompanied by studies with the Barnes maze to determine the impact of the drugs, HBO_2 and the combinations on spatial learning and memory. Introduce your work effort topic.

Objectives

We have four objectives:

Aim 1: Measure oxidative tissue damage by 5ATA HBO2 in mouse forebrain and hindbrain using a standardized battery of tests under conditions of A) Control, B) HBO_2, C) AED combinations alone, and D) HBO_2 plus AED combinations.

Aim 2: Measure basic neurotransmitter levels in mouse forebrain and hindbrain under conditions of A) Control, B) HBO_2, C) AED combinations alone, and D) HBO_2 plus AED combinations. These measurements will focus on two transmitters that are implicated in CNS O_2 toxicity, GABA levels and glutamate levels in HPLC extracts of both brain regions. Also, we will measure brain protein S100-B, glial fibrillary acidic protein (GFAP), and enolase-1 (ENO1).

Aim 3: Measure inflammatory activity in mouse forebrain and hindbrain, lung parenchyma, and mouse plasma and compare the four conditions outlined in Aims 1 and 2.

Aim 4: Measure cell death at days 1 and 7 in the brain and compare it with changes in spatial learning and memory by Barnes maze.

Methods

Groups of six freely-moving mice per group are studied in separate cages, two at a time, in a hyperbaric chamber and compressed to 5 ATA O2 at 0.7 ATA min^{-1}. The HBO_2 exposures last up to 60 min for the control mice and those treated with AEDs separately and up to 90 min for those treated with the AED combinations. Chamber temperature and relative humidity were

maintained at 23 ± 0.5° C and 60 ± 2%. All mice were continuously monitored for signs of CNS HBO_2 toxicity, and in some instances, video recordings were made for later review. HBO_2 exposures were concluded when mice exhibited tonic-clonic seizures. For Aim 1 our methods are protein oxidation by carbonyl formation (RCO), lipid peroxidation by malondialdehyde (MDA), DNA oxidation by 8-oxoguanine (OHdG), GSH/GSSG ratio, and 3-Nitrotyrosine (3-NT). For Aim 2, the methods focus on two transmitters that are implicated in CNS O2 toxicty, GABA levels and glutamate levels inHPLC extracts of both brain regions. Also, we will measure brain protein S100-B, glial fibrillary acidic protein (GFAP), and enolase-1 (ENO1). For Aim 3 the measurements will include NALP3 inflammasome protein, TNF-α, Interleukins (IL)-1β and IL-18, and NOx levels. Bronchoalvelar lavage samples will be used to measure lung injury by albumin protein translocation, LDH, soluble RAGE, cell count, and cell differential counts. Lung function is mesured ex vivo by Flexivent. For Aim 4, we are using western blotting and immunochemical localization studies for caspase-3 cleavage, necroptosis proteinsRIPK1 and 3, MLKL phosphorylation, and Tunel in the brain.

Results

Table 1: Equally-effective doses (EQDx) of AEDs predicted to increase seizure latency in HBO_2

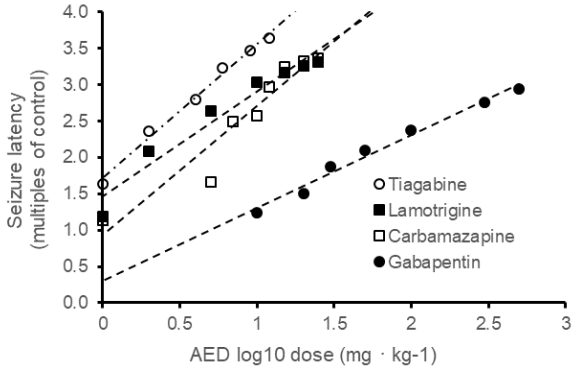
AEDs	EQD2x (mg · kg-1)	EQD2.5x (mg · kg-1)	EQD3x (mg · kg-1)	EQD3.5x (mg · kg-1)
Tiagabine	1.4 ± 0.2	2.6 ± 0.3	4.8 ± 0.6	9.6 ± 1.4
Gabapentin	30.2 ± 3.2	132.3 ± 15.7	364 ± 47.7	>1000a
Carbamazepine	4.8 ± 0.4	9.4 ± 1.1	16.4 ± 1.9	36.4 ± 4.1
Lamotrigine	3.1 ± 0.4	6.6 ± 0.7	13.2 ± 1.8	27.6 ± 3.2

Values represent EQD2-3.5x ± SEM. EQD-2x, 2.5x, 3x and 3.5x reflect doses predicted to increase seizure latencies in mice exposed to HBO2 at 5 ATA over vehicle controls by multiples of 2, 2.5, 3 and 3.5, respectively. >70% of mice exhibited altered motor coordination or balance during rotarod testing, thus these doses were not tested in HBO_2.

Table 2 Interactive antiseizure effectiveness of combined AEDs in mice exposed to HBO2

AEDs	F	EQD3x-add (mg · kg-1)	EQD3x-mix (mg · kg-1)	Interaction
TGB+CBZ	3:1	7.7 ± 0.09	5.3 ± 0.05*	Synergy
	1:1	10.6 ± 1.10	6.9 ± 0.08*	Synergy
	1:3	13.5 ± 1.70	9.7 ± 0.90	Additivity
TGB+LTG	3:1	6.9 ± 0.08	4.8 ± 0.06*	Synergy
	1:1	9.1 ± 1.10	6.1 ± 0.08*	Synergy
	1:3	11.1 ± 1.20	12.1 ± 1.30	Additivity

Values represent EQD3x-add and EQD3x-mix ± SEM. TGB, tiagabine; CBZ, carbamazepine; LTG, lamotrigine; F, fixed dose ratio; EQD3x-add, the theoretical additive doses of TGB+CBZ or TGB+LTG that increases seizure latency over vehicle control by a factor of 3; EQD3x-mix, the experimentally determined doses of TGB+CBZ or TGB+LTG that increases latency over vehicle control by a factor of 3. For each fixed dose ratio, 4-5 dose combinations were testing in HBO_2 at 5 ATA. *p < 0.05.

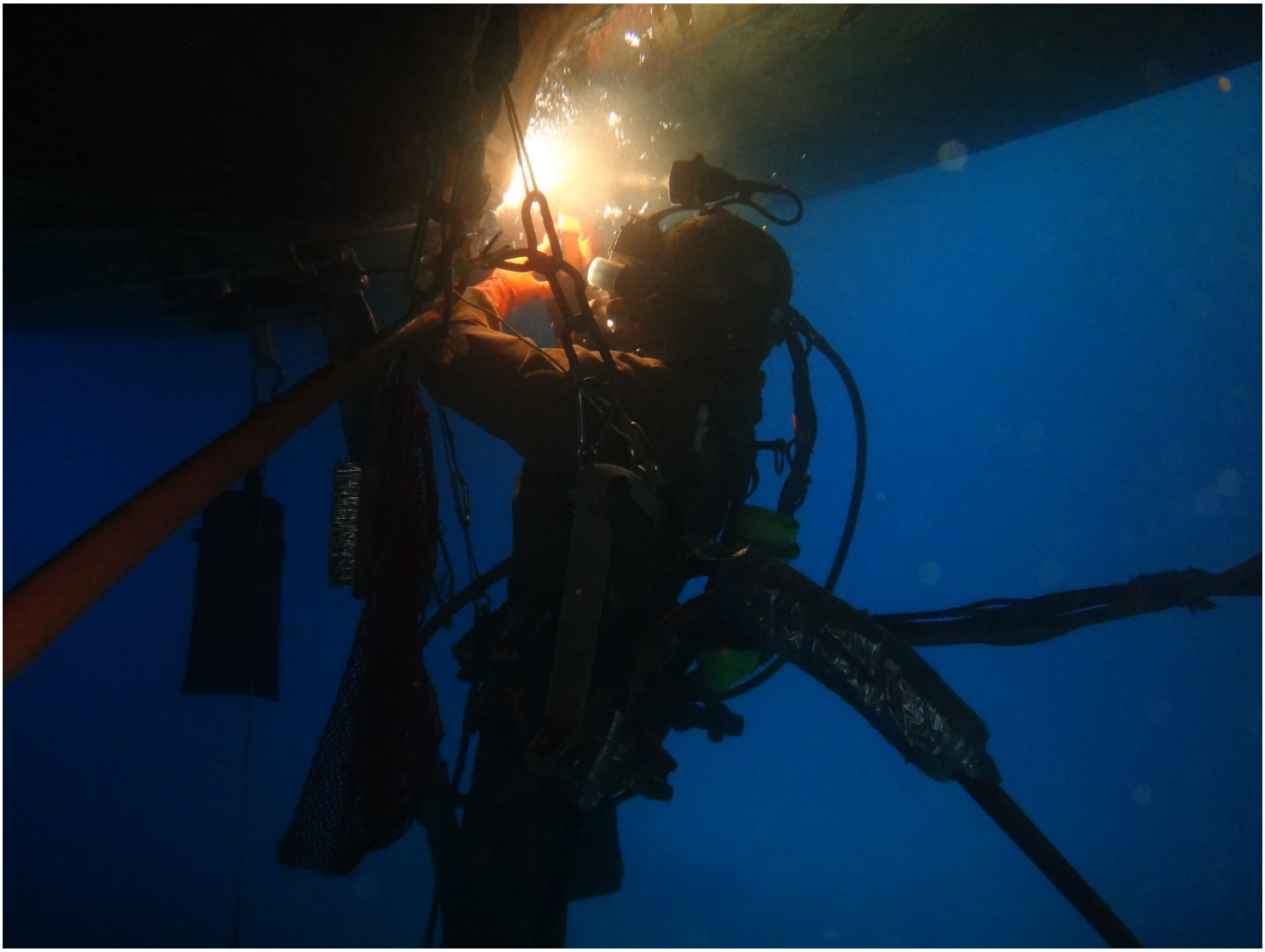


These new results provide supporting data for the use of TGB, LTG, and CMZ and excluding GBP in the ongoing biochemical studies to measure the degree of oxidant damage to neuronal and glial elements after HBO_2.

Notes:

Approved, DCN# 43-5229-19

Day 2: Wednesday, May 15, 2019



2019 ONR-NAVSEA UNDERSEA MEDICINE PROGRAM REVIEW

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: NAVSEA
Project Status: ONGOING

PROBABILISTIC MODELS OF OXYGEN TOXICITY

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Background

The Navy has a stated need for improvements in predictive models of central nervous system (CNS) oxygen toxicity. Specifically, there are Navy needs for advanced predictive models of CNS oxygen toxicity including optimization of air breaks for decompressing divers and chamber tenders. Additionally, the Navy has stated needs for continued advancements in probabilistic decompression sickness (DCS) theory, models, and methods including an improved understanding of counter diffusion in isobaric and non-isobaric exposures. The development of new CNS oxygen toxicity and probabilistic DCS models should also include algorithm development to allow these models to operate in real-time so that they may be used in diver-warn dive computers.

Objectives

One objective of this project is to develop, optimize, and evaluate models to predict the risk of CNS oxygen toxicity. Recent U.S. Navy studies have collected additional data on central nervous system (CNS) oxygen toxicity so that new efforts at creating and refining probabilistic models that predict the risk of CNS oxygen toxicity at high partial pressures of oxygen may be undertaken.

Another objective of this work is to continue our current development of decompression models that allow for mixed gas use including gas switching to accelerate decompression. We have added gas transfer affects such as counter-perfusion, counter-diffusion, and gas-phase advection to many of our probabilistic pharmacokinetic DCS models.

Methods

The so-called Harabin [1] data set is the primary oxygen toxicity data set used by the U.S. Navy for calibration of an evaluation of oxygen toxicity models. The Harabin data set is a compilation of previous high oxygen partial pressure studies with various single and repetitive exposures. The 2170 exposures resulted in 862 dive-ending oxygen toxicity events including nausea, dysphoria, headache, numbness, vertigo, twitch, visual, unconsciousness, and convulsion. In the present study, the Harabin data were analyzed and found to contain several errors which were corrected for use in the present CNS modeling efforts. A machine-readable version of the corrected data were shared with the Navy Experimental Diving Unit.

Three existing oxygen toxicity models as well as eight new oxygen toxicity models were analyzed and or optimized using the method of maximum likelihood. Significant improvement in the performance of three previously-published oxygen toxicity models was found upon re-optimization of these models.

Results

The three previously-published CNS oxygen toxicity models evaluated in this work include the Harabin 93 exponential [2], the Harabin 95 exponential [3], and the Harabin autocatalytic models [3]. Exact solutions of these three models were derived in order to substantially increase computational throughput during optimization. Of these previously existing models, a significant improvement in their performance was found upon re-optimization.

The Harabin autocatalytic model, which was derived to enable air breaks to reduce the overall risk of oxygen toxicity, is the most widely used oxygen toxicity model. However, we found that upon re-optimization, the Harabin 93 exponential model substantially outperformed the Harabin autocatalytic model.

Eight new models were derived, optimized and compared with the three Harabin models. Of the eight new models, two failed to find a single solution. Of the six new models that found solutions, none outperformed the Harabin 93 exponential model.

The results of the model optimization of 11 models considered thus far are summarized in the table below. For these results, all models were optimized on subset 2 (immersed, exercised, old) of the Harabin; data containing

275 exposures and 135 dive-ending cases of oxygen toxicity (c.f. ref [2], page 19).

Model	LL	PCNS
Harabin 93 Exp	-152.6	134.3
Howle 18C	-190.6	135.0
Howle 18D	-190.6	135.0
Howle 18E	-190.6	135.0
Howle 19H	-190.6	135.0
Harabin 95 Exp	-218.9	115.6
Howle 18A	-224.5	121.5
Harabin Auto	-266.3	115.0
Howle 18F	-325.7	35.1
Howle 18B	NA	NA
Howle 19I	NA	NA

Notes: Model optimization results for Harabin subset 2 of CNS oxygen toxicity data. For this subset, 275 exposures resulted in 135 dive-ending oxygen toxicity events.

References

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[2] Harabin, A.L. *et al*. A model for predicting central nervous system toxicity from hyperbaric oxygen exposure in man: effects of immersion, exercise, old and new data. NMRI 94-03, 1994.

[3] Harabin, A.L. *et al*. A model for predicting central nervous system oxygen toxicity from hyperbaric oxygen exposures in humans. *Toxicol Appl. Pharmacol.*, 132:19-26, 1995.

Notes:

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: ONR
Project Status: ENDING

CELLULAR MECHANISMS OF CNS OXYGEN TOXICITY DURING CO_2 RETENTION AND KETONE METABOLIC THERAPY

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Background

The goal of this 6.1 research project in rodents is to further understand how conditions that accelerate and delay onset of CNS oxygen toxicity (CNS-OT) seizures (Sz) affect each of the following phenomena that are implicated in CNS-OT: i) increased production of reactive O_2 species (ROS) in the brain stem; ii) electrical signaling by medullary neurons; and iii) hyperoxic ventilatory response (HOVR); i.e., hyperventilation. A highly effective mitigation strategy that reduces the risk for CNS-OT by delaying onset of Sz during hyperbaric oxygen (HBO_2) is ketone metabolic therapy (KMT). Conversely, CO_2 retention is a condition that increases the risk for CNS-OT by accelerating onset of Sz while HBO_2. CO_2 retention commonly occurs in Naval unique SPECWAR/SPECOPS diving operations and pressurized DISSUB emergencies.

Neurons in the caudal Solitary Complex (cSC) are studied because of their presumptive role in the neurophysiology of CNS-OT and cardiogenic pulmonary O_2 edema during HBO_2. cSC neurons also are activated by hypercapnia, orexin, and hyperoxia and presumably contribute, in part, to the HOVR that precedes Sz during HBO_2. Finally, cSC neurons also regulate gastric acid secretion, gastric CO_2 production, and lower esophageal sphincter tension (gastric CO_2 ventilation).

Objectives

Aim 1) We tested the hypothesis that KMT delays CNS-OT in part by decreasing ROS production during hyperoxia. *Modifications to Aim 1 from the original proposal*: electrophysiology experiments were put on hold due to problems encountered for making fluorescence imaging experiments under hyperbaric conditions. These problems were resolved as illustrated in Fig. 1.

Aim 2) Measure early changes in respiration prior to onset of CNS-OT using radio telemetry in freely behaving SD rats during exposure to $HBO_2 \pm CO_2$ and ±Ketone Ester (KE) at 5 ATA O_2. The goal was to determine how KMT affects the HOVR prior to onset of CNS-OT. *Modifications to Aim 2 from the original proposal*: planned experiments were delayed following a lab accident in March 2018 that shut down the USF animal dive chamber for one year. Planned experiments were recently initiated under the scope of ONR N00014-18-1-2701 (P.I. D'Agostino with Poff & Dean).

Aim 3) Determine the effects of chronic and acute CO_2 retention on sleep-wake cycles, respiration, and gastric function in freely behaving rodents.

Methods

Aim 1) Fluorescence imaging experiments were conducted in rat brain slices (400 μm thick) harvested from Sprague-Dawley (SD) rats maintained inside a hyperbaric chamber. Brain slices were loaded with the fluorescent dye dihydroethidium (2.5 μM DHE, 35-36°C; exλ/emλ 525/590nm) to measure superoxide production ($\cdot O_2^-$) from cSC cells during exposure to control O_2 (0.4 ATA) and hyperoxia (0.95, 1.95 & 4.95 ATA O_2) ± ketone salts (KS; 2&5 mM KS @ 1:1 ratio of β-hydroxybutyrate:acetoacetate).

Aim 2) SD rats were implanted with DSI 4-ET radio-telemetric modules to measure cortical electro-encephalogram (cEEG), respiratory muscle electromyogram (rmEMG), electrocardiogram (ECG) inside a hyperbaric chamber. Each animal was exposed to 5 ATA O_2 until onset of 1st & 2nd bouts of Sz. Sz were scored based on behavioral/motor Sz (scored as 0-6 to reflect increasing intensity & complexity) and absence vs. presence of increased cEEG activity. cEEG activity was analyzed by power band analyses before, during, and following each Sz.

Aim 3) SD rats were implanted with DSI 4-ET radio-telemetric modules and exposed to acute or chronic CO_2 as follows using a Biospherix chamber: chronic CO_2 (2 wk air control → 4wk × 4%CO_2 → 4wk air recovery) and measuring cEEG, neck EMG, rmEMG & ECG; and acute CO_2 (1hr × 5% CO_2 then 1hr × 10% CO_2) and measuring ECG, rmEMG, and gastric smooth muscle EMG in the antrum (gEMG) and duodenum (duEMG) of the stomach.

Results

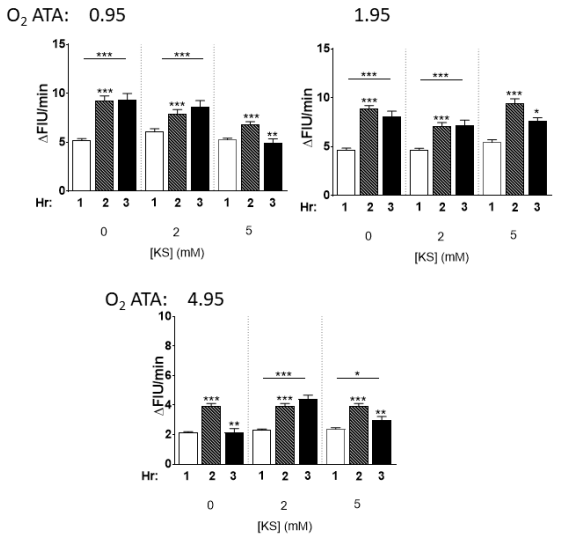

Fig.1. Effects of hyperoxia (0.95, 1.95 & 4.95ATA) ± ketone salts (KS: 2&5mM) on $\cdot O_2^-$ production in cSC cells in rat brain slices based on changes in DHE fluorescence intensity per minute (ΔFIU/min) during exposure to control 0.4ATA O_2 (Hr 1, open histogram), hyperoxia (HR 2, grey histogram), and hyperoxia + KS (Hr 3, black histogram). Hyperoxia increases $\cdot O_2^-$ production, which is significantly decreased by 5mM KS (but not 2mM KS) during all levels of hyperoxia tested (n= 49-69 cells/condition at ~10cells/slice/ rat; pooled cells/condition).

Aim 1) New experiments indicate that ROS production can also be measured in real time during exposure to HBO_2 in the mammalian CNS. Our findings show that 0.95, 1.95 & 4.95 ATA O_2 all increase $\cdot O_2^-$ production and accumulation in cSC cells. ROS production is sustained during 2 hr of hyperoxia at 0.95 & 1.95 ATA but decreases during the second hr of 4.95 ATA O_2. Not all cells in the cSC produce $\cdot O_2^-$ at the same rate during hyperoxia however. Exposure to 1 hr 5 mM KS significantly decreases $\cdot O_2^-$ production during hyperoxia at 0.95, 1.95 & 4.95 ATA in most cSC cells tested; however, exposure to 2mM KS usually did not inhibit ROS production during hyperoxia.

Aim 2) Assessing latency to Sz during exposure to HBO_2 is vital for quantifying effectiveness of KMT and other mitigation strategies. Using SD rats, we have found that the first Sz lasts ~15sec and may be underwhelming in magnitude and absent any noticeable change in cEEG (11% of dives) or motor Sz (11% of dives). 78% of animals tested exhibited both cortical Sz and behavioral/motor Sz. By contrast, the second Sz is longer in duration and more intense and follows a variable interictal period ranging from tens of sec to tens of min. Remarkably, a first electrical discharge (cEEG) does not occur in any SD rats during ≤3min preceding onset of first or second cortical Sz activity. First and second bouts of Sz also have different powerband profiles. Experiments are now underway to study effects of KMT and CO_2 rebreathing during HBO_2 on the latency to Sz under the scope of ONR N00014-18-1-2701 (P.I. D'Agostino with Poff & Dean).

Aim 3) CO_2 rebreathing modifies REM sleep, pulmonary ventilation, and gastric activity. Exposure to chronic CO_2 initially increases REM sleep which trends back towards baseline levels during the remaining 3 wk of hypercapnia. Returning to air significantly decreases REM sleep which trends back towards baseline levels during the next 3 wk of air breathing. Acute exposure to hypercapnia increases pulmonary ventilation, decreases heart rate, and slows and then abolishes regular recurring gastric bursts. Returning to air reverses the effects of acute CO_2 on integrated pulmonary and gastric ventilation. Analyses are ongoing of the effects of chronic CO_2 on pulmonary ventilation, the hypercapnic ventilatory response, and gastric bacteria.

Notes:

AN ASSESSMENT OF THE KETONE ESTER R,S-1,3-BUTANEDIOL ACETOACETATE DIESTER FOR THE INDUCTION OF KETOSIS AND DELAY OF CNS HBO_2 TOXICITY IN SWINE

LT Geoffrey Ciarlone, PhD, Aaron Hall, PhD, Richard Mahon, MD, Brian Williams, PhD

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Background

Closed-circuit underwater breathing apparatus (CC-UBA) and pure oxygen (O_2) breathing mixtures have benefitted divers by allowing for stealth during special operations missions and avoidance of the restrictions accompanying breathing inert gases, such as narcosis and risk for decompression sickness. However, extended exposure to hyperbaric O_2 (HBO_2) results in central nervous system HBO_2 toxicity (CNS-OT), which is characterized by tonic-clonic seizure, unconsciousness underwater, and death due to drowning. Current mitigation strategies target reduction of the partial pressure of O_2 and/or depth, limiting mission capability. A novel and effective mitigation strategy, such as a pharmacological agent with a minimal or acceptable side effect profile, would allow for the expansion of mission options for military divers and ensure an ongoing technological advantage versus near-peer adversaries.

Of the various pharmacological agents that have been tested to delay CNS-OT seizures, the ketone ester (KE) R,S-1,3-butanediol acetoacetate diester [BD-$(AcAc)_2$] has been shown to be particularly efficacious, delaying seizure in rodents exposed to 5 atmospheres absolute (ATA) O_2 by 574%. Here, we are transitioning the use of BD-$(AcAc)_2$ into a large animal model to determine the lowest effective dose for inducing a level of therapeutic ketosis capable of significantly delaying seizure, while also ensuring no performance deficits arise from its administration. Upon successful completion of this study, sufficient data will have been collected to definitively determine the efficacy of BD-$(AcAc)_2$ in delaying CNS-OT seizures, which will assist in the determination of whether or not to transition to human testing and eventual Fleet use to expand operational mission options.

Objectives

Aim 1: Assess pharmacokinetics of BD-$(AcAc)_2$ via analysis of blood ketone levels following acute oral administration, and determine the lowest effective dose (LED) for induction of neuroprotective levels of AcAc for delay of CNS-OT seizures.

Aim 2: Determine the efficacy of the LED identified in Aim 1 for prolongation of seizure latency in NMRC's swine model of CNS-OT following acute oral administration.

Aim 3: Assess the effects of BD-$(AcAc)_2$ on exercise capacity and cardiorespiratory function.

Methods

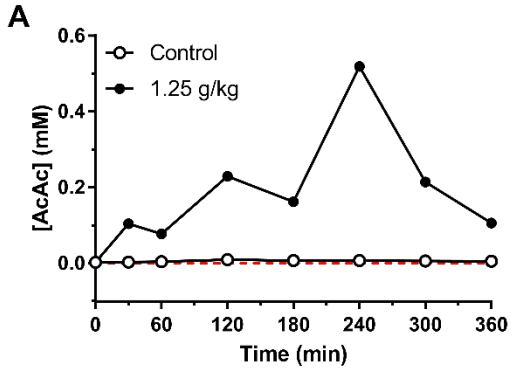
Aim 1: Swine will be anesthetized and fitted with a venous central line catheter for collection of mixed venous blood, and subsequently administered an oral dose of either water (control) or BD-$(AcAc)_2$. Blood will be collected at 0, 30, 60, 120, 180, 240, 300, and 360 minutes for GC/MS analysis of the ketones β-hydroxybutyrate (βHB) and acetoacetate (AcAc), as well as blood glucose and venous blood gases. Animals will also have breath samples collected for GC/MS analysis of acetone in breath exhalant as a non-invasive measure of ketosis. Breath will also be sampled for real-time metabolic analysis of resting O_2 consumption.

Aim 2: Using the LED from Aim 1, swine will be orally administered either water or BD-$(AcAc)_2$ and dove to 5 ATA O_2. Animals will be assessed for time to tonic-clonic seizure onset.

Aim 3: Following a week of treadmill familiarization and training, swine will be orally administered either water or the LED determined in Aim 1 and confirmed for efficacy in Aim 2, and will complete a maximal oxygen consumption (VO_2max) assessment and undergo a hypercapnic ventilatory response (HVR) test to confirm that the LED does not induce deficits in exercise performance or abnormal cardiorespiratory function.

Results

To date, two animals have been tested in Aim 1 for validation of methodology and collection methods. Fig. 1 displays mixed venous blood concentrations of AcAc (panel A) and βHB (panel B) in animals that received oral administration of 1.25g/kg BD-$(AcAc)_2$ vs. control (water) at t=0 min, n=1/treatment. Notably, both AcAc and βHB were elevated by BD-$(AcAc)_2$ administration by 30 minutes post-dosing, with ketone levels peaking at 240 minutes and remaining elevated out to the 360 min time point.



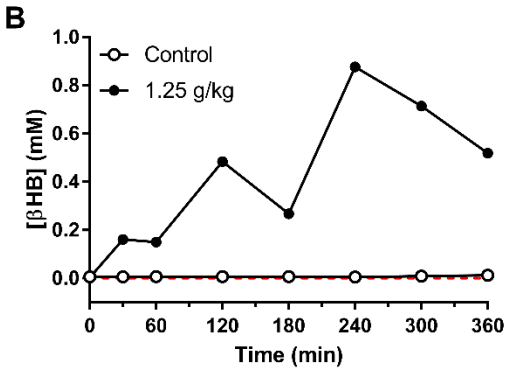


Fig. 1. Mixed venous blood concentrations of the ketones acetoacetate (AcAc, panel A) and β-hydroxybutyrate (βHB, panel B) following oral administration of either water (open circles) or BD-$(AcAc)_2$ (1.25g/kg, closed circles) at t=0 min in 30kg swine (N=1/group). *Note negligible endogenous ketone production in control animals, as expected.

Notes:

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: NAVSEA
Project Status: ONGOING

KETOGENIC DIET FOR REDUCTION OF CNS OXYGEN TOXICITY IN WORKING DIVERS

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Background

Divers breathing elevated partial pressure of oxygen (PO_2) continue to be at risk for developing CNS oxygen toxicity (CNSOT). The underlying pathophysiologic mechanisms of CNS oxygen toxicity are still not completely known. A number of physiologic factors have been investigated and found to increase the risk of oxygen toxicity. These include elevated PO2, inspired or elevated CO2, immersion, exercise/increased metabolic rate, and water temperature.

The utilization of nutritional ketosis (NK) has recently been proposed as a mechanism to protect against CNS oxygen toxicity. Children and some adults with seizures refractory to anti-epileptic drugs have been successfully treated through induction of NK achieved by consuming a diet consisting of high fat, scarce carbohydrate, adequate protein. This diet has been termed the "ketogenic diet" (KD), and has seen a surge in popularity among the general public in recent years.

It is likely the ketogenic diet expresses its antiepileptic effects through multiple physiologic mechanisms such as: direct effects of ketone bodies on GABA receptors, modulation of GABA, Glutamate and adenosine neurotransmitter systems, modulation of biogenic monoamine levels, ion channel regulation, glycolic restriction and alternative substrate utilization, direct inhibition by fatty acids, and reduction of oxidative stress. While these mechanisms are still under investigation, previous research has successfully demonstrated an increased latency to seizure in animals with elevated serum ketone levels achieved through starvation, diet modification, or ketone supplementation. The effects of NK on CNS O_2 toxicity in human subjects has yet to be studied.

Objectives

The primary aim of this study is to establish an operationally feasible means of achieving nutritional ketosis (NK), and evaluate the effect of NK on latency to CNS O2 toxicity in immersed (head-out) working divers. Secondary objectives focus on understanding the physiology of CNSOT and NK including cognitive effects, brain function and hemodynamics.

Methods

This is a randomized, investigator blind, crossover study. Fifty healthy, male and female volunteers, 18-50 years old will be selected after initial screening. Subjects will participate in two hyperbaric exposures in a randomized manner. One after consumption of a conventional diet (CD), the other after consumption of a 3-day ketogenic diet (KD). Dietary intake is supervised by a dietitian, serum and urine ketone levels will be recorded. Subjects will also consume a ketone supplement prior to the hyperbaric exposure during the ketogenic arm to support sustained ketone and energy levels throughout the experiment.

Hyperbaric exposures will be conducted with 100% O_2 at 35 fsw for up to 120 minutes during the pilot phase. This was selected based on previous work that elicited O_2 toxicity symptoms without seizures. Subjects will be submerged in 28±1°C water to the shoulders, and exercise on a cycle ergometer at 50 watts. The study will terminate upon manifestation of CNSOT signs or symptoms.

A battery of physiologic and cognitive parameters will be evaluated and compared between groups and individual case-controls which include but are not limited to: anthropometric measurements, beta-hydroxybutyrate level (BHB), changes in PaO2, PaCO2, heart rate and blood pressure measured via radial artery catheter, continuous expiratory gas sampling, respiratory rate, tidal volume, continuous electrocardiogram, standard and quantitative electroencephalogram (qEEG), cognitive performance assessment with NASA's Multi-Attribute Task Battery II (MATB-II) software, and electrodermal activity assessment will be added this year.

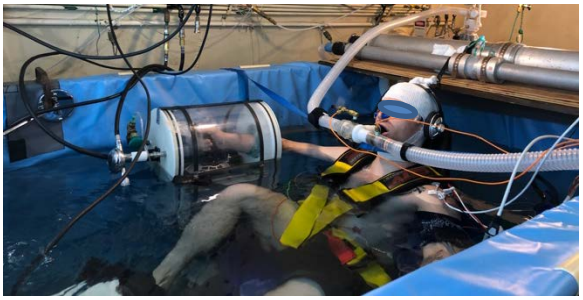


Fig.1. Subject setup in "Foxtrot" chamber

Results

The pilot phase of the protocol including 20 exposures for 10 subjects (6 male, 4 female) is complete. One subject's control dive was aborted due to mechanical issues. "Intention to treat" and "Completion" analysis were not significantly different. Subject 10's CD aborted dive time of 44.8 minutes was replaced with the group CD dive mean of 47.2 minutes for analysis on an intention to treat basis. Subject, pre-exposure BHB, dive time and symptoms are shown in Table 1 below. One dive in the CD group (6_1) had a questionable oxygen toxicity symptom of left hand tingling, which may have been a side effect of flushing the radial artery catheter. All other symptoms appeared to be true manifestations of CNS oxygen toxicity. One subject (8_2) had a convulsive episode during the KD exposure. No injury occurred and no residual effects.

Table 1. Subject BHB, Dive Time, Symptoms

SUBJ	BHB (mmol/L)	TIME (Min)	SYMPTOM
1_1	-	32.1	lightheaded
1_2	1.43	120.0	none
2_1	2.32	48.2	concentration, palpitations
2_2	-	29.2	facial twitching, difficulty speaking
3_1	1.95	78.0	diaphoresis
3_2	0.1*	76.6	diaphoresis
4_1	0.2*	35.0	tingling ascending both arms
4_2	1.56	37.6	dizzy, coordination
5_1	1.7*	50.5	leg twitching
5_2	0.1*	39.1	leg twitching
6_1	<0.18	42.5	* left hand tingling, art line symptom?
6_2	1.93	73.8	ear ringing
7_1	1.18	94.4	ear ringing
7_2	0.1*	70.3	nausea
8_1	0.28	65.3	Headache/FH pressure, tunnel vision
8_2	0.83	36.7	seizure
9_1	0.48	35.0	concentration, eyes heavy
9_2	1.80	42.5	headache - forehead pressure
10_1	<0.18	44.8**	**dive aborted, bike mechanical issue
10_2	1.98	120.0	none

Notes: * Precision-Xtra ketone meter, Duke Lab sample not collected or hemolysis prevented analysis. BHB was not collected for the control dives of the first two subjects.

Primary outcome results for BHB level and dive time are summarized in table 2. Subjects achieved the required minimum beta-hydroxybutyrate (BHB) level of 0.5 mmol/L within the prescribed three day ketogenic diet for 9/10 subjects. A fourth day on the ketogenic diet was needed for one subject.

Table 2. BHB & dive times by group, ITT analysis

	CD (n=10)	KD (n=10)	P-Value
PX arrival BHB (mmol/L)	0.1 ± 0.1	0.8 ± 0.2	0.0002*
PX arrival - pre-dive BHB (mmol/L)	0.0 ± 0.1	0.6 ± 0.4	0.0012*
Lab BHB (mmol/l)	0.3 ± 0.2	1.7 ± 0.5	0.0126*
Dive Time Average (min)	47.2 ± 17.2	70.2 ± 28.3	0.0410*
Dive Time Median (min)	40.8	62.1	
Dive Time Range (min)	29.2 - 76.6	36.7 - 120	

Notes: * Kruskal Wallis analysis. PX = Precision-Xtra Ketone Meter. Arrival BHB was taken on arrival to facility on experimental day
Pre-dive BHB levels taken after eating and ketone supplementation.

Figure 1 below depicts the latency to CNSOT (survival) for the CD and KD groups respectively in Kaplan-Meier curve format. Figure 2 represents dive times by individual subject.

Figure 1. Kaplan-Meier "Survival Curve" by group

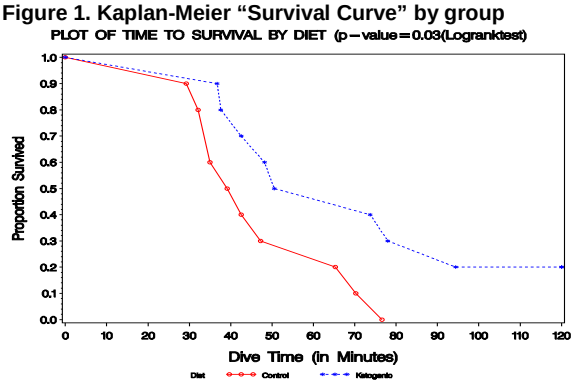
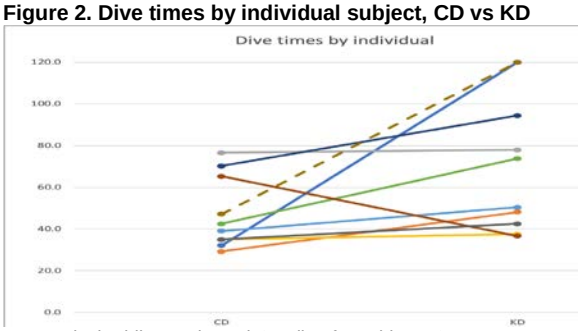


Figure 2. Dive times by individual subject, CD vs KD



Notes: dashed line = aborted CD dive for subject 10, group average substituted for CD dive per intention to treat analysis

Summary

Latency to CNSOT is modestly longer for each individual's KD dive compared to their CD dive for most subjects. A few outliers appear skew the mean times for the group as demonstrated by wide the standard deviation. While there is a statistically significant difference in dive times between the CD and KD group, we do not consider this operationally significant without additional study.

Notes:

Feasibility of electrodermal activity for detecting seizures elicited by central nervous system oxygen toxicity under the water

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Background

Previous studies have suggested that electrodermal activity (EDA), usually measured on the fingertips, can potentially be used to predict seizures, and that EDA is sensitive to sympathetic arousal not only in dry conditions, but also when subjects are fully immersed in water. The current work is to examine if EDA can be used to detect and/or predict the onset of seizures caused by central nervous system oxygen toxicity (CNS-OT) in both dry and water immersion conditions. This study will provide methods for detection and/or prediction of seizures, the most adverse side effect that divers can get when pre-breathing oxygen to thwart decompression sickness (DCS).

Objectives

The first objective of the study is to determine the feasibility of EDA for detecting seizures using rats. The rats will allow us to explore HBO_2 breathing at pressures higher than 3 Atmospheres Absolute (ATA), which causes a high seizure occurrence rate. This first objective will be in collaboration with Drs. Jay Dean and Dominic D'Agostino at the University of South Florida. The second objective is to evaluate the reliability of EDA in humans undergoing cognitive stress and mild CNS-OT. The protocol will elicit some CNS-OT manifestations, with either no or a low seizure occurrence rate. The second objective will be in collaboration with Dr. Bruce Derrick at Duke University.

Methods

The first aim of this study is to evaluate the feasibility of the EDA for predicting the onset of seizures resulting from CNS-OT caused by oxygen prebreathing at elevated pressure in rats. To accomplish this aim, an animal model based on 10 adult male Sprague-Dawley rats breathing HBO_2 in a chamber at 4, 5, and 6 ATA, in which behavioral seizures are detected though video recordings and electroencephalogram (EEG) recordings, will be used to observe the changes in EDA before and during the seizures caused by CNS-OT. This animal-model protocol was successfully used previously, for looking at the changes in the heart rate and minute ventilation under CNS-OT, and their power to predict seizures. The protocol will be implemented by the group of Dr. Jay Dean at the University of South Florida. For the proposed study, an identical protocol will be used, and EDA electrodes will be placed on the back of the animals to study the feasibility of seizure prediction using EDA. A study has shown that good EDA signals can be obtained from rats' backs.

The second aim of this study is to evaluate the feasibility of using EDA to predict CNS-OT events in humans. Specifically, we aim to measure either a non-significant change or a delayed significantly increased response in EDA activity after high-pressure HBO_2 administration to humans following a ketogenic and another HBO_2 after consumption of a normal diet (N=25); the order of diet consumption is reversed for the other subjects (N=25). For this aim, the changes in EDA in subjects (N = 50) undergoing cognitive stress at increased oxygen partial pressures and immersed (head out) in water will be observed in an experiment to be carried out in the "foxtrot" chamber pool at the Duke University Hyperbaric Center. Due to the human physiologic changes directly related to immersion in water, the risk of CNS-OT is greater when immersed, as compared to being in a dry hyperbaric chamber. The protocol that was previously used for studying the ketogenic diet for reduction of CNS-OT in working divers will be used.

Human subjects will undergo a cognitive stress test based on MATB-II software while being at elevated oxygen partial pressures in the "foxtrot" chamber pool pressurized at 2.06 ATA (35 FSW), for 30 minutes. Due to safety purposes, subjects will be seated in water to the shoulders, in a head-out position secured by a harness to prevent head submersion in the event of a convulsion or loss of consciousness.

Approximately four minutes prior to the task, participants will have one electrode per finger placed on the middle and index fingers of their non-dominant hands. The hand with electrodes will be out of the water; we may also investigate placing the EDA electrodes on the forehead.

Similar to the analysis of rats' EDA data, both time-domain and frequency-domain indices of EDA will be analyzed to observe the changes produced in the signal for subjects with ketogenic vs. normal diet.

Results

Preliminary results indicating the feasibility of measuring EDA in immersed condition can be seen in Figure 1. Figure 1 includes box plots for the obtained measures of EDA, for baseline and Stroop task stages. The Stroop task is known to induce cognitive stress as subjects were asked to mentally determine the color of a word which named a color. Fig. 1 incorporates the results of SCL, NS.SCRs, EDASympn and TVSymp for all subjects. The time-domain measures (SCL, NS.SCRs) were not significantly increased during the Stroop task, compared to baseline. The tonic component of EDA, which is used to compute the SCL, was highly variable in this study, compared to the mean. EDASympn (normalized power of the 0.045 to 0.25 Hz range) and TVSymp, our spectral-analysis indices of EDA, were significantly increased by the Stroop test, compared to their levels during the baseline stage.

Summary: If the EDA is proven to be sensitive to the autonomic changes during CNS-OT and seizures produced by HBO2 in the animal model and shown to be also reliable during increased oxygen partial pressures in humans, the technique has the potential to be used for detecting and/or predicting seizures caused by oxygen prebreathing to prevent DCS. Indices based on analysis of the EDA can be used to provide indicators of CNS-OT so that early warnings can be provided to Navy personnel and divers to avoid seizures or allow for appropriate management of the events. Moreover, the EDA has the potential to be used as a diagnostic marker of stress and/or fatigue, when used in conjunction with other measures such as heart rate variability. The long-term goal is to be able to embed EDA instrumentation and processing techniques in wearable devices that provide real-time signal analysis and produce early warning indicators of CNS toxicity and high-level stress experienced by Navy divers or other personnel. We envision the EDA being used to predict and detect not only seizures, but also dangerous levels of stress or fatigue Navy divers or personnel may experience.

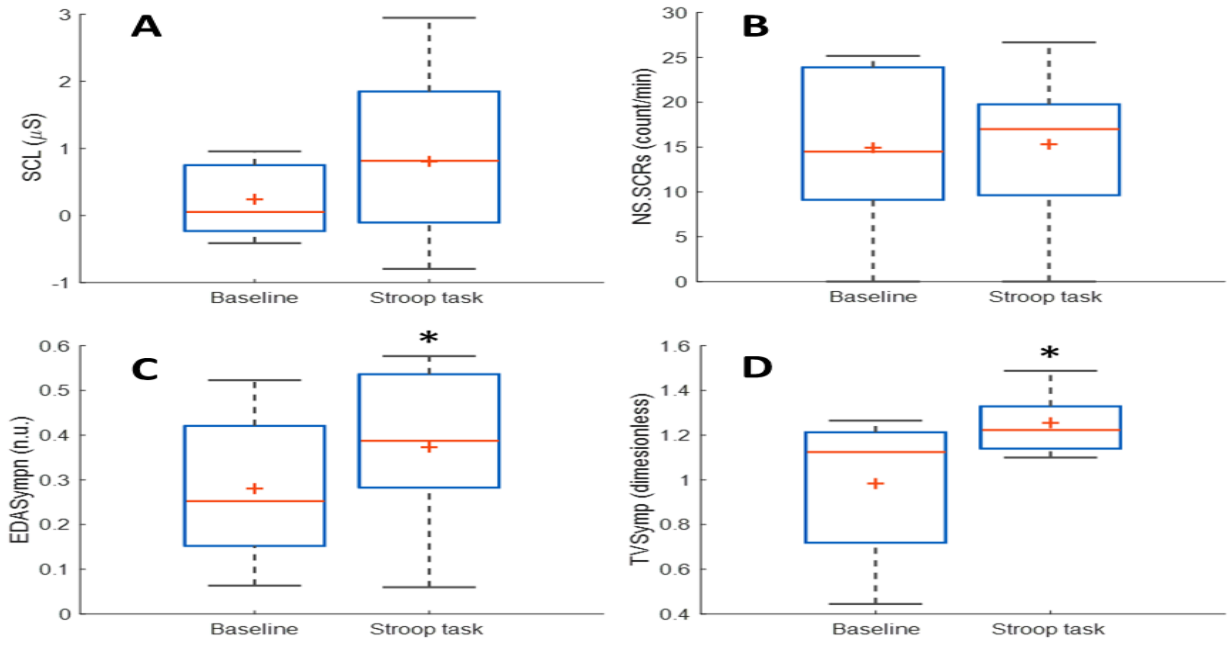

Figure 1. Box plots of the time-domain and frequency-domain measures of EDA for baseline and Stroop task stages, for N=14 subjects immersed in water. (*) denotes significant differences between stages. (A) skin conductance level (SCL), (B) non-specific skin conductance response (NS. SCRs), (C) normalized power spectra in the 0.045-0.25 Hz band (EDASympn), and (D) time-varying index of EDA (TVSymp).

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: ONR
Project Status: ONGOING

OPTIMIZING KETONE METABOLIC THERAPY AND IDENTIFYING BIOMARKERS FOR MITIGATION AND PREDICTION OF CNS OXYGEN TOXICITY: ANIMAL STUDIES

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Background

Naval Special Warfare and Special Operations diving maneuvers and submarine operations are limited by the potential for Central Nervous System-Oxygen Toxicity (CNS-OT) seizures (Sz). Several FDA-approved antiepileptic drugs (AEDs) are known to delay CNS-OT, and in general, work by suppressing CNS hyperexcitability. AEDs have adverse side effects that can compromise the safety and performance of the warfighter. Ketogenic metabolic therapy (KMT) is an alternative mitigation strategy against CNS-OT. We previously discovered that oral supplementation with the R,S-1,3-butanediol diacetoacetate ketone ester (KE) delays latency to onset of CNS-OT in the unanesthetized, freely moving SD rat model by nearly 600%. Our most recent studies (2018-2019) demonstrated that formulations of KE with ketogenic agents (KAs) like medium chain triglycerides (MCTs) offer advantages over KE alone in delaying CNS-OT (Ari et al., *Physiol. Reports,* 2019) and in producing anxiolytic effects (Ari et al., *JoVE*, 2019). It was determined that the effects of KAs are mediated, in part, via adenosine A1 receptor activation (Kovacs et al., *Front Behav Neurosci.* 2018). Thus, KMT represents a promising prevention strategy against CNS-OT, but to fully understand it's optimal, safe, and effective use in humans, several questions remain to be answered.

Objectives

Several candidate KAs are now in existence, so the optimal KA formulation and dose for delaying CNS-OT are largely unknown, as are their potential effects on cognitive or motor performance. The first goal of this project is to optimize the formulation and dosage of KA(s) that induce therapeutic ketosis for purposes of delaying CNS-OT (EEG measurement), without impairing cognitive or motor performance. Secondly, there remains a crucial need to identify early physiological predictors of CNS-OT onset, both within and outside the context of ketogenic intervention. Doing so may allow sufficient time to reduce inspired pO2 and safely avert Sz onset. Thus, the second goal of this project is to determine the effects of hyperbaric oxygen ± KA on presumptive physiological markers (e.g. respiration, body temperature) that can be monitored in real time to predict an impending oxygen Sz. Finally, the molecular mechanisms of the anti-convulsant effects of KMT in CNS-OT remain unelucidated, but are important for translating and advancing the use of KMT in humans. The third goal of this project is to study the anti-convulsant mechanisms of therapeutic ketosis by measuring expression of candidate signaling molecules in brains of animals that have been exposed to protracted HBO2 resulting in no Sz versus Sz. As designed, the proposed studies will answer crucial questions to benefit the United States Navy and field of hyperbaric medicine, thereby advancing the science and application of KMT for CNS-OT.

Methods

All experiments were performed in unanesthetized, freely behaving male Sprague-Dawley (SD) rats. Methods conducted in FY18 sought to determine the effects of acute ketosis on animal performance via quantitative behavioral testing of cognition (anxiety, learning and memory) and locomotion under ambient conditions (1 ATA normobaric air).

Performance behavior was tested through General Locomotor Activity on the Open Field (OF) and DigiGait (DG) tests. Cognitive behavior was tested through Learning and Memory Recall on the Novel Object Recognition (NOR) test. Anxiety was assessed using Light Dark (LD) and OF tests. All cohorts of animals were tested using all 4 tests over three consecutive days, apart from the 4th cohort where only the DG test was performed. The cohorts were organized in a way such that the one-time oral gavage was administered with the KE, water (control for ketosis), or no gavage (control for gavaging). The maximum number of tests given in one day was 2 interspersed with a 1.5-2 hour rest period. Testing containers and equipment were wiped down with 70% alcohol between tests to avoid remnants of rat odor or hormones from the previous animal that could influence the subsequent animal test.

Results

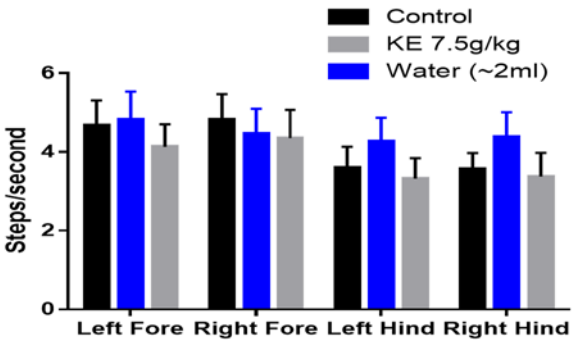

Figure 1. **KE-treated rats do not exhibit impaired motor performance.** Animal gait was quantified with multiple static and dynamic parameters using the DigiGait test. Stride frequency steps per second shown here. There were no significant differences between control, KE or water treated groups indicating that there was no impairment of KE or an effect of gavage.

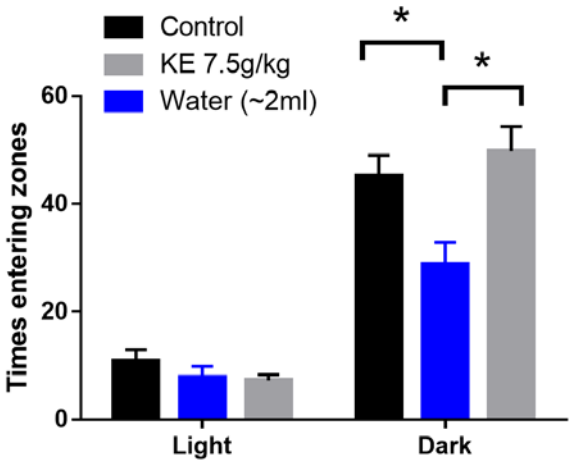

Figure 2. **KE-treated rats exhibit anxiolytic behavior** Increased duration, frequency, and shorter latency times to enter the dark zone, are said to be anxiolytic. Frequency of times animals entered zones shown here.

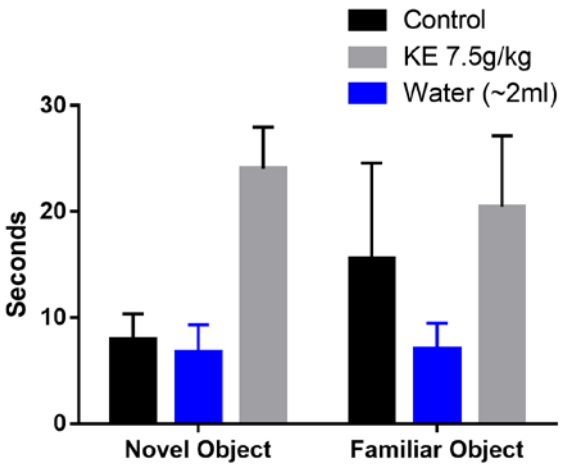

Figure 3. KE-treated rats exhibit a trend towards improved learning and memory. Learning and memory/recall capabilities of the animals are quantified with Novel Object Recognition tests. Learning is said to have occurred if the rats explore the novel object more frequently, for a longer amount of time and if they went to the novel object rapidly after the initiation of the experiment. NOR Latency to first interaction shown here.

Notes: Results demonstrate representative data from most significant findings from studies completed in FY18.

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: ONR
Project Status: ONGOING

HUMAN FUEL FOR OPTIMIZING COLD WATER PERFORMANCE

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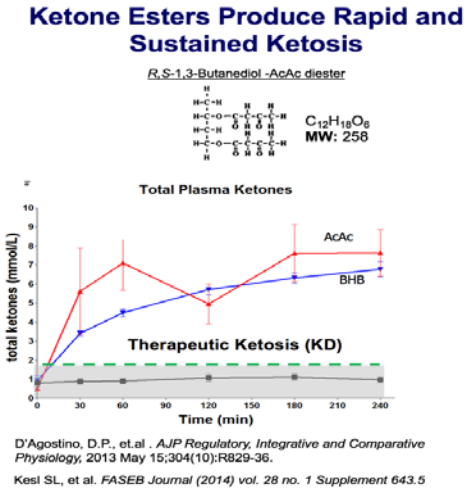
Background

Cold water operators undergo an extensive and unique set of physical, physiological, and psychological stressors during a mission. In-water transit may exceed 6 hours submerged in cold water, on rebreathers, and in a confined space. During this lengthy transit, operators must maintain vigilance over navigation, vehicle, and life support controls while exposed to the risks of mixed gas or oxygen rebreather diving, alongside hypothermia, dehydration, undernutrition, and other factors that can significantly degrade operator performance. Once the team has reached target, they must be at peak cognitive and physical readiness as they carry out their mission objective. When the mission objective is complete, the operators undertake the lengthy return trip back to the home. Optimized operator cognitive and physical performance is essential throughout all phases of a cold water mission, and safe and tolerable approach to optimizing warfighter performance and resilience will be key for future cold water operations.

The proposed study will investigate the cognitive and physical performance enhancing effects of two soon to be commercially-available, orally-administered, food-grade KE supplements. KEs causes a rapid and sustained elevation of ketone metabolites that is bioidentical to those found in food and results from rapid fat metabolism. Numerous studies have shown that ketones improve mitochondrial bioenergetics to enhance energy production more efficiently than glucose [1]. Because ketone bodies are small and water soluble, they can readily cross the blood brain barrier

and both enhance and preserve brain metabolism. Severe carbohydrate restriction and fasting induce nutritional ketosis (blood ketone level > 0.5 mmol/L) within 24-48 hrs; however, exogenous KE supplementation can induce rapid (< 30 minutes) and sustained ketosis (> 8hrs) in a predictable and dose-dependent manner [2,3].

Since ketones represent an effective metabolic fuel that readily crosses into the brain and recent research has demonstrated that ketosis improves spatial memory impairment caused by hypobaric hypoxia [7], in addition to conferring neuroprotective effects [8], it stands to reason that induction of ketosis with a KE supplement could be an optimal strategy for maximizing operator physical and cognitive resilience. Additionally, and specific to undersea cold water operational exposures, previous research has demonstrated that acute oral administration of a KE supplement significantly delayed CNS oxygen toxicity seizures [2]. Experts in underwater and



thermal physiology have also suggested that ketosis could be an approach to mitigating hypothermia in underwater operators given the increased thermogenesis and optimal adipose tissue storage associated with ketosis [9].

Methods

Task 1: Pharmacokinetic analysis of KE supplements. For this task, we will conduct a PK study with exercise for the commercially-available ketone supplement to identify optimal dosing and timing of physiological ketosis.

Task 2: Assess the effect of KE supplementation on a mission-relevant physical and cognitive performance task battery, developed in collaboration with Wright State Research Institute under the "Biotechnologies for Cold Water Performance" study. Based on the optimal dose and timing identified in Task 1 for each supplement, we will assess the effect of the KE supplements on mission-relevant performance in a subject cohort that matches the age, cognitive, and relative fitness level of the cold water operator population. In addition to performance metrics, we will evaluate core body temperature, peripheral body temperature by FLIR, and we will assess the degree of muscle glycogen depletion during the task battery.

Task 3: Transition the performance task battery and results from the KE supplementation assessment to field/dive

Results

The cold water task battery was designed based on results from operational task analysis combined with input from the cold water operational population and related human performance team leaders. The task battery was validated for its ability to induce changes in core and peripheral body temperature, manual dexterity, cognitive performance, and muscle glycogen stores. Task battery validation study data are under analysis at the time of this abstract and final results will be presented at Program Review.

Data detailing pharmacokinetics of the candidate ketone ester have been detailed from earlier studies. At the time of this abstract, evaluation of the ketone ester was in preparation following pilot assessments. Pilot data will be presented at Program Review.

We anticipate completion of the ketone ester evaluation and final study reporting no later than July 2018. The results from the cold water task battery and ketone studies will be communicated to relevant undersea performance laboratories for potential transition to field/diving studies by end of FY19.

Notes:

1) Cahill Jr, G. F. (2006). Fuel metabolism in starvation. Annu. Rev. Nutr., 26, 1-22.

2) D'Agostino DP1, Pilla R, Held HE, Landon CS, Puchowicz M, Brunengraber H, Ari C, Arnold P, Dean JB. Therapeutic ketosis with ketone ester delays central nervous system oxygen toxicity seizures in rats. Am J Physiol Regul Integr Comp Physiol. 2013 May 15;304(10):R829-36.

3) Kesl SL, Poff AM, Ward NP, et al. Effects of exogenous ketone supplementation on blood ketone, glucose, triglyceride, and lipoprotein levels in Sprague–Dawley rats. Nutr Metab (Lond). 2016; 13: 9.

4) Murray AJ, Knight NS, Cole MA, et al. Novel ketone diet enhances physical and cognitive performance. The FASEB Journal. 2016;30(12):4021-4032.

5) Egan B, D'Agostino D. Fueling performance: Ketones enter the mix. Cell Metabolism, Volume 24, Issue 3, 373 – 375.

6) Cox, PJ et al. Nutritional ketosis alters fuel preference and thereby endurance performance in athletes. Cell Metabolism , Volume 24 , Issue 2 , 256 - 268

7) Zhao, Ming et al. Ketogenic diet improves the spatial memory impairment caused by exposure to hypobaric hypoxia through increased acetylation of histones in rats. PLoS ONE 12.3 (2017): e0174477. PMC. Web. 3 June 2017.

8) Stafstrom, Carl E., and Jong M. Rho. The ketogenic diet as a treatment paradigm for diverse neurological disorders. Frontiers in Pharmacology 3 (2012): 59. PMC. Web. 3 June 2017.

9) A Study of Novel Approaches to Enhancing Warfighter Tolerance to Hypothermic Stress. Blue Sky Study Report. Editors Ken Ford and Dawn Kernagis. November 29-30, 201

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: ONR
Project Status: ONGOING

THE ROLE OF OXYGEN BREATHING ON CAROTID BODY SENSITIVITY, OXYGEN TOXICITY, AND PERFORMANCE IN DIVERS

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Background

Navy and special warfare divers are exposed to various physiological challenges during and following dive missions. In particular, blood oxygen content is elevated during dives, even at shallow depths. Exposure to high blood oxygen content could lead to maladaptation resulting in an increased risk of oxygen toxicity and carbon dioxide toxicity.

The carotid body chemoreceptors are the primary oxygen sensors in the body and are activated upon exposure to hypoxia and their activation is blunted when they are exposed to hyperoxia. The carotid body chemoreceptors are also activated during hypercapnia and, importantly, there is crosstalk between the carotid body chemoreceptors and central chemoreceptors. Blunting carotid body chemoreceptor activation during a dive due to elevated blood oxygen content could be a contributing factor for the increased risk of oxygen and carbon dioxide toxicity in Navy divers.

Navy and special warfare divers are also exposed to cold temperatures during their dive missions that cause a reduction in body temperature. Cold temperatures within the physiological range reduce *in vitro* carotid body chemoreceptor activity. Consequently, divers who are exposed to cold-water immersion for a prolonged time might be at an increased risk of oxygen and carbon dioxide toxicity due to reductions in carotid body chemoreceptor activity stemming from both cold body temperatures and high blood oxygen content.

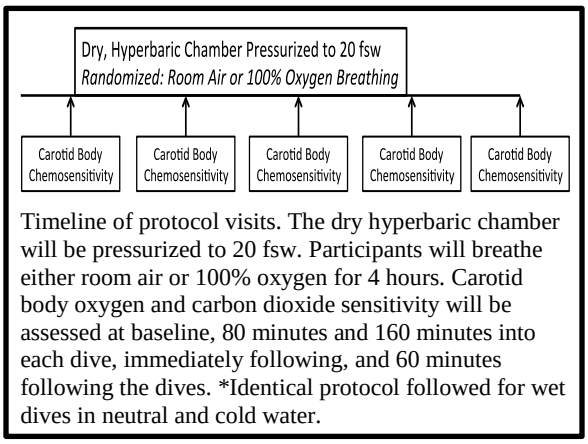
Objectives

Specific Aim 1: Determine if carotid body chemosensitivity is altered during and following a simulated dive and determine if breathing 100% oxygen during a simulated dive alters carotid body chemosensitivity when compared to breathing 21% oxygen. We hypothesize that carotid body chemosensitivity will be reduced during and following the simulated dives when compared to pre-dive values and carotid body chemosensitivity will be reduced during and following the simulated dives when divers breathe 100% oxygen when compared to breathing 21% oxygen.

Specific Aim 2: Determine if cold-water immersion alters carotid body chemosensitivity. We hypothesize that carotid body chemosensitivity will be reduced following a cold water dive when compared to a dive performed in thermoneutral water.

Methods

We will recruit two cohorts of men to participate in up to 6 visits (informed consent and screening visit (V1), baseline data collection (V2), and 4 dive visits (V3-V6)). Both certified scuba divers and non-divers will be recruited for Cohort 1 (dry diving) who will participate in V1-V4. Only certified scuba divers will be included in Cohort 2 (wet diving) who can participate in V1-V6. Our experimental set-up is shown in Fig. 1.



Timeline of protocol visits. The dry hyperbaric chamber will be pressurized to 20 fsw. Participants will breathe either room air or 100% oxygen for 4 hours. Carotid body oxygen and carbon dioxide sensitivity will be assessed at baseline, 80 minutes and 160 minutes into each dive, immediately following, and 60 minutes following the dives. *Identical protocol followed for wet dives in neutral and cold water.

Fig. 1. Experimental set-up to measure carotid body chemosensitivity (left) and the complete set-up in the hyperbaric chamber (right).

Results

We have completed data collection for Specific Aim 1 and a sample of the preliminary results (n=5) are presented in Fig 2.

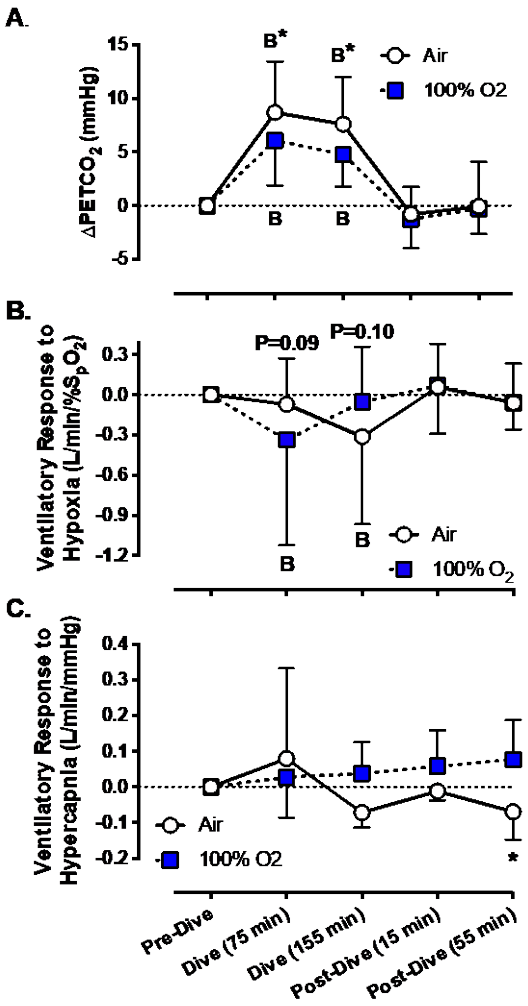


Fig 2. Preliminary results. The change from Pre-Dive values for end-tidal carbon dioxide ($PETCO_2$) (A), ventilatory response to hypoxia (B), and ventilatory response to hypercapnia (C) (n=5). B = different vs. Pre-Dive ($P<0.05$). * = different vs. 100% O_2 dive ($P<0.05$). Reported P-values are between condition comparisons.

Data extraction and analyses are ongoing as we prepare for manuscript submission in FY19. We are on track for Specific Aim 2 data collection in FY19.

Notes:

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: ONR
Project Status: NEW

EVALUATION OF THERMOREGULATORY DIFFERENCES BETWEEN INSULATIVE CLOTHING OPTIONS DURING COLD-WATER DIVING

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Background

Military divers are exposed to the extreme environment of the open sea, including extreme pressure and temperature variability, all the while being charged with operational tasks. Perhaps the most pervasive environmental factor that affects military divers is cold exposure and puts not only the operator but the mission at risk.

Objectives

To evaluate Yazbeck wetsuit for thermal protective capability during long duration cold-water exposure at various depths. To measure changes in skin and core temperature, as well as, cognitive function during a mock arctic training scenario

Methods: Active duty US Navy SEALs were recruited and participated in this effort that took place in two phases: Phase 1 was the cold pool in Hawaii (n=14) and Phase 2 (n=13) at NEDU in Florida. Dives of various duration (60-540 minutes) and depths (20-75'fsw) were conducted at 2˚C in wetsuits (16-17mm total thickness). Core, mean skin temperature, hand, foot, finger and toe temperatures were monitored as well as cognitive function (DANA).

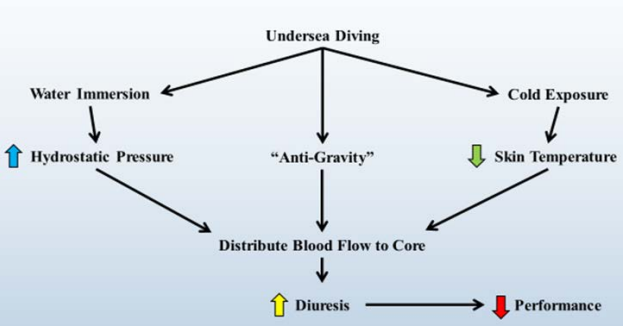

Fig.1. Simplified overview of the effects of cold-water immersion.

Results

Phase 1: There were no differences between pre- and post-evolution core temperature regardless of mission specialty or clothing selection (pre: 37.3 ± 0.6 vs. post: 36.8 ± 0.3°C; $P < 0.05$). Furthermore, when split by mission specialty, there were no differences between any time-point for PNs ($P > 0.05$). Moreover, there were no differences in core temperature at any time-point for MSs ($P > 0.05$); however, two individual MSs experienced significant decreases (< 35.0°C) in core temperature at 3.5 and 4-hours into the study (and one again at 6-hours). These two participants reached the clinical threshold for hypothermia (<35.0°C) during vigorous exercise during the land simulation portion of the evolution. Both participants were removed from the training evolution until they rewarmed their core above 35.0°C, at which time they were reintegrated into the training. Mean skin temperature, which is much more susceptible to cold, significantly decreased for all participants, regardless of position, over the 9-hour training evolution, (pre: 33.9 ± 0.9 vs. post: 28.5 ± 1.6°C; $P < 0.01$). All other skin temperature locations: face, arm, chest, thigh, calf, hand and foot, also experienced significant temperature loss ($P < 0.05$) over the course of the training evolution, regardless of position.

Phase 2: Due to the four diver per dive limit, statistical analysis was not conducted. However, core temperature was preserved across all dives ranging from 30-75 fsw. Mean skin temperature decreased as expecte. core temperature during cold water submersions and cold exposure at depths ranging from 30-75 feet sea water (fsw); however, there is a decrease in mean skin temperature, particularly in the extremities which resulted in the termination of 3 dives due to hand and/or foot temperature dropping to 10.0˚C (50.0˚F), which is the cut-off point to mitigate risk of non-freezing cold injury/nerve damage. Extremity temperature variation and severity of temperature loss can be attributed primarily to duration of dive, as opposed to dive depth or gas mix. The

coldest core temperature measured was 35.79˚C (96.42˚F) during the 75fsw dive on N2O2 post-dive when the diver was out of the water. The coldest mean skin temperature was 20.03˚C (68.05˚F) was observed post-dive during the 75fsw dive on HeO2. The coldest toe temperature was 7.2˚C (45.0˚F) was observed post-dive during the 50fsw dive on HeO2. The coldest finger temperature was 10.9˚C (51.6˚F) was observed post-dive during the 50fsw dive on HeO2. The ability to create predictive equations for the determination of physiological temperature changes based on dive depth, duration, and gas mix are in the early stages of development. These limited data suggest that with more data these predictive equations could be established and implemented. Preliminary data show trend; there are differences between the actual rates of change. The rate of core temperature change, regardless of gas mix, is plotted in Figure 7. Regression equations developed from the small sample sizes for each dive provide a glimpse of the predictive ability that may be achieved with more data. Even so, with these limited core temperature data, regardless of gas mix, under the conditions of each individual dive, the respective R^2 indicates that time accounts for 52.2 to 81.9% of the variance. When analysed by each gas for each dive depth, the R^2 values indicate that time accounts for 52.4 to 71.0% of the variance when breathing N_2O_2 and 44.8 to 80.5% of the variance when breathing HeO_2.

Thus, leading us to believe that with more data, a better fit line with higher predictive modelling may be achieved.

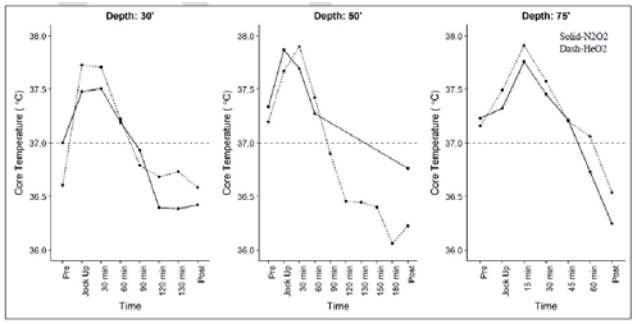

Fig.2. Core Temperature Changes over time and by depth

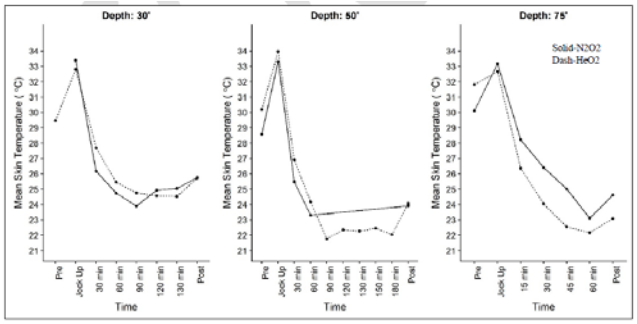

Fig.3. Mean skin Temperature Changes over time and by depth.

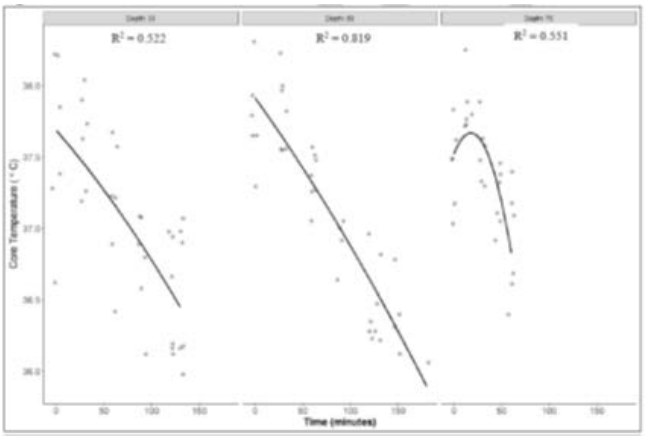

Fig.4. Rate of core temperature change by depth

Notes:

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: NAVSEA
Project Status: ONGOING

MEASUREMENT OF REGIONAL HEAT EXCHANGE USING DIRECT REGIONAL CALORIMETRY IN RESTING SUBJECTS IMMERSED IN COLD WATER

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Background

Navy Diver thermal protection, a primary concern in attempting or completing cold-water tasks, remains inadequate. Optimal heat distribution with minimal energy input is sought. Therefore, heating requirements that support thermal balance (TB) in various cold-water scenarios were quantified.

Objectives

Determine overall heating requirements and optimized regional heating strategies for mission-relevant diving operations.

Methods

Two phases were completed to 1) measure overall heating requirements when heat is provided to the diver during different immersion scenarios and 2) determine regional heating strategies that provide sufficient thermal protection while minimizing power consumption. **Phase 1** - Nine subjects participated in controlled 35°F and 50°F immersion water temperature experiments in a garment ensemble consisting of base layer, insulative undergarments, gloves, booties, and dry suit (total 1.3 Clo) together with active heating via a tubesuit heated to an inlet temperature of (89 ± 2°F or 102 ± 2°F).

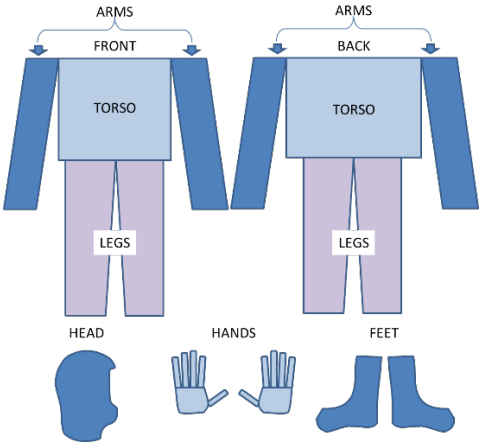

Fig.1. Six tubesuit zones: Torso, Legs, Arms, Hands, Feet, Head. Experimental scenarios are complete perfusion (phase 1) or partial combinations of these water perfused zones (phase 2). No heat was supplied to the head zone in phase 2.

Phase 2 – Eight subjects were immersed in an identical dry suit ensemble as phase 1. Tubesuit inlet temperature, 100°F; immersion water, 35°F. Independent variables were a combination of anatomical locations in which heat was applied: arms (A), legs (L), and torso (T) [hands and feet were always heated (H and F, respectively)]. Ergo, 1a) ALHF, 1b) LHF, 2a) TAHF, 2b) AHF, 3a) THF, 3b) HF.

Fig.2. Tubesuit with thermistors placed. electrical tape holds tubing in place, bandage wrap is used for T-8 placement on back as well as tubesuit legs garment support..

Results

Phase 1 - Each subject achieved thermal balance (TB, ≤ 2hrs immersion). TB was defined as temperature equilibrium where core and mean skin temperatures varied ≤ 0.2°F over 20 minutes time. No cold-effect termination criteria were achieved as skin temperatures all remained well above 53.6°F and core temperature above 95.9°F. Therefore, the 89°F tubesuit in 35°F water (coldest immersion water with minimal active heating support) was determined to be adequate for 2 hours of immersion time and demanded 236 Watts of power. The 89°F tubesuit in 50°F water used the least power at 168 Watts. The 102°F heat supplied tubesuit resulted in greater overall skin temperatures and greater retention of stored body heat at the cost of approximately double the power of the 89°F suit

when compared in the same immersion water temperature scenario.

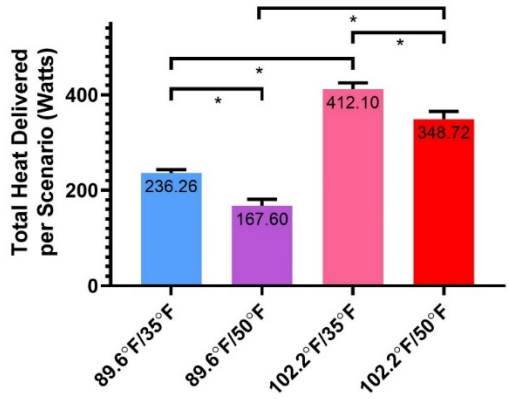

Fig.3. Heat delivered via the tubesuit per cold water scenario total Wattage. Colors in this illustration are used repetitively to identify tubesuit heat and immersion temperature scenarios. [*] = p< 0.05

Phase 2 - ALHF was the only group to reach TB and was therefore the standout "best" scenario including maintenance of core and skin temperatures. The other scenarios that did not reach TB, however, did not reach termination criteria either. Therefore, although those groups remained in a more thermally dynamic state, no absolute reason existed to remove the remaining scenarios from consideration as ideal for mission settings. Namely, the LHF arrangement supplied significantly less Wattage than did ALHF and TAHF, yet maintained core temperature and was 2nd best at maintaining mean skin temperature in a tie with TAHF. Metabolic compensation of groups AHF, THF, and HF would be a concern in the field as subjects shivered continuously and were visibly and notably tired upon surfacing.

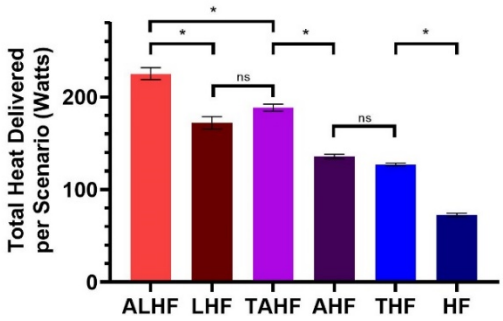

Fig.4. Heat delivered via the tubesuit per cold water scenario total Wattage. Colors in this illustration are used repetitively to identify tubesuit heat and immersion temperature scenarios. Average total heat contribution (Watts) per scenario. [*] = p< 0.05

Conclusions

Phase 1 - This line of experimentation made clear the energy demand necessary to achieve thermal balance for support of long-duration resting immersion in cold-water environments of 35 and 50°F in a dry suit garment ensemble of 1.3 Clo that is highly relevant to current field operations. All subjects achieved thermal balance (TB) and most reported as not feeling thermally challenged (e.g. uncomfortable due to cold). Thermal data showed that minimal or no extra metabolic compensation was necessary for these active heat supplemented cold-water diving scenarios. All scenarios were safe as no termination criteria were approached.

Phase 2 - When power is limited or for technologies where whole-body heating is not currently feasible (e.g. electric resistive garments), employing regional heating strategies may improve thermal protection and reduce power consumption.

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: NAVSEA
Project Status: NEW

EPIDEMIOLOGICAL ANALYSES OF U.S. NAVY DIVER SEPARATION HEALTH ASSESSMENTS

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Background

Navy divers face unique occupational stressors and extreme physical demands as part of their job. These stressors include exposure to: hydrostatic pressures, extreme temperatures, increased partial pressures of inhaled gases, contaminated water, dangerous marine life, underwater blasts and noise. Some of the short term effects of these occupational hazards have been studied, including decompression sickness, hypoxia, hypercapnia, arterial gas embolism, pneumothorax, hypothermia and high pressure nervous syndrome. Little is known, however, about the long-term health of Navy divers. This is a supplemental study to the Diver Health Epidemiology Program.

Objectives

1) Describe the overall health status of Navy divers upon separation to provide insight into past and future risk reduction measures. Catalog illnesses and injuries by prevalence. Identify correlations and potential risk factors for common or severe conditions.
2) Contribute to the ongoing broad effort of developing a longer term population-based health study of Navy divers, with the goal of better understanding the long-term health effects of diving specific occupational risks and exposures.
3) Contribute to a Navy diver SQL server database currently in the early stages of development.

Methods

Available data sources will capture the health records of approximately 10,000 current and retired U.S. Navy divers. The Naval Safety Center's Dive/Jump Reporting System (DJRS) will be used to identify divers to cross reference in other data sources. A method will be developed to identify all separation health assessments for the identified divers in DoD data systems (e.g. TMA/DHA). These records will be used to create an analytical data set incorporating dive logs, personnel files, and separation health assessments. That dataset will be used to create a report that catalogs the specific lifetime health conditions by prevalence of Navy divers at the time of separation. To support this effort, requests to access VA data will be sent and an initial analysis of the available data via a descriptive report will be produced.

Milestone/Quarter	1	2	3	4
Access Naval Safety Center's Dive/Jump Reporting System (DJRS) data, identify divers	■			
Request data from VA	■			
Link available data		■		
Test relational databases			■	
Create analytical data set, analyze data				■
Produce report				■

Fig.1. Milestones for the four quarters of the 1 year project.

Results

Results are pending. This proposal was accepted by NAVSEA in February 2019 and work has begun. The Naval Safety Center (NSC) is in possession of the NSMRL CO signed MOU to requesting access to the pertinent NSC data. BUMED has received the needed documentation for granting access to diver health records.

The views expressed in this presentation reflect the results of research conducted by the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the U.S. Government.

The study protocol was approved by the Naval Submarine Medical Research Laboratory Institutional Review Board in compliance with all applicable Federal regulations governing the protection of human subjects.

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: NAVSEA
Project Status: ONGOING

OPTIMIZING PERFORMANCE DURING TOPSIDE OPERATIONS AND DIVING AT ALTITUDE

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Background

US military ground operations can be conducted at medium and high altitude. Mission needs can require fighters to be flown immediately to altitude without time for adjustment or adaptation. One of the normal physiological compensations of altitude is hyperventilation to preserve blood oxygenation. The increase in respiratory work provides some protection against altitude-related disease but can also impair performance due to respiratory muscle fatigue. Problems can quickly develop on land with exercise, but can also be seen underwater should diving activity be required.

In previous work in our and other laboratories respiratory muscle training (RMT) has been shown to increase respiratory strength and endurance making respiration more effective and extending exercise performance as much as 68% at the surface and as much as 86% at a depth of 120 fsw. There is little information available on the decompression stress of divers working at altitude, particularly diving that begins immediately upon arrival. This proposal will examine the effects of RMT on performance during topside operations and during diving at altitude. It will also explore the decompression strain that occurs after diving at altitude by assessing venous gas bubbling after diving at 3658 m (12,000 ft) of altitude.

Objectives

To extend the findings from previous studies, this proposal will test the hypothesis that voluntary isocapnic hyperpnoea training (VIHT) will improve exercise performance during acute exposure to a simulated altitude of 3,658 m (12,000 ft). We hypothesize that improved exercise capacity of the respiratory muscles will allow the hypoxia-induced hyperventilation to be sustained for a longer time exercising at attitude. We will also determine if the performance enhancing aspects of resistance respiratory muscle training (RRMT) for divers are maintained after exposure to attitude-induced respiratory distress. Lastly, in an exploratory aim we will examine the degree of decompression stress associated with diving at altitude by examining intravascular venous gas bubbling after a dive initiated at 3600 m.

Methods

Volunteer subjects (n=30) will receive active VIHT, RRMT, or sham RMT (controls). VIHT has been selected as it primarily improves respiratory muscle endurance and has been shown to be most effective for conditions without increased gas density. RRMT has been shown to provide the greatest benefit at depth. To determine the best RMT method at altitude VIHT and RRMT will compared to each other, as well as to a control group. If RRMT provides some protection from respiratory muscle fatigue during topside operations it could become the preferred choice for divers operating at altitude.

For Aim 2, ten male divers will be recruited to complete the study. Subjects will be decompressed to a simulated altitude of 12,000 ft in our hyper-/hypobaric chamber and remain there at that pressure for one hour before beginning the dive. Subjects will enter the wet portion of the chamber and complete a simulated dive to the pressure equivalent of an actual depth of 55 fsw.

Results

14 subjects have completed Phase I and the full complement of remaining subjects is on protocol. Conditioning and procedures for Phase II are being pilot tested and will begin in June.

Notes:

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: NAVSEA
Project Status: NEW

DOES HEART RATE VARIABILITY PREDICT IMPAIRMENT OF OPERATIONAL PERFORMANCE IN DIVERS?

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Background

This application proposes to validate or repudiate Heart Rate Variability (HRV) as an operational performance indicator and early warning sign for inert gas narcosis and oxygen toxicity.

Inert gas narcosis and CNS oxygen toxicity are operational risks for all divers. Better defining the underlying human physiology of these conditions at depth will allow development of systems and practices to allow the working diver to take corrective action to avoid cognitive impairment during a dive. The recently completed NAVSEA study, N0463A-12-C-001, Hypercapnia: cognitive effects and monitoring, tested 42 human subjects fitting Navy age and fitness profiles under 20 distinct conditions of gas partial pressure and exercise by using a multi-tasking NASA flight simulator (MATB-II) to measure cognitive performance. During this study robust narcosis and oxygen-related impairment signals were observed.

Objectives

The proposed study will assess HRV's utility as an early warning sign for narcosis or oxygen toxicity related cognitive in realistic diving environments. If HRV is shown to be a reliable operational performance predictor it could aid mission planning and reduce risk. Warning devices based on HRV algorithms could be developed if indicated.

Methods

All useable data from N0463A-12-C-001's files will be analyzed. No new data will be created. There are 512 individual experimental stages in the dataset. ECG, hemodynamic and respiratory readings were digitally captured using ADInstrument's LabChart 7 Pro software (Colorado Springs, Co) using an analog-to-digital data acquisition board connected to a dedicated study computer. Each experimental stage is 5 minutes in length. Timed segments of electronic electrocardiogram or arterial line tracing data will be selected, analyzed for HRV and correlated with breathing gas partial pressures, exercise state and MATB-II scores (cognitive testing results). HRV data will be calculated from the original LabChart data files.

Electrocardiogram tracings will be manually selected for each experimental stage and saved as separate LabChart files. Arterial line tracings will be substituted where electrocardiogram tracings are not of adequate quality for analysis. Dr. Ki Chon's laboratory at the University of Connecticut (UCONN) will perform the final HRV analysis. All HRV data sent to UCONN will be de-identified. The percent change will be dichotomously categorized as narcosis and/or toxicity if there is greater than a 10% performance decrement compared to baseline or a stage failure.

Timeline events

A. Prepare Data Sources for Analysis (6 months)
- 1) Extract HRV data and link it by time interval to corresponding experimental stages
- 2) Clean and process the HRV signal, create feature vectors
- 2 define mathematical boundaries for cognitive testing outcomes
- 3 apply mathematical boundaries to assign cognitive testing outcomes for each stage

B. Assess the timing and association of HRV with Cognitive Testing Results (6 months)
- 1) create Feature Vectors (FV) of physiologic and cognitive data for every protocol stage
- 2) employ principal dynamic mode analysis of HRV segments
- 3) employ supervised machine learning algorithm

C. Final Report: (3 months)
- 1) Assess the association and timing of HRV changes with psychomotor performance
- 2) Complete a Final Report

Results and Products to be delivered:

The Navy will receive a report detailing the utility of HRV as an early predictor of narcosis and CNS oxygen toxicity under the conditions of N0463A-12-C-001. A scientific article will be submitted to a peer-reviewed journal**.** The award funding began 1/29/19. FY 2019 results are pending.

Notes:

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2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: NAVSEA
Project Status: NEW

DATA SCIENCE-DRIVEN RESEARCH TO IMPROVE OPERATIONAL GUIDANCE AND HUMAN SAFETY FOR IMMERSION IN WARM WATER ENVIRONMENTS

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Background
Through Fleet operational requirements and research performed by Navy Dive biomedical institutions, the US Navy established the need to improve the understanding, prediction, and mitigation of the physiological and cognitive effects of immersion. This research included thermoregulation experimentation and development of assessments to measure cognitive and endurance effects of exposure at varying temperatures under diving conditions.

Over a period from 2001-2003, Naval Sea Systems Command's (NAVSEA) Navy Experimental Diving Unit (NEDU) conducted extensive research in this domain, yielding several key conclusions and identifying critical knowledge gaps related to the safety of dive operations in warm water environments. As noted by NEDU, "free-swimming, unmonitored divers may exceed their physiological limits and continue to work to the point of becoming hyperthermic and incapacitated" [NEDU TR 03-11]. NEDU also provided recommendations for future investigations and improvements to operational guidance. NEDU suggested that "further investigation is needed to establish safe limits for dive operations in warm water environments," and that "[future] development is needed for a next-generation battery of tests that simulates both the cognitive and physical skills as well as the abilities required by divers in real operations (Next-generation SINDBAD)."

Objectives
The goal of this project is to use advanced data analytics methodologies to improve forecasting of overheating in divers operating in warm water environments, where water temperatures range from 94 - 101.5 °F (34.4 - 38.6 °C). Improved forecasting will enable the refinement of exposure guidance for diving, reducing risk and improving overall diver safety.

Recent literature demonstrates the utility of graph databases and graph analytic techniques to uncover previously unknown relationships in data; example biomedical applications of these techniques range from identification of interdependencies between genetics and cancers to drug repurposing. This project will leverage modern data science technologies such as graph database technology (Neo4j) and the JHU/APL SOCRATES scalable graph analytics platform to analyze existing data from immersion research conducted by NEDU. This project aims to uncover critical factors affecting physical and cognitive performance, and physical endurance of divers in warm water.

Methods
The project will be generally structured using the Systems Engineering Process and associated three-phase V model of (1) Problem Decomposition and Definition, (2) Solution Implementation, and (3) Integration.

The initial phase of the project involves collaborating with NEDU and other dive research entities to identify reports and suitable data sources for ingestion into the advanced analytics platforms. In the following two phases, data will be ingested and continuously explored and modeled using advanced analytics platforms and databases. Findings will be reported to NAVSEA.

Results

The project is underway, and initial collaborations with NEDU have been established

Notes:

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: NAVSEA
Project Status: ONGOING

DEVELOPMENT OF A PORTABLE DOUBLE-LOCK FLEXIBLE AND COLLAPSIBLE RECOMPRESSION CHAMBER FOR CONDUCTING TREATMENT IN ACCORDANCE WITH NAVY TREATMENT TABLES TO 165 FSW

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Background

This Project relates to the development of a 42-in diameter, lightweight, foldable, double lock, multi-occupant hyperbaric chamber capable of U.S. Navy treatment tables to 165 fsw, for supporting hyperbaric treatment applications in remote locations.

PCCI and SOS Hyperlite previously developed the Emergency Evacuation Hyperbaric Stretcher (EEHS) which has proven itself to be an ideal solution in applications where a compact, portable and lightweight hyperbaric chamber is required. It is in operational service with the US Navy, Army, Air Force, Special Operations, DHS-Customs & Border Protection, DHS-Coast Guard, FBI and NOAA. The major disadvantage of this compact design is that hands-on medical care is not possible. A double lock, multi-occupant chamber would overcome that problem. Current EEHS technology utilizes latest technology polymer fibers. This new technology has the advantage of being scalable, creating the potential to engineer a three occupant, dual compartment chamber, that is sufficiently economical in terms of weight and volume for use on smaller vessels and in the various remote locations around the world where US Navy divers operate.

PCCI and SOS Hyperlite made significant progress towards the development of a 42-inch diameter chamber capable of U.S. Navy treatment tables to 3 ATA under a Small Business Innovative Research contract from NOAA. That effort was advanced further towards the goal of a 42” diameter 6 ATA rated chamber under a prior BAA contract from NAVSEA.

Those contracts led to the production of a testable prototype as shown in Figure 1.

The purpose of this project is to continue the development to the point where an operational, fully USN certified system capable of supporting 3 occupants is produced and delivered.

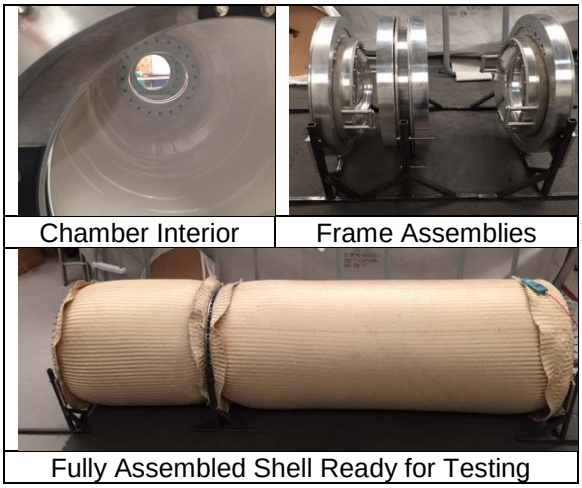

Figure 1: Current Prototype Pressure Shell

Objectives

The intent of this effort is to complete the development and commercialize a chamber to fulfill the need for a compact, portable, and light weight hyperbaric chamber designed to accommodate three occupants and which can provide the US Navy with a system that will address mission requirements and meet the needs and requirements of Navy divers. The double lock unit will allow the transfer of medics in and out at any time to perform full hands-on medical care and treatment for even the most critically ill patients, while delivering therapeutic gases through built-in breathing masks.

The unit will be capable of treatment in accordance with the Navy Treatment Tables to 165 fsw. When not in use, it will collapse and separate to a set of cases that can be readily stored and transported.

The maximum theoretical strength of the Vectran fabric tube assembly used is approximately 594 psig. By the usual rule-of-thumb, the factor used to predict actual achievable pressure is about 50% yielding a predicted maximum useable pressure of 297 psig. In prior tests, burst pressures as high as 440 psig (73.7% of max.) have been achieved at 70 ^{o}F and as high as 386 psig at 100 ^{o}F (64.6% of max.) with 360 psig at 100 ^{o}F (60.1%) being achieved consistently. In

all cases the event initiating failure was braid slippage through the clamp. One of the major sub-objectives of this project was to improve the performance of the braid clamp so that the burst pressure tests consistently achieved 386 psig or more at 100 ^{o}F, with the event initiating failure being braid failure instead of slippage through the clamp.

Another sub-objective was the development of criteria for the amounts of damage to the braid and/or bladder assemblies can sustain and still remain in service. Included was a test to demonstrate non-catastrophic failure following penetration by an M16 round while fully pressurized and the testing of a patch kit.

Methods

In order for the system to be approved for human occupancy it must meet the requirements of ASME PVHO-1. However, the materials and construction methods required represent new technologies not previously addressed by established pressure vessel codes. Consequently, a PVHO-1 Case was submitted for the material and construction methods required. It has been approved as PVHO-1, Case 18.

The major components of the system include; high strength braided fabric tube, gas-tight bladder, aluminum end frames and doors, a clamp for securing the fabric to aluminum frames, base/case system, air control system, pre-inflation supports, interior frame and ancillary items. All components require some degree of engineering and development.

The primary focus of the development efforts in this project were to a) increase the holding power in the clamps and b) reduce weight wherever possible.

Finite element analyses were used to confirm that the stresses in all metallic structural members are within allowable stresses defined by PVHO. However, for the flexible braid, FEA is not applicable. To confirm that the braid and the braid clamping system are adequately designed and that the construction processes used are reliable, the chamber was placed through a rigorous testing program with principal elements as follows:

- Establishment of MAWP by testing a number of full-size shells to a specified multiple of MAWP without failure. To achieve an MAWP rating of 180 fsw (80 psig), the number of consecutive successful proof tests that are required is as follows;

No. Tests	Ratio of Proof Pressure to MAWP	Required Proof Pressure (psig at 100 ^{o}F)	Req'd clamp holding power (lbf per inch
1	6	480	6,858
2	5.502	440	6,287
4	5.045	404	5,772
6	4.796	384	5,486
8	4.627	370	5,286
10	4.499	360	5,143
15	4.277	343	4,900

- Creep testing for a minimum of 300 hours at 3.64 x MAWP (291 psig) for a minimum of 5 successful tests.
- Cyclic hydrostatic testing to 1.5 times MAWP to a minimum of 4,000 cycles.
- Cyclic folding test to a minimum of 500 cycles
- Cold temperature tolerance test
- Off-gassing tests.

Results

The improved clamp design did not slip under any of the conditions tested. Reliable and reproducible assembly techniques were also developed. However, the proof pressure that could be achieved consistently without failure remained at 360 psig due to an increasing probability of braid failure once the test pressure passed 370 psig. As of the date of this document, 6 consecutive successful proof tests had been achieved with the Main Lock and 9 with the Entry Lock. Under Case 18 rules those support MAWP ratings of 75.1 psig (169 fsw) and 79 psig (176 fsw) respectively. The add'l tests required to reach MAWP ratings of 80 psig are pending.

The creep test, cyclic hydrostatic tests, cyclic folding tests and the cold tests all met Case 18 requirements. The off-gassing test are pending.

The M16 test produced, as expected, a set of small holes, one on each side of the chamber. The holes were sealed with patches made from bladder material and the chamber pressurized to 150% of MAWP without incident.

The results obtained to date support a Case 18 MAWP rating of 75.1 psig (169 fsw). Additional pending tests are expected to increase that rating to 80 psig.

The estimated production shell weights for the ML and EL were reduced to 331 lbs and 304 lbs respectively.

Notes:

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: NAVSEA
Project Status: NEW

DEWEY MONITOR: DEVELOPMENT OF A PULSE OXIMETER TO INDEPENDENTLY MONITOR OXYGEN LEVELS IN REBREATHER DIVERS

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Background

Rebreather diving has one of the highest fatality rates per man-hour of any activity in the world. The leading cause of death is hypoxia, typically from equipment or procedural failures. Hypoxia causes very few symptoms prior to causing loss of consciousness. Additionally, since the electronics responsible for controlling oxygen levels in rebreathers often control their alarm systems, frequently divers do not receive any external warnings prior to loss of consciousness.

Pulse oximetry is a robust, inexpensive technology with decades of use in clinical settings. It uses light to measure the oxygenation levels of a person's blood in a non-invasive manner. The hypothesis of this project was to see if pulse oximetry could provide a simple alarm system that measures the person rather than the rebreather. A previous pilot study, performed in the dry, showed that pulse oximetry provided roughly 49 seconds (± 17) warning time to a diver on a failed UBA Mk 16 with falling levels of oxygen saturation. This warning time was determined to be of practical length to take any one of a number of possible corrective actions. However, this study had limitations, such as being performed in air rather than immersed in water, having a small number of data points, and being restricted to one set of temperature and exercise conditions.

Objectives

The objectives of the current project were divided between Duke University and collaborators Naval Surface Warfare Center Panama City Division (NSWC PCD). The overall project objectives were:

1) Develop a COTS pulse oximeter for use underwater (NSWC PCD)
2) Characterize the warning time provided to a diver by the oximeter under a variety of physiologically relevant diving conditions (Duke)
3) Publish the scientific results to allow application of the measurements to other models of pulse oximeter (Duke) and enable transfer of technology (NSWC PCD).

These objectives are unchanged from the original proposal.

Methods

The test population is comprised of healthy, fit, nonsmoking human subjects ages 18-40. This population was selected to be representative of military divers.

To qualify for the study, subjects need to pass a comprehensive screening process. The screening includes a physical exam to assess fitness to dive, a pressure test in the hyperbaric chamber, a familiarization dive with the full face mask in the test pool, and a VO_{2max} exercise test to measure the subject's maximum level of oxygen consumption. Subjects are enrolled if they pass all exams, including a minimum oxygen consumption rate of 30 mL/kg/min for female subjects and 35 mL/kg/min for male subjects in the VO_{2max} test.

A single test sequence begins with the subject breathing air and riding a horizontally positioned, underwater bicycle ergometer with the pedal resistance set to target the desired rate of oxygen consumption.

The subject breathes air from an open circuit gas source (air) for 5 minutes to reach a steady state of oxygen consumption. Next, mixed expired gas is collected and minute ventilation measured, for later verification of O_2 consumption and CO_2 production rates. The subject is then switched to the mouthpiece of an Innerspace Megalodon rebreather (in Navy use as the UBA Mk 28) with a disabled oxygen addition system, so the subject will gradually consume the gas in the closed loop while continuing to exercise.

The subject will be instrumented with four oximeters: one on the forehead using a traditional attachment method, another on the forehead using the bracket designed by NSWC PCD to fit inside the mask skirt, a third on the scapula, and a fourth on the sternum. The placement of the sensors on the scapula and sternum allow for measurements for potential future sensor models at these locations; therefore, if operators from different communities want sensors at different locations, they can develop such a sensor without repeating the human testing. Arterial blood gas samples are also taken to verify the oximeter readings at key points during the study, specifically: prior to being placed on the rebreather, when the inspired oxygen partial pressure is 0.12 atm, and at the time of the test sequence termination (termination criteria are $SpO_2 \leq 80\%$, inspired $PO_2 \leq 0.08$ atm, or failure to respond to visual cue).

The first 10 subjects will experience four sequences, three of which are at surface atmospheric pressure: cold water (20°C) with 2 L/min O_2 consumption, cold water with 0.7-1.0 L/min consumption, and warm water (28°C) with 2 L/min O_2 consumption. One test will occur in cold water at a depth of 77 fsw, O_2 consumption rate 2 L/min. However, if after 10 subjects there is no depth-dependence in the warning time, the fourth test will be eliminated for the remaining 20 subjects.

Subjects will also be required to respond to a randomly blinking light to show cognitive responsiveness. “Useful warning time” will be defined as the time between 95% and 80% saturations on the forehead oximeter in the bracket, or the time from 95% to failure to respond to the light signal.

Results

The work plans for the NSWC PCD objective (design and construction of a forehead-mounted, waterproof pulse oximeter) have been completed, and the oximeters have been delivered to Duke as of the start of 2019.

The oximeters have been successfully integrated into the hyperbaric equipment at Duke, including fitting the masks to the rebreather. Human test subject recruitment and screening have been ongoing, with 7 successful screenings in March. Studies for those subjects are scheduled for completion in April and May of 2019. As of March, an additional 14 subjects have also been recruited and are scheduled for screenings in April and May.

Fig.1. Modified Megalodon rebreather. Customized to accept gas from multiple sources and accommodate two forehead pulse oximeters.

Notes:

2019 ONR Undersea Medicine & NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: ONR/NAVSEA
Project Status: ONGOING

DIVERS AUGMENTED VISION DEVICE

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Background

Military diving operations are conducted in one of the most inhospitable environments on the planet characterized by high turbidity and zero visibility. Divers have a critical need to view life support and sensor data in real time, regardless of the ambient visibility conditions. A visual display is the necessary means of providing that data to the diver, unfortunately handheld displays or displays built into sensors can be virtually useless in a high turbidity underwater environment. A see-through head-up display (HUD) integrated into a diver's facemask and dive helmet would provide such a capability.

Emerging waveguide optical display technology has the potential to provide this capability.

The Office of Naval Research (ONR), working in collaboration with Naval Sea Systems Command (NAVSEA), provided funding and tasking to Naval Surface Warfare Center-Panama City Division (NSWC PCD) for the Diver Augmented Visual Device (DAVD) Phases 1, 2, and 3 to develop this capability. Phase 1 comprised research into display technologies, down-selection of waveguide optical displays, and laboratory testing with end users.

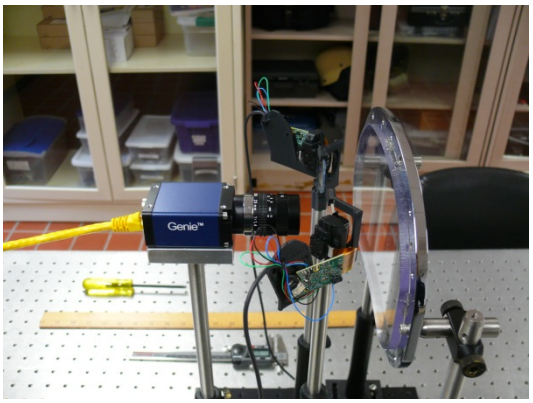

Fig.1. Waveguide Display Systems. The Waveguide optical display modules are characterized and optimized for FOV, Focal Distance, and Image Conversion Angles.

Phase 2 comprised design, development, and manufacture of two (2) initial prototype systems for limited in-water evaluation.

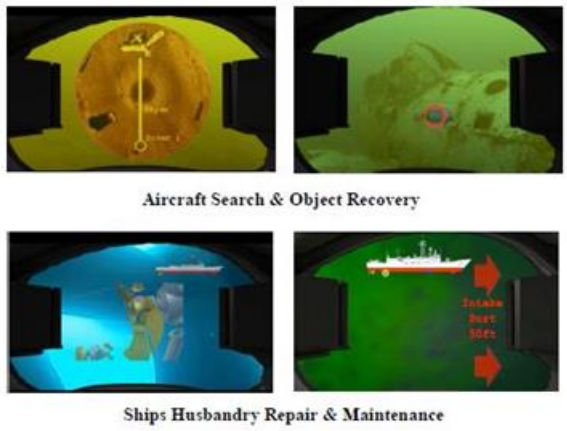

Fig.2. Laboratory AR Display Imagery. AR images are displayed on the waveguide modules for evaluation by end-users in the laboratory.

Fig.3. DAVD Ph2 for MK20 FFM. CDR Cameron Chen (CO of NDSTC) prepares to dive the DAVD prototype configured for the US Navy MK20 FFM at NDSTC.

Phase 3 (FY 2018-FY 2019) is ongoing.

Objectives
The objectives for DAVD Phase 3 are to conduct additional in-water testing and evaluation of the prototype system with military, first responder/public safety, and NASA divers; and based on the feedback on system design and functionality, complete the final design of the Phase 3 production system. To accelerate this final Phase 3 design and transition to production, a Cooperative Research And Development Agreement (CRADA) was signed with industry partner Coda Octopus Group, Inc. to jointly develop the final production version system designated DAVD Gen 1.0.

Methods
In-water test methods include representative dive missions that include navigation to various target locations, object identification & recovery, and using augmented reality overlay images for training. Feedback from divers will include readability of text and images, field-of-view, clarity of images, and overall utility for real dive missions.

Results
In-water testing of the Phase 2 prototypes was successfully completed by military divers at Mobile Diving & Salvage Unit One (MDSU-1) Joint Base Pearl Harbor. Public Safety Divers evaluation was completed by Florida State University-Panama City's Underwater Crime Scene Investigation Unit in Panama City.

Fig.4. DAVD Ph2 for KM37 Dive Helmet. Navy diver ready to test the DAVD prototype configured for the US Navy KM37 helmet at MDSU-1 Joint Base Pearl Harbor.

In-water testing by divers from NASA is scheduled for June 2019 at the Aquarius Habitat in Islamorada, Florida to determine if the DAVD system can be incorporated into training regimens for astronauts conducting extra vehicular activity (EVA) missions.

Additional planned efforts include development of a collaboration project between NSWC PCD and NASA-Johnson Space Center for the joint development of a next-generation projection based see through HUD capability for US Navy diving helmets, US Navy 1-Atmosphere Dive Suits, and NASA Extra Vehicular Activity (EVA) Space Suits.

Notes:

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: ONR
Project Status: NEW

ADVANCING DIVER-ROBOT INTERACTION CAPABILITIES

assoc. prof. Nikola Mišković; prof. Iain Anderson; Đula Nađ, PhD; Christopher Walker; Igor Kvasić; Derek Orbaugh Antillon

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Background

The underwater environment is considered hazardous for humans since they depend on technical equipment for life support. Divers have limited navigation and communication capabilities. Further, the underwater environment is under constant influence of environmental disturbances which additionally impacts diver performance.

Aiming to enhance human-machine teaming and improve decision-making speed and quality during demanding technical dives despite environmental difficulties, the A*dvancing Diver-Robot Interaction Capabilities (ADRIATIC)* project builds upon recent accomplishments in the area of diver-robot interaction and wearable sensor. Wearable sensors in the form of an intelligent diving glove and wet-suit enable continuous monitoring of divers' physiological parameters to improve overall diver safety and health. Haptic feedback and robust gesture recognition from the intelligent diving glove will allow quick interaction with the robotic diving buddy. Modular extensions to a diver-held navigator will allow autonomous propulsion while retaining data collection benefits and increasing situational awareness and safety. Collaborative motion strategies between the resulting autonomous vehicle and the diver will optimize operation and mission execution.

In addition to these naval significances, the project will have high scientific impact with investigations into advanced materials for light ergonomic wearable sensors, robust diver detection and recognition from forward looking sonars, new diver-robot interaction modalities, new acoustics localization methods, etc. These scientific advancements will reduce risk to divers and marines while increasing their operational capability.

Objectives

Based on desired impacts the project goals can be grouped into three research objectives and an applicative objective to demonstrate future feasibility of moving from basic research towards application.

1. *Diver-robot collaborative motion strategies*

The objective is focused on enabling cooperative motion between a diver and an autonomous underwater vehicle (AUV). The first aim is to exploit the forward-looking sonar for diver position and orientation detection. Strategies for safely tracking, following and swimming with the diver will be researched and tested.

2. *Posture and physiological parameter monitoring*

The objective is focused on characterizing the divers physiological state. Lightweight wearable sensors, using advanced materials, will be investigated to reduce overall burden on the diver. Collected information will be processed to quantify diver's health, strain and behavior during the dive.

3. *Novel diver-robot communication methods*

The objective is focused on data flow and interaction between the diver and AUV. Haptic actuators, elastomers and inertial sensors will be used to create an intelligent diving glove which easily adapts to the user. Diving gestures will be extended into a diving interaction language to communicate with the AUV. Use of vehicle motion for providing navigation instructions will be investigated.

4. *Diver-robot interaction feasibility evaluation*

The objective is focused on testing researched sensors and algorithms to ensure that the overall research direction has a sensible path towards end-user usability. Utilities for dry testing and training will be developed and combined with field-trials including multiple divers.

Methods

To ensure smooth project execution the objectives are subdivided into several activities. At the start of the project the focus is to assess current state-of-the art and provide a set of requirements for: a) demonstration and usage scenario, b) vehicle capabilities, c) minimal set of interaction commands, d) list of physiological parameters, e) quality-performance indicators. The requirements are revisited and tuned at least once after first large end-user trials. Part of the

requirements is opened for fine-tuning during development when diver feedback is available.
First activities include development of a *diver-robot simulator* with realistic vehicle and sensor behavior. The simulator is complemented with a virtual-reality (VR) training environment allowing early inclusion of divers within algorithm development.

Sonar image interpretation is investigated using deep learning methods on available existing close-range sonar recordings of divers. This will crystalize interesting topologies and approaches to be further developed when the vehicle is ready for real-life deployment and data collection.

Collaborative motions methods will be investigated with the diver-robot simulator. Nonlinear control methods will be combined with intelligent decision-making to ensure safe maneuvering in the diver's vicinity. Vehicle movements enabling diver navigation aiding through guidance will be investigated.

Diver physiological parameters are measured using wearable sensors. The activity uses dielectric elastomers positioned around the body to measure joint positions for pose reconstruction and respiration. Photoplethysmography will be applied to measure heart rate. Similar elastomers are used for *diver glove development*.

Gesture recognition from the glove is done by classifying hand pose and individual finger joint measurements. First implemented gestures are based on demo scenario requirements.

Gestures are transmitted through acoustic Nano-modems that are the basis of *diver-robot communication*. Robot to diver communication will investigate multiple modalities: a) haptic feedback on the glove, b) light signals, c) indication through vehicle posture.

Evaluation of feasibility and workshops for end-users will be held on multiple occasions during the project to receive timely feedback about necessary extensions or improvements to ensure good ergonomics of each sub-system.

Results

The project work-effort started in October 2018 and results achieved by 31st of March are reported in this section.
Official project meetings between partners were held in October 2018 and March 2019. Skype conferences were held to fully define the *demo scenario* and *first set of system requirements*.

Fig 1. Bench testing. The figure shows the glove recognizing a gesture, sending the command over acoustic modems to the software-in-the-loop simulation which activates the vehicle.

The initial *diver-robot simulator,* to allow testing of motion methods, was *completed*. 2.5 hours of diver recordings in the sonar were extracted and labeled for neural network training. Bench testing *glove prototype* was *developed* (Fig 1). Action chain between glove gestures and the vehicle software-in-the-loop simulation, through real acoustic communication, were bench tested.
The propulsion models for the diver navigator were designed. Sensor and electronics acquisition is in-progress and factory acceptance test is expected before end of the first year. Sonar image interpretation activity has started with positive preliminary results. Virtual-reality training environment development is in-progress.

Notes:

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: NAVSEA
Project Status: NEW

SILDENAFIL FOR PREVENTION OF IMMERSION PULMONARY EDEMA

RE Moon, JJ Freiberger, BM Keuski

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Background

Immersion pulmonary edema (swimming-induced pulmonary edema, SIPE) occurs during a dive or vigorous swim. In the Navy this usually occurs in young, healthy individuals such as SEAL and Special Warfare Combatant-Craft trainees. SIPE generally resolves spontaneously within 24 hours but it can be fatal. It is believed that SIPE is responsible for some deaths.

Depending upon severity, the prevalence of SIPE is reported in up to 60% during 2.4-3.6 km open sea swimming trials in the Israel Defense Force. At BUD/S approximately 40 cases per year (around 3%) have been reported, more commonly during winter, when it is observed in up to 5% of BUD/S trainees. Return to duty time can be up to 7 days. SIPE also affects other groups of highly fit individuals such as triathletes. In susceptible individuals it tends to recur, thus a preventive medicine would be useful for both Navy SEALs and civilians.

Special Forces trainees who experience SIPE are not automatically disqualified, and many BUD/S candidates who experience SIPE graduate to become SEALs. There must therefore be a significant number of SIPE-susceptible operational Special Warfare personnel, who might be called upon to perform a mission-critical task where onset of SIPE could affect the warfighter's mission or his life. In such situations, a medication with few side effects and no adverse effect on exercise performance such as sildenafil that could prevent SIPE in susceptible individuals would be extremely valuable.

Sildenafil is medically indicated for pulmonary artery hypertension. The drug has also been used for prevention of high altitude pulmonary edema (HAPE) in mountaineers. Use of the drug by a small number of individuals suggests that it may prevent SIPE (Martina SD, et al. Med Sci Sports Exerc. 2017;49:1755-7), but its effectiveness for SIPE prevention is unproven due to lack of systematic testing. Sildenafil has very few side effects, and in particular does not adversely affect exercise performance. Studies in our lab have shown that during submersed exercise in cold water SIPE-susceptible individuals have higher pulmonary artery (PA) and PA wedge pressures, which are reduced with a 50 mg oral dose of sildenafil.

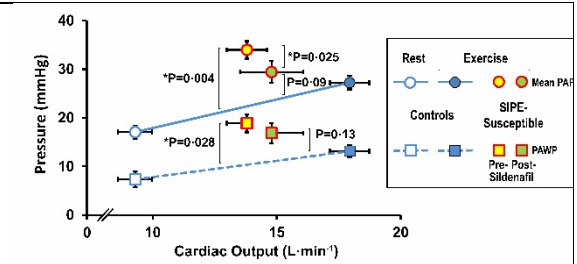

Fig. 1: Pulmonary artery PAP) and pulmonary artery wedge pressures (PAWP) during submersed exercise in 20°C water. From Moon RE, et al. Circulation. 2016;133:988-96.

During a screening test, mild SIPE manifestations occurred during a 40-minute exercise head-out immersed in 20°C water (see Fig. 2).

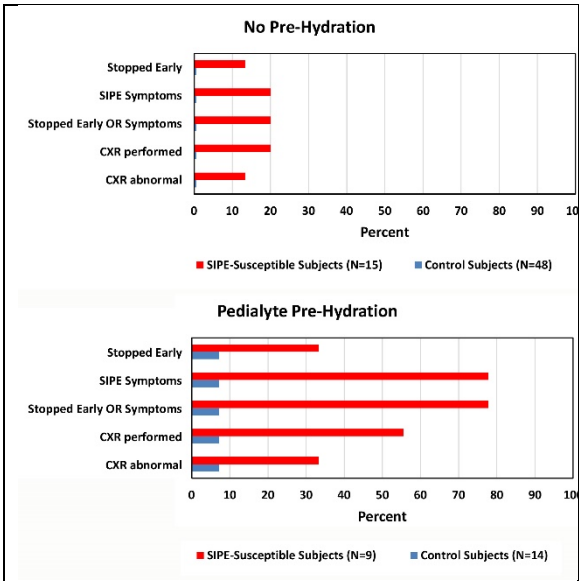

Fig. 2: Manifestations of SIPE in a cohort of individuals with a SIPE history and those without. After Pedialyte pre-hydration the majority of SIPE-susceptible individuals experienced one or more symptoms or signs of SIPE.

Availability of a drug that can prevent SIPE would provide the Navy with a useful tool that could be administered to SEALs who have experienced SIPE prior to critical missions. It would also be useful for civilians who have experienced SIPE but wish to continue with the precipitating exercise such as swimming or competing in triathlons, and also for patients with heart failure

for whom swimming induces shortness of breath or pulmonary edema.

We hypothesize that sildenafil administration to SIPE-susceptible individuals one hour before a swim in cold water will reduce or eliminate the risk of SIPE.

Objectives

The aim of this study will be to provide the Navy an FDA-approved drug that can be used to prevent SIPE. We hypothesize that a 50 mg dose of sildenafil will reduce or eliminate SIPE in susceptible individuals. We will perform a randomized placebo-controlled study in 20 SIPE-susceptible volunteers with the aim of testing the effectiveness of a standard dose of sildenafil for prevention of SIPE in susceptible individuals. The objective is to perform a placebo controlled trial of sildenafil using an established screening test (see above).

Methods

The method to be used to test the hypothesis will be a 40-minute period of exercise immersed to the neck in 20°C water following an oral fluid pre-load, a test that has resulted in SIPE manifestations in the majority of susceptible individuals. We plan to study 20 individuals who have previously experienced SIPE. Each volunteer will be tested twice. Either sildenafil 50 mg or an inactive drug (placebo) will be administered in random order prior to each exercise. For each participant exercise periods will be performed at least 7 days apart. The identity of the drug and placebo will be concealed from the investigators and the volunteers until the end of the study. The number of instances in which SIPE manifestations after sildenafil and placebo will be compared.

Results

IRB approval has been obtained and recruitment is underway.

Notes:

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: ONR
Project Status: NEW

STEM OUTREACH PROGRAM:

DESIGN OF A PASSIVE WATER SAMPLER TO TEST DIVE SITES SUSPECTED TO BE CONTAMINATED

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Background

Contaminated water is a common concern for working divers because they cannot select the locations of their dives; instead, the sites are dictated by the work needed. The current guidance for military divers regarding contamination is sparse, with little to no information on how to determine if the water at a dive site is contaminated. This paucity of guidance was the subject of discussion by Office of Naval Research and NAVSEA personnel at the May 2017 Deep Submergence Program Review, where they made a request to the researchers present to help address the many knowledge gaps in the field. Their concerns are reaffirmed by the findings of the Navy Experimental Diving Unit, which issued a written report stating they could find "no standard procedures to analyze water for potential contaminants or to assess risk related to such analyses" for either the military or commercial diving industries (1).

Current methods for sampling for biological contaminants are largely limited to "grab samples," which are single samples taken at one location in the water column, and these samples require training and expertise to perform correctly. Passive samplers provide a second option for chemical testing. These devices are commercially available and can be placed in the water several days ahead of time to test for pre-selected chemical contaminants. However, they are large, expensive, and limited to a narrow panel of chemical contaminants. No known devices currently exist to take a sample through multiple depths along a water column and allow for testing of that sample.

Fig.1. ChemCatcher passive sampler. One of the commercially available samplers to test for chemical contaminants. The contaminants must be selected in advance, and the sampler is then left underwater for an extended time period prior to retrieval and laboratory testing.

Fig.2. Water sample bottle. Most water samples are taken as "grab samples," meaning they are one sample at one location in the water column at one time. These samples are cheap to perform but require training and expertise to avoid contamination. This type of sample is then tested later in a laboratory environment.

Objectives

1) Design and build prototype water samplers that can take a water sample through the water column
2) Conduct a STEM Outreach program that will allow high school students to both test the water samplers and engage in DoD scientific research

Methods

Three undergraduate engineering students will be hired as part of this program, and they will perform the design and prototyping work over the summer of 2019. Applicants are assessed for skills related to design and manufacturing. The students who are hired will work out of the Duke University Center for Hyperbaric Medicine & Environmental Physiology, where they will have access to world-class prototyping and machining facilities on campus. They will also collaborate closely with experts in water sampling from the laboratory of Dr. Claudia Gunsch, who specializes in the characterization of water microbiomes.

The students will preliminarily test the samplers by assessing the devices' ability to take a mixed sample through a column of water of known composition in a vertical test tank, with layers stratified by salinity or colored dyes. They will also be able to test the samplers in more realistic field conditions in the bodies of water on campus, which are frequently used for scientific research. Once they have developed a working design, they will manufacture a small number of the sampler devices.

These sampler devices will then be provided to students from a local high school science class. This program provides funding for a field trip and lunch for these students to travel to a local lake and use kayaks to assess the function of the samplers. The majority of the students will be provided basic chemistry kits to test their water samples for variability such as pH and calcium content. A small number of the samples will be sent for comprehensive professional testing to examine them for unintended contamination, which will provide an assessment of the ease-of-use of the samplers.

As this project was designed to be a STEM outreach program, its scope and timeline were limited to the design of the sampler device to ensure that it could be completed by students. The current overall objective is therefore simply to allow water samples to be taken at dive sites that are suspected to be contaminated.

These samples can then be assessed later to determine if the type of protective diving equipment worn was sufficient for the water conditions, and if the diver needs preventative medical treatment. The samples can also be used to inform medical diagnosis and treatment if a diver becomes ill after a dive. Future projects have already been outlined to permit on-site testing of the samples for biological contaminants that could lead to illness, which will allow for more informed pre-dive decision-making about the level of protective equipment that is appropriate for a site.

Through this program, both the undergraduate and high school students will receive exposure to a DoD-funded STEM project that directly addresses a fleet need and also has the potential to benefit other communities with contaminated water concerns.

Results

The design work for this project is expected to begin May 2019 and as such the project has no results to report to date. The three undergraduates will be selected for hiring by mid-April.

Reference: (1) Steigleman WA. *Survey of current best practices for diving in contaminated water*. Panama City, FL: US Navy Experimental Diving Unit; 2007.

Notes:

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: ONR
Project Status: ONGOING

BIOTECHNOLOGY TO IMPROVE COLD-WATER OPERATOR PERFORMANCE

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Background

On June 2-3 2016, the Department of Defense Biotechnologies for Health and Performance – Research, Policy and Operational Applications working group held a workshop to identify warfighter biomedical and performance challenges. For each of the identified gaps, meeting participants mapped out potential biotechnology approaches to address each challenge and maximize warfighter performance.

One major product of this workshop was the Navy Undersea Operations Use Case, which was created by experts in undersea medical research and operations. The purpose of this use case was to provide an overview of operational issues faced by undersea warfighters and illustrate the potential application of novel biotechnologies to optimize warfighter performance and resilience against these issues. The Use Case focused on a Navy Special Warfare team conducting a mission via a Virginia class submarine and a SEAL Delivery Vehicle (SDV) and included submarine lock-in/lock-out operations as well as potential scenarios related to a disabled submarine.

This project explores novel neuromodulatory methods and nutritional supplementation to improve performance as well as transcriptional biosignatures to assess performance in the undersea cold-water environment.

During cold-water immersion, autonomic conflict may arise from opposing sympathetic and parasympathetic neural drivers that control the cold-water shock response and diving response, respectively (Williams et al 2015). Such autonomic conflict may cause serious cardiac arrhythmias as well as risks of morbidity and mortality (Shattock and Tipton 2012). The cardiovascular dive response consists of a decrease in heart rate, an increase in peripheral vasoconstriction, and a corresponding redistribution of blood flow to tissues. The vagus nerve mediates the parasympathetic cardiac response, while sympathetic vasomotor fibers control peripheral vascular tone. Through transcutaneous electrical stimulation of the vagus nerve (tVNS) it may be possible to reduce rates of cold-water shock and mitigate the impact of hypothermia.

In this study, we will measure the impact of tVNS treatment on cold-water dive performance, both cognitive and physical, on operators simulating an operationally relevant task, such as lock-in/lock-out, SDV transit, and/or DISSUB escape.

From the standpoint of physical performance, the vagus nerve also plays a role in thermoregulation and energy metabolism. Hepatic vagal afferents to the nucleus of the solitary tract (NTS) contribute to enhancement of sympathetic activity and stress-responsiveness, leading to hypermetabolism and hyperthermia (Szekely 2000), as well as activation of brown adipose tissue (BAT) and shivering responses that increase core body temperature (Morrison 2016).

Acute cold-stress and even mild-hypothermia have been found to negatively impact complex cognitive tasks that involve working memory, attention, task sustainment and reaction time (Paulauskas 2015). From the standpoint of cognitive performance, VNS has been shown to significantly improve memory and performance of cognitive tasks in both rats and humans (Clark 1998, 1999). In ongoing DARPA-sponsored research we have demonstrated improvements in visual task acquisition and retention (25%) and reaction times (20-25%) in a USAF population of ISR analyst trainees.

VNS has been shown to activate regions of the brain that serve as hotspots for epilepsy, including the hippocampus and amygdala (Naritoku et al., 1995). These regions of the brain are also known to be critically important for learning and comprehension, as well as cognition generally. Human subjects receiving VNS have shown specific enhancements in comprehension, leading to enhanced decision making (Ghacibeh et al., 2006). VNS has been shown to increase neuronal plasticity in humans (Hays 2015). In small animal models, VNS has been shown to enhance decision making compared to animals

receiving sham stimulation (Ogbonnaya and Kaliaperumal, 2013). VNS has also been shown to augment plasticity in the brain in general (Pena et al., 2014).

VNS is a neuromodulatory treatment with a long history of safe and effective use. It has been an FDA approved medical treatment for epilepsy and depression for over two decades. In 2017, non-invasive tVNS gained FDA approval for the treatment of migraines and cluster headaches.

Objectives

The objectives of this project include:

1. Classified biotechnology review to augment the Navy Undersea Operations Use Case.
2. Undersea operator task analysis, and development of an operationally-relevant pool-based task battery that permits rigorous evaluation of cold-water cognitive and physical performance.
3. Development of a tVNS protocol for the treatment of cold-water shock and hypothermia in the undersea operational environment.
4. Using our pool-based task battery, assess tVNS efficacy in mitigating cold-water shock and hypothermia, as well as improving cognitive performance.
5. Identification of transcriptional signatures of hypothermia and cold-water shock, as well as signatures indicative of relative susceptibility to hypothermia and cold-water shock.
6. Identification of transcriptional signatures of ketone ester supplementation in cold-water shock and hypothermia.

Methods

A classified assessment of biotechnologies will be conducted to explore biotechnologies that could mitigate the impact of hypothermia in performance of critical undersea tasks that were identified in the Biotechnologies for Health and Performance use case.

Based on our task analysis, a pool-based cold-water task battery was developed for initial, rapid assessment of biotechnologies that could optimize operator cold-water performance. This task battery was designed and validated with feedback from the cold-water operational community and associated human performance experts.

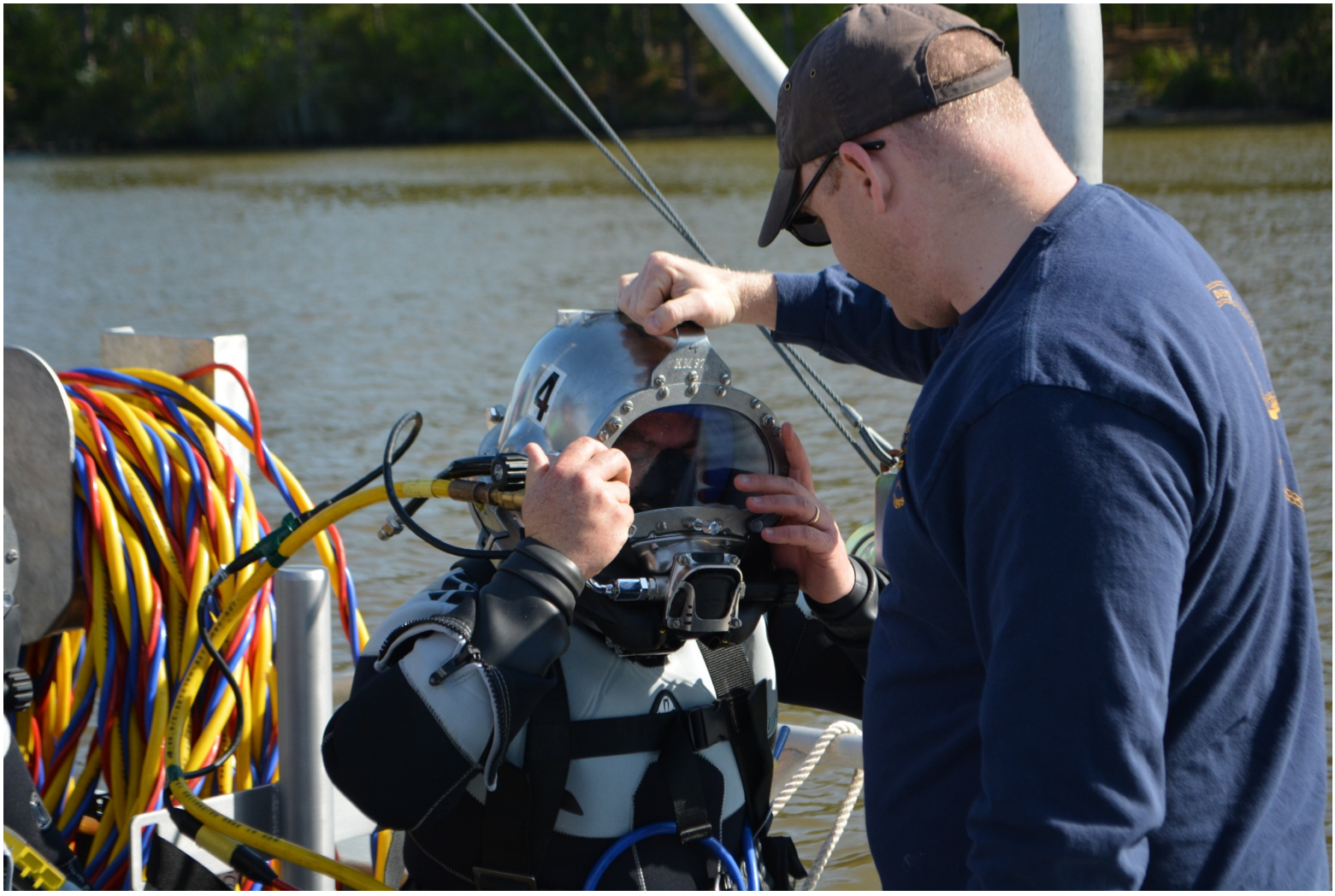
The cold-water task battery has been validated, and additional studies are planned to assess the the effect of tVNS stimulation on cold-water performance.

Results

The cold-water task battery was developed to include measurements of cognitive performance, physical performance, manual dexterity, muscle glycogen stores, molecular changes, and core and peripheral body temperature before and after cold-water immersion. This task battery is currently being used for assessment of ketone esters in the cold-water setting, and will soon be used to assess tVNS. Ongoing related DARPA-funded studies have demonstrated tVNS significantly improves the rate of learning and target detection during USAF ISR training. The methodology for these studies has been used to develop the protocol for the pending cold-water evaluations. The study team anticipates completion of the classified biotechnology assessment and tVNS studies by end of FY19.

Approved, DCN# 43-5229-19

Day 3: Thursday, May 16, 2019



2019 ONR-NAVSEA UNDERSEA MEDICINE PROGRAM REVIEW

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: NAVSEA
Project Status: ONGOING

THE EFFECTS OF DISABLED SUBMARINE STRESSORS ON SUBMARINER COGNITION

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Background

During a disabled submarine (DISSUB) scenario, submariners must survive harsh conditions aboard the DISSUB until rescue assets arrive or escape becomes necessary. During this time submariners must successfully operate while being exposed to a range of stressors originating from changes to the environment (e.g., increasing compartment temperature), their mental status (e.g., due to confinement/social isolation), and physical state (e.g., shortage of potable water and food). While experiencing these stressors, submariners must execute a host of cognitively-demanding tasks, such as reacting to emergencies, performing stay-time calculations, and making critical survival decisions.

In Year 1 of the project, we conducted an in-depth literature review to understand how the various environmental, mental, and physical stressors might impact submariners' cognitive functioning and their ability to successfully execute an escape or rescue. Results indicated that while the physiological effects of stressors are generally well understood, much less is known about how stressors may impact cognitive performance. Specifically, little research has examined the cognitive effects of stressors at an exposure duration representative of a DISSUB scenario (i.e., 3-7 days). Two technical reports detailing the findings of our reviews are undergoing review (Chabal, Bohnenkamper, Reinhart, & Quatroche, in review) or are in final preparation (Chabal, Reinhart, Bohnenkamper, Moslener, in preparation).

Objectives

The main objective for Year 2 of the project is to conduct empirical research to evaluate submariners' cognitive responses to some of the factors present in a DISSUB scenario.

Methods

Fifteen participants will undergo a 5-day DISSUB simulation during which they will be exposed to a host of environmental, mental, and physical stressors. During the DISSUB simulation, participants (groups of 2-3 at a time) will be confined to a chamber (Figure 1), except when using the head facilities. The chamber facility mimics the living conditions of typical submarine berthing areas.

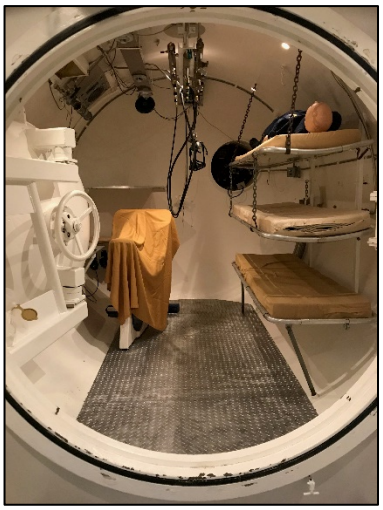

Fig.1. DISSUB simulation chamber interior. The chamber (23 ft. long and 9 ft. diameter) contains three racks for sleeping, mimicking the berthing facilities aboard a submarine.

Throughout the DISSUB simulation, participants will be required to utilize only emergency lighting sources (i.e., battle lanterns and personal flashlights), experience social isolation (i.e., no internet or phone capabilities), and consume a DISSUB-specific diet (i.e., ~1300 kcal/day diet composed of high fat content foods). In addition to these manipulated stressors, participants may experience other stressors such as caffeine withdrawal, boredom, sleep restriction, pain due to lack of mobility, and heat stress. To account for this, we will regularly monitor the atmosphere (e.g., temperature and gas levels), participants' physical states (e.g., body weight and sleep patterns), and subjective states (e.g., boredom and musculoskeletal pain) throughout the DISSUB simulation to determine the prevalence and severity of these stressors.

Throughout the DISSUB simulation period participants will undergo daily cognitive testing

including measures of attention (Psychomotor Vigilance Task; Defense Automated Neurobehavioral Assessment), memory (Digit Span Test), flexible thinking (Wisconsin Card Sorting Task), risk-taking propensity (Balloon Analogue Risk Task), and mood (Profile of Mood States). These cognitive domains were selected for their importance in a DISSUB scenario. In addition to traditional cognitive measures, participants will also complete operational assessments adopted from the DISSUB guard book, including sections on the management of toxic gases and performing stay-time calculations.

Results of the cognitive and operational assessments conducted throughout the DISSUB simulation period will be compared to baseline data collected at laboratory visits prior to the DISSUB simulation.

Results

The results of the DISSUB simulation experiment are forthcoming. All data collection will be concluded by the end of the fiscal year. All data analysis and report generation will be completed by the end of FY20.

Notes:

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: ONR
Project Status: NEW

NON-EQUILIBRIUM THERMODYNAMICS OF BIOLOGICAL MEMBRANES

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Background

This continuing proposal seeks to answer a fundamental question in biomembranes research – how do lipid bilayers form and repair pores? Understanding the behavior of biologically derived materials under different conditions of repair and growth is critical for our ability to understand the response of cells to osmotic stresses and for the design of synthetic cellular mimics. This proposal is motivated by many applications of lipid bilayers including materials research, synthetic biology, and biomedicine. In the project proposal, I had proposed the construction of a fundamental theoretical framework to probe the response of lipid bilayers using the first principles of non-equilibrium thermodynamics. This framework will be used to explore the phase space that governs pore formation and repair in membranes of increasing complexity. Our approach is different from the traditional approaches of using mechanics, fluctuations, and transport individually to describe the membrane properties. Instead of looking at different aspects of the membrane separately, we present a unifying framework based on the energy of the membrane, and derive the dynamic mechanisms that couple membrane-tension induced pore formation.

Objectives

The objectives of this proposal are as follows:

Aim 1: Identify the dynamics of swelling and pore formation in single component artificial giant unilamellar vesicles (GUVs) under osmotic differentials.

Working hypothesis: Nano-pore nucleation driven by thermal fluctuations, determines the critical tension for large pore opening in swelling GUVs. To address this aim, we will develop a non-equilibrium thermodynamic approach, starting from first principles, to identify the energetics of how pores form in GUVs with only one lipid component under osmotic stress.

Aim 2: Identify the dynamics of swelling and pore formation in multicomponent artificial GUVs under osmotic differentials.

Working hypothesis: The composition of the GUVs will change the response of GUVs to osmotic differentials. We will extend the model developed in Aim 1 to include the thermodynamics of non-ideal fluid mixing (not limited to dilute solutions) to identify how the membrane tension responds to not only externally applied stresses such as osmotic pressure but also to dynamically varying spatial organization of the membrane.

Aim 3: Assess vesicle growth by lipid incorporation in membrane hydrophobic interfaces.

Working hypothesis: Pore formation is critical for vesicle growth and works by creating hydrophobic/solution interfaces that allow lipid incorporation to the membrane, leading to an increase in vesicle size. I propose to develop models that will identify the interaction between heterogeneous membrane vesicles and lipids during pore repair and vesicle repair. It is expected that this model will aid in the design and development of biologically based repair complexes for self-repair.

Methods

In order to understand the observed swell-burst cycles, we propose a theoretical description of the system which couples mass conservation of the solvent (including osmotic influx, and leak-out through the pore), mass conservation of the solute (including diffusive and convective flux through the pore), and dynamics of the membrane (including elastic energy, membrane folding and unfolding, pore edge energy, and viscous dissipation). Based on the idea that pore nucleation event under membrane tension is a critical phenomenon of the cycle dynamics, we incorporate thermal fluctuations which drive the formation of stochastic nano-pore at the origin of large pore opening. The full model is formulated in terms of three coupled stochastic differential equations, one for the GUV radius, one for the pore radius, and one for the amount of solute. These equations are solved numerically in Matlab® (Mathworks, Natick, MA) through a Euler-Maruyama scheme.

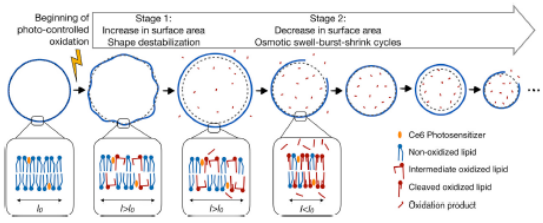

Fig.1. Proposed mechanism for dynamics of lipid vesicles dur to photooxidation.

Results

We published the first results of this model in the Biophysical Journal in 2017. Here, we showed that single lipid GUVs in 200mM sucrose hypotonic conditions exhibit cyclic behavior, characterized by the formation of large transient pores. We identified a scaling relationship between the time period of the cycle, the concentration difference, and the strain rate. These results are summarized in Figure 1. The periods of the swell-burst cycles increase with the cycle number, or in other words, the deformation rate (therefore also stress rate) decreases with each cycle. Incidentally, the maximum vesicle radii also decrease with time.

Maximum membrane deformations are measured to range between 10 and 20%. Direct observations of the pore opening/resealing events, yields an estimate of the pore life time of the order of 100 milliseconds.

One of the main features of our model is the inclusion of thermal fluctuations inducing pore nucleation. Although many studies have focused on the physics of pore nucleation in lipid bilayer membranes, to our knowledge, none have been applied to vesicle systems with multiple swell-burst cycles. Here we fill this gap, and show that thermal fluctuations trigger pore nucleation, and therefore determine the subsequent swell-burst cycle dynamics. Importantly, the stochastic basis of thermal fluctuations naturally induce a dependence of the pore opening tension to the stress rate, which is a characteristic property of lipid membranes.

We have extended this model to two other systems to study the role of (a) surfactants and (b) crowding. The solubilization of the lipid membrane due to the insertion of surfactant molecules induces a reduction of membrane surface area at almost constant vesicle volume.

In collaboration with Stephanie Bonneau's group (Sorbonne University, Paris), we showed the lipid oxidation in mitochondrial lipids can affect their permeability. This work is summarized below and was recently published in Biophysical journal. Unsaturated lipid oxidation is a fundamental process involved in different aspects of cellular bioenergetics; dysregulation of lipid oxidation is often associated with cell aging and death. To study how lipid oxidation affects membrane biophysics, we used a chlorin photosensitizer to oxidize vesicles of various lipid compositions and degrees of unsaturation in a controlled manner. We observed different shape transitions that can be interpreted as an increase in the area of the targeted membrane followed by a decrease. These area modifications induced by the chemical modification of the membrane upon oxidation were followed in situ by Raman tweezers microspectroscopy. We found that the membrane area increase corresponds to the lipids' peroxidation and is initiated by the delocalization of the targeted double bonds in the tails of the lipids. The subsequent decrease of membrane area can be explained by the formation of cleaved secondary products. As a result of these area changes, we observe vesicle permeabilization after a time lag that is characterized in relation with the level of unsaturation. The evolution of photosensitized vesicle radius was measured and yields an estimation of the mechanical changes of the membrane over oxidation time. The membrane is both weakened and permeabilized by the oxidation. Interestingly, the effect of unsaturation level on the dynamics of vesicles undergoing photooxidation is not trivial and thus carefully discussed. Our findings shed light on the fundamental dynamic mechanisms underlying the oxidation of lipid membranes and highlight the role of unsaturations on their physical and chemical properties.

Notes:

DEVELOPMENT OF AN INTERACTIVE SOFTWARE APPLICATION TO PROVIDE RECOMMENDATIONS FOR HUMAN EXPOSURE TO UNDERWATER NOISE

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Background

Unmanned underwater vehicles (UUVs) use active acoustics for navigation and communication. UUVs emit sounds that may injure nearby swimmers and divers. The Navy's expanding use of UUVs is increasing the likelihood of diver exposure to active acoustic technologies. Indeed, some Naval missions depend on human-UUV interaction to achieve success. The increasing use of UUVs near human divers amplifies the Navy's overall operational risk, creating a new requirement for rapid determination of safe acoustic exposure.

The standard operating procedure to determine safe exposures to active underwater acoustic sources is to: (1) refer to Appendix 1A of the US Navy Diving Manual, or (2) contact NAVSEA 00C for guidance on situations not specifically described in the manual. As new technologies are being tested and integrated into the fleet, the traditional generalized guidance (e.g. Appendix 1A sonar worksheet) often does not apply. Therefore, NAVSEA 00C must establish mission-tailored guidance for diver interactions. NAVSEA 00C will then contact their subject matter experts (SMEs) at the Naval Submarine Medical Research Laboratory (NSMRL) for support.

Objectives

The objective of this project is to streamline the guidance process and, thus, improve the mission-tailored support that Navy diving operations receives from NAVSEA 00C and NSMRL. To accomplish this objective, we will develop an interactive software application (App) that will provide recommendations for human exposure to underwater noise. The App will function by analyzing user input (e.g. frequency, sound level, environment) and output a standoff range that is scalable based on mission parameters and level of acceptable risk.

Methods

We will draw from our bioeffects database and expertise to build an interactive decision aid tool. Outputs from the App will not only provide noise exposure guidance, but will also include source information that will provide transparency to our rationale and thought processes. We will develop the App through a 5-step process.

Generation of Conceptual Map of Underwater Acoustic Bioeffects: Our goal is to create a visual representation (concept map) of the current state of knowledge and the gaps in information related to continuous wave underwater noise exposures. We are creating this concept map through a careful review of the literature. This review considers academic papers, technical reports, domestic and international diver underwater sound exposure research and guidelines, internal NSMRL manuscripts, and other DoD documents. Much of the relevant data is already in-house at NSMRL. The concept map will be used in conjunction with the App to help the expert user identify the specific data source used in recommendations generated by the App.

Update NSMRL's Bioeffects Database: The current NSMRL bioeffects database is a compilation of decades of underwater experimental data. These data have been previously de-identified, checked against associated experimental logs, and organized to allow examination based on multiple characteristics. This database will serve as the backbone of the App for creating psychological and physiological response predictions. In addition, we are continually updating our knowledge with new and historical data obtained from national and international partners.

Implementation of the Propagation Module: We are evaluating multiple models to determine the most appropriate for predicting sonar acoustic propagation for different scenarios. We are evaluating tradeoffs between precision, complexity, and distributability of the code within our App.

Development of the Bioeffect Relational Risk Module: The bioeffect relational risk module will predict diver responses to recorded or calculated

acoustic stimuli. Predictions of the module will be tested against experimental data from our bioeffects database. We will develop the module by focusing on the identified injury mechanisms and injury severity findings from historical in- water human and animal experiments. The dynamic risk will be predicted based on our cumulative knowledge of the bioeffects of continuous underwater noise. In addition, we will explore the literature to identify relationships between the severity of injury and quantitative measures of noise exposure. The aim of this module is to calculate the diver risk of injuries based on received acoustic waveform properties.

Development of Graphical User Interface: Our team has significant experience in the development of intuitive interfaces for a variety of applications. We will employ user-centered design processes to build the user interface. We will communicate with NAVSEA 00C throughout the design process to ensure that the recommendations generated by the App are easily interpreted and allow explicit identification of the methodology employed along with the source supporting data.

Fig.1. Example of Appendix 1A App Interface.

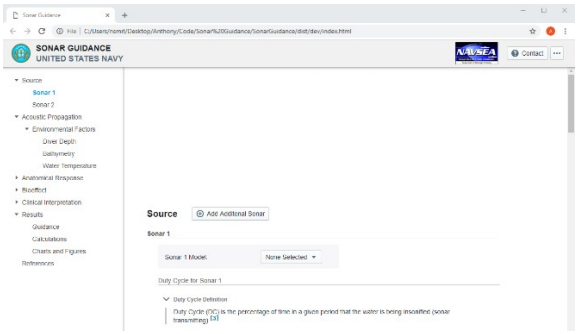

Fig.2. Example of Sonar Guidance App Interface.

Results

We have completed steps 1 and 2. Steps 3, 4, and 5 are functional components of the final App and are being worked on concurrently. The framework for the App has been created and we are in the process of populating it with content. We are on schedule and expect to have the final product completed by the end of FY19.

While testing out different software and GUI layouts, we found that we could evaluate software functionality by creating an exemplar data set. Rather than generating a random data set, we decided to create an electronic version of Appendix 1A sonar standoff worksheet as an additional App We will provide this App to NAVSEA 00C as an additional deliverable.

Notes:

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: NAVSEA
Project Status: ENDING

DEVELOPMENT OF A METHODOLOGY FOR CHARACTERIZATION OF ACOUSTIC TECHNOLOGIES OF NAVAL UUV EXISTING AND FUTURE ASSETS

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Background

As the Navy becomes increasingly reliant on underwater unmanned vehicles (UUVs) on missions, the likelihood of divers and swimmers interacting with these technologies likewise increases. Indeed, in some missions, human interactions with UUVs are critical to their success. However, there is growing concern that the noise produced by the UUVs are potentially harmful to humans.

Little is known about the full acoustic signature of these UUVs. Manufacturers of these technologies often just measure the narrow range of acoustics within which the UUVs are designed to function. Yet, this disregards acoustic distortion or other properties that may result in production of additional frequencies during operation. During shifts in frequency output, additional lower frequencies that are within human hearing range could be produced. Without verification of measurements over a broader range of frequencies, providing guidance on human interactions with these acoustic technologies is challenging. Due to the current lack of data, guidance must be overly conservative to factor in potential (but unknown) additional risks. As a result, operations can be inhibited or drastically altered to remain in compliance. More accurate guidance is needed. NSMRL is filling this need to provide recommendations to NAVSEA 00C with so many knowledge gaps.

Objectives

The objective of this project is to develop a methodology for use by active acoustic technology manufacturers to characterize the full range of sounds produced by their systems. The purpose of this effort is to expand the data collection by these manufacturers and thus provide the necessary information to develop guidance to the fleet when divers/swimmers are interacting with these technologies.

Methods

Currently, we are in the process of renting acoustic modem technologies from multiple vendors. Acoustical measurements are ongoing and will continue as we complete additional contract processes and obtain time at the testing facilities. In year one we measured equipment from Coda Octopus in the UK as well as a Kongsberg sonar system borrowed from NAVSEA 00C. Coda Octopus was chosen because NSMRL has provided diver standoff guidance to NAVSEA 00C for products from this company.

We are conducting our acoustic characterization at Naval Undersea Warfare Center Dodge Pond Acoustic Testing Facility, in Niantic, CT. This facility is utilized by the Navy and outside contractors to test underwater acoustics devices. The site has quiet conditions (ambient noise less than sea state zero) and the natural bowl-shape of the pond with a mud-filled bottom (52.5 feet in depth) minimize acoustic reflections. We will also test within NSMRL's 10 foot deep pool to characterize the acoustics in a "non-ideal", highly reflective environment.

We have created a scaffold built of flooded PVC that will surround the acoustic technology. Hydrophones capable of capturing flat responses at low and high frequencies (20 Hz-500kHz) will be attached to each corner. Our goal is to measure these frequencies to cover the range of operational acoustics and capture any lower sounds that could penetrate the range of human hearing.

As part of the development of the methodology and guidance/recommendations, we will develop appropriate protocols for collecting these types of data. Metrics/variables that we intend to measure include sound pressure level (SPL), total sound exposure level (SEL), frequency content of the intended signal, potential frequency contents of unintended signals (i.e., distortions, harmonics), temporal characteristics (i.e., continuous signal, pulsed signal, duty cycle), and source directivity. We will document the methodology process with the intent that they will become a required

component by NAVSEA 00C as part of the active acoustic technology procurement process.

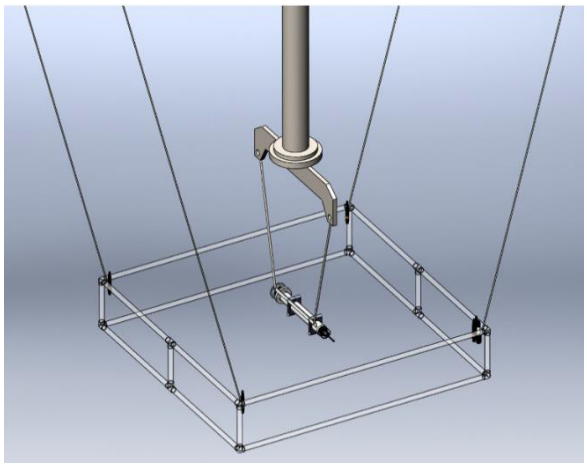

Fig.1. Example of equipment set up for characterization of sonar system acoustics.

This document will include examples of the appropriate equipment (i.e., hydrophones, amplifiers, recording equipment) to capture the needed acoustic metrics for the acoustic technology. We will recommend types of hydrophones that should be used, including considerations for bandwidth (frequency range), sensitivity, and directionality. In terms of digital recording of the signals, recommendations will be made for sampling rate and duration. A clear description of data reduction methods will also be included, documenting the appropriate metrics for describing the acoustics of the technologies. We will also discuss additional considerations, including recording environment, location of hydrophones, and ambient noise. As it is likely that most vendors will not have a "pristine" acoustic facility such as Dodge Pond at their disposal, tradeoffs will be discussed in terms of dealing with reverberant or noisy environments based on our measurements conducted in the NSMRL pool.

Results/Progress

Measurements from the Coda Octopus and Kongsberg sonar system were conducted at Dodge Pond and NSMRL in FY18. As there were multiple frequencies, scanning rates, and other equipment settings, this proved to be much more data than we originally anticipated. These measurements have been analyzed, and exemplars from both measuring environments are being used as examples in the acoustic methodology guide.

We have identified acoustic modem systems from several vendors and are currently developing contract agreements to allow us to rent the systems to collect measurements. We expect to have collected data by the time the program review occurs.

Notes:

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: ONR
Project Status: ONGOING

MITOCHONDRIAL STRESS AND CELLULAR PROTECTION IN UNDERSEA MEDICINE

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Background

Two major health threats associated with undersea medicine, especially in the scenario of submariner rescue and diving related environmental exposures are decompression sickness (DCS) and oxygen toxicity (O2Tox). DCS, O2Tox and other toxic exposures (e.g., CO poisoning). albeit through putatively distinct mechanisms, can ultimately lead to cellular dysfunction rooted in derangements of bioenergetic function. The maintenance of cellular health requires that cellular bioenergetic capacity be preserved in the face of incipient or sustained environmental challenges. Ultimately, degradation of normal mitochondrial function in response to environmental stressors such as DCS or O2Tox leads to cellular dysfunction and integrates into organ dysfunction or even death. Identifying the molecular basis of mitochondrial responses to DCS, O2Tox and other undersea medicine related environmental exposures as the bioenergetic basis of cell dysfunction enables therapeutic insights and facilitates the development of cytoprotective interventions to reduce injury from such stressors and to protect the health and readiness of Navy personnel.

Objectives

We quantified the degree of impairment of mitochondrial respiratory function and alterations in mitochondrial dynamics, especially motility, in human vascular smooth muscle cells subjected to decompression from hyperbaric conditions utilizing various gas atmospheric combinations. We also quantified the degree to which functional impairment of motility and respiration were normalized by addition of an exogenous mitochondrial fuel, a succinate prodrug NV118. We further subdivided these effects into cell perinuclear and peripheral domains. To achieve this objective required that we use both high resolution respirometry and fluorescence microscopy imaging methods to assess both alterations in cellular bioenergetic performance and mitochondrial motility. We exposed cells to hyperbaric conditions with different gas compositions used in the exposure chamber.

Methods

Cell culture: For bioenergetic assays primary human vascular smooth muscle cells were cultured in 24-well polystyrene Seahorse XF cell culture plates at a density of 60,000 cells/100µL 24 hours prior to mitochondria respiration assay experiments. For mitochondrial dynamics cells were plated at 10,000 cells per dish on fibronectin-coated MatTek 35-mm glass-bottom dishes for approximately 48 hours.

Dive conditions: Cells were examined in four groups: Control (room air, ambient pressure), Air dive (19.8% O_2, 79.2% N_2 and 1.0% CO_2); N_2 dive (4.0% O_2, 95.0% N_2 and 1.0% CO_2) and O_2 dive (84.0% O_2, 15.0% N_2 and 1.0% CO_2). Except for control, all dives were at room temperature at 4.8 atm for 1 hour. All described dives were performed in a Nat'L BD model portable hyperbaric chamber rated for 100 PSI.

Determination of mitochondrial respiration: Prior to the measurement of cellular respiration, cells were pre-treated with various dive conditions described above. Mitochondrial oxygen consumption rates (OCR) in pmol/min were evaluated in intact cells using the Seahorse XFe24 Analyzer. Select compounds were injected to obtain Routine; LEAK; Max; and residual oxygen consumption (ROX). All respiratory states were measured at three consecutive time points. The values were averaged to give a single measure of respiration for each state. Each well was considered to be a separate experiment. The time between the end of dive experiments and start of the mitochondrial respiration assay was approximately 30 minutes.

Determination of mitochondrial dynamics: We measured mitochondrial dynamics, including: (1) Net and total movement; (2) Mitochondrial number and length; (3) Rates of fission and fusion events. We performed partitioned cell mitochondrial dynamics using wide-field fluorescence microscopy and postprocessing of acquired images as was developed in our lab and published previously, including subdivision of the cell into two domains: a 3.2 µm thick perinuclear region and the remainder of the cell domain

referred to as the cell periphery.

Statistical analysis: Data were tested for normal distribution with the D'Agostino and Pearson omnibus normality test. Data are presented as mean ± SD if not indicated otherwise. Differences between dive groups were assessed using ANOVA in repeated measures. Post-hoc pair wise comparisons using Tukey Kramer t-tests to adjust for multiple comparisons were used to assess differences between groups and respiratory states. A P value of <0.05 was considered statistically significant. We computed the coefficient of determination (R2) of the probability plots as we have previously described and published. P-values comparing log distributions were computed using two-sample Kolmogorov-Smirnov tests. We also used unpaired t-tests to compare differences in net distance and fusion/fission events.

Results

Figure 1 displays the oxygen consumption rate for ATP-linked respiration and spare respiratory capacity (SRC) for each group measured as pmol/min/1000 relative fluorescence units (RFU). Key findings include that the Oxygen group had significantly lower ATP-linked respiration when compared to the other groups otherwise there was no difference between the other groups. For SRC, the Air group was significantly lower when compared to all other groups. The SRC for the Oxygen group was also significantly lower when compared to Control and Nitrogen group.

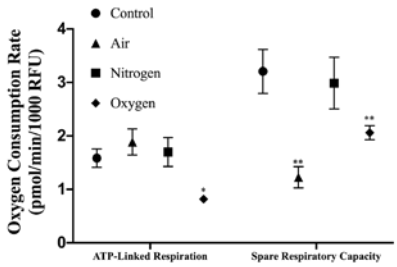

Figure 1. * The ATP-linked respiration of the Oxygen group significantly different from all groups (P < 0.05). **The SRC of both the Air and Oxygen group significantly different from the other groups (P < 0.001).

Figure 2 displays the SRC for each dive with and without cell treatment with NV118 immediately post-decompression. SRC increased in all groups following NV118.

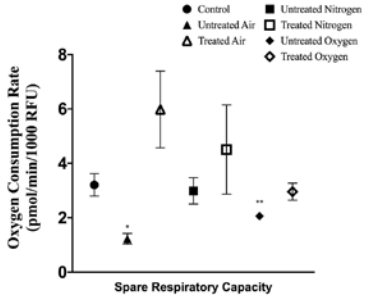

Figure 2. Values presented as mean ± SEM. *Untreated cells significantly different from treated cells in the Air group (P < 0.01). **Untreated cells significantly different from treated cells in the Oxygen group (P < 0.01).

Figure 3 shows an example of mitochondrial motility in perinuclear zone for the Oxygen dive. Motility is significantly increased in cells with decompression, and was more pronounced in the perinuclear region as opposed to the peripheral region (not shown). Importantly, when exposed to NV118 following decompression mitochondrial motility normalizes back to control conditions.

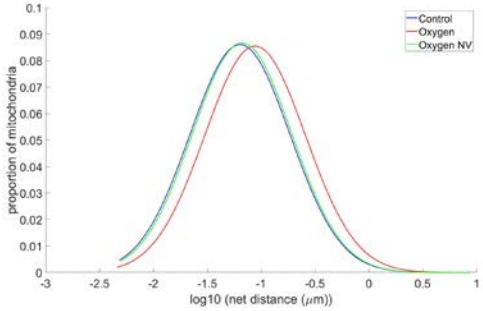

Figure 3. Histograms of mitochondrial motility for the perinuclear zone. Motility following decompression from Oxygen dive is significantly increased compared to control conditions, and addition of .NV118 following decompression renormalizes motility back to control values.

We also assessed mitochondrial membrane potential and superoxide production in cells under control and all dive conditions, with and without NV118 treatment. From these data we can construct a bioenergetic profile for the perinuclear and peripheral cell regions. Generally this demonstrates that decompression leads to increased perinuclear clustering of mitochondria and a shunting of bioenergetic capacity from the cell periphery to the perinuclear region in the face of an overall loss of cellular bioenergetics. However, the addition of NV118 immediately post-decompression leads to additional cellular protection from bioenergetics failure and helps to normalize the intracellular partitioning of mitochondrial bioenergetic capacity.

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Notes:

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: ONR
Project Status: ONGOING

LIPID COMPOSITION AND NITROGEN SOLUBILITY OF THE SPINAL CORD AND BRAIN: COMPARISIONS BETWEEN DIVING AND TERRESTRIAL MAMMALS TO PROVIDE INSIGHT TO TYPE II DCS

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Background

Marine mammals, in particular the deep diving beaked and sperm whales, may alter their diving behavior in response to anthropogenic sound, potentially increasing their risk of tissue supersaturation and bubble formation. It is also possible that altered dive behavior may have the potential to introduce a more serious form of embolism, Type II decompression sickness (DCS). Type II DCS is the more severe form of decompression illness, and the most common site of injury for this illness is the spinal cord, which has a higher incidence of damage than the brain. Type II DCS spinal cord injuries in human divers can manifest as loss of limb function, loss of lower body sensation, inability to walk, or loss of bladder and gastro intestinal control; it can persist for months, and in some cases is permanent. Marine mammals present an interesting case study for Type II DCS, as these species are adapted for diving but may nonetheless succumb to an imbalance in nitrogen gas dynamics when their normal diving regime is interrupted. Little is known about the mechanisms behind spinal cord injury in DCS. The spinal cord may be particularly vulnerable because it has a high lipid content (which presumably means correspondingly high nitrogen [N_2] solubility, although there are no data on this topic) and, in most animals, poor collateral blood supply. Contrary to the large body of literature on lipid composition of adipose tissue across species, including an abundance of information from marine mammals, we know virtually nothing about variation in spinal cord composition between diving and terrestrial mammals, nor how the various components might interact with N_2 gas. In addition, and in contrast to other animals, marine mammals have a substantial and complex circulatory arrangement around the spinal cord. It has been hypothesized that autochthonous bubbles in the spinal cord might create local pressure and occlude blood flow leading to anoxic damage and disruption to the myelin sheath. The few studies that have been conducted on DCS in spinal cords with animal models indicate that the myelin layer becomes disrupted in affected specimens. Given the data presented above, it would seem likely that the brain and spinal cord will exhibit different interactions with N_2 gas, likely as a function of both blood supply and lipid composition. Further, the substantial amount of vascular surrounding the spinal cord of marine mammals, compared to non-diving groups, may mean additional potential for interaction with N_2 gas given the proximity and extent of blood flow to this tissue.

Objectives

The objectives of is project are to (1) determine the lipid content and composition (lipid class) of the spinal cord and brain of diving mammals (shallow and deep diving cetaceans) for comparison with comparable data from terrestrial mammals (pigs); (2) compare the nitrogen solubility of the spinal cord and brain of these two groups; (3) characterize spinal cord tissue (i.e. capillary density; vascular supply) to evaluate the potential for gas exchange; (4) examine the spinal cord tissue of marine mammals for evidence of bubble formation and/or histopathologic changes.

Methods

Samples for this project were collected from archived samples from previous marine mammal strandings along the US east coast (cervical spinal cord tissue only), detailed necropsies of recent marine mammal strandings in the southeastern US (spinal cord and spinal nerve from cervical through lumbar regions, brain tissue), and Wells Pork Products (North Carolina; thoracic spinal cord tissue only). The lipid content (% wet weight) of samples was determined using a modified Folch et al. (1957) method. The lipid class composition was determined using high-performance thin-layer chromatography (HPTLC) following the methods of Vitiello and Zanetta (1978); considerable method development was required here as this is the first time the lipids in the CNS of these animals have been examined. Figure 1 is an HPTLC chromatogram of the lipid

classes present in the thoracic spinal cord of a bottlenose dolphin, shown in dark green, with a standard shown in light green.

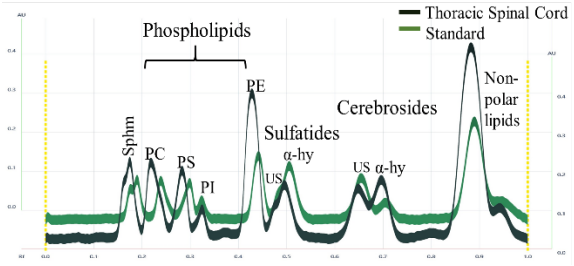

Fig.1. HPTLC chromatogram showing the lipid classes present in the thoracic spinal cord of marine and terrestrial mammals examined in this study. A representative spinal cord sample is shown in dark green and the standard is shown in light green. Sphm: sphingomyelin, PC: phosphatidylcholine, PS: phosphatidylserine, PI: phosphatidylinositol, PE: phosphatidylethanolamine, US: un-substituted, α-hy: alpha-hydroxylated.

Results

We successfully developed a method for separating and quantifying the poorly characterized lipids present in marine mammal neural tissue. The lipid content and lipid class composition of cervical spinal cord from 11 species of marine mammals (N=24 individuals) with a range of diving behaviors has been determined (Fig. 2). The cervical spinal cord of species with typically shallow typical dive profiles contained significantly less lipid (8.17±2.86%) than the cervical spinal cord of species with deeper dive profiles (10.72±2.72%; *P*=0.0008). The lipid class composition of the cervical spinal cord of these 11 species was not significantly different by typical dive profile (shallow vs. deep; Fig. 2).

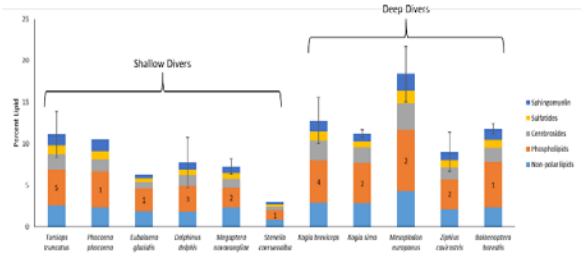

Fig.2. Lipid content of cervical spinal cord from 11 marine mammal species. The overall height of the bars represents average percent lipid by species and error bars are standard deviation. Numbers indicate sample size for each species. The colors are the percent of each lipid class. Cervical spinal cord tissue of shallow divers contained significantly less lipid than the cervical spinal cord of deep divers (*P*=0.0008), but no significant differences in lipid class composition were observed.

The lipid content of the brain, spinal cord and spinal nerves has been examined in 5 marine mammal species longitudinally throughout the body (cervical to lumbar regions; Fig. 3). Thoracic spinal cord and brain samples from three domestic pigs were also available. There was no significant difference in the percent lipid of spinal cord and brain tissue by species or region of the body, mostly due to high variability within and between species (*P*>0.05 for both species and region of the body). HPTLC data indicated no significant difference in the lipid class composition by species or body region.

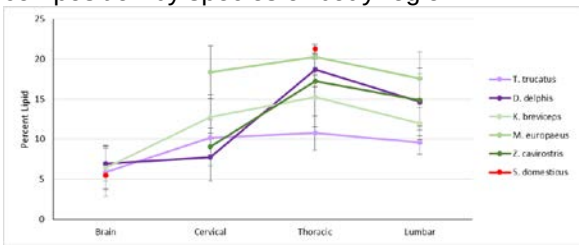

Fig.3. Lipid content of brain and spinal cord, longitudinally, in 5 marine mammal species and pig. Purple lines=shallow diving species; green lines=deep diving species; pig samples in red. Error bars = standard deviation. There was no significant difference in % lipid or lipid class composition by species or body location (*P*>0.05).

Within an individual, nerve tissue from the lumbar region contained significantly more lipid than nerve tissue from the thoracic region (*P*=0.0003). Despite the homogeneity in the lipid class composition within a tissue, the lipid class composition of spinal cord, spinal nerve, and brain tissue were all significantly different from each other (Fig. 4; *P*=0.001, global R= 0.34). This may be representative of the different functions for these tissues. The roles that differences in lipid class composition may play in nitrogen gas absorption will be investigated in FY 19 as will microvasculature of these tissues.

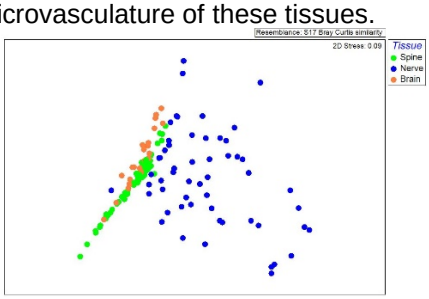

Fig.4. Lipid class composition by tissue type. Spinal cord tissue is shown in green, spinal nerve tissue is shown in blue, brain is shown in orange. The lipid class composition of these three tissue types were significantly different (*P*=0.0003, global R=0.34).

Notes: ***Hurricane Florence caused significant damage to the UNCW campus. Although the Koopman lab was not directly affected, UNCW's Microscopy facility was shut down and only recently have parts of it been restored. Consequently the microvasculature portion of our study (objective 3) has been delayed; we anticipate starting in August 2019.***

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: ONR
Project Status: ONGOING

AUTONOMIC ACTIVITY AND WATER IMMERSION

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Background

The hydrostatic pressure during water immersion, including autotransfusion of fluid from the extravascular space to within the vascular compartment, affects several physiological systems that potentially influence the risk of oxygen and carbon dioxide toxicity. Specifically, the autonomic nervous system reflexively modulates cardiovascular and ventilatory responses to many physiological challenges but little is known about activation of the autonomic nervous system during water immersion.

Circulating catecholamines (i.e. epinephrine and norepinephrine) have been shown to be reduced during thermoneutral water immersion. This indicates that sympathetic activity is reduced during water immersion; however, circulating catecholamines are not a direct measure of autonomic activity. Microneurography is the gold standard technique used in humans to directly measure bursts of sympathetic nerve activity (Fig 1.). Directly measuring sympathetic nerve activity during water immersion will provide us with new information regarding cardiovascular and ventilatory control that might contribute to the development of oxygen and carbon dioxide toxicity.

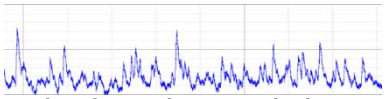

Fig.1. Example of muscle sympathetic nerve activity obtained from microneurography. This recording shows sympathetic nerve burst activity during simultaneous metaboreflex and mechanoreflex stimulation in dry conditions.

In addition to the hydrostatic pressure that elicits changes in systemic physiology, Navy divers routinely breathe hyperoxic gas mixtures during missions. Breathing hyperoxia reduces sympathetic activity, heart rate, blood pressure, and ventilation at rest and during exercise on land, but it is not known how water immersion affects these responses. Similarly, Navy divers might also experience carbon dioxide scrubber failures while using a rebreather. This causes the inspired fraction of carbon dioxide to rise. An increase in arterial carbon dioxide stimulates the sympathetic nervous system to increase ventilation, heart rate and blood pressure, which contrasts the physiological effects of water immersion. In this context, the autonomic nervous system does not seem to respond appropriately to hypercapnia during water immersion which is supported by the evidence that the pressure of end tidal carbon dioxide rises during head out water immersion.

To further investigate the physiological responses during water immersion, most Navy operations also involve exercise. Mechanoreceptors and metaboreceptors are both activated and contribute to the reflex control of the cardiovascular and ventilatory systems during exercise. However, breathing hyperoxia and hypercapnia alter the normal mechanoreceptor and metaboreceptor driven responses when on land. Thus, it is important to understand if breathing hyperoxia and hypercapnia during water immersion influences the metaboreflex and mechanoreflex.

Objectives

Specific Aim 1: Determine if sympathetic nerve activity is altered by breathing 100% oxygen air compared to air breathing during both thermoneutral and cold-water immersion conditions. We hypothesize that: 1) cold water immersion will increase sympathetic nerve activity compared to thermoneutral water immersion during air breathing and 2) breathing 100% oxygen air during thermoneutral and cold-water immersion will reduce sympathetic nerve activity compared to the air breathing conditions.

Specific Aim 2: Determine if sympathetic nerve activity is altered by breathing hypercapnic air compared to air breathing during both thermoneutral and cold-water immersion conditions. We hypothesize that breathing hypercapnic air during thermoneutral and cold-water immersion will increase sympathetic nerve activity compared to the air breathing conditions.

Methods

Specific Aim 1: Healthy participants will complete four randomized 4-hour head out water immersion trials. One trial will be performed in thermoneutral water (35°C) while breathing air, one trial will be performed in cold water (25°C) while breathing air, one trial will be performed in thermoneutral water while breathing 100% oxygen, and one trial will be performed in cold water while breathing 100% oxygen. Static handgrip exercise followed by circulatory occlusion will be performed before entering the water, within 10 minutes of entering the water, every hour during immersion, and post-immersion to assess activation of the mechanoreflex and metaboreflex. Muscle sympathetic nerve activity will be continuously recorded along with cardiovascular (i.e. blood pressure, heart rate, limb blood flow, cerebral blood flow) and ventilatory (ventilation, partial pressure of end-tidal carbon dioxide) data.

Specific Aim 2: Healthy participants will complete four randomized 4-hour head out water immersion trials. One trial will be performed in thermoneutral water (35°C) while breathing air, one trial will be performed in cold water (25°C) while breathing air, one trial will be performed in thermoneutral water while breathing hypercapnic air, and one trial will be performed in cold water while breathing hypercapnic air. Hypercapnic air will be administered progressively throughout the head out water immersion trials (begin breathing air with 0.5% carbon dioxide and increasing by 0.5% every 30 minutes). Static handgrip exercise followed by circulatory occlusion will be performed before entering the water, within 10 minutes of entering the water, every hour during immersion, and post-immersion to assess activation of the mechanoreflex and metaboreflex. Muscle sympathetic nerve activity will be continuously recorded along with cardiovascular (i.e. blood pressure, heart rate, limb blood flow, cerebral blood flow) and ventilatory (ventilation, partial pressure of end-tidal carbon dioxide) data.

Results

In FY18, we built our custom made rapid-filling water immersion tank (Fig. 2). This tank allows us to perform complex autonomic measurement techniques, such as microneurography, during water immersion. Data collection on 12 participants who are currently enrolled in various stages of testing for Specific Aim 1 and data extraction are ongoing.

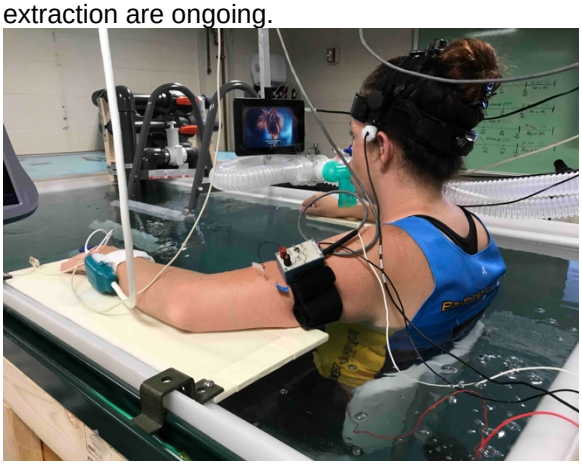

Fig. 2. Experimental Set-up. The custom-built immersion tank allows us to perform complex physiological measurements, such as muscle sympathetic nerve activity in the radial nerve, beat to beat arterial blood pressure, arterial oxygen saturation, middle cerebral artery blood velocity, ventilation, oxygen consumption, and end-tidal carbon dioxide during head out water immersion.

Notes:

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: NAVSEA
Project Status: NEW

EXPLORATION OF MEDICAL RESPONSE STRATEGIES TO OPTIMIZE SURVIVAL OF ESCAPEES FROM A DISABLED SUBMARINE (DISSUB)

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Background

Few USN submarine sinkings have occurred since World War II and none since the loss of USS Scorpion (SSN 589) in 1968 — testament to the safety of modern submarine designs and operations. The intervening years have however seen further international losses and continuing occurrence of mishaps that could result in the disablement and sinking of the submarine (a "DISSUB event"). The risk and high cost of a DISSUB event, together with commitment to international response efforts, make Submarine Escape and Rescue (SER) capability a core safety requirement for submarine operating nations. This was further emphasized by the recent loss of the ARA San Juan S-42.

Recent US Navy SER policy and investment in recent years have focused on the rescue aspects of response. Rescue is the only means of survival in DISSUBs beyond physiological escape depth and is the safer and preferred method of saving life in most situations. It is, however, essential to have an effective escape system, because survivors may be forced to conduct an escape before rescue forces arrive on site, or when operational or engineering constraints render rescue impossible.

Crew survival in DISSUB events depends upon many variables, one of which is a rapid and appropriate medical response, with assets capable of providing appropriate levels of medical care in a complex and remote mass casualty situation. Definition and mitigation of shortfalls in current US Navy DISSUB survival, SER and medical response capability are current Joint Force, Navy and Submarine Force (SUBFOR) priorities.

Research efforts have focused on hardware solutions for the DISSUB environment and survivor decompression strategies. Examining the epidemiological features of DISSUB events and formulating reliable estimates of casualties and threats to the Health Service Support system are critical to effective planning of medical resource requirements.

Prolonged Field Care (PFC) principles offer a novel approach to DISSUB medical response capability gaps. PFC is an established NATO Special Operations Forces (SOF) concept for optimizing survival in austere delayed medical evacuation conditions. It is an existing US capability with potential for extension beyond the operational land environment.

This work extends an FY17 DSBD-funded effort that focused on the application of prolonged field care principles to DISSUB rescue and surface abandonment, to triage of submariners following DISSUB escape scenarios.

Objectives

To secure still pending approval for publication of the Technical Report associated with the FY17 work effort (A Critical Review of Casualties from Non-Combat Submarine Incidents and Current US Navy Medical Response Capability with specific focus on the Application of Prolonged Field Care to Disabled Submarine Survival and Rescue). Lessons learned from mitigation of associated PAO issues will be applied in the approach to the current work, to ensure more streamlined transition.

To undertake a critical review of the likely biomedical evolution and existing medical response capability in DISSUB escape scenarios to define (1) An updated risk assessment and casualty estimate for any DISSUB escape event to which the US Navy may be called upon to provide assistance; (2) Optimal and existing medical response capabilities; (3) Skill and equipment advances with potential to address capability gaps (with particular focus on PFC techniques); and (4) Recommendations for updating of US Navy DISSUB onboard and surface medical concept of operations CONOPS or Independent Duty Corpsman (IDC) training.

Methods

We will conduct a narrative literature review of the published, unclassified historical and scientific evidence and current CONOPS, and collaborate

with relevant USN and Allied Force SMEs, to define the risks to survival of DISSUB escapees, and inform a casualty estimate and medical capability gap analysis. Resulting recommendations for risk mitigation will be further supported by discussion with implementation authorities, including NUMI, BUMED Undersea Medicine, NAVSUBSCHOOL (PSET) and SUBFOR / SUBPAC Fleet Medical Officers.

Results

This is a new start and completed data analysis is limited. Preliminary findings from historical data and literature review are described below.

Based on historical evidence and current submarine design, NSMRL believes that any DISSUB event will result in rising pressure and slow but unstoppable flooding of the survivor compartment which will require survivors to escape before the minimum rescue force arrival time. This risk is further compounded by current shortfalls in rescue capability, and supports parallel efforts to optimize escape capability.

The feasibility of through water escape has been demonstrated to 240 feet from an actual DISSUB and to 600 feet experimentally. History has shown that, even since the advent of rescue capability in 1939, escape remains a significant means of survival in catastrophic submarine accidents (Table).

Method of egress	Incidents and vessel type		Numbers attempting	Survivors	Last event
Surface abandonment	Nuclear	4	291	207	2003
	Diesel-electric	31	736	685	1988
	Total	**35**	**1027**	**892**	**2003**
Escape	Nuclear	2	92	85	1989
	Diesel-electric	9	212	135	1988
	Total	**11**	**304**	**220**	**1989**
Rescue	Nuclear	0	0	0	N/A
	Diesel-electric	1	33	33	1939
	Total	**1**	**33**	**33**	**1939**

Notes: Method of international DISSUB survivor egress since 1939.

There are three situations which have to be considered in submarine escapee survival: (1) Survival in the submarine before escape, (2) Survival during the ascent, and (3) Survival on the surface. Escapees from a disabled sunken submarine face a plethora of hazards from the compromised conditions inside the submarine and the cold, immensely pressurized environment outside it. Potential threats to survival include trauma, toxic gases, nitrogen narcosis, carbon dioxide poisoning, thermal stress, barotrauma, arterial gas embolism (AGE), decompression sickness (DCS), cold water immersion and drowning. Escape gear must help submariners survive these diverse threats, while minimizing the operator error to which it has been historically prone. History has additionally demonstrated the considerable importance of sea survival capability and early on-scene recompression assets for optimization of DISSUB escapee survival and morbidity risk.

Review of historical data is ongoing. Casualty estimates will additionally be supported by existing risk models and simulated DISSUB escape data, including the published findings of experimental trials, escape exercises and Pressurized Submarine Escape Training (PSET) experience, which offer a valuable source of additional biomedical outcome data. The biomedical risks associated with both individual and mass escape technologies will be evaluated.

The authors are involved in a number of NSMRL initiatives to optimize DISSUB escapee survival:

- GUARDBOOK updates to improve the ease and reliability of survivor stay-time calculations.
- Recent GUARDBOOK procedural walkthrough aboard USS Colorado.
- Review and update of existing DISSUB escape DCS risk models.
- Consideration of the medical implications of design modifications to the Columbia Class escape trunk to allow escape while the boat is lying at an angle.

Notes:

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: NAVSEA
Project Status: ONGOING

HYPERTHERMIA AND HYPOHYDRATION IN A DISABLED PRESSURIZED RESCUE MODULE

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Background
In the event of disabled submarine (DISSUB), a Pressurized Rescue Module (PRM) may be deployed. The PRM is a small pressurized vehicle capable of holding the rescued sailors and safely delivering them to the surface. Safe deployment of the PRM is dependent on understanding and mitigating the possible challenges if failures were to occur. If the PRM were to become disabled, the environmental conditions could quickly challenge the health and safety of those inside the PRM. For instance, because of the high number of sailors in a small space, the magnitude of increases in humidity and air temperature during a blower failure are predicted to rise to upwards of 35°C and the air will quickly become saturated with water, raising humidity to >95% relative humidity (RH). With a heat index of ~68°C, such conditions can challenge temperature regulation, potentially overwhelming the capacity for heat loss via sweating and cutaneous vasodilation. As a result, the thermal environment in the disabled PRM can, in theory, become dangerous very quickly.

To our knowledge there are no models, or data to base then on, capable of accurately predicting the magnitude of hyperthermia (i.e., increases in core body temperature) and/or hypohydration (i.e., reductions in body fluids occurring subsequent to sweating) in a nearly saturated (>95% RH) environment with air temperatures in excess of 33°C for up to 24 h. Furthermore, because of reductions in the evaporative efficiency of sweat at increased ambient pressures, these dangerous thermal conditions could become exacerbated in the event of a failed pressurization system in the PRM. However, to our knowledge there are no models capable of accurately predicting the magnitude of hyperthermia and/or hypohydration in a hyperbaric, humid and warm environment for up to 24 h.

Objectives
Empirical data are needed to determine the magnitudes of hyperthermia and hypohydration in a disabled PRM at 1 ATA and at a depth of 20 feet of seawater (fsw, 1.6 ATA) to be used to develop predictive models.

We will examine the following Aims:
Aim 1: Determine the magnitudes of hyperthermia and hypohydration incurred in a warm and humid disabled PRM scenario at 1 ATA for up to 24 h.
Aim 2: Identify the magnitudes of hyperthermia and hypohydration incurred in a warm and humid disabled PRM scenario at 20 fsw for up to 24 h.

Methods
Fifteen healthy, physically active young males (18-40 y) will complete each Aim. In both Aims, subjects will first complete a screening trial, during which informed consent is obtained and inclusion/exclusion criteria are reviewed. In both Aims, subjects will then complete three experimental trials in which they will be exposed to a 32°C, 33°C, and 35°C, 95% RH environment. The order of these trials is conducted in a randomized, counterbalanced manner. Each trial is separated by at least 1 week to prevent adaptation to hyperthermia and hypohydration. Subjects are not able to drink any fluids during these exposures given the lack of fluid availability in PRMs. Subjects will wear a standard uniform of long pants, a short sleeve shirt and athletic shoes, and will be in the seated position. They arrive at the laboratory euhydrated, normothermic, and having avoided exercise, alcohol, and caffeine for at least 12 h.

Primary instrumentation and measurements:
Instrumentation and measurements are the same in both Aims. Core temperature is measured via an ingestible temperature capsule. Skin temperature is measured as at 12 locations. These data will be collected before exposure and every 10 min thereafter for monitoring purposes. Body weight (following towel drying of sweat) is measured before exposure and every 60 min thereafter. Venous blood and urine samples are obtained before, at half way, and at the end of the exposures. Blood and urine samples will be measured for indices of hydration status.

Primary calculations: Percentage changes in body weight loss are calculated to provide an accurate measure of acute changes in total body water. The rate of change in core temperature and percentage changes in body weight over the final hour of exposure are used to predict the magnitudes of hyperthermia and hypohydration that are expected to occur over 12 and 24 h. From these data, the volume of fluid predicted to stave off dangerous levels of dehydration (>4% loss of body weight) will be calculated.

Results

We have complete data sets from 10 subjects, and data collection is currently ongoing in 5 others. Preliminary analyses of the primary dependent variables as they relate to Aim 1 are presented.

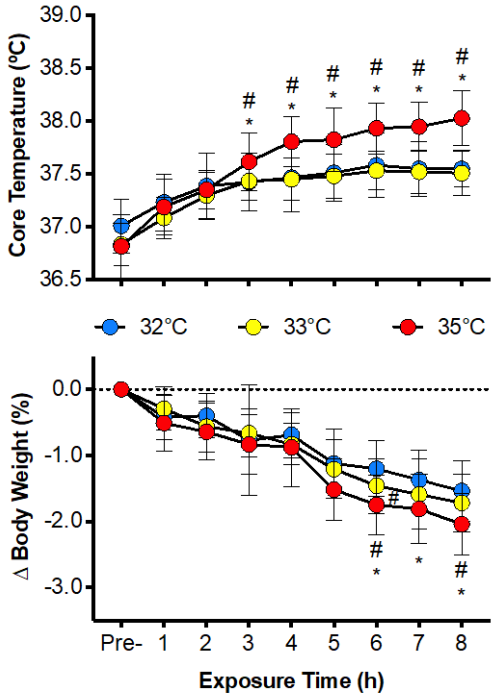

Fig.1. Core temperature (top) and percent changes in body weight (bottom) during 8 h exposure to 32°C, 33°C, and 35°C, 95% RH, 1 ATA environments. Mean ± SD, n=10, # different from 32°C (P<0.05), * different from 33°C (P<0.05).

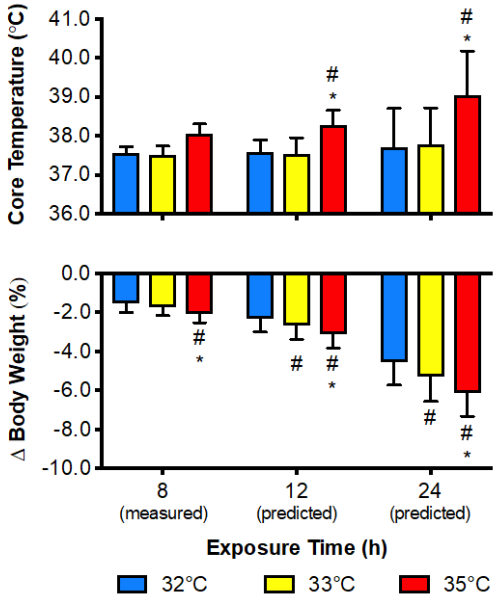

Fig.2. Measured core temperatures (top) and percent changes in body weight (bottom) after 8 h of exposure to 32°C, 33°C, and 35°C, 95% RH, 1 ATA environments, and predicted changes in these variables over 12 and 24 h. Mean ± SD, n=10, # different from 32°C (P<0.05), * different from 33°C (P<0.05).

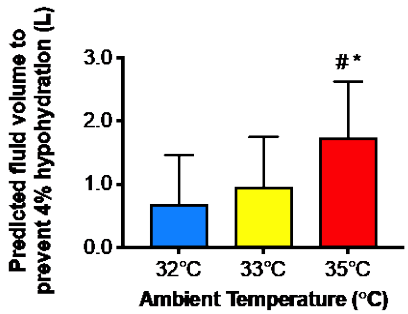

Fig.3. Predicted fluid volume required to prevent 4% hypohydration over 24 h exposure to 32°C, 33°C, and 35°C, 95% RH, 1 ATA environments. Mean ± SD, n=10, # different from 32°C (P<0.05), * different from 33°C (P<0.05).

Conclusions: Over 8 h of exposure to 32°C, 33°C, 35°C, 95% RH, 1 ATA environments the magnitudes of hypohydration and hyperthermia are dependent on ambient temperature. Projecting forward, both hypohydration and hyperthermia are likely to be problematic when exposures approach or exceed 35°C over 24 h or longer. It is estimated that to stave off dangerous levels of hypohydration (>4% loss of body weight) each sailor should have available ~1.8 L of fluid.

Notes:

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: NAVSEA
Project Status: ENDING

IMPROVING SAFETY OF SUBMARINE ESCAPE AND RESCUE FROM SHALLOW DEPTH

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Background

In a submarine rescue, personnel in the disabled submarine (DISSUB) may have been exposed to increased ambient pressure for a prolonged duration, often more than 24 hours. Such exposure to increased pressure can result in saturation of the body's tissues with dissolved gases, mostly nitrogen (N_2), when breathing air as in a submarine. Decompression of personnel during submarine escape can be very rapid with a "drop-out" ascent to the surface in minutes. Rapid "drop-out" and other accelerated decompressions from the submarine can provoke significant gas bubble injury to the body's tissues known as decompression sickness (DCS). Forms of DCS can involve life-threatening tissue injury to the central nervous system (CNS-DCS) and to the respiratory system (RDCS), the chokes, with pulmonary edema reducing gas exchange in the lungs and generating foam, which obstructs the airways. Each serious form of DCS carries a potentially high risk of morbidity/mortality. Potentially fatal decompression outcomes require the ethical use of an animal model to investigate the risk, course, natural history of serious DCS, and efficacy of DCS treatment modalities. Experimental decompression findings in a large animal model (i.e. sheep) are essential for understanding serious DCS and developing practical risk management procedures to minimize fatal outcomes in submarine escape and rescue.

Objectives

The UW sheep model of the decompressed human used to evaluate risk and preventive measures to lower the risk of serious DCS in submariners escaping from their disabled submarine. Specifically, the objectives of this proposal is to investigate: 1) risk in various decompression scenarios, 2) accelerated rates of decompression within defined limits of chosen risk, and 3) potentially efficacious therapeutic interventions with oxygen to minimize potentially fatal decompression outcomes. Use of a large-animal model is important in these experiments, because humans and large animals have similar gas uptake and elimination rates as well as potentially injurious bubble formation, which scale to the 3/4 power of body mass. Adult sheep, with similar body weights to that of adult humans have decompression injuries and DCS incidences similar to those in decompressed humans.

Methods

We did study “drop-out” decompression from 60 fsw with 90 min oxygen pre-breath (OPB) and 60 min surface interval to establish the baseline response. Then we did decompression from 70 fsw to 60 fsw with rate of 2 feet/min, and provided 90 min OPB before “drop-out”, and 30 min surface interval period. We closely observed the sheep for clinical signs of DCS during the surface interval, and then 24 hours after initial decompression.

Fig.1. Ultrasound bubble detection. Pre-cordium Spenser scores at surface, and 1-h time points.

Results

Six adult female sheep (mean weight 92.2 kg ± 7.9 SD) underwent dry chamber air exposure at 60 feet of sea water for 24 hours. Animals breathed chamber air with oxygen maintained at 21% and CO_2 < 0.05 surface equivalent percent. At 24-h they were provided a 90-min oxygen pre-breath, and then decompressed at 30 feet/min, a Navy fleet diving standard ascent rate for human exposures. We closely observed the sheep for clinical signs of DCS during the surface interval of 60 min. All six sheep survived drop-out decompression with Type I, all showed frank limb bends and most developed dysbaric osteonecrosis (DON). Gross pathology confirmed bone MRI DON lesions.

Another six adult female sheep (mean weight 85.2 kg ± 12.9 SD) underwent dry chamber air exposure at 70 feet of sea water for 24 hours. At 24-h they were slowly decompressed to 60 fsw with rate of 2 feet/min, and provided a 90-min oxygen pre-breath at 60 fsw, and then decompressed at 30 feet/min. We closely observed the sheep for clinical signs of DCS during the surface interval of 30 min, and then 24 hours after initial decompression. All six sheep survived drop-out decompression from 70 fsw with Type I, and all showed frank limb bends.

Sheep drop-our pressure, fsw	Incidence of DCS during 30 and 60 min surface interval, %				
	DCS Type I	Limb bends	CNS-DCS	RDCS	Lethal
60 fsw (90 OPB, 60 min SI)	100	100	0	0	0
70 fsw (90 OPB, 30 min SI)	100	100	0	0	0

Fig. 2. Results of drop our decompression.

Notes:

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: ONR
Project Status: ONGOING

LIGHTWEIGHT ATMOSPHERIC DIVING SUITS - N13A-T029

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Background

Today's Atmospheric Diving Suits are large, cumbersome, rigid enclosures designed primarily to keep a diver alive at the high pressures of depth, with flexibility and maneuverability sacrificed due to the difficulty of its primary goal. The Navy is seeking a new light-weight ADS design, and/or new joint types and appendages to change that paradigm. Midé in partnership with a cross functional team at Massachusetts Institute of Technology (Center for Ocean Engineering (OE), Naval Construction and Engineering Program (2N), and the Man-Vehicle Lab (MVL)) endeavored to create a next generation Atmospheric Diving Suit and novel appendages to solve these challenges.

Objectives

The Navy is seeking a new light-weight ADS design. This design must be less than 400 lbs on ship; and be able to self-propel using legs and fins. This Light-Weight ADS (LW-ADS) will allow a larger variety of launch craft (small boats) and enable the system's use in broader mission capability. The primary objective of this Phase II STTR is to create a unique ADS joint and resulting appendage(s) capable of surviving at 1000 ft depth, while also providing a great deal of maneuverability. Near term goals are to focus on a joint that can survive 1000 ft depth pressure (~450 Psi), while also providing angular motion greater than 60 degrees with minimal diver effort.

Methods

To make a revolutionary ADS, you must start by rethinking the ADS joints themselves. This project has centered on developing a novel flexible joint, which uses an internal support structure that accommodates and balances external hydrostatic pressure loading during deflection. This joint offloads the user during operation and allows for increased joint flexibility, which will allow the diver to move in a more natural bending motion than compared to existing ADS. A three pronged approach has been used to develop the joint. The first studying the human body and its kinematics, the second analyzing how the hydrostatic loading affect the joint at various depths, the third looking at advanced materials (including shape memory alloys, fibers, rubbers, and composites) to construct the flexible joint and other surrounding rigid suit structures.

Regarding human body and kinematics studies, the project began with utilizing MIT MVL's concept of "lines of non-extension" (LoNE). The "lines of non-extension" (LoNE) are lines along the body that do not strain during articulation. MIT MVL have long studied LoNE in developing mechanical counter pressure space-suit designs. The concept is that by creating exo-skeleton like sub structure along LoNE the team can reduce joint complexity to two dimensions, while still allowing full range of motion. For this project MIT has developed internal software codes to map LoNE on the human body using a methodology based on Digital Image Correlation (DIC) to capture the skin strain fields at a very close 1 mm^2 resolution.

As part of this effort the MIT team also performed a human factors pool test in an existing ADS 1200 commercial suit (thanks to commercial partners Phoenix International). The testing instrumented the diver's arms with IMUs and pressures sensors to assess suit usability, diver mobility and exertion under typical tasking.

Fig.1. Existing ADS Pool Test

Midé has primarily used FEA and Matlab based calculation models to design and evaluate joint and structural designs (composites and elastomers). In Phase I Midé used an internal 300 psi hydrostatic pressure chamber to perform initial survivability evaluation of the shell and joint prototypes. Midé has used the same chamber to test Phase II prototypes; however, final testing of the full scale joint was completed at NSWC Panama City's Hydrospace lab, using a Midé built fully submersible robotic test arm, corresponding

instrumentation panel, control box, and custom control software (designed in Labview). In parallel to the hydrospace lab high pressure testing, a qualitative test protocol for a human factors evaluation of the Phase II joint under low pressure (lab tank test), has been carried out at MIT, and a follow up low pressure test tank was built at Mide.

Results

In Phase II MIT MVL has expanded its DIC modeling method to analyze LoNE maps of various size people, and off body LoNE maps (representing how a thick suit may influence the LoNE), and has begun analyzing more complex joints such as shoulders. MIT 2N completed an ADS mission study, developed a Navy dive database website, and compiled user exertion data from the ADS1200 suit pool tests. MIT OE has designed a human factors test protocol a small tank test fixture, and completed qualitative low pressure flexion tests.

Midé has incorporated lessons learned from numerous areas and developed a one of a kind joint concept capable of fully offloading the diver during joint deflection. Midé first built and tested a ½ scale prototype to prove feasibility of the joint concept. After the proof of concept, Midé successfully designed the full scale version of the joint, and fabricated several for testing.

Fig.2. Midé Phase II Full Scale Joint Fabricated

Midé then designed and fabricated a robotic test arm, which is capable of being fully submerged such that the joint can be tested and flexed at various angles at high hydrostatic pressure. The outer shells of the test arm are rated to 2500 Psi, a custom electrical control umbilical and tubing/plumbing all protrude from the arm and out to the "surface" through bulkheads in the test chamber walls.

After initial shake-out testing at Midé's test tank (~200 psi), the joint and robotic test arm system were shipped to NSWC Panama City's Hydrospace lab for testing down to 1000 fsw (~450 Psi).

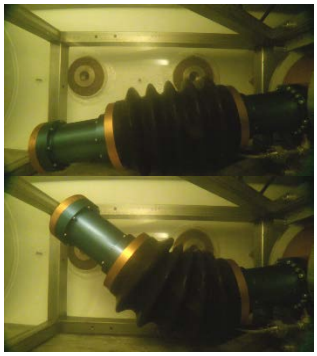

Fig.3. Joint being tested at NSWCPCD, arm flexing up to ~55 degrees.

After successful testing at PCD, Midé designed and built a test arm featuring crude rotary joints, and our "membrane" elbow joint. This hybrid arm (rotary + membrane joint) allows for a greater range of motion (more degrees of freedom). Mide then built a low pressure lab test tank to evaluate the motion of the arm (similar to a pool test). Current progress is to revise the elbow joint internals for smoother function across depths, and improve the rotary design by reducing joint friction (or incorporate existing rotary joints if available).

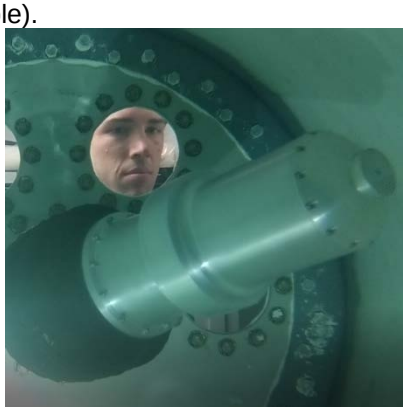

Fig.4. Prototype test arm being tested in low pressure Midé test tank

Notes:

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: ONR
Project Status: Ongoing

CONTAMINATED WATER RESEARCH

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Background

Water pollution is a global issue. With relatively few exceptions most water bodies contain some level of contaminant loading burden (biologic and/or chemical). While forward operation sites located in Latin America, Asia, and Africa pose the greatest risk of exposure, with in some cases 70% of their water resources receiving an impaired/ poor condition rating[1], the probability of exposure to contaminates of concern within developed countries is still high[2,3] According to the 2017 US EPA Water Quality Inventory Report to Congress[2], at least 43% of US water resources are listed as impaired or unable to support at least one of their designated uses. This number rises to an average of 76% when comparing the total number of sites monitored to total number of sites listed. In particular, and of specific relevance to Navy Divers, some of the highest numbers of impaired sites listed (coastal water estuaries, embayment, great lakes and shore lines) are associated with types of waterbodies our troops would frequent for routine operations. As a result the potential exposure to contaminated waters during both day-to-day operations and special mission critical operations poses a serious problem for divers.

In addition, many military operations rely on quality of water. As such, the DoD as a whole views water pollution as a potential health and safety risk for our armed forces[4] making it imperative to anticipate, understand, evaluate, and address the potential threat. As a result, proper preparation, identification of potential contaminants of concern, and monitoring of contaminated water, remain issues of concern for the Navy, Marines and forwardly deployed organizations needing safe water resources.

While there have been funded efforts, which have focused on the improvement of protective equipment and the development more real-time in situ contaminants of concern sensors, most Federal and DOD funding has focused on emergency response planning and water purification due, in part, to current limiting (e.g. reagent-dependent, target-specific, and time consuming) aspects of available technology. Additionally, these methods lack a quantitative approach and capacity to sort through a complex, heterogeneous environment. While the ability to evaluate and address the potential threat, via improvements to sensors and PPE equipment, continues to improve, our ability to anticipate and understand the threat remains a critical issue.

Objectives

The goals of this effort are: (1) to provide the Contaminated Water Diving (CWD) community a framework for addressing data gaps that impact how to broadly evaluate CWD sites characteristics and potential risk characterization to divers; (2) to inform CWD operation from a legacy pollutant perspective and current site conditions; (3) provide a means of addressing site specific or emergent contaminants of concern; (4) enable dive scenarios to be defined by site specific characteristics and (5) provide a platform through which key stakeholders can establish communication and collaborations focused on mitigating potential adverse health effects related to diving in contaminated waters.

Methods

This presentation will describe what has been accomplished to date and emergent data gaps that have been identified through previous efforts.

Future efforts will focus on the current state of the science related to CWD in marine environments, such as industrial harbors and waterways, small harbors and ports, and open ocean environments.

A workshop is being planned that will focus on understanding past, present, and future initiatives related to marine environmental water quality contaminants, safe exposure limits, and marine environmental scenarios. The focus of the workshop will be to continue developing solutions for addressing CWD issues that are scientifically based, realistically achievable, and designed to protect diving personnel from

potential adverse health effects related to diving in contaminated waters.

Results

Results of previous efforts will be combined with workshop results to develop a framework for addressing identified data gaps that impact how to broadly evaluate CWD site characteristics and potential risk characterization to divers. This will inform CWD operations from a legacy pollutant perspective, and in addition will provide a means for addressing site-specific or emergent contaminants of concern.

Notes:

(1) The US EPA defines an impaired / poor water quality condition as "unable to support one or more of the uses designated for them by the state, such as fishing or swimming" on a global context a poor rating would indicate the water is not safe for drinking

(2) US EPA 2017. National Water Quality Inventory: Report to Congress. EPA 841-R-16-011, 22pp.

(3) UNEP 2016. A Snapshot of the World's Water Quality: Towards a global assessment. United Nations Environment Programme, Nairobi, Kenya. 162pp

(4) DoD Instruction 6490.03, "Implementation and Application of Joint Medical Surveillance for Deployments

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: NAVSEA
Project Status: ENDING

TRANSFER OF DUKE DIVE TRIAL TO U.S. NAVY

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Background

Decompression studies (or non-decompression studies that required decompression) have been conducted at the Duke Hyperbaric Center from the late 1960s to present. Some, particularly the earliest, could not be done today due to safety concerns or funding limitations, and some were statistically underpowered by current analytical standards.

Objectives

The objective of this project is to consolidate these studies in ANMRI dive profile format with DCS case descriptions and transfer these data to the U.S. Navy for use in probabilistic DCS model calibration and evaluation. The data, when combined with existing U.S. Navy human dive trial data, will expand the scope of existing decompression studies, improve statistical power, and might aid investigation of factors affecting DCS risk.

Methods

Original handwritten chamber logs, dive notes, DCS event and treatment records were consulted to generate dive trial data in ANMRI format. Studies for which some or all of the data had previously been reduced to ANMRI format were checked against the original logs for accuracy and corrected if needed. Missing dive profiles were added, and other missing data such as DCS symptom onset times [1] were added or corrected.

From DCS incident case reports and treatment records, incidents were reassessed using a standardized protocol [2]. In several cases, as a result of reassessment, full DCS events were downgraded to either marginal or no DCS. Further, some cases of marginal DCS were downgraded to no DCS. No cases in this study resulted in an event being upgraded from either no DCS or marginal DCS to full DCS, however, in at least one case DCS event originally classified as type I was reassessed as type II. Additionally, a DCS case that was absent from the original reporting of the data was located and that case was added to the corrected data.

A custom software interface, written in the C# computer language using a previously developed DCS modeling software solution, was developed in order to expedite the creation of the text-based ANMRI format data from handwritten dive logs. This interface allowed for simplified entry of the time/depth history of a given profile as well as breathing gas and gas switch information, DCS outcome information, and symptom onset times, if any. For some of the dive profiles additional text-based information was included in the ANMRI file.

Results

The following studies were delivered to the U.S. Navy in ANMRI format with supporting information:

(a) MK15: Dry resting and wet exercising N_2-0_2 and He-0_2 dives using the MK15 UBA. 1395 exposures resulting in 10 cases of marginal DCS and 16 cases of DCS.
(b) Fife: Deep air dives some with O2 decompression. 26 exposures with no cases of marginal or full DCS.
(c) SIO_2: Dives with O_2 surface intervals. 235 exposures with 1 case of marginal DCS and 2 cases of DCS.
(d) NOAA: Dives with O_2 surface intervals. 89 exposures and 1 case of DCS.
(e) Ascent Rate: Single air dives with ascent rate of either 10fsw/min or 60fsw/min. 289 exposures with 1 case of marginal DCS and 4 cases of DCS.
(f) Project Dive Exploration: Open water air and N_2-O_2 single and repetitive dives. 122129 exposures with 38 cases of DCS. Marginal DCS cases were coded as non-events for this study.
(g) PSI-Augmented NMRC 99-02: Perceived Severity Index data added to NMRC 99-02 [3,4] database.

References

[1] Weathersby, P.K. *et al.* Predicting the time of occurrence of decompression sickness. J. Appl. Physiol. 72(4): 1541-1548.
[2] Freiberger, J.J. *et al.* Consensus factors used by experts in the diagnosis of decompression

illness. Aviat. Space Environ. Med. 75:1028-1028. 2004.
[3] Temple, D. J. *et al.* The dive profiles and manifestations of decompression sickness cases after air and nitrogen-oxygen dives. Volume I. Data set summaries, manifestation descriptions, and key files. NMRC 99-02 (Vol. I). 1999.
[4] Temple, D. J. *et al.* The dive profiles and manifestations of decompression sickness cases after air and nitrogen-oxygen dives. Volume II. Complete profiles and graphic representations for DCS events. NMRC 99-02 (Vol. II). 1999.

Notes:

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: ONR
Project Status: Ending

MICROPARTICLES, PLATELET-NEUTROPHIL AGGREGATION AND DECOMPRESSION SICKNESS

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Background

We hypothesize that circulating microparticles (MPs, 0.1- 1 µm diameter vesicles produced by all vascular cells) are the proximal cause for organ injuries following decompression stress because they initiate an inflammatory response.

Objectives

1) Elucidate MPs production mechanisms.
(2) Evaluate methods to ameliorate MPs injuries.
(3) Determine the fate of MPs *in vivo*.

Methods

Biochemical studies using human and murine platelets and neutrophils in *ex vivo* gas exposures, several rodent models, and studies of human volunteers. Procedures include analyses of cells and MPs by flow cytometry and a variety of biochemical assays to assess free radical generation, cytoskeletal modifications, and inflammatory mediator production.

Results

Aim 1(a) We have now demonstrated that events occur *in vivo* matching the basic studies we previously have done *ex vivo.* That is, we previously showed that MPs are generated in neutrophils and platelets at high pressure (versus on decompression) via oxidative stress due to high gas pressure (J Biol. Chem. 289: 19931, 2014; Free Rad Biol Med 101: 154, 2016) and the biochemical pathway is linked to the NLRP3 inflammasome so MPs are packaged with inflammatory interleukin (IL)-1β. During the past budget period we published a paper (J Appl Physiol 125: 1339-1348, 2018) showing that mice exposed to 790 kPa generate MPs containing high concentrations of IL-1β during the 2 hour exposure, and particles increase still more in the hours following decompression (Figure 1).

Aim 1(b) More recently, we have shown the same events occur in healthy humans exposed to simulated dive profiles. In collaboration with the Canadian Navy, blood from research subjects exposed in hyperbaric chambers to air pressure equal to 18 meters of sea water (msw) for 60 minutes, or 30 msw for 35 minutes, were obtained prior to-, or at the conclusion of pressure exposures, and 2 hours post-decompression. At 18 msw (n=15) MPs increased by 1.8-fold, and IL-1β by 7.0-fold ($p<0.05$, repeated measures ANOVA on ranks). At 30 msw (n=16) MPs increased by 2.5-fold, and IL-1β by 4.6-fold ($p<0.05$), and elevations persisted after decompression. Figure 2 shows results from 30 msw studies.

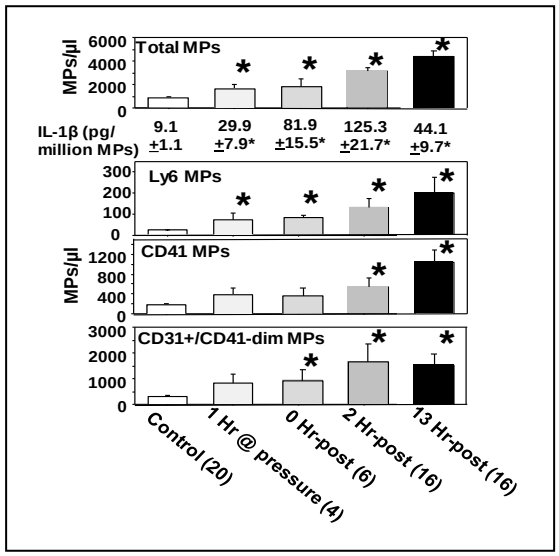


Fig.1. Mice exposed to 100 psi for 2 hours generate MPs (top panel) with high intra-MPs IL-1β concentration during pressure and after decompression. Panel 2 shows MPs expressing neutrophil specific CD66b, Panel 3 shows MPs expressing platelet-specific CD41, and the lowest panel shows a combination of protein markers indicative of particles derived from endothelial cells. Data are mean ± SE, *p<0.05 ANOVA.

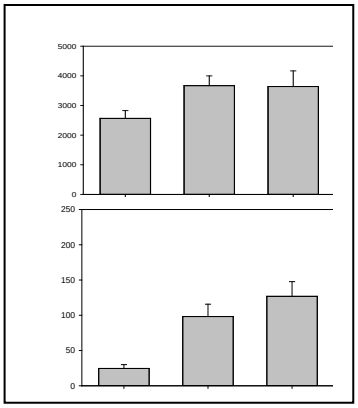


Figure 2. Changes in blood-borne MPs (top frame) and IL-1β content (lower frame) show elevations during pressure exposure and persistent elevations post-decompression. Data are mean ±SE, n=16 divers, *p<0.05).

Aim (2a). In a recent paper we demonstrated that vascular damage due to high-pressure generated, IL-1β enriched MPs can be blocked by treatment with anti-IL-1β antibodies and by anakinra, an FDA approved IL-1β receptor antagonist (JAP 125: 1339-1348, 2018). Figure 3 shows vascular damage in multiple organs in the first panel (mice treated with a control IgG) and antagonism with treatments in panels 2 and 3.

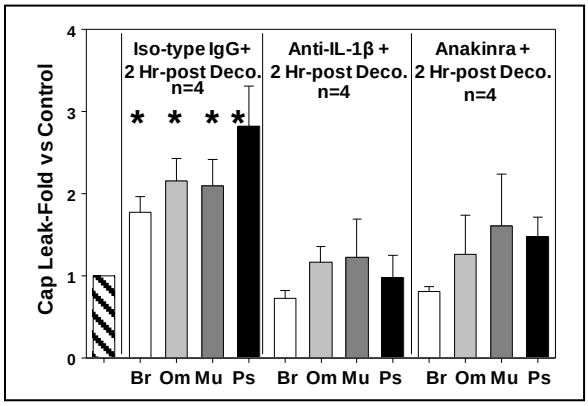
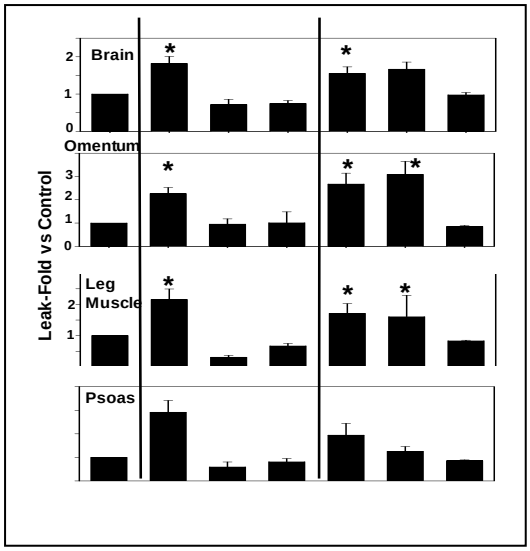


Figure 3. Vascular leakage of 2 x 10^6 Da rhodamine-labeled dextran. Mice were euthanized 2 hours after exposure for 2 hours to 790 kPa air. Extravasation of dextran in brain, omentum, leg skeletal muscle and psoas was evaluated and normalized to data from unpressurized control mice. Data are mean ± SE, * indicates significantly different from control, p<0.05, ANOVA, n=4.

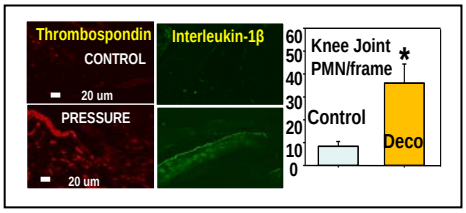
Aim 2(b). Because hyperbaric oxygen (HBOT) is used to treat DCS, an obvious question is whether HBOT ameliorates MPs-induced damage in the DCS murine model. We recently reported that HBOT given as prophylaxis prior to high pressure exposure, or as treatment post-decompression impedes MPs production, inflammasome formation and IL-1β production (J Appl Physiol (1985). 2019 Feb 14. doi: 10.1152/japplphysiol.01109.2018). Figure 4 shows inhibition of vascular leak by HBOT.

Figure 4. HBOT inhibits extravasation of dextran in brain, omentum, leg skeletal muscle and psoas (same procedures as used in Figure 3). HBOT exhibited marked inhibition in mice euthanized at 2 hours post-decompression whether it was administered prior to, or after decompression. Benefit was incomplete when administered post-decompression, however, as mice euthanized 13 hours post-decompression exhibited capillary leak in many organs.



Aim 3(a). We have carried out studies looking for joint inflammation in the murine model. Figure 5 shows increased synovial lining thrombospondin, a protein that will bind phosphatidylserine (as is present on the MPs surface), a parallel increase in IL-1β, and increases in joint space neutrophils in decompressed mice. Preliminary studies involving intravenous administration of PEG-Telomer B indicate that we can impede these inflammatory events by lysing MPs.

Figure 5. Mouse knee joint thrombospondin and peri-articular IL-1β immediately after 2 hours at 100 psi pressure, and joint PMN.



Notes:

2019 ONR Undersea Medicine and NAVSEA Deep Submergence Biomedical Development Program Review Sponsor: ONR
Project Status: ONGOING

DIVER BIOMETRIC DEVICE USING NOVEL PHYSIOLOGICAL SENSORS

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Background

This project aims to develop a Diver Biometric Device (DBD) that will provide the Navy an underwater physiological monitoring capability and enable medical research, and performance and health assessments. The proposed design consists of a torso-worn suite of sensors that measures a diver's physiological vital signs (electrocardiogram (ECG), heart and respiration rates, skin temperature, core temperature, and blood oxygenation), and body orientation and activity parameters (Inertial Motion Unit (IMU) that consists of 3D acceleration, gyroscope, and Magnetometer). The DBD will record this raw physiological data and produce relevant physiological metrics and alerts. It will save the data and equipment parameters to removable internal memory.

The DBD is intended for use by Navy divers to record physiological data for medical research and incident analysis. Research enabled by the device will focus on topics such as understanding hyperbaric exposures and associated diseases, human performance assessment, and development of real-time decompression algorithms. The device is intended for use in a variety of operating conditions, including controlled laboratory environments, studies in open water, and during training and possibly missions of Navy divers. Because no integrated underwater physiological monitoring device currently exists, development of the DBD is expected to advance current capabilities of the Navy.

Objectives

The principal objective of the project is to develop an easy-to-use multimodal sensor suite that operates underwater without the need to waterproof the diver. The first objective of this project is to design, develop, and test ECG, respiration, and skin temperature sensors (based on existing QUASAR sensors) that can function underwater. A waterproof electronics module with environmental sensors will also be designed and built, and integrated with the physiological sensors into a wearable chest belt. The complete prototype will be constructed and then tested on divers in an ocean environment with the help of our partners at Scripps Institution of Oceanography.

In later phases of the project, data interpretation algorithms will be developed, and additional physiological measurement capabilities like core temperature and pulse oximetry will be added. The final system will be tested on divers in a Navy underwater lab.

Methods

In Q1 & Q2 of 2018 a testbed system was used to take measurements on divers in a 10 m deep salt water pool using an off the shelf waterproof housing and electronics and sensors that existed at QUASAR at the time (Fig. 1). That data was analyzed in Q2 & Q3 2018 to understand the constraints and noise generated through motion artifacts of actual divers performing a dive-mission relevant movement protocol.

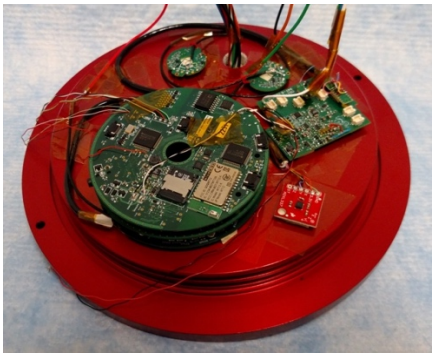


Fig.1. DBD Testbed System. This testbed system used electronics from QUASAR's dry electrode EEG headsets.

From Q2 2018 to Q2 2019 the DBD system design was developed and a prototype was produced. The capacitive ECG sensors of the DBD underwent a major revision to make them easier to produce and to lower system noise. The main electronics board was designed, and initial firmware was coded.

Results

With two capacitive sensors placed in a Lead I configuration across the chest, the Testbed system was able to detect the full ECG

morphology underwater including the QRS complex, as well as P and T waves.

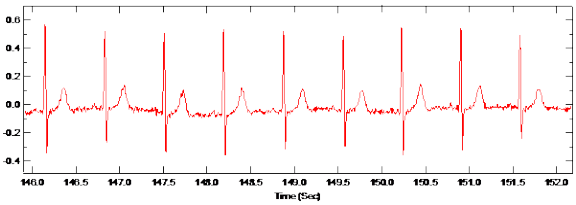


Fig.2. ECG waveforms acquired in salt water with subject wearing a commercially available wetsuit.

To simulate the conditions of a diver working underwater, a protocol was developed that included swimming, finning, sitting, kneeling, and bending within a 10 m saltwater pool. Data gathered on the testbed system shows that R-peaks are robust to motion, even when the user bends torso or fins quickly, as shown below.

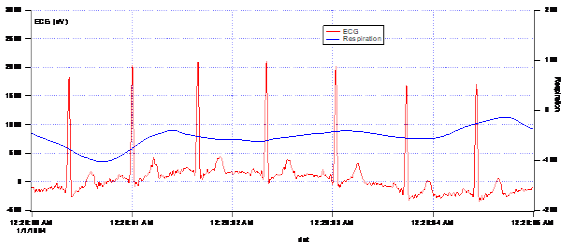


Fig.3. ECG During Fast Finning. Red line shows ECG, blue line shows expansion of the chest (respiration).

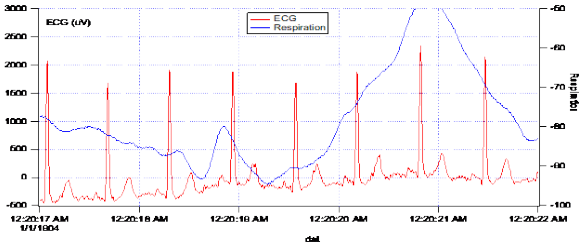


Fig.4. ECG During Torso Bend. Red line shows ECG, blue line shows expansion of the chest (respiration)..

To analyze the overall ability of the DBD Testbed to reliably resolve heartbeats during motion, we analyzed the ECG data gathered from our test divers using our R-peak detection algorithm. Over the course of 3 diver sessions and 7000 heartbeats, our algorithm correctly identified over 99.7% of all R-peaks with reference to visual inspection. These results suggest that reliable heartrate (HR) and heartrate variability (HRV) metrics should be obtainable with the DBD.

In addition to taking data with the testbed system, the DBD design was refined over the course of the past year. This required designing and implementing all mechanical and electrical design for the main module and the ECG sensors. In Q1 2019 the first physical DBD prototype was completed and assembled. Overall, we are ahead of schedule and anticipate having the DBD fully integrated and preliminarily tested by the end of May 2019.

A core design principle in the development of the DBD has been to focus on diver comfort, which led to dividing the electronics into separate rigid sections contained within a conformable elastomeric main belt. This DBD design thus features a central waterproofed housing where the main electronics and IMU sit, and a custom rubber-like front band that retains and protects the capacitive ECG sensors, the skin temperature sensor, the battery module, and the respiration sensor.

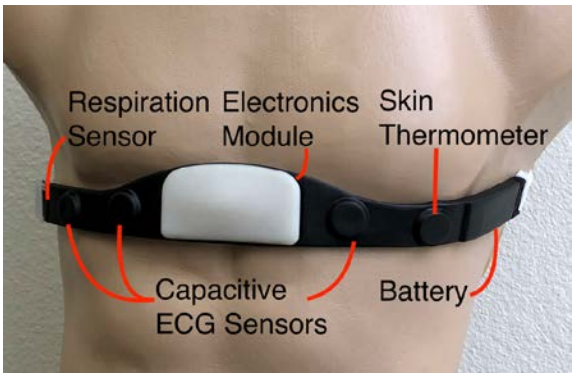


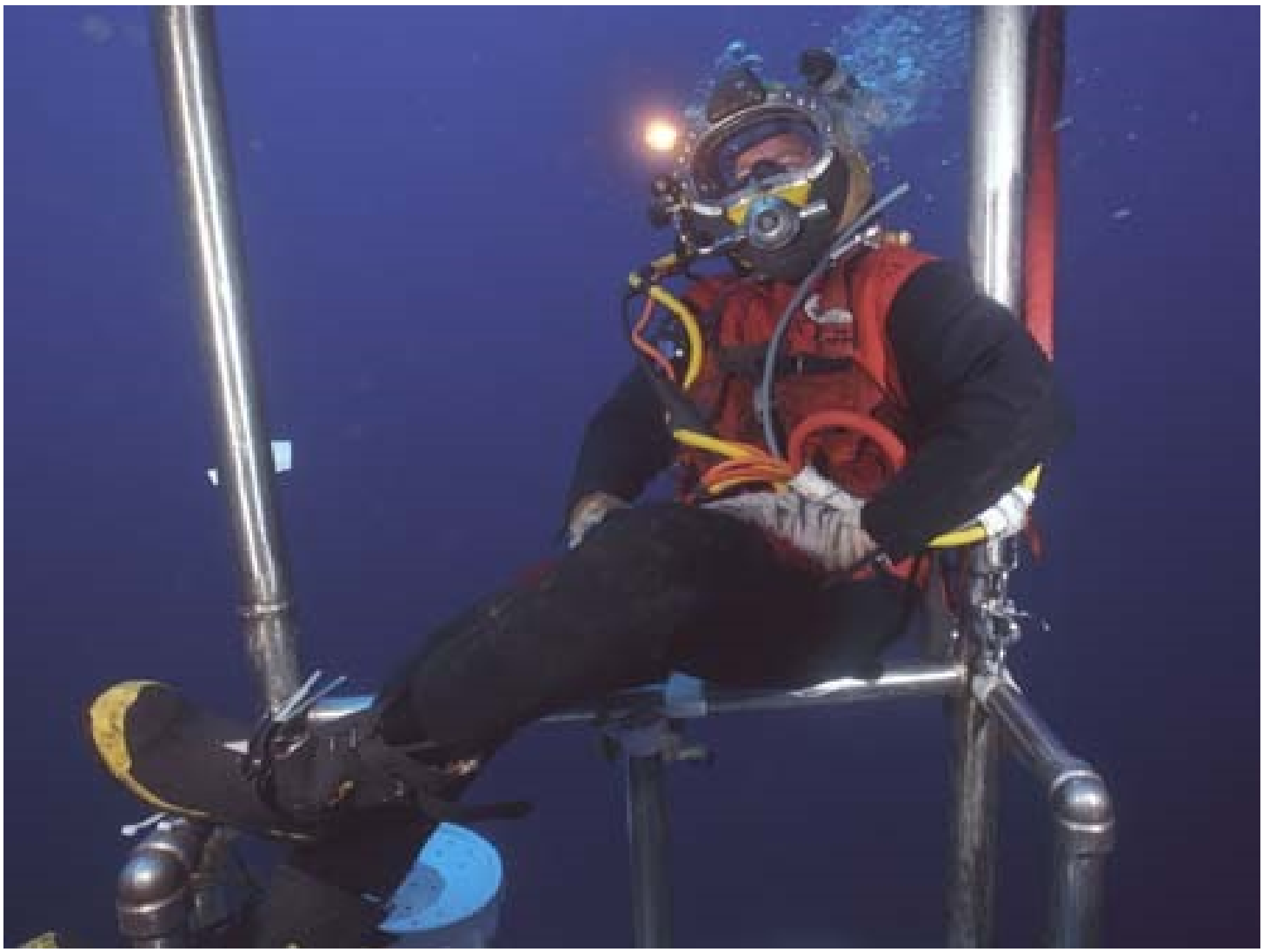
Fig.5. DBD Prototype. Photograph of the first fully integrated prototype of the DBD system

The end goal of this project is to create a rugged system that integrates with dive computers to provide real-time physiological metrics and alerts to Navy divers and command, as well as for commercial divers. To date we are on track to finalize and test the first DBD prototype in Q2 2019, and we will run tests with 10-20 divers in Q2 & Q3 2019. Once completed, this will bring the current project phase to a close at TRL 6.

If granted, the options phases of this project will allow us to finalize the DBD design and enhance it by extending its capabilities with the addition of: core body temperature, pulse oximetry, real time processing of physiological signals, interfacing to commercial dive computers for real-time metrics and alerts, as well as the opportunity to test it at NEDU and NHRC to achieve TRL 7.

Notes:

Approved, DCN# 43-5229-19



Thank you for Participating in the 2019 ONR-NAVSEA Undersea Medicine Program Review