Protocol

Hyperbaric Oxygen Therapy for Post Traumatic Stress Disorder - a Pragmatic, Double Blinded Randomized Trial

Primary Investigator and physician in charge: Dr. **Carmel Kalla**

This study will be conducted at the Israeli Naval Medical Institute, Haifa.

Box 22, Rambam Healthcare Campus, P.O. 9602, Haifa 3109601

Name	**Degree**	**Role**	**Telephone**	**E-mail**	**Signature**
Carmel KALLA	MD	Primary Investigator, Head of Clinical Psychiatric Unit, IDF-MC	058-6139999	carmelkalla@gmail.com	
Orly KNOLL	MD, MHA	Co-investigator, Head of INMI	050-2061629	orlyrofe@gmail.com	
Dror OFIR	PhD	Co-investigator,	052-9280655	drorof@gmail.com	
Ivan GUR	MD	Co-investigator,	054-2555655	ostyly@gmail.com	
Yinnon MATSLIAH	MD	Co-investigator	052-9455428	ymatsliah@gmail.com	

Abstract

Post-traumatic stress disorder (PTSD), affecting approximately 6% of the general population and up to one-third of individuals exposed to combat zones and disasters, is a significant contributor to morbidity and mortality among IDF personnel. Hyperbaric oxygen therapy (HBOT), in which patients breathe oxygen at a partial pressure higher than 1 atmosphere in a hyperbaric chamber, has been investigated in the context of treating a wide range of neuropsychiatric disorders, including PTSD and mild traumatic brain injury (mTBI). Four controlled studies conducted in patients with mTBI, about half of whom also suffered from PTSD, have yielded conflicting conclusions regarding the potential efficacy of hyperbaric therapy. A single study involving approximately 30 patients with PTSD without mTBI demonstrated significant clinical improvement; however, it was characterized by several methodological limitations—chief among them the absence of blinding or a placebo control. None of the studies conducted to date have reported long-term findings (beyond one year), included patients with a short duration of symptoms (“early PTSD”), or included female participants. The proposed study aims to conduct a prospective, double-blind, controlled investigation of the biological effect of hyperbaric therapy in PTSD, continuously throughout the hyperbaric treatment course, at the end of treatment, and during a substantial follow-up period of two years after treatment completion. We intend to include adult participants who are capable of providing informed consent and who meet DSM-5 diagnostic criteria for PTSD. To maintain a pragmatic study with high external validity, exclusion criteria will be limited to those indicating risk (concrete suicidality, or a history of manic or psychotic disorder) or factors likely to impair treatment efficacy (incompatibility with hyperbaric chamber treatment, inability to complete the full treatment protocol, or pregnancy). Participants who miss a substantial number of treatments (five consecutively or one-third of the total treatments) will be withdrawn from the study.

The primary outcome measure will be the CAPS-5 questionnaire. In addition, PTSD symptom questionnaires, surveys assessing cognitive, executive, and affective functioning, and health-related quality of life will be administered. An exploratory outcome will focus on sleep quantity and quality and physiological monitoring using wearable devices, currently considered the most promising biomarker in the context of PTSD. Following enrollment and the provision of informed consent, balanced randomization will be performed with respect to covariates previously described as potential confounders (such as age, duration, and severity of symptoms, …). Participants will receive 60 hyperbaric treatments, five days per week, at either 2.0 atmospheres or 2.5 atmospheres. Both the participants and the evaluating clinical staff will be blinded to treatment allocation.

Background

Post Traumatic Stress Disorder

Post Traumatic Stress Disorder is a significant public health concern.(1) Affecting over 6% of the general population, lifetime prevalence may be as high as 33% in high risk populations - including veterans, combat zone residents and areas affected by natural disasters. (2) Manifested clinically as significant hyperarousal, hypervigilance, intrusive thoughts and flashbacks, and a myriad of sleep disturbances, PTSD is an important cause of morbidity and mortality. (3) Comorbid affective disorders reach a prevalence of over 50%. Over 20% of patients with PTSD have concomitant substance use disorders. Suicidal ideation is reported in as many as 50% of persons struggling with PTSD. Death by suicide is at least twice as common in individuals with PTSD compared to matched controls. Disability and loss of income affect over 90% of patients. (4) Despite several well researched treatment approaches, including various psychotherapeutic interventions aided by pharmacotherapy and biofeedback, the prognosis of PTSD remains poor. (5) The economic burden of PTSD exceeds 232 billion $ annually in the United States alone. (6) The risk of PTSD in Israel is estimated to increase three- to ten-fold following the attack of October 7th, 2023.(7)

Disruptions in frontolimbic circuits have been stipulated as important culprits in PTSD pathogenesis. Monoaminergic dysregulation in the infralimbic cortex is accepted to be a core mechanism in PTSD pathogenesis. In an animal model (8) HBO therapy restored hypothalamus-pituitary-adrenal axis activity in the infralimbic cortex in a rat model. Thioreduxin reductase has been shown to be deregulated in the hippocampus of single prolonged stress exposed rats.(9)

Hyperbaric Oxygen Therapy

Defined as the administration of breathing oxygen at partial pressures exceeding 1 atm, hyperbaric oxygen (HBO) therapy has been attempted in various neurological and psychiatric impairments.(1,10,11) Hyperoxemia, with PaO_2 as high as 1800mmHg under 3.0 ATA of pure

O_2, is the most important mechanistic path by which HBO alters physiology.(12) Hyperoxemia leads to hyperoxic vasoconstriction, increased tissue oxygen concentration (up to 400 mmHg) and decreased reactive oxygen species (ROS) formation. Alternating between normal and supraphysiological PO_2 has been shown to induce local transcriptional, paracrine and systemic changes, modulating mitochondrial and cellular function and promoting the release of hypoxia inducible factors (HiFs). (1)

While generally safe, potential complications can be divided into those originating in pressure shifts, and those attributed to increased PO_2. As a consequence of Boyle's law, changing the pressure gradient on a confined gas will result in a proportional change in its volume. Thus, gas pockets that are not fully ventilated may experience volume shifts during the inherent pressure shifts in the hyperbaric chamber. This will result in considerable shear forces, often resulting in tissue damage, also known as barotrauma. Ear barotrauma is most often reported, at the rate of 1-5%. Sinus barotrauma is reported at 0.5-0.6%, with no serious cases of lung barotrauma or air embolization reported in any of the currently available elective HBO therapy literature.(1,13) The increased PO_2 may result in CNS oxygen toxicity (COT), often manifested as myoclonus, visual or other focal neurological impairments, and seizures. Reported incidence associated with elective HBO therapy is estimated below 0.5:10,000 treatments. All of these potential adverse events are fully reversible.(12)

HBO Therapy for PTSD

The majority of prospectively randomized studies were conducted in subjects with mild traumatic brain injury (mTBI), in whom PTSD was reported in 30-60% of cases. (1) A recent pooled estimate of 4 RCTs (11,14) (15) (16) totaling 252 patients found no significant effect of HBO therapy compared with sham (PCL Md of 0.61, 95% CI [−7.75, 8.96], p = 0.38). (1) The only trial reporting outcomes over 1 month after HBO therapy termination (14) reported no significant difference between the HBO therapy and the sham control groups at six months (PCL Md 4.1, 95%C.I.[-11.9, 3.7], p=0.29). This is in stark contrast with the significant improvement observed at the end of the 60 consecutive HBO therapy sessions (PCL Md of

12.3, 95%C.I.[-21.4,-3.1], p=0.01). At 12 months, PCL scores were actually worse in both groups (mean PCL 5.8 (±11.8) higher than baseline in the HBO therapy and 5.8 (±13.3) in the sham control, p=0.99). Longer (2 and 3 years post treatment termination) follow up was conducted in a small subgroup (40% and 14% of the original cohort, respectively). In another study focused on patients with fibromyalgia and childhood sexual assault, somatization, anxiety and depression levels reduced by 50% following 60 daily treatments compared to no change in controls receiving psychotherapy alone.(10)

To our knowledge, only one RCT thus far focused on PTSD patients with no mTBI. (17) Of 35 patients allocated 1:1 to 60 daily HBO therapy sessions at 2.0 ATA for 90 minutes each, 14/18 completed the HBO therapy (three removed due to problems equalizing, one opted out after 20 sessions) and 15/17 completed follow up of no sham control. While Clinician-Administered PTSD Scale (CAPS-5) scores were similar at baseline (46.6±11.5 vs 49.5±10.7), a significant reduction was observed after 12 weeks of therapy (28.5±17.4 vs 51.5±8.4, group by time ANOVA F=30.6, p<0.001). Significant improvements were also demonstrated in BSI and BDI scores (F = 5.72, p = 0.024 and F = 7.65, p = 0.01). Twenty two (78.6%) of the original 28 patients completing HBO therapy in this study were re-evaluated a median of 704 (±230) days after treatment termination. Mean CAPS-5 scores were unchanged compared to those obtained immediately after treatment (26.6±14.4 vs 28.6±16.7, p = 0.745) and both were significantly lower than pretreatment scores (47.5±13.1, p<0.001) (18).

A safety concern unique to PTSD is the potential to aggravate symptomatology during treatment. Observational data collected during the above mentioned study (19) suggests surfacing of new trauma related memories is not uncommon, occurring in 35.7% of patients after 30.5±13.2 treatments on average. No increased risk of suicide ideation or behaviour was recorded in this study.

Mild Traumatic Brain Injury

Mild traumatic brain injury (mTBI) is often defined as the occurrence of neurological symptoms (including a brief loss of consciousness) following a trauma to the head, without significantly prolonged decrease in consciousness (Glasgow Coma Scale of 13-15) or amnesia (if present, shorter than 24 hours). (11) A major concern in military medicine, mTBI is a complication of up to 30% of battlefield injuries. (20) Co-occurrence with PTSD is common: as many as 10-20% of veterans with PTSD have mTBI, and up to 50% of those suffering from mTBI develop PTSD. Phenotypically similar to PTSD, mTBI can be complicated by prolonged post-concussion symptoms, including headaches, cognitive impairments, affective disorders and sleep disturbances. (21) A recent meta-analysis of participant level data from four studies (totaling 254 patients) reported a significant improvement in verbal memory (CVLT-II Md of 3.8; 95% CI [1.0, 6.7], p=0.01), a marginally significant improvement in the PTSD checklist score (Md -2.7 points, 95% CI [-5.8, 0.4], p=0.09), and a trend towards improvement in the Rivermead Postconcussion Symptoms Score (Md -2.3, 95% CI [-5.6, 1.0], p=0.18). (21)

Well described in the treatment of mTBI, animal studies demonstrated HBO therapy to improve tissue oxygenation, induce stem cell proliferation and decrease inflammation. (22) Case series in humans have demonstrated improved cerebral blood flow and decreased markers of CNS inflammation. (4)

Limitations of current knowledge

Isolating the true biological effect of HBO on PTSD has been the cornerstone of the scientific controversy surrounding the role of HBO therapy in psychiatry. Namely, neutralizing participation and placebo effects on the one hand, while providing no increased pressure or PO_2 on the other, has been the main limitation of studies conducted thus far. Sham protocols that involve hyperbaric air exposure result in increased PO_2 (albeit usually <1.0 atm).(14,15) In a single study using hypoxic (10.5% O_2) nitrox at 2.0 ATA,(16) there was still exposure to increased ambient pressure. Thus, proponents of HBO explain the lack of effect observed in these studies to be the result of unjustified exposure to hyperbaric conditions in the control group.(23)

Conversely, the effect reported in studies opting to avoid any hyperbaric chamber-setting exposure in the control group could be easily attributed to participation and placebo effects. (1) In this approach, neither participants nor observers can be reasonably blinded. Beyond these effects, the behavioral modifications inherent to the intervention (but not the passive control) group - i.e. attending daily treatments, the strong and intense interpersonal interaction during 2-3 hours of treatment (with other patients and the chamber attendant) daily, completing questionnaires and assessments, etc - may have a direct effect on PTSD symptoms. Finally, the selection of patients proving to be able to complete a full HBO course (typically 40-60 daily encounters), not applied in the control group, can be an important source of bias when gauging the effect of HBO in passively controlled PTSD studies. These concerns are further supported by the only RCT examining both sham (1.2 ATA of air) and no hyperbaric intervention controls in patients with mTBI: improvement was similar in both the HBO and sham groups, both superior to the no hyperbaric intervention group.(15)

Beyond these cardinal concerns, previous studies have been very restrictive in including relatively healthy, chronic (>1-4 years duration), and overwhelmingly male patients. There have been no head to head comparison of different treatment protocols and HBO dosing thus far.

Goals

Our primary goal is to examine the effect of HBO therapy dose on PTSD symptoms.

Specific Secondary Aims

- Assessing the long-term effects of HBO in PTSD
- Examining the effect modification of mTBI on the therapeutic effect of HBO in PTSD
- Describing in detail trends in PTSD symptoms throughout the treatment period (as opposed to before and after assessments only), thus assessing the optimal duration of therapy.

Study Hypotheses

- Symptoms of PTSD will reach peak improvement following 40-60 HBO treatments compared to sham pressurized controls
- This improvement will wane over the coarse of the ensuing 2 years of follow up, with the mean difference of PTSD measurements (e.g. CAPS-5 scores) decreasing.
- The improvement will be more significant in patients with a shorter duration of symptoms
- The improvement will be more significant in patients suffering from concomitant mTBI
- The improvement will reach a plateau after about 40 treatments

Materials and Methods

Study Design

This is a double blinded, prospectively randomized, controlled, pragmatic study. The pragmatic design allowing for a myriad of additional therapy approaches, and in particular - stepping up or down pharmaco- and psycho- therapy during the trial, as well as widely applicable inclusion and exclusion criteria, aims to answer previous concerns raised of the external validity of contemporary clinical HBO research. (20)

Population and Setting

Patients referred by the Combat Reactions Unit or the Rehabilitation Division at the Ministry of Defense for hyperbaric treatment evaluation at the Israeli Naval Medicine Institute (INMI) from September 1st, 2024, until December 31st, 2027, will be considered for this study. A dedicated advertisement will be placed at military treatment centers, as outlined in Appendix B and form 10 adjacent to this protocol. Only potential participants calling the investigator at their own discretion will be approached. After completing a fitness for hyperbaric chamber evaluation and provided inclusion and exclusion criteria are met, patients will be offered to participate in the study. An investigator that is not part of the treating medical team will offer an in-depth explanation of the existing knowledge, possible side effects and the right to refuse participation

before and at any moment during the study. Reasonable time (at least 12 hours) will be given for pondering and additional questions, before signing an informed consent. The investigator providing explanations and requesting informed consent will not be a member of the treatment team and will not wear any attributes of rank or insignia during the entire explanation and informed consent process.

Inclusion Criteria

- Age over 18 years and up to 80 years
- Ability to understand the researcher's explanation and give informed consent.
- Of any sex or gender (unlike previous studies, a significant effort will be made to include women in this study)
- A diagnosis of PTSD according to DSM-5 criteria with a severity that warrants discharge from military service.

Exclusion Criteria

- The lack of ability to attend 120 daily (5 times/week) treatments at the INMI
- Current or past psychotic disorder
- Evidence of active suicidal ideation
- Evidence of past or present manic disorder
- Difficulty equalizing pressures, including pulmonary emphysema, significant sinusoidal obstruction, eustachian tube dysfunction, or an pneumothorax that is not drained.
- Known or suspected pregnancy.

Primary Outcome

- The change in the Clinician-Administered PTSD Scale (CAPS)-5 score at the end of the treatment series compared to the baseline score

Secondary Outcomes

- The change in the CAPS-5 score two years after inclusion in the study relative to the baseline score
- Changes in the Beck's Depression Inventory (BDI) score
- Changes in the score of the Pittsburgh Sleep Quality Questionnaire (PSQ)
- Changes in the State Trait Anxiety questionnaire score
- Changes in the post-traumatic disorder score in the CAPS-5 continuously over time
- Changes in the Basic Symptoms Inventory (BSI)
- Changes in Executive Function Questionnaire (BRIEF-A)

Exploratory Outcomes

- Sleep continuity index
- Average blood pressure while awake and asleep over 24 hours
- Psychomotor Vigilance Test (PVT)

Research Site

This research will be conducted at the Israeli Naval Medicine Institute (INMI), Israeli Navy, Haifa.

Study Duration

Enrollment over two years (1.9.2024-31.12.2027), follow-up for another two years, data analysis, processing and publication for up to one year. A total of five years.

Equipment and Materials

The treatment will be carried out in a HAUX STARMED multi-place pressure chamber (by HAUX LIFESUPPORT SYSTEMS, Cuxhaven, Germany) containing three sections (12+2+6 places, respectively). Ambulatory blood pressure measurement will be performed by an ambulatory blood pressure monitor (ABPM) model F11 (by SUNTECH). Measurement of sleep continuity and physiological monitoring will be performed using a combined pulse-oxymetry and accelerometry wearable device such as the Oura ring (Oulu. Finland).

Detailed Research Plan

Week (-3) to 0: PRE ENROLLMENT

- Review of the electronic healthcare registry (EHR) by the primary investigator
- Eligibility check (inclusion and exclusion criteria)
- A designated investigator will approach the patient by phone and offer a general description of the study procedure and duration, as well as clearly stating the voluntary nature of the study and the ability to refuse or withdraw consent at any point
- Office visit #1, during which a detailed explanation of the study procedure, the ⅓ probability of sham treatments necessitating a further 60 therapeutic sessions, and potential side effects (including barotrauma and CNS oxygen toxicity) will be offered. In addition a pressure-chamber fitness test (including full medical history, physical examination and otoscopy during valsalva) will be completed.
- The patient will be offered at least 12 hours before signing informed consent, during which time a full copy of the informed consent, as well as a telephone number of the explaining investigator (with an invitation to ask any further questions)
- Obtaining a written and signed informed consent by an investigator that is not a part of the treatment team
- Childhood Trauma Questionnaire (STQ)
- Structured Clinical Interview for DSM-5 (SCID)
- A roll in (baseline) physiological monitoring (including ABPM and sleep monitoring)

week 0: randomization

- Randomization at a ratio of 1:1 (2.0_{ATA}:2.5_{ATA}). The randomization will be performed using the Frane algorithm (24,25) For random allocation, covariate adaptive randomization (CARAT) will be used in the R software,(26) taking into account covariates that have previously been associated with the likelihood of improvement in post-traumatic stress disorder,(1) including:
  - Duration of symptoms
  - Age

○ Severity of baseline functioning (baseline CAPS-5 score)

○ The presence of additional psychiatric morbidity, including the use of addictive substances

○ Presence of mTBI

○ The baseline index of the primary and secondary outcome questionnaires (CAPS-5, BDI, PSQ, STA, PCL-5, BSI, BRIEF-A)

○ Potential competing therapies, including the following:

PTSD specific therapy	Collected (and balanced) parameters
Conversational therapy	Primary approach (CBT, psychodynamic, mixed), frequency (N of sessions/month)
Pharmaco - therapy	Drug class (SSRI, SNRI, TCA, Cannabinoids, Hallucinogens, Other), daily dose, duration of therapy
Experimental therapy	E.g. vagal stimulation (date, proceedure preformed)

Week 1-12: Daily hyperbaric treatment (five treatments per week)

● HBO 10 = 2.0_{ATA} Treatment group: compression to 2.0 ATA for up to fifteen minutes, breathing pure oxygen for 45 minutes, breathing air for 5 minutes, breathing pure oxygen for 45 minutes, decompression with a safety stop for 20 minutes. The choice of 2.0 ATA peak PO_2 was made in view of previously raised theoretical concerns of cerebral hypoxic vasoconstriction at higher PO_2s,(23) and the fact this was the peak pressure chosen in the only PTSD oriented RCT thus far.(17)

● HBO 15 = 2.5_{ATA} Treatment group: compression to 2.0 ATA for up to fifteen minutes, breathing pure oxygen for 45 minutes, breathing air for 5 minutes, breathing pure oxygen for 45 minutes, decompression with a safety stop for 20 minutes.

- Weekly measurement: asking each patient “are you in a treatment group?”, “what pressure do you think you have reached?”, as well as physiological (ABPM and sleep) monitoring
- Bi-weekly measurement (every ten treatments): CAPS-5, BDI, PSQ, STA
- Measurement at the beginning and end of the period only: BRIEF-A, EQ-5D

Week 26: Long-term outcome 1

For all participants: the main and secondary outcome questionnaires (CAPS-5, BDI, PSQ, STA, PCL-5, BSI, BRIEF-A), Physiological monitoring (ABPM and sleep), full medication history, PVT, PrePulse Inhibition test.

Week 52: Long-term outcome 2

For all participants: the main and secondary outcome questionnaires (CAPS-5, BDI, PSQ, STA, PCL-5, BSI, BRIEF-A), Physiological monitoring (ABPM and sleep), full medication history, PVT, PrePulse Inhibition test.

Week 78: Long-term outcome 3

For all participants: the main and secondary outcome questionnaires (CAPS-5, BDI, PSQ, STA, PCL-5, BSI, BRIEF-A), Physiological monitoring (ABPM and sleep), full medication history, PVT, PrePulse Inhibition test.

Week 104: Long-term outcome 4 and study completion

For all participants: the main and secondary outcome questionnaires (CAPS-5, BDI, PSQ, STA, PCL-5, BSI, BRIEF-A), Physiological monitoring (ABPM and sleep), full medication history, PVT, PrePulse Inhibition test.

Removal Criteria (any of the following):

- As per the subject’s request, at any point, for any (or no) reason whatsoever.
- Loss of more than five consecutive treatments for any reason
- Loss of more than 20 cumulative treatments for any reason

- CNS oxygen toxicity

Prespecified Subgroup Analysis

Expansive inclusion criteria and a pragmatic research design are our guiding principles, as we believe this approach to result in externally valid and clinically impactful results. At the same time, a separate analysis of the following a priori defined subgroups will expand our understanding of the effect of hyperbaric oxygen in specific subtypes of PTSD:

Duration of Symptoms: There is much evidence, both in animal models and in observational studies in humans, regarding the substantial difference in neuropathophysiology in young stroke patients versus patients with chronic PTSD. The definitions in the literature are not consistent, but similar to several previous studies we will define the subgroup of early PTSD as patients who started hyperbaric treatment less than a year from the onset of symptoms, and chronic PTSD as the duration of symptoms over 4 years before the start of hyperbaric treatment.

Mild Traumatic Brain Injury (mTBI): Previous studies have shown significant improvement in traumatic brain injury symptoms under hyperbaric therapy. In view of this evidence, we anticipate that a possible therapeutic effect of hyperbaric oxygen will be increased in patients with concomitant TBI.

Statistical Analysis

Standard descriptive statistics will be used to summarize population characteristics. Comparing pre and post treatment values, as well as between groups (2.0_{ATA} vs. 2.5_{ATA}, subgroups) we will use a chi-square test for categorical variables, Mann-Witney U test for nonparametric variables and student's unpaired t-test for normally distributed continuous variables. Tukey's correction was applied when applicable to adjust for multiple comparisons. Categorical variables will be described using proportions and percentages, non-parametric variables with median and interquartile range (IQR) and normally distributed continuous variables as mean with standard deviation (SD). A 2-sided $Pv<0.05$ will be considered statistically significant for all tests. A mixed model for repeated measures will be constructed for the primary, as well as every of the

secondary and exploratory outcomes. Included will be covariates found to significantly predict the outcome at question in a univariate analysis. All calculations will be performed using R version 4.4.1.

Sample Size and Power

Based on previously published data, we estimate the primary outcome (CAPS-5 score) to have a standard deviation of around 12 points. In view of previously published results and our hypothesis as detailed above, we will not assume homoscedasticity. Given the lowest effect reported thus far in a positive study was a mean difference of 12 CAPS-5 points,(18) and in line with the accepted definition utilized in most previous studies,(18) we will define a clinically significant imrovement at >10 CAPS-5 points. Aiming to maintain a power of at least 80% to detect such a difference at an alpha of <0.05 and a 1:1 allocation our minimal estimated sample size is 38 participants in the intervention group and 19 participants in the control group. Allowing for the previously reported 70% participation and an additional ~20% attrition rate, numbers that are consistent with our own experience (e.g. treating acute acoustic trauma), we will need to approach at least 102 patients during the study period. A full monte carlo based code for these calculations is provided in Appendix A. (17)

Data Handling

All data collection will be carried out solely by the investigators outlined below. A designated clinical research form (CRF) using microsoft excel will be created to handle all data collected. All identified data will be saved on a designated unclassified (בלמ"ס) password protected computed. Access will be restricted to the abovementioned investigators. The CRF will be encrypted by a 128 bit RAS protocol. Removing all identifiers (including dates - to be replaced by time intervals, names, addresses, ID, military ID and phone numbers) and coding the information will be performed by Mj. Dror Ofir, PhD, who'll be keeping the coding key. Handling coded information will be performed on a separate, unclassified, password protected and designated computer, access to which will be restricted to investigators handling data analysis as detailed below.

Data Visualization

We expect the following key metrics to be collected. Tables hereby presented are a rough, preliminary estimate of the data we strive to collect.

Table 1 - Basic Cohort Characteristics

Variable	Intervention N=2x	Control N=x	P-value

Age (Mean, SD)
Females (N, %)
Background Medical History (N, %)
  Asthma
  Allergic rhinitis
  Smoking
  Hypertension
  Dyslipidemia
  Siezures
Chronic Medication (N, %)
  Antihypertensives
  Lipid Lowering Drugs
  GLP-1 or glucose lowering
  Anticonvulsants			

Psychiatric History
  Duration of PTSD Symptoms (median, IQR)
  Known Comorbidity (N,%)
  Substance use
  Affective dis.
  Personality dis.
  Family history of psychosis			

Baseline Questionaires (median, IQR) CAPS-5 STQ SCID BDI PSQ STA PCL-5 BSI BRIEF-A			

Baseline Physiology (mean, SD)
  Sleep duration
  Mean daytime, sleeping and overall BP
  Daytime, sleeping and overall HRV			

Table 2 - Primary Outcome

Sampling TIme	Baseline	14 days	28 days	42 days	56 days	70 days	84 days	6 months	12 months	18 months	24 months	Unadjusted MLM	MMLM
Intervention Group CAPS-5 (median, IQR)												p-value	P-value
Control Group CAPS-5 (median, IQR)												p-value	P-value

Scientific Importance and Clinical Significance

While HBO proponents point towards reported trends of PTSD symptoms amelioration under HBO therapy, (23) skeptics underline the limitations of scarce prospectively randomized studies (a total of 30 patients thus far), narrow selection (chronic PTSD, often excluding females and mTBI) and the lack of appropriate placebo control. Empirically, thus far the role of HBO in the treatment of PTSD has not been sufficiently validated. A recent meta-analysis of HBO therapy in PTSD deemed the conduction of a well designed (sham) control in a prospectively randomized trial to be the most important milestone in the clarification of this question.(21)

We believe the following additional considerations to further justify the ethical importance and scientific novelty of this proposed study:

- Thus far, females have been excluded, or at the very least neglegably represented in all research examining HBO for PTSD. Our study aims to include as many women as possible.

- Our understanding of the long term effect of HBO is extremely limited (thus far we have data of only 40 patients)(27). This is the first study, to our knowledge, a-priory designed to include a long term follow up.
- We hope physiological metrics collected, and in particularly - sleep data, will help to further elucidate effects remaining obscure when relying solely on self reported, questionnaire based outcomes.
- Our pragmatic design aims to include the great majority of PTSD patients, including those with a relatively short duration of symptoms (the absolute majority of patients described thus far have been suffering from PTSD for many years prior to inclusion). Studying potential interventions in this physiologically distinct subgroup is imperative in view of the national disaster befallen upon our nation on with the tragic events of October 2023.

Ethical Considerations

We identify a few important caveats when considering the potential risks of this study:

- Central Oxygen Toxicity (COT) - manifested as visual and neurological changes, and rarely - seizures. This condition is rare (point incidence of 1:10,000 treatments)(1) and completely treatable with the discontinuation of hyperbaric oxygen. A recent study found no evidence of long term sequelae in patients with documented COT. In view of the very low risk and lack of any evidence of long term harm, we believe the risk of COT to be justifiable.
- Barotrauma - the combined risk or around 5% is pertaining to minor injuries, fully cured spontaneously within 2-3 days of pressure changes abstinence. Available evidence(13), as well as our combined experience of over 40 years, has no documented evidence of serious barotrauma occurring in the highly monitored and controlled setting of HBO therapy.

- Memory resurfacing - the only available study examining potential psychiatric side effects (19) suggests trends towards increased memory resurfacing, without any signals of increased suicidal ideation or intent. We take these potential, albeit yet to be substantiated, concerns very seriously, and intend to have a dedicated hotline directly to the board certified psychiatrist (and the PI) should the patient, their next of keen or the treatment team have any concerns. Notwithstanding these concerns, we believe the benefits of clarifying the true effect of a potential treatment outweigh these risks.

Funding

This work will be partially supported by the Israeli Ministry of Defence, the Division of Veteran Rehabilitation, grant nu 2024-0189 (totallin 150,000 NIS, of which 130,000 NIS are allocated for perishables (e.g. breathing gasses, breathing masks and tubing) and 20,000 NIS are reserved for statistical analysis and publication expenses).

Access and Delegation of Responsibility

Name	Allowed Roles and Access	Duration	Signature	PI's Signature
Carmel Kalla	Approaching potential recruits, providing explanations, questionnaire administration, Physiological monitoring, Raw CRF	1.9.2024-31.8.2027		
Orly Knoll	Approaching potential recruits, providing explanations, fitness to dive examination, HBO administration. CRF completion,	1.9.2024-31.8.2027		
Dror Ofir	obtaining informed consent, Physiological monitoring, full access to the CRF and the coded information.	1.9.2024-31.8.2027		

Ivan Gur	Approaching potential recruits, providing explanations, fitness to dive examination, Physiological monitoring, HBO administration, CRF completion, Coded data and Statistical analysis	1.9.2024-31.8.2027		
Yinnon Matzliach	Approaching potential recruits, providing explanations, fitness to dive examination, HBO administration. CRF completion,			

Limitations

Challenges, potential technical issues and biases. The research requires coordination with busy participants who are not always free. At the same time, the importance of the post-traumatic disorder and the lack of other good treatments justify in our view an excessive investment in the recruitment and retention of research subjects with an important potential therapeutic method such as hyperbaric therapy.

Appendix A - sample size simulation

```
set.seed(123)  # For reproducibility

# Parameters
effect_size <- 10   # Difference in means
std_dev <- 12         # Standard deviation
power <- 0.8        # Desired power
alpha <- 0.05       # Significance level

# Simulation Function
calculate_power <- function(n) {
  p_values <- replicate(10000, {
    group1 <- rnorm(n*2, mean = 0, sd = std_dev)
    group2 <- rnorm(n, mean = effect_size, sd = std_dev)
    t.test(group1, group2)$p.value
  })
  mean(p_values < alpha)
}

# Find Sample Size
n <- 10  # Initial guess
while (calculate_power(n) < power) {
  n <- n + 1
}

cat("Required sample size per group:", n)

n/0.70/0.80
```

Appendix B - Recruitment Add:

For a medical study involving volunteers suffering from PTSD (a condition also known in Hebrew as "combat stress reaction"):

Volunteers are needed for a study titled: "Hyperbaric Oxygen Therapy for Post Traumatic Stress Disorder - a Pragmatic, Double Blinded Randomized Trial."

The study will take place at: The Institute of Marine Medicine.

Address: 8 HaAliya HaShniya St., Haifa (Haifa Naval Base, Bat Galim, opposite to Rambam Medical Center).

A medical study is being conducted on the topic: The effect of hyperbaric chamber treatment on PTSD.

Study description: Volunteers who are interested in participating in the study will receive a detailed explanation from a designated researcher, undergo an evaluation for suitability for hyperbaric chamber treatment, and complete a series of assessments (including questionnaires and measurements of heart rate and blood pressure). Participants will then be randomly assigned to receive either hyperbaric chamber treatment or placebo (sham) treatment, without the participants or the research staff being aware of the assignment. Participants will then be asked to attend a series of 60 treatments (five treatments per week for 12 weeks). Participants assigned to the placebo group will be able to complete the full course of 60 treatments after the study concludes. During the treatment period, weekly assessments will be conducted (including questionnaires, heart rate measurements, and blood pressure measurements).

Those interested in participating are invited to contact 054-255-5655 at any time.

Bibliography

1. Parr NJ, Anderson J, Veazie S. Evidence Brief: Hyperbaric Oxygen Therapy for Traumatic Brain Injury and/or Post-traumatic Stress Disorder. Washington (DC): Department of Veterans Affairs (US); 2021.
2. Andrews SR, Harch PG. Systematic review and dosage analysis: hyperbaric oxygen therapy efficacy in the treatment of posttraumatic stress disorder. Front Neurol. 2024 May 31;15:1360311.
3. Mozayeni BR, Duncan W, Zant E, Love TL, Beckman RL, Stoller KP. The National Brain Injury Rescue and Rehabilitation Study - a multicenter observational study of hyperbaric oxygen for mild traumatic brain injury with post-concussive symptoms. Med Gas Res. 2019;9(1):1–12.
4. Harch PG. Systematic review and dosage analysis: hyperbaric oxygen therapy efficacy in mild traumatic brain injury persistent postconcussion syndrome. Front Neurol. 2022 Mar 17;13:815056.
5. Bennett MH. Evidence brief: hyperbaric oxygen therapy (HBOT) for traumatic brain injury and/or post-traumatic stress disorder. Diving Hyperb Med. 2018 Jun 30;48(2):115.
6. Davis LL, Schein J, Cloutier M, Gagnon-Sanschagrin P, Maitland J, Urganus A, et al. The economic burden of posttraumatic stress disorder in the united states from a societal perspective. J Clin Psychiatry. 2022 Apr 25;83(3).
7. Mayer Y, Shiffman N, Bergmann E, Natoor M, Khazen S, Lurie I, et al. Mental Health Outcomes of Arab and Jewish Populations in Israel a Month after the Mass Trauma Events of October 7, 2023: A Cross-Sectional Survey of a Representative Sample. Psychiatry Res. 2024 Jun 15;339:116042.
8. Lin C-C, Huang K-L, Tung C-S, Liu Y-P. Hyperbaric oxygen therapy restored traumatic stress-induced dysregulation of fear memory and related neurochemical abnormalities. Behav Brain Res. 2019 Feb 1;359:861–70.
9. Peng Y, Feng S-F, Wang Q, Wang H-N, Hou W-G, Xiong L, et al. Hyperbaric oxygen preconditioning ameliorates anxiety-like behavior and cognitive impairments via upregulation of thioredoxin reductases in stressed rats. Prog Neuropsychopharmacol Biol Psychiatry. 2010 Aug 16;34(6):1018–25.
10. Hadanny A, Bechor Y, Catalogna M, Daphna-Tekoah S, Sigal T, Cohenpour M, et al. Hyperbaric Oxygen Therapy Can Induce Neuroplasticity and Significant Clinical Improvement in Patients Suffering From Fibromyalgia With a History of Childhood Sexual Abuse-Randomized Controlled Trial. Front Psychol. 2018 Dec 17;9:2495.
11. Harch PG, Andrews SR, Rowe CJ, Lischka JR, Townsend MH, Yu Q, et al. Hyperbaric oxygen therapy for mild traumatic brain injury persistent postconcussion syndrome: a randomized controlled trial. Med Gas Res. 2020;10(1):8–20.
12. Jones MW, Brett K, Han N, Wyatt HA. Hyperbaric Physics. StatPearls. Treasure Island (FL): StatPearls Publishing; 2023.

13. Wolf EG, Prye J, Michaelson R, Brower G, Profenna L, Boneta O. Hyperbaric side effects in a traumatic brain injury randomized clinical trial. Undersea Hyperb Med. 2012 Dec;39(6):1075–82.

14. Weaver LK, Wilson SH, Lindblad AS, Churchill S, Deru K, Price RC, et al. Hyperbaric oxygen for post-concussive symptoms in United States military service members: a randomized clinical trial. Undersea Hyperb Med. 2018;45(2):129–56.

15. Miller RS, Weaver LK, Bahraini N, Churchill S, Price RC, Skiba V, 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 Jan;175(1):43–52.

16. Cifu DX, Hart BB, West SL, Walker W, Carne W. The effect of hyperbaric oxygen on persistent postconcussion symptoms. J Head Trauma Rehabil. 2014 Feb;29(1):11–20.

17. Doenyas-Barak K, Catalogna M, Kutz I, Levi G, Hadanny A, Tal S, 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 Feb 22;17(2):e0264161.

18. Doenyas-Barak K, Kutz I, Levi G, Lang E, Beberashvili I, Efrati S. Hyperbaric Oxygen Therapy for Veterans With Treatment-resistant PTSD: A Longitudinal Follow-up Study. Mil Med. 2023 Jul 22;188(7–8):e2227–33.

19. Doenyas-Barak K, Kutz I, Lang E, Levi G, Efrati S. Memory surfacing among veterans with PTSD receiving hyperbaric oxygen therapy. Undersea Hyperb Med. 2023;50(4):395–401.

20. Hoge CW, Jonas WB. The ritual of hyperbaric oxygen and lessons for the treatment of persistent postconcussion symptoms in military personnel. JAMA Intern Med. 2015 Jan;175(1):53–4.

21. Hart BB, Weaver LK, Gupta A, Wilson SH, Vijayarangan A, Deru K, et al. Hyperbaric oxygen for mTBI-associated PCS and PTSD: Pooled analysis of results from Department of Defense and other published studies. Undersea Hyperb Med. 2019;46(3):353–83.

22. Schimmel S, El Sayed B, Lockard G, Gordon J, Young I, D'Egidio F, et al. Identifying the target traumatic brain injury population for hyperbaric oxygen therapy. Int J Mol Sci. 2023 Sep 27;24(19).

23. Doenyas-Barak K, Kutz I, Lang E, Merzbach R, Lev Wiesel R, Boussi-Gross R, et al. The use of hyperbaric oxygen for veterans with PTSD: basic physiology and current available clinical data. Front Neurosci. 2023 Oct 25;17:1259473.

24. Bugni FA, Canay IA, Shaikh AM. Inference under Covariate-Adaptive Randomization. J Am Stat Assoc. 2018 Jun 28;113(524):1784–96.

25. Kang M, Ragan BG, Park J-H. Issues in outcomes research: an overview of randomization techniques for clinical trials. J Athl Train. 2008

Jun;43(2):215–21.

26. Ma W, Ye X, Tu F, Hu F. carat : An *R* Package for Covariate-Adaptive Randomization in Clinical Trials. J Stat Softw. 2023;107(2).

27. Hart BB, Wilson SH, Churchill S, Deru K, Weaver LK, Minnakanti M, et al. Extended follow-up in a randomized trial of hyperbaric oxygen for persistent post-concussive symptoms. Undersea Hyperb Med. 2019;46(3):313–27.