Hyperbaric Oxygen Therapy (HBOT) Pilot Study
Preliminary Report

for 2020 Senate Bill 72

Prepared by Purdue Neurotrauma Group, Purdue University, West Lafayette, IN

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Contents

1 Project Summary

Hyperbaric oxygen therapy (HBOT) is administered inside a treatment chamber and provides the patient with 100 percent oxygen at high atmospheric pressures. The effectiveness of the HBOT in treating neurological diseases is not yet well established. Significant evidence suggests that HBOT is beneficial in the treatment of certain mental disorders such as post-traumatic stress disorders (PTSDs) associated with mild traumatic brain injury (mTBI). This study examines the treatment response of military veterans with clinically diagnosed PTSD that undergo HBOT using advanced magnetic resonance imaging (MRI). This is one of the first studies to use MRI data for HBOT evaluation. Study participants are scanned at the baseline, post 20 dives and 40 dives, and at the 3-month follow-up post therapy. The proposed mechanism of action of HBOT in PTSD/mTBI is that increasing oxygenation of blood and tissues results in the improvement of neuronal functioning by the reactivation of metabolic and neuronal pathways. Study participants have demonstrated statistically significant changes in major neurometabolites evaluated with magnetic resonance spectroscopy (MRS). Additionally, changes have been observed in CSF flow and survey metrics. The main aim of the study is to assess HBOT using more objective measures compared to survey data, i.e., MRI metrics, and to inform healthcare professionals and prospective patients about the treatment options available.

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3 Introduction

We are observing a post-traumatic stress disorder (PTSD) veteran population undergoing independent and elective hyperbaric oxygen therapy (HBOT) at State-contracted hospitals by doing MRI scans to track the health outcomes of sustained treatments of HBOT. Our MRI research will not be conducting, endorsing, modifying, informing, nor interfering with the elective HBOT treatments of the PTSD population. Raw data or patient information will only be shared between Purdue and the State-contracted hospital organization if the participant signs release forms. We will assess a variety of magnetic resonance imaging (MRI) metrics. MRI will be performed at the Purdue MRI Facility using several pulse sequences, including high-resolution (1-mm isotropic resolution) structural (T1-weighted, T2-weighted) images, diffusion tensor MRI (DTI) to evaluate white matter microstructural integrity, resting-state functional MRI (rs-fMRI) to determine functional connectivity and investigate the impact of HBOT on each participant's baseline measures and the brain's default mode network. Further, magnetic resonance spectroscopy (MRS) is performed to measure relative concentrations of metabolic levels within brain volumes, e.g., N-acetyl aspartate reflects neural density and integrity, choline as a membrane turnover marker, myo-inositol a as glial cell marker. We perform perfusion MRI to measure cerebral blood flow, cerebral blood volume, and the delivery of oxygen from blood to brain tissues. Two surveys- Central Nervous System Vital Signs (CNSVS) and Purdue Neurotrauma Group Basic Health Assessment/Questionnaire (PNGBHQ)–are collected prior to each MRI session;

Surveys

1) CNSVS:
   a)Verbal Memory Test
   b)Visual Memory Test
   c) Stroop Test
   d) Reasoning Test
   e) Alcohol Use Disorders Identification Test (AUDIT) SF-10
   f) Depression, Anxiety and Stress Scale (DASS) SF-21
   g) Drug Use Questionnaire (DAST) SF-20
   h) Epworth Sleepiness Scale (ESS) SF-8
   i) Medical Outcomes Survey (MOS) SF-36
   j) PTSD Checklist for DSM-5 (PCL-5)

Several participants have indicated triggers post collecting the CNSVS survey data, therefore we have concluded CNSVS survey collection.

2) PNGBHQ: This is a basic health questionnaire to eliminate other confounding factors in the study such as medication or additional therapy that could interfere with our findings.

Specific Aims/Objectives

HBOT has shown positive data in treating the neurophysiological causes associated with PTSD in military veterans. However, currently published research is limited to survey metrics for assessing the efficacy of the therapy. HBOT works by subjecting the patient to higher atmospheric pressures and increasing the concentration of oxygen. This increased pressure and oxygen uptake are intended to help get oxygen to critical regions of the brain to help the body heal brain injury through natural processes. HBOT's long history and widespread use across the medical community for wound care align with this extended application of the therapy. The primary objective of our study is to use our research findings to inform federal, state, Department of Defense (DoD), and Department of Veteran Affairs (VA) representatives who are making decisions regarding funded veteran care. A positive finding in research could lead to veterans having HBOT as a covered therapeutic option for those who are already saturated with drug-based solutions to diagnosed PTSD and/or mTBI. U.S. Food and Drug Administration (FDA) approved HBOT for 13 uses, none of which includes treatments for mental health conditions, including mTBI or PTSD. This pilot study would also springboard future clinical trials by proving a rigorous testing template for tracking progress through MRI modalities. The scope of our research is to characterize the effects of HBOT in a veteran population with PTSD and/or mTBI. We will provide an unprecedented and comprehensive examination through advanced MRI metrics including diffusion tensor imaging (DTI) to evaluate white matter microstructural integrity, functional MRI (fMRI) to determine functional connectivity (or observe diminished hyper-connectivity), magnetic resonance spectroscopy (MRS) to measure relative concentrations of metabolic levels within PTSD-specific localized brain volumes, and high-resolution (1-mm isotropic resolution) T1 and T2-weighted images for detailed structural and volumetric analysis. To provide consistency with existing studies,

each MRI scan session is accompanied by two surveys: CAPS-5, PC-PTSD-5 and Purdue Neurotrauma Group Basic Health Assessment (PNGBHQ). The first two assess recent PTSD symptoms while the latter is a basic health questionnaire to eliminate other confounding factors in the study. Each patient will receive five 1-hour long MRI scans: baseline, post twenty dives, post forty dives, 6-month post therapy, and 1-year post therapy. Patients will receive forty 1-hour long HBOT "dives" at 1.5 atmosphere absolute (APA) at an HBOT provider with adequately trained and certified staff. Potential discoveries from the study include finding quantifiable imaging-based biomarkers indicating a benefit of HBOT for treating individuals with PTSD and/or mTBI. Diffusion-weighted MRI (DWI) will be employed to query white matter integrity throughout the brain. We expect white matter healing to be indicated by increased fractional anisotropy (FA) and decreased mean diffusivity (MD). fMRI will be used to first compare the healthy population identifiability with the PTSD population identifiability. Identifiability is determined by 248 regions of interest which are each compared to one another within a functional connectome matrix using principal component analysis. We expect to observe extensive hyper-connectivity as a baseline measure and a positive outcome would be more normalized network connectivity in post therapy measurements when compared to a healthy population. MRS data will identify statistically significant change in commonly studied brain metabolites. The three surveys will not serve as an overall reportable metric, but as an internal cross verification of results only or (in the case of the health questionnaire) to eliminate potential outlier cases from statistical modeling.

The aim of the study is to characterize the effects of HBOT in a veteran PTSD population by assessing MRI data including diffusion-tensor MRI (DTI) and fMRI measures of veteran brain function and health. Our longitudinal study at Purdue will perform MRI of these veterans and a group of healthy volunteers as a control for MRI scanner acquisition variability. A potential discovery of this study is finding quantifiable imaging-based biomarkers indicating a benefit of HBOT for treating veterans with PTSD, with possible association with TBI, which has been shown to be a promising option for this vulnerable population. This study will investigate potential biomarkers for PTSD.

Diffusion-tensor MRI (DTI) will be employed, specifically diffusion tensor imaging (DTI), to query white matter integrity throughout the brain. We expect white matter healing to be indicated by increased fractional anisotrophy (FA) and decreased mean diffusivity (MD).

rs-fMRI will be used to first compare the healthy population identifiability with the PTSD population identifiability. Identifiability is determined by 248 regions of interest which are each compared to each other within a functional connectome matrix using principal component analysis. We expect initially that PTSD patients will be more "identifiable" as their injuries will uniquely set them apart since certain regions of the brain are making up for the deficient connections in others. Our objective will be to track whether the unique "barcode" (please note that this "barcode" does not in any way identify the person, it only serves to identify the scanned brain) associated with PTSD patients dwindles over time to a more normalized and homogeneous state. We expect to observe larger changes in PTSD participants within the 248 regions of interest captured by the functional connectome weighted adjacency matrix. Observing a decrease over successive treatments of these larger, identifiable differences would be an indication of improved functional connectedness within the participants' resting-state brain. A recent review of MRI studies in PTSD reinforces the hypothesis that we should be able to see more distinct differences in the rs-fMRI.

MRS has been used to investigate the metabolic window on a wide range of biochemical processes are extremely diverse. MRS was the first tool that demonstrated biological changes in mental health patients, namely imbalances in brain metabolism, changing the stigma of mental health. However, integration of the evidence for altered in vivo metabolite levels across PTSD population are lacking. This study evaluates unedited spectroscopy of clinically diagnosed PTSD participants undergoing HBOT. Four brain regions related to the underlying PTSD symptoms have been investigated for assessment of alterations in cognitive-affective processing such as lack of extinction of the fear response, flashbacks, heightened physiological responses to trauma cues, and general hyperarousal symptoms and hypervigilance.

Perfusion MRI: It is well known that brain trauma can cause vascular/microvascular damages to the brain. A non-invasive MR sequence, namely arterial spin labeling (ASL), has been widely used to assess the cerebral blood flow in various brain diseases. In this study, we will perform 3D ASL MRI (GE scanner) on the subjects to measure the vascular responses/recoveries under the hyperbaric oxygen treatment.

The surveys will not serve as an overall reportable metric, but as an internal cross verification

of results only or (in the case of the health questionnaire) to eliminate potential outlier cases from statistical modeling.

Background and Significance

All previous studies evaluating the efficacy of HBOT for PTSD utilized a variety of patient survey data for conducting analysis. This observational study will employ MRI-based analysis (above) to examine changes within a PTSD (with possible associated TBI, per IRB-2019-51) population as it is treated in a typical HBOT facility by hospital personnel. This will provide analysis of a physical-metric dataset as a supplement to existing surveys. Our study is one of the first ones doing MRI assessments and therefore the potential benefit of having a positive result greatly outweighs the risks of MRI, as the current standard of care relies heavily on a variety of prescription drugs. Additionally, exploring an alternative, non-invasive, mode of treatment for PTSD, especially for disabled veterans, is inline with the current objectives of the VA, https://www.research.va.gov/topics/ptsd.cfm: "VA researchers are advancing the understanding of PTSD and its effects, developing and testing treatments for the condition, and working to find ways to prevent PTSD from occurring after trauma. Ongoing studies range from investigations of the genetic or biochemical foundations of the disorder to evaluations of new or existing treatments."

Research Hypotheses

The research question is whether HBOT has any health outcomes as observed over 12 months before, during, and after elective treatments for veterans diagnosed with PTSD. Based on an extensive literature review which included a number of studies specific to combat induced PTSD in veterans, our hypothesis is that we will see some initially statistically significant improvement across all metrics but that this benefit will begin to diminish after a year or more after the last treatment. We will explore this research question through magnetic resonance imaging (MRI) based biomarkers of brain function and connectivity, supplemented by psychological survey data, to determine if there is a statistically significant improvement in symptoms related to PTSD, clinical outcomes, or brain function. We hypothesize that:

For DTI: 1. At baseline, veterans diagnosed with PTSD exhibit lower FA and higher MD, compared to controls. 2. After 12 months of HBOT treatment, veterans diagnosed with PTSD will exhibit statistically equivalent FA and MD compared to controls.

For rs-fMRI: 1. At baseline: We expect patients to exhibit high identifiability as brain trauma tend to be uniquely wired based on participant specific trauma. We expect this to be true for PTSD as well. 2. After 12 months of HBOT treatment: We expect patients to become more normalized in the rs-fMRI metrics mentioned in the Aim/Objectives and Summary section over the course of treatment but we expect that the high identifiability will return after the elective treatments session comes to a close.

For MRS: 1. At baseline, veterans diagnosed with PTSD exhibit decreased N-acetylaspartate (NAA) and choline (Cho) brain metabolites compared to other timepoints. 2. After 12 months of HBOT treatment, veterans diagnosed with PTSD will exhibit statistically nominal NAA.

For perfusion MRI: 1. At baseline, veterans diagnosed with PTSD exhibit lower regional cerebral blood flow and cerebral blood volume, compared to controls. 2. After 12 months of HBOT treatment, veterans diagnosed with PTSD will exhibit statistically regional cerebral blood flow and cerebral blood, compared to controls.

List the approximate duration in the fashion below.

How long will participants be asked to be in the study? 12-16 months

Number of Visits = 6

Minutes or Hours per visit = 1 hour + minimal time for surveys

Single Day or Multiple Days? - Multiple Days

Total number of months until all data are collected = 12

Specific Study Procedures

Individuals will be offered an opportunity to participate in our MRI study by State contracted hospital staff as they are approved to receive elective HBOT sessions. Volunteer participants for our study will be screened at Purdue University by confirming their ability to consent (through the Montreal Cognitive Assessment) and their ability to safely have an MRI scan. We will then obtain consent for future MRI scans and Surveys. Having been cleared, participants for our study will undergo the first of six (6) MRI scans to be performed over the next year. Prior to commencement of their concurrent HBOT, each patient will undergo an MRI session. We will also collect voluntarily disclosed health

information via the PNGBHQ survey. This same survey will be employed before each MRI session as well to track any correlating factors that might pollute data for the PTSD analysis (such as changes in behavior for tobacco, alcohol, and drug use). The individual will then receive forty (40) HBOT treatments, one hour each. These treatments will be administered by State contracted hospitals daily on weekdays. No Purdue researchers will be involved with or inform the hospital HBOT protocols. Following commencement of the HBOT, patients will undergo five (5) follow-up MRI sessions at the Purdue Engineering MRI Facility (West Lafayette, IN) and will be asked to self-identify they they have continued to meet their regularly scheduled HBO therapy sessions. The first set of follow-up sessions will take place during the HBOT treatment protocol. Patients will undergo an MRI session every four (4) weeks, intended to coincide with the 20th and 40th treatments.
The second set of follow-up sessions will serve as a longitudinal assessment, taking place three (3), six (6) and twelve (12) months after the final HBOT. MRI session protocol: All subjects will undergo the same MRI scanning. Procedures for each MRI session: The subject will review the Consent form and ask any questions to the research personnel. The subject will privately change clothes into a clean, non-metallic hospital patient gown. The subject will then be provided disposable ear plugs and advised to insert them in order to mitigate loud noises during the MRI scan. The subject will lie on their back in the MRI scanner and their head will be positioned within the commercial head receive coil array, e.g., NOVA Medical 32-channel head receive array. We will collect three types of MRI data during this project. First, we will acquire anatomical MRI to obtain the 3D structure of the subject's brain tissues. This step includes routine preparation scans (i.e., localizer and calibration), a T1-weighted sequence, and a T2-weighted sequence. Second, we will acquire diffusion-tensor imaging (DTI) data using the standard GE Healthcare diffusion tensor imaging (DTI) sequence. Third, we will acquire resting-state functional MRI using the standard GE Healthcare sequence. GE Healthcare MRS and perfusion sequences would be performed subsequent to the rs-fMRI sequence. We will also acquire MRS data using sLASER sequence and high order shimming in four (4) different brain regions. Lastly, we will acquire perfusion MRI data, also known as arterial spin labelling.

Throughout the MRI session, the subject will be offered brief (1-3 minute) breaks between scans, also serving to provide an opportunity to communicate any concerns to the researcher personnel. Prior to each scan, research personnel will relay specific instructions to the subject and provide a cue when the next scan is about to begin.

The entire MRI session should be completed within 60 minutes. Upon completion of scanning, the subject will walk out of the MRI room and privately change back into their clothes. Their patient gown will be deposited in the on-site dirty laundry hamper.

4 Functional Analysis

Hyperbaric oxygen therapy (HBOT) has shown some promise in improving symptoms of post-traumatic stress disorder (PTSD) in past studies. The project we are working on focuses on utilizing functional-MRI data from PTSD patients to investigate the effects that this treatment has on cerebral blood dynamics. We hypothesize that HBOT will generally increase the cerebral blood perfusion, leading to reduced blood transit time at certain brain regions and increased coherence of blood dynamics. To interpret the fMRI data (series of low resolution 3-D images taken every few seconds, which is called Repetition Time, or TR), we performed several processing steps in a program called FSL. First, the raw data was preprocessed to obtain "4-dimensional" images where the fourth dimension represents time. Preprocessing also yielded subject displacement (i.e., motion artifact) profiles as seen in Figure 1, which display absolute and relative head movement of subjects as a function of TR while they are inside the MRI. These profiles were examined carefully to ensure that the images will not be blurred due to abnormal spontaneous head movement. If a large motion artifact was detected (as shown in Figure 1), we will truncate the sections with motion artifacts and keep a continuous section that is free of the artifacts. In the case of Figure 1, the first 40 and the last 60 or so 3D images will be cut out so that only the smooth portion remains for further processing. In this study, each patient undergoes a baseline fMRI scanning session before treatment. Each successive session is done after 20 additional HBOT dives at 1.5 atm of pure O2. All the subjects' displacement profiles were then characterized and sorted to determine which subjects should be prioritized for further analysis. Figure 2 (left) shows a table containing the information on data quality from the patient data we have collected so far. Since PTSD can be triggered by certain unpleasant stimuli, the cramped and loud nature of the MRI scanner caused many subjects to have involuntary movements during sessions. Thus, only a few subjects yielded usable data over the course of 3 sessions, specifically subjects 13 and 15. The other patients' data will be treated with much more rigorous motion-artifact correction before further analyses. These data sets were then processed in MATLAB to calculate "delay times" for each voxel. The voxel-wise delay time represents the amount of time it takes blood to arrive at each voxel of the image relative to the global mean of fMRI data. For example, if a voxel has a delay time of +3 seconds, it means that the oxygenated blood arrived at that voxel about 3 seconds after the average delay times of all voxels in the image. The MATLAB program also creates a 3-D delay map from these values; an example of this can be seen below in Figure 3. The delay maps are visibly different with each session. Each successive scan reveals fewer voxels with high absolute delay times (turquoise and yellow) and more voxels with delays closer to zero (dark blue and red) relative to the global mean. This is consistent with the hypothesis that HBOT will reduce delay times and increase delay coherence. However, this is largely a qualitative observation. More data is needed for the robustness of the study. In addition to 3-D maps, we have also generated histograms of delay values to directly compare the distributions of each session with the baseline. The distributions are also consistent with the observations from the delay maps, as there are more voxels with delay times closer to zero with each session. Subject 13 yielded plots that closely resemble those of subject 15. Although we have seen some interesting results that are relatively consistent with our hypothesis, it is impossible to draw conclusions from this preliminary analysis due to the small sample size. In the future, it should be emphasized to patients that it is of utmost importance to remain still during the scans to maximize the data quality. We plan to continue collecting fMRI data from patients in further sessions and repeat this process with more subjects to gain insights into the efficacy of hyperbaric oxygen therapy.

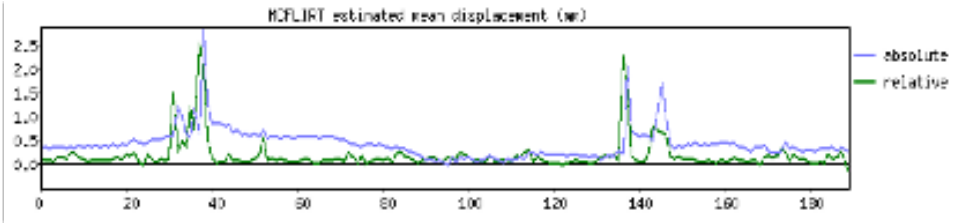

Figure 1: MRI movement profile of one subject displaying absolute (blue) and relative (green) movement in mm over time.

Session01	Subject	Movement Profile	Quality
	HBOT0301	HBOT0301_movement	Usable
	HBOT1501	HBOT1501_movement	Usable
	HBOT1801	HBOT1801_movement	Usable
	HBOT0201	HBOT0201_movement	Usable, maybe truncate last 150 TRs
	HBOT1001	HBOT1001_movement	Usable, truncate last 50 TRs
	HBOT1201	HBOT1201_movement	Usable, truncate last 50-70 TRs
	HBOT1301	HBOT1301_movement	Usable, truncate last 30
	HBOT1601	HBOT1601_movement	Usable, truncate first and last 20
	HBOT1701	HBOT1701_movement	Usable, truncate last 50
	HBOT1901	HBOT1901_movement	Usable, truncate last 50
	HBOT2001	HBOT2001_movement	Usable, truncate first 20-50, small spike at 100
	HBOT2101	HBOT2101_movement	Usable, maybe truncate
	HBOT0401	HBOT0401_movement	Several spikes, probably unusable
	HBOT1101	HBOT1101_movement	Unusable
Session02	HBOT0602	HBOT0602_movement	Usable, truncate at 140
	HBOT0702	HBOT0702_movement	Usable, truncate at 95. Spike
	HBOT1302	HBOT1302_movement	Usable, maybe truncate first 60
	HBOT1402	HBOT1402_movement	Usable, truncate at 140
	HBOT1502	HBOT1502_movement	Usable, truncate at 140
	HBOT1702	HBOT1702_movement	Usable, some movement
	HBOT0402	HBOT0402_movement	Maybe usable if truncated at 450
	HBOT1602	HBOT1602_movement	Maybe usable, truncate first 60
	HBOT1902	HBOT1902_movement	Unusable
	HBOT1102	HBOT1102_movement	Unusable
Session03	HBOT1403	HBOT1403_movement	Usable
	HBOT0703	HBOT0703_movement	Usable, truncate first 70
	HBOT1103	HBOT1103_movement	Usable, truncate at 140
	HBOT1303	HBOT1303_movement	Usable, maybe truncate first 80
	HBOT1503	HBOT1503_movement	Usable, truncate at 150
	HBOT1703	HBOT1703_movement	Probably usable, truncate?
	HBOT0403	HBOT0403_movement	Unusable
	HBOT0603	HBOT0603_movement	Unusable
	HBOT1603	HBOT1603_movement	Unusable
Session04	HBOT0404	HBOT0404_output	Usable, truncate at 130
	HBOT0604	HBOT0604_movement	Likely unusable

Figure 2: Subject list and displacement profiles sorted by lowest absolute displacement. Green represents subjects with the least movement, while red is the highest and generally unusable for processing.

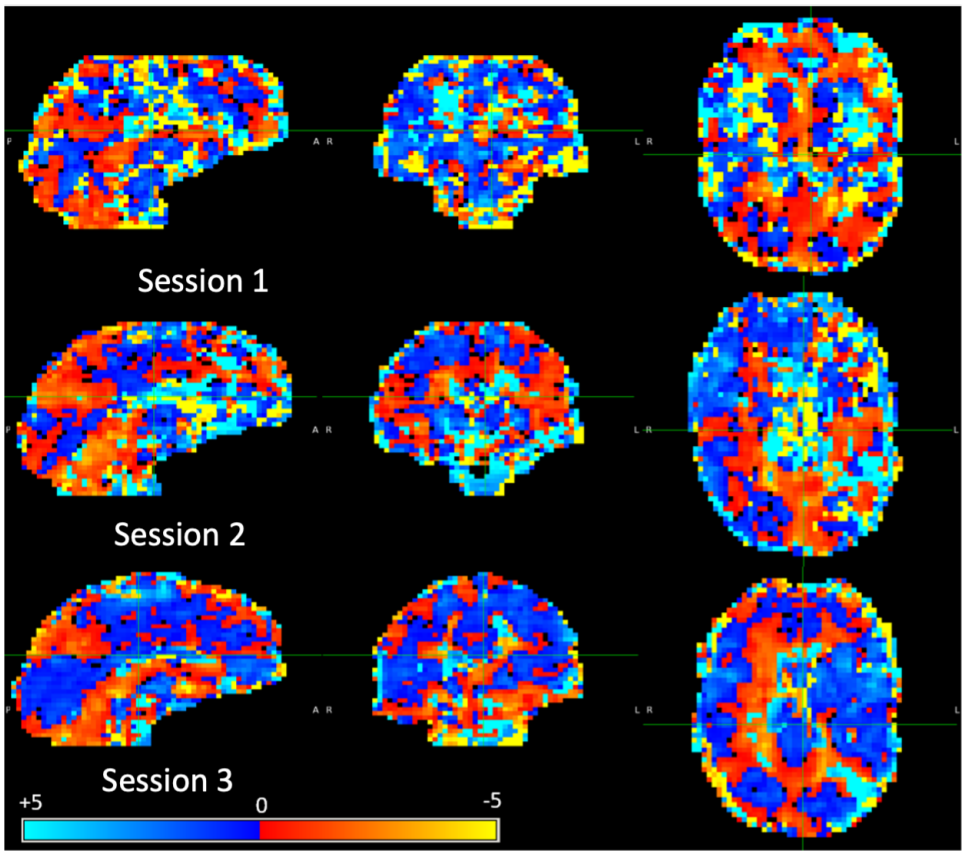

Figure 3: 3-D delay maps of subject 15 viewed in FSLeyes over the course of 3 sessions. The color bar displays the delay times of each voxel in seconds relative to the global mean delay.

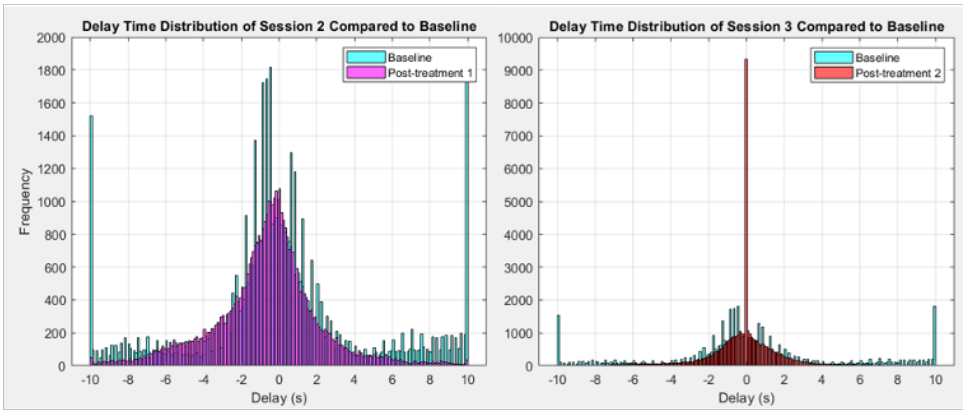

Figure 4: Session 2 and 3 delay time distributions overlayed with baseline distribution of subject 15.

5 Magnetic Resonance Spectroscopy

Magnetic Resonance Spectroscopy is a noninvasive technique used for measuring biochemical changes in the tissue. MRS can be conducted as a part of routine magnetic resonance imaging (MRI) on any commercially available MRI scanners. While MRI identifies the anatomical changes by obtaining contrast images, MRS creates a spectrum displaying the types and quantity of chemicals from a small region of the brain. By excluding the overwhelming signals from water and fat, we can quantify clinically relevant biomarkers. The applications of MRS to investigate the metabolic window on a wide range of biochemical processes are extremely diverse. The international MRS Consensus Group has recently documented the clinical utility of MRS, for diagnostic and prognostic purposes, in common disorders of the central nervous system [6]. MRS was the first tool that demonstrated biological changes in mental health patients, namely imbalances in brain metabolism, changing the stigma of mental health.

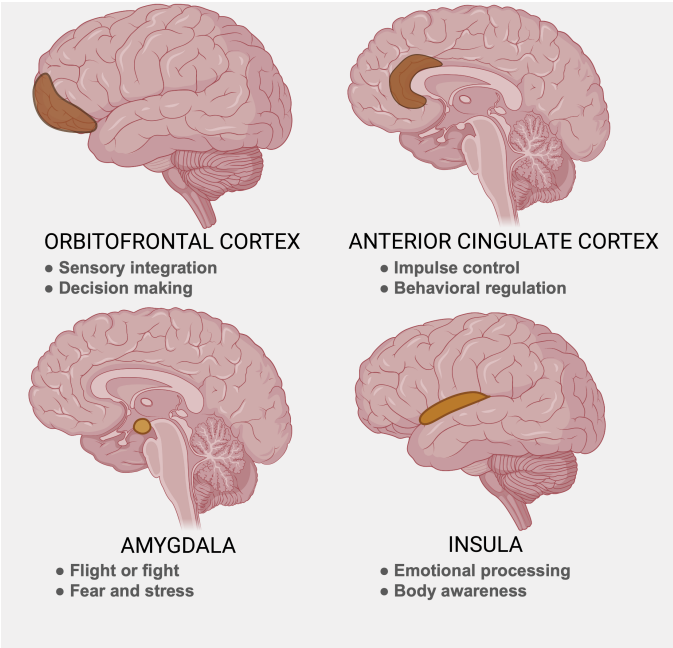

Figure 5: Brain regions that play an important role in PTSD include orbitofrontal cortex, anterior cingulate cortex, amygdala, and insula.

Therefore, the aim of this study was to utilize spectroscopy as an investigational tool to assess neurometabolic changes during and post hyperbaric oxygen therapy. MRS data was acquired at the baseline, post twenty dives, post forty dives, and three months follow-up. Total of eleven participants are evaluated along this timeline. Four brain regions related to the post-traumatic stress disorder (PTSD) were evaluated using MRS as shown in Figure 5. Orbitofrontal cortex was chosen due its role in decision making and sensory integration. Anterior cingulate cortex was chosen as it regulates impulse control and regulate behavior. Insula regulates emotional control and response, as well as body awareness. Lastly and most importantly, amygdala controls flight or fight state by cautioning body about fear and stress. Data was acquired using highly optimized pulse sequence, semi-Localized by Adiabatic Selective Refocusing (sLASER). This pulse sequence provides single-shot full-intensity signal with clean localization and minimal chemical shift displacement error (CSDE) due to the high bandwidth adiabatic full-passage (AFP) pulses. Pairs of AFP pulses in sLASER further suppress J-evolution and prolong apparent transverse relaxation times (T2) [2]. sLASER, when combined with voxel based static B0 and transmitted B1 calibration routines, provides neurochemical profiles with high data reproducibility at 3T [1].

A nonhomogeneous field causes line broadening and frequency shifts in MRS, therefore optimization of magnetic field homogeneity (shimming) is crucial for MRS as shown in Figure 6. Optimized acquisition using echo time (TE) 35ms, repetition time (TR) 2000ms, 64 averages, 2048 data points, and volume of interest (VOI) 20x20x20 mm3 allows quantification of major metabolites from spectra

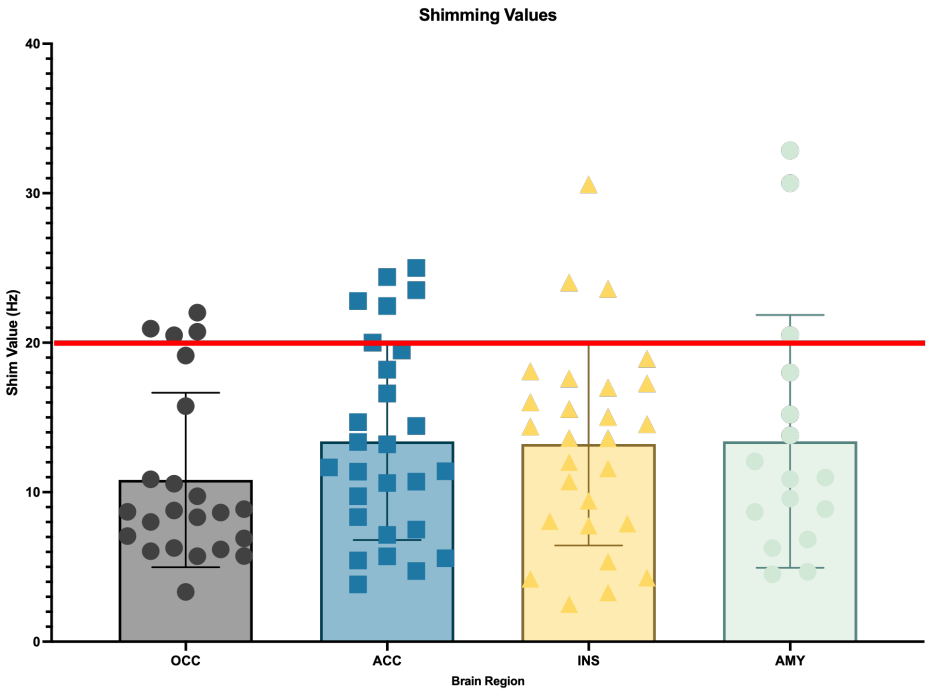

Figure 6: Data quality can be expressed using shimming values that are collected during each scan. Red line is a threshold for acceptable values. Higher values represent line broadening which limits metabolite distinction on a spectrum.

averaged over five minute acquisition time. Data was analyzed as shown in Figure 7. Osprey, an all-in-one software suite for state-of-the art processing and quantitative analysis of in-vivo MRS, was used for preprocessing that consists of the alignment of individual averages, averaging, polarity correction, residual water removal, linear baseline correction, and eddy current correction [5]. Furthermore, voxel coregistration and segmentation was also completed in Osprey utilizing statistical parametric mapping (spm). Lastly, metabolite quantification was completed in LCModel. N-acetyl aspartate (NAA), creatine (Cr), choline (Cho), myo-inositol (Ins), and glutamine+glutamate (Glx) were reported using water referenced quantification and corrected for cerebrospinal fluid (CSF). All statistical analysis (Shapiro-Wilk normality test, paired ttest, Grubbs outlier test) was performed using STATA SE.

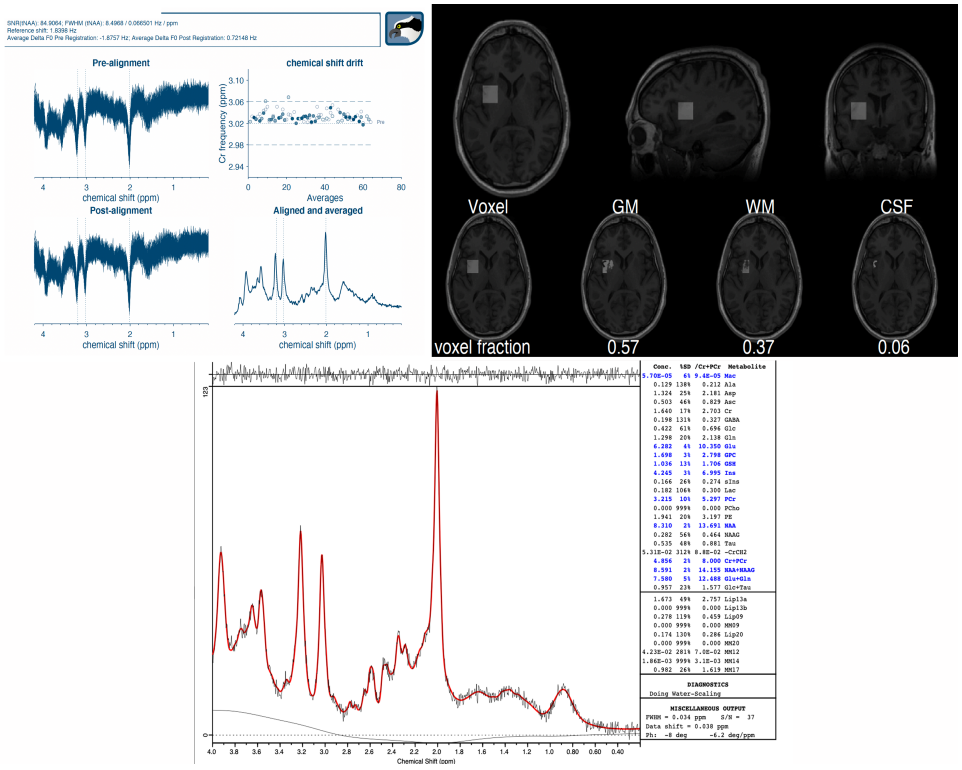

Figure 7: Data analysis pipeline using Osprey and LC Model. A) The top left panel shows the individual averages prior to frequency-and-phase alignment. The bottom left panel shows them after frequency-and-phase alignment. The top right panel shows a scatter plot of the maximum of the 3.02 ppm Cr/PCr signal over the course of the acquisition for both pre- and post-alignment. Finally, the bottom right panel shows the aligned, averaged and referenced spectrum after eddy-current correction and residual water removal. This spectrum will be passed on to the Fit module for modeling. B) Top image shows voxel coregistration in the brain. Bottom image displays the voxel mask next to the contributions from grey matter, white matter, and CSF, along with the fractional volume estimates for each tissue. These values will be used during quantification to account for tissue-specific effects of relaxation, tissue water content, and metabolite content. C) LC Model is a gold standard used for fitting and quantifying metabolites. However, preprocessing steps A) and B) are necessary to quantify correct metabolite concentration. The right panel shows the signal amplitudes normalized by the water amplitude.

Each brain region had specific neurometabolic changes as shown in Figure 8. Different research study conducted at Purdue University has shown tCho decrease in PTSD population compared to the healthy age and gender matched controls as seen in Figure 9. tCho functions as a powerful neuroprotectant and is a precursor to the neurotransmitter acetylcholine which plays a critical role in memory formation and the creation and maintenance of synapses [4]. Interestingly in orbitofrontal cortex tCho is statistically increased after 40 dives compared to the baseline which indicates potential recovery.

Insula is a brain region with the most neurometabolic changes followed by the HBOT. As previously mentioned, insula has a core role in supporting subjective feeling states. It can also regulate the introduction of feelings into cognitive and motivational processes. We are reporting statistically significant changes of mIns in insula after 20 dives compared to the 40 and 3 month follow up. mIns is one of the most abundant metabolites in the human brain located mainly in glial cells and functions as an osmolyte. Increased mIns may reflect a chronic inflammatory response due to sustained astroglial activation impeding axonal regeneration. Furthermore, we observe significant changes in tCr after 20 dives compared to the 40 and 3 month follow up. tCr is an important organic compound acting as intracellular high-energy phosphate shuttle and energy storage. While located in most cells where it plays its main roles in energy metabolism and cryoprotection, Cr is highly concentrated in

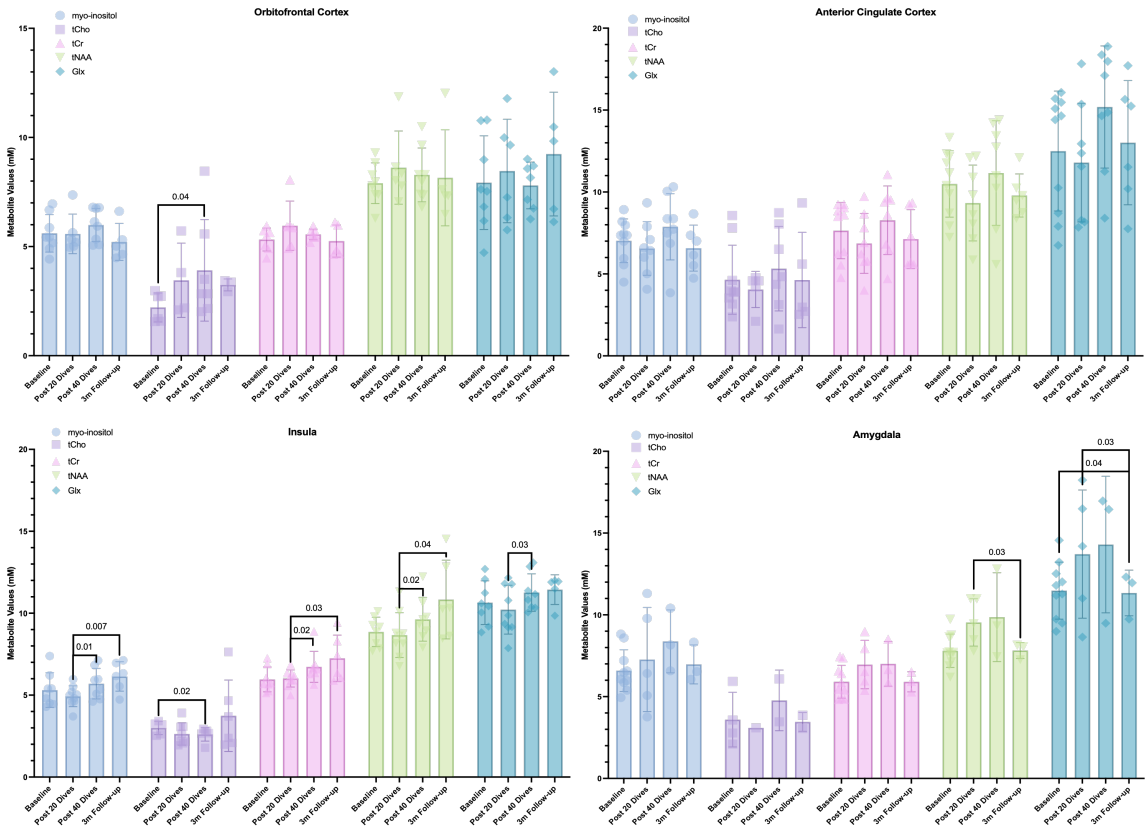

Figure 8: MRS Data analysis plots for each brain region. In orbitofrontal cortex, tCho was statistically increased after 40 dives compared to the baseline. There were no statistically significant changes in anterior cingulate cortex. In insula, all the metabolites showed change after 20 dives. In amygdala, tNAA and Glx underwent changes post HBOT therapy.

brain tissues, where it also acts in osmoregulation and neurotransmission. Additionally, tNAA, the most visible metabolite on MRS spectrum, which is present exclusively in the nervous system and it is a marker of the number of viable neurons, follows the same trend as tCr and mIns. Lastly, the combination of glutamate (Glu) and glutamine (Gln) concentrations that due to similar resonance has to be reported as Glx is increased after 40 dives compared to the post 20 dives timepoint. Glu is the most abundant excitatory neurotransmitter in the brain and plays a fundamental role in learning and memory. Glu is released by pre-synaptic neurons, and it is rapidly converted to Gln in astrocytes. Gln released from astrocytes is converted back to Glu, as part as a Glu/Gln cycle that is essential to the normal functioning of brain cells. While some studies have reported increase in Glx to be indicator of neurological disorders, studies have also shown increase in Glx following positive therapy response [3].

Amygdala regulates emotions, such as fear and aggression, and it is also involved in tying emotional meaning to our memories, reward processing, and decision-making. We report decrease in tNAA, neuronal integrity marker, at the 3 months follow up compared to the post 20 dives. Additionally, we report statistically significant decrease in Glx at the 3-month follow-up compared to the post 20 dives. Both findings in amygdala could be an indicator of the importance of therapy continuation.

No statistically significant changes were observed in anterior cingulate cortex.

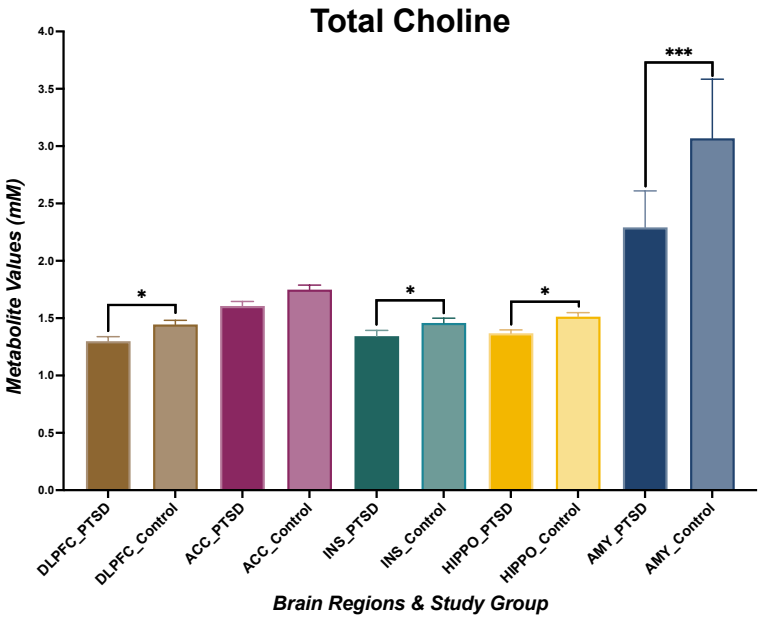

Figure 9: PTSD participants have decreased total Choline in five different brain regions compared to the healthy age and gender matched controls.

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6 MRS Measurement of Lactate in the Brain

Lactate and its role in the human body have long been the subject of great interest and controversy. Traditionally, lactate is considered to be an anaerobic metabolism product. If the brain is unable to obtain enough oxygen, or if it is unable to deal with enough oxygen, the concentration of lactate increases significantly, which is considered a marker of cell deficiency and poor neurological results.

While normally to measure lactate in vivo using MRS, TE of 144 or 288ms is optimal. However, when lactate is abundant the brain, it can be quantified at short TE. Lactate is observed as a doublet at 1.33 ppm as shown in Figure 10.

With limited sample size, we pooled MRS data for lactate across all brain regions for each MRI session. Some of the participants did not have increased lactate which is why standard deviations in Figure 11 are quite large. We have seen a steady, but not statistically significant, decrease in lactate after 20 dives and 30 dives. Interestingly, at 3 months follow-up we see slight increase in lactate highlighting the importance of therapy continuation.

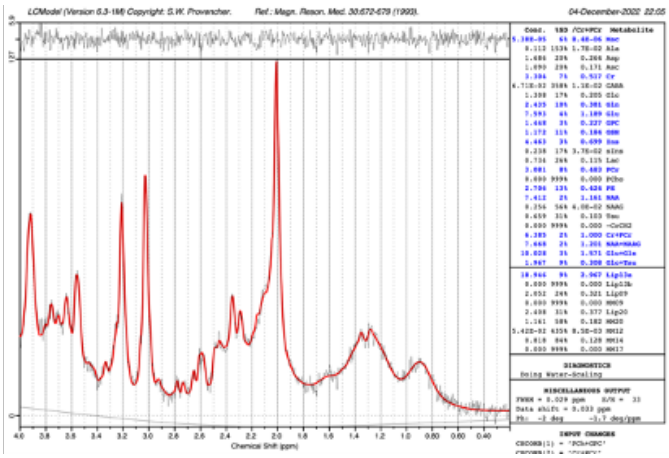

Figure 10: MRS Spectrum of high resolution data with signal to noise ratio (SNR) 33 shows lactate peak at 1.33pm as a doublet.

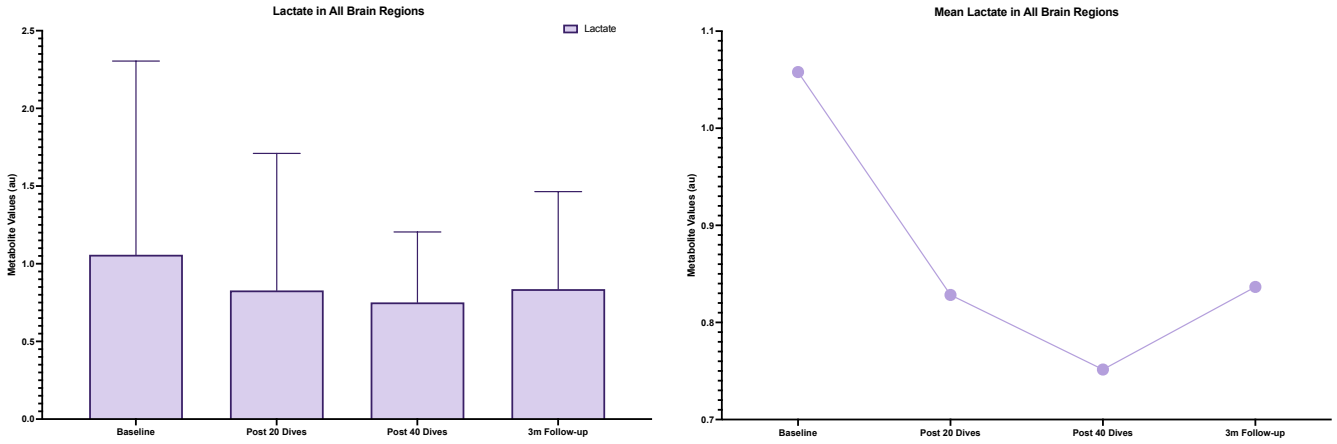

Figure 11: The graph shows largest lactate mean at the baseline, and decrease post 20 and 40 dives. However, there is a slight increase at the 3 months follow-up.

7 CSF Flow

Cerebrospinal fluid (CSF) is made by tissue that lines the ventricles in the brain and it is vital for normal brain function as shown in Figure 1. CSF flows in and around the brain and spinal cord to help cushion them from injury and provide necessary nutrients. Changes in composition, flow, or pressure can cause various neurological symptoms. The first stage of CSF secretion is likely to be passive due to plasma ultrafiltration from leaky capillary networks to connective tissue. Subsequently, the secretion of CSF is considered to be mainly an active process that involves several ion channels. This creates an osmotic gradient, which attracts water to the ventricles [12]. As expected, HBOT increases CSF flow, providing brain with the metabolites observed in the MRS section as shown in Figure 2. Unlike MRS, where only voxel of interest was segmented, using Statistical Parametric Mapping (spm) software, CSF was quantified for the whole brain [9]. Firstly, the whole brain was segmented into white matter, grey matter, and CSF. Secondly, total brain volume was calculated, and lastly, using the ratios from the first step, CSF volume was calculated. Histogram in Figure 3 shows increase in CSF from the baseline to post 20 dive and 40 dives, followed by the slight decrease in CSF flow post therapy at the 3-month follow-up. To better visualize the alterations plot in Figure 4 shows the trendline of the CSF change.

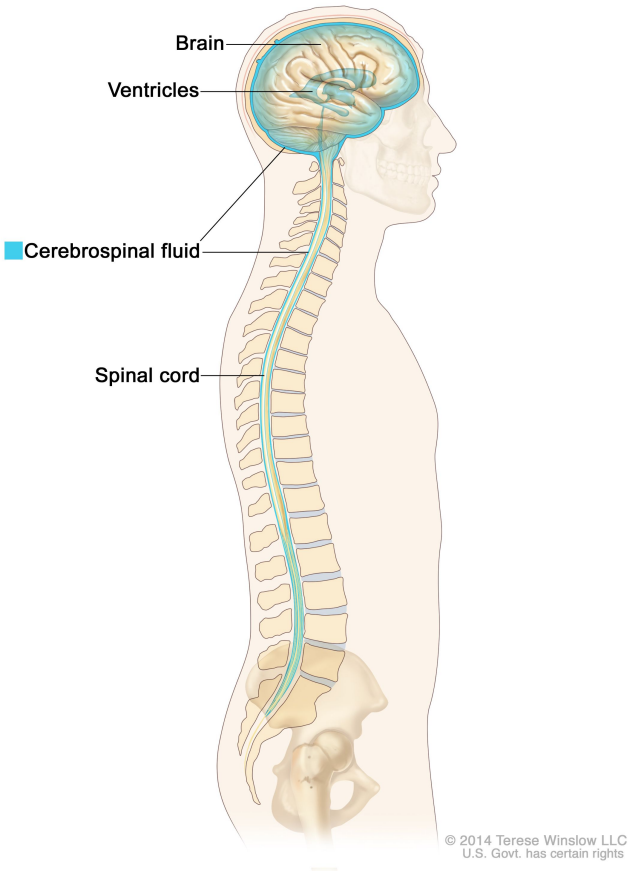

Figure 12: The cerebral spinal fluid (CSF) is displayed in blue, provides diagnosis information, allows the treatment of certain diseases, and opens up opportunities for research on neurological diseases [11].

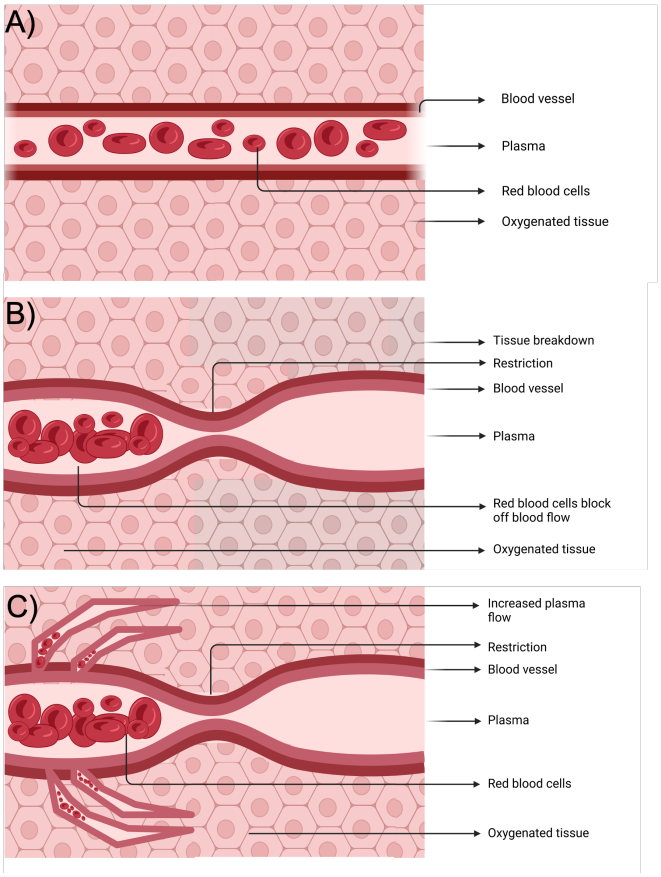

Figure 13: Clinical applications of HBOT are extremely diverse [10]. However, there is very limited literature investigating benefits of HBOT in brain. A) Normal blood flow, can often be restricted. B) When blood vessels are restricted, plasma can still flow, but oxygen delivery to the surrounding tissue is obstructed causing tissue breakdown. C) Following the HBOT, oxygen under pressure forces more oxygen into the tissue encouraging new blood vessels to grow and regenerating tissue.

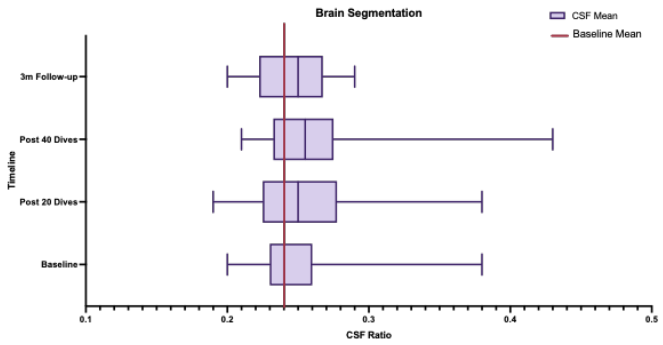

Figure 14: At the baseline CSF flow was the smallest, and it was followed by the increased flow post 20 dives, and even larger increase post 40 dives. At the 3 month follow-up, CSF flow has decreased close to the post 20 dives timepoint.

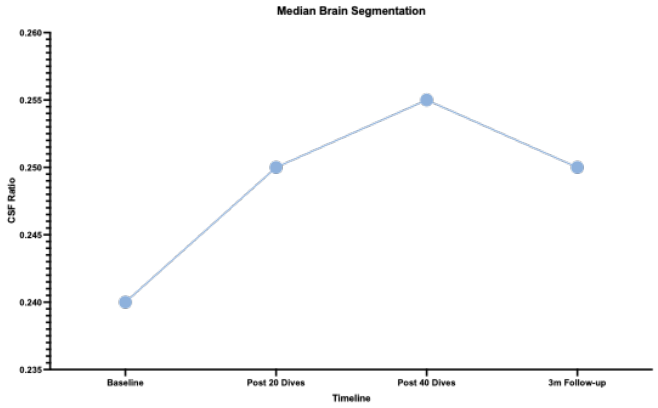

Figure 15: The plot above illustrates trend of the CSF flow fluctuations pre, during, and post therapy.

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8 Survey Data

While survey data was collected to compare our findings with previous studies that have only collected surveys to assess HBOT, several participants where triggered by the surveys and therefore we had to conclude their collection. However, when analyzed the data, we noticed obvious trends, but none of the data was statistical significant due to the incomplete sample size.

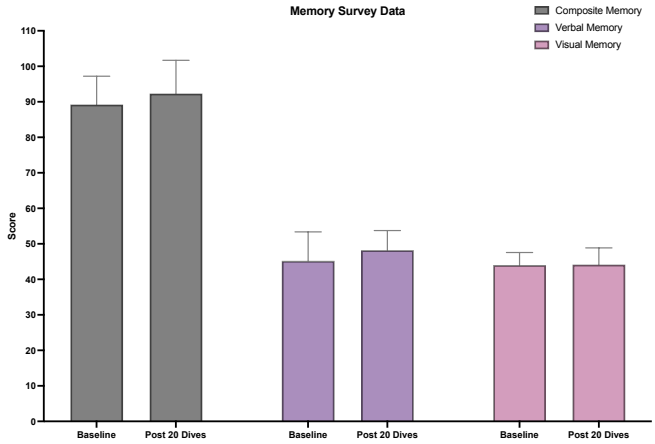

Figure 16: CNS Vital Signs includes parallel tests of verbal memory (word list learning) and visual memory (figure learning). The tests are virtually identical, but one uses words as stimuli, the other, geometric shapes. Additionally, composite memory test is calculated as the mean of three domain z-scores (episodic memory, executive function, and attention processing.) Data shows increased trend in composite,verbal, and visual memory.

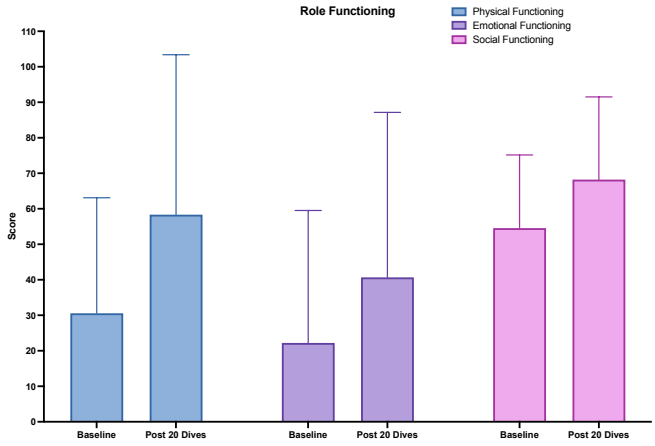

Figure 17: As part of the Medical Outcomes Survey there is a set of generic, coherent, and easily administered quality-of-life measures. These measures rely upon patient self-reporting and are now widely utilized by managed care organizations and by Medicare for routine monitoring and assessment of care outcomes in adult patients.Plot shows increase in physical, social, and emotional functioning post therapy.

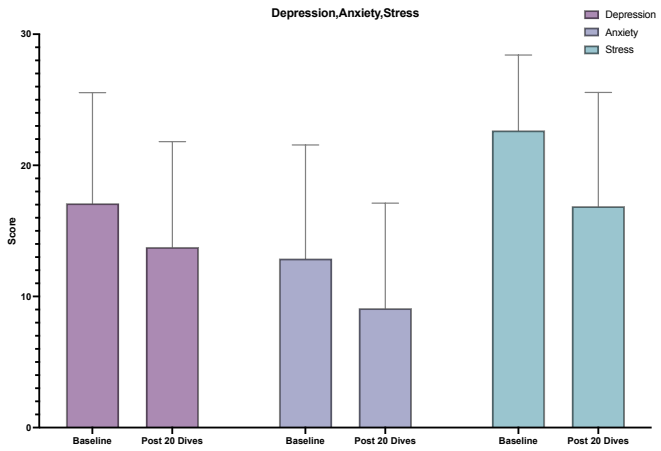

Figure 18: This Questionnaire is a short version (21 item) of a 42-item self report instrument designed to measure three related negative emotional states: depression, anxiety and tension/stress. Data shows, decrease trend in depression,anxiety, and stress post HBOT therapy.

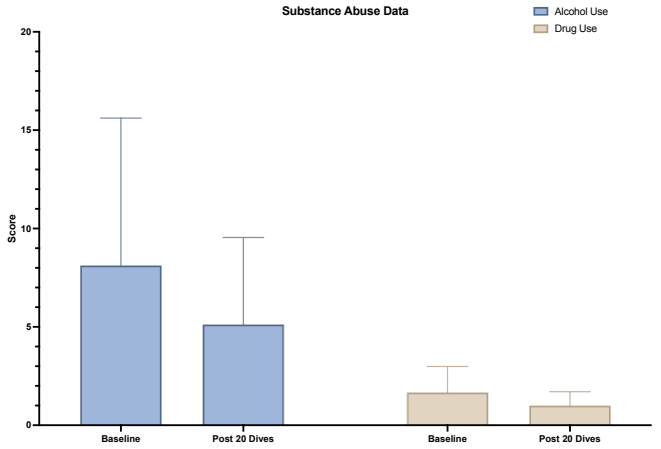

Figure 19: There is still no clear determination of which cluster of PTSD symptoms is most closely associated with substance abuse. As observed in this plot, but drug and alcohol abuse have decreased post HBOT.

9 Discussion

While the study is still ongoing, our preliminary data indicate positive effect of hyperbaric oxygen therapy on PTSD/mTBI participants. This is mostly observed in reported neurometabolic changes identified using MRS. Different brain regions, that are associated with various PTSD symptoms, underwent different fluctuations. One surprising trend is that in insula all the metabolites (mIns, tCr,tCho,NAA,Glx) post-20 dives had worse results (based on current understanding of these metabolites) compared to the baseline. This can be due to several reasons; our leading hypothesis is the possibility that before therapy reaches peak efficacy, the brain requires some length of assimilation time before metabolite levels stabilize.
The majority of the collected data, including CSF flow, lactate in the brain measurements, and MRS, highlight the importance of the therapy continuation. CSF flow has linearly increased at the post 20 and 40 dives timepoints compared to the baseline, but there was a slight decrease in the CSF flow at the 3 month follow-up.
Additionally, lactate has decreased at the post 20 and 40 dives timepoints compared to the baseline, but increased at the 3 month follow-up.
Both of these findings indicate importance of HBOT therapy continuation.

10 Additional Program Considerations

This section compiles notes recorded by the initial study coordinator within the Purdue Neurotrauma Group, and reviewed by the current study coordinator and Primary Investigator. These considerations are primarily of a practical nature, e.g., lessons learned that may be useful to review before planning or performing future studies of a similar nature. Further, some practical notes regarding specific but anonymous study participants are recorded. Please note any specific opinion is that from the study coordinator and deemed potentially relevant by the Primary Investigator. Any stated opinions do not reflect scientific conclusions of the Purdue Neurotrauma Group nor an institutional position of Purdue University.

10.1 Study Challenges

The organizational structure, study population, and COVID-19 each had some form of impact on the execution and timeline of the pilot study. The following reviews challenges observed by the Purdue Neurotrauma team for the consideration of future studies.

10.1.1 Recruitment and retention

Despite the small number of participants required for the pilot study, recruitment was difficult. Firstly, the original HBOT contract only included an HBOT facility at Clark Memorial in Jeffersonville, IN, in the southwest corner of the state along the border, which was the only provider applicant to the first version of the pilot study program. When Purdue's bid was accepted for the MRI and analysis portion, this created a 3 hour drive for study participants between Purdue's MRI facility and Clark's HBOT chamber. This made commitment to the MRI scan protocol difficult for participants. Additionally, Clark Memorial's location along the border in a relatively low density area made remote recruitment difficult despite news paper releases and posted flyers at local veterans organizations and hospitals. After HBOT facilities in the Indianapolis region were secured by IDOH, recruitment became much easier. COVID-19 and civil protests also impacted study recruitment and retention, with some individual participants or would-be participants expressing concerns about traveling long distances during this time. While we do not have access to patient records, our portion of the study indicates that at least five participants started HBOT dives after the initial MRI scan but did not complete the MRI protocols due to physical or mental health concerns. Of those, three are known to have completed their HBOT dives but not the scan protocol. These numbers may be higher as the program continues or with additional hospital statistics. Currently 25 individuals have started the program by attempting the first MRI scan. Of these, 11 are currently projected to complete the MRI portion of the study end-to-end. One has completed the end-to-end study. Additionally, two subjects have dropped out with significant MRI data completed and can be pooled for the initial HBOT results. On this current track, we have 11 (projected) + 1 (complete) = 12 whole data sets projected. Utilizing

the two partial data sets leaves only one participant left to be recruited for the pilot study, assuming no further attrition from the program.

10.1.2 Mental Health

Due to the scope of the study, participants were selected from a pool of Indiana veterans who had TBI and/or associated PTSD. Necessarily, individuals entered the program with mental health needs which may or may not have been concurrently and sufficiently addressed by a mental healthcare professional. Even in the presence of proper care, PTSD related mental health events or emergencies can occur at any time during the pilot study. We observed one such event in which the participant exhibited a threat of suicide in relation to COVID-19 and limittaion to continue their dives due to quarantine requirements. A full synopsis of events was given to IDOH as hospital HBOT provider subcontracts worked internally to resolve risk mitigation for mental health events and emergencies moving forward. Mental health assessments became required for participation at some facilities, but not all. This created a barrier for some individuals who had to wait more than a month to be seen by a mental healthcare professional (despite having mental health records from the VA) prior to being seen for HBOT dives.

Additionally some participants reported duress from the noises and sounds in the MRI machine itself. One individual withdrew from the study after they were unable to continue exclusively due to these noises. Another was unable to be scanned due to the mental stress of confinement. Others expressed concern for fellow veterans whose PTSD may be triggered by the loud repetitive noises which may emulate blade rotors or automatic weapons fire for some individuals. While Purdue Neurotrauma did screen for individuals with those potential triggers, it is possible that some participants may be unaware or conceal potentially adverse reactions to the machine prior to the MRI scan. The Purdue team installed headphones, which alleviate the problem greatly but not entirely.

10.1.3 Contract Structure

It should be noted that a program like the HBOT pilot study is a very ambitious but uncommon research endeavor mandated by the state legislature. This led to unconventional approaches in trying to fill the research goals of the program. While Purdue led the multi-organization coordination, the team was not responsible for subcontracting the HBOT providers. This relieved the burden of drafting subcontracts from the research team, but a subcontracting structure stemming from within a centralized clinical research institution is a more commonly employed approach which may be used in future or follow-on studies. Purdue was also brought in after the initial contracts with the first HBOT facility had been signed, which set a precedent contract structure which was initially disadvantageous to the HBOT provider at Clark Memorial Hospital. For example, prior to Purdue's involvement, Clark was responsible for research analysis and reporting, which fall outside the scope of their clinical HBOT facility. Eventually, Clark Memorial withdrew. Changing the contracts required a legislative amendment to the funding bill, which significantly delayed the research and recruitment side of the study.

10.1.4 Participant Agreements

One of the participants in the program complained that they believed they were entitled to travel reimbursement to and from the HBOT site. While the participation agreements are clear, it should be stressed that any and all costs, including travel, should be thoroughly reviewed with subjects prior to participation, including a break down of the organizational structure and procedures should erroneous billing issues arise.

10.1.5 Physical complications

The exact number of physical complications related to HBOT is unknown to the Purdue Neurotrauma team since participants are treated within the hospital network. One individual dropped from the study due to stress triggers in the MRI machine.

10.2 Considerations for Future Studies

10.2.1 Centralized Contracting

Future studies could benefit from centralizing the coordination and accountability by creating subcontracts for HBOT from the lead coordinating organization. This would also help centralize issues for participants as they arise. For example, in the currently scheme, individuals currently have to schedule MRIs with one organization and dives with another. There should also be a streamlined process for participants in the study so they do not get lost in the healthcare system and unknowingly surrender their insurance information for erroneous billing.

10.2.2 Mental Health Management

Regardless of the inclusion of study elements in the future application of HBOT for PTSD, a centralized mental health professional should be provided for all study participants who can not only assess the benefits of the program, but their overall mental well-being to ensure the best chance of success within any HBOT protocol.

10.2.3 Providing Non-study HBOT

The State could immediately provide funds for Indiana veterans with TBI and/or PTSD to access HBOT outside the study, given that they are approved and monitored by the HBOT providers. While collecting and analyzing data is important to learning more about this new therapy option, participation in the MRI study does not necessarily have to be a requirement or hindrance to accessing HBOT. This could mitigate the PTSD triggers from the MRI machine used in the study for some individuals and ameliorates ethical issues wherein an individual may feel compelled to participate in the research portion of the pilot program so as to more immediately receive HBOT.

11 Conclusion

This report was produced on January 1, 2023, and has been drafted for the Indiana State Department of Health (ISDH) and the Indiana State legislature. We would like to thank ISDH personnel, all of the HBOT providers and subcontractors, General James Bauerle, and Purdue University for their support.

Questions and Inquiries can be directed to the program's Primary Investigators, Dr. Joseph Rispoli and Dr. Yunjie Tong.