ABSTRACT: HBOT for Blast Injured Veterans

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Source
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Abstract
This is a preliminary report on the safety and efficacy of 1.5 ATA HBOT in military subjects with chronic blast-induced mild-moderate traumatic brain injury (TBI)/post-concussion syndrome (PCS) and post-traumatic stress disorder (PTSD). Method: 16 military subjects received forty 1.5 ATA/60 minute HBOTs in 30 days. Symptoms, physical and neurological exams, SPECT brain imaging, neuropsychological and psychological testing were completed before and within one week after treatment. Results: subjects experienced reversible middle ear barotrauma (5), transient deterioration in symptoms (4), and reversible bronchospasm (1); one subject withdrew. Post treatment testing demonstrated significant improvement in: symptoms, neurological exam, Full scale IQ (+14.8 points; p<.001), WMS IV Delayed Memory (p=.026), WMS-IV Working Memory (p=.003), Stroop Test (p,<.001), TOVA Impulsivity (p=.041), TOVA Variability (p=.045), Grooved Pegboard (p=.028), PCS symptoms (Rivermead PCSQ: p=.0002), PTSD symptoms (PCL-M: p<.001), Depression (PHQ-9: p<.001), Anxiety (GAD-7: p=.007), quality of life (MPQoL: p=.003), and self-report of percent of normal (p<.001),SPECT coefficient of variation in all white matter and some gray matter ROIs after the first HBOT, and in half of white matter ROIs after 40 HBOTs, and SPECT statistical parametric mapping analysis (diffuse improvements in regional cerebral blood flow after 1 and 40 HBOTs). Conclusion: Forty 1.5 ATA HBOTs in one month was safe in a military cohort with chronic blast-induced PCS and PTSD. Significant improvements occurred in symptoms, abnormal physical exam findings, cognitive testing, and quality of life measurements, with concomitant significant improvements in SPECT.

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Additions to manuscript are in green, rearranged portions are in yellow, and deletions are in blue with strikethrough.

J of Neurotrauma

A Phase I Study of Low Pressure Hyperbaric Oxygen Therapy for Blast-Induced Post Concussion Syndrome and Post Traumatic Stress Disorder

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Abstract

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Keywords: HBOT, TBI, PCS, PTSD, SPECT

Introduction

Blast-induced traumatic brain injury (TBI) and post-traumatic stress disorder (PTSD) are diagnoses of particular concern in the United States because of the volume of affected servicemen and women from the conflicts in Iraq and Afghanistan. A Rand Report (Tanielian and Jaycox, 2008) estimates that 300,000 (18.3%) of 1.64 million military service members who have deployed to these war zones have PTSD or major depression and 320,000 (19.5%) have experienced a TBI. Overall, approximately 546,000 have one of the three diagnoses and 82,000 have symptoms of all three [Note: symptoms of TBI refer to the post-concussion syndrome (PCS)]. The frequency of the combined diagnoses in Veterans of mild TBI and PTSD has recently been estimated between 5-7% (Carlson, 2010). With a probable diagnosis of mild TBI the combined diagnosis incidence rises to 33-39% (Carlson, 2010). A Walter Reed Army Institute of Research post-deployment survey of 4618 soldiers reported 15.2% of injuries had a history of loss of consciousness or altered mental status (Hoge et al., 2008). That study also found that 43.9% of those with history of loss of consciousness and 27.3% of those with history of altered mental status met criteria for PTSD.

Separating the two diagnoses of PCS and PTSD, however, has been very difficult. When adjustments were made for PTSD and depression by logistic regression analysis, the only symptom unique to mild TBI was headache. Due to this difficulty in differentiation we made no attempt to separate the two diagnoses in this study based on symptoms.

Evidence-based treatment for PTSD exists, but problems with access to and quality of treatment have been problematic in the military setting (Tanielian

and Jaycox, 2008). Treatment of the symptomatic manifestation of mild TBI, the PCS, is limited. Treatment consists of off-label use of FDA blackbox labeled psychoactive medications, counseling, stimulative, and adaptive strategies. There is no effective treatment for the combined diagnoses of PCS and PTSD. This purpose of this study is to explore feasibility, safety, and treatment effects of hyperbaric oxygen therapy on PCS and PTSD.

Hyperbaric Oxygen Therapy (HBOT) is a medical treatment that uses greater than ambient pressure oxygen as a drug by fully enclosing a person or animal in a pressure vessel and then adjusting the dose of the drug to treat pathophysiologic processes of diseases. (Harch, Neubauer 1999). At 2.0-3.0 atmospheres absolute (ATA) HBOT is a reimbursed treatment for approximately 15 diagnoses (Gesell, 2009; Centers, 2006). HBOT has not been applied to PTSD to our knowledge while the evidence for its effectiveness in PCS is scant. Since 1989 we and others have investigated the application of a lower pressure protocol of HBOT, HBOT 1.5 ATA, to patients with a variety of chronic neurological disorders (Harch et al., 1994; Neubauer et al., 1994; Harch et al., 1996a; Harch and Neubauer, 1999; Golden et al., 2002; Harch and Neubauer, 2004a; Harch and Neubauer, 2009b, Harch and Neubauer, 2009c), based on initial studies done by Neubauer (Neubauer, 1990) in chronic stroke. Few of the chronic TBI patients had PCS from mild TBI, blast-induced PCS, or blast-induced PCS with PTSD. We published the first application of HBOT 1.5 to chronic blast-induced PCS with PTSD in 2009 (Harch and Fogarty, 2009a).

Oxygen toxicity (U.S. Navy, 2008; Clark, 1993) is a concern in any HBOT study. The most severe manifestation is seizure. A study of HBOT on sub-acute moderate to severe TBI at 2.0 ATA (Lin et al., 2008) reported a 9% seizure rate.

At doses less than 2.0 ATA, side effects and toxicity in chronic brain injured patients have been noted only with prolonged courses of HBOT, i.e., 70-500 treatments (Harch, 2002).

We report the safe application of a 29-day treatment course of 1.5 ATA HBOT to 16 U.S. servicemen with mild-moderate blast-induced PCS or PCS with PTSD and note a biphasic response with transient worsening of symptoms in 4 of the 16 subjects followed by improvement as treatment continued. These Veterans experienced symptomatic, physical, cognitive, affective, and brain blood flow improvements.

Materials and Methods

Study Design and Protocol: The design is a pilot proof-of-concept study with pre and post testing and no control group. Subjects completed a history and physical exam by the P.I., clinical interview by the neuropsychologist, psychometric testing, symptom and quality of life questionnaires, baseline SPECT, first HBOT the following day, and repeat SPECT three hours after the first HBOT. Subjects commenced twice/day, five day/week 1.5 ATA/60 minute total dive time HBOT until 40 HBOTs were completed. Within five days of final HBOT subjects underwent repeat focused history, physical exam, psycho-metric testing, questionnaires, and SPECT.

Inclusion Criteria: Subjects had to be 18-65 years old, with one or more mild-moderate TBIs characterized by loss of consciousness due to blast injury that was a minimum of one year old and occurred after 9/11/01. They had to have a prior diagnosis of chronic TBI/PCS or TBI/PCS/PTSD by military or civilian specialists, with an absence of acute cardiac arrest or hemorrhagic shock at time

of TBI, Disability Rating Scale (Rappaport et al., 1982) of 0-3, negative urine toxicology screen for drugs of abuse, negative pregnancy test in females, otherwise good health, and less than 90% on the Percent Back to Normal Rating Scale, PBNRS, (Powell et al., 2001).

Exclusion Criteria: Subjects were screened out of the study with pulmonary disease that precludes HBOT (e.g., bronchospasm unresponsive to medication, bullous emphysema), unstable medical conditions that are contraindicated in HBOT (e.g. severe congestive heart failure or heart failure requiring hospital emergency evaluation or admission in the previous year), severe confinement anxiety (e.g., patients who require anesthesia conscious sedation for MRI), participation in another experimental trial with active intervention, high probability of inability to complete the experimental protocol (e.g. terminal condition), previous HBOT, history of hospitalization for past TBI, stroke, non-febrile seizures, or any seizure history other than seizure at the time of TBI, past or current history of mental retardation (baseline FSIQ ≤ 70), alcohol or drug abuse (MAST or DAST >3), pre-/post-TBI history of systemic illness with impact on CNS (P.I.'s decision).

Symptom and Physical Exam Scoring: Subjects constructed a prioritized symptom list and answered neurological and constitutional symptom questions from the P.I.'s standard questionnaire (see Appendix). Abnormal components of the physical exam were videoed and then replayed before the final exam after HBOT for comparison. After the 40th HBOT subjects judged all symptoms as "better", "worse", or "same" and the P.I. did the same for the physical exam abnormalities. Exam items inadvertently omitted on retesting were scored as unchanged. Six months following the last HBOT subjects were queried by phone

about the status of their prioritized symptom list. Each subject was asked to rate each symptom as better, worse, or the same compared to the status of that symptom before any HBOT.

Psychometric Testing: Table 1 lists neuropsychological and psychological, quality of life, screening, diagnostic tests, and the schedule of administration. The choice of tests was guided by past experience with pre and post testing for HBOT effects in chronic TBI. IQ testing was included instead of more easily measured variables, like reaction time, because of a concern that the measures reflect social relevance and the reported deficits from injury, such as frontal lobe (attention, executive function, motor speed, decision speed, and working memory), general intellectual ability, memory, PCS symptoms, quality of life, and affective symptoms (anxiety, depression). Practice and test/retest effects were minimized by choice of tests or where possible using alternate tests, e.g. WAIS-IV on pre-test and WASI post-test. All tests were outcome tests except the WTAR, Green, MAST, DAST, and CES. The original screening PBNRS is defined in Table 1. It was expanded at psychometric test sessions to include cognitive, emotional, and physical domains and each subject asked to rate his/her current percent of premorbid normal function in each domain. Prior diagnoses of TBI/PCS and PTSD were confirmed or refuted by using clinical interviews, symptom lists, the Rivermead Post Concussion Symptoms Questionnaire [PCS: ≥3 on at least 3 questions,(Sterr, 2006)], the PTSD Checklist-Military (Total=>/=50, Tanielian and Jaycox, 2008; Andrykowski, 1998) and DSM-IV criteria for the diagnoses.

SPECT Brain Blood Flow Imaging: Subjects underwent SPECT brain blood flow imaging performed by a single technologist on a Picker Prism 3000 XP Triple-Head gamma camera system before, within 4 hours after the first HBOT, and

within 48 hours after the 40th HBOT. Subjects were placed on a gurney in the supine position, head of bed elevated 30 degrees, in a designated quiet low light area of the nuclear medicine department. Heparin lock IV catheter was placed and after at least 15 minutes of no speech or movement ~25 mCi of ^{99m}Technetium Ethyl Cysteinate Dimer (ECD) was injected and followed with a 10cc normal saline flush. The patient remained quiet and motionless for another 55 minutes and then was placed supine on the scanning couch. The head was secured with tape to the head cradle and the subject was aligned with an overhead laser. Acquisition entailed a 360 degree rotation with 40 stops, 20 seconds/stop on a 128 x 128 matrix, using low energy high resolution fan beam collimators. Cine was viewed for gross motion artifact and the study immediately repeated if the image was motion degraded.

Processing was performed by a single off-site experienced nuclear technologist. Mild motion artifact was corrected with Picker motion attenuation software. Raw data was processed by transverse reconstruction using 360 degree filtered back projection and a ramp filter, followed by a LoPass filter, order 2.2. Cutoff was taken at the intersection of the best fit LoPass filter and noise on the power spectrum graph. Per file attenuation correction and best fit ellipse were applied. Images were oblique reformatted with slice thickness at 4 mm (2 pixels), aligned, and off-center zoom applied (20 cm^2 area). Images were presented in all 3 orthogonal planes.

SPECT Texture Analysis: Transverse processed images were analyzed by author EFF (unblinded to study and scan sequence) to capture the pre/post HBOT SPECT pattern change from heterogeneity to homogeneity (Figure 1) that we have observed in previous HBOT treated patients (Harch, 1996a; Harch,

2009a; Harch and Neubauer 2009c). Osirix™ DICOM software was used to perform a first order texture analysis of count histograms (Dougherty, 1996). In previous HBOT treated blast cases the pattern shift (apparent normalization) corresponded to a relative reduction in high flow areas and relative increase in low flow areas (Figure 1 insets) or narrowing of the count histogram that was registered as a reduction in SD and CV (see below).

Images were oriented and aligned by visual inspection. A single transverse slice was taken above the level of the deep gray matter in the centrum semiovale of each patient's three SPECT brain scans. A circular region of interest (ROI) was chosen of sufficient size, 0.781 cm^2, to fit within the cortical boundary of the baseline (first) scan. Five cortical and two white matter ROIs were selected in each hemisphere. The cortical ROIs were placed along template ray-lines cast at 30 degree angle intervals on either side of the anatomic center point in the image, assigning 0 degrees to 12:00 on a clock face of the transverse slice. The white matter ROIs were placed along the 60 degree and 120 degree ray-lines from the center point. Thus, the template ray-lines for the left and right hemispheres were at 1 o'clock and 11:00, respectively, for the 30 degree ray, 2 o'clock and 10:00 for the 60 degree ray, etc. To aid best fit visualization for placement of the ROI on the 2nd and 3rd scans the pre-HBOT baseline image was individually fused in Osirix™ to the 2nd and 3rd scans. If the template ROIs landed across the cortical junction with white matter or across obvious focal metabolic lesion margins when first placed by whole scan best-fit fusion they were adjusted along the ray line to sample appropriate scan to scan concordant tissues.

For each ROI mean number of counts/pixel (MCP), standard deviation of counts/pixel (SD), and coefficient of variation (CV) (standard deviation as a percent of mean) of counts/pixel were measured for all three scans for each patient. Group averages for each ROI of mean counts/pixel, standard deviation of counts/pixel, and coefficient of variation were taken for each scan's ROIs and differences compared from baseline to post-1 and post-40 HBOT scans. Statistical analysis was performed as described below. A decrease in CV was the primary SPECT outcome.

SPECT Statistical Parametric Mapping Analysis: Differences in ECD uptake were analyzed using SPM8 software (Wellcome Department of Cognitive Neurology, London, UK) implemented on the Matlab platform (MathWorks Inc., Sherborn, MA) by authors DA and DVT. Author DVT performed all analyses and was asked to compare scan A to scan C, analyze for change, significance of change, and then direction of change starting with a p value for each voxel of < 0.01. He was blinded to all details of the scans, including context (clinical study), patients/subjects, normal vs. injury, treatment or not, one vs. multiple groups, location, etc., and expectation of change or direction of change in the scans. DVT was then asked to perform a similar comparison of Scans B to A and C.

The images were spatially normalized using a twelve parameter affine transformation followed by non-linear deformations (Ashburner, 1999) to minimize the residual sum of squares between each scan and a reference or template image conforming to the standard space defined by the Montreal Neurological Institute (MNI) template. The original image matrix obtained at 128x128x29 with voxel sizes of 2.16mm x 2.16mm x 6.48mm were transformed and resliced to a 79x95x68 matrix with voxel sizes of 2mm x 2mm x 2mm consistent with the MNI template. Images

were smoothed using an 8mm full width half maximum isotropic Gaussian kernel. Within subject comparisons were performed by pairwise t-test between first and second scan and between the first and third scan. Anatomical locations of the significant statistical parameter maps were identified by registering clusters using the Anatomical Automatic Labeling (AAL) atlas (Cyceron).

HBOT: Hyperbaric oxygen therapy was performed in monoplace hyperbaric chambers. Patients were compressed and decompressed at 1-2 psi (pound per square inch) on 100% oxygen, the rate depending on patient comfort and preference. Depth of pressurization was 1.5 ATA. Total dive time was 60 minutes. Treatments were twice/day, 5 days/week with a 3-4 hour surface interval between treatments. Protocol goal was 40 HBOTs.

Statistical Analysis: Values of psychometric tests were acquired pre and post 40 HBOTs and SPECT parameters were acquired pre, post-1 HBOT, and post-40 HBOTs. For each SPECT ROI at each time point mean, standard deviation, median, range (minimum and maximum), and 95% confidence interval around the estimated mean were calculated for mean of counts/pixel, standard deviation of counts/pixel, and CV of counts/pixel. Changes in psychometric and SPECT parameters between pairs of time points (pre-HBOT to post-1-HBOT, pre-HBOT to post-40-HBOTs, and post-1-HBOT to post-40-HBOTs) were similarly summarized, with the inclusion of a p-value indicating whether or not the mean change was significantly different from zero. The p-values were obtained by the paired Student t test if the changes were nearly normally distributed, or by the non-parametric Wilcoxon Signed-Ranks test if the changes were significantly non-normally distributed, by the Anderson-Darling test. Line graphs of the CV were also prepared, showing the mean value (Y axis) of the parameter for each

ROI (X axis) for each of the three time points (separate lines), along with vertical error bars around each plotted point indicating ± 1 standard error of the mean (SEM). One subject who withdrew before completion of treatment and post-treatment testing was included in the demographic data and safety/feasibility analysis, but excluded from the per protocol analysis (outcome testing).

Results

Subjects: Eight active duty and eight recently retired servicemen were self-referred or referred by their military commanders/physicians. Fourteen subjects had pre-study diagnoses of TBI/PCS with PTSD and two subjects had TBI/PCS. Pre-study diagnostic evaluations and criteria were not available to the study authors. All subjects underwent brain MRI in the military prior to treatment. All subjects gave informed consent and enrolled in LSU IRB #7051.

Demographics of Sample: Sample demographics are reported in Table 2. Sixteen subjects were enrolled. One subject withdrew from the study due to complications described below. Since he did not complete post-treatment testing he was included in the demographic data, but excluded from all data analyses. All subjects were male and averaged: thirty years old, 2.8 years post TBI, loss of consciousness of 2 minutes (excluding 2 subjects with 4.5 and 9h), 6 years of service, 2.7 blast TBIs, RPCSQ 39, PCL-M 67, Mast 2.1, DAST .6, DRS 1.6, and 39 HBOTs in 29 days. Loss of consciousness was estimated by each patient and the P.I. based on events at time of injury and bystander reports to patient. All sixteen subjects satisfied the RPCSQ and DSM-IV criteria for PCS. Fifteen of sixteen subjects met the PCL-M threshold for PTSD (≥ 50); the remaining subject scored 48. All sixteen subjects met the DSM-IV criteria for PTSD.

MRI Results: Results were obtained from patient recollection of results and medical records when available. Twelve of sixteen subjects had normal MRIs of the brain. Two subjects were normal except for arachnoid cysts. Another had an abnormal MRI that was later repeated at the VA and reported as normal. A final subject had an abnormal MRI, but the abnormality was not recalled by the subject.

Safety of the HBOT Protocol: Mild reversible middle ear barotrauma (MEBT) occurred in five subjects, four of these in the setting of upper respiratory infections at 8, 27, 27, and 30 HBOTs, requiring protocol breaks of 5 days, termination of protocol, 1 day, and 16 days, respectively. The fifth subject experienced no protocol break. All were treated with systemic decongestants with or without topical decongestants. Four of the five resumed treatment and successfully finished the protocol. The fifth subject experienced a series of problems that included a delay to scanning and treatment secondary to a scanner malfunction, followed by shortness of breath, beginning with the first HBOT, that was incident to each HBOT and increased during his time in the chamber. Pre/post HBOT peak flow reductions were measured, he was medicated to symptom relief with albuterol pre-each HBOT, and showed a reduction in bronchospasm and shortness of breath with subsequent HBOTs. His bronchospasm was felt to be due to the low humidity oxygen environment of the monoplace chamber. This subject subsequently experienced an upper respiratory infection (URI), (MEBT), and bullous myringitis at 27 HBOTs. Because of the delay to testing caused by the scanner repair the subject's

schedule could not accommodate a protocol break to resolve the URI/MEBT and finish the protocol. He withdrew from the study and returned home.

Four of the sixteen subjects reported a transient deterioration in some of their symptoms: two with mood swings/emotional lability at 20 and 10 HBOTs, one with worsened headaches at 19 HBOTs, and one with increased depression at 22-25 HBOTs. Treatment was continued and the symptoms resolved over the course of the next 4-6 HBOTs. There were no other untoward side-effects. Specifically, we found no evidence of oxygen toxicity (U.S. Navy, 2008; Clark, 1993).

Effectiveness of HBOT for Chronic Blast TBI/PCS and PTSD: Effectiveness of HBOT was measured across multiple domains: symptoms, physical exam, psychometric testing, quality of life, and SPECT.

Symptoms and physical exams: Twelve of fifteen subjects (80%) reported improvement in a majority of their symptoms on their prioritized symptom list after HBOT. Eleven of fifteen subjects (73%) reported improvement in a majority of symptoms on the primary author's standard symptom questionnaire. Response to HBOT according to specific symptoms is recorded in Table 3 which combined symptoms from the prioritized list and the primary author's questionnaire. Headache, sleep disruption, short-term memory loss, cognitive problems, decreased energy, self-characterized "PTSD symptoms" or "nightmares" (grouped as PTSD symptoms; "PTSD symptoms" were not further queried or defined since PTSD symptomatology was quantified for all subjects in the PCL-M.), short temper/irritability, mood swings, imbalance, photophobia, and depression, which were present in a majority of subjects, were improved in

44-93% of the subjects. Patients with decreased hearing, tinnitus, and arthralgias reported minimal change: 20, 37, and 0% improvement, respectively.

On physical exam all fifteen subjects were found to have improved on a majority of their abnormal findings. Imbalance and incoordination were the most common abnormal physical exam findings (See Table 4). Patients experienced improvement in 87-100% of these findings. In addition, 64% (7/11) of subjects who were on psychoactive or analgesic prescription medication before HBOT decreased or discontinued their medication usage during HBOT; 11% of those on analgesic medication (1/9) increased analgesic medication. Psychoactive medications pre-HBOT were as follows: Selective Serotonin Reuptake Inhibitors/Serotonin Norepinephrine Reuptake Inhibitors/Aytpical Anti-psychotics/Atypical anti-depressants (9 subjects), anxiolytic/hypnotics (8), anti-convulsants (5), anti-migraine (4), narcotics (3), vasodilators (2), muscle relaxants (2), anti-histamine/anti-emetics (1), cholinesterase inhibitor (1), stimulants (1). Nine subjects were taking more than one medication, 1 subject was taking one medication, and five subjects were on no psychoactive medications.

At six month phone followup 11/12 subjects (92%) who reported improvement on the majority of the symptoms on their prioritized symptom list after 40 HBOTs maintained this improvement. One of the three subjects who did not report initial improvement now reported improvement in the majority of symptoms on his prioritized list.

Psychometric Testing, Affective, TBI/PCS Symptom, and Quality of Life Measures: Change from Pre to Post HBOT on the neuropsychological outcome variables is shown in Table 5. Significant improvement was recorded on 7 of 13

measures at p<0.05 to p<0.001level and beyond. Global intellectual function and measures of frontal lobe executive function (Working Memory and Stroop Test) showed the largest improvements. The Full Scale IQ increased nearly a full standard deviation, an average of 14.8 points from 95.8 to 110.6 (p<0.001). The WMS-IV Working Memory Index increased 9.9 points from a pre-HBOT average of 97.0 to a post-HBOT average of 106.9 (p=0.003). The Stroop Color/Word Interference score improved 11.1 points from a mean of 84.3 to 95.3 after HBOT (p<0.001).

Change in memory was slightly smaller but significant and clinically meaningful on the WMS-IV. The WMS-IV Delayed Memory Index increased 9.2 points from 97.7 to 106.9 (p=0.026). The Rivermead Paragraph subtest showed a decrease from the pre-HBOT of 9.5 units recalled to a post-HBOT score of 7.5 units recalled (p=0.049).

Improvement in attention was found on several measures but not on all. The TOVA measures of Impulsivity (p=0.041) and Variability (p=0.045) both showed significant increases from pre to post-HBOT. The TOVA Inattention and Reaction Time measures improved a few points from pre to post-HBOT but neither was significant.

Only one measure of motor speed and fine-motor coordination (Grooved Pegboard for the dominant hand) showed a significant improvement (7.9 points, p=.028). Dominant hand motor speed (Finger Tapping Test) increased from a standard score of 90.9 to 98.6 but failed to reach significance (p=.174). Neither the Finger Tapping Test nor the Grooved Pegboard pre to post-HBOT scores were significant for the nondominant hand.

Table 6 presents the pre and post-HBOT changes for outcome variables of emotional recovery from PTSD, anxiety, and depression, symptoms of post-concussion, and the subjects' ratings on the percent to which they felt back to normal for cognitive, physical, and emotional functioning. All 8 variables showed a significant improvement from pre to post-HBOT. On the PTSD Check List-Military, the average score dropped 20.3 points from 67.4 to 47.1 ($p<0.001$). After HBOT eight of fourteen subjects no longer met the PCL-M threshold criteria for diagnosis of PTSD. The Rivermead Post Concussion Symptoms Questionnaire average score dropped 15.6 points from 39.7 pre-HBOT to 24.1 after treatment ($p=0.0002$). Together, these two measurements indicated a major improvement in the symptoms of PTSD and PCS. Consistent with these findings, the subjects report a significant drop in depression (PHQ-9; $p<0.001$) and anxiety (GAD-7; $p=0.007$), and a concomitant increase in their perceived quality of life ($p=0.003$). One component of the PHQ-9 addressed suicidal ideation on a four point scale: 0=none, 1=several days of last 2 weeks, 2=more than 1/2 days of last 2 weeks, and 3=nearly every day. The suicidal ideation component of the PHQ9 improved after treatment by an average of 0.40 +/- 0.63 score points. This improvement was significant by the Wilcoxon test ($p=0.048$). As a group, the subjects felt that they were less than 50% back to normal for cognitive, physical, and emotional function when they started treatment. They reported a mean increase of 17.4 points for cognitive function ($p=0.002$), 19.5 points for physical function ($p<0.001$), and 28.8 points for emotional function ($p<0.001$), increases of 39%, 45%, and 96%, respectively.

SPECT Brain Blood Flow Imaging: SPECT regional cerebral blood flow (rCBF) indices are presented in Table 7: (MCP), (SD), and (CV) of counts/pixel in each

region of interest (ROI). (MCP), (SD), and (CV) were compared from first scan (pre HBOT) to after 1 HBOT and after 40 HBOTs, and from after 1 HBOT to after 40 HBOTs. Significant changes are registered in Table 8. The CV data, a primary outcome SPECT measure, from all three scans is graphed in Figure 3 for the right hemisphere and Figure 4 for the left hemisphere.

SPECT demonstrated significant increases in (MCP) in the right hemisphere only from baseline to post-1 HBOT (30, 120, and 150° gray matter and 120° white matter ROIs); there were no significant changes from baseline to post-40 HBOTs. In the left hemisphere SPECT demonstrated significant increases in (MCP) from baseline to post-1 HBOT (30, 60, 120, and 150° gray matter ROIs and 60 and 120° white matter ROIs) and from baseline to post-40 HBOTs (120° gray matter and 60 and 120° white matter ROIs).

SPECT demonstrated significant decreases in the SD of counts/pixel in the right hemisphere only from baseline to post-1 HBOT (90 and 150° gray matter ROIs and 60° white matter ROI); there were no significant changes from baseline to post-40 HBOTs. However, there were significant increases (a reversal of effect) from post-1 to post-40 HBOTs (60 and 150° gray matter ROIs). In the left hemisphere SPECT demonstrated significant decreases in the SD of counts/pixel only from baseline to post-1 HBOT (30 and 150 ° gray matter ROIs). There were significant increases (reversal of effect) from baseline to post-40 HBOTs (60 ° white matter ROI) and post-1 to post-40 HBOTs (30 and 150° gray matter ROIs).

SPECT demonstrated significant reductions in the CV of counts/pixel in the right hemisphere from baseline to post-1 HBOT (60, 90, and 150° gray matter and 60 and 120° white matter ROIs) and from baseline to post-40 HBOTs

(60° white matter ROI). There were significant increases (reversal of effect) from post-1 HBOT to post-40 HBOTs (60, 90, and 150° gray matter ROIs). In the left hemisphere SPECT demonstrated significant reductions in the CV of counts/pixel from baseline to post-1 HBOT (30, 60, 90, 120, and 150° gray matter ROIs and 120° white matter ROI) and from baseline to post-40 HBOTs (120° white matter ROI). However, there were significant increases (reversal of effect) from post-1 HBOT to post-40 HBOTs (30 and 150° gray matter ROIs and 60° white matter ROI).

SPM Results: Initial statistical parametric maps with significance set at $p < 0.01$ with family wise error correction (FWE) for multiple comparisons showed diffuse improvements in brain blood flow throughout the brain from cerebellum to frontal cortex after 1 HBOT (Figure 5). In order to separate clusters into discrete anatomical location the significance level was raised to $p < 0.001$ with FWE. This analysis revealed eighty-five clusters significantly improved after 1 HBOT. The eleven most significant regions of change occurred in the precentral, temporal, thalamic and occipital regions and are displayed in Table 9 and Figure 2 (the eleventh was included because of its location in the motor area). There were no significant differences when comparing the second (after 1 HBOT) and the third (after 40 HBOTs) scans at this level of significance. However, when comparing the third scan to the baseline scan the significance level threshold had to be raised to $P< 0.0001$ with FWE to achieve cluster separation into discrete anatomical areas. At this level of significance fifty significant clusters were identified (Table 10 and Figure 3). The most significant change was in the right frontal region after 40 HBOTs.

To compare significant increases in brain blood flow after 1 HBOT to changes after 40 HBOTs a significance level of $P<0.001$ was chosen. Cortical

maps of these analyses demonstrate more widespread significant increases in brain blood flow after 40 HBOTs (Figure 6 4).

To illustrate the overlap of brain areas with increased brain blood flow after 1 HBOT that also show increased brain blood flow after 40 HBOTs the analysis after 40 HBOTs ($p< 0.001$) was repeated using the clusters affected by 1 HBOT ($p< 0.01$) as a mask (Figure 7). Seventy-five significant clusters were discovered with the top ten most significant shown in Table 11 and Figure 5.

A separate analysis tested the hypothesis that (rCBF) in the hippocampus should improve after HBOT given symptomatic and measured WMS memory improvements. The changes after 1 HBOT were compared to the changes after 40 HBOTs (Figure 8 6). After one HBOT significant changes ($P<0.001$) were seen in hippocampal regions on both sides of the brain. The most significant changes were seen in a cluster in the inferior lateral left hippocampus ($t = 17$; $K_E = 93$; coordinates -28,-8 -24) followed by a cluster in the superior medial left hippocampus ($t= 14.86$, $K_E = 139$; coordinates -22,-28,-6). The largest cluster was seen in the right medial hippocampus ($t=12.69$; coordinates 24,-22-16). After forty HBOTs the significant changes in the hippocampus remained on both sides of the brain ($p<0.001$). The most significant changes in hippocampal rCBF were seen in the lateral right hippocampus ($t= 23.95$; $K_E = 626$; coordinates 42,-18,-18) followed by left medial hippocampus ($t= 14.81$; $K_E =366$, coordinates -20,-20,-16).

Discussion

Safety of the HBOT Protocol. In this preliminary report of the effect of forty HBOTs on blast-induced chronic mild-moderate PCS and PTSD we observed that

HBOT 1.5 ATA is safe with no major side effects or complications. Although the number of subjects is small, this lack of major side effects is consistent with ours and others' previous experience with similar low pressure HBOT in patients with more severe chronic TBI (Harch et al., 1994; Neubauer et al., 1994; Harch et al.,1996a; Harch and Neubauer, 1999; Golden et al., 2002; Harch and Neubauer, 2004a; Harch and Neubauer, 2009b and c), but differs from a report by Lin (Lin et al., 2008) on HBOT in moderate to severe TBI where 9% of the patients experienced seizures. The dosage of HBOT in the Lin study was 2.0 ATA for 1.5 hours at depth for 20 treatments compared to our 1.5 ATA for 60 minutes total treatment time. The Lin seizure rate is 300 times the seizure frequency in the general HBOT population at 2.4-2.5 ATA (Clark, 2009) and 30 times the seizure rate at 2.45 ATA in acutely carbon monoxide poisoned patients (Hampson et al., 1996). The greater seizure frequency in the Lin study is likely due to the combination of more severe brain injury, earlier treatment, no air breaks during HBOT, and the dose of 2.0 ATA for 1.5 hours. Seizures at 1.5 ATA have only been reported with prolonged series of treatment and much greater numbers of HBOTs (Harch, 2002) than employed in the present study.

Reversible MEBT occurred in five of sixteen subjects. Most of these occurred during the prodromal and early clinical phase of acute URIs. URI incidence is an uncommonly recorded adverse event in hyperbaric oxygen therapy, but twice/day dosing is also atypical for chronic hyperbaric indications. It is not our preferred dosing schedule, but was chosen due to limitations of time, resources, finances, and out of state location in this subject population. The mild immunosuppression of HBOT (Rossignol, 2007) and and twice/day dosing may have contributed to the 25% URI rate.

Four of the subjects (25%) experienced a transient deterioration in symptomatology at approximately 20 HBOTs. This has not been reported previously in hyperbaric medicine to our knowledge in the debilitated typical elderly population receiving HBOT at much higher pressures for chronic wound conditions. Symptoms consisted of marked worsening of headaches in one patient, increase in mood swings and emotional lability in two patients, and worsened depression in a fourth patient. These symptoms occurred at the halfway point, approximately 20 HBOTs, and were managed non-pharmacologically. Simultaneously, many of the other patients reported an overall improvement in symptoms at this point. We speculate that this mid-point in the protocol represents a transition in brain wound adaptation/transformation to the repetitive effects of intermittent hyperoxia. Due to the self-limited course of this deterioration and the final response to the full course of treatment we conclude that there is no justification for cessation of HBOT during this transition.

Effectiveness of HBOT for Blast TBI and PTSD. The remarkable findings in this study were the significant improvements in self-reported symptoms, physical exam changes, PCS symptoms, perceived quality of life questionnaires, affective measures (general anxiety, depression, suicidal ideation, and PTSD), cognitive measures (memory, working memory, attention, FSIQ), and SPECT brain blood flow imaging. The magnitude of improvement was consistent across all domains measured. These findings were mirrored by a reciprocal reduction or elimination of psychoactive and narcotic prescription medication usage in 64% of those subjects for whom they were prescribed. Spontaneous

improvement as an explanation for all of these findings is inconsistent with the natural history of PCS and PTSD 2.8 years after injury.

Reduction in headaches and increase in FSIQ/cognitive function evidenced effectiveness of HBOT 1.5 in the treatment of blast TBI/PCS cerebral wounds. Headache is a marker of blast-induced PCS and distinguishes PCS from PTSD (Hoge et al., 2008). In our study 13/15 (87%) patients reported a substantial reduction in headaches during the 30 days they received HBOT. A reduction in headache and improvement in PCS symptoms (39% reduction in RPCSQ, p=0.0002) is consistent with the treatment of the extra-cerebral marker of PCS as well as the associated underlying biological injury caused by TBI. This biological wound is established in our subjects due to their loss of consciousness (Lidvall, 1975; Symonds, 1962).

FSIQ increased 14.8 points to 110.6 ($p<0.001$). As a global measure of cognitive function this increase is consistent with the patient's self-reported 40% cognitive improvement, the global nature of blast brain injury, and the global improvement in blood flow on SPECT. affords a global measurement of cognitive function in a single test. A reduction in FSIQ is consistent with the global blast injury mechanism. The estimated pre-morbid FSIQ for our subjects was 105, yet the pre-HBOT FSIQ was measured at 95.8. While in the normal range, subjects reported that they were only at 50% of their pre-injury normal level cognitively before HBOT. Post-HBOT FSIQ increased 14.8 points to 110.6 ($p<0.001$) and the subjects felt that they had improved 40% cognitively from their injured state, thus validating their complaint of reduced cognitive ability from the blast injury. It is possible that sSome of the IQ increase could be explained by purported inaccuracy WASI FSIQ overestimation of the WASI (Axelrod, 2002) which

overestimated FSIQ compared to the WAIS-III in a clinically heterogeneous psychiatric patient sample, but the WASI has been validated in other adult heterogeneous clinical samples (Ryan, 2003; Hays, 2002).., but oOur study was performed on a relatively homogenous patient group. in which criticisms from a heterogeneous group may not apply. The consistency of our findings despite different ways of measuring (WASI and PBNRS) argues against a significant contribution from a WASI flaw and is consistent with a conclusion that the HBOT did improve overall cognitive functioning.

Memory and frontal lobe function (simple sustained attention, working memory, and more complex attention) improved from what would appear to be "average" or "normal" levels to what the subjects considered to be more their "normal" levels. Simultaneously, subjects reported that their PCS symptoms were reduced significantly (Rivermead PCS Questionnaire) significantly decreased by 39% the change from 39.7 to 24.1 represents a major reduction (39%) in symptoms ($p=0.0002$) over a 30-day period. Since the PCS complaints and reduced cognitive abilities had been present for an average of 2.8 years, spontaneous recovery is very unlikely. Our results are very similar to cognitive improvements in a controlled chronic severe TBI HBOT study (Golden, 2006) and case report (Hardy, 2007). While only 26% of the subjects were TBI patients in the Golden study 35 HBOTs in 35 days caused a significant 7.19 point increase in Stroop Color/Word score compared to normal and chronic brain injury controls, both of whom had similar 30-35 day test/retest intervals. Test/retest effect across 1 and 2 week intervals is 3.83 points (Franzen, 1987). The combined effect of Golden and test/retest (7.19 + 3.83 = 11.02) is nearly

identical to the 11.1 point increase in our study. Our subjects achieved an 11.1 point increase.

Changes in motor speed and fine motor coordination reached significance on only one of four measures, the Grooved Pegboard for the dominant hand, while the P.I. recorded improvements on coordination in 90-100% of subjects who had abnormalities on baseline testing. Possible explanations for this discrepancy include: 1) testing of different size and groups of muscles (finger/hand for the psychometric tests vs. entire upper and lower extremities on physical exam); 2) investigator bias/non-blinding; 3) qualitative (physical exam) vs. quantitative (psychometric) testing; 4) small n in the study.

The Rivermead Behavioral Memory (RBM) Paragraph Delayed Recall was the sole significant negative cognitive outcome. The RBM is only one subtest of a larger test and was added because the test offered alternative forms of the paragraph for retesting purposes. The negative result may be a function of the limited range of the test, unequal difficulty of the different paragraphs, small n, problems with sustained attention immediately after our intensive HBOT schedule, or a true negative effect of HBOT on this component of memory.

The SPECT findings were as impressive as the cognitive improvements and were consistent with the bi-hemispheric increases in SPECT regional cortical blood flow reported by Neubauer and Golden (Golden, 2002). SPECT demonstrated statistically significant improvements after HBOT in both similar and different parameters using two different analytical methods. Both texture and spm analyses showed consistent and significant improvements in blood flow after 1 and 40 HBOTs compared to baseline, no significant difference in blood flow between 1 and 40 HBOTs, yet . The spm analysis revealed

considerable overlap of the areas with improved blood flow after 1 and 40 HBOTs. Spm also revealed more widespread significant increases in blood flow after 40 versus 1 HBOT (more voxels and brain regions) compared to baseline and compared to texture analysis which showed the opposite, less ROIs with significant increases in blood flow after 40 versus 1 HBOTs, despite a generalized non-significant increase in MCP (blood flow) from 1 to 40 HBOTs. This discrepancy was due to an increased variance in blood flow after 40 HBOTs vs. 1 HBOT that is evident on the reversal of SD and CV improvements in primarily gray matter ROIs from 1 to 40 HBOTs (2/4 right and left hemisphere white matter ROIs maintained the improvement in SD and CV after 40 HBOTs that were seen after 40 HBOTs). Some of the increased variance might be explained by the timing of imaging (within 4 hours after the 1st HBOT and 48 hours after the 40th HBOT), and the intensive twice/day, 5d/week HBOT schedule. This increased variance is not captured on spm due to the different analytical and statistical methods.

Interestingly, the increased variance after 40 hbots was not seen in The combination of intense HBOT and imaging within 48 h after 40th HBOT may account for some of the variance in MCP changes, SD, and CV after 40 HBOTs.

Texture analysis also demonstrated significant SPECT improvements with decreases in SD and CV (normalization of pattern) after 1 HBOT with partial regression of most of these improvements after 40 HBOTs, except in 2/4 right and left hemisphere white matter ROIs. The loss of the improved SD and CV from 1 to 40 HBOTs is consistent with the increased variance in flow measurements after 40 HBOTs.

The differential effect of 40 HBOTs on white vs. gray matter is consistent with a biological effect of repetitive HBOT 1.5 ATA on the primary injury site in mild-moderate TBI, the white matter (Lipton, 2009; Kraus, 2007).

Significant improvements in SPECT occurred after both 1 and 40 HBOTs, however by historical precedent and design symptoms, cognition, and QoL were only tested after 40 HBOTs . The symptomatic, cognitive, and QoL improvements evolved over the course of the treatment and no subject claimed significant symptomatic improvement after the first HBOT. The dichotomous findings of SPECT improvement after 1 and 40 HBOTs and neurological function after 40 HBOTs only, and the differential effect of 40 HBOTs on white vs. gray matter SPECT texture analysis strongly suggest different physiologic effects of one and 40 HBOTs on injured brain at different points in the treatment process. Furthermore, the differential effect of 40 HBOTs on white vs. gray matter is consistent with a biological effect of repetitive HBOT 1.5 ATA on the primary injury site in mild-moderate TBI, the white matter (Lipton, 2009; Kraus, 2007). Some of the SPECT findings may also be due to imaging at different times after HBOT (within 4 hours after the 1st HBOT and 48 hours after the 40th HBOT), and the intensive twice/day, 5d/week HBOT schedule. The combination of intense HBOT and imaging within 48 h after 40th HBOT may account for some of the variance in MCP changes, SD, and CV after 40 HBOTs.

An unexpected finding was the confirmation of reduction in PTSD that was symptomatically observed in our first published case of PCS/PTSD (Harch, 2009a). In the present study subjects achieved a 30% reduction in PTSD scores in a 30 day period. A biological substrate for this HBOT effect is difficult to identify. Symptomatically, combat blast-induced PCS is inextricably interwoven

with blast-induced PTSD. PCS and PTSD share some common biological pathways, processes, and anatomy in the brain (Kennedy et al., 2007). The hippocampus, in particular, is a pathological target in both PCS (Umile, 2002) and PTSD (Woon, 2008; Bremner, 2007; Wang, 2010). HBOT treatment of hippocampal PCS injury may explain some of the observed effect on PTSD symptom reduction in our study.

Explanatory mechanisms for the HBOT effects are numerous. Neubauer (Neubauer, 1990) demonstrated that increased brain blood flow after a single HBOT in chronic cerebral ischemia (The Neubauer Effect) predicted subsequent neurological improvement with repetitive HBOT. Ischemia is a known pathological process in traumatic brain injury (Gaetz, 2004). Focal ischemia causes a post-transcriptional metabolic/protein synthesis impairment to neurons, termed the "ischemic" freeze (Hossman, 1993). The first HBOT may override this "ischemic freeze" consistent with Siddiqui's demonstration of improved oxygen capacitance of non-CNS ischemic tissue (Siddiqui, 1997). The increase in blood flow on SPECT after 1 HBOT in our study may reflect this reversal of impaired protein synthesis . Simultaneously, it may test vascular reserve capacity similar to the Wada test (Vorstrup, 1988).

The global improvements in brain blood flow after 1 HBOT in our subjects were associated with improved function after 40 HBOTs, thus supporting the Neubauer Effect's prediction of neurological improvement. Spm analysis demonstrated considerable overlap of the areas with improved blood flow after 1 HBOT with those after 40 HBOTs, indicating that the areas identified on SPECT by the Neubauer Effect are likely those responsible for neurological improvement after 40 HBOTs. We have demonstrated the Neubauer Effect in

severe chronic TBI patients (Harch et al., 1994; Neubauer et al., 1994; Harch et al.,1996a; Harch and Neubauer, 1999; Harch and Neubauer, 2004a; Harch and Neubauer, 2009b and c), along with a pattern shift on SPECT after the first HBOT. The pattern shift consists of normalization (relative decrease in high and increase in low blood flow)-(Harch, et al 1996a; Harch and Neubauer, 1999; Harch and Neubauer, 2004b) that is captured by a reduction in SD and CV in this study. The first HBOT would not be expected to improve function, however, due to delivery to blast-induced white matter degenerated brain (Bauman, 2009).

The increased blood flow on SPECT, variance in MCP change, and improved neurological function after 40 HBOTs suggests a set of mechanisms different from those after 1 HBOT. We propose that these mechanisms are the typical trophic mechanisms of HBOT in chronic non-central nervous system wounds (Gesell, 2009). Repetitive HBOT stimulates angiogenesis in chronic non-CNS wounding (Marx, 1990), most likely by genomic effects (Godman, 2009) and has been shown to increase blood vessel density in injured hippocampus in our chronic rat TBI model where the progenitor of this HBOT protocol was tested (Harch, 2007). HBOT-induced increased hippocampal blood vessel density in this model highly correlated with improved spatial learning and memory. In our subjects SPECT spm analysis showed significant improvements in blood flow in hippocampus while our subjects achieved significant gains in memory. These blood flow and memory improvements in our subjects are consistent with a trophic effect of HBOT on chronic brain wounding in the hippocampus and possible healing/reinnervation of denervated tissue (Bauman, 2009).

Other mechanisms may contribute to the HBOT effects in our study. A single hyperbaric oxygen reoxygenation causes prolonged excitability and neural

plasticity of hippocampal neurons after exposure to hypoxia (Garcia, 2010), consistent with the Neubauer Effect generated in this study. Repetitive HBOT has shown increased neurogenesis and cerebral blood flow in chronic global ischemia (Zhang, 2010). Zhang administered repetitive HBOT 30 days after ischemic insult, very similar to the 50 day delay in our animal model (Harch, 2007). Neurogenesis has been shown to occur in association with angiogenesis (Palmer, 2000). As mentioned above, angiogenesis is a known trophic mechanism of HBOT and may be responsible for the increased blood vessel density in our animal model (Harch, 2007). HBOT has also been shown to cause release of bone marrow stem cells into the peripheral circulation (Thom, 2006). Peripheral stem cells are known to cross the blood brain barrier (Mezey, 2003).

The limitations of the present study were lack of confirmation of post-injury brain MRI results in some subjects, unblinded investigators (except for the SPECT brain imaging spm analysis) and lack of a control group. Lack of brain MRI findings confirmation in a few subjects could confound study results only by inadvertent inclusion of non-clinically apparent neurological disease that was manifest on MRI alone. We believe this is a very remote possibility: these young men were highly fit pre-military, underwent regular fitness evaluations while in the military, and had no premorbid disqualifying conditions. All symptomatology commenced with the incident blast and was present continuously since the blast. Routine late MRI evaluations in mild-moderate TBI are usually negative, consistent with the majority of the scans in our subjects. We presume the few missing data points would similarly be normal or non-contributory.

Investigator bias and placebo effects possibly contributed to the magnitude of some of the effects we measured, but are unlikely to account for the majority of the effects or the consistency and magnitude of the effects across all domains, particularly SPECT. Investigator bias could be present in the P.I.'s symptom and physical exam recording and in SRA's neuropsychological testing, but it does not explain the significant SPECT findings where separate independent analyses, one of which was blinded, were performed by EFF in North Dakota and DA and DVT in California. None of the SPECT co-investigators interacted with the subjects and they performed their analyses months after the subjects had completed their final imaging Importantly, the blinded SPECT analyst, DVT, produced the most significant statistical results.

Placebo effects cannot be entirely ruled out, however, there are multiple arguments against this notion. Treatment effect size in two meta-analyses of randomized placebo-controlled trials vs. observational studies performed on the same treatments has been shown to be very similar (Concato, 2000; Benson, 2000). This suggests that placebo effects are overestimated in observational studies such as ours. Placebo effects on many of the cognitive measures in our study have been reported to be smaller than the changes we found with HBOT: (Doraiswamy, 2007: FSIQ, WMS Visual Immediate and Delayed Memory), (Calabrese, 2008: Stroop Reaction Time), (Jorge, 2010: Stroop Color/Word raw score). Placebo effects reported on SPECT in psychiatric disease, healthy individuals, and neurological disease have shown focal changes in regional cerebral blood flow (Beauregard, 2009), most commonly in the inferior frontal gyrus, striatum, and rostral anterior cingulate cortex (Jarcho, 2009). The global diffuse changes we measured have not been reported. In addition, it is highly

improbable that a placebo effect could account for the multiplicity of differential changes on SPECT after 1 and 40 HBOTs using two different forms of mathematical/statistical analyses. Lastly, the parallel improvements in memory scores and hippocampal blood flow are inconsistent with a placebo effect.

Test/retest practice effects could explain some of the cognitive improvements, however, practice effects do not fully explain our measured increases for seven reasons: 1) practice effects on the WAIS-III FSIQ over a mean 34.6 day retest interval have been shown to be 2.0-3.2 points across all age groups, 6 points in the 16-29 year old group, and decrease with age; our subjects averaged 30 years old (Tulsky and Zhu, 1997). They have also been shown to increase 6 points over three or six months retest times (Basso, 2002). Six points is 41% of the measured FSIQ increase on the WAIS-IV in our subjects; 2) the bulk of practice effects occur on the first retest (Bartels et al., 2010; Falleti, 2006) and our subjects had been cognitively tested at least once before our pre-HBOT testing session, Second and third retest (third and fourth tests) effects should have been smaller than six points; 3) Working Memory has been shown to be amongst the most resistant to practice/retest effects (Basso, 2002; Bartels, 2010). Our subjects averaged a 9.9 point statistically significant improvement; 4) Practice effects are usually studied in normal individuals with intact memory function. Intact memory is a prerequisite for learning/practice effects. In individuals with impaired memory function, such as our subjects, practice effects may be less (Basso, 2002); 5) we used the alternate form WASI for the post-treatment I.Q. test in order to minimize practice effects.; 6) Stroop Color/Word score increase in a controlled HBOT study of chronic brain injury produced results similar to ours (Golden, 2006); 7) Stroop Color/Word test/retest effects

across 1 and 2 week intervals are 3.83 points (Franzen, 1987); our increase was 11.1 points.

It is also unlikely that placebo effects could cause statistically significant widespread positive improvement in SPECT, using two different methods of analysis. Texture analysis of SPECT showed differential effects in the two hemispheres, all lobes, between one and 40 HBOTs, significant changes on 33% of all measurements, 75% of which were positive and 25% negative, and which occurred differentially on mean counts, standard deviation, and coefficient of variation /pixel after 1 and 40 HBOTs. Spm analysis showed similar widespread improvements in brain blood flow and considerable overlap of regions with improved brain blood flow after both 1 and 40 HBOTs, a highly improbable phenomenon. In contrast spm showed the greatest improvements after 40 HBOTs while the most significant changes on texture analysis were after 1 HBOT. This disparity strongly suggests different physiologic effects of 1 and 40 HBOTs on brain blood flow that are differentially captured by the two different analytical methods. Lastly, spm demonstrated matching significant improvements in hippocampal rCBF that were consistent with the improvements in WMS memory scores. This is inconsistent with a placebo etiology.

Additionally, placebo effects on SPECT reported in psychiatric disease, healthy individuals, and neurological disease have demonstrated a variety of focal changes or clusters of focal changes in regional cerebral blood flow (Beauregard, 2009) with the inferior frontal gyrus, striatum, and rostral anterior cingulate cortex commonly involved (Jarcho, 2009). Global diffuse changes have not been reported. The SPECT findings in our subjects consist of diffuse changes in rCBF with a change in visual pattern from heterogeneity to homogeneity. This

is similar to the bihemispheric increases in SPECT rCBF reported by Neubauer in a diverse group of 50 chronically brain injured children and adults treated with HBOT between 1.25 and 2.5 ATA (Golden, 2002). Our SPECT findings correspond to a reduction in the coefficient of variation that is consistent with a progression to the pattern of brain blood flow in normal humans at rest, namely a narrow range of brain blood flow. It is also consistent with a global effect on the brain that is reinforced by the additional spm analysis and the global improvements in all of our outcome instruments.

Our results were achieved with half (40 HBOTs) of our normal protocol (80 HBOTs) on an accelerated twice/day schedule The protocol was truncated at 40 HBOTs and compressed to twice/day treatments due to time and fiscal constraints. Through clinical experience, clinical research, and an animal pilot study that compared sham HBOT, 40, and 80 HBOTs (Harch, 1996b) we found greater cognitive, and blood flow improvements (animal model, Harch, 2007) and clinical and blood flow improvements (human cases) with 80 HBOTs, but the cases were primarily chronic moderate-severe TBI (vide supra). Neubauer and Golden (Golden, 2002) reported progressively greater blood flow in a case series of chronic severe brain injured patients receiving 70 low pressure HBOTs. Recently, Wright and Zant (2010) reported the effectiveness of our HBOT 1.5 protocol in two airmen with blast-induced PCS, using 40 and 80 HBOTs (for persistent symptoms after 40 HBOTs). Our subjects finished HBOT with partial improvement in their symptoms. It is likely that additional HBOT would be beneficial.

In conclusion, application of a lower pressure protocol of forty HBOTs at 1.5 ATA to a sixteen subject cohort of military subjects with blast-induced

chronic PCS and PTSD was found to be safe. One fourth of the subjects experienced transient clinical deterioration halfway through the protocol and one subject did not finish. Simultaneously, as a group the fifteen subjects experienced notable improvements in symptoms, abnormal physical exam findings, cognitive testing, PCS and PTSD symptom questionnaires, quality of life questionnaires, depression and anxiety indices, and SPECT brain blood flow imaging that are inconsistent with the natural history of PCS 2.8 years post injury. The symptomatic improvements were present at six month phone followup in 92% of subjects who reported improvement after 40 HBOTs. More objective psychometric testing and SPECT imaging were not performed to confirm durability of the HBOT treatment effect. Sixty-four percent of the patients on psychoactive and narcotic prescription medication were able to decrease or eliminate usage of these medications. This data is preliminary and needs confirmation with larger numbers of subjects or with a stronger design such as a randomized or Bayesian study.

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Author Disclosure Statement

Dr. Harch owns a small consulting company called Harch Hyperbarics, Inc. which has no contracts.

Dr. Andrews: No competing financial interests exist.

Juliette Lucarini, R.N: Tenant in common ownership of Harch Hyperbarics, Inc.

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Table 1.

	Pre	**Post**	**Domain Measured**
Combat Experience Scale (Keane et al., 1989)	X	-	Combat experience
Green Word Memory Test (Lesak et al., 2004)	X	-	Effort
Wechsler Test of Adult Reading (Wechsler, 2001)	X	-	Estimate premorbid IQ
Michigan Alcohol Screening Test (MAST, Revised, 2009)	X	X	Alcohol use
Drug Abuse Screening Test (Gavin et al., 1989)	X	X	Drug use
Percent Back To Normal Rating (Powell et al.,	X	X	Rating Recovery

2001)-"Current percent of normal premorbid level of function"			
Rivermead Post Concussion Symptom Quest. (King et al., 1995)	X	X	PCS
PTSD Checklist-Military (PTSD, 2009)	X	X	Rating PTSD
Wechsler Adult Intelligence Scale-IV (WAIS-IV, 2009)	X		IQ
Wechsler Abbreviated Scale of Intelligence (WASI, 2009)		X	IQ
Test Of Variables of Attention (Greenberg, 1996)	X	X	Attention
Stroop Test (Lesak et al., 2004)	X	X	Attention, Executive Function
Finger Tapping Test (Reitan et al., 1993)	X	X	Motor Speed
Grooved Pegboard (Reitan et al., 1993)	X	X	Motor Coordination
Wechsler Memory Scale – IV (WMS-IV, 2009)	X	X	Memory, Ex Function
Rivermead Paragraph Memory (Wilson et al., 1985)	X	X	Memory
Perceived Quality Of Life (Patrick et al., 1988)	X	X	Satisfaction
Patient Health Questionnaire – 9 (Kroenke et al., 2001)	X	X	Depression
Generalized Anxiety Disorder – 7 (Spitzer et al., 2006)	X	X	Anxiety

List of Psychometric Measures, When Administered, and Domain Measured

Table 2. Subject Demographic Characteristics. Numerical variables are summarized as mean and range (minimum to maximum).

Patient Characteristic	**Safety Population (All Enrolled Subjects)**
Number of subjects	16
Sex	All male
Average age (years)	30 (21 – 45)
TBI-to-HBOT Interval (years)	2.8 (1.25 – 4.75)
Duration of loss of consciousness (LOC)-(minutes)	Mean = 2.0 for 13 Subjects (1 – 10 mins); excluding 2 subjects (4.5 hrs and 9 hrs)
Service at time of LOC (years)	6.0 (1 – 17)
# blast TBI's with LOC or altered LOC	2.7 (1 – 7)
RPCSQ Score (scale: 0-64)	39 (27-47)
PCL-M Score (scale: 17-85)	67 (48-84)
HBOT's / day	39 HBOT's (27 – 40)/29 days (16 – 43)
MAST score (scale: 0 – 22)	2.1 (0 – 3)
DAST score (scale: 0 – 20)	0.6 (0 – 3)
Disability Rating (scale: 0 – 30)	1.6 (.5 – 3)

PBNRS pre study	43% (5-72.5)
Pre-TBI Estimated I.Q. (Average)	104.9
Years of Education (Average)	12.9

Table 3. Symptom Changes (15 subjects). Combined symptoms from subjects' prioritized symptom list and primary author's standard questionnaire.

Symptom	**Better (%)**	**No Change (%)**	**Worse (%)**
Headache	**87** (13/15)	13 (2/15)	0
Sleep Disruption	**75** (9/12)	25 (3/12)	0
Short-term Memory	**92** (11/12)	8 (1/12)	0
Cognition	**93** (14/15)	7 (1/15)	0
Energy Level	**87** (13/15)	13 (2/15)	0
"PTSD Symptoms"-sic, (P) or Nightmares (N)	**50** (2/5)-P (2/3)-N	50 (3/5)-P (1/3)-N	0

Short-temper/Irritability	**82** (9/11)	18 (2/11)	0
Mood Swings	**87** (13/15)	13 (2/15)	0
Imbalance	**55** (6/11)	45 (5/11)	0
Fine motor Incoordination	**75** (3/4)	25 (1/4)	0
Decreased Hearing	20 (2/10)	**80** (8/10)	0
Tinnitus	37 (3/8)	**63** (5/8)	0
Depression	**93** (13/14)	7 (1/14)	0
Arthralgias	0	**100** (5/5)	0
Photophobia	**44** (4/9)	**44** (4/9)	11 (1/9)

Table 4. Abnormal Physical Finding Changes (15 subjects).

Abnormal Physical Finding	**Better (%)**	**NoChange (%)**	**Worse %**
Tests of Balance: Tandem Gait	**100** (14/14)	0	0
Romberg (Eyes closed, hands at sides, feet together x 30 sec.)	**93** (14/15)	7 (1/15)	0
Unterberger exam (arms outstretch, eyes closed, marching in place x 30 sec.)	**87** (7/8)	13 (1/8)	0
Tests of Coordination: Finger to Nose Heel to Shin	**91** (10/11) **100** (5/5)	9 (1/11) 0	0 0
Dysdiadochokinesis	**100** (5/5)	0	0
Rapid Finger Tapping	**90** (9/10)	10 (1/10)	0
Motor Tests: Focal Weakness-upper or lower extremity	**71** (5/7)	29 (2/7)	0

Deep Knee Bend-Strength and Stability	**75** (6/8)	12.5 (1/8)	12.5 (1/8)
Tremor	**100** (5/5)	0	0
Sensory Tests: Focal Hypesthesia	**89** (8/9)	11 (1/9)	0

Table 5. Pre to Post HBOT Change for Neuropsychological Outcome Variables (significant values in red).

Outcome Variables[1]	**Pre-HBOT Mean +/-SD (15) Median (Range)**	**Post-HBOT Mean +/-SD (15) Median (Range)**	**Pre:Post Diff +/-SD 95% CI[2]**	**Signif of Pre to Post [3]**
Full Scale IQ[4]	95.8 +/-8.4 98 (80-106)	110.6 +/-10.3 110 (97-129)	14.8 ± 7.4 CI: 10.7 to 18.9	**p<0.001**
Delayed	97.7 ± 13.3	106.9 ± 15.4	9.2 ± 14.3	**p=0.026**

[1] All scores reported in Standardized Scores except for the Rivermead Paragraph Memory subtest
[2] CI=Confidence Interval
[3] p values are by the paired Student t test, unless the data was not normally distributed, in which case the non-parametric Wilcoxon Signed-Ranks test was used.
[4] Pre-HBOT was Wechsler Adult Intelligence Scale-IV and Post-HBOT was Wechsler Abbreviated Scale of Intelligence

Memory (WMS-IV)	94 (76-125)	107 (80-142)	CI: 1.3 to 17.1	
Rivermead Paragraph	9.5 ± 2.4 (15) 10 (6 – 14)	7.5 ± 3.6 (15) 8 (2 – 13)	-2.1 ± 3.7 CI: -4.1 to -0.0	**p=0.049**
Working Memory (WMS-IV)	97.0 ± 13.6 91 (85-131)	106.9 ± 13.1 105 (88-127)	9.9 ± 10.3 CI: 4.1 to 15.6	**p=0.003[5]**
Stroop Color/Word Interference	84.3 ± 12.2 80 (65-108)	95.3 ± 12.8 94 (67-118)	11.1 ± 9.2 CI: 6.0 to 16.2	**p<0.001**
TOVA[6] Inattention	73.3 ± 29.6 (15) 86 (40 – 107)	75.8 ± 27.2 (15) 85 (40 – 107)	2.5 ± 22.8 CI: -10.1 to 15.2	p=0.514
TOVA Impulsivity	89.6 ± 24.9 (15) 90 (40 – 123)	98.6 ± 23.1 (15) 107 (40 – 118)	9 ± 16.2 CI: 0.0 to 18.0	**p=0.041**
TOVA Reaction Time	93.1 ± 22.5 (15) 99 (53 – 120)	99.1 ± 14.6 (15) 103 (70 – 123)	5.9 ± 19.3 CI: -4.8 to 16.6	p=0.254
TOVA Variability	64.4 ± 28.7 45 (40-111)	75.3 ± 24.6 80 (40-111)	10.9 ± 20.2 CI: -0.2 to 22.1	**p=0.045**

[5] np=non-parametric Wilcoxon Signed-Ranks test
[6] TOVA= Test of Variables of Attention

FingerTap Dominant H	90.9 ± 18.3 (15) 93 (55 – 118)	98.6 ± 15.0 (15) 98 (75 – 130)	7.7 ± 20.7 CI: -3.8 to 19.2	p=0.174
FingerTap NonDominant	90.0 ± 21.5 (15) 95 (40 – 118)	94.0 ± 25.2 (15) 91 (40 – 130)	4 ± 18.5 CI: -6.2 to 14.2	p=0.416
Grooved Pegbrd Dom	88.9 ± 19.8 (15) 88 (55 – 124)	96.8 ± 18.8 (15) 98 (65 – 129)	7.9 ± 12.4 CI: 1.0 to 14.7	**p=0.028**
Grooved Pegbrd NonD	84.0 ± 22.0 (15) 85 (40 – 120)	87.3 ± 22.8 (15) 85 (40 – 118)	3.3 ± 15.3 CI: -5.2 to 11.8	p=0.423

Table 6. Significance of Pre to Post HBOT Change for Psychological Outcome Variables.

Outcome Variables	**Pre-HBOT Mean +/-SD (15) Median (Range)**	**Post-HBOT Mean +/-SD (15) Median (Range)**	**Pre:Post Diff +/-SD 95% CI**	**Signif of Pre to Post**
Rivermead PCS	39.7 +/-6.0 40 (27-47)	24.1 +/-12.6 26 (0-42)	-15.6 ± 12.8 CI: -22.7 to -8.5	**p=0.0002**

PCL-M	67.4 ± 10.5 68 (48-84)	47.1 ± 16.0 46 (24-69)	-20.3 ± 18.2 CI: -30.4 to -10.2	**p<0.001**
PHQ-9 Depression	16.6 ± 4.9 18 (5-24)	8.2 ± 4.7 7 (2 - 17)	-8.4 ± 7.4 CI: -12.5 to -4.3	**p<0.001**
GAD-7 Anxiety	12.7 ± 5.8 14 (4-21)	7.9 ± 5.3 7 (0-21)	-4.8 ± 5.8 CI: -8.0 to -1.6	**p=0.007**
Perceived QOL	81 ± 37 74 (29-154)	114 ± 36 125 (42-161)	33 ± 36 CI: 13 to 53	**p=0.003**
% Back to N: Cognitive	49.7 ± 17.0 50 (20 – 85)	68.9 ± 20.0 75 (30 – 95)	19.2 ± 17.9 CI: 9.3 to 29.1	**p<0.001**
% Back to N: Physical	46.7 ± 22.2 45 (10 – 85)	67.5 ± 18.5 70 (25 – 90)	20.9 ± 16.3 CI: 11.8 to 29.9	**p<0.001**
% Back to N: Emotional	32.3 ± 19.9 30 (5 – 80)	63.2 ± 20.5 65 (30 – 90)	30.9 ± 21.7 CI: 18.8 to 42.9	**p<0.001**

Table 7. MCP, SD, and CV Counts/Pixel SPECT brain blood flow in Right (R) and Left (L) Hemisphere ROIs of a single transverse slice in the centrum semiovale

[30, 60, 90, 120, and 150° Gray (G) matter and 60 and 120° White (W) matter] before HBOT (Pre), post 1 HBOT (P1), and post 40 HBOTs (P40).

	MCP	MCP	MCP	SD	SD	SD	CV	CV	CV
ROI	Pre	P1	P40	Pre	P1	P40	Pre	P1	P40
R30°G	1,433 ±263	1,611 ±274	1,609 ±399	58.1 ±24.9	53.6 ±11.8	69.0 ±37.0	4.07 ±1.61	3.38 ±0.74	4.28 ±1.95
R60°G	1,451 ±284	1,635 ±326	1,655 ±324	54.2 ±22.7	51.3 ±15.3	70.5 ±24.2	3.72 ±1.25	3.23 ±1.02	4.33 ±1.36
R90°G	1,354 ± 263	1,500 ±305	1,504 ±302	53.0 ±17.2	41.3 ±17.6	59.0 ±30.0	3.99 ±1.32	2.75 ±0.96	3.94 ±1.72
R120°G	1,416 ±248	1,604 ±294	1,605 ±311	56.0 ±31.0	51.5 ±23.6	57.4 ±22.1	3.82 ±1.71	3.13 ±1.03	3.57 ±1.06
R150°G	1,501 ±253	1,740 ±329	1,767 ±348	71.0 ±34.0	64.0 ±35.0	81.0 ±43.0	4.66 ±1.72	3.73 ±1.88	4.59 ±2.13
R60°W	820 ±156	899 ±171	983 ±235	59.0 ±22.7	41.6 ±16.0	44.1 ±19.8	7.30 ±2.61	4.58 ±1.56	4.61 ±2.07
R120°W	761 ±162	851 ±218	838 ±219	62.7 ±25.7	48.8 ±18.9	55.0 ±29.3	8.15 ±2.83	5.87 ±2.19	6.80 ±3.60
L30°G	1,405 ±260	1,593 ±305	1,603 ±338	83.0 ±32.0	56.2 ±18.3	95.0 ±44.0	6.12 ±2.70	3.60 ±1.20	6.30 ±3.40
L60°G	1,367 ±274	1,558 ±309	1,614 ±351	85.0 ±43.0	68.8 ±22.5	79.3 ±28.0	6.40 ±3.70	4.41 ±1.05	5.23 ±2.68
L90°G	1,338	1,516	1,581	62.9	52.1	55.3	4.72	3.37	3.62

	±238	±319	±355	±28.6	±20.6	±18.5	±1.97	±0.90	±1.37
L120°G	1,385 ±246	1,601 ±331	1,637 ±309	58.0 ±23.0	42.0 ±12.4	60.0 ±35	4.30 ±1.96	2.67 ±0.74	3.58 ±1.77
L150°G	1,536 ±250	1,728 ±336	1,747 ±318	70.2 ±29.7	45.9 ±17.1	69.6 ±29.2	4.65 ±1.91	2.70 ±0.95	4.04 ±1.76
L60°W	818 ±120	998 ±292	1,013 ±254	56.7 ±26.1	56.0 ±50.0	68.0 ±30.0	6.90 ±3.00	5.13 ±2.59	6.91 ±2.82
L120°W	745 ±117	882 ±136	922 ±248	63.4 ±19.1	53.8 ±18.6	56.8 ±29.9	8.58 ±2.53	6.15 ±2.01	6.20 ±3.10

Table 8. Significant changes in MCP, SD, and CV Counts/Pixel from pre-HBOT to after the first HBOT (PP1; post 1 HBOT minus pre), pre-HBOT to after 40 HBOTs (PP40; post 40 HBOTs minus pre), and post first HBOT to post 40 HBOTs (P1,40; post 40 HBOTs minus post 1 HBOT), in right (R) and left (L) hemisphere ROIs at 30, 60, 90, 120, and 150° of gray (G) matter and 60 and 120° of white (W) matter of a transverse SPECT slice in the centrum semiovale. Positive changes were assigned to significant increases in MCP and decreases in SD and CV and are shaded blue. Near positive significant changes in MCP, SD, and CV are shaded green. Negative changes were assigned to decreases in MCP, and increases in SD and CV and are shaded red. Numerical figures are p values. Note differences in right and left hemisphere MCPs post first and 40th HBOT, significant reductions

in the SD and CV after the first HBOT in both gray and white matter and in the white matter only after 40 HBOTs while a reversal of this effect, significant increases, were seen in the SD and CV between the 1st and 40th HBOT in mostly gray matter and one white matter site.

Measure-ment	MCP PP1	MCP PP40	MCP P1,40	SD PP1	SD PP40	SD P1,40	CV PP1	CV PP40	CV P1,40
R-30-G	0.038	0.170	.0.987	0.471	0.389	0.174	0.098	0.772	0.152
R-60-G	0.052	0.120	0.861	0.441	0.056	0.012	0.012	0.204	0.018
R-90-G	0.076	0.217	0.967	0.020	0.282	0.053	<0.001	0.865	0.010
R-120-G	0.031	0.134	0.991	0.616	0.895	0.539	0.155	0.604	0.294
R-150-G	0.015	0.051	0.825	0.035	0.302	0.017	0.008	0.879	0.044
R-60-W	0.068	0.055	0.244	0.011	0.080	0.715	0.002	0.007	0.964
R-120-W	0.045	0.261	0.804	0.057	0.476	0.510	0.011	0.107	0.934
L-30-G	0.028	0.098	0.936	0.001	0.388	0.003	<0.001	0.821	0.004
L-60-G	0.025	0.059	0.630	0.210	0.582	0.192	0.048	0.127	0.679
L-90-G	0.069	0.066	0.563	0.185	0.417	0.557	0.009	0.068	0.463
L-120-G	0.025	0.040	0.756	0.052	0.842	0.151	0.012	0.240	0.082
L-150-G	0.041	0.075	0.874	<0.001	0.937	0.003	<0.001	0.180	0.001
L-60-W	0.014	0.034	0.884	0.599	0.050	0.083	0.055	0.989	0.030
L-120-W	0.014	0.003	0.568	0.237	0.545	0.714	0.029	0.037	0.943

Table9. Top eleven clusters of voxels showing significant increases in brain blood flow after 1 HBOT compared to baseline scan. Significance level raised to $p < 0.001$ with FWE.

Increases in rCBF Post 1st HBOT					
	Cluster Size		Coordinates		
Brain Area	**K_E**	**T**	**X**	**Y**	**Z**
1. Precentral Left	946	30.18	-32	-22	56
2. Temporal Lobe Left	615	29.64	-62	-26	10
3. Precuneus Right	1663	27.81	22	-52	24
4. Thalamus Right	89	25.15	-10	-6	-8
5. Post Central Right	211	24.44	42	-30	66
6. Occipital Left	427	23.36	-20	-72	20
7. Lingual Left	50	23.22	-20	-68	-10
8. Temporal Inferior to Mid-Lateral Right	103	22.66	44	-2	-36
9. Temporal Inferior to Mid-Lateral Left	266	22.33	-52	-64	-4
10. Frontal Inferior Triangle Left	75	22.24	-48	26	8
11. Superior Motor Area Right	134	22.09	12	16	50

Table 10. Top ten clusters of voxels showing significant increases in brain blood flow after 40 HBOTs compared to baseline scan. Significance level raised to $p < 0.0001$ with FWE.

Increases in rCBF Post 40th HBOT					
	Cluster Size		Coordinates		
Brain Area	K_E	T	**X**	**Y**	**Z**
1. Frontal Mid to Mid Orbital Latera Right	314	39.94	38	42	6
2. Occipital Superior to Calcarine Right	7126	38.78	22	-78	28
3. Temporal Pole Superior to Insula Left	112	34.14	-34	6	-18
4. Temporal Superior to Post Central Left	438	33.8	-55	-12	4
5. Cerebellum to Temporal Inferior Left	1146	33.47	-22	-76	-40
6. Calcarine Left	212	29.99	-6	96	4
7. Frontal Inferior Triangle to Mid Lateral Orbital Left	370	27.79	-36	42	0
8. Parietal Superior to Inferior Right	401	27.11	34	-52	58
9. Precuneus to Para Central Lobule Right	693	26.89	10	-44	54
10. Superior Motor Area Left	228	26	-2	18	52

Table 11. Top ten clusters of voxels showing significant increases in brain blood flow common to brain scans after 1 HBOT ($p < 0.01$ with FWE) and 40 HBOTs ($p < 0.001$ with FWE).

Increases in rCBF Post 40 HBOTs Masked by Scan Post 1 HBOT

	Cluster Size		Coordinates		
Brain Area	**K_E**	**T**	**X**	**Y**	**Z**
1. Occipital Superior to Temporal Right	1581	38.78	22	-78	28
2. Temporal Superior Left	514	33.8	-56	-12	4
3. Temporal Right	360	31.84	52	-32	-20
4. Precentral Left	917	29.4	-44	-2	40
5. Parietal Superior Right	11	26.2	34	-52	60
6. Cuneus to Occipital Left	405	24.04	-12	-86	16
7. Cerebellum Left	71	22.88	-38	-58	20
8. Cerebellum to Lingual Right	24	22.02	14	-56	10
9. Post Central to Supra-Marginal Right	203	21.51	36	-32	68
10. Rolandic Operculum Right	85	21.11	-60	-18	12

Harch.Legends

Figure 1. Visual demonstration of SPECT (gray scale) pattern change from heterogeneity (pre-HBOT, left) to homogeneity (post one HBOT, right) in sample transverse centrum semiovale slice. Inset histogram of each image shows counts in the white matter elliptical ROI (Entire centrum white matter ROI was used for

demonstration purposes only. Actual ROIs are seen in Figure 2). Note the broader range of counts in the pre-HBOT scan vs. the narrower concentration of counts post 1 HBOT. Visually, this is appreciated best in the cortical rim.

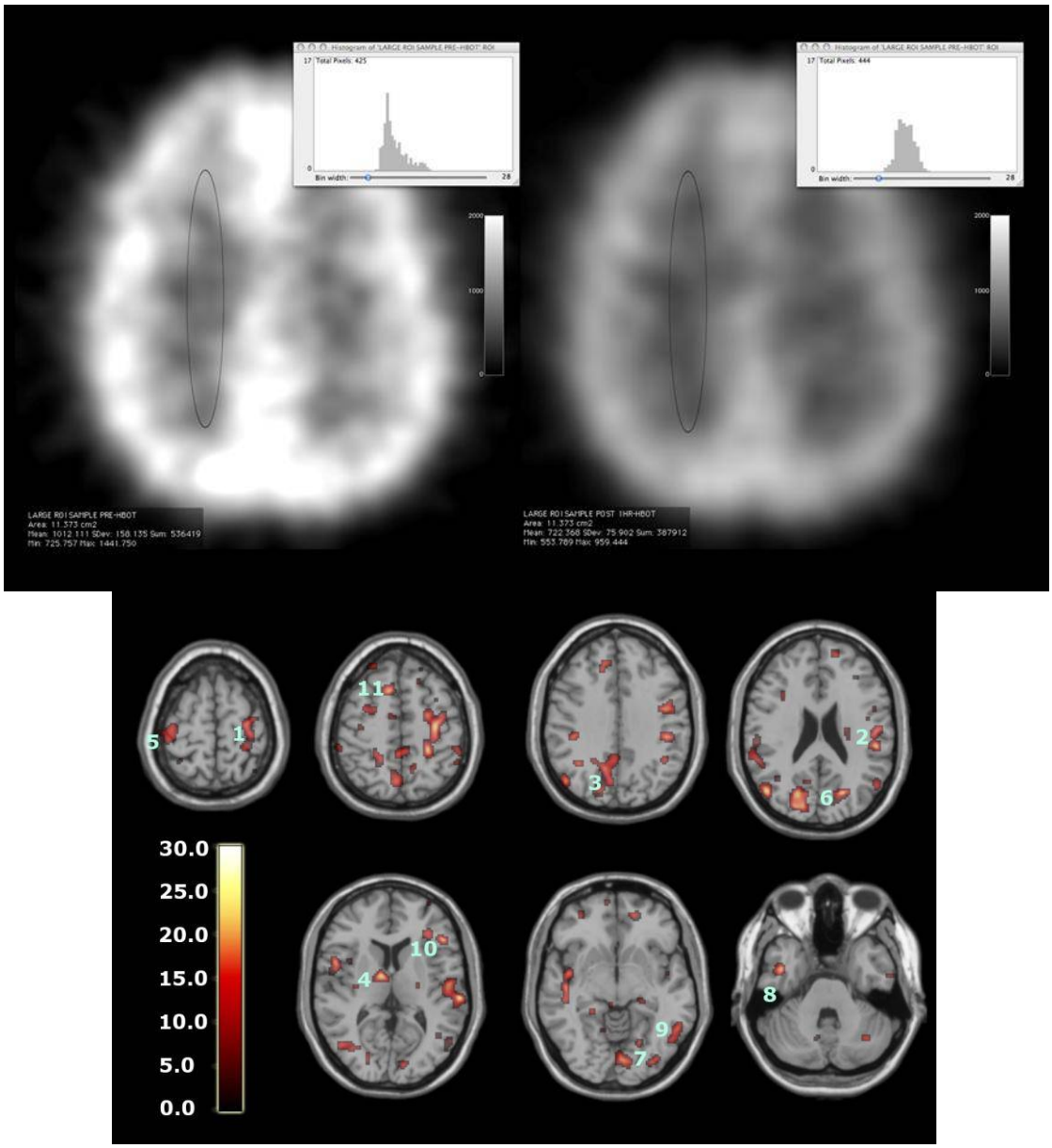
Figure 2. Fusion of significant SPECT clusters after 1 HBOT with standard reference MRI T1 transverse image**.** Numbers correspond to the top eleven significant clusters at the $p < 0.001$ level labeled in Table 9, numerically in order from highest T value to lowest. Significant clusters incidentally occurring on the same slices are also depicted. (Color bar shows relative amplitude of rCBF improvement).

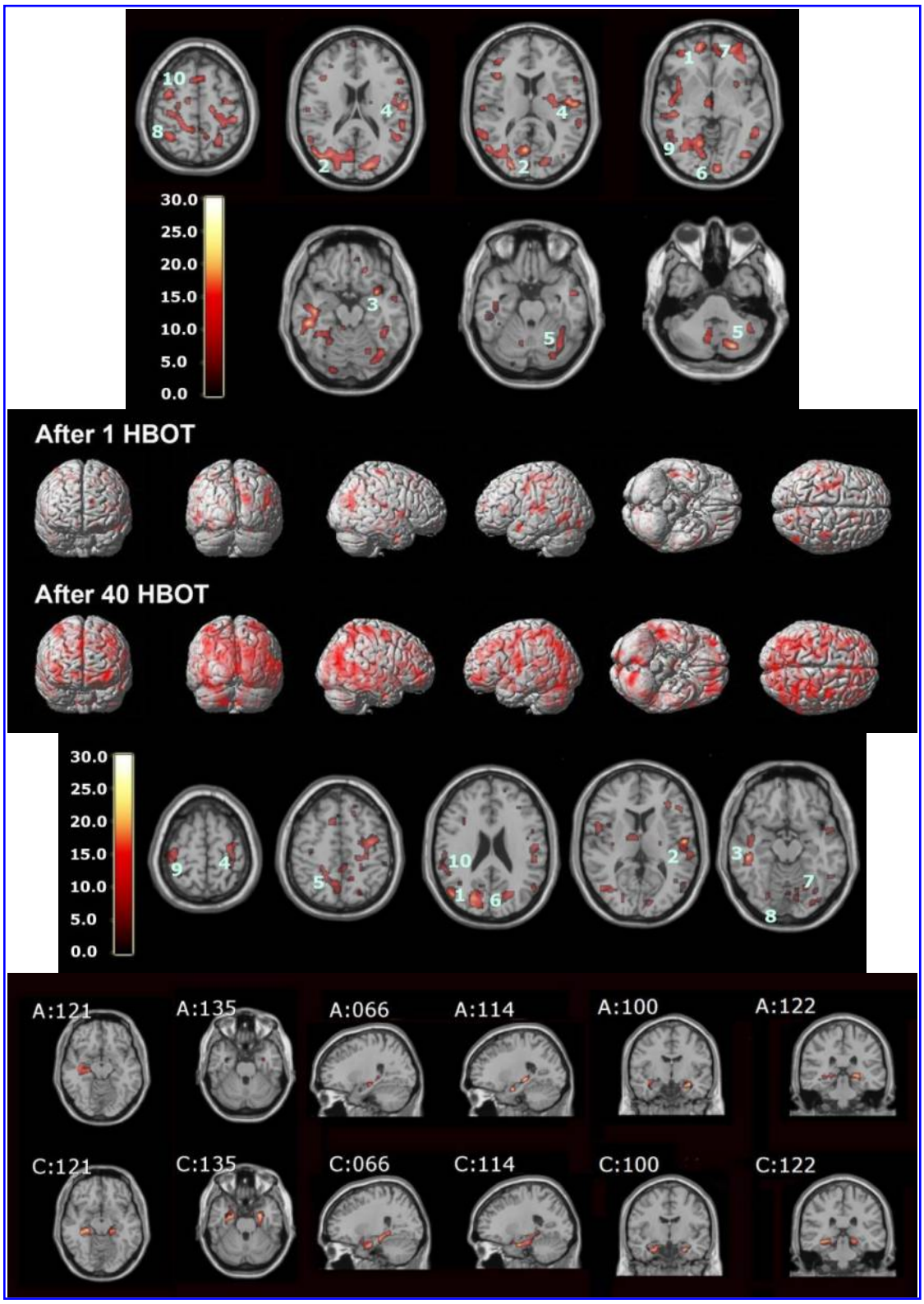
Figure 3. Fusion of significant SPECT clusters after 40 HBOTs with standard reference MRI T1 transverse image. Numbers correspond to the top ten significant clusters at the $P < 0.0001$ level labeled in Table 10, numerically in order from highest T value to lowest. Significant clusters incidentally occurring on the same slices are also depicted. (Color bar shows relative amplitude of rCBF improvement).

Figure 4. Cortical Views from front, back, right, left, inferior, and superior aspects show effects of 1 HBOT (top row) and 40 HBOTs (bottom row) at a significance level of $p < 0.001$. Significant increases are in red.

Figure 5. Fusion of significant SPECT clusters after 40 HBOTs masked by clusters after 1 HBOT with standard reference MRI T1 transverse image. Numbers correspond to the top 10 significant clusters in Table 11 at the $P < 0.001$ when masked inclusively by results after one scan at the $p < 0.01$ level, numerically ordered from highest to lowest T value. Significant clusters incidentally occurring on the same slices are also depicted. (Color bar shows relative amplitude of rCBF improvement).

Figure 6. Fusion of significant SPECT hippocampal increases in rCBF with standard reference MRI T1 transverse, sagittal, and coronal slices after 1 HBOT (Row A) and 40 HBOTs (Row C), $p < 0.001$ with FWE.





Harch. APPENDIX

Standardized Questionnaire:

1. Energy level on 1-10 scale (10 was pre-LOC energy level, 0 is inability to get out of bed).
2. Weight change since injury.
3. Mood swings.
4. Irritability/short temper.
5. Mood, 1-10 scale (10 is happiest in life, 0 is not wanting to live).
6. Cranial and cranial nerve symptoms: headache, dizziness, visual symptoms, loss of hearing, tinnitus, vertigo, change in smell/taste, trouble talking, enunciating, swallowing, chewing.
7. Sensory symptoms: numbness, tingling.
8. Motor: focal or generalized weakness.
9. Incoordination: fine motor (hands/fingers), gross motor (tripping, stumbling, imbalance).
10. Cognitive: trouble thinking/grasping ideas, organizing thoughts, decreased speed of thinking, confusion, problems following directions/instructions, difficulty expressing thoughts/word-finding, forgetfulness, misplacing/losing things, problems remembering old information or new information, losing one's place in thought or conversation or while driving, going blank, staring episodes, feeling suddenly lost or disoriented, concentration/attention problems, difficulty writing, family or friends commenting on change in personality or behaviour.
11. Joint pain or swelling.
12. Incontinence of bowel or bladder.

Neurological exam:

1. Cranial nerves: II-XII.
2. Deep tendon reflexes upper and lower extremities.
3. Motor: tone, mass, tremor, deep knee bend, strength, tiptoe and heel walking.
4. Sensory: pin and touch in the four extremities.
5. Gait: normal, tandem (slow and fast).
6. Pathological reflexes: glabellar, snout, palmomental, grasp, suck, root, Hoffman, Babinski, clonus.
7. Cerebellar: Romberg, finger tapping speed/rhythm, elbow flexion check response, finger to nose testing, heel to shin gliding, rapid alternating hand movements-palm/dorsal hand thigh slapping.