Effect of Short Periods of Normobaric Hyperoxia on Local Brain Tissue Oxygenation & Cerebrospinal Fluid Oxidative Stress Markers in Severe Traumatic Brain Injury

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Preliminary evidence suggests that $PbtO_2$ values of ≤ 15 mm Hg may be suggestive of brain tissue hypoxia. Accordingly, many neurotrauma intensive care units attempt to maintain the $PbtO_2 \geq 20$ mm Hg based on the belief that this intervention will increase availability of oxygen in the brain for metabolism, and will avoid periods of brain tissue hypoxia with a 5 mm Hg buffer range. In clinical practice, one approach to managing a low $PbtO_2$ (< 20 torr) is to increase the delivered fraction of inspired oxygen (FiO_2). It remains unclear whether this therapy has risks as it also has the potential to increase oxidative stress. To determine if short periods of normobaric hyperoxia (2h) affect oxidative stress markers and antioxidant defenses, cerebrospinal fluid (CSF) was assessed in adults [n=11, (9 male, 2 female), mean age 26±1.8 yrs], with severe TBI (Glasgow Coma Scale score 6±1.4) before, during, and after a FiO_2=1.0 challenge. Markers of oxidative stress including lipid peroxidation (F_2-isoprostane [ELISA]) and protein oxidation (protein sulfhydryls [fluorescence]) and markers of antioxidant defenses including total antioxidant reserve (AOR) [chemiluminescence] and glutathione [fluorescence] were evaluated in CSF. Physiological parameters, [intracranial pressure (ICP), mean arterial pressure (MAP), cerebral perfusion pressure (CPP), $PbtO_2$, arterial oxygen content (pO_2)] were assessed at the same time points, using a 30 minute average prior to each FiO_2 change. Mean (±SD) $PbtO_2$ and PaO_2 levels significantly changed for each time point, [before 27.3±7.4,

173.1±51.4; during 93.9±58.1, 385.5±108.3; and after 29.3±13.0, 171.8±45.1] a FiO_2 challenge, (p=.04; .01), respectively. Oxidative stress markers, antioxidant reserve defenses and physiological parameters did not significantly change for any time period. These preliminary findings suggest that brief periods of normobaric hyperoxia improve oxygen levels without producing local oxidative stress in brain tissue. Additional studies are required to examine extended periods of normobaric hyperoxia and application of treatment during periods of critical $PbtO_2$ levels.

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PREFACE

This work was performed with a multidisciplinary approach, interacting with neurosurgery, critical care medicine, trauma, nursing and respiratory therapy. In addition, the basic laboratory support at the University of Pittsburgh is extraordinary. I could not have chosen a better environment for my research.

I would like to thank all of my committee members and co-authors for their endless reviews of my dissertation and manuscript. Without their mentoring and guidance, this work would not be as concise as it is. Thank you to my advisor, Dr. Leslie Hoffman, who would not allow me to procrastinate any longer, and gave me the additional support to complete my dissertation.

I would also like to extend my gratitude to all of the families who agreed to have their loved ones participate in this important research project. This is such a difficult time for families of traumatic brain injury patients. The unselfish, altruistic human nature to further research and help future victims of head injury is truly amazing. I was very pleased to find the results of my dissertation project produced no potential detrimental effects for the markers that I chose to examine.

Most importantly, I wish to thank my family for their endless love and support. My parents provided a loving home and always encouraged my academic pursuits. As grandparents, they provided the best daycare for my three children as I balanced work and school. Thanks to

my brother and sister for being great academic role models and providing a positive, competitive environment. Thanks to my husband Dave who always picked up the pieces when I was too stressed out through these MANY years of schooling. And thanks to my three beautiful children who would play independently while I finished my long hours of writing.

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1.0 INTRODUCTION

Traumatic brain injury (TBI) is a major public health concern, with 1.5 to 2 million people affected each year in the United States (Langlois et al., 2003). It is estimated that 50,000 individuals per year suffer a severe TBI. Among the subset of individuals who experience a severe TBI, mortality is estimated to be 20%, with 50-70% of the survivors experiencing permanent neurological disabilities that limit their return to school or the workforce (Torner, 1999). The majority of TBI victims are less than 30 years of age at the time of their injury, resulting in an estimated loss of productive years that is greater than estimates for cancer and cardiovascular disease combined. Annual costs for the acute, rehabilitative, and home care of TBI patients are estimated to be $4 million (Torner, 1999), with annual lifetime costs of TBI, including lost productivity, estimated at $60 billion (Finkelstein, 2006).

Although the statistics are daunting, it has been shown that neurological improvement can occur even in the most severely injured sub-population (King, Carlier, & Marion, 2005). TBI mortality and morbidity is a combination of two phases of injury, primary and secondary. Primary injury in TBI results from the direct impact of the external force which can damage brain parenchymal tissue and axons. Advances in automotive technology and law enforcement of safety adherence have decreased the incidence of TBI and the severity of the primary injury at the time of the accident (McGwin, Metzger, & Rue, 2004; Metzger, McGwin, MacLennan, & Rue, 2004; Stewart et al., 2003). Secondary injury after TBI occurs as a result of additional

insults to the brain that ultimately worsen neurological outcome. The two most severe secondary insults, hypotension and hypoxemia, can exponentially compound neurological recovery and mortality (Rosner & Daughton, 1990). Of concern, ischemic damage has been found in over 50% of post-mortem tissue from TBI patients following autopsy (D. I. Graham et al., 1989). In addition to low cerebral blood flow and oxygenation, biochemical cascades are also set in motion that cause tissue damage and cell death (Bareyre, Wahl, McIntosh, & Stutzmann, 1997; Busto, Dietrich, Globus, Alonso, & Ginsberg, 1997; Clark et al., 1997; Dixon, Bao, Long, & Hayes, 1996; S. H. Graham, Chen, & Clark, 2000; Hayes, Yang, Whitson, & Postmantur, 1995; Kochanek et al., 1995; Raghupathi & McIntosh, 1996). Three biochemical pathways, in particular, have been implicated as key participants in this cascade of secondary injury, glutamate excitotoxicity, calcium overload, and oxidative stress (Juurlink & Paterson, 1998). The ultimate goal of acute management of patients following TBI is to minimize or prevent secondary injury from occurring and thus improve neurological outcome.

Within the last decade, treatment of TBI has become more standardized with the adoption of the *Guidelines for Management of Severe Traumatic Brain Injury* ("Guidelines for the management of severe traumatic brain injury," 2007). Implementation of these guidelines begins at the scene of the accident with rapid triage and stabilization of the patient, and includes management in a designated neurotrauma intensive care unit (NTICU) during the acute post injury phase, with the common thread being the prevention of hypotension and hypoxemia. The two parameters currently used to monitor the potential contribution of blood pressure and brain perfusion during the acute post-injury phase are mean arterial pressure (MAP) and cerebral perfusion pressure (CPP). Systemic hypoxemia is measured using arterial oxygen saturation (SaO_2), arterial partial pressure of oxygen (PaO_2), and hemoglobin content (Hg).

Brain tissue oxygenation ($PbtO_2$), a recent addition to monitoring, can also be continuously assessed, providing the critical care team an *in vivo* tool to directly quantify changes in brain oxygenation at the local level. The ability to monitor $PbtO_2$ is emerging as an important advancement in critical care management, as very low $PbtO_2$ values are associated with poor neurological outcome and death (Valadka, Gopinath, Contant, Uzura, & Robertson, 1998). Currently, there is no management standard for treatment of low $PbtO_2$ values; however, some critical care teams treat falling or low $PbtO_2$ values by increasing the fraction of inspired oxygen (FiO_2) through the ventilator. Although effective in increasing $PbtO_2$ values, and presumably oxygen delivery to the brain, there is concern that this therapy may increase secondary injury within the injured brain (Longhi & Stocchetti, 2004). Studies in children (Bayir et al., 2002) and adults (Wagner et al., 2004) have shown that antioxidant reserves are markedly depleted and biomarkers of oxidative stress are increased in the acute period after TBI, potentially placing the already vulnerable brain at risk for secondary injury. In experimental studies, hyperoxia has increased artificially induced apoptotic neuronal death and increased protein carbonyls (Kaindl et al., 2006).

Increased brain tissue oxygen concentrations, theoretically, could disrupt the normal reducing homeostasis within the cell and thus paradoxically increase free-radical production and free radical-mediated tissue damage. Reactive oxygen species (ROS) produced during normal metabolism are involved in enzymatic reactions, mitochondrial electron transport, signal transduction, activation of nuclear transcription factors, gene expression and the antimicrobial action of neutrophils and macrophages (Bayir, 2005; Halliwell, 1989). The accumulation of free radicals is prevented by an active reducing environment which is maintained by antioxidant enzymes and substances, such as glutathione and ascorbate. Oxidative stress and subsequent

oxidative injury will occur in an environment of an altered reduction state or depletion of antioxidants (Halliwell, 1992). Lipid membranes are the most sensitive to free radical accumulation and, as such, alterations in their biochemistry are early indicators of oxidative damage. *F_2-isoprostane* is a marker of lipid peroxidation. Protein damage may also result from free radical damage, with the loss of protein sulfhydryl groups (Levine, Moskovitz, & Stadtman, 2000). Loss of *protein sulfhydryls* is an indication of protein oxidation. Disruption of antioxidant processes can be assessed by measurement of the antioxidant, *glutathione,* and measurement of *total antioxidant reserve* (Tyurin et al., 2000). By measuring changes in markers of oxidative stress, (e.g., F_2-isoprostane and protein sulfhydryls), and antioxidant defenses, (e.g., glutathione and total antioxidant reserve), in relation to a FiO_2 challenge, it will be possible to gain further insight into the biochemical consequences of normobaric hyperoxia in the acute period after a severe TBI.

1.1 PURPOSE

The purpose of this study was to examine the effect of a brief period of normobaric hyperoxia on physiological parameters and biochemical markers of oxidative stress and antioxidant defenses in patients admitted to a Level 1 trauma center who have sustained a severe TBI.

1.2 SPECIFIC AIMS

Specific Aim 1: To determine the effect of a short period of normobaric hyperoxia, via a FiO_2 challenge (100% oxygen administered for 2 hours) on key physiological values: local brain tissue oxygenation ($PbtO_2$), global brain perfusion (cerebral perfusion pressure [CPP]), and intracranial pressure (ICP) after severe TBI.

. **H 1.1**: A FiO_2 challenge will significantly increase $PbtO_2$, providing evidence for improvement of brain oxygenation.

H 1.2: A FiO_2 challenge will significantly decrease ICP, thus improving CPP, providing evidence for improvement of cerebral perfusion.

Specific Aim 2: To determine the effect of a short period of normobaric hyperoxia via a FiO_2 challenge on biochemical markers of oxidative stress and antioxidant defenses in CSF after severe TBI.

H 1.1: A FiO_2 challenge will significantly increase biomarkers of oxidative stress (as measured by increased F_2-isoprostane and decreased protein sulfhydryls) in CSF, providing evidence that normobaric hyperoxia treatment exacerbates oxidative stress injury in compromised brain.

H 1.2: A FiO_2 challenge will significantly decrease biomarkers of antioxidant reserves (as measured by glutathione and total antioxidant reserve) in CSF, providing evidence that normobaric hyperoxia treatment places the injured brain at risk for oxidative stress injury.

1.3 DEFINITION OF TERMS

Severe TBI: TBI is defined as a closed head injury due to blunt trauma resulting in intracranial injury and permanent or temporary cognitive and physical impairment. The severity of the TBI is determined by the level of consciousness, specifically assessed by the Glasgow Coma Scale (GCS) score (Teasdale & Jennett, 1974). The GCS score ranges from 3-15, with 15 being a normal level of consciousness. In this study, a severe TBI was defined as a GCS score of ≤ 8, not following commands, within 24 hours of injury, and without the influence of alcohol, sedatives or paralytics.

Independent Variable:

FiO_2 challenge: In the multi-trauma patient, FiO_2 is temporarily increased (FiO_2 0.90) during the resuscitative period in the emergency room. After transport to the NTICU, the FiO_2 is decreased to $\leq .60$ as quickly as tolerated while maintaining the arterial oxygen saturation > 94% and PaO_2 > 90mm Hg. In this study, a FiO_2 challenge was defined as a 2 hour period when the FiO_2 delivered by the mechanical ventilation was increased to 1.0. The FiO_2 challenge was performed 24-48 hours after admission to the NTICU and a minimum of 2 hours after stabilization of FiO_2 $\leq .60$. This time point was selected to avoid the initial trauma-related increase in oxidative stress markers described by Bayir (Bayir et al., 2002; Wagner et al., 2004), and yet sample the injured brain at a time when $PbtO_2$ monitoring would normally be occurring.

Dependent Variables:

Physiological Parameters: $PbtO_2$, ICP, mean arterial pressure (MAP) and CPP values were continuously collected via the patient's monitor, and minute data downloaded to a patient data

server that is stored by the Brain Trauma Research Center. For each study time point (before, during, and after FiO_2 challenge), the final; 30 minutes of data collection prior to CSF sampling was averaged to provide a comparison to the CSF collection time for each of the three collection points.

$PbtO_2$ was measured by an oxygen sensor placed in the brain parenchyma after severe TBI diagnosis (usually within 24 hours of injury). The values obtained are thought to reflect availability of oxygen at the cellular level, with an increase in $PbtO_2$ indicative of an increase in oxygen supply to the tissue. In this study, $PbtO_2$ was measured using a Licox® brain oxygenation probe (Integra Neurosciences™) placed within the brain parenchyma as standard clinical management for severe TBI patients at this institution.

ICP was measured by a pressure monitor placed within the lateral ventricle of the brain that was connected to a catheter (extraventricular drain [EVD]) with a dual purpose of pressure readings and drainage of CSF for sampling and treatment. Continuous pressure readings were obtained when the tubing system was in the closed position. In this study, ICP was defined as the pressure reading obtained from this continuous monitoring system.

MAP was calculated as [2 (diastolic blood pressure) + systolic blood pressure]/ 3. Arterial blood pressure was measured by a catheter placed in the radial artery that was connected to a pressure monitor. The calculation of MAP was transformed within the patient monitor (Hewlett Packard/Philips Medical Systems, Bothell, WA) and displayed as a digital value. In this study, MAP was defined as the continuous calculated reading obtained from the patient monitor.

CPP was calculated as MAP – ICP, and is a measurement of the blood pressure gradient that dictates blood flow to the brain governing subsequent oxygen and nutrient delivery. The calculation of CPP was performed within the patient monitor and displayed as a digital value. CPP was recorded continuously when ICP and MAP monitoring are simultaneously being performed. In this study, CPP was defined as the continuous calculated reading obtained from the patient monitor.

Oxidative Stress was defined as an abnormal state of disequilibrium between oxidation and reduction states. This may be a result of excessive accumulation of ROS from oxidation or the decrease in reduction potential resulting from a decrease in antioxidant defenses. This study utilized two markers of oxidative stress:

F_2-Isoprostane: The brain has an inherent high metabolic rate with a physical makeup predominantly of polyunsaturated fatty acids in neuronal membranes. When arachidonic acid-containing lipids are oxidized by free radicals, the products include F_2-isoprostanes, a family of prostaglandin F_2-like compounds. These are stable, reliable markers of *in vivo* oxidative stress (Roberts & Morrow, 2000) that can be detected in biological fluids such as CSF (Bayir et al., 2002; Morrow, Minton, & Roberts, 1992). In this study, *F_2-Isoprostane* was measured using a commercially available enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI), with a detection threshold of 5 pg/ml.

Protein Sulfhydryls: Thiols are especially sensitive to ROS attack. In the basal state, levels of S-thiolated proteins are extremely low, constituting <1% of the total protein levels. A decrease in protein sulfhydryls reflects an increase in oxidative stress damage

(Chai, Hendrich, & Thomas, 1994), from loss of protein thiols. In this study, *protein sulfhydryls* were measured using a fluorescence assay technique (Langmuir et al., 1996).

Antioxidant Defense was defined as the normal biochemical reduction reaction in response to oxidation. Antioxidant defenses correct imbalances between oxidation and reduction states in cells. A decrease in antioxidant defenses and/or an accumulation of reactive oxidative species (ROS) may result in oxidative stress. This study utilized two markers of antioxidant defenses:

Glutathione (GSH) is a tripeptide, L-γ-glutamyl-L-cysteinyl-glycine, with a molecular weight of 307. It is the major regulator of the intracellular reduction state (Meister, 1992; Meister & Anderson, 1983). GSH is the antioxidant present in the highest concentrations in cells of all organs, including the brain. GSH protects the brain against ROS and is involved in the disposal of peroxides. CSF levels of GSH are reflective of the amount of antioxidant properties present within the brain. In this study, *glutathione* content was estimated using a fluorescence assay technique (Langmuir et al., 1996).

Total Antioxidant Reserve (AOR) was defined as the pool of antioxidants present in a sample that have a net result in reducing oxidants. This pool contains water soluble antioxidants, of which 90% are composed of glutathione, ascorbate, and urate. A known amount of peroxyl radicals was added to the CSF sample, and the amount of radicals scavenged was determined and represents the total endogenous antioxidants. This value was compared to standard, known values. In this study, *total antioxidant reserve* in CSF was estimated using a chemiluminescense assay (Tyurina et al., 1995).

1.4 CONCEPTUAL FRAMEWORK

In the normal basal state of aerobic metabolism, an equilibrium exists between oxidation and reduction (Figure 1). Oxidation results in the generation of ROS and their intermediates. Reduction reduces the ROS and intermediates to less toxic components, and thus mitigates oxidative damage. Oxidative stress results when this balance is disrupted with increased oxidation or decreased reduction. Several factors can compound this balance, such as hereditary factors, disease processes, and the external environment.

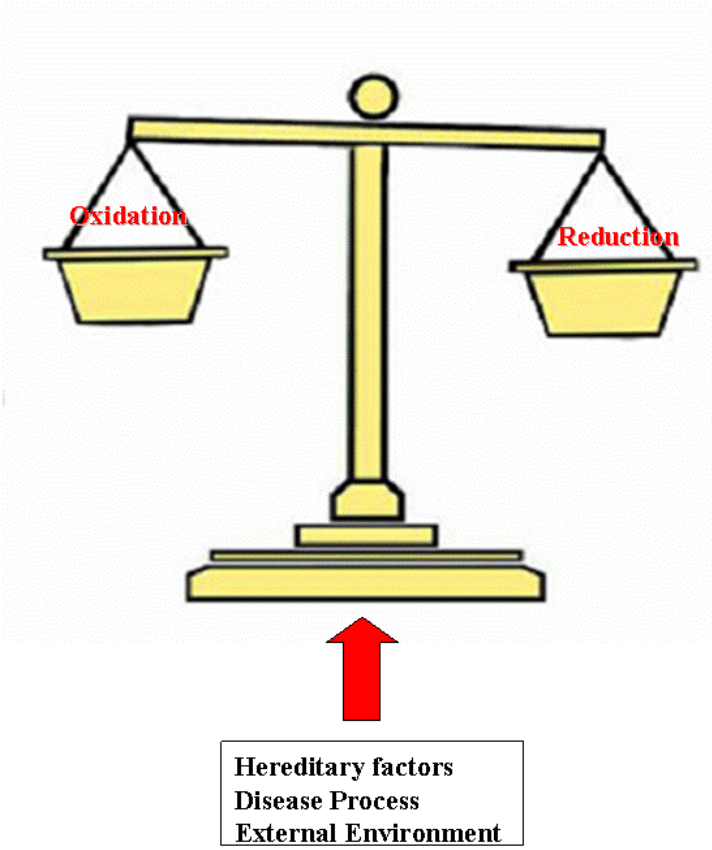

Figure 1: Normal equilibrium between oxidation and reduction

The conceptual framework for this proposed study is illustrated in Figure 2. Following a severe TBI, to maximize a favorable neurological outcome, it is imperative that secondary injury is minimized in the acute post-injury phase. When brain tissue oxygenation is threatened to ischemic levels, as in a $PbtO_2$ level < 20 mm Hg, FiO_2 is raised by the critical care team. This study was designed to evaluate the effect of increasing the FiO_2 concentration to 1.0 on the physiological ($PbtO_2$, CPP, and ICP) and the biochemical (oxidative stress and antioxidant reserve markers) response to this change. The goal was to examine the impact of normobaric hyperoxia treatment on measures of tissue oxygenation and oxidative stress. This approach was

chosen to determine if the emerging intervention (normobaric hyperoxia, an increase in the FiO_2) is producing beneficial or detrimental actions on recovery of the injured brain after severe TBI.

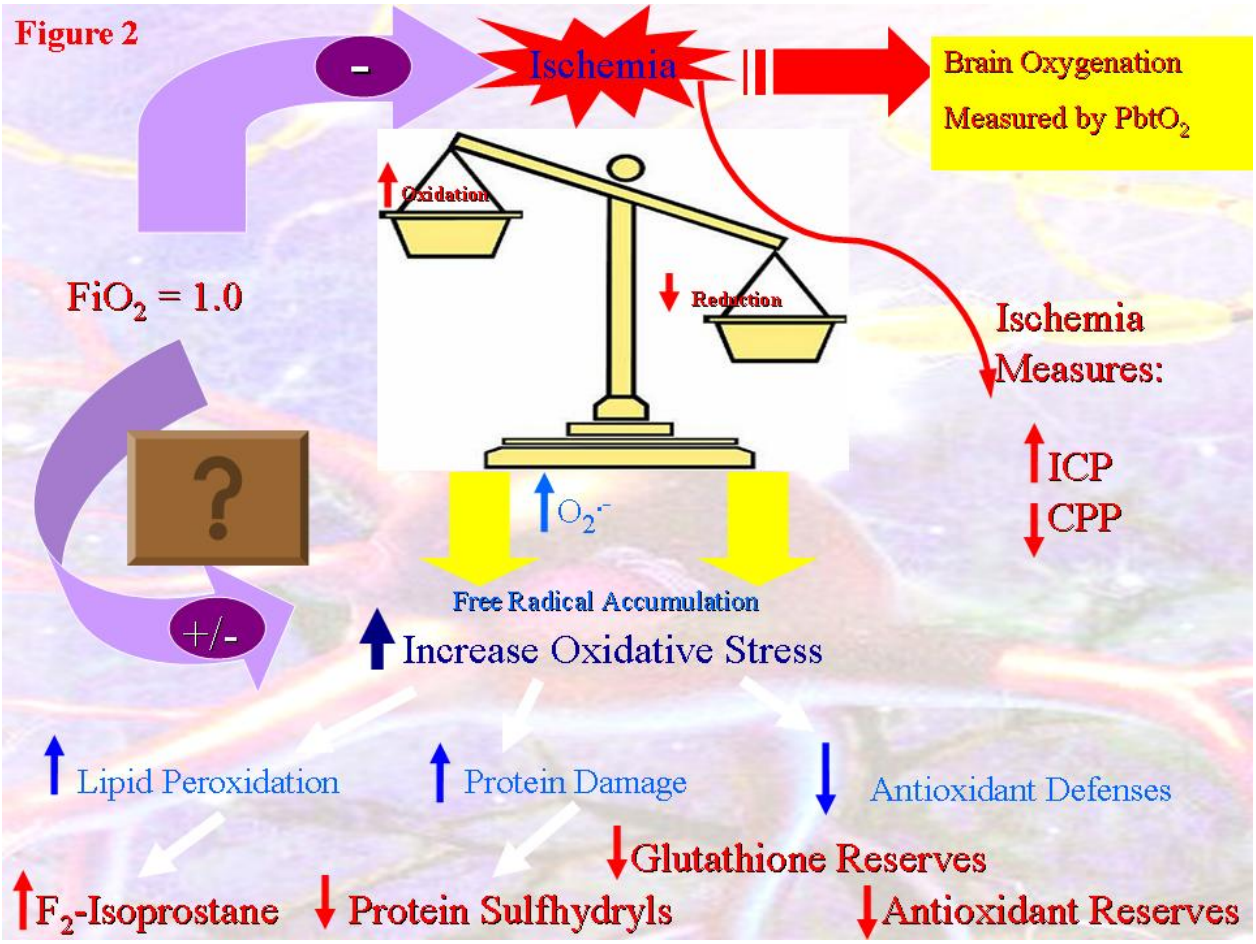

Figure 2: Conceptual Framework Schematic

1.5 SIGNIFICANCE

Prevention and minimization of secondary injury during the acute post-injury phase is the goal of the critical care team caring for patients with severe TBI. Standard clinical orders are written with set parameters to maintain physiological vital signs with the goal of avoiding any episodes

of hypotension or hypoxemia. Continuous cerebral and systemic hemodynamic monitoring in a specialized NTICU allows specialized care of severe TBI patients. The importance of this specialized nursing vigilance is evident in a study by Rosner et al (Rosner & Daughton, 1990) who identified that even one episode when the CPP decreases to < 50 mm Hg can double the risk of morbidity and mortality (Rosner & Daughton, 1990). Further, mortality has been shown to increase approximately 20% for each 10 mmHg decrease in CPP. Although CPP and ICP are the 'gold standards' for neurocritical care monitoring, it is also recognized that brain tissue hypoxia and/or infarction can occur with no corresponding change in ICP or CPP (Stocchetti et al., 1998).

Recent advances in neurological monitoring include the continuous measurement of $PbtO_2$, thus enabling the neurosurgical team an *in vivo* tool to directly visualize changes in brain oxygenation. Since cerebral oxygenation is the most critical function of cerebral blood flow, additional monitoring of this parameter is likely to be important to direct therapy in severe TBI patients who are at greatest risk for ischemic sequelae. Valadka, et al (1998) reported that the longer a patient's $PbtO_2$ values are <15 torr, the greater the likelihood for death. In this study, the critical threshold value was 6 torr (Valadka et al., 1998). Stiefel (2005) compared the neurological outcome of patients having $PbtO_2$ monitoring in addition to ICP monitoring versus historical controls with ICP monitoring alone, and reported a 25% improvement in favorable outcomes (Stiefel et al., 2005). Although this study has been criticized for ignoring potentially confounding factors that may have affected neurological outcome, use of this monitoring parameter is increasing nationwide.

Although many institutions have implemented $PbtO_2$ monitoring and institute various treatment modalities when the $PbtO_2$ values decrease, the *Guidelines for Management of Severe Traumatic Brain Injury* ("Guidelines for the management of severe traumatic brain injury,"

2007) have deemed this monitoring as a class III option with no current standardization of treatment targeting low $PbtO_2$ values. Increases in arterial oxygen delivery (caused by an increase in the FiO_2) readily increase $PbtO_2$ values, and a 'sliding scale' to obtain a $PbtO_2 > 20$ torr is often targeted in some institutions. However, the effect of normobaric hyperoxia has not yet been examined in relation to oxidative stress and antioxidant reserve in the setting of severe TBI. It is possible that paradoxically increasing $PbtO_2$, may place the injured brain at additional risk for secondary damage.

The project described herein is innovative as it is the first, to our knowledge, that has evaluated the implications of using this new neurological monitoring device (Licox®, Integra Neurosciences™, Plainsboro, NJ) in the clinical setting of severe TBI in relation to biomarkers of oxidative stress that are uniquely measured as a battery at the University of Pittsburgh. This project is on the cutting edge of nursing research as bedside nurses monitor changes in $PbtO_2$, and independently, or in collaboration with the respiratory care team, alter FiO_2 concentrations to adjust $PbtO_2$ within the desired range.

2.0 LITERATURE REVIEW

2.1 TRAUMATIC BRAIN INJURY

TBI is a public health concern, with 1.5 to 2 million people affected each year in the United States (Langlois et al., 2003). It is estimated that 50,000 individuals per year suffer a severe TBI. In this severe TBI subset, a 20% mortality rate has been reported, with 50-70% of these survivors having permanent neurological disabilities that limit their return to school or the workforce (Torner, 1999). Annual costs for the acute, rehabilitative and home care of TBI patients are estimated to be $4 billion (Torner, 1999). Factoring in the loss of productivity due to the young population that is affected, this estimate increases dramatically to $60 billion in the United States per year (Finkelstein, 2006). Quality of life is affected, not only for the individual, but also for the family.

During the late 1980's, surveys conducted by the Agency for Health Care Policy and Research, Department of Health and Human Services indicated that treatment provided for TBI patients varied across hospitals throughout the United States. Ten years later, a survey that involved 261 trauma centers throughout the United States reported the same findings (Ghajar et al., 1995). To address this nationwide problem, ten prominent neurosurgeons specializing in neurotrauma responded by conducting an extensive literature review of studies that tested the efficacy of commonly used therapies for the acute care of patients with severe TBI ("Guidelines

for the management of severe head injury. Brain Trauma Foundation, American Association of Neurological Surgeons, Joint Section on Neurotrauma and Critical Care," 1996). In 1995, this effort resulted in the publication of the first evidence-based *Guidelines for the Management of Severe Head Injury* by the Brain Trauma Foundation, in collaboration with and endorsement from the American Association of Neurological Surgeons (Bullock et al., 1996). This document provided guidelines for consistent acute care of patients with severe TBI and has since been endorsed by The American Association of Neurologic Surgeons, the World Health Organization Neurotrauma Committee, and the New York State Department of Health. The *Guidelines* have been integrated into the European Brain Injury Consortium (Maas et al., 1997) and distributed to all members of the American Association of Neurological Surgeons. They are updated periodically with the most current version in 2007 ("Guidelines for the management of severe traumatic brain injury," 2007). The principles elucidated in the *Guidelines* provide intensive treatment strategies but allow adjustments for individualized care of the injured brain and non-central nervous system injuries. While a major step in improving care of the patient who experiences a TBI, understanding of the complex circumstances which contribute to secondary injury following this injury remains incomplete.

2.2 SECONDARY INJURY IN TBI

There are two types of injury that occur after TBI, primary and secondary injury. Primary injury occurs as a consequence of the impact of the external force and produces injury by directly damaging brain parenchymal tissue or stretching axons. In addition, the primary injury sets in motion a biochemical cascade which causes additional injury, as shown in laboratory and clinical

studies (Bareyre et al., 1997; Busto et al., 1997; Clark et al., 1997; Dixon et al., 1996; S. H. Graham et al., 2000; Hayes et al., 1995; Kochanek et al., 1995; Raghupathi & McIntosh, 1996).

Three biochemical pathways have been implicated as key participants in this biochemical cascade of secondary injury: glutamate excitotoxicity, calcium overload, and oxidative stress (Juurlink & Paterson, 1998) (Figure 3). Under ischemic conditions, cerebral blood flow, oxygen and glucose delivery are decreased, resulting in calcium accumulation, the release of glutamate, and free radical generation, all of which may result in apoptosis, or cell death, if not reversed.

Figure 3

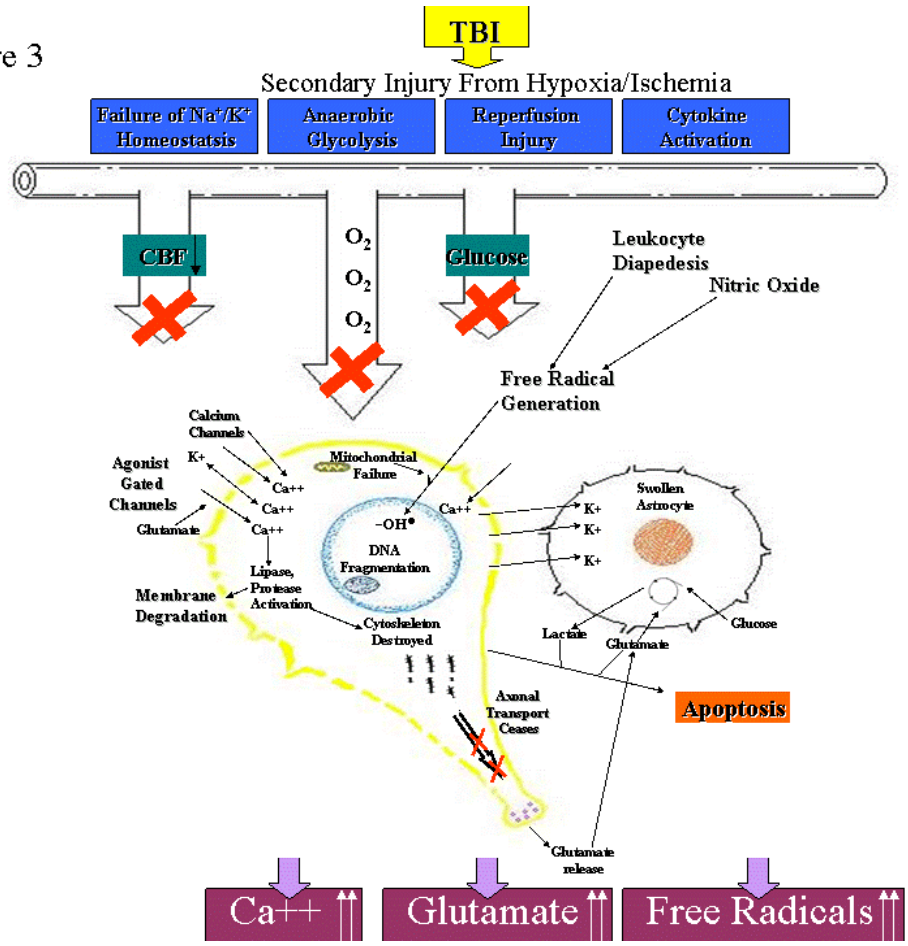

Figure 3: Cellular Responses Following Secondary Injury: Schematic illustrating the cellular response to TBI during an ischemic event, if the process is not reversed [adapted from a published review (Zauner, Daugherty, Bullock, & Warner, 2002)].

The ultimate goal of the acute care treatment after TBI is to minimize and prevent secondary injury from occurring and thus improve neurological outcome. A major focus of research to minimize the impact of secondary injury relates to improving the understanding of biochemical responses. Prior studies have shown that biochemical mediators are released during periods of ischemia and also as a consequence of primary and secondary insults to the brain. These mediators are thought to play a major role in influencing the recovery process (Bullock et al., 1998). Various neurochemicals have been implicated in the cascade of events that cause secondary injury, with the most common being the neurotoxic build-up of extracellular concentrations of the excitatory amino acid (EAA) glutamate.

Glutamate concentrations have been shown to increase in laboratory studies examining functional outcome following TBI (Faden, Demediuk, Panter, & Vink, 1989; Globus, Alonso, Dietrich, Busto, & Ginsberg, 1995; Katayama, Becker, Tamura, & Hovda, 1990; Matsushita, Shima, Nawashiro, & Wada, 2000) and in humans after TBI (Bullock, 1994; Bullock, Zauner, Woodward, & Young, 1995; Palmer, Marion, Botscheller, Bowen, & DeKosky, 1994; Stover et al., 1999). Glutamate toxicity depends on the energy state of the nervous tissue, with greater toxicity in low glucose and low oxygen environments (Novelli, Reilly, Lysko, & Henneberry, 1988), a finding that suggests a key role during ischemic periods. Poor neurological outcome, defined as 6-month Glasgow Outcome Scale (GOS) score of 1-3 (dead, vegetative state or severe impairment), has been shown to correlate with increased extracellular glutamate levels (Bullock, 1994). However despite the strong correlational evidence for glutamate toxicity and poor outcomes, efforts to attenuate this increase have shown success in animal models (Lewen, Fredriksson, Li, Olsson, & Hillered, 1999; Okiyama, Smith, White, Richter, & McIntosh, 1997;

Phillips, Lyeth, Hamm, Reeves, & Povlishock, 1998) but not in clinical trials (Doppenberg & Bullock, 1997).

Glutamate toxicity may also occur in conjunction with two additional biochemical cascades, calcium overload and oxidative stress, leading to additive or multiplicative effects on neurological outcome following a TBI. Although glutamate receptors have been implicated as the major pathway contributing to toxic calcium influx resulting in neuronal death (Choi, 1988), calcium derangement can occur under other conditions and pathways.

Under normal physiological conditions, neurons tightly regulate calcium homeostasis. In abnormal conditions, such as TBI, calcium influx occurs through damaged channels which results in excessive intracellular accumulation. This excessive intracellular calcium causes further cellular and mitochondrial damage with the breakdown of proteins, swelling of the mitochondrial, damaging the DNA leading to programmed cell death, or apoptosis.

An active focus of current research involves identifying the interplay and triggering of these cascades and minimization of target effects. Oxidative stress, described below, is also a cause of biochemical disarray.

2.3 CEREBRAL ISCHEMIA AND BRAIN OXYGENATION IN TBI

Systemic insults caused by the multi-trauma nature of the overall injury can also impair cerebral blood flow and oxygenation with the development of hypotension and hypoxemia. These consequences can exponentially compound adverse effects from the initial injury and potentiate the effects of secondary injury (Chesnut, Marshall, Klauber et al., 1993). Of concern, ischemic

changes have been found in over 50% of post-mortem tissue from TBI patients (D. I. Graham et al., 1989), suggesting that ischemic changes are common. The ultimate goal of the acute care treatment after TBI is to minimize and prevent secondary injury from occurring and thus improve neurological outcome.

The regulation of CBF is controlled by cerebral metabolism, levels of carbon dioxide and oxygen, CPP, and neurogenic mechanisms. CBF is relatively higher in the brain, compared to other organs and is approximately 50 ml/100g/minute. In the uninjured brain, the CBF is tightly controlled; however, temporary regional increases do occur during certain tasks, such as controlled movements, and with CBF and glucose utilization. Following TBI, patients have been observed to experience an 'uncoupling' of CBF and metabolism that leads to the development of cerebral ischemia (Bergsneider et al., 2000).

Marion (Marion, Darby, & Yonas, 1991) studied 32 severe head-injured adults having a total of 61 cerebral blood flow studies utilizing the xenon-computerized tomography method (Gur, Good, Wolfson, Yonas, & Shabason, 1982), and found that immediately after a TBI, the human brain enters a hypoperfusion state (< 32.9 cc/100gm/min) that may last for 24 to 48 hours (Marion et al., 1991). Studies in experimental models have shown up to a 74% reduction in CBF during the hypoperfusion state (Hendrich et al., 1999). This period of hypoperfusion may cause ischemic changes that are detrimental to the recovering brain (Golding, Robertson, & Bryan, 1999). Additional studies have shown that this hypoperfusion state may persist in non-survivors (Hlatky, Contant, Diaz-Marchan, Valadka, & Robertson, 2004; Marion et al., 1991) whereas in survivors, it is often followed by a state of hyperemia or 'luxury flow' (Marion et al., 1991).

Bouma et al (Bouma, Muizelaar, Choi, Newlon, & Young, 1991) examined early CBF defined as the first 6 hours after injury using intravenous ^{133}Xe and related the results to

differences in arteriovenous oxygen content and neurological outcome in 186 TBI patients (Bouma et al., 1991). Twenty-four severe TBI patients (13%) experienced a global CBF of < 18 ml/100gm/min, defined as the threshold for infarction, during their course of monitoring. The neurological outcome for this ischemic group of patients was compared to a group of nonischemic patients (n=160). Findings indicated a trend for poorer outcomes and higher mortality rates (p<.04). This study exemplifies the importance of interventions to prevent cerebral ischemia with the goal of improving neurological outcome.

Chesnut et al (Chesnut, Marshall, Klauber et al., 1993) examined the prevalence of secondary insults, namely hypotension and hypoxia, in patients enrolled in the Traumatic Coma Data Bank (TCDB) to determine their relationship to outcome. Hypotension was present in 35% of 717 patients in the TCDB with an associated 150% increase in mortality (Chesnut, Marshall, Klauber et al., 1993). In a related study, these investigators prospectively examined early hypotension (injury through resuscitation; n=717) and late hypotension (intensive care unit; n=493) in head-injured patients within the TCDB (Chesnut, Marshall, Piek et al., 1993). Early hypotension occurred in 35% (n=248) and late hypotension occurred in 32% (n=156) of this cohort. Hypotension doubled the mortality rate if occurring early after injury, and tripled poor outcome (death or vegetative) if occurring later. Logistic regression modeling was used controlling for age, admission GCS motor score, hypoxia, and extracranial trauma. One episode of hypotension occurred in 117 patients and 66% of these patients died or did not recover beyond a vegetative state (Chesnut, Marshall, Piek et al., 1993). Conversely, Rosner (Rosner, Rosner, & Johnson, 1995) reported that all patients (n=158) with severe TBI who received intense management with a protocol to maintain a CPP > 70 mm Hg achieved favorable outcomes (Rosner et al., 1995).

A concern arose after CPP targeted therapies resulted in an increased usage of pressors to artificially raise the CPP predisposing to possible side effects. A prospective randomized trial to assess this concern was performed by Robertson, et al (Robertson et al., 1999) by comparing the effects of a CPP vs. ICP targeted acute care management in 189 adult severe TBI patients. The risk for ischemia, defined by jugular bulb desaturation was decreased by 2.4 fold in the CPP targeted group; however, overall neurological outcome was unchanged. In addition, a 5-fold increase in the incidence of acute respiratory distress syndrome (ARDS) was seen in the CPP targeted group, cautioning the non-judicious usage of this treatment. Additional studies have since been performed lending evidence that autoregulation determines the effectiveness of CPP directed therapy. A prospective study by Howells, et al (Howells et al., 2005) of 131 severe TBI patients receiving CPP directed therapy resulted in improved neurological outcome only if intact regulation was present. Improved neurological outcome in those receiving ICP directed therapy was increased if defective autoregulation was seen (Howells et al., 2005). Individualized therapy is emerging as a means of improving outcomes in defined subsets of patients.

These and other studies have demonstrated the adverse effects of cerebral ischemia in the acute period after severe TBI (Gopinath et al., 1994; Muizelaar & Schroder, 1994; Rosner et al., 1995); however the target 'ischemic' level may be different for individual patients, and care must be taken not to generalize all TBI patients within the same treatment parameters. Additional monitoring parameters, such as $PbtO_2$, may assist in individualized patient directed care.

2.4 NORMOBARIC HYPEROXIA IN TBI

With the introduction of the Licox® probe, it became possible to measure regional brain tissue oxygenation and titrate FiO_2 to achieve the desired values. The probe is commonly inserted in the uninjured white matter of the brain following clinical confirmation of severe TBI and $PbtO_2$ is continuously monitored generally for the first 5 days after injury. In individuals with severe TBI, the normal range for $PbtO_2$ has been reported to be 25-30 torr (Maas, Fleckenstein, de Jong, & van Santbrink, 1993). A threshold value for survival after TBI was reported to be 5 torr (Maas et al., 1993). In a study that examined $PbtO_2$ values and outcome, a pattern emerged of poor outcomes antecedent with lower $PbtO_2$ values (Doppenberg, Zauner, Watson, & Bullock, 1998). In this study, a $PbtO_2$ of $\leq$19 torr was associated with poor outcome. In a pivotal study by Valadka (Valadka, Gopinath et al., 1998), 43 severe TBI patients had $PbtO_2$ monitoring for an average of 85 ± 41.8 hours. The subsequent $PbtO_2$ data was examined for an ischemic threshold that resulted in death within 3 months after injury. The data suggested that the longer a patient's $PbtO_2$ values are <15 torr, the greater the likelihood for death, with a critical threshold value of 6 torr (Valadka, Gopinath et al., 1998). The ischemic threshold of < 20 torr was established as a suggestion based mainly as a result of the above described work of Valadka (Valadka, Goodman, Gopinath, Uzura, & Robertson, 1998; Valadka, Gopinath et al., 1998). Many institutions, such as the University of Pittsburgh, treat low or falling values to maintain a $PbtO_2$ > 20 torr, avoiding periods of $PbtO_2$ < 15 torr.

To avoid low $PbtO_2$ values, a common practice in the NTICU is to utilize normobaric hyperoxia to increase the amount of dissolved oxygen in plasma to hopefully diffuse to the injured tissue. Although the dissolved oxygen in plasma contributes minimally (2-3%) to the total arterial oxygen content (PaO_2), it is the driving force to move oxygen to tissues. After a

TBI, the injured brain has been shown to display areas of variable blood flow, glucose utilization and edema. Although a normal PaO_2 may be observed, a low $PbtO_2$ may also be present. Cerebral blood volume (CBV) may increase to 'rescue' this edematous site by delivering increased substrate (oxygen and glucose), thus detrimentally increasing ICP to the rest of the brain. By increasing the oxygen content of the cerebral blood flow (by increasing the FiO_2), more substrate will ultimately be delivered to the injured sites, with a subsequent decrease in CBV demand (if CBF autoregulation is intact), and thus a decrease in ICP. Clinical evidence for this physiological theory are minimal and conflicting, as shown by the following studies interested in determining if changes in $PbtO_2$ are translated into improvements in CPP. In a study by Kiening that enrolled 23 head-injured patients, an increase of CPP from 32 +/- 2 to 67 +/- 4 mmHg significantly improved $PbtO_2$ by 62% (13 +/- 2 to 21 +/- 1 mmHg) (Kiening et al., 1997). In a similar study, with a sample of 7 severe TBI patients, it was observed that when CPP was < 60 mm Hg, $PbtO_2$ decreased (Bruzzone, Dionigi, Bellinzona, Imberti, & Stocchetti, 1998). In contrast, in a study of 9 patients (7 subarachnoid hemorrhage, 1 meningeoma, and one severe TBI) there was no effect on $PbtO_2$ when CPP was increased to > 60 mm Hg (Stocchetti et al., 1998). This may suggest that a change is observed in response to an increase in CPP only if the minimal oxygenation threshold is not met or that CBF autoregulation may have been variably affected by TBI in these patients.

Several additional studies have examined the effect of normobaric hyperoxia on biochemical markers of injury. In an experimental study (Singhal, Wang, Sumii, Mori, & Lo, 2002), rats were randomized to receive hyperoxia (FiO_2=1.0) or normoxia (FiO_2 =.30), using a 2-hour filament occlusion and 1-hour reperfusion of the middle cerebral artery. Histological studies examining the infarct volumes at 24 hours after injury showed 70% (total) and 92%

(cortical) reduction in the hyperoxia group, compared to the normoxia group. In addition, levels of oxidative stress were evaluated using three indirect methods. Evan's blue dye extravasation was quantified to assess blood-brain barrier damage. Heme oxygenase-1 (HO-1), a heat shock protein inducible by oxidative stress, was assessed using Western blot techniques. Levels of protein carbonyl formation were assessed by immunoblot technique. No differences were found between the hyperoxia and normoxia groups. These results provided evidence, in an animal model, that hyperoxia treatment during focal cerebral ischemia-reperfusion may be neuroprotective, and does not increase oxidative stress.

In the clinical setting, Menzel et al (Menzel et al., 1999) conducted a study that examined the effect of normobaric hyperoxia on physiological measures of ischemia, and extracellular markers of substrate delivery, namely lactate and glucose in 14 head-injured patients (Menzel et al., 1999). In this study, the FiO_2 was changed from .35 to .60 and then 1.0 over a 6 hour period, during the initial 24 hours after injury. Hyperoxia did not have an effect on CPP, ICP or extracellular glucose levels. However lactate, a biochemical marker of anaerobic metabolism, decreased by 40%. Increased lactate has been shown to be a measure of anerobic metabolism and is associated with poor outcome (DeSalles et al., 1986). A superior choice of biomarkers to assess anaerobic metabolism would have been the lactate/pyruvate ratio which is impervious to the dilutional effects of edema, and more evident of changes in the lactate and pyruvate disequilibrium (Persson & Hillered, 1992). Three-month neurological outcome was compared to the *oxygen reactivity* (increase in $PbtO_2$), with those patients exhibiting a lower response having more favorable outcomes than those with higher oxygen reactivity (0.4 ± 0.2 versus 0.9 ± 0.6, $p<.02$). These reactivity changes are small, although apparently significant to outcome, and

provide evidence that this normobaric hyperoxia treatment was safe when used in the acute period in the adult TBI population.

In a similar study, Magnoni et al (Magnoni et al., 2003) altered the FiO_2 (3 hours at 1.0) in eight patients with severe TBI with subsequent measurement of lactate, pyruvate, lactate/pyruvate ratio and extracellular glucose levels. There was a significant decrease in both lactate ($p < 0.01$) and pyruvate ($p < 0.05$); however, there was no change in either the lactate/pyruvate ratio or glucose levels, providing evidence that the treatment did not improve or worsen aerobic metabolism at the cellular level (Magnoni et al., 2003). Both of these studies assessed the effect of hyperoxia treatment during periods of normal oxygen availability. Biochemical changes may only occur when an ischemic threshold is met or approached. An experimental study in a model of focal ischemia in rats using hyperoxia treatment during the ischemic-reperfusion period attenuated neurological deficits at 24 hours post-injury and histological damage 14 days post-injury (Flynn & Auer, 2002).

A collaborative study between two well-known trauma centers specializing in the care of TBI patients (Medical College of Virginia and University of Bern, Switzerland), prospectively examined the effect of early normobaric hyperoxia (1.0 FiO_2 within 6 hours of admission, for a duration of 24 hours) on extracellular biomarkers of cerebral metabolism and neurological outcome (Tolias et al., 2004). Fifty-two patients treated in this fashion were compared to 112 historical controls matched by initial GCS score and ICP in the initial 8 hours after admission. Glucose extracellular levels increased ($p = 0.001$) in the FiO_2=1.0 group; however, glutamate and lactate significantly decreased ($p < 0.005$). ICP in the treatment group was significantly lower also (12.13 ± 0.75 mm Hg vs 15.03 ± 0.8 mm Hg, $p < 0.005$). Three and six month neurological outcome between the groups was not significantly different. This study provides

encouraging safety data for the usage of normobaric hyperoxia after severe TBI population. Biochemical markers of brain metabolism improved with normobaric hyperoxia treatment. ICP, although significant between groups, was not outside the normal range for either group, and outcome measurements were not significant between groups.

In summary, although there are compelling physiologic rationale for the potential benefits of normobaric hyperoxia as a treatment to improve brain oxygenation ($PbtO_2$), there is minimal empirical evidence for its usage with only a small number of studies that have attempted to measure the effects of this therapy. There are no clinical studies to date that have assessed the impact of this treatment on biomarkers of oxidative stress.

2.5 OXIDATIVE INJURY IN THE BRAIN

Medical therapy, such as that involved in using $PbtO_2$ monitoring to ensure a target value, involves the administration of high concentrations of oxygen. Excessive administration of oxygen can result in tissue damage as the result of the formation of reactive oxygen intermediates and peroxidation of membrane lipids. There are two categories of the negative effects of excessive oxygen therapy: direct toxicity by highly reactive free radicals, the cause of bronchopulmonary dysplasia, and indirect toxicity from a maladaptive physiologic response to oxygen (i.e. retinopathy). Premature infants are particularly sensitive to these effects, as a result of their greatly impaired antioxidant defenses (Weinberger, Laskin, Heck, & Laskin, 2002). In the adult, oxygen toxicity is manifested as acute respiratory distress syndrome (ARDS), and the relationship between increases in FiO_2 and oxidative stress in the lung is firmly established (Gutteridge & Halliwell, 2000). In the normal brain, large increases in tissue oxygenation, as

with hyperbaric oxygen, can provoke brain injury and seizures (Huang et al., 2000; Kleen & Messmer, 1999). However, it is unclear if high oxygen concentrations cause the same type of damage in brain as that seen with ARDS patients and premature infants.

In the normal brain, there is a high rate of oxidative metabolic activity that mandates the need for high levels of antioxidants. Dysregulation of these processes, as in trauma, presents a potential detrimental release of oxidants, with a corresponding disruption in the antioxidant properties. Antioxidants within the brain are classified into two groups: enzymes and low molecular weight antioxidants (LMWA). Several different enzymes have been shown to protect the brain during ischemic injury, such as superoxide dismutase (SOD) (Chan, 1996; Watson, 1993). LMWA's, such as ascorbic acid, are also extremely instrumental in minimizing oxidative stress. As with failed N-methyl-D-aspartate (NMDA) receptor blockade in clinical trials (to minimize glutamate toxicity), many efforts to provide therapeutic attempts to scavenge free radicals have proven unsuccessful in human TBI (Marshall et al., 1998; Marshall & Marshall, 1995), although successful in experimental animal models (Hall, 1987; Young, Wojak, & DeCrescito, 1988; Zuccarello, Marsch, Schmitt, Woodward, & Anderson, 1989). These findings suggest that there may be a combination of biochemical cascades that are negatively affected by the pathophysiology of TBI. This combination may include oxidative stress and free radical formation.

Reactive oxygen species produced during normal metabolism are involved in enzymatic reactions, mitochondrial electron transport, signal transduction, activation of nuclear transcription factors, gene expression and the antimicrobial action of neutrophils and macrophages (Bayir, 2005; Halliwell, 1989). The accumulation of free radicals is prevented by an active reducing environment which is maintained by antioxidant enzymes and substances,

such as glutathione. Oxidative stress and subsequent oxidative injury will occur in an environment of altered redox state or depletion of antioxidants (Halliwell, 1992). Lipid membranes are the most sensitive to free radical accumulation and, as such, are an early indicator of oxidative damage. *F_2-isoprostane* is a measurement of lipid peroxidation. Protein damage may also result from free radical damage, with the release of thiol groups (Levine, 1985). An decrease in *protein sulfhydryls* is an indication of protein oxidation. Disruption of the antioxidant processes can be assessed by measurements of the antioxidant enzyme *glutathione* and the *total antioxidant reserve* (Bayir et al., 2002; Tyurin et al., 2000). By measuring changes in markers of oxidative stress, e.g., F_2-isoprostane and protein sulfhydryls, and antioxidant defenses, e.g., glutathione and total antioxidant reserve, in relation to a FiO_2 challenge, it will be possible to gain further insight into the biochemical consequences of normobaric hyperoxia in the acute period following a severe TBI.

Oxidative stress has been assessed in both the pediatric (Bayir et al., 2002) and adult population (Bayir et al., 2004; Wagner et al., 2004) of severe TBI patients in studies at the University of Pittsburgh. 87 cerebrospinal samples from 11 children with severe TBI were compared to 8 controls for markers of antioxidant reserve and oxidative stress (Bayir et al., 2002). A biochemical marker of oxidative stress, F_2-isoprostane was significantly increased compared to controls ($p < .05$). In addition, antioxidant defenses, total antioxidant reserve and glutathione levels, were decreased after day 1 ($p < .05$). This study was the first comprehensive study examining an impressive battery of oxidative stress and antioxidant reserve in clinical TBI. Even with a small sample size, significant differences were found in the biochemical markers assessed.

A similar study assessed the relationship between markers of oxidative stress and antioxidant defenses and gender, age and therapeutic hypothermia in 199 cerebrospinal samples from 68 adult TBI patients (Bayir et al., 2004). A significant gender effect was found ($p < 0.018$) with females exhibiting a 2-fold higher level of F_2 isoprostane, providing evidence that female hormones may provide a neuroprotective effect against oxidative stress. F_2 isoprostane was also significantly associated with the presence of hypoxemia ($p = .04$). This suggests the possibility that toxicity from hyperoxia could be greater in males.

The above two studies are evidence that a comprehensive battery of biochemical markers of oxidative stress and antioxidant reserve are readily assessed at the University of Pittsburgh and provide very robust results even in small sample sets.

3.0 METHODS

3.1 DESIGN

A test-retest repeated measures design was utilized to assess the relationship between a normobaric hyperoxia challenge (100% oxygen administered for 2 hours) and measurements of key physiologic values and biochemical markers of oxidative stress and antioxidant defenses in 11 adult patients who have experienced a severe TBI. Physiologic measures ($PbtO_2$, MAP, CPP, ICP) and biochemical markers of oxidative stress (F_2-isoprostane, protein sulfhydryls) and antioxidant defenses (glutathione, total antioxidant reserve) were measured before, during (2 hours after induction of the FiO_2 challenge, and after (2 hours following) FiO_2 challenge. Demographic data (age, initial GCS, and gender) were obtained to describe the sample and identify possible confounding variables. Each patient served as their own control.

3.2 SAMPLE AND SETTING

Subjects were 11 patients with severe TBI admitted to a Level 1 trauma center located in a tertiary care institution. Based on data from the institution, approximately 55-60 patients with TBI were admitted and treated per year. Inclusion criteria for this study were:

1. Age 16-45

2. Initial Glasgow Coma Scale (GCS) score of ≤ 8, without the influence of pharmacologic agents, alcohol, or seizure activity
3. Positive computed tomography (CT) scan for TBI
4. Placement of extraventricular drain (EVD) per standard of care ICP monitoring and CSF drainage and sampling
5. Placement of $PbtO_2$ probe (Licox®, Integra Neurosciences™) per standard of care $PbtO_2$ monitoring
6. Signed written consent from next-of-kin for the IRB approved protocol
7. $FiO_2 \leq 0.6$ or PaO_2/FiO_2 ratio ≥ 200

Exclusion criteria were:

1. Age or GCS other than above
2. Family unavailable for written consent
3. Inability to place EVD or $PbtO_2$ probes
4. Need for $FiO_2 > 0.6$ or PaO_2/FiO_2 ratio < 200
5. Enrollment in any other research interventional trial

Patients who required a $FiO_2 > 0.6$ or who have a PaO_2/FiO_2 ratio of < 200 were excluded to increase the likelihood of detecting a difference in oxidative measures and avoid patients who have an increased risk of pulmonary complications. The upper age range was set at 45 years to avoid enrolling older patients who may have unreported lung disease. Children less than 16 years of age were not enrolled due to the increased risk of hyperoxia on the immature brain and because of the difference in brain recovery in adults and children.

3.3 STANDARD MEDICAL CARE

All patients enrolled in this study received standard medical care (Table 1). The standardization of clinical care from pre-hospital to emergency room triage to acute NTICU care to rehabilitative care has been refined to minimize individualized physician ideologies. *The Guidelines for Management of Severe Traumatic Brain Injury* (produced by the Brain Trauma Foundation and endorsed by the American Association of Neurological Surgeons) were used as the basis for pre-hospital and critical care of these patients ("Guidelines for the management of severe traumatic brain injury," 2007).

Table 1: NTICU Management for Severe TBI Patients

Pre-hospital Management:	Initial evaluation, triage by certified paramedics
	Pre-intubation GCS whenever possible
	Early intubation for all patients with GCS ≤8 due to head injury
	Use of short or intermediate acting paralytic agents
	Restricted use of hyperventilation therapy
	Early (pre-hospital) notification of neurosurgery
Emergency Department Management:	Advanced Trauma Life Support Protocol: rapid restoration of normal blood pressure, oxygenation
	Determination of post-resuscitation GCS prior to the administration of sedating or paralytic medications
	CT of head immediately after hemodynamic and pulmonary resuscitation, or if patient requires emergent chest or abdominal surgery, CT of head post-surgery
ICU Management	
Physiological Monitoring:	ICP monitoring with ventriculostomy
	Continuous blood pressure monitoring via indwelling arterial catheter
	Central venous catheter for monitoring intravascular volume and maintenance of normothermia (36.5^{o}C)
	Licox® catheter inserted for monitoring of brain tissue oxygenation ($PbtO_2$) and temperature
	Rectal temperature probe for monitoring core body temperature
Baseline Physiological Parameters:	
	ICP < 20 mm Hg
	Mean arterial pressure >90 mm Hg
	Cerebral perfusion pressure >60 mm Hg
	Central venous pressure 8-15 cm H_2O
	$PbtO_2$ > 20 mm Hg
	Arterial pCO_2 33-37 mm Hg
	Hematocrit >28%
	CT scan within 24 hours with CT perfusion (contraindicated with iodine allergy)
Management of Elevated Intracranial Pressure – A "Stepwise" Approach:	
	Systemic pharmacological paralysis and sedation
	Intermittent CSF drainage
	Bolus Mannitol/Lasix therapy or 3% hypertonic saline therapy
	Reduce arterial pCO_2 to 30-32 mm Hg
	Decompressive temporal lobectomy and/or hemicraniectomy in select patients
	Pentobarbital coma 600-1000 grams loading dose, 60 mg/hour drip titrated for ICP control and cardiac suppression
Management of Hypotension – A "Stepwise" Approach:	
	If initial CVP is < 5 mmHg and/or systolic blood pressure is < 90 mmHg:
	Albumin 5% - 500 ml IV bolus, may repeat x 1
	Normal saline solution – 1000 ml IV bolus, may repeat x 1
	Refer to severe head injury fluid resuscitation protocol guidelines

At the scene, both ground and air emergency medical personnel were educated to perform rapid and precise care of all severe TBI patients with the goal of stabilization and immediate transfer to a Level 1 trauma hospital. This involved pre-intubation assessment of severity of injury (GCS), early intubation, restricted use of hyperventilation to ensure proper oxygenation and blood flow, and selection of short or intermediate acting paralytic agents to be able to quickly re-examine the neurological status.

Once severity of injury was confirmed in the emergency room department by a trained neurotrauma resident with consultation with the attending neurosurgeon and clinical nurse coordinator, the patient was transferred to the NTICU, with standing orders. All standing orders were focused on the minimization or prevention of secondary injury occurring within this acute recovery period, with an emphasis on oxygenation and blood flow.

A physiatrist evaluated all patients within 24 hours of admission and a comprehensive program of physical and occupational therapy as appropriate was initiated. The management of all aspects of the patient's trauma care was coordinated through daily rounds with the neurosurgeon, trauma surgeons, critical care intensivist, physiatrists and the clinical nurse coordinator.

3.4 MEASUREMENT OF STUDY VARIABLES

3.4.1 Measurement of $PbtO_2$

Licox® (Integra Neurosciences™, Plainsboro, NJ) brain oxygenation probes were placed as standard management for all severe TBI patients to monitor the partial pressure of white matter

brain tissue oxygenation. The system consists of a probe, introducer and sensor. The *$PbtO_2$ sensor* is a closed polygraphic cell located at the tip of the completely closed polyethylene catheter body. The sensor samples a sensitive area concentrically around the tip (13 mm^2). It consists of 2 polarographic electrodes within a polyethylene tube, bathed in a buffered solution with a pH of 7.4. (Figure 4). These electrodes are manufactured with one made of silver and one of gold that are connected to the electrical contact pins in the connector housing. Once placed, the connectors are attached to the monitor for continuous recordings (Figure 5).

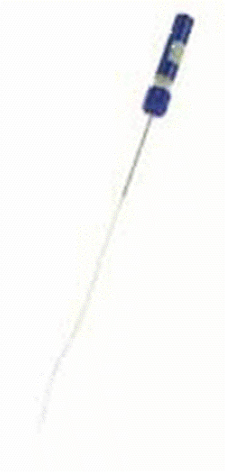

Figure 4: Illustration of an individual sensor (temperature or oxygen) that is placed within the 3-port Licox® system.

Figure 5: Illustration of a Licox® monitor. The front ports connect to the patient's inserted catheters, and the back ports download the continuous data to the patient data monitor.

The accuracy of $PbtO_2$ measurements is dependent on temperature, with every 1^oC change, a 4% oxygenation sensitivity change occurs. In order to accurately measure $PbtO_2$, the brain tissue temperature is measured continuously and concurrently with the $PbtO_2$ to adjust for changes in brain temperature. The value of the data obtained is ultimately dependent on the site of placement within the brain.

The *introducer* of the Licox® has three ports that are labeled according to the probe that it houses: the oxygenation ($PbtO_2$) probe, the temperature probe, and a middle port that may be used for a parenchymal pressure probe, or closed if another monitoring device is used for ICP readings, such as in this study with the use of a separate ventriculostomy. The system incorporates a bolt that is secured to the skull in a drilled hole. The introducer is securely screwed into the bolt after all probes are inserted (Figure 6).

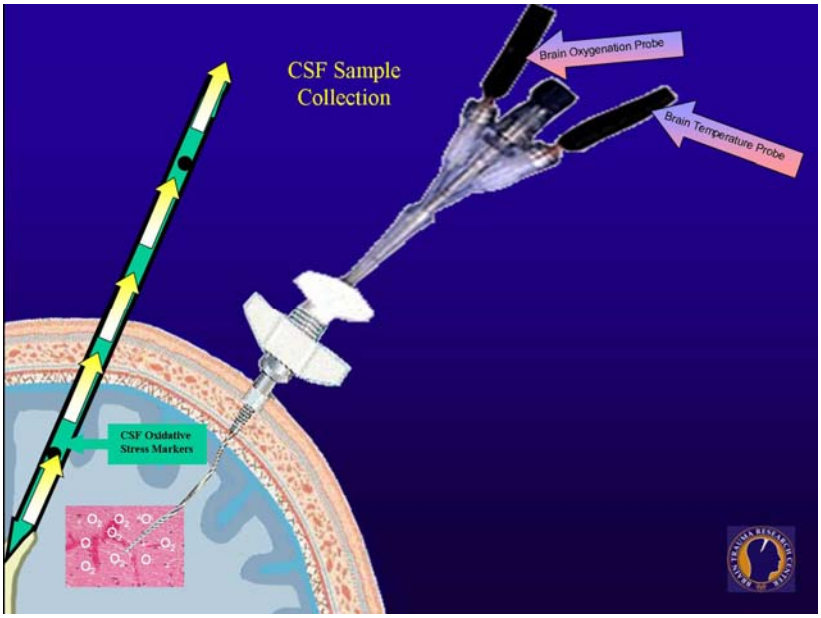

Figure 6: Illustration of Licox® insertion into the white matter of the brain to sample brain tissue oxygenation. Also shown is the ventriculostomy inserted within the lateral ventricle to drain CSF and for CSF sample collection.

The Licox® oxygen probe (diameter 0.8 mm, length 460 mm, oxygen sensitive area of 13 mm^2) is inserted within the bolt system to a fixed approximate depth of 35 mm below the dura into the brain parenchyma contralateral to the primary site of injury. Calibration for the oxygen and temperature sensors is performed by the company prior to shipping, and a 'smart card' calibration is provided with each catheter set. The catheters cannot be used without the matching 'smart card'.

Insertional Trauma. The tissue that is affected surrounding the probe is reported to be a thickness of 70 μm to 500 μm. Local microtrauma can occur with insertion, with up to a 20% risk (unpublished data, University of Pittsburgh). Tissue lacking oxidative metabolism does not provide oxygen readings; therefore, only viable tissue will provide $PbtO_2$ values. Values

obtained 2 hours immediately after insertion are deemed unreliable due to insertional trauma artifact (Valadka, Gopinath et al., 1998; van Santbrink et al., 2003).

Accuracy. Integra Neurosciences™ reported the following accuracy rates determined at continuous $PbtO_2$ measurement at 37°C: 0-20 mm Hg: +/- 2 mm Hg; 20-50 mm Hg: +/- 10%; and 50-150 mm Hg: +/- 12%. The recommended duration of use is 5 days as accuracy diminishes with length of probe use.

Care to Ensure Proper Functioning. The neurotrauma residents, the research nurse and the patient's primary nurse were trained in troubleshooting problems with the Licox® catheter system. In addition to continuous monitoring, the primary nurse entered the $PbtO_2$ and temperature data into the bedside electronic flow-sheet. If the $PbtO_2$ was below 20 torr, the attending physician was notified. After an equilibration time of 1 hour, the catheter was tested for responsivity by increasing the FiO_2 to 100% for 5 minutes. The corresponding increase in $PbtO_2$ values should occur within that time frame if the catheter was placed within a viable area of tissue.

Care to Minimize Infection. To minimize the risk of infection, the probes were placed under sterile conditions in the emergency department, operating room or NTICU, and the duration of monitoring was limited to five days.

3.4.2 Measurement of Additional Physiological and Ventilatory Variables

FiO_2: All eligible patients were intubated and managed on a ventilator secondary to the severity of the brain injury, with the goal of ventilatory management being the maintenance of PaO_2 saturation > 94%. Following baseline assessment and sample collection, the FiO_2 was changed

from baseline to 1.0 for the 2 hour testing period. PaO_2 was measured before, during and after-FiO_2 challenge to ensure that the oxygen supply was increased.

CPP was measured as the difference between MAP and ICP. This number was automatically calculated from data acquired from the patient monitor.

ICP was continuously monitored in all eligible patients due to the severity of the TBI. An EVD was placed by the neurotrauma resident within the first 24 hours of injury. In the majority of subjects, the EVD was placed under sterile conditions at the bedside in the NTICU. In some instances, the EVD was placed in the emergency room or the operating room. The target for placement was the lateral ventricle, confirmed by drainage of CSF and a computed tomography (CT) scan within 24 hours of placement. The target ICP was < 20 mm Hg. Continuous pressure readings were obtained by connecting the system to the bedside monitor. A waveform was displayed with a digital value that was downloaded every minute.

MAP was calculated as [2 (diastolic blood pressure) + systolic blood pressure]/3. MAP was continuously monitored in all eligible patients due to the severity of the TBI. A radial arterial line was placed by the critical care intensivist within 24 hours of injury. Continuous pressure readings were obtained by connecting the system to the bedside monitor. A waveform was displayed with a digital value that was downloaded every minute. Target MAP was > 90 mm Hg. MAP was collected for confirmation of the calculation of CPP values.

3.4.3 Measurement of Oxidative Stress Markers

F_2-isoprostane was measured in CSF samples by a commercially available enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI) with a detection limit of 2 pg/ml. To control for the presence of F_2-isoprostane in normal conditions, each patient acted as their own

control since normal baseline values and values after TBI are highly variable. If samples were not stored properly, oxidation could continue after the sample was taken, thus artificially elevating the values. Caution was taken to properly store these samples at –80^{o}C as soon as the patient's study was completed. In addition, two sets of standards were run along with the assay of tests samples: one set in standard buffer, and one with CSF-spiked with known amounts of F_2-isoprostane.

Protein sulfhydryls were measured in CSF samples using a maleimide reagent, ThioGlo-1 (Convalent Associates, Inc., Woburn, MA), that produced a fluorescent product upon reaction with sulfhydryl groups (Langmuir et al., 1996). Levels of protein sulfhydryls were determined after adding 4 mM SDS to the CSF sample. A Cytoflur 2350 fluorescence plate reader (Millipore Corporation, Marlborough, MA) was used to detect fluorescence using an excitation wavelength of 388 nm and an emission wavelength of 500 nm. This data were exported from the spectrophotometer using Cytoflur software.

3.4.4 Measurement of Antioxidant Defenses

Glutathione levels were measured in CSF utilizing the assay described under protein sulfhydryl measurement. GSH content was estimated by the immediate fluorescent response observed upon addition of ThioGlo-1 to the CSF sample. The response was compared to a standard curve obtained by the addition of known amounts of GSH (.04- 4 μM) to 50 mM disodium phosphate buffer (pH 7.4) containing 10 μM ThioGlo-1, to extrapolate sample values.

Total Antioxidant Reserve (AOR) was measured in CSF samples by a chemiluminescense assay produced in the presence of luminol and a source of peroxyl radicals, previously described by Tyurina, et al (Tyurina et al., 1995). For this assay, a water-soluble azo-initiator, AAPH, was

used to produce peroxyl radicals at a constant rate. Based on the known rate of peroxyl radical generation by AAPH, the amount of these radicals scavenged by endogenous antioxidants with the sample being assayed, CSF levels were determined. A Microlite ML 1000 microtiter plate luminometer (Dynatech Labs, Chantily, VA) was used for the final determinations of these amounts.

3.5 RESEARCH PROCEDURES

1. Recruitment and Informed Consent. This project was approved by the University of Pittsburgh Internal Review Board (IRB) under protocol #0407147 (Appendix A). All potential subjects were unable to sign informed consent, therefore proxy consent was required. The patient's next-of-kin was identified by the emergency room social worker. These family members were approached initially by the attending neurosurgeon or the resident-on-call, made aware of their loved one's health status and briefed on the research study. If potential subject's next-of-kin wishes for more information, the investigator further explained the study and obtained written consent.
2. Subject confidentiality was a concern and the following measures were taken to prevent identity disclosure. CSF samples collected were aliquotted and coded with a unique identifying number, different from the patient's medical record number or social security number. All study data were coded with the same unique identifying number in order for these to be matched to the samples. The demographic information of the patient was stored on a password protected computer in a locked office. All signed consents were stored in a locked filing cabinet in a locked office.

3. Extraventricular drain and Licox® probes were placed as standard management as soon as severe TBI diagnosis was verified. The FiO_2 challenge was performed within 24-48 hours of injury, to minimize initial confounding values related to the primary injury; > 8 hours of Licox insertion to minimize confounding values related to possible insertional trauma and > 2 hours from any ventilator setting change.
4. A baseline arterial blood gas was taken to ensure that proper oxygenation was occurring (PaO_2 > 90 mm Hg) and to correlate with the initial $PbtO_2$ value.
5. The initial FiO_2 value was recorded.
6. Baseline physiological data ($PbtO_2$, CPP, ICP and MAP) were recorded every 10 minutes through the study period [before, during (2 hours of increased FiO_2 treatment) and after (2 hours post-FiO_2 treatment)]. In addition, minute data were acquired from the patient monitor and 30 minute averages were matched to the CSF sample for each time point.
7. A sterile CSF ventricular sample was drawn (3cc) per NTICU policy, centrifuged, aliquoted and transferred to a $-80^{O}C$ freezer for future batch analysis.
8. The FiO_2 was increased to 1.0 for 2 hours. During this 2 hour period, the investigator monitored the subject for physiological changes (ICP, MAP, CPP, $PbtO_2$) and nursing activity (turning, suctioning, etc) that might influence the data. All efforts were taken to minimize or delay unnecessary nursing activity.
9. After 2 hours, a second CSF and arterial blood sample were taken ('during' sample), and the FiO_2 was returned to baseline.
10. Two hours after the study was completed, a third corresponding CSF and arterial blood sample was taken ('after' sample) to ensure that no long term effects have occurred.

11. Each CSF sample was date and time stamped. The CSF samples were centrifuged and then aliquoted and transferred to cryoprotectant tubes and stored at –80^{o}C for later analysis. Samples were labeled with a unique identifying number to maintain patient confidentiality.
12. Processing of samples for oxidative stress and antioxidative reserve biomarkers was performed on batch samples to optimize the performance of assays and supplies.
13. Strict sterile technique was used when withdrawing specimens.

3.6 DATA MANAGEMENT

All physiological data ($PbtO_2$, CPP, MAP, and ICP) were manually recorded every 10 minutes. Time from the patient monitor (Hewlett Packard/Philips Medical Systems, Bothell, WA) at the start of the FiO_2 intervention was recorded, as well as the return to the baseline setting. In addition, all physiological data acquired by the patient's monitor were downloaded at a rate of 1 data point/minute, time and date stamped, and stored within the Brain Trauma Research Center's main database. Validation checks were performed by the PI to ensure that the observed written vital signs are equivalent to the computer generated data. If the validation was approved, the minute data was averaged for the last 30 minutes of the before, during and after intervention time (FiO_2 challenge) to match the CSF samples.

3.7 STATISTICAL ANALYSIS

Overall Design: The statistical design was a within subject, repeated measures design using a generalized linear model (GLM). Univariate analysis of variance (ANOVA) was utilized to assess the relationship between FiO_2 increases and physiological parameters, as well as biochemical oxidative stress and antioxidant reserve biomarkers. Descriptive statistics and bivariant analyses (both parametric and nonparametric based on the distribution) were used. All data were screened for accuracy of input, missing data and outliers. All statistical procedures were performed using the statistical program SPSS (version 12). Statistical significance was determined by a *p* value less than 0.05. Sample size was not based on a power analysis as results will be used to provide preliminary data for a larger study.

Analysis Plan: For the preliminary analyses, descriptive statistics were performed for all variables, including mean, standard deviations and standard error of the mean. The primary statistical analysis consisted of a univariate within subjects ANOVA with one within-subjects factor. The independent variable was the time of normobaric hyperoxia treatment, and the outcome measures were physiological variables ($PbtO_2$, ICP, CPP) and biochemical marker levels (F_2-isoprostane, protein sulfhydryls, glutathione, and antioxidant reserve).

GLM Univariant Assumptions: The within-subjects effect was analyzed in GLM with the assumption of two conditions being met: 1) equal variances and 2) uncorrelated variables. Equal variances are tested by summing the error sums of squares for each of the transformed variables. The resulting variances were homogeneous. The correlation between the transformed scores was tested by summing the sums of squares for the new transformed variables. Ideally, there would be no significant correlation between the transformed scores. If there was a significant correlation, there would have been an overestimate of the strength of the

relationships, and a correction factor would be applied. The Mauchly test of sphericity tested for both of these assumptions at the same time. Although all of the assumptions are met in the theoretical models, they are not always met in the clinical environment. The test can still be robust if the assumptions were not significantly violated. If the assumption of sphericity was not met, the Type 1 error rate would have been inflated, and the null hypothesis may have been rejected unjustly. The Huynh-Feldt Epsilon correction formula would have been used in the case of the sphericity assumption not being met.

Covariates for this study were severity of injury, described as initial GCS, and demographic characteristics of the population studies, such as age, gender and race. Statistics were limited in the number of variables to be controlled for within the final statistical model due to the small sample size.

4.0 CHAPTER 4

The results and discussion of findings from this study are presented below in the format of a manuscript submitted to Journal of Neurotrauma. The entire manuscript follows, beginning with the introduction section. The bibliography is incorporated within the dissertation document itself.

4.1 INTRODUCTION

The current management of severe traumatic brain injury (TBI) in the acute care setting focuses on prevention, early detection and treatment of secondary injury through therapy designed to maintain adequate cerebral perfusion and intracranial pressure control ("Guidelines for the management of severe traumatic brain injury," 2007). Recent technological advances allow brain tissue oxygenation ($PbtO_2$) to be assessed continuously, providing an *in vivo* tool that allows rapid detection of changes in response to the intrinsic evolution of damage, effect of secondary insults and/or application of preventive or reactive therapies. In patients with severe TBI, Valadka, et al. reported that the longer the time a patient experienced a $PbtO_2$ of $\leq$ 15 torr, the greater the likelihood of death. Additionally, any decrease in $PbtO_2$ to < 6 torr, regardless of its duration, was associated with an increased mortality (Valadka, Gopinath et al., 1998). Subsequently, various treatment modalities to raise the $PbtO_2$ above these thresholds, such as

therapeutic hyperoxia, began to be implemented for patients with severe TBI, albeit with limited empirical evidence. In clinical practice, one approach to managing a low $PbtO_2$ (< 20 torr) is to increase the delivered fraction of inspired oxygen (FiO_2). Although effective in increasing $PbtO_2$, and presumably oxygen availability in the brain, the impact of hyperoxia on secondary injury mechanisms, such as oxidative stress, is not well understood.

Diffusion of oxygen into injured brain tissue may be limited in the setting of brain edema and decreased cerebral blood flow (Hlatky, Valadka, Gopinath, & Robertson, 2008), and thus high levels of dissolved oxygen in arterial blood may facilitate oxygen delivery. In a clinical study comparing patients receiving 24 hours of 100% oxygen to historical controls, normobaric hyperoxic therapy was shown to decrease lactate levels and the lactate/pyruvate ratio, increase glucose levels, decrease intracranial pressure (ICP) and improve outcome. In a prospective clinical study utilizing positron emission tomography (PET), normobaric hyperoxia treatment enhanced cerebral metabolism in "at risk" tissue with a complimentary small reduction in the lactate/pyruvate ratio, but no global changes were seen (Nortje et al., 2008). A study comparing ICP and $PbtO_2$ monitoring and treatment, including hyperoxia, vs. ICP monitoring and treatment alone, reported a favorable and dramatic improvement in neurological outcomes in the group with additional $PbtO_2$ monitoring (Stiefel et al., 2005).

However, other evidence points to a negative effect of hyperoxia treatment on the injured brain. There exists concern about the use of hyperoxia due to the potential of this therapy to elicit an oxidative stress response (Longhi & Stocchetti, 2004). Kaindl et al reported increased neuronal apoptotic death and protein carbonyls in mice subjected to hyperoxia (Kaindl et al., 2006). Additional evidence in animal models of ischemia and reperfusion suggests a potential powerful, deleterious effect of hyperoxia in early post-ischemic reperfusion of brain (Vereczki et

al., 2006), possibly via oxidative post-translational modification and inhibition of the key mitochondrial enzyme, pyruvate dehydrogenase by peroxynitrite (Bogaert, Rosenthal, & Fiskum, 1994). Similarly, studies in children (Bayir et al., 2002; Wagner et al., 2004) and adults (Bayir et al., 2002; Wagner et al., 2004) have shown that, with management strategies, antioxidant reserves are markedly depleted and biomarkers of oxidative stress are increased in cerebrospinal fluid (CSF) in the acute period after TBI. Accordingly, a treatment strategy involving the use of high concentrations of oxygen to achieve a target $PbtO_2$ could theoretically, place the already vulnerable brain at risk for secondary injury. It is of clinical interest to determine if any harmful effects occur with the use of therapeutic normobaric hyperoxia to increase a low or normal $PbtO_2$ value.

The purpose of this study was to examine the effect of a brief, controlled period of normobaric hyperoxia (100% oxygen administered for 2 hours) on (1) the key physiological values of local brain tissue oxygenation ($PbtO_2$), intracranial pressure (ICP), global brain perfusion (cerebral perfusion pressure [CPP]) and systemic blood pressure (mean arterial pressure [MAP]); and (2) biochemical markers of oxidative stress (as measured by F_2-isoprostane, and protein sulfhydryl) and antioxidant defenses (as measured by reduced glutathione and antioxidant reserve) in CSF of patients admitted to a Level 1 trauma center who sustained a severe TBI.

4.2 MATERIALS AND METHODS

4.2.1 Subjects

Under the scope of an approved institutional review board protocol, we prospectively studied 11 adult patients (18-45 years of age) with severe TBI who were admitted to our Level 1 trauma center. Severe TBI was determined in the emergency room by the attending neurosurgeon using the following criteria: 1) post-resuscitative Glasgow Coma Scale (GCS) score ≤ 8, not following commands and without the influence of pharmacologic agents, alcohol or paralytics; and 2) positive computer tomography (CT) scan for severe TBI diagnosis. Entry criteria for the study were: 1) $FiO_2 \leq 0.6$ or PaO_2/FiO_2 ratio > 200; 2) external ventricular drain (EVD); 3) brain tissue oxygenation ($PbtO_2$) probe (Licox®, Integra Neurosciences™); 4) informed consent from the legal authorized representative; 5) controlled ICP (≤ 25 mm Hg) and $PbtO_2$ (≥ 15 mm Hg) parameters at the time of the FiO_2 challenge; and 6) ability to begin data collection within 48 hours of injury. Patients who required a $FiO_2 > 0.6$ or had a PaO_2/FiO_2 ratio of ≤ 200 were excluded to maximize the ability to detect a difference between standard therapy and normobaric hyperoxia therapy on oxidative measures and to avoid severe impairments in oxygen tension. In addition, patients were excluded if they exhibited prolonged (> 30 minutes) hypotension and hypoxia prior to hospital admission, had an unknown injury time, or an injury time > 24 hours prior to arrival in the emergency department, or were clinically brain dead. The upper age limit was set at 45 years to avoid enrolling older patients with unreported lung disease.

4.2.2 Critical Care Management

Intensive care management was provided within a dedicated neurotrauma intensive care unit with care guided by standardized protocols consistent with the *Guidelines for Management of Severe Traumatic Brain Injury* ("Guidelines for the management of severe traumatic brain injury," 2007). All clinical orders are focused on minimization or prevention of secondary injury occurring within this acute recovery period, with an emphasis on cerebral oxygenation and blood flow. MAP endpoint goals were between 90-110 mm Hg, $PaCO_2$ between 33-37 mm Hg, central venous pressure (CVP) between 8-15 mm Hg; and CPP > 60 mm Hg. ICP values > 20 mm Hg were aggressively managed using a step-wise protocol that included pharmacologic paralysis and sedation, continuous CSF drainage, osmotic therapy (bolus mannitol or continuous hypertonic saline infusion), transient escalation of hyperventilation and barbiturate infusion. The $PbtO_2$ probe (diameter 0.8 mm, length 460 mm, oxygen sensitive area of 13 mm^2) was placed approximately 35 mm below the dura into the brain parenchyma of the frontal lobe, contralateral to the primary site of injury to provide sampling from a normal-appearing site that, consequently, should represent an uninjured brain region at risk for secondary injury. As local microtrauma can occur with insertion, and levels of a number of mediators may be increased in CSF during the initial period of sampling, a 24-hour interval was allowed between probe insertion and the normobaric hyperoxia trial (Valadka, Gopinath et al., 1998; van Santbrink et al., 2003).

4.2.3 Normobaric Hyperoxia Trial

Arterial blood gas (ABG) and sterile CSF ventricular samples were obtained 30 minutes prior to the trial and defined as 'Before Trial'; immediately after the normobaric hyperoxia trial (FiO_2 increased to 1.0 for 2 hours), representing the period during the trial and defined as 'During Trial'; and 2 hours following the return to baseline FiO_2 settings, representing the period following the trial and defined as 'After Trial'. Physiological parameters (ICP, MAP, CPP, $PbtO_2$) were continuously monitored and data collected every minute via a data acquisition server linked to the patient's monitor (Hewlett Packard/Philips Medical Systems, Bothell, WA). The last 30 minutes of data collection prior to each CSF sampling was averaged to provide a comparison to the CSF collection interval for each of the three collection points. During the data collection period, nursing activity was minimized and notations were made of any nursing activity that might influence the data obtained, e.g., turning, suctioning, etc. The duration (2 hrs) of the normobaric hyperoxia trial was chosen to provide sufficient time to produce changes due to oxidative stress to the brain (if present) but not prolonged to cause potential damage if biomarkers were markedly changed by the trial.

4.2.4 CSF Sample and Marker Assays

To obtain CSF, the EVD was placed in the closed position for 15 minutes and a fresh CSF sample obtained via the proximal access port. All CSF samples were immediately placed on ice, centrifuged, transferred to a cryoprotectant tube and stored in a -80^{o}C freezer for future batch analysis.

F_2-isoprostane was measured in CSF by a commercially available enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI) with a detection limit of 2 pg/ml. *Protein sulfhydryls* were measured in CSF samples using a maleimide reagent, ThioGlo-1 (Convalent Associates, Inc., Woburn, MA), that produces a fluorescent product upon reaction with sulfhydryl groups (Langmuir et al., 1996). Levels of protein sulfhydryls were determined after adding 4 mM SDS to the CSF sample. A Cytoflur 2350 fluorescence plate reader (Millipore Corporation, Marlborough, MA) was used to detect fluorescence using an excitation wavelength of 388 nm and an emission wavelength of 500 nm. *Glutathione* levels (GSH) measured in CSF utilizing the assay described above. GSH content was estimated by the immediate fluorescent response observed upon addition of ThioGlo-1 to the CSF sample. The response was compared to a standard curve obtained by the addition of known amounts of GSH (.04- 4 μM) to 50 mM disodium phosphate buffer (pH 7.4) containing 10 μM ThioGlo-1, to extrapolate sample values. *Total Antioxidant Reserve (AOR)* was measured in CSF samples by a chemiluminescense assay produced in the presence of luminol and a source of peroxyl radicals, previously described by Tyurina, et al (Tyurina et al., 1995). For this assay, a water-soluble azo-initiator, AAPH, is used to produce peroxyl radicals at a constant rate. Based on the known rate of peroxyl radical generation by AAPH, the amount of these radicals scavenged by endogenous antioxidants with the sample being assayed, CSF levels were determined. A Microlite ML 1000 microtiter plate luminometer (Dynatech Labs, Chantily, VA) was used for the final determinations of these amounts.

4.2.5 Statistical Analysis

Descriptive statistics and bivariant analyses (parametric and nonparametric based on the distribution) were used. Data were screened for accuracy of input, missing data and outliers and statistical procedures performed using SPSS (version 12), with a statistical significance determined *a priori* at $p < 0.05$. Multivariate analysis of variance (MANOVA) was utilized to detect significant changes in physiological variables ($PbtO_2$, MAP, ICP, CPP) and biochemical marker levels (F_2-isoprostane, protein sulfhydryls, glutathione, and total antioxidant reserve) over time. If significant results were obtained in the MANOVA procedure, appropriate post hoc paired Student *t*-tests were performed. Significance values were adjusted for multiple comparisons according to Bonferroni, if required.

4.3 RESULTS

4.3.1 Subject Demographics

Subjects (9 male, 2 female) were 26 ± 1.8 years of age, 91% Caucasian, with an initial GCS score of 6 [3,8] (Table 2).

Table 2: Study Population Characteristics

Gender, % male	**81.8%**
Race, % Caucasian	**81.8%**
Age **Mean ± SEM**	**26 ± 1.8**
GCS on admission **Median [range]** **GCS 3-5** **GCS 6-8**	**6 [3,8]** **36.4%** **63.6%**

Values are mean ± SEM or median [range] where appropriate
Definition of abbreviations: SEM = standard error of the mean; GCS = Glasgow Coma Scale score.

4.3.2 Physiological Results

The initial FiO_2 was 0.50 ± 0.09 (range 0.40 - 0.60). All baseline physiologic parameters were within normal ranges. Compared to baseline, the normobaric hyperoxia challenge increased PaO_2 (173.1 ± 51.4 to 385.5 ± 108.3 mmHg) and $PbtO_2$ (27.3 ± 7.4 to 93.9 ± 58.1 mm Hg), as expected. The MANOVA demonstrated a significant overall effect for normobaric hyperoxia on PaO_2 (F = 38.9; d.f. = 2, 18; $p < .0001$) and $PbtO_2$ (F = 15.4, d.f. = 2, 20; $p < .0001$). Normobaric hyperoxia significantly improved PaO_2 (t= 7.2; $p < .0001$) and $PbtO_2$ (t= 4.1; $p <$

.0001) from baseline values, with a corresponding significant decrease in PaO_2 (t=-6.0; $p <$ .0001) and $PbtO_2$ (t= -3.8; $p <$ .0001) when the ventilatory setting was changed back to the baseline FiO_2 setting. No significant resultant changes were seen from before- FiO_2 challenge to after FiO_2 challenge for PaO_2 and $PbtO_2$. No significant changes were seen in ICP, MAP, or CPP (reported in Figure 7).

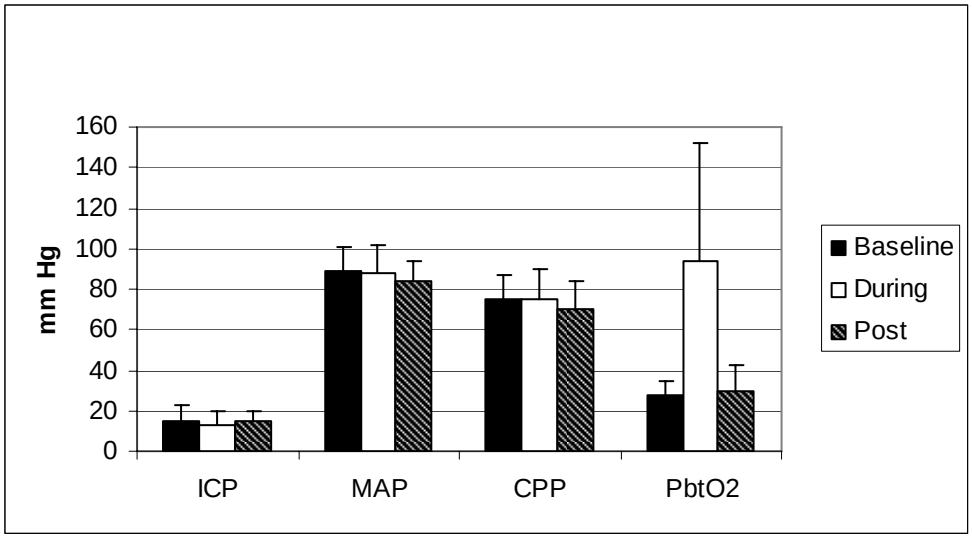

Figure 7: Effect of normobaric hyperoxia trial on physiological parameters before, during and after 2 hour normobaric hyperoxia trial. Definitions of abbreviations: ICP = intracranial pressure; MAP = mean arterial pressure; CPP = cerebral perfusion pressure; $PbtO_2$ = brain tissue oxygenation. Error bars are SEM for each parameter. Note that PaO_2 values are not shown, refer to text.

4.3.3 Biomarker Results

Markers of oxidative stress (F_2-isoprostane, and protein sulfhydryls; Figures 8 and 9) and antioxidant reserve (glutathione and total AOR; Figures 10 and 11) did not significantly change

at any study time points. Notably, AOR, a sensitive marker of anti-oxidant defenses (Bayir et al., 2002), did not demonstrate even a trend toward reduction, and no substantial trends were seen for any marker vs baseline measures.

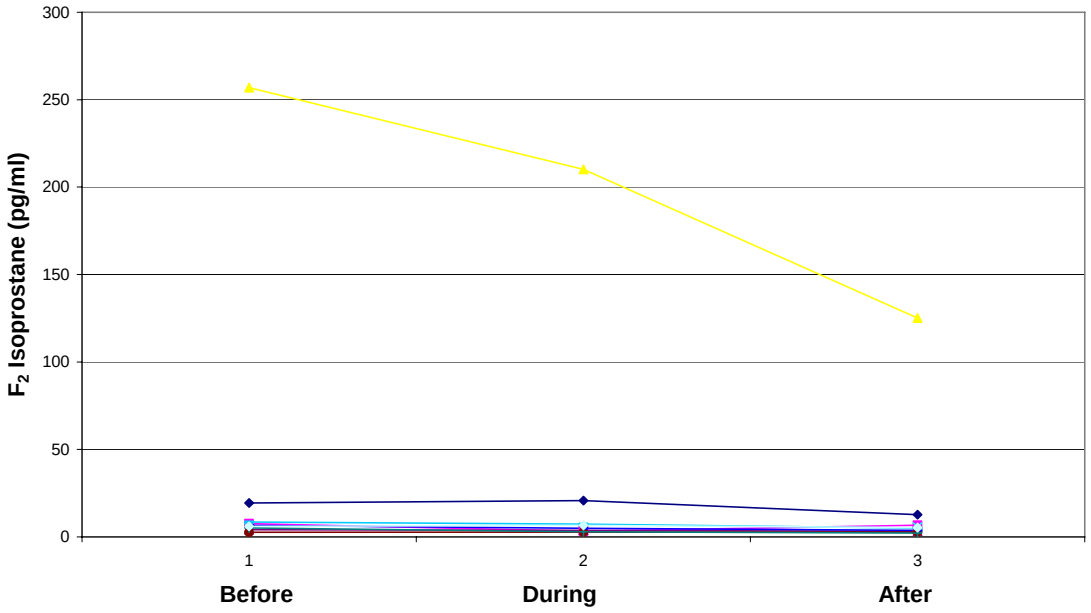

Figure 8: Effect of a normobaric hyperoxia trial on F_2 isoprostane levels in CSF. Individual patients depicted by line graph. Overall F_2 isoprostane concentration did not significantly change from before, during or after trial.

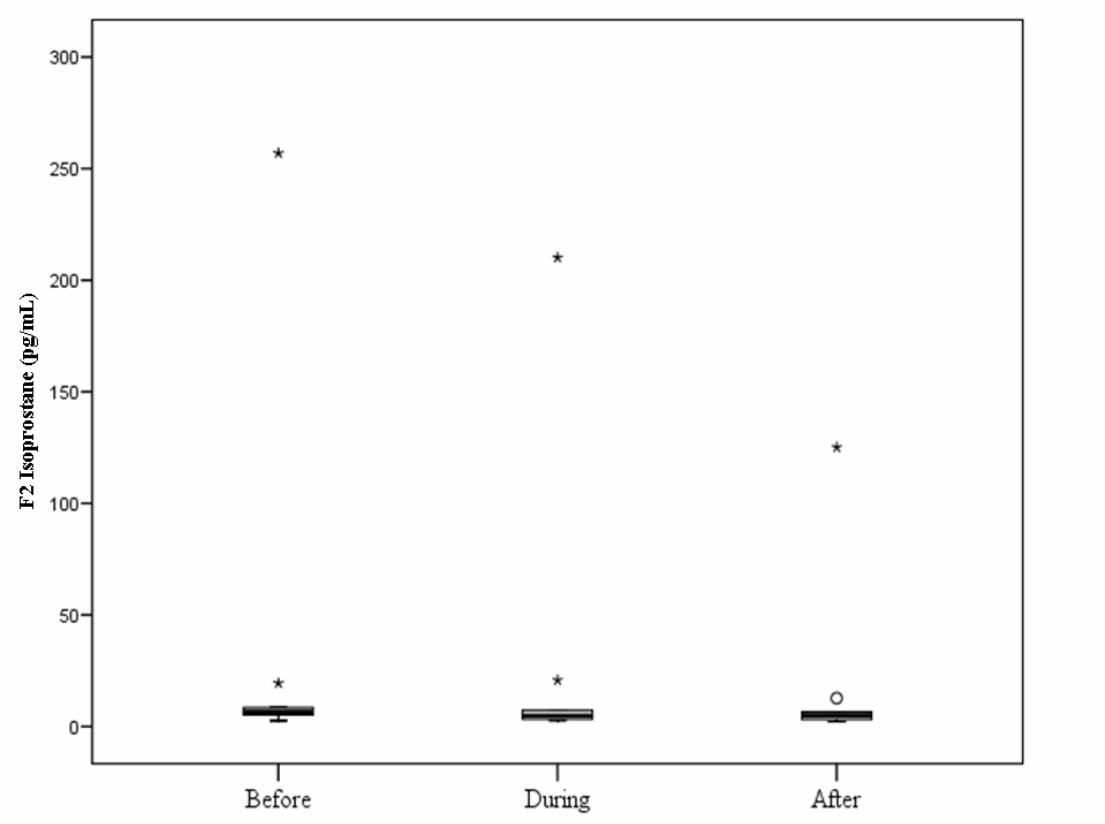

Figure 9: Effect of a normobaric hyperoxia trial on F_2 isoprostane levels in CSF. Overall F_2 isoprostane concentration did not significantly change from before, during or after trial. Patients grouped and reported in a box plot format (o symbol depicts outlier value; * symbol depicts extreme outlier value).

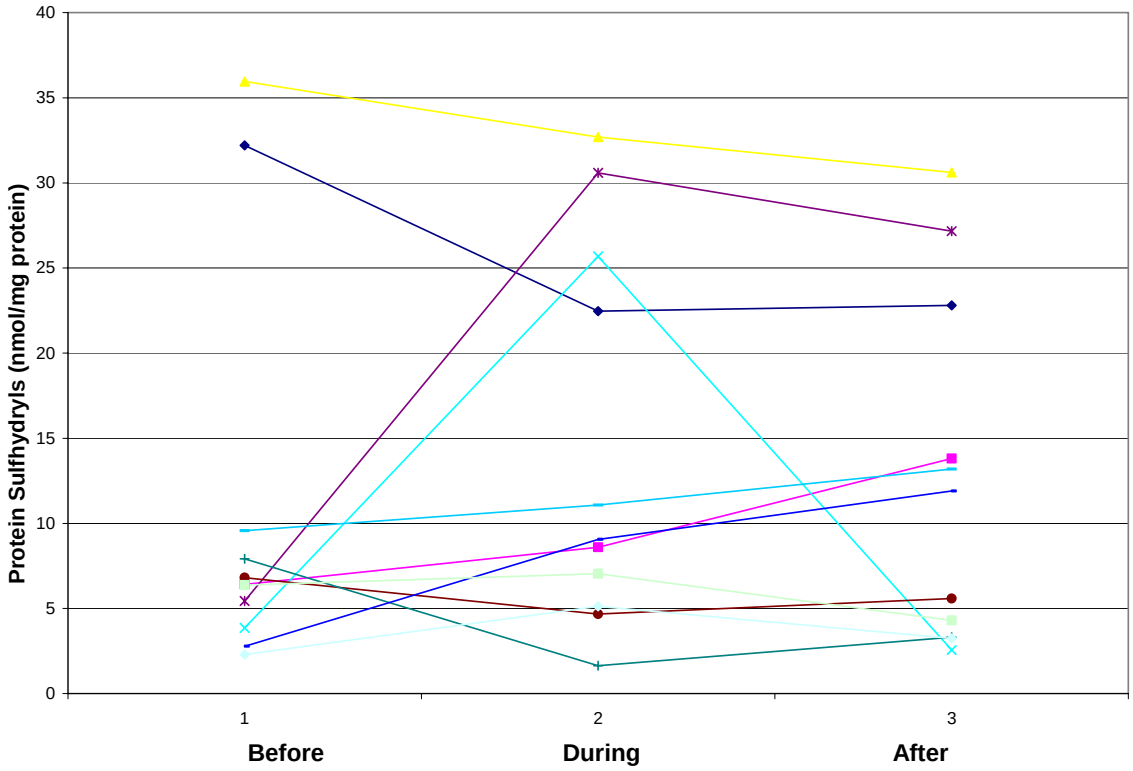

Figure 10: Effect of a normobaric hyperoxia trial on content of protein sulfydryl concentrations in CSF. Overall concentrations of protein sulfydryls did not significantly change from before, during or after trial. Individual patients depicted by line graph.

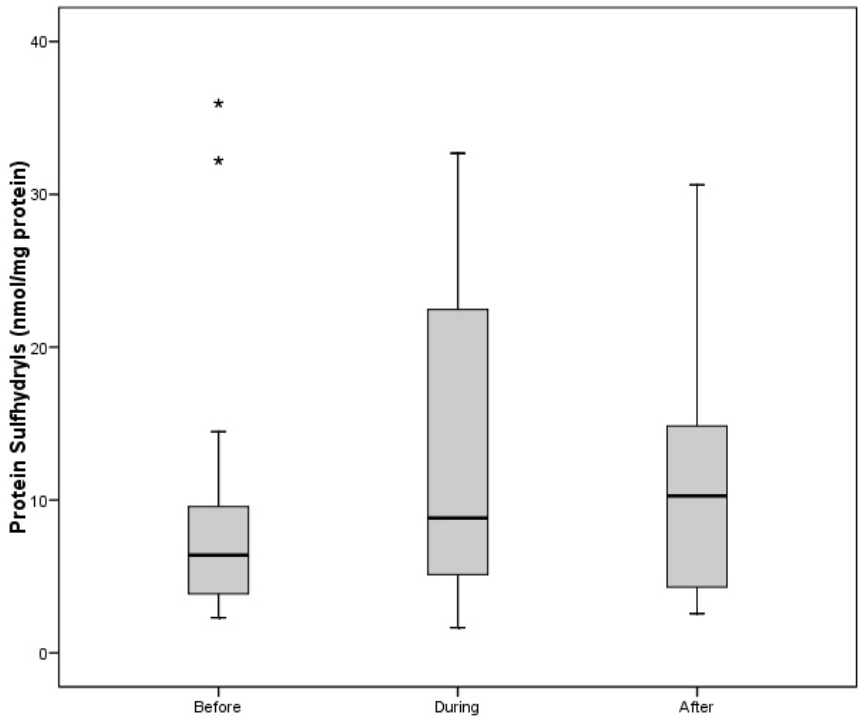

Figure 11: Effect of a normobaric hyperoxia trial on content of protein sulfydryl concentrations in CSF. Overall concentrations of protein sulfydryls did not significantly change from before, during or after trial. Patients grouped and reported in a box plot format (* depicts extreme outlier).

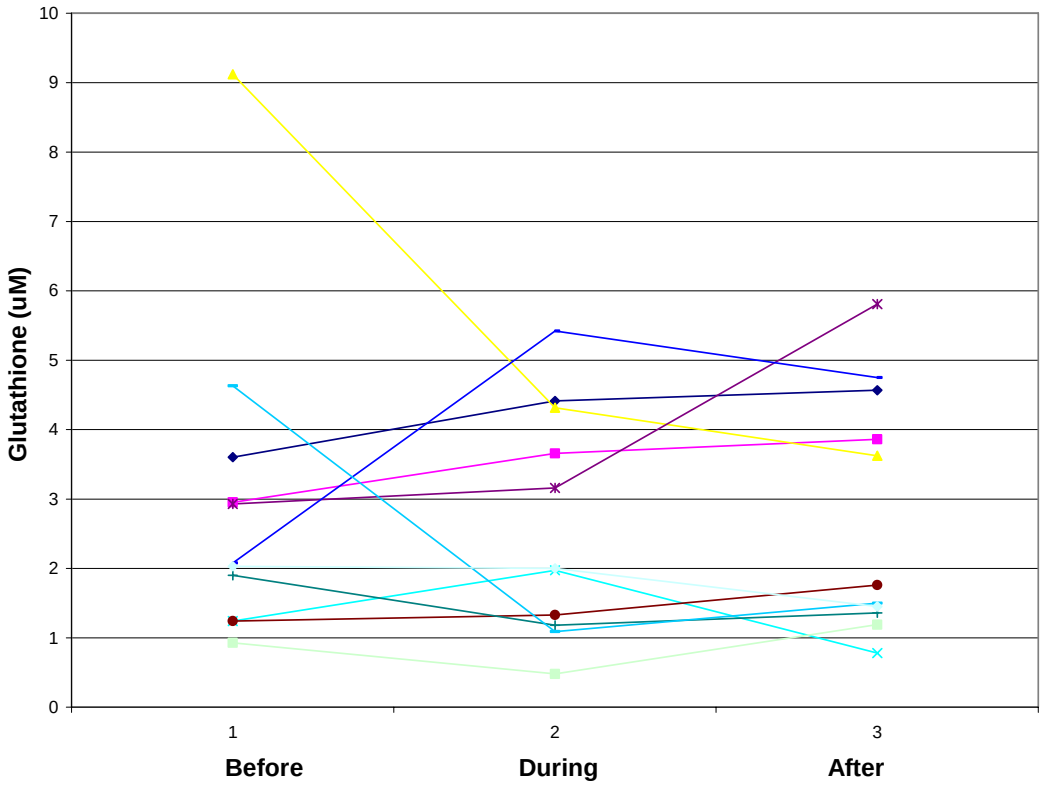

Figure 12: Effect of a normobaric hyperoxia trial on glutathione concentrations in CSF. Overall concentrations of glutathione did not significantly change from before, during or after trial. Individual patients depicted by line graph.

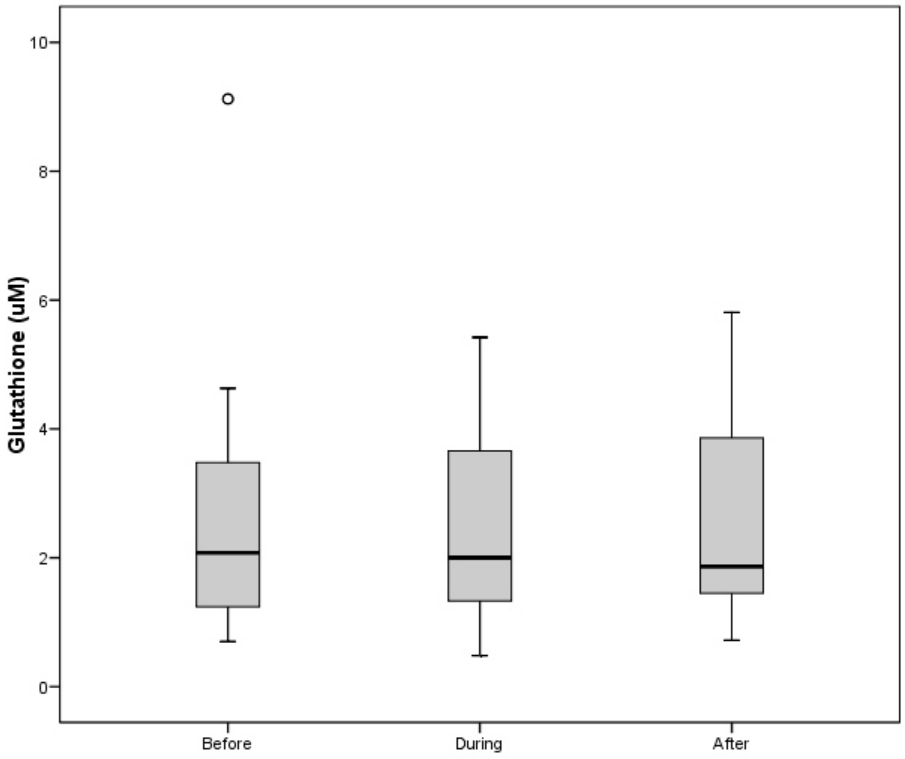

Figure 13: Effect of a normobaric hyperoxia trial on glutathione concentrations in CSF. Overall concentrations of glutathione did not significantly change from before, during or after trial. Patients grouped and reported in a box plot format (o symbol depicts outlier value).

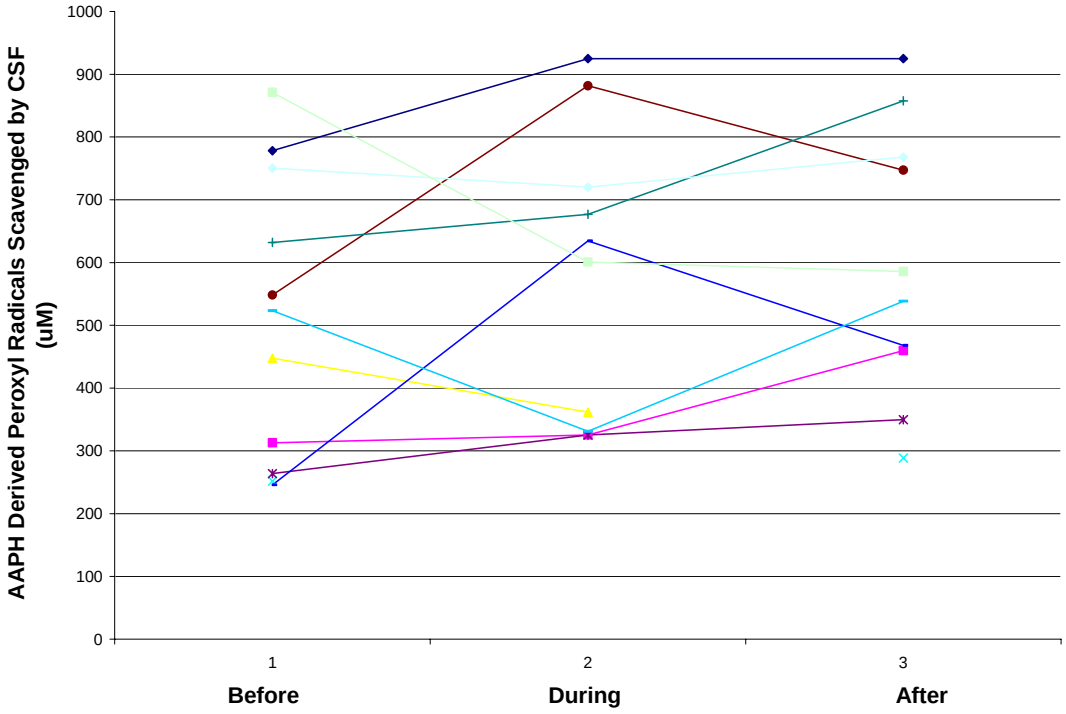

Figure 14: Effect of a normobaric hyperoxia trial on total antioxidant reserve concentrations in CSF. CSF was subjected to free radical attack from peroxyl radicals generated from AAPH in the presence of a reporter molecule, luminol. Overall concentrations did not significantly change from before, during or after trial. Individual patients depicted by line graph.

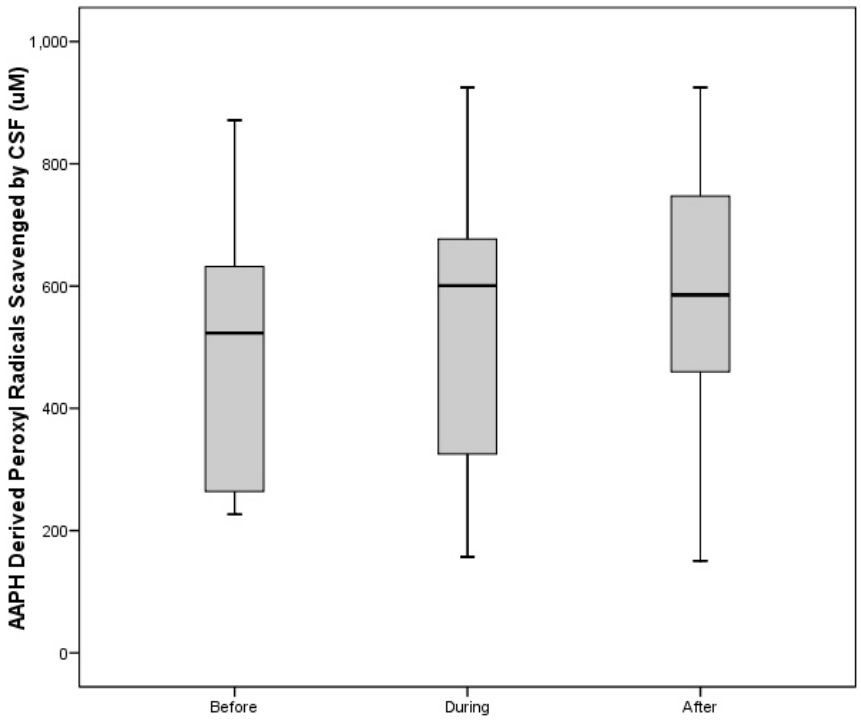

Figure 15: Effect of a normobaric hyperoxia trial on total antioxidant reserve concentrations in CSF. CSF was subjected to free radical attack from peroxyl radicals generated from AAPH in the presence of a reporter molecule, luminol. Overall concentrations did not significantly change from before, during or after trial. Patients grouped and reported in a box plot format.

4.4 DISCUSSION

This interventional study is an initial examination of the effect of a brief (2 hour) controlled period of normobaric hyperoxia on oxidative stress in an adult severe TBI population, similar to a setting of ischemia/reperfusion damage. The present data demonstrates that raising FiO_2 enhanced $PbtO_2$ without apparent risk to a short period of normobaric hyperoxia when measuring CSF oxidative stress and antioxidant defense markers. This clinical study is the first to examine

the effect of normobaric hyperoxia on a battery of oxidative stress biomarkers in CSF. The lack of response in the oxidative stress biomarker battery of the present study, in conjunction with the previously reported improvement of cerebral metabolism markers of the lactate/pyruvate ratio (Nortje et al., 2008; Tolias et al., 2004) lends support that short durations of normobaric hyperoxia are potentially safe in this population.

Although the sample size was small (n=11), we chose a homogenous sample in certain aspects to minimize the variability in secondary injury pathways that might cause a concurrent increase in oxidative stress biomarkers. To control for variations in therapy, all patients were treated with a strict standard protocol for TBI based on the *Guidelines for Management of Severe Traumatic Brain Injury* ("Guidelines for the management of severe traumatic brain injury," 2007). No patients were in crisis situations for ethical considerations, since the use of normobaric hyperoxia is controversial. No patients were experiencing refractory intracranial hypertension, low CPP or low $PbtO_2$.

We have previously shown that severe TBI in children and adults results in the compromise of antioxidant defenses and the exacerbation of free radical-mediated lipid peroxidation (Bayir et al., 2002; Wagner et al., 2004). The potential of compromised antioxidant defenses suggests that an intervention, such as normobaric hyperoxia may place the injured brain at increased risk for secondary damage in the acute phase of recovery, as a consequence of increased free radical production. Although this study resulted in no significant changes for physiological parameters (CPP, MAP, ICP) or biomarkers for oxidative stress and antioxidant reserves, caution must be taken to extrapolate this to the larger TBI population. The baseline values for $PbtO_2$ were 28.5 ± 5.4 mm Hg, and ICP were 14.7 ± 4.9 mm Hg, representing normal oxygenation and pressure values. The resulting non-significant changes in physiology (other

than increases in $PbtO_2$) and biomarker analyses that resulted from the FiO_2 increase to 1.0 may not represent the practice of increasing the FiO_2 when the $PbtO_2$ is < 20 mm Hg, or the ICP is > 20 mm Hg. We cannot rule out the possibility that under such conditions, normobaric hyperoxia may induce oxidative stress, due to the presence of ongoing excitotoxicity, inflammation, activation of xanthine oxidase, or other mechanisms known to mediate oxidative stress. Also, the normobaric hyperoxia trial was performed at least 24 hours after $PbtO_2$ probe insertion to avoid sampling biomarkers resulting from local microtrauma or the initial trauma insult. Normobaric hyperoxia instituted under these early conditions may have an additive effect, with proximity to TBI, ischemia or other secondary injury pathways such as excitotoxicity. Although it has been theorized that patients experiencing a low $PbtO_2$ due to low cerebral blood flow in the damaged region may benefit the most from normobaric hyperoxia (Hlatky et al., 2008), additional studies examining patients with low $PbtO_2$ values and a more extended period of treatment are needed to confirm the absence of adverse events. In addition, because the immature brain is known to be at an increased risk for oxidative damage and there are recognized differences in pathophysiology and outcomes, these findings need to be confirmed in pediatric patients.

The method of CSF sampling chosen was to examine a global representation of the brain under hyperoxia therapy, as opposed to local sampling that microdialysis can offer for specific aims such as pericontusional responses. The injured areas of the brain may respond more or less significantly to this treatment and need to be examined.

The duration of treatment chosen was brief, with the goal of not causing prolonged oxidative stress damage if a dramatic effect with treatment was demonstrated. Because we noted a lack of effect, longer exposure periods need to be examined, as well as long-term neurological

outcomes. Some centers use this treatment transiently while others are using this continuously. Although a brief period of normobaric hyperoxia was employed in this current study, an immediate and drastic increase in $PbtO_2$ occurred, with an average increase of over 200%. In addition, in prior studies, assessment of oxidative stress markers is capable of demonstrable, rapid responses, shown by marked increases in F_2 isoprostane early after TBI (Bayir et al., 2002; Wagner et al., 2004). The data herein indicate some individual variability exists across subjects in response to normobaric hyperoxia treatment; however no substantive trends were observed. In patients having an increase in one variable, there was no consistent increase across other measured variables; hence normal physiologic variability is the most likely explanation for our findings. One subject was shown to be an extreme outlier for F_2 isoprostane; however, the values decreased over time, and did not significantly increase with the normobaric treatment, suggesting that some individuals inherently have a high basal level of oxidative stress after TBI.

Frequently following TBI, with the development of cerebral edema, diffusion of oxygen to tissue can be compromised and thus there is a theoretical target for increased oxygen delivery via raising the FiO_2. Subsequently, hyperoxia treatment is being used transiently to increase low $PbtO_2$ values. It is also believed that the increase in oxygen delivery to compromised regions will produce a compensatory decrease in cerebral blood volume and thus a reduction in intracranial pressure via autoregulation. Although this study did not show an effect of the examined physiological variables, sampling during a crisis situation (ie, intracranial hypertension, hypoxia, etc) may have produced different results and additional studies are needed.

4.5 CONCLUSION

This study is the first to examine prospectively the effect of short periods of normobaric hyperoxia on CSF oxidative stress markers. Short periods of normobaric hyperoxia significantly increased $PbtO_2$ but did not significantly change physiological parameters (ICP, CPP, and MAP) that may influence neurological outcome or CSF markers of oxidative stress and antioxidant reserves in TBI adult patients. This supports the safety of the application of short periods of normobaric hyperoxia after severe TBI in the adult population.

APPENDIX A

University of Pittsburgh

Institutional Review Board

3500 Fifth Avenue
Ground Level
Pittsburgh, PA 15213
(412) 383-1480
(412) 383-1508 (fax)

MEMORANDUM

TO: David O. Okonkwo, MD
FROM: Christopher Ryan, PhD, Vice Chair
DATE: July 20, 2007
SUBJECT: IRB #0407147: The Effect of FiO_2 Elevation on Local Brain Tissue Oxygenation ($PbtO_2$) and Oxidative Stress in Patients with Severe Traumatic Brain Injury

The renewal with modifications of the above-referenced proposal has received expedited review and approval by the Institutional Review Board under 45 CFR 46.110 (8). This protocol is closed to accrual and all protocol interventions are complete.

Approval Date: July 18, 2007
Renewal Date: July 17, 2008

Please note that it is the investigator's responsibility to report to the IRB any unanticipated problems involving risks to subjects or others [see 45 CFR 46.103(b)(5) and 21 CFR 56.108(b)]. The IRB Reference Manual (Chapter 3, Section 3.3) describes the reporting requirements for unanticipated problems which include, but are not limited to, adverse events. If you have any questions about this process, please contact the Adverse Event Coordinator at 412-383-1504.

The protocol and consent forms, along with a brief progress report must be resubmitted at least one month prior to the renewal date noted above as required by FWA00006790 (University of Pittsburgh), FWA00006735 (University of Pittsburgh Medical Center), FWA00000600 (Children's Hospital of Pittsburgh), FWA00003567 (Magee-Womens Health Corporation), FWA00003338 (University of Pittsburgh Medical Center Cancer Institute).

Please be advised that your research study may be audited periodically by the University of Pittsburgh Research Conduct and Compliance Office.

CR:dj

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