NARRATIVE:
LOW PRESSURE HYPERBARIC OXYGEN THERAPY IN THE TREATMENT OF POST CONCUSSION SYNDROME AND CHRONIC TRAUMATIC BRAIN INJURY (Paul G. Harch, M.D.)

BACKGROUND:

I. THE IMPACT OF TRAUMATIC BRAIN INJURY (TBI). TBI is a disorder of major public health significance. Each year in the United States there are 100 new cases/100,000 population and 52,000 deaths with the highest incidence in the 15-24 and over 75 years old age groups[1]. Most patients survive and add to an increasing prevalence of patients with chronic TBI, estimated at 2.5-6.5 million individuals in 1998[1]. TBI is the leading cause of long-term disability in children and young adults[1]. Patients experience disruption in health, cognition, behavior, emotional function, social function, work, school, and family life with a resultant substantial economic toll[1]. This economic toll was estimated in 1998 to be $9-10 billion/year for acute care and rehab of new cases[1]. Lifetime costs of care are much higher and remarkably underestimated by exclusion of lost earnings, costs to social service systems, and the value of the time and foregone earnings of family members who care for persons with TBI[1]. One source estimated the direct and indirect costs at $56 billion in 1995[2].

While the above figures are significant, they grossly underestimate the scope of the problem for at least two reasons: 1) Reporting bias against mild TBI. The aforementioned figures are based exclusively on information about hospitalized patients and those who die before hospitalization, i.e., moderate and severe TBI. These groups represent only 15-25% of the total TBI population[3,4], and 2) Significant under diagnosis of mild TBI[1]; 20-40% of patients with mild TBI never seek medical attention[5]. More accurate figures over a decade ago for mild TBI were 180/100,000 population[6]. Consequently, mild TBI has been characterized as a "hidden epidemic.[7]" Since 2001, however, this "hidden epidemic" has been augmented by U.S. war veterans from Iraq and Afghanistan with TBI. The Pentagon estimates that 11% to 28% of combat troops may have been exposed to bomb blasts and suffered at least mild traumatic brain injury with as many 400,000 potentially injured.[8] At Walter Reed Army Medical Center 30% of Iraq and Afghanistan casualties have been diagnosed with mild, moderate or severe traumatic brain injury[8]. The impact of these figures on military readiness, the voluntary service, return to combat, combat effectiveness, and military families are inestimable and of major public concern.

TBI survivors include patients that span the entire spectrum of TBI and TBI disability: mild, moderate, and severe. TBI is a graded injury with degree of injury, pathological findings, and disability proportional to the magnitude of force impacting the head (see discussion below). Progressively greater force causes greater pathological damage which is composed of elements of injury found in less severe forms of TBI (axonal injury) as well as the pathology specific to more severe levels of injury (parenchymal hemorrhage, extra-parenchymal hemorrhage, etc.). As a result, conclusions drawn about treatment of the pathology and disability of mild TBI should also be applicable to more severe degrees of TBI. Despite different definitions of mild TBI[9], 15-29% of the mild TBI population have appreciable complaints 6 months after injury[10]. These ongoing symptoms have been termed the Post-Concussion Syndrome (PCS)[11], and this syndrome is associated with a high degree of morbidity and unemployment[12]. At one year the incidence declines slightly to 10-15%, but many of these individuals are at risk for developing the persistent PCS[3], a syndrome of organic and psychiatric pathology[3]. Both of these syndromes, collectively referred to as PCS in this application, have long-term cognitive, social, emotional, and psychological dysfunction[1,3,13] that our proposed treatment may address. Since both civilian and military mild TBI patients and mild TBI sequellae patients with post-concussion syndrome (PCS) comprise the largest, most visible, and controversial of TBI patients this discussion and scientific argument will focus on them, while the study will include both mild and moderate TBI patients.

There is no cure for the residual effects of TBI and PCS despite the application of a wide range of rehabilitation strategies, including cognitive rehabilitation[14,15,16], pharmacologic therapy[17,18,19], and others[1]. In fact, treatment failures are common 3-6 months post injury[20]. A 1998 NIH Consensus Development Conference on Rehabilitation of Persons with Traumatic Brain Injury reported that "a critical analysis of the

literature on TBI rehabilitation yields only a few studies that suggest effectiveness under limited conditions."[1]. They felt that a major limitation of TBI rehabilitation research was the narrow focus on restorative approaches which were either adaptive or enabling. Little or no attention has been directed to the problem of biological repair of the underlying chronic TBI wound. A recent review of TBI research since the 1998 Consensus Conference shows that wound repair has still not been addressed[21]. Moreover, many of the 30 recommendations in this followup review culled from the NIH Consensus regarding TBI rehabilitation research are addressed by our proposed study which will use low pressure HBOT to repair the chronic CNS wounds of TBI[22-27].

II. PATHOPHYSIOLOGY OF TBI. TBI is a complicated heterogeneous diffuse cerebral insult characterized by primary mechanical disruption of tissue[28-31] and secondary injury from ischemia[32], hypoxia[30,33,34], edema[30,35,36], vasospasm[37,38], neurochemicals[39,40],and reperfusion injury[35,41]. Both acute and chronic injury exists on a spectrum with resultant tissue pathology that is proportional to the severity of injury[42,43,44]) and is commonly classified as mild, moderate, or severe. Mild TBI has been defined in multiple ways[9,12,45,46,47,48] using various combinations of signs, symptoms, and laboratory criteria. The lack of a consensus definition[49] has led to wide variability in research findings due to inclusion of patients with different degrees of mild TBI. The underlying problem appears to be the inability to readily document a specific pathological correlate of mild TBI. Because of the lack of an anatomic barometer the characterization of chronic symptoms resulting from mild TBI suffer from the same problem. Specifically, there has been a long-standing organic vs. functional argument[3,50] over the existence or degree of anatomical pathology for persistent symptoms after mild TBI, the post-concussive syndrome[48].

In the past 20 years opinion has shifted more in the direction of organic injury in mild TBI[12,46,50,51,52,53]. Multiple studies have reported gray and white matter injury in animal and human acute mild TBI [28,31,42, 44,54,55,]. The pathological findings have also been captured on anatomic and functional imaging studies and the imaging abnormalities felt to act as surrogate markers of tissue injury. In a non-impact TBI pig study[56], for example, histological areas of damage were highly correlated with abnormal T2 MRI findings. This ability to image tissue injury in TBI is dependent on the sophistication of the imaging modality. As the sophistication of the imaging modality has increased the incidence of abnormalities in acute mild TBI has simultaneously increased. For example, CT has had poor sensitivity for mild TBI[57,58] while MRI[57,59,560,61,62], magnetization transfer imaging (MTI)[63], magnetic resonance spectroscopy(MRS)[63,64,65], and SPECT[51,66-75] have demonstrated a greater incidence of abnormalities. This increased sensitivity was mirrored by the findings in Kimura's[56]swine model. Magnetization transfer ratio analysis of conventional MRI imaging in normal areas of T2 signal showed a significant correlation with anatomic white matter damage. In essence, both anatomic and functional imaging findings are increasingly felt to represent tissue injury. A recent review of imaging in TBI has reaffirmed these conclusions and the application of functional imaging with SPECT, PET (positron emission tomography), and MRS to both acute and chronic TBI[76]. A more in depth review of magnetic resonance imaging modalities and their application to TBI is provided below in the Methods section.

The acute pathology of mild TBI matures with time and results in downstream synaptic loss[42,77], nerve cell loss[44,54,78,79], and overall tissue loss[80]. The underlying organic pathology in mild TBI can be unmasked by hypoxic stress[81]. In this study asymptomatic mild TBI patients demonstrated a significant decrement in neuropsychological performance compared to baseline testing when subjected to the hypoxia of an equivalent 3,800 meter elevation. These results implied an organic compromise of the functional reserve capacity of the injured brain[82]. Such a reduction in reserve capacity is supported by Hofman et al[51] who demonstrated atrophy of the brain months after mild TBI and McAllister's findings of changed activation circuitry patterns on functional MRI to increasing working memory processing loads in mild TBI patients 1 month post injury[83]. Reduced reserve capacity has also been shown in studies of multiple concussion patients[84,85] Those patients with an acute concussion after one or more non-disabling prior concussions displayed evidence of previously unidentified/unappreciated damage from the prior concussions[84]. The net conclusion of this pathological and imaging literature is that mild TBI causes organic brain injury that is persistent.

Despite the evidence for organic pathology in acute mild TBI, identification of the pathological substrate for symptoms or signs has been difficult. Loss of consciousness (LOC), however, appears to be a marker of acute tissue injury that also correlates with subsequent tissue loss in both animals and humans. Jane identified

the pathology of seven day old animal concussion characterized by loss of consciousness as brainstem white matter injury[77]. Kotapka[86] demonstrated that similar transient unconsciousness (less than 15 minutes) caused hippocampal lesions in 46% of animals from 4 hours to 15 days post injury. In humans LOC has been linked to both imaging abnormalities and tissue injury. Both Jenkins[57] and Hofman[49] have shown brain lesions on MRI in 100% of patients with loss of consciousness of less than 5 minutes, and 57% with Glascow Coma Scale (GCS) of 14-15 and less than 20 minutes loss of consciousness, respectively. In Hofman's study all of the patients with acute MRI or SPECT findings showed brain atrophy on repeat MRI 6 months later manifest by an increased ventricle/brain ratio, indicating primarily white matter loss. It appears from these studies that acute MRI or SPECT findings in patients with mild TBI and LOC seemed to be surrogate markers for tissue injury sufficient to produce infarct. Similarly, MacKenzie[87] showed whole brain atrophy on repeat MRI at least 3 months apart in mild to moderate traumatic brain injury. Those patients with LOC had greater atrophy. Neuronal loss has also been found on post mortem examination of patients with "concussion" by both Lidval[78] and Symonds[79]. In summary, acute loss of consciousness in mild TBI is associated with abnormal imaging findings and tissue injury that results in later tissue loss. Because of the data establishing organicity of LOC in mild TBI LOC will be an inclusion criterion in our study.

A significant proportion of mild TBI patients will have persistent symptoms one year after injury, which represents PCS. One author's review reports 8-32% of subjects with residual headaches, 4-25% with memory loss, and 19-25% with dizziness[53] while another suggests 7-8% with symptoms and 14% disabled from work[88]. At least two sources[48,53] define the PCS in terms of significant head trauma, symptoms, neuropsychological deficits in memory or attention, and social or occupational dysfunction. Similar to the acute symptoms in mild TBI the late symptoms and neuropsychological abnormalities in PCS have been shown to correlate with imaging abnormalities. Lewine[88] has demonstrated that 65% of patients with persistent post-concussion symptoms had abnormal magnetic source imaging. The imaging abnormalities suggested at least partially reversible or compensated injury since they directly correlated with symptom resolution. Hofman[51] found that slower reaction times in patients with persistent neurocognitive symptoms significantly correlated with abnormal MRI findings at 6 months post mild TBI. Voller[61] reported that 25% of patients with very mild TBI (GCS 15) and significant impairment in verbal memory, arithmetic ability, and psychomotor reaction time six weeks post injury had abnormal MRI's. Kesler and colleagues[90] noted a significant correlation of memory and intellectual impairment several years post all severities of TBI with the number of abnormalities on MRI and quantitative MRI individually and the combination of MRI, quantitative MRI, and SPECT. The incidence of abnormalities on each imaging modality varied between 51 and 62%. Neuropsychological impairment has also been shown to correlate with MRS abnormalities. In a collection of TBI patients of varying severities, MRS at an average of 53 days post TBI revealed that the NAA/creatine ratio in white and gray matter was significantly associated with composite neuropsychological dysfunction[91]. The authors felt that MRS measurements reflected the behavioral manifestations of neuronal dysfunction.

Similarly, neuropsychological deficits have been correlated with SPECT and PET abnormalities one month to years post TBI. Baulieu[92] found that all patients with neuropsychological deficits one year post TBI had SPECT abnormalities eleven months earlier. Memory deficits specifically correlated with left brainstem/ and basal ganglia/cerebellar ratios. Laatsch[93], in a small series of patients, reported SPECT and neuropsychological deficits in 100% of patients an average of 20 months post mild-moderate TBI (GCS 11-15). They noted that the SPECT deficits generally correlated with the neuropsychological impairments and improved on statistical parametric mapping (SPM) analysis as the neuropsychological deficits improved during cognitive rehabilitation therapy. A similar correlation between neuropsychological abnormalities and SPECT abnormalities has been demonstrated by Ichise[94] in both mild and major TBI patients referred for neurorehabilitation 6 months post injury. Umile[95] documented verbal or visual memory deficits in 95% and functional imaging (PET, SPECT) findings in 90% of mild TBI patients with persistent post-concussion symptoms. 75% of these patients had abnormal medial temporal lobe results on PET and SPECT. Correlation was established between neuropsychologic testing and functional imaging, but was not consistent across the entire group. MRI and/or CT scans at the time of injury were normal in 75% of patients. The conclusion from the literature on the sequelae of TBI and imaging is that persistent symptoms, i.e. PCS, and psychometric abnormalities resulting from mild TBI correlate with and are the reflection of organic injury to the brain acutely

and chronically These findings, taken together with the data referenced above suggesting an organic basis for LOC in TBI, dictate that our study set the lower threshold for inclusion at mild TBI patients with acute LOC who also have demonstrated neuropsychological impairment.

III. PSYCHOMETRIC TESTING. Neuropsychological measurement has been used as the gold standard in the differential diagnosis and functional evaluation of cognitive and neurobehavioral outcomes following TBI[96,97]. Neuropsychological evaluation has been able to demonstrate good clinical correlation with brain imaging techniques, such as the SPECT, functional MRI, and PET[95] as described above. A definite correlation has also been shown between symptom severity following mild TBI or PCS and performance on a battery of cognitive tasks[98], suggesting that patients with more pronounced TBI or PCS symptoms performed worse in cognitive tasks than controls and mild TBI patients with fewer PCS symptoms. This is consistent with the pathology of TBI described above where greater severities of TBI result in greater tissue damage.

A number of standard psychometric tests have been identified in the literature[99,100-102], which validly and reliably measure those areas of cognitive function most often impaired as a result of mild to moderate TBI. Most of these measures have been recommended as valid outcome measures in TBI recovery or clinical trials research[96,97,100] The areas of cognitive and neurobehavioral function usually impaired in mild TBI include measures of executive function and working memory[103], short-term memory[104], attention and concentration, speed of information processing[105], reaction time, concept formation and problem-solving, and other measures of frontal and prefrontal cortex insult, such as emotional and behavioral changes and loss of control[99, 171, 97.100]. Since TBI is a whole brain injury with a diffuse heterogeneous pattern of injury that varies between individuals the measures chosen for this study reflect the both the extant literature and the wide range of common cognitive abnormalities seen in TBI.

IV. NEUROLOGICAL, NEUROBEHAVIORAL, EMOTIONAL, SOCIAL, AND FUNCTIONAL OUTCOME OF TBI. The diffuse and focal injuries of TBI result in neurological, neurobehavioral, social, and emotional deficits in addition to the well-documented cognitive deficits. This is due to injury to the areas of brain subserving non-cognitive neurological function, behavior, emotion, and social interaction as well as the translation of cognitive deficits to function in these domains. Multiple studies have documented these other deficits and the long-term functional outcomes of TBI[97,106-110]. While these deficits have been commonly registered for moderate and severe TBI they have also been documented in mild TBI[111-113]. Multiple different instruments have been employed to measure the various outcomes and capture quality of life reduction post TBI. The instruments in this study (described below) were chosen to register the broad range of persistent dysfunction in TBI and its impact on quality of life.

V. HYPERBARIC OXYGEN THERAPY. Hyperbaric oxygen therapy (HBOT) is the use of greater than atmospheric pressure oxygen as a drug to treat basic pathophysiologic processes/states and the diseases in which they are manifest[26]. HBOT has drug effects on both acute and chronic tissue pathophysiology[114]. Chronically, HBOT is a trophic drug that exerts its effects in non-healing wounds that are often characterized by shallow perfusion gradients. The prototypical chronic wound model of HBOT is the head and neck soft tissue and osteoradionecrosis wound caused by external beam radiation. Repetitive HBOT in this model results in angiogenesis and healing[115,116], yet the intervening mechanisms have been poorly understood until recently. In the past 10 years these steps have been elucidated in a variety of basic science studies that have identified HBOT as a direct or indirect DNA signaling agent[117-120]. Through the action of repetitive intermittent exposure to hyperoxia DNA is signaled to begin transcription of genes that control wound repair and cause trophic tissue changes. This mechanism is affected through the pharmacological properties of pressure induced dissolution of large amounts of molecular oxygen in plasma according to Henry's Law[121]. Unfortunately, despite the plethora of studies on basic HBOT effects in acute CNS in animals and humans and chronic non-CNS pathophysiology in humans there have been no studies on chronic CNS pathophysiology in animals until the open focal cortical contusion model[27] (see below) which underpins this application.

The best known drug effects of HBOT are those associated with its use in the acute treatment of decompression illness (DCI), namely bubble compression, bubble dissolution, and the alleviation of ischemia and hypoxia[121,122]. However, Harch and colleagues have previously observed potential effects in chronic injury while treating Gulf of Mexico divers with acute DCI[123-127]. Divers with weeks to months delay to recompression responded similarly to more acute cases. Moreover, repetitive HBOT (tailing treatment) in these

late presentation divers resulted in the same effect as repetitive HBOT in acute cases. Lastly, clinically stable divers with residual brain injury from DCI had a significant positive response to a lower pressure protocol of HBOT[126] that had been previously applied to patients with chronic stroke[128,129] and multiple sclerosis[130,131]. In the past 20 years low pressure HBOT (less than 2 ATA) has been applied to patients with chronic brain injury of a variety of etiologies, including coma[132], TBI[133,134], natural gas/carbon monoxide poisoning[135,136], global ischemia[137], near-drowning[22,26,138], CP[22,139-145], and [CP, TBI, global ischemia/anoxia, stroke, Lyme's Disease, and "other"][144]. The majority of these studies have reported positive results.

A. Preliminary Studies

1. Initial Experience with Low Pressure HBOT, Chronic Brain Decompression Illness, and SPECT.
The clinical experience with the three groups of divers described above was confusing and contrary to the dogma in diving medicine which presumed that separated inert gas was the sole pathological target of hyperbaric recompression. The finding of delayed treatment benefit prompted a review of the U.S. Navy animal literature on brain decompression illness which revealed that the majority of small bubbles directly presented to the brain via carotid injection passed through the brain vasculature within three to five minutes[146-48]. The bubble passage resulted in mechanical endothelial injury and a secondary inflammatory cascade characterized by reperfusion injury, ischemia[123-127,149-151], hypoxia, edema[152] and wbc infiltration.[153,154] All of this literature, an additional study on acute treatment of DCI[155], and our findings led to the supposition that much of the benefits of treatment of acute human cerebral DCI (beyond the first hour of injury, the timeframe and experience upon which U.S. Navy treatment was based) was not a consequence of treating separated gas, but of addressing reperfusion injury and ischemia[126]. More importantly, we hypothesized that the tailing treatment of both acute and delayed presentation cases and retreatment of cases with chronic neuro-cognitive residual was primarily altering the sequelae of subacute/chronic ischemic brain injury[123-127,149-151] and treating the microscopic residual effects of mechanical bubble injury and inflammation.[156]

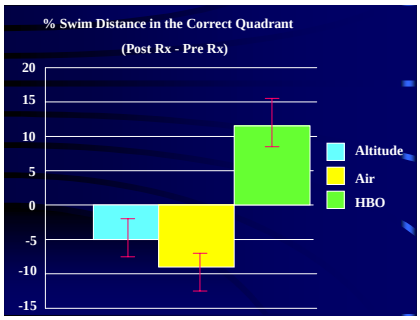
2. Application of LPHBOT and SPECT to Chronic TBI-Initial Experience. In 1990 the low pressure HBOT protocol used in the chronic divers was applied to a small IRB-approved study of two boxers with dementia pugulistica who experienced transient improvement in dizziness, and mild sustained improvements in behavior, affect, and psychometric testing, respectively (unpublished data). These encouraging results led to a study of patients with a wide spectrum of chronic brain disorders who were evaluated and treated with low pressure HBOT. The study tested whether 40-treatment blocks of low pressure HBOT could improve symptoms, abnormal physical exam findings, performance on cognitive tests, and rCBF deficits seen on SPECT brain imaging in patients with a minimum one year old brain injury. In addition, the study tested whether the rCBF response to a single HBOT treatment would identify perfusion deficits that improved after the single HBOT and thus could predict symptom/exam/ cognitive/SPECT improvement after a 40 HBOT treatment course, perhaps in concert with the "Idling Neuron" hypothesis.[157] In other words, the study attempted to see if SPECT identified injured areas of brain that could be rehabilitated with repetitive HBOT (SPECT was used as a surrogate marker and indexing tool because of previous success with this in a stroke case[157] and divers[123-5]).

While a number of CNS disorders were studied, the experience in mild to moderate chronic TBI was nearly as successful as the treatment of the divers[125,126]. We speculated that the similarity of responsiveness to low pressure HBOT in the two groups was due to: 1) the presence of microscopic ischemic foci in both conditions (primary white matter vascular injury with secondary ischemia in decompression sickness[156], direct white matter injury[42,44] with secondary ischemia in mild TBI, 2) the small size of the perfusion gradient wounds in both conditions (heterogeneous pattern on SPECT[123-127,149-151]), and 3) the predominance of ischemic relative to infarcted tissue (normal anatomic imaging in both DCS and mild TBI). Mechanisms of action, however, were completely unknown.

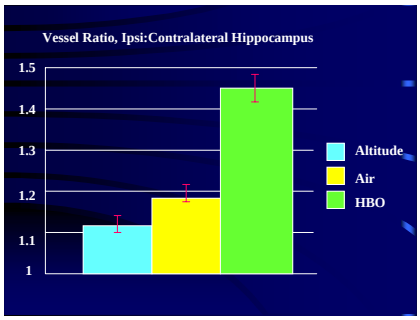
3. Animal Confirmation of HBOT in Chronic TBI. In 1995 duplication of the human HBOT/TBI experience and demonstration of the known angiogenic effect of HBOT in chronic wounds was sought in animals. Harch and Kriedt applied the human protocol used in divers and TBI patients above to a group of 12 rats in a controlled study of chronic traumatic brain injury[158]. This open focal cortical contusion model[159] causes a cortical infarct and a microscopic hippocampal contusion that partially mimics the acceleration/deceleration injury of blunt head trauma and blast injury/concussion of mild TBI. The hippocampal injury manifests as a deficit in spatial memory [159] which is again similar to the memory deficits seen in mild TBI. Fifteen days after

injury the infarct to the cortex is complete and the injury to both cortex and hippocampus is considered chronic[159]. The human low pressure HBOT protocol (above preliminary studies) applied forty-five days post injury produced statistically significant relative increases in hippocampal blood vessel density that correlated with simultaneous improvement in learning/spatial memory (hippocampal function), essentially, a partial reversal of the traumatic hippocampal injury. The increase in blood vessel density was consistent with the known angiogenic effect of HBOT in chronic shallow perfusion gradient wounds cited above[115-6].

The pattern of relative increase in vessel density to injured hypometabolic rat brain tissue corresponded closely to the pattern of improvement noted on SPECT brain imaging in the above cases where the overall regional rCBF progressed from a heterogeneous pattern to a homogeneous pattern. To exclude the possibility that these findings were a Type II error, the experiment was replicated with 60 rats in 2001; the results were statistically stronger[27] and recently published[160] To our knowledge this is the first ever demonstration of improvement of chronic animal brain injury of any etiology. More importantly, however, it reaffirmed the case series experience noted above in humans with TBI using the same human protocol of HBOT, suggested a generic effect on common microscopic tissue pathology and shallow perfusion gradient wounds in divers and mild TBI patients, was consistent with the known trophic effect of HBOT in chronic wounds, angiogenesis, and argued very strongly that the improvements seen in both the animals and humans could be extended to humans in a controlled trial. The results of this study are shown below in the following two bar graphs.



Above Figure:
Improved Spatial Learning. (Left to right): Blue are altitude control rats (5,595 feet-Albuquerque), Yellow are sham air dive rats at sealevel, and Green are sealevel HBOT rats. Bar graph denotes percent swim distance in the correct quadrant on the Morris Water Task (Spatial Learning Function).



Above Figure:
Blood vessel density ratio, ipsilateral to contralateral, of contused hippocampus. Color code is same as opposite figure.

Another important facet of the animal model confirmation of effectiveness of low pressure HBOT in chronic mild TBI was that the experiment accelerated the possibility of HBOT application to humans by reversing the normal FDA scientific proofing process. Traditionally, new therapies proceed from in vitro experimentation to small animal experiments, large animal experiments, primate experiments, foreign clinical trials, and finally United States clinical trials. In our case the discovery was made serendipitiously by extending and modifying HBOT in a traditional human application of HBOT, cerebral decompression sickness, collateralizing the findings to other neuropathologies, namely TBI, applying it extensively to humans in a prospective pilot trial, accumulating additional human case experience to fine-tune the dosing of HBOT, and then confirming the human experience in an animal model using the original human dose. This “reverse FDA” sequence argues strongly against the need for any further immediate animal confirmation and for rapid proofing in the human arena. Essentially, we have inadvertently shortened the time from discovery to application. A controlled clinical trial at this point will bring this therapy to rapid deployment in the U.S. military and civilian population as demanded by this award track.

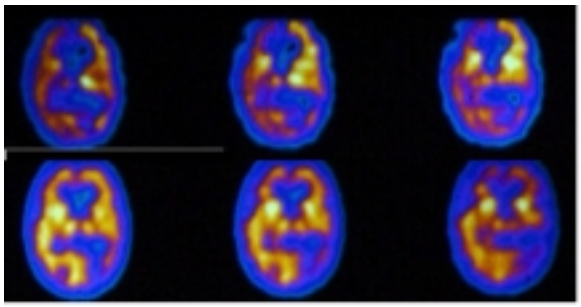


Figure 1. Case A pre (top) and post (bottom) HBOT

4. Cumulative Experience of LPHBOT and SPECT in Chronic Human TBI. Over the past 17 years we have treated approximately 70 patients with varying severities of chronic (greater than one year post injury) traumatic brain injury in a case series format using each patient as his/her own control. Patients underwent history and physical exam, video exam for obvious physical deficits, psychometric testing when possible, and other baseline outcome testing. The patients were studied with blocks of 40 HBOT's with repetition of baseline testing and SPECT brain imaging after each block of HBOT. In the context of an unfunded pilot study, only SPECT imaging was performed on all patients since the predictive ability of SPECT was one of the primary hypotheses of the study. Preliminary analyses indicate that the great majority of patients responded positively with lasting symptomatic, cognitive, and rCBF improvements. These improvements were seen regardless of severity of chronic TBI, level of disability, or length of time from injury to treatment. One case was treated 44 years after TBI that occurred in infancy[161] Qualitative changes (visual interpretation) on SPECT have been substantial (see Figure 1). In most of these cases the effect of HBOT is a smoothing of the trauma induced heterogeneous pattern of flow to the more normal homogeneous pattern of brain blood flow. The pattern of change was exhibited by patients with both normal and abnormal anatomic imaging, indicating an effect on both microscopic and macroscopic tissue injury. The SPECT brain imaging improvements were the most consistent and seem to be independent of etiology or severity of TBI[22,23,26].

Although symptomatic improvements were not recorded using standardized questionnaires in this pilot study, most of the patients with mild TBI were able to return to school, work, and/or increase their post-injury level of function and performance. The cognitive gains in the mild TBI patients were measured by psychometric testing or SAT scores pre and post HBOT. Some of these gains were significant. An example is a 48 y.o. female with "complicated" mild TBI who was involved in an MVA that resulted in LOC of less than 1 minute . GCS in the ER was 14 for 16 hours. Repetitive acute CT imaging was negative. Two years later the patient was evaluated for short term memory dysfunction, problems with attention and concentration, and inability to organize her thoughts that prevented return to work (Workers' Compensation Claim). The patient commenced a course of 40 1.5 ATA/60 minute HBOT's over the next 30 days and experienced improvement in her cognitive deficits that facilitated return to work and completion of her Masters' degree. Repeat psychometric testing showed a 40 percent improvement in working memory scores that was consistent with improvement on repeat SPECT.

5. *Additional Evidence of LPHBOT in Chronic TBI.* The above experience of HBOT in chronic TBI is also consistent with a case report of low pressure HBOT and SPECT brain imaging in the treatment of chronic TBI[24]-(symptomatic gains, no psychometric testing) and our controlled study of HBOT, psychometric testing, and SPECT brain imaging conducted at the Transitional Learning Community and the University of Texas Medical Branch, Galveston[25]. In this study the patients improved symptomatically, on SPECT, and showed a trend of improvement on psychometric testing. A group of normal patients subjected to HBOT and SPECT showed no significant SPECT changes. A much larger retrospective case series of HBOT in chronic brain injury (primarily TBI in the adult cohort) showed improvements in SPECT rCBF as patients received a minimum of 70 HBOT's at varying doses of HBOT[144]. The study also demonstrated that the SPECT improvements only occurred after a minimum of 15-50 HBOT's, consistent with this P.I.'s experience above. This minimum dosage was confirmed by a recent case report[162] where 20 HBOT's 1 year after TBI showed improvements in sensorimotor and electrophysiological measures that were not durable. One year after the first series of HBOT's the patient received 60 HBOT's with durable sensorimotor, electrophysiological, and neuropsychological improvements. These two reports together reaffirmed the P.I.'s experience above with sequential blocks of 40 HBOT's and the design of the present study for two blocks of 40 HBOT's.

6. Summary. This application is the culmination of 17 years of investigation that began with the clinical observation that the standard of care in the treatment of divers with brain decompression illness (DCI) was inconsistent with the purported underlying pathogenesis and pathophysiology. This led to the impression that much of the treatment of acute cerebral DCI was treatment of acute ischemic/hypoxic brain injury. The

experience with the three groups of divers above suggested that the pathological target of HBOT in these groups was subacute and chronic ischemia/hypoxia. Given that brain injury of diverse etiologies shares common pathological processes[163], particularly the inflammatory reaction, the successful treatment of these divers was extended to patients with other neurological disorders that featured similar microscopic tissue pathology. We theorized that the basis for improvement of these brain "wounds" might in fact be treatment of the microscopic version of the macroscopic shallow perfusion gradient chronic wound model of external beam radiation injury in which HBOT has been so successful[115,116]. The neurological condition which most closely duplicated the success and size of tissue pathology in the divers was the PCS of mild TBI. When the application of low pressure HBOT to PCS, and more severe degrees of brain injury, achieved similar results to the divers that was verified by the same clinical, neuropsychological, and SPECT instruments a retrograde approach was attempted by seeking an animal model to test and validate the human experience. In 1996 and again in 2001 the human findings were reaffirmed and then replicated, respectively, in a rat model of chronic traumatic brain injury.[160]

While the exact mechanisms of action of low pressure HBOT in chronic brain injury are unknown and largely speculative, the clinical results are not. This application proposes to investigate the human and animal findings in a rigorous human trial, to attempt to identify sensitive outcome measures, and to correlate functional brain anatomy and true anatomy with functional improvement/outcomes. Positive results of this study would lead to immediate translation to the clinical arena, particularly the military TBI population, and thus improve patients and dramatically reduce the economic, societal, and personal burden of this condition. In addition, it would spawn further larger clinical studies with HBOT in TBI, including subacute TBI, and studies in combination with other therapies.

Thus, this proposal calls for a clinical trial of low pressure HBOT in the treatment of mild (PCS) to moderate chronic traumatic brain injury that employs psychometric testing, SPECT brain imaging, MRI, MRS, MR Diffusion Tensor Imaging (MRDTI), and quality of life assessments, including return to work, school, or previous level of function as outcome measures. The study will test the hypothesis that 40 low-pressure HBOTs administered in a one month period to patients with one to three year old post-concussion syndrome from mild to moderate TBI can improve symptoms, psychometric test scores, SPECT brain imaging, MR imaging, quality of life, and return patients to school, work, or previous level of function. It will also test the effects of 40 vs. 80 HBOT's. The study seeks to correlate changes in these outcome measures and see if the surrogate imaging measures and QOL assessments correlate with the more accepted psychometric testing to identify the most sensitive outcome measure. That measure would be used as the single outcome in further trials. In addition, correlation of QOL changes with psychometric test changes and the correlation of both of these with SPECT SPM and MRI changes will be sought to demonstrate anatomic specificity of HBOT induced effects on brain injury. Specifically, changes in the medial temporal lobe on imaging (SPECT, MRS, MRDTI) will be sought to correlate with potential changes with memory function in the temporal lobes.[164-5.]

Hypotheses:

1. A twenty day course of 40 low-pressure HBOT's can significantly improve cognitive function in mild TBI patients with PCS and chronic symptomatic moderate TBI patients.
2. 80 HBOT's will have a greater benefit than 40 HBOT's.
3. Improvement in cognitive function in chronic TBI patients will correlate with psychometric testing, imaging changes (SPECT, MRI, MRS, MRDTI), quality of life (QOL), and neurobehavioral outcome measures.

Specific Aims:

1. Our primary specific aim is: to determine whether a twenty day course (40 treatments) of low pressure HBOT generates significant improvement in cognitive function in mild TBI patients with PCS and chronic symptomatic moderate TBI patients.
2. A second aim is to determine whether an additional 40 HBOT's (80 total) achieves further cognitive improvement in this population of chronic TBI patients.
3. A third aim is to determine if improvements in cognitive function can be measured by psychometric testing and, if such changes are observed, whether they correlate with SPECT, MRI, MRS, MRDTI, QOL, and neurobehavioral outcome measures, including return to school or work. Specifically, using SPECT, MRI,

MRS, and MRDTI we will attempt to determine if improvements in memory on psychometric testing correlate with changes in temporal lobe function/anatomy on imaging.
4. A fourth aim is to see if improvements in cognitive function induced by HBOT are durable and result in return to school, work, or premorbid level of function.
5. A fifth aim is to determine which of psychometric testing, SPECT, MRI, MRS, MRDTI, QOL, or neurobehavioral outcome measures is the most sensitive outcome to use in future research with HBOT.

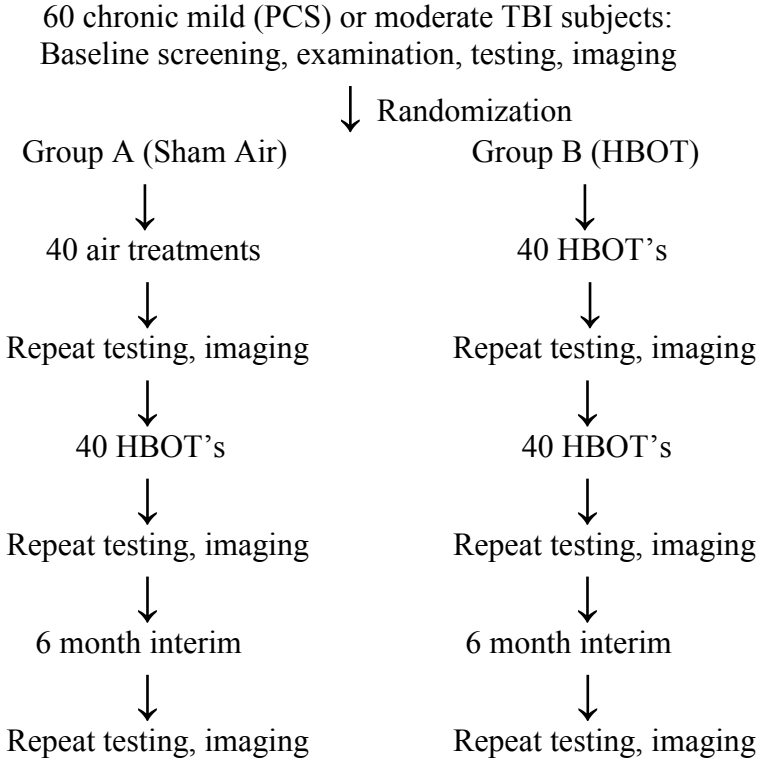
Research Strategy:

The research design is a 2 phase randomized prospective controlled double-blinded trial that will evaluate efficacy of two different doses of the drug HBOT in chronic mild TBI (PCS) and moderate TBI. The study will use a two-group parallel repeated measures design to compare the mean difference from baseline in psychometric testing, SPECT brain imaging, MRI hippocampal volume measurements, MRS hippocampal NAA, choline, and creatine ratios, MRDTI, and QOL assessments in 60 chronic TBI patients (30 mild TBI PCS and 30 moderate TBI). The primary response variables will be the difference from baseline on psychometric test scores. The subjects will be civilian TBI patients and military veterans who sustained a TBI in the recent Iraq or Afghanistan Wars. All patients will be at least 1 year and less than 4 years post TBI. Half of the groups will be mild and half will be moderate TBI. The 60 patients will be randomly divided into two groups of 30 patients each, an experimental low pressure HBOT group and a sham air treatment group. They will be stratified by mild or moderate and the civilian subjects also by litigation or disability claim.

The study will consist of two phases that run sequentially on each patient. The first phase is the randomized controlled double-blinded portion. After screening, testing, and imaging the subjects will receive either 40 sham air treatments or 40 low pressure HBOT's on a twice/day, 5d/week schedule. This will take one month to complete. In the principal investigator's 17 year experience and in the results of the first animal pilot trial[158] with 12 rats (sham air group, 40 HBOT group, and 80 HBOT group) this schedule has been well tolerated and produced measurable durable improvements. At the end of this first month of treatment both groups will undergo repeat testing with all measures, and thus address the first hypothesis and first aim. The experimental design for the first component of this study was derived from our clinical experience and the advice of a scientific advisory board that was assembled in 2004. The board included: 1) Dr. Gaylan Rockswold, professor of neurosurgery, University of Minnesota and Hennepin County Medical Center. Dr. Rockswold was chairman of the advisory board. He has performed the most rigorous, technically exacting, and productive studies of HBOT in acute severe traumatic brain injury[166-7]. 2) Dr. Nicholas Bazan is professor of neurosciences and chairman of the LSU Neurosciences Center of Excellence. He is an ophthalmologist and basic science researcher whose specialty is molecular biology and mechanisms of brain injury. 4) Dr. Ed Morse is professor of behavioral medicine and psychiatry at LSU and Tulane Universities' Schools of Medicine and an epidemiologist who is expert in behavioral testing of the substance and alcohol abuse populations. 5) Dr. George Howard is professor and chair of Biostatistics at the University of Alabama, Birmingham. He is an expert in statistics and stroke epidemiology. 6) Dr. Ken Adams is professor and chair of the department of psychology at the University of Michigan who is expert in the neuropsychological testing of TBI. 7) Dr. Russell Van Dyke is professor of pediatrics and infectious diseases at Tulane. He is a clinical trials specialist who has performed a number of pediatric AIDS studies. All of these individuals are senior NIH funded researchers.

The second phase commences immediately following the above outcome testing. Since the drug HBOT causes permanent trophic anatomic changes (see above discussion of HBOT mechanisms) and associated durable clinical changes a crossover design is precluded. As a result, the sham air group will proceed to 40 HBOT's while the original HBOT group will receive another 40 HBOT's on a once/day, 5d/week schedule. This schedule has again been found to be well tolerated while a twice/day schedule has resulted in extreme fatigue and even neurological/cognitive regression in some subjects. At the completion of the second phase of HBOT all subjects will again be retested with all outcome measures. The data will address the second hypothesis and second aim by inter-group comparisons and intra-group comparison of the 80 HBOT group. In addition, the sequential reception of 40 sham air treatments and 40 HBOT's by the original air group will address Hypothesis 1 and Aim 1 with a self-to-self intra-group comparison.

Six months after the final HBOT in both groups all subjects will repeat all testing measures and correlation analyses will be performed to test Hypothesis 3 and address Aims 3, 4, and 5. A summary of this design is as follows:



The above design will facilitate testing of efficacy of the minimum number of HBOT's (40) found to produce permanent improvements in humans. In addition, it will afford evaluation of two different doses of HBOT, 40 vs. 80 HBOT's. In the original pilot animal study of 1996 with only 4 animals in each of 3 groups the 80 HBOT group was found to have non-significant cognitive improvement greater than the 40 treatment group, but nearly twice the blood vessel density. These findings dictated the design of the confirmatory animal study of 2001[160] which used 20-22 animals in each group and 80 HBOT's vs. 80 sham air treatments. The animal results duplicated the human clinical results that were generated over 5.5 years in the prospective pilot human study that evaluated sequential blocks of 40 HBOT's. Each successive block of 40 HBOT's was commissioned upon demonstration of clinical and imaging and/or psychometric testing of patients after completion of the previous block of 40 HBOT's. The general experience that needs confirmation in this study is that 80 HBOT's produced greater and more measurable improvements than 40 HBOT's. The design of this study will accomplish that. Finally, the retesting with outcome measures after the six month interim period will answer the question of durability of the treatment.

I. Methods:

A. Summary Protocol, Inclusion and Exclusion criteria: Subjects will be 18-45 year old adults with the PCS or residual neurocognitive symptoms from a single moderate TBI that is 1-3 years old who are in categories 0-3 on the Disability Rating Scale[168]. The upper age limit of 45 was chosen to maximize the number of military candidates by including older individuals in the reserves and minimize the effects of aging on imaging findings and rehabilitation potential. The upper limit of 3 years post TBI is somewhat arbitrary, but the majority of the principal investigator's experience in chronic TBI has been within this time period. The upper three categories of the DRS were chosen to attempt to obtain the largest population of chronic TBI survivors, minimize heterogeneity, and include the groups of patients with the least amount of gross brain tissue loss from the primary injury. This population represents the groups that have the greatest potential to return to work, school, or near/complete previous level of function. Patients will be questioned regarding hyperbaric oxygen therapy absolute contraindications and relative contraindications as well as other inclusion and exclusion

criteria which includes a minimum loss of consciousness at the time of injury. They will then be consented and screened with the Rivermeade Head Injury Questionnaire[169] If the subjects score 9 or higher on the Rivermeade they will complete the Addiction Severity Index[170] to exclude substance abusers, the PTSD checklist[171] to rule out PTSD, the Test of Memory Malingering[172], the Green Word Memory Tes[173], and the Wechsler Test of Adult Reading[174] to estimate premorbid IQ.

Subjects will proceed to full psychometric evaluation with the tests to be described below. Only those patients with cognitive deficits will be included in the study. Those subjects will be urine drug tested and tested for pregnancy then complete QOL questionnaires that are also described below. They will undergo neurological exam, hyperbaric medicine exam, and imaging with MRI, MRS, MRDTI, and SPECT. They will be randomized to HBOT or air and be blinded to treatment group. Only the hyperbaric technician will be knowledgeable of treatment group. Subjects will begin Vitamin C, E, and multivitamin supplementation for cofactor and antioxidant support and undergo 40 treatments: the air group will receive 40 sham air treatments and the HBOT group 40 1.5 atmospheres absolute (ATA)/60 minutes bid, 5d/week treatments. Within the next 4-6 weeks maximum the subjects will have repeat psychometric test battery, QOL questionnaires, neurological exam, urine drug testing, pregnancy testing, and imaging. Following the interim testing period subjects will proceed to the second phase with 40 HBOT's at 1.5 ATA/60 minutes, once/d, 5d/week for both groups. All exams/tests/imaging completed after the first 40 treatments will be repeated and a 6 month interim period begin from the last HBOT. At 6 months repeat exam/tests/imaging will be performed as well as questioning regarding return to work or school.

1. *Inclusion Criteria:*
   a. Adults, 18-45 years old. The study will target this younger age group to avoid many of the exclusion criteria, capture the school and work population, and minimize the effects of aging that feature reduced brain reserve capacity[82]. Children will be excluded since the study is a small trial and their inclusion would necessitate stratification which would demand a larger n.
   b. Single mild TBI that is a minimum of one and maximum of three years old and was characterized by loss of consciousness. Will require medical report or field report of loss of consciousness.
   c. Absence of acute cardiac arrest or hemorrhagic shock at time of TBI that would cause a global ischemic insult on top of TBI.
   d. Absence of intracranial neurosurgery post-TBI.
   e. Disability Rating Scale of 0-3.
   f. Score of 9 or higher on the Rivermeade Head Injury Questionnaire.
   g. Negative Addiction Severity Index (Severity score of less than 2)
   h. Negative PTSD checklist.
   i. Negative Test of Malingering Memory.
   j. Negative Word Memory Test (Effort score 90% or higher).
   k. Minimum cognitive deficits on psychometric testing of either: 1) current or pre-HBOT attentional (EFS Color-Word Interference Test) and/or copy memory (on the Rey Complex Figure Test-Recall) deficits during eligibility screening that fall less than or equal to one-half standard deviation below their estimated premorbid Full Scale IQ as measured by the Wechsler Test of Adult Reading[174].
   l. Negative urine toxicology screen for drugs of abuse.
   m. Negative pregnancy test in females.
   n. Otherwise good health.
2. *Exclusion criteria:*
   a. Pulmonary disease that precludes HBOT (e.g., bronchospasm unresponsive to medication, bullous emphysema).
   b. 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).
   c. Severe confinement anxiety (e.g., patients who require anesthesia conscious sedation for MRI).
   d. Pregnancy.
   e. Other pre-TBI neurological or major psychiatric diagnoses.
   f. Pre or post TBI history of substance abuse.

g. Pre or post TBI history of alcoholism.
h. Participation in another experimental trial with active intervention.
i. High probability of inability to complete the experimental protocol (e.g. terminal condition).
j. Previous HBOT.
k. Multiple TBI's in the previous 1-3 year inclusion period.
l. History of hospitalization for past TBI, stroke, nonfebrile seizures, or any seizure history other than seizure at the tme of TBI.
m. Past or current history of mental retardation (baseline FSIQ $\leq$ 70).
n. Pre-/post-TBI history of systemic illness with impact on CNS (P.I.'s decision).
o. History of malingering or motivation problem related to litigation.

B. Screening and excluding malingering: Eligibility screening and neuropsychological tests will be administered to each participant by a psychometrist or neuropsychologist, who will administer all pre- and post-HBOT measures to prevent intra-study fluctuations in measurement methods and data quality. The psychometrists will be supervised by the study neuropsychologist, Susan Andrews, Ph.D; all will remain "blind" to participants' treatment group assignments for the duration of the study. The screening measures will identify confounding disorders or conditions, such as malingering and/or somatoform disorders that are frequently identified in patients with a history of mild TBI[101-2].

The neuropsychological tests in this study are utilized for four purposes: 1) to determine eligibility for this study, 2) as pre-tests to measure each participant's baseline level of neuropsychological functioning including: intellectual functioning, memory, executive abilities, psychomotor speed and coordination, and psychosocial/adaptive functioning prior to HBOT 3) as post-tests to measure the effects of HBOT on the neuropsychological measures listed above, 4) to measure constructs which serve as moderators for the effects of HBOT, including IQ, personality, and adaptive functioning. Although practice effects are observed on many tests, the impact of practice effects should be comparable for both treatment groups. We will minimize these effects by utilization of tests with alternative forms, such as the Rivermeade Behavioral Memory Test and the Rey Auditory Verbal Memory Test. Many other tests do not have large practice effects, such as the Test of Variables of Attention, the Finger Tapping Test, and certain subtests of the Wechsler Memory Scale-III (Faces I and II, Digit Span, Spatial Span, Executive Function System (EFS) Design Fluency, Verbal Fluency).

Since TBI has both focal and diffuse distribution of injuries different patterns of cognitive, neurobehavioral, and adaptive functional impairments are found that contribute to heterogeneous courses and outcomes. In our experience HBOT has differential effects on these impairments that vary individually. To capture the heterogeneity of both the TBI and HBOT response, we will use multiple pre- and post-treatment neuro-behavioral and adaptive functional measures. All evaluations will be completed within the same week of both pre- and post-HBOT functional imaging to permit relatively contemporaneous correlational analyses between pre- and post- neuropsychological, neurobehavioral, or adaptive functional outcome change scores and corresponding pre- and post-HBOT treatment functional imaging change scores.

1. Screening for TBI Cognitive Deficits: **The Rivermead Post Concussion Symptoms Questionnaire**[169] is a questionnaire that measures the severity of post concussion symptoms in TBI and that has been shown to reliably identify those patients with chronic cognitive deficits[98].

2. Inclusion and Exclusion Criteria: the **Addiction Severity Index**[170] is a standardized measure of lifetime and current substance or alcohol abuse or dependence. A severity score greater than 1 will be used to exclude patients from this study with either past and/or current histories of significant substance/alcohol dependence or abuse. Higher severity scores are associated with more severe addictive symptoms on this measure, which could confound evaluation of any HBOT treatment effect.

Disability Rating Scale[168] is a well characterized instrument that reliably reflects general outcome in moderate to severe TBI and has been shown to correlate with electrophsiologic measures of brain injury[175]. It will be used to exclude the moderate to severely disabled and track outcomes on the Level of Functioning and Employability Items, the two scales shown to be sensitive to change 2-5 years post TBI[176].

3. Premorbid or Baseline IQ: **The Wechsler Test of Adult Reading**[174] is a measure of estimated premorbid Full Scale IQ that is co-normed with the WAIS-III[177]and WMS-III[178]. In the WTAR, participants are asked to

read a list of 50 words out loud, which have irregular pronunciations. It was developed on the rational that reading recognition is relatively stable skill in the presence of cognitive decline associated with head trauma.
4. Malingering Measures: **The Word Memory Test**[179] measures both verbal memory and effort. Scores on the immediate recognition, and delayed recognition trials are used to measure effort. This test has been used extensively in TBI research to identify patients who amplify deficits for secondary gain[180-3] It has been shown to be effective in mild brain injury where poor effort is extremely high[184]. The WMT will be one of the primary screening tools in this study.
The Test of Memory Malingering[172] (TOMM) is a brief visual recognition test used to discriminate memory malingering from memory impairment. The test involves two learning trials and an optional retention trial. The TOMM will be used to screen for effort using the standard cutoff scores of 45 or better on Trial 2.
C. Psychometric Testing: Subjects will be tested for intelligence, memory, learning, attention, executive function, and psychomotor speed/coordination.
1. Intellectual Function: **The Wechsler Adult Intelligence Scales-III**[185](WAIS-III) is a comprehensive measure of intellectual functioning. It consists of 14 subtests that measure different cognitive abilities: The WAIS-III generates an estimate of general intellectual functioning, or Full Scale IQ, and also provides four indices or factor scores, including: Verbal Comprehension, Perceptual Organization, Working Memory, and Processing Speed. The WAIS-III will be used to obtain patients' IQs before and after HBOT treatment phase, given the perceived importance of IQ scores in assessing outcomes on the effect of HBOT on WAIS-III FSIQ, VIQ, or PIQ scores in patients with TBI.
2. Memory Functioning: **The Rivermeade Behavioral Memory Test**[186](RBMT-E) was developed to provide measures that could be directly related to the behavioral effects of impaired memory. The test comes in 4 parallel forms, which is particularly a benefit as it will offer another alternative means of measuring paragraph or logical memory, in addition to the Wechsler Memory Scale-III, which does not offer parallel forms. This should reduce practice effects. The memory problems of TBI patients are brought out by this test[186].

The Wechsler Memory Scale-III[178](WMS-III) is a well-known battery of declarative and verbal and nonverbal learning and memory tests. It was co-normed with the WTAR[174], which will permit detection of potential declines in learning and memory, as measured by the WMS-III when compared to each patient's estimated premorbid intellectual functioning based on his/her own WTAR FSIQ standard scores[174]. We will use an abbreviated version of the WMS-III to reduce the burden for patients because of our multiple pre-/post-HBOT treatment outcome measures. The abbreviated WMS-III version will include immediate and delayed recall/recognition recall trials for the Logical Memory subtests and immediate and delayed recall trials for the Faces subtests[178]. The full WMS-III takes 60 minutes to complete and the abbreviated version will take approximately 30 minutes. Several of the subtests of the WMS-III appear more sensitive to the effects of mild TBI than the WAIS-III[187].

The Rey Complex Figure Test[188] (R-CFT) is a task in which participants copy a complex figure and are later tested for recall and recognition of the figure. This a measure of complex visuo-constructive copy skills that have been recommended for TBI research[96,97]. Higher scores on this measure reflect better design copying performance. The *Rey Complex Figure Test*-Recall and Recognition Recall trials[188] are measures of uncued and cued complex design recall. Design copy and recall trials have been recommended for TBI research[97]. The additional potential value of a delayed cued recognition recall trial that has become available has not been well studied in prior TBI research, but could contribute to differentiating so-called uncued design retrieval deficits as compared to so-called design memory-related consolidation, storage, or forgetting deficits, resulting from TBI-related impairments. Thus, we will include measures contrasting uncued recall and recognition recall trials in this study. Higher uncued recall and cued recognition recall scores on these measures reflect better retrieval and/or consolidation[188].

The Rey Auditory Verbal Learning Test[189] (R-AVLT) is a memory test which provides a measure of immediate auditory memory, learning curve, retroactive and proactive interference, delayed memory, and recognition. The subject is presented with a 15-word list, followed by an immediate recall test. The list and recall test, are presented 5 times, then a second 15-item word list is presented followed by a recall test for the original 15 words. A retention test is usually given 30 minutes later. There are multiple alternate forms of this test, which will cut down on the practice effect. TBI patients can show a reduced recall for each trial but will

demonstrate a learning curve and some loss on delayed recall but a near normal performance on the recognition trial, indicating a significant verbal retrieval problem. These patients also tend to make a few intrusion errors[190]. The R-AVLT has also been effective in predicting psychosocial outcome after TBI[191].

3. Measures of Attention and Executive Functioning: **The Test of Variables of Attention***[192]* (TOVA) is a computerized continuous performance measure of sustained visual attention, signal detection, and information processing speed[192]. This test presents visually presented targets and non-targets and requires the participant to press a button to respond to targets. It yields measures of inattention, impulsivity, response speed, and variability of response speed. TBI patients with processing speed deficits are identified with the TOVA[99].

The Delis-Kaplan Executive Function System*[193]***.** (D-K EFS). The D-K EFS consists of nine tests that measure a wide spectrum of verbal and nonverbal executive functions. These tests are chosen to measure executive function in TBI patients because of the years of collected data indicating the relevance of these tasks in delineating frontal executive dysfunction[99]. Five of the nine tests will be used in this study to provide an estimate of abilities associated with prefrontal and frontal lobe functioning including: the Verbal Fluency Test (VFT), the Design Fluency Test (DFT), the Color-Word Interference Test (CWIT), the Trail Making Test (TMT), and the Tower Test.

The Verbal Fluency Test[193] (VF) measures fluency or generative skill to produce specific words beginning with specified individual letters (i.e., EFS Letter Fluency) or specified semantic categories (i.e., EFS Cognitive Fluency). This task also includes a measure of alternating attention, or switching, during which the participant must switch back and forth between naming items from two categories. This test provides scaled scores for total correct responses, switching accuracy, set-loss errors, and repetition errors. Verbal fluency tasks have been previously used and recommended in TBI research[97]. Higher scores on these measures reflect better verbal fluency or generative skills.

The Design Fluency Test[193] was also co-normed with the EFS Category and Letter Fluency tests that are described above. This test measures fluency or generative skills in producing novel designs that has been previously used and recommended in TBI research[194]. This measure also includes a switching condition, which requires participants to alternate attention while completing the task. This test yield scaled scores for total correct figures, switching accuracy, set loss errors, and repetition errors. Higher scores on this measure reflect better nonverbal design fluency or generative skills. All three of these fluency measures are sensitive to TBI as well as educational experience, necessitating common norm-referenced sample standard scores to determine if the sensitivity of any fluency or other so-called specific executive function measures (e.g., EFS Trail Making and Color-Word Interference Tests) are related to mild TBI.

The *Color-Word Interference Test*[99] measures suppression of more automatic verbal response (reading) to generate a conflicting response (naming dissonant print colors). The typical Stroop task requires patients to name color patches initially, then to read names of colors presented in black ink, and then to suppress reading color names printed in dissonant ink in order to name the ink color. An additional modified trial on this version of the Color-Word Interference Test requires patients to switch between naming the dissonant ink colors and reading the conflicting words, with this kind of modification previously reported to be more sensitive to cognitive inhibitory and flexibility deficits associated with Mild TBI[195]. This measure generates scores for participant response speed on color, word, inhibition, and inhibition switching trials. Higher correct scores on the Color-Word Interference test reflect better performance. Given the frequent association of attention deficits associated with mild TBI and, more specifically, PCS[48], the Color-Word Interference Tests will be given during eligibility screening to identify patients with disproportionate attention deficits (i.e., standard scores greater than or equal to one-half standard deviation below their estimated premorbid FSIQ based on their obtained FSIQ on the WTAR[174].

The Trail Making Tests are measures of rapid mental shifting and flexibility that reflect modifications of earlier Trail Making tests which had been previously recommended as valid outcome measures of the impact of TBI[97]. This version of the TMT includes separate tasks to measure visual scanning, number sequencing, letter-sequencing, number-letter sequencing, and motor speed, to measure

how these factors are related to performance. Higher scores reflect better performances on these attentional-related tests.

The Tower Test[99] requires the participant to move disks of varying size, from small to large, across three pegs to build a designated tower in the fewest possible moves. This measure generates scaled scores representing achievement or number of moves, mean first-move time, time-per-move ratio, and move accuracy ratio.

4. Psychomotor Speed and Coordination: **The Grooved Pegboard**[196] **test** (GPT) is a measure of rapid unilateral manual motor coordination that has been recommended in TBI outcome research[97]. This timed task requires participants to place pegs in a pegboard. Quicker response times indicate better performance, with performance of each hand measured.

The Finger Tapping Test[197] (FTT) is a measure of rapid unilateral manual motor speed that has been recommended in TBI outcome research[97]. This task requires the participant to tap a lever for seven 10" trials with the index finger of each hand.

D. Neurobehavioral and Quality of Life (QOL): Subjects will complete a variety of these instruments.

1. The Ruff Neurobehavioral Inventory[198] (RNI) is a recently developed measure of behavioral, emotional, and adaptive functioning that has built-in validity scales to measure any potential intentional or unintentional efforts by patients to over- or under-report neurobehavioral symptoms. It has been included in this study instead of the better known and validated Neurobehavioral Rating Scale-Revised (NBR-R)[199] because the NBR-R lacks validity scales to detect either any patient intentional or unintentional reporting tendencies toward over- or under-reporting neurobehavioral symptoms. Given the importance of adequately assessing the potential efficacy of HBOT treatment, the use of measures of patient reporting biases, as is available on the RNI, is necessary to assess the validity of patient self-report "styles," both before and after HBOT treatments. Higher scores on subtests from this measure reflect greater neurobehavioral or adaptive functional disturbances[198].

2. *Iowa Collateral Head Injury Interview*[200] (Iowa). The Iowa contains 21 items, which assess the presence of psychosocial symptoms often associated with frontal lobe damage, especially damage secondary to closed head injury[200]. The symptoms measured include: absentmindedness, indecisiveness, non-spontaneity, perplexity, apparent low motivation, disorganization, inflexibility, poor planning, failure to learn from experience, poor judgment, non-reinforcing attitude, risk seeking, disinhibition, impulsivity, stimulus-bound behavior, impolitic speech, immaturity, neutral affect, poor insight, poor empathy, and self-centeredness. It is administered to a collateral informant, usually the partner or spouse of the head-injured party.

3. *Personality Assessment Inventory*[201] (PAI) The PAI is an objective self-report measure of psychological functioning and personality[201] that has been used in TBI[202]. The questionnaire is comprised of 344 items. It requires a 4th grade reading level. This questionnaire generates 4 validity scales that measure response style and impression management. The 11 clinical scales primarily assess axis 1 disorders, or psychopathology, and features of Borderline Personality Disorder and Antisocial Personality Disorder. This measure also provides 5 treatment scales, and 2 interpersonal scales.

4, *The Rivermead Post-Concussion Symptoms Questionnaire (see above).*

5, The *Functional Status Exam*[203] (FSE) is a measure of patient-reported perceptions of their limitations in physical (e.g., personal care, ambulation, and travel), social (functioning at work or school, home, and community), psychological (cognitive and behavioral), and financial status domains. This measure has been validated in prior TBI outcome research[203-4]. This scale has been shown to be more closely correlated with severity of injury than the Short Form-36[204], and, as a result, we will not use the Short Form-36 in this study. Higher scores in each of these adaptive functional domains reflect higher levels of functional limitations or deficits.

6. *The Modified Perceived Quality of Life Scale*[205] (MPQOL is a measure of the degree of personal satisfaction with one's level of functioning across several activities of daily living. Higher scores in the MPQOL scale reflect higher perceived satisfaction with current level of functioning. Thus, this scale measures "positive" affective-related ratings about functioning while the FSE[203] measures "negative" affective-related functional limitations.

7. *The "Percent-Back-to-Normal" rating*[206] (PBN) is a global measure of patient self-reported recovery or the degree to which the patient perceives she or he falls between no post-TBI recovery (i.e., 0% = not at all back to normal) and complete post-TBI recovery (i.e., 100% = complete recovery or back to normal).

Pre-/Post-Measurement Schedules for Randomized,

Eligibility Screening, Moderator, & Outcome Measures	**Inclu-sion Cri-teria**	**Pre-test**	**Post 40 Rx's**	**Post 80 Rx's**	**Post 6 mos. F/U**
Eligibility Screening					
Rivermead Post-Conc Sx Question.	**X**	**X**	**X**	**X**	**X**
Wechsler Test Adult Read(>70ss)[#]	**X**	**X**			**X**
Word Memory Test (valid scores)	**X**	**X**			**X**
Test of Memory Malingering (>/=45)	**X**	**X**			**X**
Addiction Severity Index	**X**				
Moderators					
WAIS-III Full Scale IQ (>/=85)	**X**	**X**			
Age, Education, & Work History		**X**			**X**
Iowa Collateral Head Injury Interview		**X**			**X**
Personality Assessment Inventory		**X**			**X**
Litigation/Disability status	**X**	**X**			**X**
Outcomes					
SPECT Imaging/MRI/MRS/MRDTI		**X**	**X**	**X**	**X**
Neurology Exam		**X**	**X**	**X**	**X**
Neuropsychological Battery:					
Verbal/Nonverbal Cognition					
WAIS-III VIQ/PIQ/FSIQ		**X**	**X**	**X**	**X**
Learning/Memory					
Rivermead Beh Memory parag Alt Forms		**X**	**X**	**X**	**X**
Wechsler Memory Scale-III		**X**	**X**	**X**	**X**
Rey AVLT Alternate Forms		**X**	**X**	**X**	**X**
Rey Complex Figure Test-Recalls		**X**	**X**	**X**	**X**
Attention/ExecutiveContr/Working Memory					

Test of Variables of Attention[#]		**X**	**X**	**X**	**X**
EFS Color Word Test[#]		**X**	**X**	**X**	**X**
EFS Verbal Fluency Test		**X**	**X**	**X**	**X**
EFS Trail Making Test		**X**	**X**	**X**	**X**
EFS Tower Test		**X**	**X**	**X**	**X**
EFS Design Fluency Test		**X**	**X**	**X**	**X**
Manual Motor					
Grooved Pegboard		**X**	**X**	**X**	**X**
Finger Tapping Test		**X**	**X**	**X**	**X**
Adaptive Behavioral					
Functional Status Exam		**X**	**X**	**X**	**X**
Ruff Neurobehavioral Inventory		**X**	**X**	**X**	**X**
Modified Perceived Quality of Life		**X**	**X**	**X**	**X**
Percent-Back-to-Normal Rating		**X**	**X**	**X**	**X**
Iowa Collateral Head injury Interview		**X**			**X**

E. Return to school or work: This will be assessed by simple questioning. Subjects will be asked if they have returned to work or school and, if so, if they returned to the previous employment or a different job, and if the new job is commensurate with the old job. If returned to school they will be asked if they are taking courses of equal subject matter or less and how they are performing. Return to premorbid level of functioning will be assessed with the PBN.

F. Neurological examination: The neurological exam will be performed by the study neurologist Dr. Barton. She will screen the patients for confounding neurological disease and perform a neurological exam that emphasizes balance and gait, two functions that have been found to be abnormal on physical exam in patients with mild or moderate chronic TBI[207-8].

G. Hyperbaric medicine exam: This exam will be performed by the P.I. It will be another layer of screening of the subject for overall fit to the study as well as assessment of hyperbaric medicine exclusions. Patients will also be instructed in how to clear their ears during chamber pressurization.

H. Imaging: Baseline studies will include chest X-ray for hyperbaric medicine screening then SPECT brain imaging. After immediate processing of the SPECT brain the patient will have a sequence of MRI scans. Baseline 3.0 Tesla magnet MRI of the brain will be the first study followed by MRI with Gadolinium. The MRI will then be coregistered with SPECT to focus on the temporal lobes where MR Spectroscopy will be focused. Temporal lobe atrophy is a consequence of TBI[209] This study will be followed by MRDTI to focus on white matter tracks in the temporal lobes and entire brain.

1. MRI: There are a number of MR pulse sequences that have been employed for assessment of Post-Traumatic Brain Injury (PTBI), including: traditional structural sequences, e.g. T1-weighted, T2-weighted, and T2 FLAIR; T2*-weighted GRE; Diffusion-weighted imaging; Magnetization Transfer Imaging; Diffusion Tensor Imaging as applied to White Matter Tracts[76]; and proton MR Spectroscopy.

The traditional structural MR sequences are of limited sensitivity in those patients with a mild degree of TBI, on the order of 43% to 68%[59,210,51,211] T2*-weighted images have been shown to be more sensitive for the detection of hemosiderin deposits, the chronic form of hemorrhage, thus inferred to reflect the degree of DAI (diffuse axonal injury) as well, though their correlation with long-term outcome has not been established[212-14]. Similarly, Diffusion-Weighted Imaging (DWI) is sensitive to acute and subacute infarcted tissue, a marker of DAI in the appropriate imaging and clinical context in the short term[215-7], but are not so sensitive as T2 FLAIR

sequences are for more long-term tissue changes nor as sensitive as T2*-weighted images are for the detection of hemosiderin deposits.

2. *Magnetization Transfer Imaging* (MTI) yields tissue contrast different from traditional MR pulse sequences by applying off-resonance RF energy to saturate bound protons (i.e. those in macromolecules that do not, per se, emit RF energy once RF pulses are ceased) that subsequently undergo a chemical exchange with unbound "free" water protons and are thus unable to emit RF energy, yielding a quantifiable measure of the structural integrity of tissue known as MTR (magnetization transfer ratio)[218]. While this technique has found useful applications in IV Gadolinium-enhanced brain imaging and assessment of demyelinating disease, e.g. multiple sclerosis, there is a poor correlation with clinical outcome thus far in patients with PTBI[76]. In one study where areas of normal-appearing white matter were assessed, MR proton spectroscopy was more sensitive in distinguishing between PTBI patients with good outcomes compared to poor outcomes than was the MTR, which detected abnormalities in such areas in only 20% of those patients with poor outcomes[63]. We will not be using MTI.

3. *Diffusion Tensor Imaging* (MRDTI), though described over a decade ago[219], has only recently become commercially available, further expanding upon the principle of Diffusion-weighted imaging[220-1]. The technique is able to generate maps of white matter (WM) tracts within the brain, now amenable to appropriate interpretation with the development of atlases depicting normal human WM tracts[222-4]. These WM tract images convey not only the direction of various tracts but also their respective size, which is a graphic means of representing a particular MRDTI parameter known as Fractional Anisotropy (FA)[225]. FA has been shown to correlate well with measures of verbal fluency[226], response time to visual target detection[227], abnormalities in those afflicted with focal cortical dysplasia[228], and age-related slowing of memory retrieval[229]. MRDTI has been shown to reflect WM abnormalities in patients with severe head injuries from 11 months to 9 years post-trauma[230-1]. Our own review of the literature since one done in a recent article published in September 2007[232], which demonstrated a correlation between FA and cognitive impairment in certain WM tracts, concurs that MRDTI appears to be a promising imaging modality in those patients with mild PTBI[233] but that no studies beyond 6 months post-injury have as yet been done to confirm this potential. As expected, one study has already demonstrated that MRDTI employing a 3 Tesla field strength MR unit improved depiction of most fiber tracts relative to those seen on a 1.5 Tesla field strength MR unit employing the same healthy volunteers in a direct comparison[234].

4. *Magnetic Resonance (Proton) Spectroscopy* (MRS) offers in vivo neurochemical information. Within brain tissue and aside from water, the three largest chemical peaks[235] are: N-acetylaspartate (NAA), a quantitative marker of neuronal health; Choline, a marker of inflammation which increases in cell proliferation (e.g. neoplasm)[236]; and Creatine, which along with phosphocreatine, is related to metabolism[237]. All three of these biochemical peaks have been found to change over time, usually reaching a stable level by 6 months following the traumatic event[238].

MRS findings have been shown to be sensitive and correlate with neuropsychological function and functional outcomes[239-41]. At this time, the sensitivity of proton MRS has not yet been sufficiently established in those patients with chronic but mild TBI[242,64,65,243]. However, there is at least one study, employing FDG-PET imaging in patients with long-term behavioral and cognitive deficits following mild traumatic brain injury, which did demonstrate a correlation between local metabolic abnormalities and neuropsychological test results[244]. Thus, MRS might be able to detect such abnormalities if future studies are done with this subset of PTBI patients.

With respect to quantifying MRS results, the amplitudes of the peaks of the various chemical entities are expressed in arbitrary units, which are, per se, not useable. The peak amplitudes are dependent on multiple technical factors, including the design and efficiency of the transmit/receive head coil, the pulse sequence employed, and the control parameters comprising the sequence chosen. Thus, the spectroscopic peaks are necessarily expressed through the use of ratios. Creatine is typically used for this purpose since it is relatively invariant and uniform in normal brain tissue[245]; however, it is not known if Creatine is stable in physically traumatized brain tissue, though there is some evidence to support that the Creatine concentration decreases in those neurons which are relatively hypermetabolic and increases in those which are relatively hypometabolic[246-7]. Thus, the use of Creatine as a reference peak, ideally, would be adjusted for relative differences in local

cerebral metabolism, such as may be determined by the metabolic component of Neurolite uptake in the SPECT portion of this study proposal. For this reason, the use of MR perfusion imaging is proposed in order to deconvolute the metabolic component of Neurolite uptake from its perfusion component. In turn, MR perfusion imaging and MRDTI should be more precise if the findings on T2*-weighted images are used to control for potentially confounding adjacent hemosiderin deposits that can artifactually diminish calculated flow[248] and fractional anisotropy, respectively.

5. MRI Materials and Methods: All MR scans are to be performed on a General Electric 3.0 Tesla magnet, HDX software platform, with official interpretation of the images rendered by two board-certified neuroradiologists with current CAQ (certificates of advanced qualification.) Each patient will have a peripheral IV heplock in place, initially for administration of the radiotracer for the Nuclear Medicine Neurolite SPECT scan of the brain; later in the day near the end of the MR study, IV administration of a standard 0.1 mmol/kg dose of Magnevist (not to exceed a maximum volume of 20 ml) will be administered via a power injector for the MR brain perfusion scan.

The MR imaging protocol, including an interview of the patient by an MRI technologist for screening purposes, will consist of the following pulse sequences, including localizer images to aid, along with the fiducial markers (vitamin E capsules) placed on the patient's head during the initial planar imaging phase of the nuclear medicine study; this should facilitate image fusion and subsequent multi-modality analysis. Imaging planes listed are tentative and designed to optimize visualization of the Papez circuit of white matter tracts, the hippocampi and amygdalae as well as facilitate coregistration with the Neurolite SPECT scan. The total time from the patient's entering the MR scanner unit room to exit will be no longer than 1 hour. Ordering of sequences is optimized to maintain SAR (specific absorption rate) of RF pulses applied to the patient's head well within safety limits:

1. 3-plane localizer GRE (Gradient Recalled Echo), used to prescribe locations for the next two series.
2. Straight Axial Echo Planar Diffusion-weighted Images, which also include T2-weighted, ADC and exponential ADC images through the entire brain.
3. True Sagittal T1-weighted FSPGR (gradient echo) images through the entire brain interleaved, which will facilitate prescribing locations for all subsequent series.
4. True Coronal T2 FLAIR FSE through the entire brain.
5. True Axial T1-weighted FSPGR images through the entire brain.
6. True Coronal Diffusion Tensor Echo Planar Images of the entire brain.
7. True Coronal thin slice 3D T1-weighted SPGR acquisition through the hippocampi and adjacent structures only.
8. True Coronal 2D proton Multi-Voxel Spectroscopy – sequence TBA of the hippocampi, amygdalae, and corpus callosum with additional voxels obtained through each individual patient's additional structural abnormalities (and contralateral side counterparts) as appreciated on the preceding sequences.
9. True Coronal dual echo T2*-weighted images with 1st TE in range of 6-10 msec and 2nd TE in range of 30-35 msec of the entire brain.
10. True Axial (i.e. along predefined planes according to the fiducial markers) Single Shot FSE T2 FLAIR
11. True Coronal GRE dynamic Perfusion images with IV Gd+ to generate regional Cerebral Blood Volume and Mean Transit Time Images, from which regional Cerebral Blood Flow images are, in turn, calculated.
12. Post-contrast True Axial T1-weighted FSPGR images through the entire brain.

6. SPECT.

Brain SPECT Data acquisition: Subjects will be seated in a dimly lit testing room, eyes open, and auditory input representing only background white noise. Fiducial markers will be placed at the lateral canthomeatal line to aid alignment with MRI. Following a 15 min acclimation period, 25-30 mCi of ^{99m}Tc ECD will be administered intravenously. After a 40 min delay to permit clearance of background scalp and blood pool radiotracer activity, the subject will be positioned in the scanner. SPECT scanning will ensue for a duration of 25 min on a GE Milennium MG camera, horizontal resolution 9 mm**.** Projection images will be acquired using low energy high-resolution parallel hole collimators positioned at less than 15 cm from the axis of rotation in a 128x128 matrix in

three-degree increments for 20 min. When acquisition is complete the study is examined for motion artifacts . If needed the acquisition will be repeated for the same or for a shorter time to insure a motion free acquisition. Image reconstruction is performed in the transverse domain using back-projection with a ramp filter. Three dimensional post reconstruction filtering will be applied. After reconstruction an attenuation correction is done, using a Chang first-order method. The reconstructed images will then be transferred to a Windows based SEGAMI workstation for producing a comprehensive color display using 21 discrete shades of color and a 40% threshold. This display includes the conventional orthogonal slices supplemented by a fourth sequence of temporal slices (along the temporal long axis), and then a set of automatically obtained volume rendered images displayed in four fixed levels of thresholding. In addition, a set of eight 3D functional surface displays, normalized to the Talairach space, were obtained via an adaptation of the Neurostat automatic software. For the evaluation of follow-up images (post HBOT) as well as for direct comparison with MRI-s, an automatic realignment method (based on the Mutual Information algorithm) is available, within the same software package. The image display described above is in routine use for several years at the University of Illinois Medical Center in Chicago IL and at the Neuroscience Center in Deerfield IL.

Image Processing for SPM : Image analysis will consist of three components: image normalization, image coregistration, and 3-dimensional voxel-wise statistical analyses. Intra-subject normalization of image count density corrects for the difference in administered dose of ^{99m}Tc ECD between sessions and for differences in global CBF across conditions. This is accomplished by ratioing to whole brain count density in all data sets. The resultant probe count ratio is then used as a scaling factor to equate the count density among image sets. Inter-subject normalization (correcting for global variability in tracer uptake between subjects) is accomplished at the same time as intra-subject normalization by scaling whole brain counts in all subjects across all conditions to 100, yielding both intra- and inter-subject normalized images. Data are then automatically resliced, normalized and coregistered. A visual check by the operator is used to validate the coregistration.

Statistical Analyses: Data analyses will be performed using the Statistical Parametric Mapping package (SPM) for Windows, provided by the Welcome Dept of Imaging Neuroscience in London , on a high speed Windows work station . Images will be spatially normalized to Talairach space. and smoothed using a 10 mm full width half maximum (FWHM) Gaussian filter. Analyses employ 3-dimensional voxel-specific assessments of the t value as representative of the change in level of the different group means. The distribution of t values across all voxels is mapped, and a threshold based on the t value for the experiment's degrees of freedom is used to identify voxels participating in the response to chronic HBOT, to distinguish between responses in the active and sham-treated groups as a whole. The voxel-based t-statistic define the regions of response to treatment. Since these voxels represent both real responses and random parts of the null set t distribution, we search the "t image" voxels for a neighborhood association. We assume that voxels from the null set t distribution are randomly distributed in space, and can be removed by requiring that "acceptable" voxels have neighbors that also meet the selected t threshold. Regions of significant activation identified on the t images are corrected for the large number of t tests performed, the lack of independence between voxels, and the resolution of the processed images using a modification of a statistical technique based on Gaussian random field theory suggested by Worsley[249] Further, only areas that exceed 50 contiguous voxels will be evaluated unless the structure identified is itself of small volume (e.g. amygdala). Remaining significant voxels are mapped onto a model brain to produce a parametric statistical image that identifies response location. The final "parametric image" reveals those voxels whose relative rCBF differed most following treatment, between groups. The result is an image of anatomic zones (clusters) which contribute to significant between-group differences. Following the identification of significant clusters, post hoc analyses will be conducted. The primary data to be analyzed will be both the amplitude and neuroanatomic extent of functional responses (clusters), expressed as %change in rCBF (delta rCBF), and cluster volume (delta vol) within a t-image (SPM) defined region. . Potential covariates will include psychometric, MRI/MRS/MRDTI imaging, QOL measures, gender, age, and severity of initial trauma.

SPECT Reading: The individual baseline Brain SPECT-s will be evaluated separately as well as compared with each of the post HBOT SPECTs. Readers will be blinded to all clinical information. The 10-step color format will be used to grade the entire brain (normal or abnormal) and specific regions: temporal and frontal lobes, anterior and posterior cingulate, basal ganglia, pons, and cerebellum. Greater than or equal to 10% reductions or increases in relative flow will be considered significant. In addition, the regions will be qualitatively scored at baseline as

decreased, normal, or increased brain blood flow with comparisons to subsequent scans as showing deterioration, no change, or improvement overall and in each region. Qualitative readings will be compared to semi-quantitative readings and then to SPM analysis and finally other imaging and outcome measures.

*7. HBOT***:** Both subject groups will be treated in single person acrylic "see-through" chambers, a Sechrist 3200 (32 inch inside diameter) or Perry Sigma Plus (40 inch inside diameter), that are equipped with concealed wall mounted gas switching panels. Only the chamber operator will be aware of the identity of the treatment gas. The 32 inch Sechrist chamber will accommodate 90% of all patients. The 10% who are claustrophobic will be offered the Sigma Plus. Patients will be instructed pre-treatment on how to equalize the pressure in their middle ears. Once inside the chamber, just after closure of the chamber door and immediately before pressurization, the subject will be instructed to perform the first Valsalva maneuver to pressurize the middle ear space before the initial 2 pounds per square inch (psi) start-up pressurization of the chamber that attains air-tight "seal" of the chamber. This maneuver expands the middle ear space and brings the ear space to neutral volume after the compression effect of the initial 2 psi seal pressurization.

Hyperbaric Oxygen Group: Pressurization will proceed with 100% oxygen at 1.0 pounds per square inch (psi) per minute, the lowest pressurization rate, to 1.5 ATA (atmospheres absolute) or 7.35 psi and will take approximately 7 minutes. During the entire pressurization the subject will be continuously instructed to "clear his/her ears" using various pressure equalization techniques that they have learned and practiced before chamber entry. Inability to pressure equalize the middle ear space will immediately truncate pressurization until the subject can equalize pressure. Pressurization will then resume until the final depth of 1.5 ATA is achieved. The subject will be notified when he/she is at treatment depth. The subject will remain at depth for approximately 45 minutes and the subject will be informed of the onset of depressurization which will occur at the same rate as pressurization. The subject will again be instructed to pressure equalize the middle ear space, however, barotrauma is minimized during decompression since gas expansion in the middle ear space passively vents through the Eustachian Tube to the pharynx. Total dive time will be 60 minutes. The subject will be queried regarding pain and untoward symptoms after this and all subsequent treatments.

Sham Air Treatment Group: After the initial pressurization on air the chamber pressure will be pressurized to 3-4 psi over 1-2 minutes then immediately decompressed over the same time period to minimal chamber pressure to give the subject the sensation of ear pressurization and ear pressure equalization in the equivalent amount of time that the HBOT group is pressurized. During this time the subject will be continuously instructed to equalize pressure in the middle ear space in attempt to confuse the subject about the sensation of pressurization and, thus, maintain blinding of treatment group. Once the chamber pressure is back to minimal pressurization the subject will be notified that he/she is at treatment depth. The subject will remain at the 1 psi level until the 52 minute mark of the dive and the pressurization/depressurization procedure at the beginning of the treatment will be repeated to fool the subject into believing that he is being decompressed. During this pressurization/depressurization the subject will be continuously instructed to equalize pressure in his middle ears. At 60 minutes the chamber door will be opened, ending the sham treatment. Subject will be queried regarding pain and untoward symptoms after this and all subsequent treatments**:**

I. Analyses (Statistics):

*1. Sample Size Estimation***:** Sample size projection for this study are based on a series of 4 chronic TBI patients, three of whom had mild TBI and one severe TBI, who completed a variety of psychometric tests before and after HBOT treatments. A common psychometric test was sought in the patients' batteries. The Wechsler Memory Scale-Delayed Memory standard scores[178] was a psychometric test in which all four patients had completed and was used to provide an approximate sample size projection. Since a true control group was lacking in this series of patient data, we assumed that a control group would demonstrate minimal practice effects. The effect size observed was assumed to continue and was of sufficient clinical interest to be used as the basis for the estimation of the minimum sample size. Sample sizes of at least 25 in each group would insure that the effect size of interest (or larger) could be detected using two-group ANOVA model with at least 80% probability (power). As a cautionary note, it should be noted that these projections are based on a very small sample size, assumptions of little or no change in a hypothetical untreated group, and the fact that the overall change in memory was significantly influenced by the performance of a single patient who experienced a dramatic improvement in memory after HBOT. As a result, the power analysis can only be considered a crude

estimate of the sample sizes necessary to detect a significant improvement in the single cognitive function of memory. For this reason and the inclusion of patients with moderate TBI the sample size was increased to 60.
2. Statistical Methods: This study will randomize 60 patients each into one of two treatment sequences (completely at random) in which one group of patients will receive two courses of 40 HBOT in succession, while the other group of patients will receive 40 sham air treatments followed by 40 HBOT in succession. In both groups patients will be measured at baseline, and after 40 treatments, 80 treatments, and 6 months follow-up. Measurements will be made on individual brain injury patients that will include numerous cognitive, neuropsychological and imaging measures at baseline and at each post-treatment time point as described above. These tests include a variety of symptom questionnaires that are to be filled out by the patients, a neurological exam and SPECT brain blood flow imaging. We will also perform MRI, MRDTI, and MRS of the temporal lobes looking at ratios of NAA, chorine, and creatine (3 ratios).

Because of the anatomic heterogeneity of traumatic brain injury, there is heterogeneity of symptoms, cognitive deficits, and emotional/psychological effects. Some patients may have greater or lesser deficits in different cognitive functions for near identical injuries. Assuming these deficit functions are due to injuries to specific areas of the brain and hyperbaric oxygen would simultaneously treat all areas of injured brain, greater or lesser improvements will occur in these functional domains. Differences in pre/post treatment average test scores between treatment groups will be compared similar to the aforementioned memory function to determine the scope of the effect of HBOT on each psychometric test and group psychometric performance measures.

Baseline characteristics will be assessed with the following measures: Rivermeade Post-Concussion Inventory, Addiction Severity Index, PTSD Checklist, TOMM, Green WMT, and the WTAR. Although this is a randomized clinical trial, we will compare baseline levels of these variables to insure that our treatment groups are comparable. This will primarily involve comparison of mean (or median) total scores (or sub-scale scores) for each of the inventories using simple two-sample independent t-tests. The purpose of these comparisons is to verify baseline comparability of the treatment groups, and not for inference.

The primary aim of the study is to determine whether treatment with HBOT significantly improves cognitive functioning. Therefore, the primary analysis variable will compare cognitive functioning between treatment groups. The following response measures provide assessments for cognitive functioning and quality of life indicators: WAIS-III, R-AVLT, R-CFT, Paragraph Memory from the RBMT-E, TOVA, D-K EFS, DFT, EFS TMT, EFS CWIT, GPT, FTT, EFS Tower Test. Iowa Collateral Head Injury Interview, FSE, MPQOL Scale, PBN, RNI, and the PAI. Since there are several variables that assess cognitive functioning, we will employ the sum testing method of O'Brien[250]. This method calls for the conversion of all psychometric test scores in the complete battery on a given individual to z-scores and sums of these z-scores and obtains a single composite value for each individual to represent cognitive functioning. In the event the distribution of this measure does not reasonably approximate normality, we will use the alternate non-parametric method described by O'Brien. The analysis of composite scores (O'Brien) will be augmented with an analysis of individual measures. A secondary analysis will be performed to compare MRS and MRI volume measurements in the temporal lobes between treatment groups. These analyses will involve comparing mean (median) ratios of NAA, choline and creatine between treatment groups.

All statistical tests will be performed using a Type I error rate of α=0.05. Confidence intervals will be constructed using a 95% confidence coefficient. A longitudinal response model that includes effects for treatment, time, and severity of injury will be used to model changes from baseline in the primary and secondary response variables (reference: Applied Longitudinal Analysis by Fitzmaurice[251]. This model is sufficiently flexible to allow comparisons both between and within treatment groups on changes from baseline.

II. Problem Areas:

A. Secondary gain, litigation, malingering: This will be addressed by screening measures and limiting half of the civilian subjects to those who are not in litigation or worker disability claims.

B. Test/retest learning effect on psychometric testing: will employ measures that minimize learning effect, however, at least for the first phase of the experiment a learning effect will be in both groups and so will be able to be quantified in the control group.

C. Small sample size: the effects of small sample size will be minimized somewhat by the number of imaging and test measures employed. If there are positive effects of HBOT there should be proof of same on at least

some measures and lack of proof on others. In addition, the O'Brien statistical method should help amplify the small sample size by individually proofing or not proofing each case. Intra-group comparisons before and after air and oxygen treatments (air group) and before and after 40 and 80 oxygen treatments will add another layer of statistical strength, although they are partially compromised by unblinding.

D. Complications: historically these have been minimal in adults with chronic TBI. Therefore, drop-out rate should be very small.

E. Drop-out rate: should be minimized by the fact that all subjects will receive treatment at some point in the protocol.

F. Excessive amount of testing: yes, but need to maximize avenues of proofing since time is of the essence. Can't do initial pilot over 4 years then a randomized controlled trial over 4 years. Results are needed now. All the cognitive testing should be done in about 10-12 hours. Imaging shouldn't take more than 3 hours. Patients will already be at the scanner for SPECT. With heparin lock in place, after the 25 minute scan they can walk to the adjacent room and undergo all the MRI sequences in less than 60 minutes.

G. Confinement anxiety: Severely claustrophobic patients will be screened by the hyperbaric physician before the Rivermead questionnaire. All others should be able to tolerate HBOT with the 40 inch Perry chamber. Xanax, as needed, will help the remaining few you still have problems.

H. Maintaining blinding of subjects: Only the hyperbaric technician will know treatment group. All investigators will be blinded to subject groups. Gas panels will be concealed to blind patients, however blinding of patients during the first 40 treatments will be difficult. Hyperbaric pressurization results in obvious middle ear space pressure sensations; these must be induced in both groups of subjects to prevent them from knowing their treatment group assignment. Unless attempts are made to deceive the sham air treatment group into believing they are receiving hyperbaric oxygen unblinding will occur. An alternate method is to use a pressure control where both groups receive 1.5 ATA of pressure. The problem with this type of control is that the air group receives a 50% increase in oxygen level with each treatment. A 30% increase in the Collet[141] study on HBOT in CP has been shown to cause durable clinical motor and cognitive improvements in pediatric brain injury. Even using hypoxic mixtures of air (14% oxygen) at depth cannot ablate the increased oxygen exposure during compression and chamber washout at 1.5 ATA of the normal air used to compress the chamber. While this 15 minutes seems like an inadequate amount of surplus oxygen to cause oxygen DNA signaling no one has studied the minimum dose (depth-time exposure) to cause oxygen signaling. As such, a pressure control group could experience a treatment effect. The method for inducing the middle ear pressure changes in the sham group is to provide a mild pressurization/depressurization at the beginning and end of the treatment that is enough to cause the patient to clear their ears and thus simulate the pressurization at the beginning and depressurization at the end of an HBOT.

After the first phase of the experiment no one will be blinded, but the efficacy portion of the study will have been completed. Given the expectation, based on the past 17 years experience of treating chronic brain injury and the now worldwide experience with the same in children, that there will be a relative lack of serious side effects associated with the use of hyperbaric oxygen, unblinding of technicians and investigators should rarely be required. If a complication arises from hyperbaric treatment the treating physician can treat it without knowing the subject's group. Serious side effects will be treated blindly by the P.I., but removal from the study will be at the discretion of the DSB. Since the P.I. is not collecting data if he were to become aware of the subject's treatment group in the course of evaluation of the complication this will not be communicated to the subject or data collecting investigators. Additional blinding maneuvers will include: 1) the use of external ear "planes", the soft rubber plugs that modulate middle ear pressure changes and help prevent middle ear barotraumas, 2) minimization of all but necessary personnel in the vicinity of the chamber to prevent visualization of the chamber pressure gauges, 3) covering the pressure gauge on the control console with a small piece of cardboard or similar material to mask the gauge, and 4) excluding the examining neurologist, neuropsychologist, and other investigators from the hyperbaric unit. The effectiveness of the blind will be evaluated by asking the participant whether or not they feel that they received treatment and recording their answer on a data form. At the conclusion of the first, 40th treatments, and after the final neuropsychological and QOL testing the participant will be asked to pick his/her treatment groups.

III. Data and Safety Monitoring Board:

An independent group of physicians can be established through DoD. The P.I. will help with selection of members of this board.

IV. Alternative Methods and Approaches:

We know of no alternative treatment methods. An effective reproducible treatment for chronic brain injury of any type has never been documented until the animal study by the principal investigator. In addition, we know of no pilot data of any modality in chronic brain injury. The only alternative to this study is to directly address the entire spectrum of TBI by performing an uncontrolled unblinded pilot trial of HBOT in chronic TBI of all degrees of severity. This is problematic, however, because it would cause significant delay (years) to a definitive efficacy study and not service one of the mandates of this award track which is to deliver an effective therapy that can be rapidly deployed to military veterans. While the present study is projected to extend over 4 years it could possibly be accelerated to 3 years, depending on the speed of recruitment of subjects. It may even be shortened if interim analysis of the first phase of the trial shows a benefit to HBOT that would ethically terminate the first phase.

Alternative outcome measures are numerous, however, not necessarily superior. The obvious imaging alternative would be PET, but was not chosen for this study for a variety of reasons. The P.I. has 17 years experience with SPECT and HBOT combined and has one of the largest cohorts (75 patients) of SPECT normals in the country. These normals have been personally recruited, interviewed, neurologically characterized with a detailed neurological questionnaire, and scanned using Neurolite on a high resolution triple-head Picker scanner. This cohort will be used for the spm analysis of Dr. Pavel. PET brain is not readily available in the New Orleans area, the P.I. is not familiar with the PET radiologists and the quality of their work, and the PET facilities, to the P.I.'s recent investigations, have not recruited their own normals. In addition, since the MRI and SPECT will be done at the same facility fusion and co-registration of images will be possible that would not with PET. Lastly, the information provided by the combination of MR studies and SPECT will give superior information to PET due to the ability to image white matter tracts and provide the convenience to the patient of one-stop imaging with on-site radiologist oversight of all of the imaging at once.

V. Recruitment of Subjects:

Subjects will be recruited through multiple avenues. The P.I. has a relationship with New Orleans area television stations due to multiple previous features on hyperbaric medicine, carbon monoxide poisoning, stroke research, and other projects. Our group anticipates media presentations to recruit subjects for this project. We also have a relationship with the Veterans Association hospital and staff in New Orleans (across the street from Charity Hospital) through VA neuropsychologist John Mendoza. The P.I. has had a working relationship with Dr. Mendoza for 17 years. The P.I. is also connected to the veterans groups in the New Orleans area through association with prominent military such as General Hunt Downer and the past president of the American Foreign Legion who recently supported a collaborative TBI/HBOT project of the P.I. at a veterans' hearing in New Orleans in September. The P.I. and co-investigators will announce the study and recruit subjects through their departments, the LSU Neurosciences Center of Excellence, LSU and Tulane Schools of Medicine, rehabilitation hospitals in the New Orleans area and professional contacts in the New Orleans area. Study neuropsychologist Suzie Andrews is well connected in the New Orleans Metro area with neuropsychologists through her 20+ years of practice and past presidency of the New Orleans Neuropsychological Association.

Print, radio advertisements/announcements, and website announcements (P.I.'s, the International Hyperbaric Medical Association, the American College for the Advancement of Medicine, the American Association of Health Freedom, International Hyperbarics Association, and others) will also be used to recruit subjects. Announcements will be sent out through the VA system and military channels (as allowed) as well as veterans groups. TBI associations and support groups in the New Orleans area and nationally will be targeted for presentations and announcements.