Hyperbaric Oxygen Therapy (HBOT) Pilot Study Report

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

Contents

1 Project Summary

Hyperbaric oxygen therapy (HBOT) is a medical treatment that involves breathing pure oxygen at higher-than-normal atmospheric pressure levels. While the effectiveness of HBOT in treating neurological diseases is not well established, there is significant evidence that it can be beneficial in treating certain neurological disorders and conditions, such as post-traumatic stress disorder (PTSD) or mild traumatic brain injury (mTBI). In this longitudinal pilot study, magnetic resonance spectroscopy (MRS) was utilized to evaluate if increasing oxygenation of blood and tissues instigated by HBOT mediates neuroplasticity or neurometabolic changes in individuals with clinically diagnosed PTSD/mTBI. 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. Despite the small sample size, the study provided valuable insights into the neurometabolic changes associated with HBOT.

The study examines the treatment response of military veterans with clinically diagnosed PTSD who undergo HBOT, utilizing advanced magnetic resonance imaging (MRI) to assess changes. This pioneering research employs MRI to evaluate the effects of HBOT, capturing data at baseline, after 20 and 40 dives, and at a three-month follow-up post-therapy. Significant neurometabolic alterations were observed in the orbitofrontal cortex (OFC), insula, and amygdala, with the insula showing the most notable changes. These findings suggest that HBOT may initiate neuroplasticity processes, offering new insights into its potential as a treatment for PTSD and mTBI. The alterations in major neurometabolites, measured via MRS, were statistically significant. The study contributes to the expanding evidence base supporting HBOT's efficacy for these conditions, proposing that enhanced oxygenation of blood and tissues improves neuronal function by reactivating metabolic and neuronal pathways.

2 Purdue Personnel List

2.1 Faculty investigators

Dr. Joseph Rispoli, jrispoli@purdue.edu — Primary Investigator (2019 - 2023.7)

Dr. Yunjie Tong, tong61@purdue.edu — Primary Investigator (2023.7 - Present)

Dr. Eric Nauman, enauman@purdue.edu — Co-Investigator
Dr. Thomas Talavage, tmt@purdue.edu — Co-Investigator

2.2 Coordination

Antonia Susnjar, asusnjar@purdue.edu — (October 2022 - Present)
T. Arthur Terlep, tterlep@purdue.edu — (October 2019 - October 2022)

2.3 Technicians

Antonia Susnjar, asusnjar@purdue.edu Gianna K Nossa, gnossa@purdue.edu
T. Arthur Terlep, tterlep@purdue.edu

2.4 Analysts

Antonia Susnjar, asusnjar@purdue.edu; Ho-Ching Yang, yang1399@purdue.edu; Gianna K Nossa, gnossa@purdue.edu; Bradley Jacob Fitzgerald, fitzge45@purdue.edu; John Robert Morris, morri519@purdue.edu; Vidhya Vijayakrishnan Nair, vijayak0@purdue.edu; Yukai Zou, Y.Zou@soton.ac.uk;
T. Arthur Terlep, tterlep@purdue.edu

3 Introduction

Traumatic brain injury and post-traumatic stress disorder

Traumatic brain injury (TBI) occurs when an external force such as an impact, blast wave, or projectile injures the brain. This type of injury may lead to temporary or lasting brain function impairments and diagnosis is often challenging due to the subtle neuroanatomical changes that are not always detectable with standard imaging tools.[1–3] TBIs are classified by their severity, damage characteristics, and the cause. In the United States, TBIs lead to the highest rates of death and disability. Each year, about 2.8 million Americans suffer a TBI, contributing to roughly 50,000 deaths annually.[4,5] Among neurological disorders, TBI is the most prevalent and presents a considerable public health challenge.[6] It is increasingly seen as a chronic condition that can result in prolonged neurocognitive issues and an elevated risk of neurodegenerative diseases, affecting daily activities, work, and social interactions.[7] Typically, TBIs involve widespread brain damage from axonal shearing and contusions, which can trigger further complications like edema and ischemia, impeding the brain's ability to heal and causing ongoing oxygen deprivation in brain tissues.[1] The financial impact of TBI is also significant, costing the U.S. economy between $48.3 billion and $60 billion annually. [5]

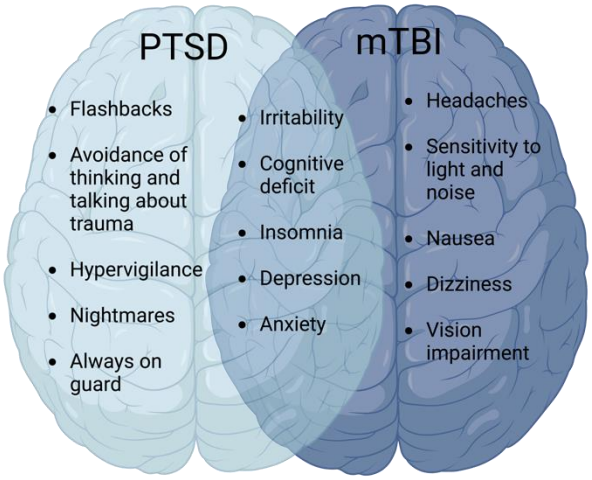


Figure 1: *This diagram illustrates the symptoms that are common to both Post-Traumatic Stress Disorder (PTSD) and Traumatic Brain Injury (TBI), as well as the symptoms that are unique to each condition.*

Moreover, there's a strong link between TBI and PTSD, with TBI victims more likely to develop PTSD. Individuals suffering from both conditions typically experience more severe symptoms and face a complex recovery process. Symptoms common to both PTSD and TBI include headaches, dizziness, fatigue, irritability, anxiety, depression, and cognitive difficulties like memory loss and confusion, see Figure 1. PTSD affects millions annually, especially disabled veterans who face additional hurdles in accessing effective treatments.[6,8] Studies highlight a notable association between PTSD and higher suicide rates among veterans, with female veterans facing particularly high risks.[9] Despite the widespread occurrence of PTSD and TBI, current treatment strategies predominantly concentrate on managing symptoms rather than tackling the root causes of these conditions. [10]This approach can provide temporary relief but often fails to address the fundamental issues that contribute to long-term impairment and decreased quality of life. As a result, many individuals experience recurrent problems, and the underlying brain injuries or psychological

trauma may go untreated, leading to chronic conditions. [11,12] This highlights a critical gap in the treatment landscape for PTSD and TBI, underlining the pressing need for the development and implementation of more comprehensive treatment modalities that not only alleviate symptoms but also promote long-term healing and recovery.

Novel therapies for TBI and PTSD

Recent advancements in both technology and neuroscience have led to the development of innovative therapies that offer hope for more effective and personalized treatment approaches. For example, Transcranial Magnetic Stimulation (TMS) is a non-invasive procedure that uses magnetic fields to stimulate nerve cells in the brain. TMS has shown potential in reducing symptoms of depression and anxiety in PTSD patients. Similarly, transcranial Direct Current Stimulation (tDCS) applies a small electrical current to stimulate specific parts of the brain. However, the efficacy can vary widely among individuals, and optimal parameters for treatment (such as intensity and frequency) are not yet standardized. Additionally, virtual reality has gained a lot of traction in the recent years, and it is used to create controlled, immersive environments where patients can confront traumatic memories under the guidance of a therapist, potentially reducing PTSD symptoms but not all patients are comfortable with or respond to virtual scenarios, which can affect treatment outcomes.

While not novel, HBOT is a non-invasive therapy involving the delivery of oxygen at high pressures in a controlled chamber. HBOT principle of action is very simple as it is shown in figure 2 since it helps to increase the amount of oxygen blood can carry.[13] An increase in blood oxygen restores normal levels of blood gases and tissue function to promote healing and fight infection. By increasing the pressure, significantly more oxygen can be dissolved into the bloodstream which can assist in healing. Although the FDA approves HBOT for various applications such as carbon monoxide poisoning, radiation and diabetic wounds or severe anemia, its use in mental health, specifically for conditions like mTBI or PTSD, is not yet approved. [14–19]HBOT enhances blood and tissue oxygenation, potentially improving brain function by stimulating metabolic and neural pathways. However, its effectiveness in treating neurological conditions and bringing about structural brain changes is still debated, partly due to a shortage of studies that adequately demonstrate how it affects brain metabolism and plasticity.[20,21]

To bridge this knowledge gap, this study employs MRS to explore HBOT's impact on veterans with PTSD and/or TBI. MRS is a non-invasive technique that uses standard MRI equipment to measure the levels of specific brain metabolites in vivo. This study investigates the biochemical effects of HBOT. Ultimately, our research aims to better characterize HBOT's effects on a veteran cohort and broaden the understanding of viable treatment options among healthcare providers and potential patients.

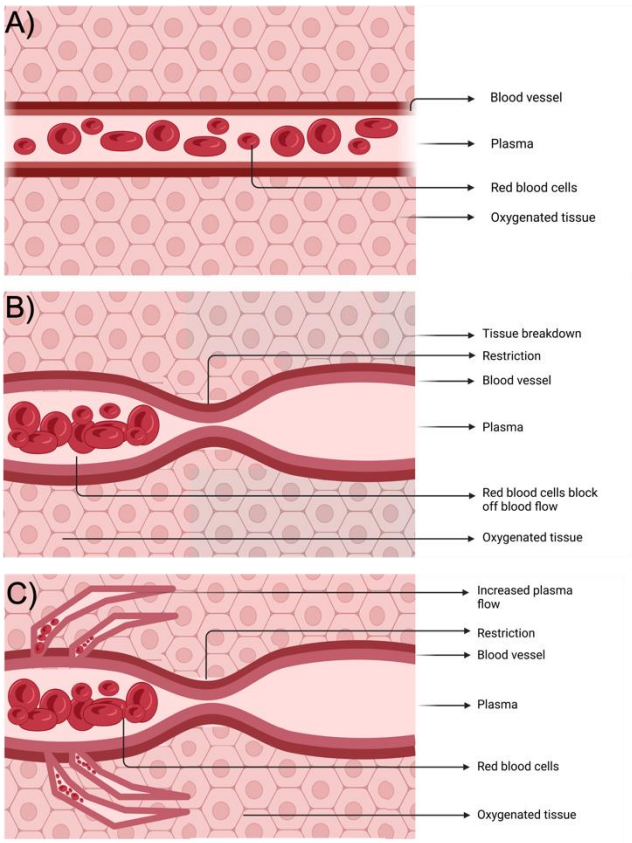


Figure 2*: Clinical applications of HBOT are extremely diverse. However, there is very limited literature investigating benefits of HBOT in brain.*

A) Normal blood flow can often be restricted due to injury or disease.

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.

4 Study Design

We have collected a data from thirteen (13) military veterans (13 males, average age 46.2 ±10.6 years) with clinically diagnosed PTSD or traumatic brain injury (TBI) who underwent forty (40) HBOT dives at hospitals and clinics contracted by the State. Even though study participants received HBOT therapy at three different clinics, protocol was standardized and optimized at each location. Every dive was an hour long at 1.5 atm and each participant completed forty dives. We have performed MRI scans at the Purdue MRI Facility before starting the HBOT, and immediately following the last 40th dive to document the long-term effects of HBOT. Before each MRI session, we collect data from two surveys—the Central Nervous System Vital Signs (CNSVS) and the Purdue Neurotrauma Group Basic Health Assessment/Questionnaire (PNGBHQ)—to further enrich our understanding of each veteran's health status in relation to their HBOT treatment.

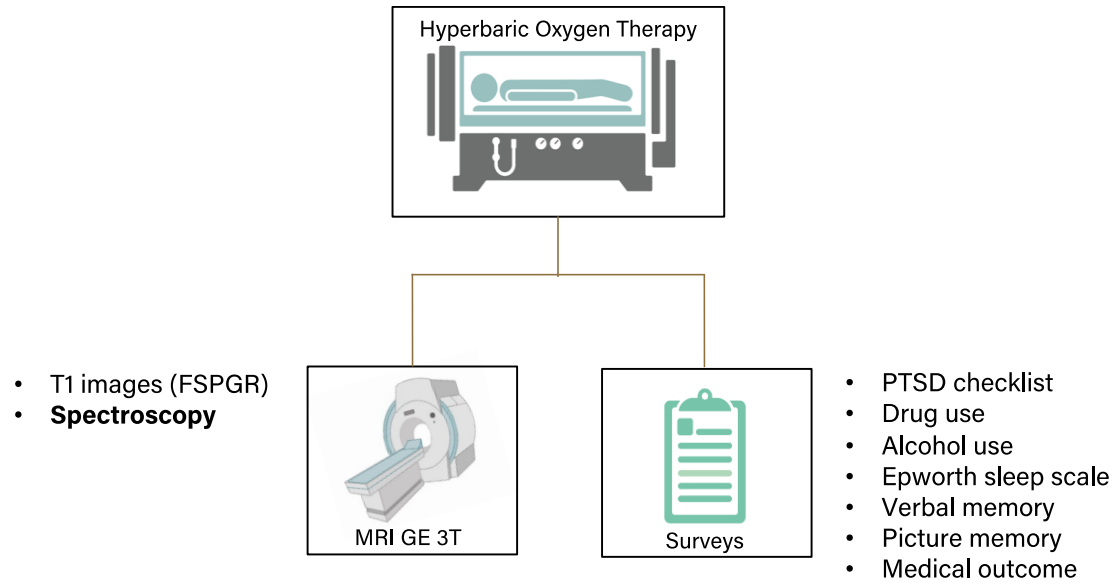


Figure 3: *Above diagram shows research design for the evaluation of HBOT in military veterans with clinically diagnosed PTSD and/or mTBI using Magnetic Resonance Spectroscopy paired with CNSVS survey data.*

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)

However, several participants experienced distress after completing the CNSVS survey, leading us to discontinue its use. This situation underscores why MRI metrics are preferable as they are more objective and less invasive for evaluating veterans' mental health.

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.

Imaging protocol

This study was approved by Purdue University's Institutional Review Board and all study participants provided informed written consent. A 3 T General Electric Discovery MR750 (Waukesha, WI) system (software Dv26.0) was used with a 32-channel brain array coil (Nova Medical; Wilmington, MA). The MRI assessments conducted included various pulse sequences: high-resolution structural images (1-mm isotropic resolution in both T1-weighted image using a 3D spoiled gradient recalled echo sequence (TR/TE=5.7/1.976ms)), MRS to measure the relative concentrations of metabolites within brain tissues, such as N-acetyl aspartate for neural health, choline as a marker of cell membrane damage, and myo-inositol indicating active injury. MRS data were collected using the semi-adiabatic localization by adiabatic selective refocusing (sLASER) sequence (TE/TR 35/2000ms, 64 averages, 2048 data points, and volume of interest (VOI) 20x20x20 mm3). 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). sLASER, when combined with voxel based static B0 and transmitted B1 calibration routines, provides neurochemical profiles with high data reproducibility at 3T.[22,23] While we say that the scanner strength is 3T, in reality magnetic field is inhomogeneous with some parts being 2.98T, while others are ~ 3.03T. In MRS, "shimming" is a crucial step to ensure the quality and reliability of the acquired data obtained by making magnetic field uniformed. Therefore, "B0 shimming" refers to the process of adjusting and homogenizing the magnetic field (B0) within the area of interest in the brain. 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 8. B0 shimming for each VOI was achieved by optimizing the second order shim terms using B0DETOX – software obtained from Columbia University that reduces B0 imperfections that lead to line broadening in MRS.[23] Since GE scanner utilize full width half maximum (FWHM) of the water signal (real part), shimming values below 30 Hz are deemed acceptable. Average shimming values for each brain region are showed in Figure 4. This serves as a quality assurance check for the acquired data. Four brain regions: anterior insula, orbitofrontal cortex, anterior cingulate cortex, and amygdala were investigated.

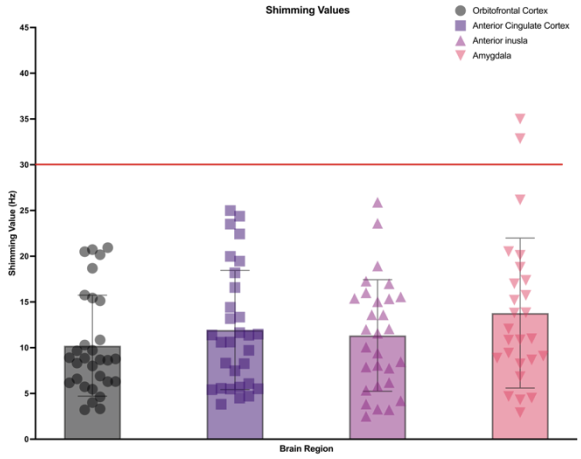


Figure 4*: Data quality can be expressed using shimming values that are collected during each scan. Red line is a threshold for acceptable values for GE scanner used in this study. Higher values represent line broadening which limits metabolite distinction on a spectrum. As shown in the plot, only 2 data points had poor shimming and were excluded from the further quantification and analysis.*

Magnetic Resonance Spectroscopy (MRS)

MRS operates on the same principles as a standard MRI and can be conducted using any regular MRI scanner without needing extra equipment. However, instead of merely displaying the brain's structure, MRS also provides detailed information about the chemical composition of different tissues as shown below in Figure 5.[24,25] Essentially, it combines the visual capabilities of an MRI with the analytical power of a laboratory, offering a comprehensive view of both the anatomy and the biochemical environment within the brain.

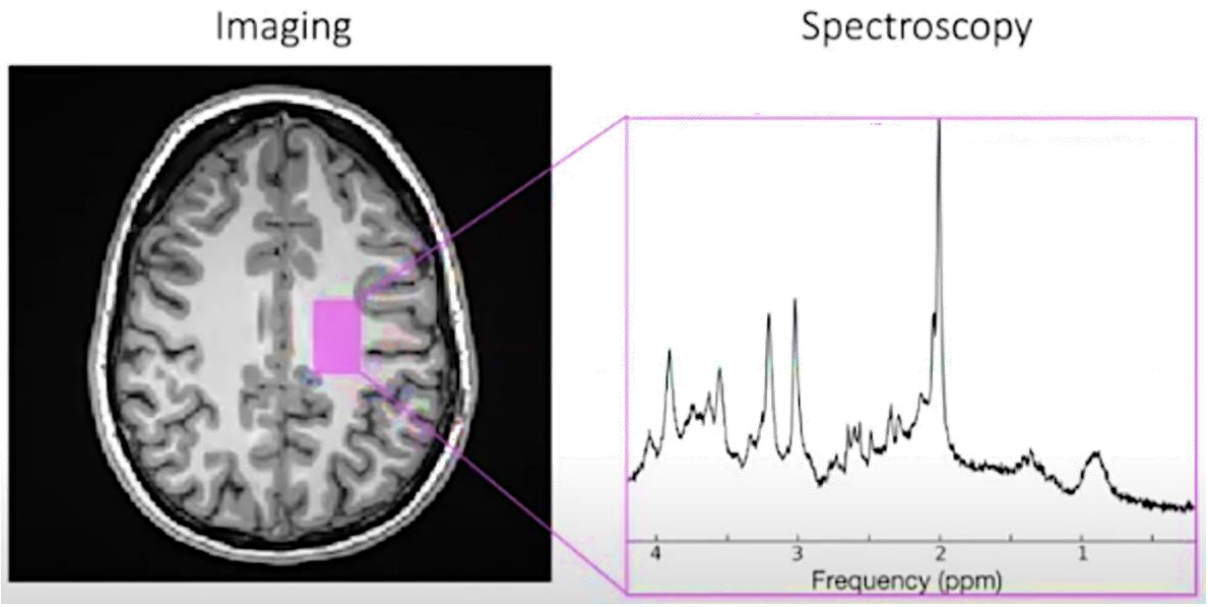


Figure 5. *Imaging signal comes from water and fat protons, while spectroscopy signal comes from protons in other chemical enviroments.*

By filtering out the dominant signals from water and fat, MRS can accurately measure neurometabolites and neurotransmitters, which serve as crucial indicators of brain function and health. MRS signal in figure 6 below is displayed as a spectrum, is composed of a signal from many different metabolites.[24–26]

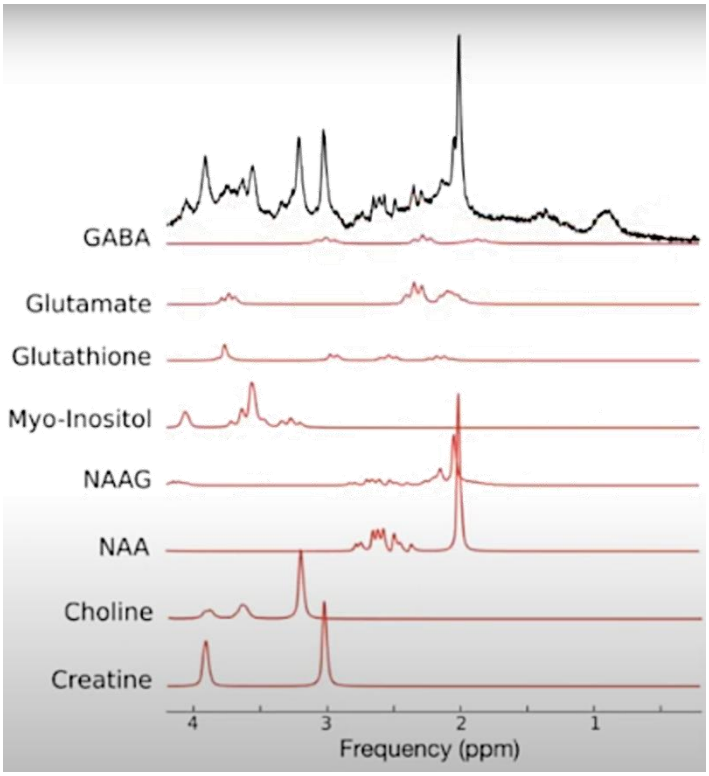


Figure 6. *Each metabolite is identified by a unique and highly reproducible frequency distribution. These differences in frequency are caused by the differences in chemical composition of neurometabolites. Neurometabolic changes are brain region specific, which is why it is crucial to identify clinically relevant regions of interest (ROI). Most ROI measurements typically range between 8-27 mL*^{3} *providing a window to biochemical composition of the region.*

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.[25,26] MRS was the first tool that demonstrated biological changes in mental health patients, namely imbalances in brain metabolism, changing the stigma of mental health. MRS has diverse applications in investigating the metabolic window of a wide range of biochemical processes including

neurological diseases including neurological disorders such as Alzheimer's disease, schizophrenia, postpartum depression, and others.[26,27] Figure 7 illustrates the widespread adoption and utilization of MRS beyond the confines of specialized research facilities.

Figure 7. *The figure showcases the geographic distribution and variety of settings in which MRS technology is being implemented, emphasizing its diverse role of clinical and diagnostic applications worldwide. With the recent advancements in MRS acquisition and processing, the widespread use of MRS has increased with total of 1870 ongoing clinical trials. Emerging MRS studies have provided compelling evidence indicating that neuroinflammation and oxidative stress are prominent features observed in the early stages of TBI. Therefore, monitoring MRS metabolites as biomarkers holds promise for assessing HBOT treatment response.*

MRS Metabolites:

- ***N-acetylaspartate (NAA)*** is often used as a marker of neuronal health and density, decreased levels of NAA are commonly observed in areas of the brain affected by TBI. This reduction suggests neuronal loss or damage. However, increase in total N-acetylaspartate (tNAA) suggests recovery post injury.

- ***Choline (Cho)*** is associated with cell membrane turnover. Elevated levels of choline can indicate an increase in cell membrane breakdown and repair, a common occurrence in both TBI and areas of the brain affected by PTSD, reflecting ongoing repair processes.

- ***Creatine (Cr)*** is typically measured to assess cellular energy metabolism. While changes in creatine levels are not always specific, they can help provide a baseline for cellular energy status, which can be disrupted by injury or stress-related disorders.

- ***Myo-Inositol (mI)*** is often increased in conditions involving glial cells (supportive cells in the brain), higher myo-inositol levels can indicate glial proliferation or activation, which is a response often seen in areas of the brain recovering from trauma or involved in neuroinflammatory processes.

- ***Glutamate and Glutamine (Glx)*** are excitatory neurotransmitters. Alterations in their levels can be indicative of changes in neurotransmitter activity following TBI or in PTSD, where imbalances in neurotransmitter systems may occur.

Metabolite	Myo-inositol	Choline	Creatine	Glutamate	N-acetyl aspartate
Acronym	Ins	tCho	tCr	Glx	NAA
Function	Glial marker	Membrane marker	Energy marker	Neuro transmitter	Neuronal marker
Trend	⇧	⇩	⇧	⇧	⇩

Table 1. *Trend of neurometabolic change in TBI.*

Brain regions

- ***The anterior cingulate cortex (ACC)*** The ACC is a part of the brain located in the frontal lobe and it is divided into two are functionally distinct regions: the dorsal ACC (dACC) and the ventral ACC (vACC). The dACC is involved in cognitive control, conflict monitoring, and error detection, while the vACC is involved in emotional processing, empathy, and social cognition. We have acquired MRS data exclusively from the dACC to gain better understanding of neurometabolic changes that could have an impact on attention, working memory, and decision-making.

- ***Orbitofrontal Cortex (OFC)*** The OFC is involved in regulating emotional responses to stimuli and has been implicated in the extinction of fear-based or trauma-related memories.

- ***Insula*** The insula is a region of the brain that is involved in a range of functions, including interception (the perception of internal bodily sensations), emotion processing, and social cognition. Recent studies have suggested that alterations in the insula function may contribute to the heightened emotional reactivity and hyperarousal symptoms that are common in PTSD.

- ***Amygdala*** The amygdala is involved in the processing of emotions, particularly fear and anxiety, as well as the formation and consolidation of emotional memories. It receives input from various sensory systems and sends output to other brain regions to initiate the physiological and behavioral responses associated with fear and anxiety, known as the "fight or flight" response. Dysfunction in the amygdala has been implicated in several psychiatric disorders, including anxiety disorders, depression, and PTSD. Amygdala is extremely challenging to shim and therefore obtain reliable MRS data as shown in Figure 8. This is in part due amygdala's location close to the skull base, which can result in susceptibility artifacts that can interfere with MRS signal quality. These artifacts arise from differences in magnetic susceptibility between the skull and brain tissue, which can cause distortions in the magnetic field that affect the MRS signal.

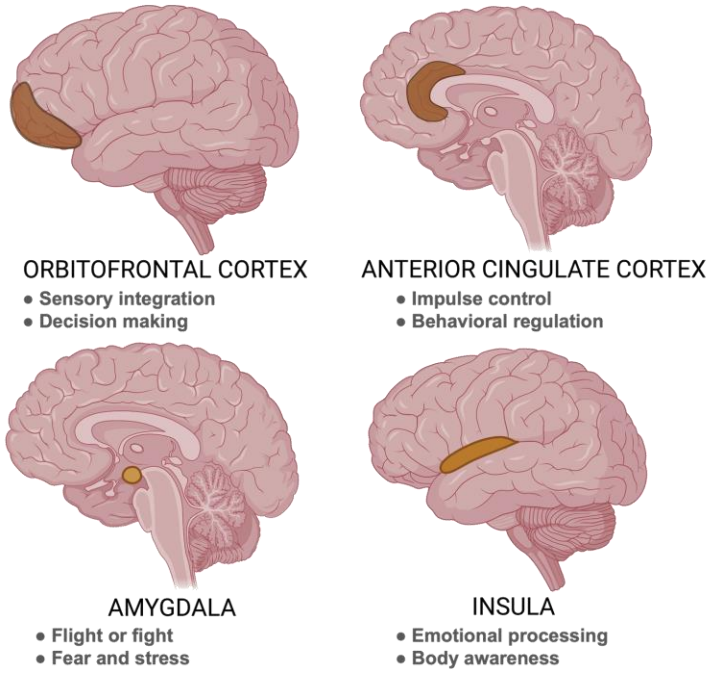


Figure 8*: Key brain regions involved in PTSD symptoms include the orbitofrontal cortex, anterior cingulate cortex, amygdala, and insula.*

Specific Aims/Objectives

HBOT has shown promise in addressing the neurophysiological underpinnings of PTSD in military veterans, according to preliminary data. However, existing studies primarily rely on survey metrics to evaluate efficacy, which may not fully capture the therapy's impact. HBOT involves exposing patients to higher atmospheric pressures and increased oxygen levels, aimed at enhancing oxygen delivery to critical brain regions to facilitate natural healing processes.

Despite its extensive use in wound care, the U.S. Food and Drug Administration (FDA) has not approved HBOT for treating mental health conditions, including PTSD or mTBI. This pilot study aims to provide comprehensive evidence to federal, state, and military health authorities, such as the Department of Defense (DoD) and the Department of Veterans Affairs (VA), about the potential benefits of HBOT for veterans.

The research will assess HBOT's effects on veterans with PTSD and/or mTBI using advanced MRS to measure metabolic levels in targeted brain areas and high-resolution MRI scans for structural analysis. Consistency with previous studies is maintained by incorporating two survey assessments during each MRI session: the CNSVS for recent PTSD symptoms and the Purdue Neurotrauma Group Basic Health Assessment (PNGBHQ) to rule out other variables. Our primary hypothesis is that HBOT will increase tNAA, a marker of neuronal integrity, indicating improved health across brain regions. This study will also establish a rigorous framework for future trials, setting the stage for extensive clinical research.

5 Data Analysis

Survey

The Central Nervous System Vital Signs (CNSVS) survey toolbox has been utilized to detect even minor neurocognitive impairments or improvements in a variety of neuropsychiatric and non-

neuropsychiatric conditions.[28] When incorporated into a neurocognitive benchmark, it facilitates "Quality Care" practices such as tracking treatment response efficacy, assessing diverse neurocognitive profiles of psychiatric or neurological disorders and treatments, and generating significant amounts of useful data for research and publication purposes. Specific list of surveys was selected for this study based on the previously published literature. Assessment included verbal memory test, visual memory test, alcohol use disorders identification test (AUDIT) SF-10, depression, anxiety, and stress scale (DASS) SF-21, drug use questionnaire (DAST) SF-20, Epworth sleepiness scale (ESS) SF-8, medical outcomes survey (MOS) SF-36, and PTSD Checklist for DSM-5 (PCL-5).
While we collected survey data using the CNSVS to compare our findings with previous studies on HBOT, some participants were triggered by the questions and had to be excluded from the analysis and further collection. Survey data was collected for all seven participants at the baseline and post 20 dives.

Magnetic Resonance Spectroscopy

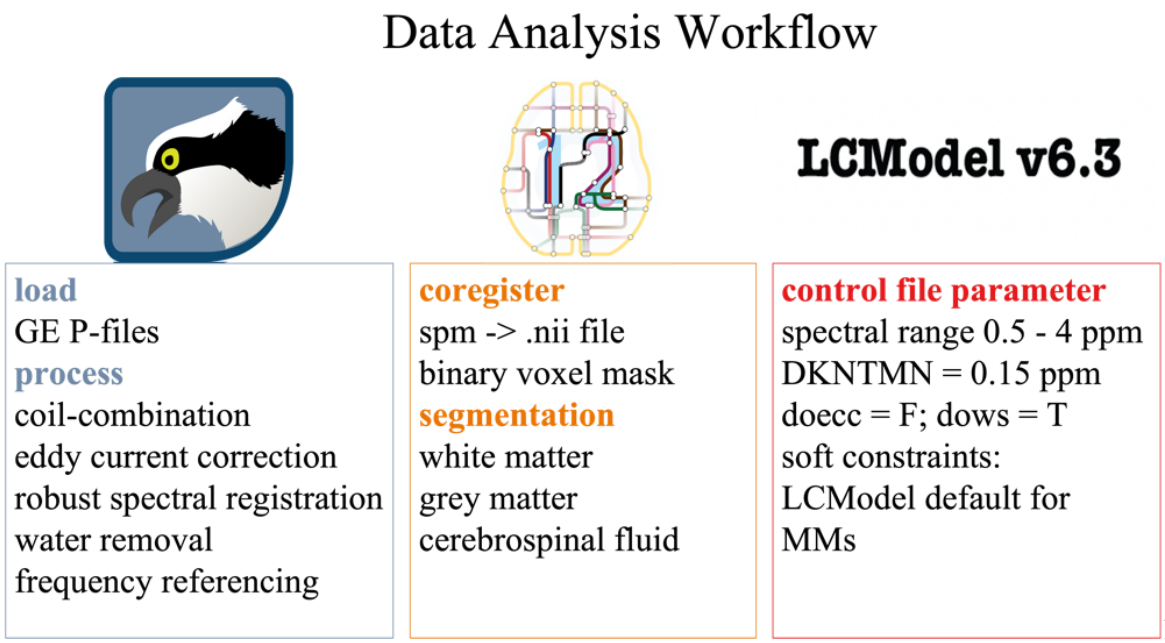


Figure 9: *Data analysis workflow for Magnetic Resonance Spectroscopy data.*

Osprey v2.4.0, 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. [29]Furthermore, voxel coregistration and segmentation was also completed in Osprey utilizing statistical parametric mapping (spm12). Lastly, metabolite quantification was completed in LC Model V6.3-1B. NAA, creatine (Cr), choline (Cho), myo-inositol (Ins), and glutamine+glutamate (Glx) were reported using water referenced quantification and corrected for cerebrospinal fluid (CSF).[30] Data analysis workflow was illustrated in Figure 9. All statistical analysis (Shapiro-Wilk normality test, Grubbs outlier test, 2-way ANOVA test) was performed using GraphPad Prism v9.2.0.6. Data was considered statistically significant for p-values below 0.05. Plots were created in Graph Pad Prism.

6 Results

Survey Results:
Although survey data was gathered to align our findings with prior studies that solely utilized surveys

to evaluate HBOT, the survey process triggered adverse reactions in several participants, necessitating an early termination of data collection. Despite this, the analysis of the available data revealed noticeable trends. However, the majority of the findings lacked statistical significance, attributed to the reduced sample size resulting from the discontinued surveys.

The CNSVS assesses cognitive functions through parallel tests for verbal and visual memory. The verbal memory test utilizes a word list learning approach, while the visual memory test employs geometric shapes for figure learning. Additionally, the composite memory score is calculated as the mean of z-scores from three domains: episodic memory, executive function, and attention processing. Data shown in Figure 10A illustrates an increasing trend in composite and visual memory scores. Notably, verbal memory showed a significant increase ($p = 0.05$).

The Depression, Anxiety, and Stress Scale (DASS) SF-21 is a condensed version of the original 42-item questionnaire, designed to measure the three interrelated emotional states of depression, anxiety, and tension/stress. According to data presented in Figure 10B, there is a noticeable decrease in these symptoms following HBOT therapy.

While it remains uncertain which PTSD symptoms are most closely linked to substance abuse, recent studies highlight the role of the insula—a critical area for body awareness—in processing drug-related cues and influencing drug cravings and relapse. As shown in Figure 10C, the data from both the AUDIT SF-10 and DAST SF-20 surveys indicate a declining trend in substance abuse following HBOT therapy.

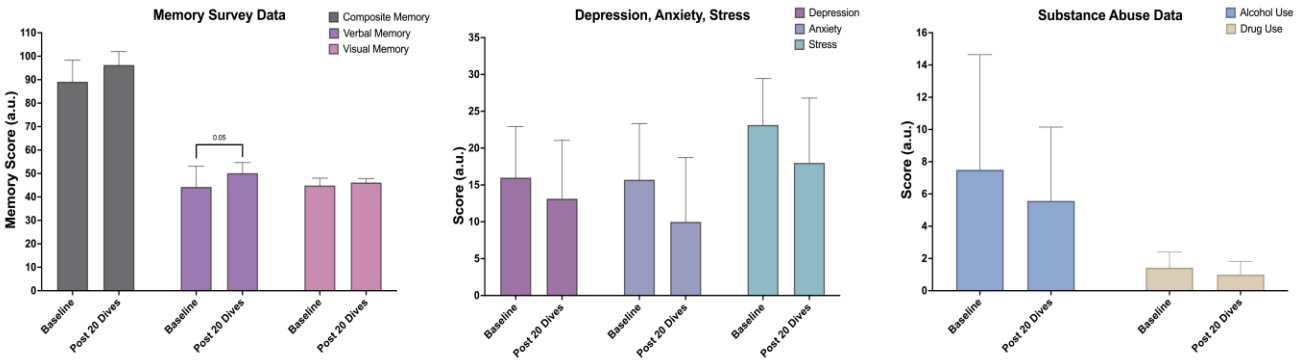


Figure 10. *A) The CNSVS test measures verbal and visual memory using similar tests, but with different stimuli (words vs geometric shapes). The results of these tests, along with tests for executive function and attention processing, are used to calculate a composite memory score. The results show an increase in the composite and visual memory scores, while verbal memory scores showed a significant increase ($p = 0.05$). B) The DASS SF-21 questionnaire is a shorter version of a self-report measure that assesses depression, anxiety, and stress. The results presented indicate a decreasing trend in depression, anxiety, and stress following HBOT treatment. C) Data from both the AUDIT SF-10 and DAST SF-20 questionnaires indicate a decreasing trend in substance abuse following HBOT treatment.*

Magnetic Resonance Spectroscopy Results

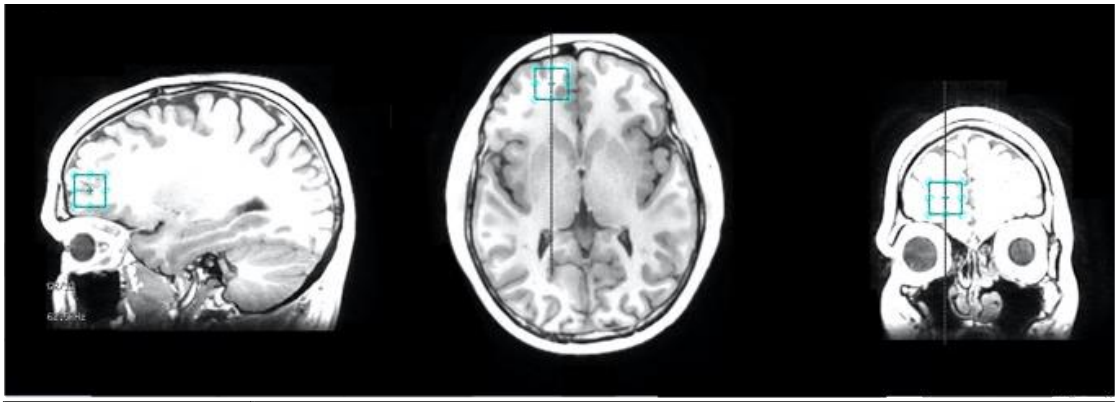
- ***Orbitofrontal Cortex***

The orbitofrontal cortex (OFC) is a region of the brain located in the frontal lobes, involved in the cognitive processing of decision-making. It plays a critical role in interpreting and managing emotional reactions to environmental stimuli. This area is particularly significant in the context of psychological conditions like PTSD, where its function becomes crucial for modulating emotions and behaviors associated with fear and anxiety.

Normally, the OFC helps individuals learn from emotional experiences and use this information to guide future behavior, a process often disrupted in those with PTSD. Research suggests that the OFC helps to understand and integrate the idea that a previously threatening stimulus is no longer a danger, essentially "updating" the emotional significance attached to that memory. This extinction process involves multiple brain areas, but the OFC's role is to dampen the response of the amygdala, another brain region that processes fear.

When the OFC functions effectively, it can inhibit the amygdala's tendency to activate stress and fear responses, promoting a more balanced emotional state. An increase in NAA levels, detected via MRS ($p = 0.0138$) in the OFC, can be particularly indicative of functional changes in this region. NAA is considered a marker of neuronal health and density. In the context of PTSD and the OFC, increased levels of NAA may reflect improved neuronal function or recovery, suggesting that the neurons in the OFC are better able to regulate emotional responses and engage in the extinction of fear memories.

Similarly, choline is a vital component of phospholipids, essential for the formation and maintenance of cellular membranes. An increase in choline levels post-treatment could indicate increased activity in cell membrane synthesis and repair. This is especially relevant in treatments aimed at recovery from neurological damage, where neuronal health and functionality are being restored or enhanced. The observed increase in choline could reflect efforts to repair damaged neuronal structures, indicating positive recovery following HBOT therapy. This change suggests that the brain is adapting and possibly healing the neural circuits involved in trauma responses.



Brain Region: OCC	myo-inositol			tCho			tCr			tNAA			Glx		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Baseline	6.53	0.82	12	2.08	0.50	11	5.09	0.56	11	7.10	0.96	12	9.13	1.96	10
Post 40 Dives	5.61	0.72	11	3.67	1.78	12	5.88	0.57	10	8.47	0.96	12	7.50	1.11	9

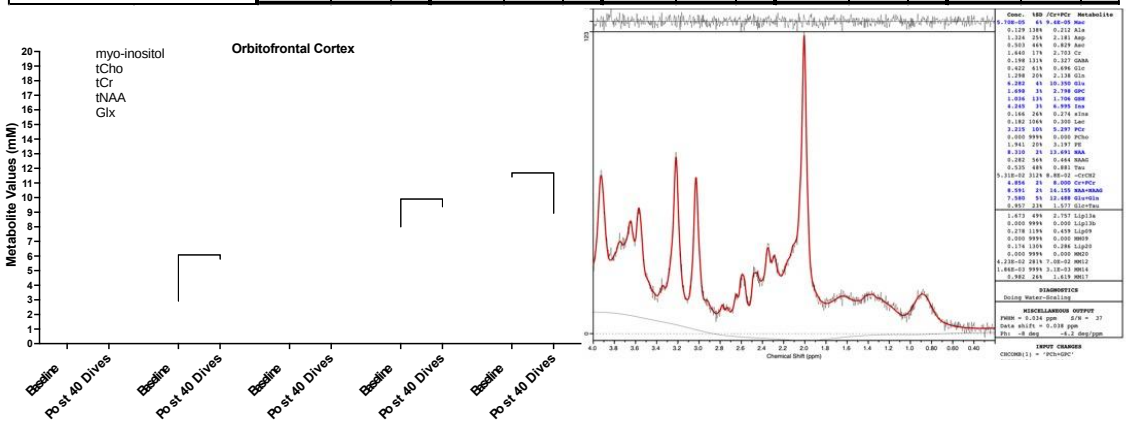
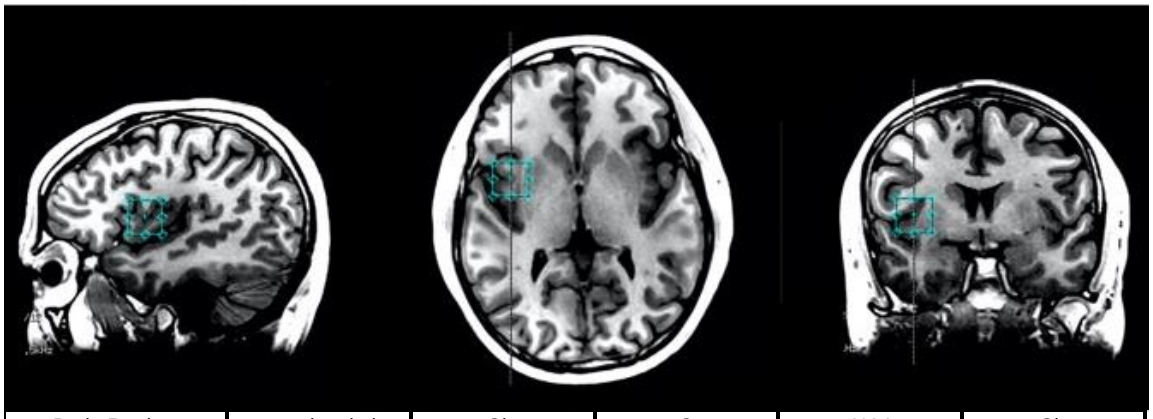


Figure 11. *The figure illustrates the positioning of the voxel in sagittal, axial, and coronal planes. MRS data was collected from the voxel highlighted in blue, and the associated spectrum displaying quantified metabolites is provided. The table includes the mean, standard deviation, and number of*

participants for each metabolite, with this data also represented graphically. Statistical analysis revealed significant changes in total choline (tCho; (p = 0.004), total N-acetylaspartate (tNAA; p = 0.0138), and glutamate/glutamine (Glx; p = 0.0087).

- ***Insula***

The insula is a critical region of the brain with several important functions, making it vital for a wide range of neural processes related to emotion processing, perception, and bodily awareness. Myo-inositol is considered a marker of glial activity and can indicate changes in astrocytic function. A decrease in myo-inositol levels in the insula, might suggest a reduction in glial activation or a decrease in inflammatory response. This could be interpreted as a positive treatment outcome, potentially indicating reduced inflammation or normalization of glial function. Additionally, creatine is involved in energy metabolism, serving as an energy buffer in the form of phosphocreatine. An increase in creatine levels indicate enhanced energy reserves or increased cellular energy demand. This might reflect an adaptive response to increased neuronal activity or recovery processes within the brain tissue. Finally, just like in the OFC, increase in tNAA might reflect neuronal recovery or regeneration, or it could indicate improved functionality of neurons in the insula.



Brain Region: Insula	myo-inositol			tCho			tCr			tNAA			Glx		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Baseline	6.17	0.86	13	2.80	0.43	8	5.74	0.53	13	8.07	0.87	13	10.30	1.28	13
Post 40 Dives	4.92	0.87	13	3.01	0.63	10	6.93	0.74	13	9.69	1.30	13	10.54	1.58	11

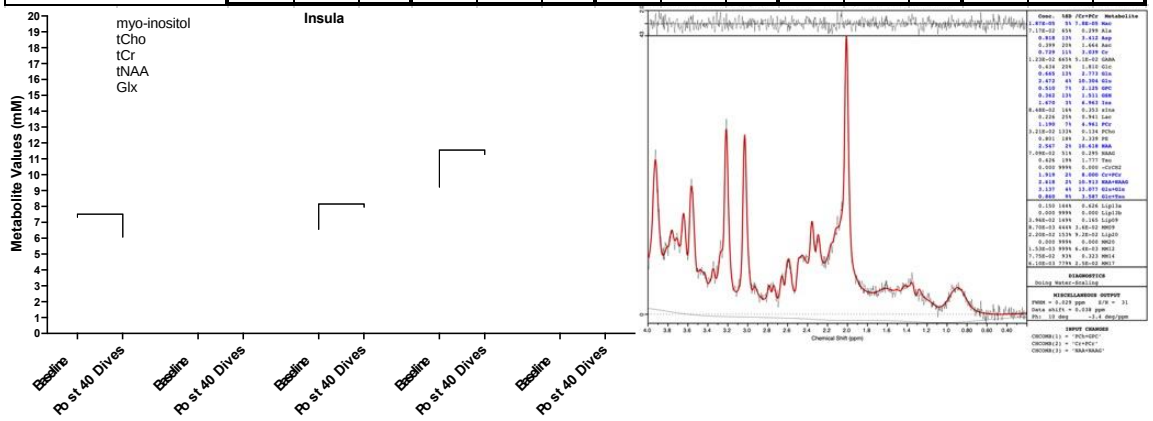
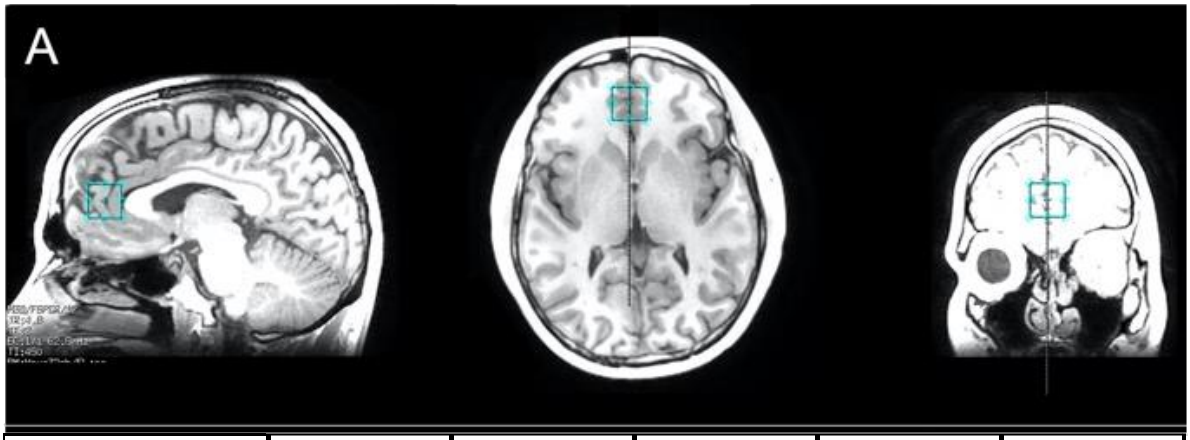


Figure 12. *The figure illustrates the positioning of the voxel in sagittal, axial, and coronal planes. MRS data was collected from the voxel highlighted in blue, and the associated spectrum displaying quantified metabolites is provided. The table includes the mean, standard deviation, and number of participants for each metabolite, with this data also represented graphically. Statistical analysis revealed significant changes in myo-inositol (p = 0.008), total creatine (tCr; (p = 0.0127), total N-acetylaspartate (tNAA; p = 0.0003).*

- ***Anterior Cingulate Cortex***

The anterior cingulate cortex (ACC) performs a wide range of cognitive and emotional functions including error detection and decision making. Glutamate is the primary excitatory neurotransmitter in the brain, involved in almost all aspects of normal brain function, including cognition, memory, and learning. Glutamine, on the other hand, is a precursor and byproduct of glutamate in the brain's metabolic pathways. Glx, often measured in MRS, represents combined signals of glutamate and glutamine. Increased Glx indicated enhanced neurotransmission which could enhance synaptic transmission and improve neural communication. This might result in better cognitive functioning and emotional regulation. While not statistically significant, this is coupled with increased tCr – an energy metabolism marker.



Brain Region: ACC	myo-inositol			tCho			tCr			tNAA			Glx		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Baseline	7.88	2.02	8	4.20	1.60	10	7.64	1.72	10	10.19	1.97	10	15.19	3.72	8
Post 40 Dives	7.03	1.33	10	4.24	1.38	9	8.28	2.10	8	11.27	3.00	9	11.55	3.41	11

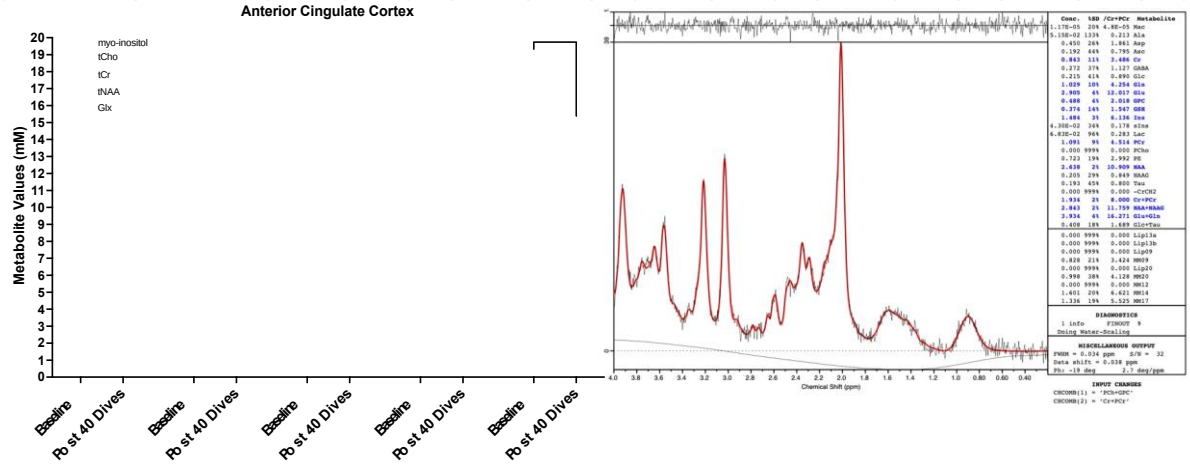
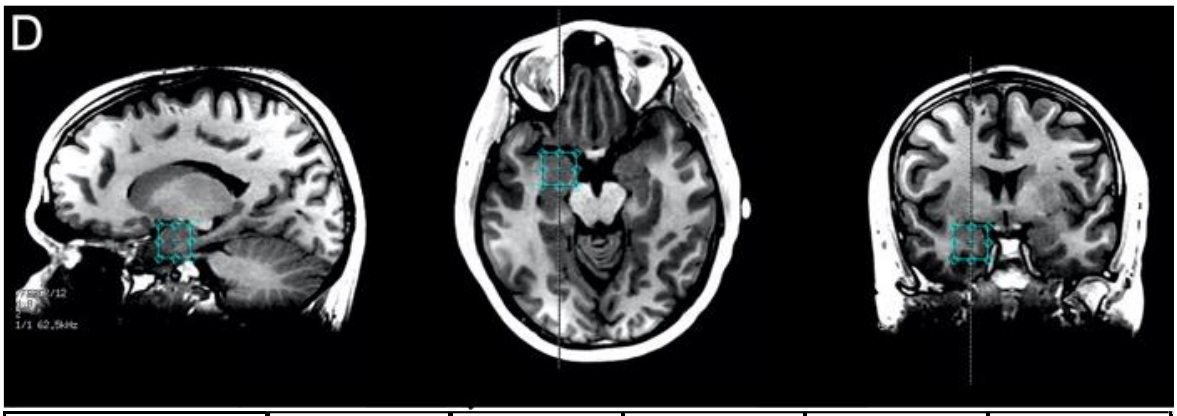


Figure 13. *The figure illustrates the positioning of the voxel in sagittal, axial, and coronal planes. MRS data was collected from the voxel highlighted in blue, and the associated spectrum displaying quantified metabolites is provided. The table includes the mean, standard deviation, and number of participants for each metabolite, with this data also represented graphically. Statistical analysis revealed significant changes only in the glutamate-glutamine marker often combined as Glx (p = 0.0067).*

- ***Amygdala***

The amygdala is a critical structure in the brain that plays a role in processing fear and aggression. While we did not observe any significant changes in the amygdala following HBOT treatment, trend of increased tNAA and Glx are good indicator of positive treatment response trajectory.



Brain Region: Amygdala	myo-inositol			tCho			tCr			tNAA			Glx		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Baseline	6.59	1.28	10	3.60	1.67	4	5.91	1.00	10	7.80	1.02	10	11.48	1.74	10
Post 40 Dives	7.68	1.63	6	4.11	1.35	4	6.46	1.12	6	8.84	2.07	6	12.82	3.22	6

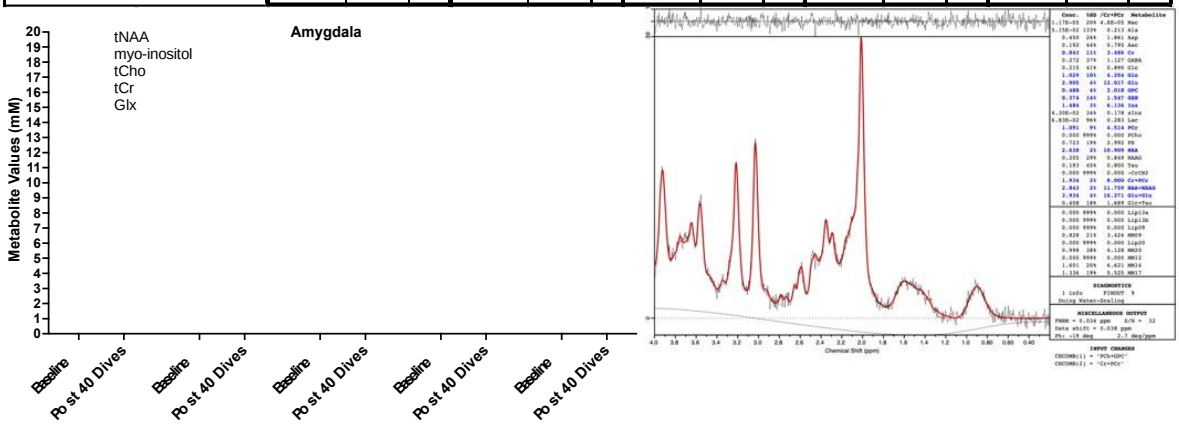


Figure 14. *The figure illustrates the positioning of the voxel in sagittal, axial, and coronal planes. MRS data was collected from the voxel highlighted in blue, and the associated spectrum displaying quantified metabolites is provided. The table includes the mean, standard deviation, and number of participants for each metabolite, with this data also represented graphically. Statistical analysis revealed no significant changes in amygdala.*

- ***tNAA- Neuronal Integrity marker***

An increase in tNAA following HBOT in the brain regions OFC, insula, ACC, and amygdala presents a significant indicator of potential treatment benefits. The similar trends observed in these brain regions suggest that HBOT may facilitate a widespread neuroprotective or neurorestorative effect. By increasing oxygen availability and potentially enhancing mitochondrial function, HBOT could be promoting neuronal recovery and reducing inflammation, which in turn improves overall brain health and functionality. These changes are particularly beneficial in the treatment of conditions like PTSD and TBI, where neuronal damage and dysfunctional neural circuits are prevalent.

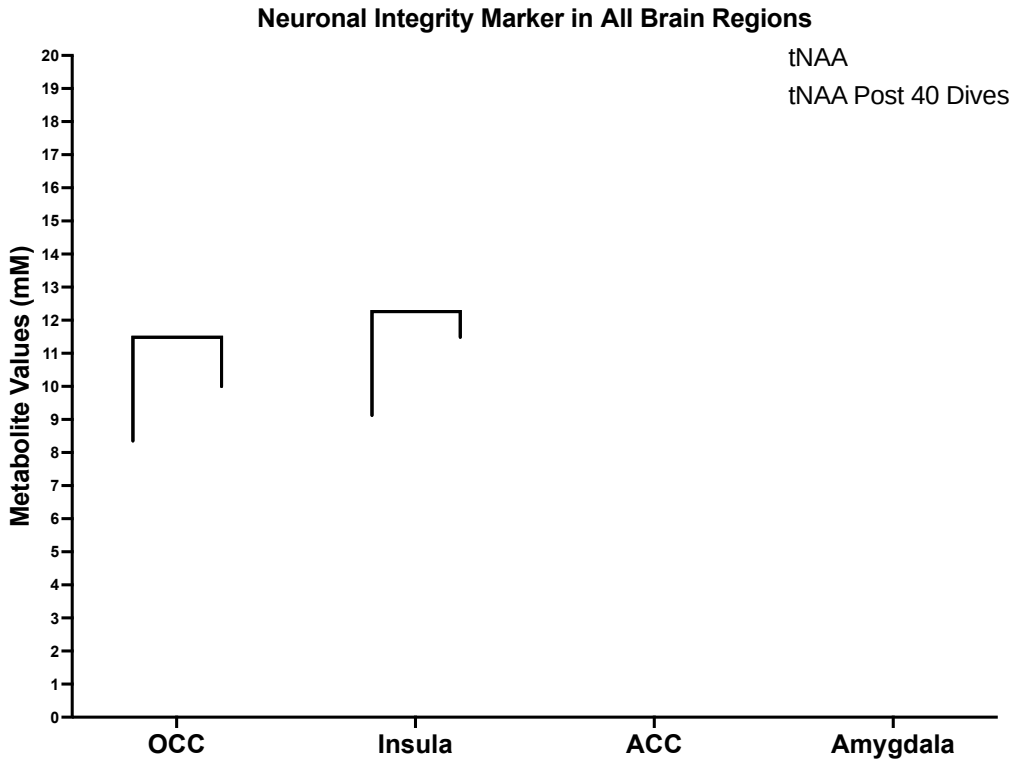


Figure 15. *This figure illustrates the statistically significant changes in the neuronal marker for health integrity, tNAA, in two brain regions: the orbitofrontal cortex (OFC) and the insula. Although no statistical changes were observed in the anterior cingulate cortex (ACC) and the amygdala, an increasing trend was noted in these regions.*

Study limitations

The current study faces several significant limitations that merit careful consideration. First, the small sample size, coupled with the exclusion of female participants, constrains the generalizability of our results. As a pilot study, this research would greatly benefit from an expanded cohort to facilitate a more detailed exploration of the neurometabolic changes prompted by HBOT. Increasing the sample size would not only bolster the reliability of the results but also enhance our understanding of how these changes manifest across a more diverse population.

Second, the absence of a control group in the study design compromises the strength of the conclusions that can be drawn. This limitation introduces potential biases that could affect the interpretation of the efficacy and impact of HBOT. To mitigate this, a supplementary study is required—one that includes a control group consisting of healthy individuals matched by age, gender, and smoking status. This control study would provide a baseline against which the neurochemical alterations observed in the treatment group can be compared, thus clarifying the specific effects of HBOT.

Lastly, while the immediate benefits of HBOT are noted, the durability of these effects over time remains uncertain. There is a pronounced need for extended follow-up studies to determine the long-term sustainability of the therapy's benefits. Such studies would help ascertain whether the improvements noted post-treatment persist and what, if any, long-term care or additional sessions may be necessary to maintain the benefits observed in patients. This aspect is crucial for developing guidelines on the optimal use and duration of HBOT in therapeutic settings.

7 Discussion

Background and Significance

Despite its limitations, this study stands out as one of the initial clinical investigations that utilize MRI metrics, specifically single voxel spectroscopy, to enhance our understanding of neuroplasticity processes. Disabled military veterans, who constitute a vulnerable group with restricted treatment options, often find survey-based research triggering, as observed in our study. This highlights the importance of using objective imaging modalities for drawing reliable conclusions. As noted in the introduction, the absence of quantitative metrics from advanced imaging modalities remains a contentious issue, hindering the broader application of HBOT in treating neurological disorders and neurotrauma. This study conducted meticulous data acquisition and processing, adhering to expert consensus, to explore biochemical processes during and after HBOT. Historically, evaluations of HBOT's efficacy for PTSD have relied on diverse patient survey data. Pursuing an alternative, non-invasive treatment aligns with the current objectives of the VA, which focuses on advancing PTSD research, developing and testing treatments, and striving to prevent PTSD post-trauma through studies ranging from genetic or biochemical bases to evaluations of novel or existing treatments.

In terms of imaging techniques, the debate within the MRS community about the preferred method for single voxel localization persists. However, sLASER was selected for its ability to handle small voxel sizes and target challenging regions effectively. This technique offers single-shot full-intensity signals with precise localization and minimal chemical shift displacement errors, thanks to the high bandwidth adiabatic full-passage pulses that also suppress J-evolution and extend apparent transverse relaxation times (T2). Our application of sLASER, combined with voxel-based static B0 and transmitted B1 calibration routines, has produced neurochemical profiles with high reproducibility at 3T, as evidenced by the high-quality data shown in Figure 4, which includes only two outliers in the amygdala.

The primary findings of this study reveal significant neurometabolic alterations in the OFC and insula, particularly noting an increase in tNAA (a marker of neuronal integrity) following 40 dives. Although limited literature exists on these specific changes, we propose that they may reflect neuroplasticity induced by HBOT. The increase in tNAA could signify improved neuronal metabolism or recovery, which is significant considering the crucial roles of the OFC and insula in regulating emotions and processing socio-emotional information. These metabolic changes may potentially improve cognitive and emotional functions, often impaired in individuals with PTSD and TBI. ***Therefore, these findings provide preliminary evidence that HBOT can induce beneficial neuroplastic changes within key brain regions involved in emotional regulation and social interaction.*** They also suggest that HBOT may help restore neural circuits damaged by trauma, thereby aiding in emotional and cognitive recovery. However, as the existing literature on the specific effects of HBOT on the OFC and insula is limited, our findings pave the way for further research.

This thorough collection and scientific analysis of MRS data not only provide insights into the potential benefits and risks associated with HBOT but also contribute to the developing treatment paradigm for PTSD and mTBI. The positive outcomes noted at the end of therapy underline the promise of HBOT as an effective treatment approach. Further studies are essential to fully assess the long-term efficacy and safety of HBOT for these conditions.

8 Supplementary Material

1. Hardware	
a. Field Strenght [T]	3T
b. Manufacturer	General Electric
c. Model (software if available)	Discovery 750MR
d. RF Coils (nuclei, transmit/receive,number of channels, type, body part)	1H, 32ch head coil, transmit/receive
e. Additional hardware	N/A
2. Acquisition	
a. Pulse sequence	sLASER (Ralph Noeske, PhD)
b. Volume of interest (VOI) locations	Anterior cingulate cortex (ACC), Orbitofrontal cortex (OFC), Insula (ins), Amygdala (amy)
c. Nominal VOI size [mm3]	20 x 20 x 20 mm3
d. Repetition time (TR), echo time (TE) [ms]	35/2000ms
e. Total number of averages per spectrum	64
f. Additional sequence parameters	Datapoints: 2048
3. Data analysis methods and outputs	
a. Analysis software	Osprey v2.4
b. Processing steps deviating from Osprey	N/A
c. Output measure	CSF corrected concentration (mM)
d. Quantification references and assumptions, fitting model assumptions	Basis set list: LCModel:sLASER (CMRR), MM basis functions/soft constraints: LCModel default Fitting method: LCModel v6.3 with DKNTMN = 0.15
4. Data quality	
a. SNR (NAA), linewidth (NAA) [Hz]	See Figure 2. for shimming values
b. Data exclusion criteria	Shapiro-Wilk normality test, Grubbs outlier test, paired ttest, SNR < 3, FWHM > 0.1ppm
c. Quality measures of postprocessing model fitting (Cramér- Rao lower bound)	CRLB <20

Supplemental Table 1. *Summary following minimum reporting standards in MRS. This study has followed all of the recommended consensus papers within the MRS field to ensure validity and allow reproducibility of this study.*

Author	Title
Oz et al. (2014)	Clinical proton MR spectroscopy in central nervous system disorders
Mullins et al. (2014)	Current practice in the use of MEGA-PRESS spectroscopy for the detection of GABA
Wilson et al. (2019)	Methodological consensus on clinical proton MRS of the brain: Review and recommendations
Near et al. (2021)	Preprocessing, analysis and quantification in single-voxel magnetic resonance spectroscopy: experts' consensus recommendations
Lin et al. (2021)	**Minimum reporting standards for in vivo magnetic resonance spectroscopy (MRSinMRS): experts' consensus recommendations**
Cudalbu et al. (2021)	Contribution of macromolecules to brain 1H MR spectra: Experts' consensus recommendations
Choi et al. (2021)	Spectral editing in 1H magnetic resonance spectroscopy: Experts' consensus recommendations
Maudsley et al. (2021)	Advanced magnetic resonance spectroscopic neuroimaging: Experts' consensus recommendations
Meyerspeer et al. (2021)	31 P magnetic resonance spectroscopy in skeletal muscle: Experts' consensus recommendations
Kreis et al. (2020)	Terminology and concepts for the characterization of in vivo MR spectroscopy methods and MR spectra: Background and experts' consensus recommendations
Juchem et al. (2020)	B0 shimming for in vivo magnetic resonance spectroscopy
Kršśák et al. (2020)	Proton magnetic resonance spectroscopy in skeletal muscle: Experts' consensus recommendations
Andronesi et al. (2021)	Motion correction methods for MRS: experts' consensus recommendations
Tkac et al. (2021)	Water and lipid suppression techniques for advanced 1H MRS and MRSI of the human brain: Experts' consensus recommendations
Lanz et al. (2021)	Magnetic resonance spectroscopy in the rodent brain: experts' consensus recommendations

Supplemental Table 2. *This study has followed all of the recommended consensus papers within the MRS field.*

Brain Region	Session	Metabolite																							
OCC		myo-inositol													tCho										
	Baseline	7.165	5.433	6.679	7.424	6.955	5.866	5.422	7.91	5.936	6.899	5.732	6.949		1.56		2.98	1.7	2.73	2.71	1.58	1.9	1.78	2.01	1.76	2.202
	Post 40 Dives	5.056	6.754	5.083	5.785	6.801	5.929	6.133	5.342	4.92	4.81	5.042			2.16	3.5	5.57	2.84	8.45	2.84	2.02	2.72	2.889	3.108	3.765	4.2
Insula																										
	Baseline	5.029	5.613	7.697	5.5	6.977	6.818	7.57	6.204	5.969	5.969	5.402	6.181	5.321	2.52	3.41			3.26		3.2		2.61	2.53	2.22	2.624
	Post 40 Dives	5.082	5.967	4.747	4.113	6.833	5.333	5.923	4.6	4.03	4.65	4.34	4.18	4.21	1.79	2.93	2.68			2.65	2.82	2.79	3.7	3.543	3.21	3.983
Amygdala																										
	Baseline	6.562	7.204	6.069	8.594	4.941	5.39	6.464	8.819	5.861	5.985					5.93	3.57		2.1		2.78					
	Post 40 Dives	10.407	8.155	6.578	8.321	6.06	6.533									6.08	3.46	3.86	3.05							
ACC																										
	Baseline	10.04	8.371	3.848	6.864	7.72	8.374	10.315	7.485							3.16	7.81	3.97	3.57	5.91	2.88	4.6	3.53	2.38	4.2	
	Post 40 Dives	5.95	7.442	7.356	6.991	7.98	5.554	8.921	7.362	8.197	4.502				5.74	2.83	3.13		4.37	5.64	3.04	6.5	3.43	3.463		

Brain Region	Session	Metabolite																							
OCC		tCr													tNAA										
	Baseline	4.906	5.646	5.945	5.06		5.497	5.729	4.474	4.274	4.431	5.033	5.02		7.77	7.96	7.39	8.83	6.29	7.39	8.29	6.3	6.105	6.22	6.07	6.53	
	Post 40 Dives	5.181	5.936	5.692		5.425	5.544	5.691	5.433	6.423	6.433	7.01			7.97	9.64	7.4	10.5	7.8	8.3	7.37	8.3	8.09	8.034	9.544	8.745	
Insula																										
	Baseline	6.182	6.09	4.999	5.901	6.353	5.559	5.315	5.902	6.848	5.387	5.103	5.504	5.492	8.75	8.79	6.77	8.15	10.1	8.17	8.62	7.9	7.431	7.521	7.278	8.113	7.326
	Post 40 Dives	6.283	6.833	6.637	8.885	6.681	6.577	7.349	6.58	6.342	6.738	6.326	7.902	7.002	9.62	9.76	8.6	12.2	9.31	10.6	8.95	7.34	11.3	10.23	8.193	10.2	9.603
Amygdala																										
	Baseline	6.414	6.576	4.834	7.451	5.451	4.822	4.908	7.375	5.862	5.4				8.66	9.72	6.19	7.58	7.67	6.89	7.13	8.75	7.697	7.743			
	Post 40 Dives	8.512	6.651	5.851	5.922	5.282	6.514								12.8	9.33	7.45	8.15	8.06	7.26							
ACC																										
	Baseline	6.244	8.617	9.241	6.353	8.506	5.54	9.057	9.236	8.851	4.777				8.72	11.8	12.3	9.9	13.3	7.93	10.4	9.17	11.15	7.236			
	Post 40 Dives	11.07	9.635	4.712	6.454	9.527	6.815	9.395	8.601						12.7	14.2	5.58	10.2	14.4	7.72	13.4	11	12.18				

Brain Region	Session	Metabolite												
OCC		Glx												
	Baseline	6.179												
	Post 40 Dives	7.153	8.159		9.006	8.697	6.259	8.575	6.755	6.21	6.71			
Insula														
	Baseline	9.354	12.16	7.867	10.79	11.78	9.13	9.867	9.159	11.8	11.51	10.42	10.39	9.677
	Post 40 Dives	10.054	10.37	11.17	13.09	12.85	11.37	10.814	10.34	9.043	8.867	7.935		
Amygdala														
	Baseline	14.562	13.22	11.19	12.52	8.987	12.01	9.74	11.27	9.493	11.8			
	Post 40 Dives	16.963	16.45	9.488	11.96	12.31	9.738							
ACC														
	Baseline	18.883	17.11	8.407	11.26	17.98	14.65	18.373	14.86					
	Post 40 Dives	10.256	15.7	16.08	8.705	15.09	6.744	8.472	12.41	9.648	8.9	15.09		

Supplemental Table 3. *Raw data for each brain region and each participant in the study.*

9 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 practical, including lessons learned that may be useful for planning or conducting future studies of a similar nature. Some practical notes regarding specific but anonymous study participants are also recorded. Please note that any specific opinions expressed are those of the study coordinator and deemed potentially relevant by the Primary Investigator. These opinions do not reflect the scientific conclusions of the Purdue Neurotrauma Group nor the institutional position of Purdue University.

9.1 Study Challenges

The organizational structure, study population, and COVID-19 each impacted the execution and timeline of the pilot study. The following challenges were observed by the Purdue Neurotrauma team for the consideration of future studies.

9.1.1 Recruitment and retention

Despite the small number of participants required for the pilot study, recruitment was difficult. The original HBOT contract included only an HBOT facility at Clark Memorial in Jeffersonville, IN, which created a three-hour drive for participants between Purdue's MRI facility and Clark's HBOT chamber. This distance made commitment to the MRI scan protocol difficult for participants. Additionally, Clark Memorial's location in a relatively low-density area complicated remote recruitment efforts, despite newspaper releases and posted flyers at local veterans' organizations and hospitals. Recruitment improved significantly after HBOT facilities in the Indianapolis region were secured by IDOH. In total, 29 individuals started the program by attempting the first MRI

scan, and 13 completed all forty dives.

9.1.2 Participant Retention

The study encountered substantial obstacles in maintaining participant retention, largely due to logistical challenges from the COVID-19 pandemic and the considerable geographic distances between participants' homes and the HBOT clinics associated with Purdue. These factors increased the risk of participant attrition despite efforts to mitigate issues through travel reimbursements, flexible scheduling, and regular phone call check-ins. The study population, primarily veterans with PTSD, added another layer of complexity. The frequent mood fluctuations and varying mental health states inherent to PTSD could unpredictably affect participants' ability to consistently engage with the study. These psychological variables influenced the overall progress of the research and significantly impacted retention rates. This combination of logistical and psychological challenges necessitated a highly adaptive approach to participant management and study execution, underscoring the need for tailored strategies to support this vulnerable group throughout the research process.

9.1.3 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 out of the study due to stress triggers in the MRI machine, and several participants required ear tubes to continue with the dives.

9.2 Considerations for Future Studies

9.2.1 Centralized Contracting

Future studies could benefit from centralizing coordination and accountability by creating subcontracts for HBOT from the lead coordinating organization. This would help centralize issues for participants as they arise. For example, in the current scheme, individuals must 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.

9.2.2 Mental Health Management

To enhance the effectiveness of HBOT protocols for PTSD, it is crucial to incorporate a centralized mental health professional within the treatment framework. This specialist will assess the therapeutic benefits and monitor the overall mental well-being of all study participants to prevent triggers and episodes like those observed during CNSVS survey collection. Integrating professional oversight will maximize participant safety and the success of the HBOT treatment.

10 Acknowledgement

This report was produced on April 22, 2024, and has been drafted for the Indiana Department of Health (IDOH) and the Indiana State Legislature. We would like to thank IDOH personnel, all of the HBOT providers and subcontractors, General James Bauerle, and Purdue University for their support.

11 References

1. B, Newberg A. Neuroimaging in traumatic brain imaging. *NeuroRx*. 2005;2(2):372-383. doi:10.1602/neurorx.2.2.372
2. Byrnes KR, Wilson CM, Brabazon F, et al. FDG-PET imaging in mild traumatic brain injury: A critical review. *Front Neuroenergetics*. 2014;6(JAN). doi:10.3389/fnene.2013.00013
3. Patel JB, Wilson SH, Oakes TR, Santhanam P, Weaver LK. Structural and volumetric brain MRI findings in mild traumatic brain injury. *American Journal of Neuroradiology*. 2020;41(1):92-99. doi:10.3174/ajnr.A6346
4. Veterans Brain Injury Center. DoD numbers for traumatic brain injury worldwide-totals. Published online 2019:0-4.
5. Center VBI. DoD numbers for traumatic brain injury worldwide-totals. Published online 2019:0-4.
6. *Evaluation of the Disability Determination Process for Traumatic Brain Injury in Veterans*.; 2019. doi:10.17226/25317
7. VanItallie TB. Traumatic brain injury (TBI) in collision sports: Possible mechanisms of transformation into chronic traumatic encephalopathy (CTE). *Metabolism*. 2019;100S:153943. doi:10.1016/j.metabol.2019.07.007
8. Koenen KC, Ratanatharathorn A, Ng L, et al. Posttraumatic stress disorder in the World Mental Health Surveys. *Psychol Med*. 2017;47(13):2260-2274. doi:10.1017/S0033291717000708
9. Karl A, Werner A. The use of proton magnetic resonance spectroscopy in PTSD research-Meta-

analyses of findings and methodological review. *Neurosci Biobehav Rev*. 2010;34(1):7-22. doi:10.1016/j.neubiorev.2009.06.008
10. Steenkamp MM, Litz BT, Hoge CW, Marmar CR. Psychotherapy for military-related PTSD: A review of randomized clinical trials. *JAMA - Journal of the American Medical Association*. 2015;314(5):489-500. doi:10.1001/jama.2015.8370
11. Marmar CR, Schlenger W, Henn-Haase C, et al. Course of posttraumatic stress disorder 40 years after the Vietnamwar findings from the national Vietnam veterans longitudinal study. *JAMA Psychiatry*. 2015;72(9):875-881. doi:10.1001/jamapsychiatry.2015.0803
12. Weaver LK, Wilson SH, Lindblad AS, et al. Hyperbaric oxygen for post-concussive symptoms in United States military service members: A randomized clinical trial. *Undersea and Hyperbaric Medicine*. 2018;45(2):129-156. doi:10.22462/03.04.2018.1
13. Hachmo Y, Hadanny A, Abu Hamed R, et al. Hyperbaric oxygen therapy increases telomere length and decreases immunosenescence in isolated blood cells: a prospective trial. *Aging*. 12(22):22445-22456. doi:10.18632/aging.202188
14. Boussi-Gross R, Golan H, Fishlev G, et al. Hyperbaric oxygen therapy can improve post concussion syndrome years after mild traumatic brain injury - Randomized prospective trial. *PLoS One*. 2013;8(11). doi:10.1371/journal.pone.0079995
15. Efrati S, Fishlev G, Bechor Y, et al. Hyperbaric Oxygen Induces Late Neuroplasticity in Post Stroke Patients - Randomized, Prospective Trial. *PLoS One*. 2013;8(1). doi:10.1371/journal.pone.0053716
16. Weaver LK, Wilson SH, Lindblad AS, et al. Hyperbaric oxygen for post-concussive symptoms in United States military service members: A randomized clinical trial. *Undersea and Hyperbaric Medicine*. 2018;45(2):129-156. doi:10.22462/03.04.2018.1
17. Keim LW, Koneru S, Ramos VFM, et al. Hyperbaric oxygen for late sequelae of carbon monoxide poisoning enhances neurological recovery: Case report. *Undersea and Hyperbaric Medicine*. 2018;45(1):83-87. doi:10.22462/01.02.2018.11
18. Miller RS, Weaver LK, Bahraini N, et al. Effects of hyperbaric oxygen on symptoms and quality of life among service members with persistent postconcussion symptoms: A randomized clinical trial. *JAMA Intern Med*. 2015;175(1):43-52. doi:10.1001/jamainternmed.2014.5479
19. Doenyas-Barak K, Kutz I, Lang E, et al. The use of hyperbaric oxygen for veterans with PTSD: basic physiology and current available clinical data. *Front Neurosci*. 2023;17:1259473. doi:10.3389/fnins.2023.1259473
20. Hayes JP, Hayes SM, Mikedis AM. Quantitative meta-analysis of neural activity in posttraumatic stress disorder. *Biol Mood Anxiety Disord*. 2012;2(1):9. doi:10.1186/2045-5380-2-9
21. Doenyas-Barak K, Catalogna M, Kutz I, et al. Hyperbaric oxygen therapy improves symptoms, brain's microstructure and functionality in veterans with treatment resistant post-traumatic stress disorder: A prospective, randomized, controlled trial. *PLoS One*. 2022;17(2):e0264161. doi:10.1371/JOURNAL.PONE.0264161
22. Gruetter R. Automatic, localized in Vivo adjustment of all first-and second-order shim coils. *Magn Reson Med*. 1993;29(6):804-811. doi:10.1002/mrm.1910290613
23. Juchem C, Cudalbu C, de Graaf RA, et al. B0 shimming for in vivo magnetic resonance spectroscopy: Experts' consensus recommendations. *NMR Biomed*. 2020;(May):1-20. doi:10.1002/nbm.4350
24. Zhu H, Barker PB. MR Spectroscopy and Spectroscopic Imaging of the Brain. *Methods in Molecular Biology*. 2011;711:203-226. doi:10.1007/978-1-61737-992-5_9
25. Wilson M, Barker PB, Bolan PJ. Spectroscopy of the Brain : Review and Recommendations. 2020;82(2):527-550. doi:10.1002/mrm.27742.A
26. Öz G, Alger JR, Barker PB, et al. Clinical proton MR spectroscopy in central nervous system disorders. *Radiology*. 2014;270(3):658-679. doi:10.1148/RADIOL.13130531
27. Hwang JH, Choi CS. Use of in vivo Magnetic resonance spectroscopy for studying metabolic diseases. *Exp Mol Med*. 2015;47(2):e139-8. doi:10.1038/emm.2014.101

28. Gualtieri CT, Johnson LG. Reliability and validity of a computerized neurocognitive test battery, CNS Vital Signs. *Archives of Clinical Neuropsychology*. 2006;21(7):623-643. doi:https://doi.org/10.1016/j.acn.2006.05.007
29. Oeltzschner G, Zöllner HJ, Hui SCN, et al. Osprey: Open-Source Processing, Reconstruction & Estimation of Magnetic Resonance Spectroscopy Data. *J Neurosci Methods*. 2020;343:108827. doi:10.1016/J.JNEUMETH.2020.108827
30. Near J, Harris AD, Juchem C, et al. Preprocessing, analysis and quantification in single-voxel magnetic resonance spectroscopy: experts' consensus recommendations. *NMR Biomed*. 2021;34(5). doi:10.1002/NBM.4257