Longitudinal Evaluation of Mild Traumatic Brain Injury: a H-MRS Study

Elijah George[1,2], Steve Roys[1,3], Jiachen Zhuo[1], Chandler Sours[4], Jacqueline Janowich[1], Teodora Stoica[1], and Rao Gullapalli[1]

[1]*Magnetic Resonance Research Center, University of Maryland School of Medicine, Baltimore, MD, United States,* [2]*Bioengineering, University of Maryland, College Park, MD, United States,* [3]*Diagnostic Radiology & Nuclear Medicne, University of Maryland School of Medicine, Baltimore, MD, United States,* [4]*Program in Neuroscience, University of Maryland School of Medicine, Baltimore, MD, United States*

Introduction: Up to 5 million Americans are currently living with Traumatic Brain Injury (TBI) related disabilities. More so, the overwhelming majority of viable TBI patients (75 %) are deemed "mild" with most injuries occurring as non-hemorrhagic and or microscopic likely rendering them undetectable by Computed Tomography (CT) or conventional MRI (Magnetic Resonance Imaging). The bulk of the pathologies experienced in mild traumatic brain injury (mTBI) arise from "secondary injury" and the sequelae include diffuse axonal injury (DAI), reactive inflammation, edema, apoptosis, excitotoxicity, mitochondrial dysfunction, ischemia and neuro-metabolic alterations[1,2]. Such physiological and biochemical changes occurring at the cellular level are hardly detected by conventional imaging or structural MR techniques. The accurate assessment of mTBI therefore necessitates a deeper understanding of changes at the molecular level which leads to changes in the biochemical process and precede any discernible macroscopic changes at the tissue level *in vivo*. Magnetic Resonance Spectroscopy (MRS) is one such modality that has the potential of providing a non-invasive means for evaluating metabolic changes that occur at the cellular level in mTBI patients.

The aim of the current study is to carry out a longitudinal evaluation of mTBI by monitoring metabolic markers of mTBI and their evolution over time such that the findings realized herein will aid improved clinical evaluation of the pathology. We combined neuro-metabolic information with neuropsychological test (NPT) data for the purpose of understanding how the current metabolic state affects ongoing cognitive capability, and to determine the efficacy of neuro-metabolic information acquired acutely in predicting outcome of mTBI patients.

Methods: For the current mTBI study, only participants with a GCS of 13-15 were used in the analysis. In addition, participants with a history of neurological and psychiatric illness, stroke, brain tumors or seizures were excluded from this study. Patients were examined acutely (< 10 days post injury), sub acutely (~1 month post injury) and chronically (~6 months post injury). MR examinations were carried out on a Siemens Tim-Trio 3T MRI scanner and included a T1-weighted-MPRAGE scan for anatomical reference and a Proton Magnetic Resonance Spectroscopy Imaging (^{1}H-MRSI) scan. The ANAM[3] (Automated Neuropsycological Assessment Metrics) group of subtests was employed to carry out Neuropsycological assessment of the subjects. The ANAM is a neurocognitive test that was developed by the US military to test a number of cognitive domains including attention, concentration, reaction time, memory, processing speed, decision-making and executive function. It is a computer based assessment and consists of a battery of seven subtests. Metabolite quantification was performed using LCModel[4]. The regions analyzed for metabolic alterations due to mTBI included the *Putamen*, *Periventricular White Matter, Centrum Semiovale* and the *Thalamus*. A two-tailed independent samples *t*-Test was used to determine group differences in metabolite-ratio values between mTBI patients and the healthy control group. A linear regression analysis was used to determine the correlations between NPT data and neuro-metabolic information.

Results: The major findings realized in this study are: (i) Significant decreases ($p < 0.05$) and decreasing trends ($p < 0.1$) in N-Acetyl Aspartate to Creatine ratio (*NAA/Cr*) at the acute time point in a number of regions for the mTBI group when compared to controls, with marginal recovery at later time points. (ii) Significant decreases ($p < 0.05$) and decreasing trends ($p < 0.1$) in total Choline to Creatine ratio (*Ch/Cr*) at all time points in the mTBI group in a number of regions when compared to controls. (iii) Significant decreases ($p < 0.05$) in *Cr* levels at the acute time point with gradual recovery at later time points in the mTBI group when compared to controls. (iv) Strong positive linear correlations ($p < 0.05$) between *NAA/Cr*, *Ch/Cr* and *Cr* levels in various anatomical regions and concurrent NPT scores for the mTBI group at all time points (v) Strong positive linear correlations ($p < 0.05$) between acute *NAA/Cr* values in regions coinciding with the thalamus and chronic NPT scores.

	Right Thalamus	Left Thalamus	Thalamus
Code Substitution Throughput	$r = 0.370$ $p = 0.109$	$r = 0.531$ $p = 0.016$	$r = 0.474$ $p = 0.035$
Match to Sample Throughput	$r = 0.428$ $p = 0.059$	$r = 0.477$ $p = 0.033$	$r = 0.472$ $p = 0.035$
Math Throughput	$r = 0.503$ $p = 0.024$	$r = 0.342$ $p = 0.140$	$r = 0.436$ $p = 0.055$
Simple Reaction Time Throughput	$r = 0.474$ $p = 0.035$	$r = 0.427$ $p = 0.060$	$r = 0.467$ $p = 0.038$
Simple Reaction Time 2 Throughput	$r = 0.450$ $p = 0.047$	$r = 0.542$ $p = 0.014$	$r = 0.518$ $p = 0.019$
Weighted Throughput Score	$r = 0.528$ $p = 0.017$	$r = 0.584$ $p = 0.007$	$r = 0.581$ $p = 0.007$

Figure 1. Table showing correlation of acute NAA/Cr measurements in the left thalamus, right thalamus and average of both left and right thalami with results of different ANAM subtests conducted at the chronic time point. Statistically significant correlations ($p<0.05$) are shown in red ink.

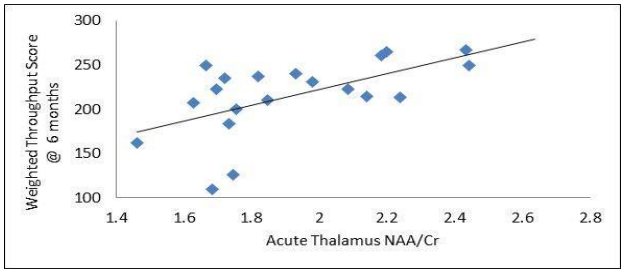

Figure 2. Scatter plot showing linear correlation ($r = 0.581$ *and* $p = 0.007$) of Weighted Throughput scores at the chronic time point with the average of acute NAA/Cr measurements in the left and right thalamus. The weighted throughput score combines results of all the ANAM subtests into a single value

Discussion: The occurrence of decreased NAA/Cr levels observed herein agrees well with previously reported changes in NAA/Cr following TBI[2]. Trauma induced disruption of neuronal integrity gives rise to mitochondrial dysfunction and a resulting compromise in NAA synthesis[5]. The recovery of NAA towards normative levels in the sub-acute and chronic time points is also well documented in literature[1,5] suggesting that the irreversible loss of neurons is not the only reason for NAA depression but also the aforementioned mitochondrial dysfunction experienced by disrupted yet viable cells that are able to recover after trauma. The lowering of Ch/Cr levels observed with the mTBI group (in spite of concomitant decreases in Cr concentration levels) across most time points does not particularly conform to findings in previous mTBI studies. However, significantly lowered levels of Ch have been observed in experimental models of TBI at early time points[1] and stroke[6]. Our observation of reduced choline suggests trauma induced depletion of the plasma membrane, as it is well documented that Ch is a marker of membrane metabolism[7]. The "mild" nature of injury however might not require the desperate inflammatory response that often leads to glial cell proliferation and the resulting popularly-observed Ch increase. Cr levels have been shown to deviate from normative values in the acute stages of TBI[8]. In the current study, a significant decrease in Cr was observed at acute time points in regions coinciding with the *centrum semiovale* for the mTBI group when compared to healthy controls. This decrease in Cr could possibly be due to reduced cerebral perfusion well documented in TBI[9] leading to an inadequate supply of Cr to the brain parenchyma. The occurrence of consistently strong positive correlations between NAA/Cr values in regions coinciding with the thalamus and NPT scores suggests that the cellular integrity of the thalamic nuclei plays a strong role in overall brain function. Previous evidence has suggested that the thalamus has connections to the entire cerebral cortex[10]. The strong correlation between thalamic NAA/Cr at the acute stage and NPT data at 6 months suggests that thalamic changes could prove to be a valuable imaging marker for mild TBI patients.

References: **[1]** Schumann MU, et al. J Neurotrauma. 2003 Aug;20(8):725-43 **[2]** Cecil KM, et al. J Neurosurg. 1998 May;88(5):795-801. **[3]** Kane RL, et al. Arch Clin Neuropsychol. 2007 Feb;22 Suppl 1:S115-26. **[4]** Provencher SW. Magn Reson Med. 1993 Dec;30(6):672-9 **[5]** De Stefano N, et al. Magn ResonMed. 1995 Nov;34(5):721-7 **[6]** Malisza KL, et al. Biochem Cell Biol. 1998;76(2-3):487-961998 **[7]** MR Garnett, et al. Brain. 2000 Jul;123 (Pt 7):1403-9 **[8]** Lin AP, et al. Brain Imaging Behav. 2012 Jun;6(2):208-23 **[9]** Ragan DK, et al. J Cereb Blood Flow Metab. 2012 Sep 12. doi: 10.1038/jcbfm.2012 **[10]** Behrens TE, et al. Nat Neurosci. 2003 Jul;6(7):750-7