Changes in Glutathione Concentration in Athletes with Repetitive Head Injury Using *in vivo* Magnetic Resonance Spectroscopy

Molly Charney

Briarcliff High School

Table of Contents

List of Figures and Tables

Review of Literature

Over the past decade, head injury has emerged as a world-wide epidemic. An estimated 1.6 to 3.8 million sports related head injuries occur per year (Langlois, 2006). For the purpose of this study, mild traumatic brain injury (mTBI) is defined as a blow to the head resulting in altered brain function. Youth, high school, collegiate and professional contact sports athletes, namely football players, are extremely susceptible to concussive and sub-concussive blows on a daily basis. The cumulative effects of head injury have been noted for many years, but more recently have been shown the have more serious long-term effects (Lin, 2012; Baugh, 2012; Goldstein, 2011; McKee, 2009).

In the 1920's dementia pugilistica was documented in a population of boxers. The disease was commonly referred to as "punch drunk syndrome" due to the confused state of the boxers after repetitive head injury (McKee, 2009). More recently, Bennet Omalu of the University of Pittsburgh documented Chronic Traumatic Encephalopathy in a deceased National Football League player (2001). Chronic Traumatic Encephalopathy, commonly known as CTE, is defined as a neurodegenerative disease caused by blunt force to the head. Since its documentation in 2001, CTE has emerged as a public health problem for contact sports athletes. However, there has been minimal research on CTE, how to diagnose it, and how to ultimately slow down the progression of symptoms (McKee, 2009; Baugh, 2012).

The clinical symptoms of CTE include cognitive, mood and behavioral changes. People with CTE develop executive dysfunction, depression, irritability, impulse control problems, aggression and substance abuse (Baugh, 2012). These clinical symptoms culminate in dementia. A potential timeline of events has been developed by researchers

at Boston University for the clinical progression of CTE (McKee, 2009). First, the individual participates in contact sports and sustains multiple hits to the head. The person then starts to develop the typical clinical symptoms of CTE about a decade after they retire from contact sports. These clinical symptoms worsen over time until the person experiences dementia (McKee, 2009). The cognitive and emotional changes of people with CTE have resulted in accidental deaths, substance abuse and suicide (Baugh, 2012). Not everyone who sustains head injury develops CTE but everyone who has developed CTE has sustained head injury. Therefore, head injury is necessary but not sufficient for developing CTE (Lin, 2012). It is therefore important to understand the underlying pathological changes that occur in CTE.

The neuropathology of CTE is distinct from other dementias. The gross changes seen in the brain include general atrophy, atrophy of the temporal, frontal and medial lobes as well as atrophy of the hippocampus, and mammillary bodies (Baugh, 2012). A cavum septum pellucidum and the dilation of the 2nd and 3rd ventricles are also frequently seen (Stern, 2011). Microscopically, CTE is characterized by neurofibrillary tangles (NFTs), which are formed by the phosphorylation of tau, neuritic threads and glial tangles (Baugh, 2012). Tau proteins stabilize the microtubules of neuronal axons and thus, when the tau becomes phosphorylated and ineffective, dementias can arise. NFTs are seen in other dementias such as Alzheimer's disease (AD). In CTE, the tau deposits are arranged in a perivascular form and the NFTs are very dense in the neocortex (Baugh, 2012). TAR DNA-Binding Protein 43 (TDP-43), a protein marker of motor neuron disease (MND), has also been seen to accumulate in regions of the brain as well

as the spinal cord. TDP-43 neuropathology in the spinal cord has resulted in the formation of a subset of CTE that includes the pathology of MND (McKee, 2010).

Currently, CTE can only be diagnosed after death and therefore, the goal of future research is to be able to diagnose CTE while the patient is still alive. Goldstein et al. detected similarities between the clinical symptoms seen in blast-exposed war veterans and football players who had sustained multiple hits to the head (2012). The neuropathology seen in the brains of military veterans was also similar to the neuropathology seen in CTE. Mice were exposed to blast injury in the lab and analyzed for cognitive and brain pathology. The mice experienced cognitive dysfunction and memory impairment. The pathology seen in the brains of the mice two weeks after injury was very similar to the pathology seen in the brains of people with CTE (Baugh, 2012). This experiment tied together CTE and war-related blast injury, increasing the importance of the detection of CTE due to the larger population affected by the disease (Goldstein, 2012). Immunoexcitotoxicity (IET) is a possible mechanism to explain the manifestation of CTE (Blaylok, 2011). Glutamate (Glu) is the most abundant neurotransmitter in the brain, but it is excitotoxic in higher than normal concentrations. The interaction between glutamate receptors and cytokine receptors cause a hyper reactive response of microglia, glial cells that are active in immune defense in the blood brain barrier. Microglia release neurodestructive elements in defense. After head injury, microglia can become fixed in a state where they continually release neurodestructive elements (Blaylok, 2011). Blaylok et al. proposed that the inefficiency of the microglia and IET might be the mechanism behind the onset of CTE. An association between the apolipoprotein E (ApoE) allele and the onset of CTE has been proposed. The E4 allele

has already been connected to TBI and AD (Jordan, 1997; Ponsford, 2011). Although, some mechanisms have been proposed, there is no definite known mechanism behind CTE (Goldstein, 2012; Blaylok, 2011).

Alzheimer's disease shares many similarities clinically and pathologically to CTE. Clinically, the patients undergo similar deterioration mentally and both diseases culminate in dementia. Pathologically, phosphorylated tau accumulates in the brains of CTE and AD patients. A major difference between the pathology of AD and CTE is that a large marker of AD is the accumulation of beta-amyloid (AB). Beta amyloid is very rarely seen in CTE. The NFTs are also much denser in CTE than AD. While there are these differences, some similarities have led scientists to investigate biomarkers already used in AD for use in CTE. Han et al. used biomarkers to provide evidence for the Jack model of sequence for AD. The sequence established that tau and AB were the first changes to be seen, followed by brain functional changes. Next, structural changes and then declines in cognition occur (Han, 2012). This sequence may be similar to the sequence of changes in CTE.

Magnetic Resonance Spectroscopy (MRS), in combination with conventional MRI, can be used as an imaging biomarker to diagnose AD. MRS is an imaging method used to determine the biochemical make-up of human and animal organs (Mandal, 2006). MRS serves as an in vivo "virtual biopsy" that has possible diagnostic value (Lin, 2012). MRS utilizes conventional MRI machinery but analyzes the data differently in order to produce a spectrum of metabolites. Each peak on the MRS spectrum represents a specific metabolite concentration in the region of interest. The main metabolites observed by 1H MRS are described in Table 1. A decrease in the metabolites N-acetyl aspartate

(NAA) and an increase in myo-inositol (mI) are seen in MRS scans of AD patients (Mandal, 2006). MRS also has possible implications for diagnosing CTE.

Metabolite	
N-acetyl aspartate (NAA)	Marker of neuronal density
Lipid	In the brain as membranes but are not visible until released ex: trauma
Lactate	Indicator of hypoxia
Creatine (Cr)	Internal reference marker
Choline (Cho)	Membrane marker and marker of diffuse axonal injury
Glutamate/Glutamine (Glx)	Glutamate is an excitotoxic neurotransmitter, tightly couples with glutamine
Myo-Inositol (mI)	Glial and astrocyte marker, similar to choline

Table 1: Important MRS detectable metabolites and the meaning of their presence in the brain.

1H MRS has been applied to mTBI in order determine the chemical changes and recovery after head injury. It was found that after mTBI NAA decreased immediately after injury and did not recover six months later and glu also decreased immediately after injury but recovered, and mI was not changed immediately after injury but was impaired after 6 months (Henry, 2011). Vagnozzi found that brain NAA levels did not return to normal until 30 days after injury (2008). Vagnozzi scanned subjects after double concussions, and found a slower recovery of NAA than the recovery in single concussion subjects. The NAA concentration in the double concussion patients took about 15 days longer to recover if it did recover fully in the study time of 3-45 days (Vagnozzi, 2008).

It has been documented that multiple head injuries could decrease NAA concentration as well as increase Glx and Cr concentrations in the white matter over the long term (Vagnozzi, 2008, 2010; Henry, 2010; Lin, 2012). Knowing the metabolic changes of people with CTE would help to diagnose the disease while they are alive and asymptomatic. In a study conducted by Lin et al., NAA concentrations decreased by as much as 20%, while Cho increased and Glx increased in the brains of athletes with probable CTE (Lin, 2010).

There has been less research on the metabolite, glutathione (GSH), but it holds the possibility of identifying the mechanism of CTE as well as developing a diagnostic metabolic profile for the disease. Glutathione is an antioxidant, composed of cysteine, glycine and glutamate, that exhibits a positive role in the brain. Glutathione serves to detoxify the brain by acting as a reducing agent and transforming free radicals as well as hydroperoxides (Dringen, 2000). Glutathione is found in an oxidized form (GSSG) and a much more common reduced form (GSH). In several neurodegenerative diseases including, AD, Parkinson's disease, and ALS, a reduction in GSH has been observed (Martin, 2009). The goal of the following research is to determine if there are differences in glutathione concentration between former professional football players and healthy controls. The eventual goal of this research is to be able to diagnose CTE before patients begin to experience clinical symptoms as well as document the mechanism of neurodegeneration in CTE.

Research Question

Does a difference in glutathione concentration between retired NFL players with a history of repetitive head injury and former professional athletes without a history of repetitive head injury exist?

Hypotheses

H_{1A}: There is a difference in the concentration of GSH between former NFL players with a history of repetitive head injury and age matched controls.

H_{1B}: There is no difference in the concentration of GSH between former NFL players with a history of repetitive head injury and age matched controls.

Methods

Phantoms

Three different phantom solutions were prepared and scanned in the 3T Siemens Verio MRI scanner. The first set of phantoms contained six phantoms, each with stable concentrations of NAA (10mM), Cr (8mM), Glu (10mM) and Gln (5mM). The phantoms also contained an increasing concentration of GSH (0.3, 0.5, 0.7, 1.5, 3 and 5 mM). The second set of phantoms contained six phantoms with stable concentrations of GSH (1.5 mM), NAA (10mM), Cr (8mM) and Gln (5mM) and increasing concentrations of Glu (0, 1.5, 5, 10, 15, 20 mM). The last set of phantoms contained stable concentrations of Cr (5mM) and GSH (1.5mM) as well as increasing concentrations of Gly (0.5, 1, 5 mM).

Subjects

The subject group (n=62) consisted of former professional football players with a history of repetitive head injury and symptoms associated with CTE. The subject group had a mean age of 54.6 years +/- 7.7 years (range 40-70 years old). All subjects had over 10 years of college and professional football play. The control group (n=10) consisted of former professional athletes with no history of repetitive head and no clinical symptoms associated with CTE. The control group had a mean age of 57.7 years +/- 9.1 years (range 45-69 years old).

MR Spectroscopy

Subjects, controls and phantom solutions were scanned using a 3T clinical MR scanner (Siemens TIM Verio). Localized shimming was performed with automatic adjustment of first- and second-order shim gradients by using the automatic three-dimensional B_0 field mapping technique (Siemens). Next, manual shimming was performed in order further refine the magnetic field. Single voxel, proton MRS was performed on each subject and control. The point resolved spectroscopy sequence (PRESS) was applied with an initial echo time of 35 milliseconds, repetition time of 1.5 seconds, 1024complex data points, and 128 averaged acquisitions. An unsuppressed water spectrum of 16 averages was also acquired in the same region to serve as a water reference for post-processing. The voxel of interest was 2x2x2 cm^3 and part of the posterior cingulate gyrus. The posterior cingulate gyrus was chosen as the voxel of interest due to the homogeneity of the region as well as documented metabolic changes in

head injury. The metabolic changes seen after head injury in the posterior cingulate gyrus have also been predictive of long-term outcome (Lin, 2012).

Data Processing

The files acquired during the MRS scan were saved as .rda files. The water reference file as well as the subject's .rda file was needed for LC Model processing. LC Model or the linear combination model is a spectral processing software that combines the individual metabolites to form a 1D spectrum. LC Model uses basis sets in order to model the in vivo data in order to quantify the metabolites from a spectrum (Figure 1). GSH quantification was completed for each subject and control. The Cramer-Rao Lower Bound (CRLB) is a data reliability indicator and represents the percent standard deviation for each individual metabolite. Data with a %SD less than 20% is considered acceptable.

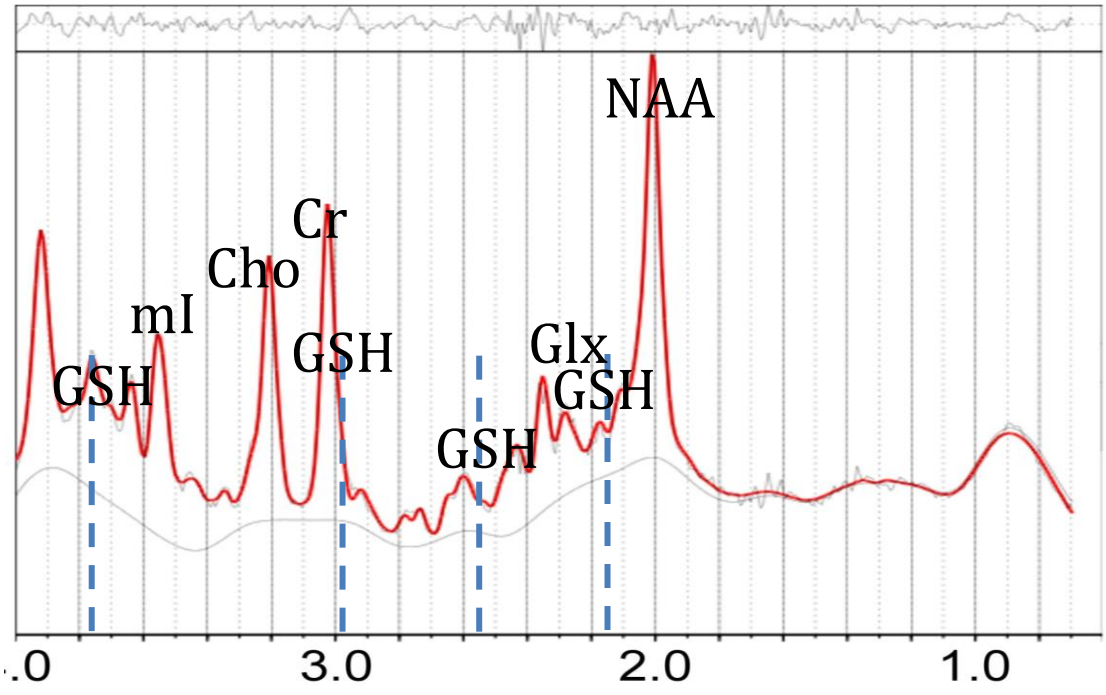

Figure 1: The above image displays the spectrum of metabolites produced by LC Model.

This reliability is equivalent to a 95% confidence interval. Any data points of extreme nature were eliminated.

Statistical Analysis

Due to the large discrepancy between the size and age of the NFL and controls groups, 10 NFL players (with the exact age or no more than one-year difference) were randomly selected and blinded to GSH results in order to create age-matched pairs with the 10 controls. There has been evidence of age related changes in GSH concentrations (Suh, 2004) and therefore age matching the controls and NFL players should have reduced this influence. Paired student t-tests using two-tails was performed in these two cohorts. Linear Regressions were run for each phantom set, plotting the concentration placed in the solution by the GSH/Cr and Glu/Cr ratios.

Results

The glutathione levels of the NFL athletes were significantly decreased ($p=0.0164$) compared to that of the control athletes with no history of repetitive head injury. A linear regression was run in order to determine if overall, age related changes in GSH were seen. The linear regression line had a slope of 0.0006, almost zero (Figure 2). This shows no increase or decrease of GSH due to the age of the athlete. The decreased GSH in the NFL athletes was found as a result of the age matched t-tests.

The phantoms created of increasing glutamate as well as increasing GSH showed overall that LC Model was able to detect GSH. In the phantoms with stable GSH and increasing glu, it was found that LC Model detected the increase in glu and reported stable values of GSH, except for one data point, which was a severe outlier.

The %SD for glu was above 20% for three of the six phantoms. The %SD for GSH in the increasing glu phantoms was under 20 for all but one phantom, which was a severe outlier in the data set. The stable values of GSH in the increasing glu phantoms show that LC Model can differentiate glu and GSH. In the phantom set with increasing levels of GSH and stable levels of glu, the GSH/Cr steadily increases while the glu/Cr measurement is variable but with a clear positive slope. Three of the six %SD of GSH in the increasing GSH phantoms was above 20%.

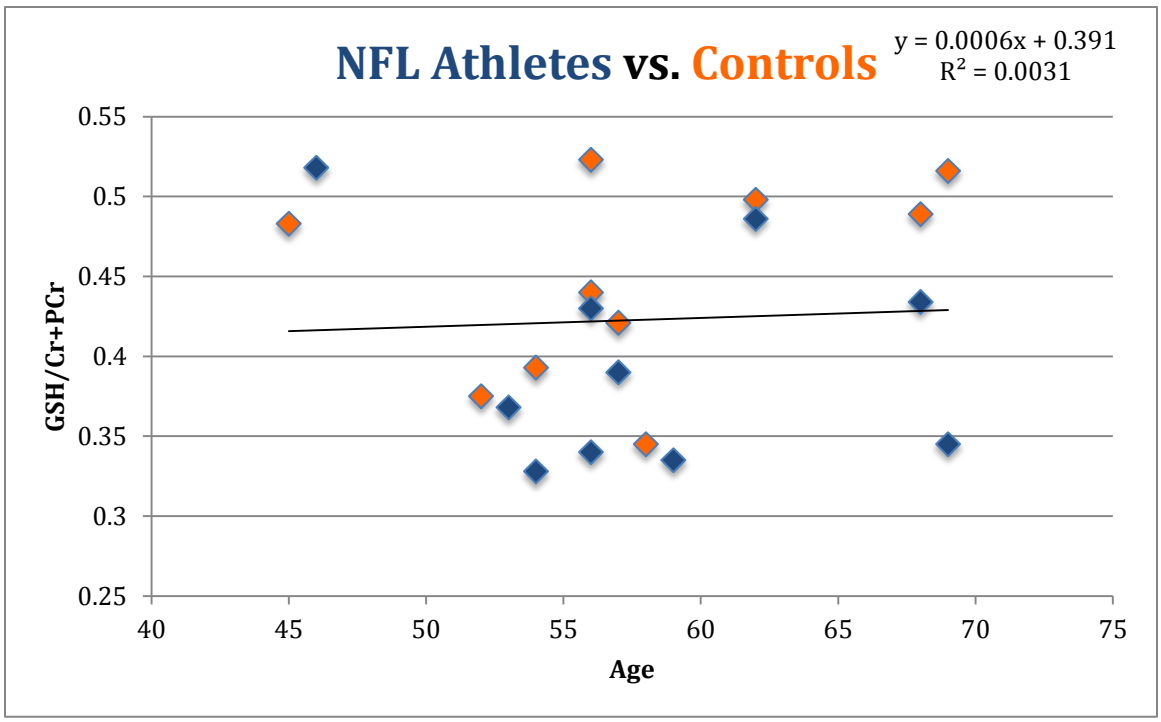

Figure 2: The above graph shows the GSH concentration of the NFL players and controls related to age. The linear trend line has a slope of about zero.

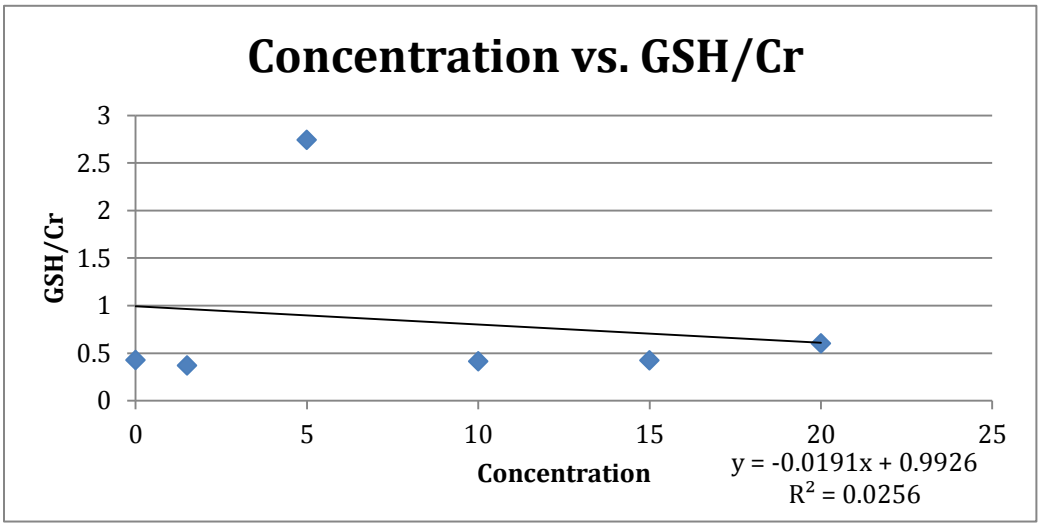

Figure 3: The above graph shows the GSH in the phantom set with increasing Glu. The GSH generally is stable besides the 5mM outlier.

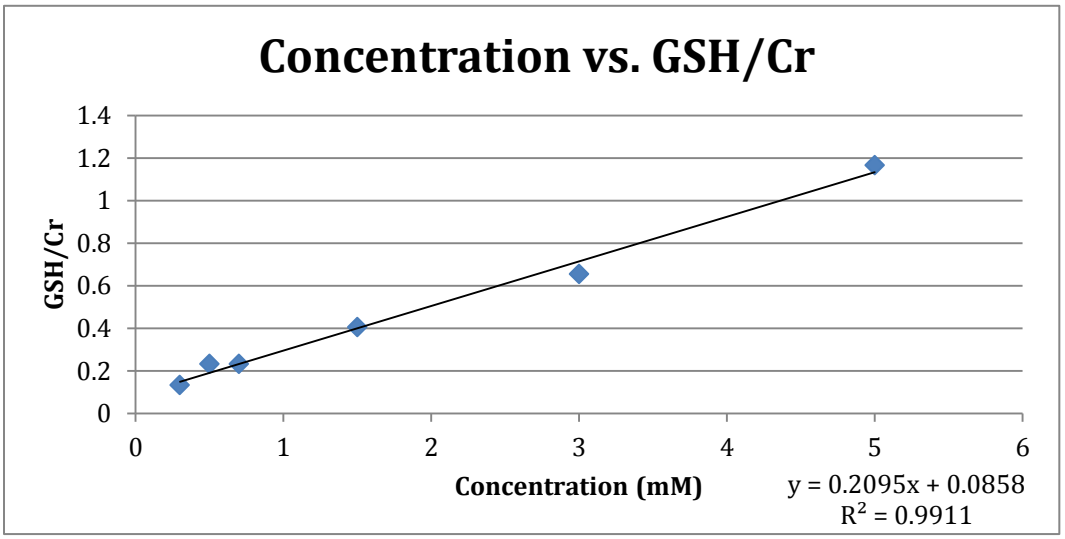

Figure 4: The above graph shows the increasing GSH concentration in the phantom sets with stable metabolites and increasing GSH.

Discussion

It was first shown in this study that LC Model, a respected and tested model of quantitative MRS analysis, does not work perfectly with phantom solution data. In the phantom set of increasing GSH, after the processing the data it is seen that there seems to be an increase in glu as well. Glu concentration was not increased in the phantom and should not have been increased in the data analysis. This may show that LC Model cannot finitely distinguish between GSH and glu. In the opposite scenario, where GSH was stable and glu increased, after post processing, GSH was seen to be stable and glu increased. Therefore, it is unclear whether the abnormalities seen in the phantom set with increasing GSH is due to noise, problems with LC Model's processing of phantoms, or LC Model's inability to process phantom data as well as it processes human data. The third possible reasoning is somewhat expected because LC Model was created from *in vivo* human data, while the phantoms are supposed to model this scenario. This is a limitation of using LC Model for quantitative phantom analysis. LC Model has been shown to be reliable for quantitative analysis of *in vivo* human data (Provencher, 1993).

This study also shows that the GSH concentration in the brains of former NFL athletes is lower than that of former professional athletes without histories of head injury. When the GSH/Cr+PCr ratio of age and sex matched controls was compared to that of the NFL athletes there was a significant difference ($p=0.0146$). The GSH/Cr of the NFL athletes was decreased compared to the controls. GSH is a marker of neuroinflammation, as its role in the brain is to reduce reactive oxygen species and hydro peroxides in order to detoxify the brain. In AD, there is a buildup of tau protein, which has been linked to oxidative stress (Bains, 1997). The Jack Model of AD suggests that beta amyloid is the

first change seen in the brain and is the trigger for the rest of neurodegeneration (Han, 2012). Beta amyloid is absent from CTE and therefore the mechanism may be different between the two neurodegenerative diseases.

Head injury, including subconcussive injury, causes oxidative stress (Tyurin, 2000). Oxidative stress has been known to cause a cysteine deficiency (Droge, 2005). Cysteine is one of the three components of GSH and is most commonly the limiting reactant of GSH formation. A decrease in cysteine would cause a decrease in the amount of reduced GSH available in the brain (Richie, 1988). The brain would then be unable to combat the oxidative stress caused by head injury; tau tangles may build up, causing the pathology observed in CTE. This hypothesis has not yet been proposed for CTE. Blaylok in 2011 hypothesized that CTE may be caused by immunoexcitotoxcity. This is when microglia become fixed in a neurodestructive state due to head injury and the interaction between glutamate and cytokine receptors. While, the mechanism of CTE is still unknown, a decrease in GSH may lead to further hypotheses and studies relating neurodestruction in CTE to oxidative stress.

Due to the small number of controls, only a small proportion of the NFL data could be used. This is a limitation of the study. Prior research has shown that GSH decreases with age (Suh, 2004). When a linear regression was performed within the combined data set of NFL players and controls, it was found that there was approximately zero relationship between age and GSH concentration as shown by the very low r^2 (0.003) and slope (0.0006) values calculated in the linear regression. Therefore, the decrease in GSH seen in the NFL players is not purely due to age related

changes. When examining the matched pairs, the NFL players generally had a lower GSH concentration then the age matched equivalent control.

The detection of GSH using in vivo MRS has many applications in psychiatric and neurodegenerative diseases. In this case, the reduction in glutathione in the brain's of former NFL players may indicate that neuroinflammation is a primary mechanism of CTE. Showing evidence of neuroinflammation using other imaging methods such as diffusion tensor imaging would help to solidify this hypothesis. Changes in GSH can be determined quickly and reliably with MRS and could be useful in diagnosing of CTE, determining the mechanism behind the disease as well as eventually treating the symptoms.

Conclusion

This study investigated the use of magnetic resonance spectroscopy and LC Model to measure glutathione concentrations in vivo. MRS and LC Model were used in order to determine if there was a difference in the GSH concentrations between former NFL players with possible CTE and healthy controls. It was found that the GSH levels in the former NFL players were significantly reduced ($p=0.0146$). In the future, an expansion of this study implementing the use of other methods to measure neuroinflammation could help in determining the mechanism for Chronic Traumatic Encephalopathy. Discovering the mechanism of CTE would be beneficial to those suffering from the disease, as it would open up the field for diagnosis technology as well as treatment to slow down the symptoms of the disease.

References

Bains, J. S., & Shaw, C. a. (1997). Neurodegenerative disorders in humans: the role of glutathione in oxidative stress-mediated neuronal death. *Brain research reviews*, *25*(3), 335–58.

Baugh, C.M. et al., (2012). Chronic traumatic encephalopathy: neurodegeneration following repetitive concussive and subconcussive brain trauma. *Brain imaging and behavior*, 6(2), pp.244–54.

Blaylock, R.L. & Maroon, J., (2011). Immunoexcitotoxicity as a central mechanism in chronic traumatic encephalopathy—A unifying hypothesis. *Surgical neurology international*, 2, p.107.

Chen, T. S., Richie, J. P., & Lang, C. a. (1989). The Effect of Aging on Glutathione and Cysteine Levels in Different Regions of the Mouse Brain. *Experimental Biology and Medicine*, *190*(4), 399–402.

Dringen, R. (2000). Metabolism and functions of glutathione in brain. *Progress in neurobiology*, *62*(6), 649–71.

Fan, P., Yamauchi, T., Noble, L. J., Ferriero, et al. (2003). Age-Dependent Differences in Glutathione Peroxidase Activity after Traumatic Brain Injury, *20*(5), 437–445.

Gardner, A., Iverson, G.L. & McCrory, P., (2013). Chronic traumatic encephalopathy in sport: a systematic review. *British journal of sports medicine*, pp.1–8.

Gavett, B.E. et al., (2011). Clinical appraisal of chronic traumatic encephalopathy: current perspectives and future directions. *Current Opinion in Neurology*, 24, pp.525–31.

Goldstein, L.E. et al., (2012). Chronic traumatic encephalopathy in blast-exposed military veterans and a blast neurotrauma mouse model. *Science Translational Medicine*, 4(134), p.134ra60.

Han, S.D. et al., (2012). Beta amyloid, tau, neuroimaging, and cognition: sequence modeling of biomarkers for Alzheimer's Disease. *Brain imaging and behavior*, 6(4), pp.610–20.

Henry, L.C. et al., (2011). Metabolic changes in concussed American football players during the acute and chronic post-injury phases. *BMC Neurology*, 11(105)

Iverson, G.L. et al., (2004). Cumulative effects of concussion in amateur athletes. *Brain Injury*, 18(5), pp.433–443.

Kaiser, L. G., Matson, G. B., Iltis, I., et al. (2011). 1H MRS detection of glycine residue of reduced glutathione in vivo, *J Magn Reson*. *202*(2), 1–14.

Kantarci, K., (2007). 1H magnetic resonance spectroscopy in dementia. *The British journal of radiology*, 80 Spec No, pp.S146–52.

Kraus, M.F. et al., (2007). White matter integrity and cognition in chronic traumatic brain injury: a diffusion tensor imaging study. *Brain : a journal of neurology*, 130(Pt 10), pp.2508–19.

Kroshus, E. et al., (2013). NCAA concussion education in ice hockey: an ineffective mandate. *British journal of sports medicine*, 1, pp.1–8.

Lin, A. P. et al., (2012). Metabolic imaging of mild traumatic brain injury. *Brain imaging and behavior*, 6(2), pp.208–23.

Lomnitski, L. (1999). Antioxidant mechanisms in apolipoprotein E mice prior to and following closed head injury, *Biochimica et Biophysica acta. 1453*, 359–368.

Martin, H. L., & Teismann, P. (2009). Glutathione--a review on its role and significance in Parkinson's disease. *Federation of American Societies for Experimental Biology*, *23*(10), 3263–72.

Mckee, A. C., Cantu, R. C., Nowinski, C. J., et al. (2009). Chronic Traumatic Encephalopathy in Athletes: Progressive Tauopathy following Repetitive Head Injury. *J Neuropathol Exp Neurol*, *68*(7), 709–735.

McKee, A.C. et al., (2010). TDP-43 proteinopathy and motor neuron disease in chronic traumatic encephalopathy. *Journal of neuropathology and experimental neurology*, 69(9), pp.918–29.

McKee, A.C. et al., (2013). The spectrum of disease in chronic traumatic encephalopathy. *Brain : a journal of neurology*, 136(Pt 1), pp.43–64.

Omalu, B., Hammers, J.L., et al., (2011a). Chronic traumatic encephalopathy in an Iraqi war veteran with posttraumatic stress disorder who committed suicide. *Neurosurgical focus*, 31(5), p.E3.

Omalu, B., Bailes, J., et al., (2011). Emerging histomorphologic phenotypes of chronic traumatic encephalopathy in American athletes. *Neurosurgery*, 69(1), pp.173–83; discussion 183.

Pereira, F., & Oliveira, C. R. De. (2000). Oxidative glutamate toxicity involves mitochondrial dysfunction and perturbation of intracellular Ca 2 + homeostasis, *Neuroscience Research. 37*, 227–236.

Provencher, S.W. (1993). Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magnetic Resonance in Medicine,* 30(6): pp. 672-679.

Seichepine, D.R. et al., (2013). Profile of self-reported problems with executive functioning in college and professional football players. *Journal of neurotrauma,* 30(14), pp.1299–304.

Shenton, M.E. et al., (2012). A review of magnetic resonance imaging and diffusion tensor imaging findings in mild traumatic brain injury. *Brain Imaging Behav*, 6(2), pp.137–192.

Spiotta, A.M., Bartsch, A.J. & Benzel, E.C., (2012). Heading in soccer: dangerous play? *Neurosurgery*, 70(1), pp.1–11; discussion 11.

Stern, R. A., Riley, D. O., Daneshvar, D. H., et al. (2011). Long-term consequences of Repetitive Brain Trauma: Chronic Traumatic Encephalopathy. *PM & R : the journal of injury, function, and rehabilitation*, *3*(10 Suppl 2), S460–7.

Suh, J. H., Shenvi, S. V, Dixon, B. M., et al. (2004). Decline in transcriptional activity of Nrf2 causes age-related loss of glutathione synthesis, which is reversible with lipoic acid. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(10), 3381–6.

Talavage, T.M., Nauman, E.A. & Yoruk, U., (2010). Functionally-Detected Cognitive Impairment in High School Football Players Without Clinically- Diagnosed Concussion. *Journal of Neurotrauma* (765), pp.1–46.

Tavazzi, B. et al., (2007). Temporal Window of Metabolic Brain Vulnerability To Concussions. *Neurosurgery*, 61(2), pp.390–396.

Terpstra, M., Henry, P.-G., & Gruetter, R. (2003). Measurement of reduced glutathione (GSH) in human brain using LCModel analysis of difference-edited spectra. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*, *50*(1), 19–23.

Tyurin, V.A. et al., (2000). Oxidative stress following traumatic brain injury in rats: quantitation of biomarkers and detection of free radical intermediates. *Journal of neurochemistry*, 75(5), pp.2178–89.

Vagnozzi, R. et al., (2010). Assessment of metabolic brain damage and recovery following mild traumatic brain injury: a multicentre, proton magnetic resonance spectroscopic study in concussed patients. *Brain : a journal of neurology*, 133(11), pp.3232–42.

Acknowledgements

This research could not have been completed without the help of several people including:

Dr. Alexander Lin- Clinical Spectroscopist, Brigham and Women's Hospital/Harvard Medical School, Helped with research direction, data analysis, and interpertation of results as well as provided data from an ongoing CTE study. Dr. Lin also made suggestions for improvements for my research paper and oversaw the subjects' MRS scans.

Michael Inglis- Briarcliff High School Science Research teacher

Dr. Robert Stern- Professor of Neurology and Neurosurgery, Boston University (Center for the Study of Chronic Traumatic Encephalopathy, responsible, with the rest of his team, for recruitment, clinical evaluations, and coordination of subjects.

Dr. Martha Shenon- Professor and Director, Psychiatric Neuroimaging Lab at Brigham and Women's Hospital, Director of MRS and MRI portion of the CTE study

Ms. Huijun Liao- Research coordinator, Brigham and Women's Hospital/ Harvard Medical School. Scanned and collected data on GSH phantom and GSH/Glu phantom sets. Taught me how to run the MRS scan as well as assisted in safety training for the lab and MRI machinery.

Sai Merugumala- Research Assistant, Brigham and Women's Hospital/Harvard Medical School, Helped acquire MRS data, assisted with technology and post processing

Daniel Rodriguez- Research Trainee, Brigham and Women's Hospital/Harvard Medical School, Assisted with data collection and processing as well as taught me how to post process the data

Dr. Luke Wang- Anesthesiologist, Boston Children's Hospital, Assisted in MRS scan acquisition