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Original Articles

A Longitudinal Proton Magnetic Resonance Spectroscopy Study of Mild Traumatic Brain Injury

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Abstract

Despite the prevalence and impact of mild traumatic brain injury (mTBI), common clinical assessment methods for mTBI have insufficient sensitivity and specificity. Moreover, few researchers have attempted to document underlying changes in physiology as a function of recovery from mTBI. Proton magnetic resonance spectroscopy (^{1}H-MRS) was used to assess neurometabolite concentrations in a supraventricular tissue slab in 30 individuals with semi-acute mTBI, and 30 sex-, age-, and education-matched controls. No significant group differences were evident on traditional measures of attention, memory, working memory, processing speed, and executive skills, though the mTBI group reported significantly more somatic, cognitive, and emotional symptoms. At a mean of 13 days post-injury, white matter concentrations of creatine (Cre) and phosphocreatine (PCre) and the combined glutamate-glutamine signal (Glx) were elevated in the mTBI group, while gray matter concentrations of Glx were reduced. Partial normalization of these three neurometabolites and *N*-acetyl aspartate occurred in the early days post-injury, during the semi-acute period of recovery. In addition, 17 mTBI patients (57%) returned for a follow-up evaluation (mean = 120 days post-injury). A significant group×time interaction indicated recovery in the mTBI group for gray matter Glx, and trends toward recovery in white matter Cre and Glx. An estimate of premorbid intelligence predicted the magnitude of neurometabolite normalization over the follow-up interval for the mTBI group, indicating that biological factors underlying intelligence may also be associated with more rapid recovery.

Key words: creatine; mild traumatic brain injury; glutamate-glutamine signal; recovery; spectroscopy

Introduction

Traumatic brain injury (TBI) has been termed a "silent epidemic" by the Centers for Disease Control (2003), consistent with the annual incidence rates of mild TBI (mTBI), recently estimated at 503 per 100,000 (Bazarian et al., 2005), and 653 per 100,000 (Ryu et al., 2009). Commonly used clinical diagnostic procedures are insufficiently sensitive. Traditional means of neuropsychological assessment are inadequate for distinguishing mTBI from other common comorbid conditions such as post-traumatic stress disorder (Brenner et al., 2010), and substance abuse (Lange et al., 2007). Conventional computed tomography (CT) and magnetic resonance imaging (MRI) evaluations conducted in the emergency department setting are only able to detect abnormalities in 20% of patients (Smits et al., 2008). Therefore, developing neuroimaging methods capable of identifying the abnormalities occurring post-mTBI has long been recognized as critical for accurate diagnosis, and to advance our conceptual understanding of the underlying pathophysiology (Bigler, 2008).

An emerging neuroimaging literature now offers hope that the silent lesion of mTBI, which should be evident during the semi-acute stage (e.g., the first 3–4 weeks) of injury, can be accurately detected and described from multiple perspectives. Abnormalities of the diffusion properties of white matter have been reported by several research teams, though there is substantial disagreement as to the nature and even the direction of these findings (Inglese et al., 2005; Lipton et al., 2009; Mayer et al., 2010; Wilde et al., 2008). Abnormal blood oxygen level dependent (BOLD) signals assessed with functional MRI (fMRI) during cognitive processing have been identified in mTBI patients compared to healthy controls (Mayer et al., 2009; McAllister et al., 2006; Smits et al., 2009), as

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have altered patterns of regional connectivity (Mayer et al., in press). Another neuroimaging method that holds great promise in mTBI research is proton magnetic resonance spectroscopy (^{1}H-MRS).

^{1}H-MRS, which has been predominantly used in studies of moderate to severe TBI, provides an assessment of several important neurometabolites that are likely altered by brain trauma. The *N*-acetyl aspartate (NAA) signal, often measured as the sum of NAA and the weaker and poorly resolved *N*-acetyl-aspartyl glutamate (NAAG) signal, is widely construed as a marker of neuronal integrity (Moffett et al., 2007), and its concentration is reduced in moderate and severe TBI (Friedman et al., 1999). The choline (Cho) signal, comprised largely of signals from the membrane lipid metabolites phosphorylcholine and glycerol phosphorylcholine, is typically elevated in TBI due to membrane breakdown and/or repair (Brooks et al., 2000). A third commonly measured spectroscopic signal reflects concentrations of both creatine (Cre) and phosphocreatine (PCre; Andres et al., 2008). These molecules are in equilibrium with the cell's main energy currency, adenosine triphosphate (ATP), but at clinical field strengths their ^{1}H-MRS signals are not well resolved. Therefore, though the Cre:PCre ratio may change with energy status, the combined Cre-PCre signal has been assumed to be constant, and has often been used as the denominator in ratios expressing relative NAA or Cho concentrations. However, recent studies (Hattingen et al., 2008; Inglese et al., 2003; Munoz et al., 2008), including our previous report on mTBI (Gasparovic et al., 2009), call into question the assumption of unchanged Cre concentration in disease states, rendering interpretation of ratio scores problematic.

Govindaraju and colleagues (Govindaraju et al., 2004) reported the first ^{1}H-MRS study to specifically focus on semi-acute mTBI, using whole-brain spectroscopic imaging (^{1}H-MRSI). Fourteen patients within 1 month of injury were compared with 13 controls. NAA/Cre was significantly reduced in 2 of 25 regions examined (parietal white matter regions), while Cho/Cre was increased in two other regions, both in the occipital lobes. Vagnozzi and colleagues (Vagnozzi et al., 2008) studied 10 non-professional athletes who sustained mTBI during a sporting event and compared them to five healthy controls. NAA:Cre and Cho:Cre ratios were obtained from left and right hemisphere frontal lobe white matter with single-voxel ^{1}H-MRS at 3, 15, and 30 days post-injury. Reductions in NAA:Cre were noted in the mTBI group in the first few days post-injury, with normalization occurring by day 30. No group differences were evident in the Cho:Cre ratio.

A second comprehensive analysis of whole-brain NAA, Cho, and Cre abnormalities was recently conducted with ^{1}H-MRSI on 29 mild to moderate mTBI patients (mean of 29 days post-injury, range 7–100 days), and 52 controls (Govind et al., 2010). The TBI sample was broken down into two groups on the basis of lesion volume, rather than by traditional Glasgow Coma Scale (GCS) or American College of Rehabilitation Medicine (ACRM) criteria. The less severe injury group ($n = 16$) included five individuals with GCS scores in the moderate range of injury using more traditional criteria (e.g., ACRM). This group showed reduced white matter NAA in all brain regions except the frontal and temporal lobes, increased Cho in the left frontal and temporal lobes, and increased Cre in the temporal lobes bilaterally. Gray matter NAA was significantly decreased in the right frontal and bilateral occipital lobes, gray matter Cho was significantly increased in the right temporal and left frontal and parietal lobes, and gray matter Cre was increased in the left and right temporal lobes.

Finally, our group recently evaluated neurometabolite concentrations in gray and white matter from a supraventricular slab of brain tissue using ^{1}H-MRSI in 10 semi-acute (mean = 11 days post-injury) mTBI patients, and 9 healthy controls (HC) matched for age, sex, and education (Gasparovic et al., 2009). In addition to measuring the neurometabolites described above, we assessed the combined glutamate-glutamine (Glx) signal, as these neurometabolites are likely candidates for disruption in brain injury (Hinzman et al., 2010). The mTBI group had significantly greater concentrations of white matter Cre and lower levels of gray matter Glx, with a non-significant trend noted toward elevated white matter Glx. Variation in neurometabolite concentrations was related to variation in executive function, as well as emotional distress. No group differences were evident in premorbid intellectual ability, which is an important consideration, as ^{1}H-MRS concentrations are often related to variations in cognitive function (Jung et al., 1999, 2009). Results from this study highlighted the differing patterns of changes in gray versus white matter metabolites following mTBI, and raised the possibility that mTBI may be characterized by a set of somewhat different neurometabolic changes than those seen in moderate to severe TBI (Andres et al., 2008; Brooks et al., 2000; Friedman et al., 1999).

As evidenced by the studies referenced above, methodological differences (e.g., chronicity and severity of injury, ^{1}H-MRS acquisition method and region of interest, inclusion criteria, nature of the control sample, and the use of ratios versus absolute measurements and relatively small sample sizes) among studies have hampered the emergence of a clear picture of the neurometabolic abnormalities and their significance in the semi-acute stage post-mTBI. Further, experts in the field stress the need for large-scale longitudinal neuroimaging studies that are capable of capturing the dynamic nature of mTBI over the 3- to 6-month period during which clinical recovery typically occurs (Belanger et al., 2007; McCrea et al., 2009). However, to date there has been only a single study that has prospectively examined alterations in neurometabolites in a human population (Vagnozzi et al., 2008).

The two primary goals for the current study were therefore to (1) replicate our findings regarding mTBI abnormalities in particular neurometabolite concentrations (Cre and Glx) in a larger sample, and (2) evaluate neurometabolite changes over time, through analysis of relationships between initial levels and time post-injury (i.e., cross-sectional study), and through analysis of 3- to 5-month follow-up data (i.e., longitudinal study). Given the previous findings in both mild (Govindaraju et al., 2004; Govind et al., 2010; Vagnozzi et al., 2008) and moderate to severe TBI (Brooks et al., 2000; Friedman et al., 1999), a secondary goal was to explore relationships between NAA and Cho concentrations across patients and controls.

Methods

Participants

Thirty-two patients with mTBI (18 females) and 32 HC (18 females) participated in the study. Of those, 2 mTBI patients

and 2 HC were excluded from the analyses secondary to problems with data acquisition. Thirty mTBI (17 females; 27.30 ± 9.52 years old; 12.90 ± 2.37 years of education), and 30 gender-, age-, and education-matched HC (17 females; 26.87 ± 9.24 years old; 13.42 ± 2.10 years of education) were included in the final sample (see Table 1 for patient characteristics). All mTBI patients were recruited from the University of New Mexico Hospital and Presbyterian Health Services Emergency departments.

Inclusion criteria for the mTBI group were based on the American Congress of Rehabilitation Medicine criteria, including a Glasgow Coma Scale score of 13–15 (at first contact with medical staff), post-traumatic amnesia (if present) limited to 24 h, and loss of consciousness (if present) limited to 30 min in duration. Mild TBI and HC participants were excluded if there was a prior history of neurological disease, major psychiatric disturbance, history of additional closed head injury with more than 5 min loss of consciousness, attention-deficit/hyperactivity disorder, learning disorder, or a history of substance/alcohol abuse or dependence. The Institutional Review Board at the University of New Mexico approved all study protocols, and all participants provided written informed consent prior to enrollment.

Table 1. Clinical Characteristics of the mTBI Group

Age	*Sex*	*Days post-injury*	*Etiology*	*AAN*	*CT/MRI findings*	*Visit 2*
23	F	21	Fall	3		Y
19	M	14	MVA	3		NE
23	F	5	Assault	1		Y
20	F	11	MVA	3		Y
25	F	20	MVA	3		Y
41	M	10	Fall	2		NE
33	M	20	Sports injury	3		NE
18	M	16	Fall	2		NE
19	F	18	Fall	3		N
23	M	17	Fall	3		N
49	M	7	Falling object	3		Y
21	F	14	MVA	3		Y
22	F	6	Fall	3		N
23	M	11	Collision	3		Y
20	F	14	MVA	3	Right sylvian fissure subarachnoid hemorrhage (CT)	Y
48	F	19	Fall	2		Y
27	F	2	Assault	2		Y
18	M	16	Sports injury	1		NE
24	M	13	Fall	3	Right supratentorial subdural hematoma (CT)	Y
50	F	7	MVA	3		Y
41	F	17	Fall	3		Y
37	F	3	Fall	3		Y
24	M	10	Fall	3		N
21	F	20	Fall	3		N
28	F	11	Assault	1		N
24	F	7	Assault	1		Y
22	M	27	Collision/ fall	3		Y
30	M	16	Falling object	3		N
23	F	11	Assault	2		N
23	M	11	Fall	1		Y

For visit 2, Y = returned, N = not returned, and NE = not eligible at the time of this report.

AAN, American Academy of Neurology rating; CT, computed tomography; MRI, magnetic resonance imaging; MVA, motor vehicle accident; mTBI, mild traumatic brain injury.

Four of the mTBI subjects were being prescribed narcotic medications for pain related to the accident at the time of their visit. One patient was taking a prescribed antidepressant (venlafaxine), and one was taking a prescribed anti-anxiety medication (alprazolam). All mTBI patients were evaluated clinically (mean day post-injury $= 13.14 \pm 5.45$), and with brain imaging (mean day post-injury $= 13.13 \pm 5.90$) within 21 days of injury. The maximum time allowed between the clinical and imaging sessions was 1 week, though it was typically much shorter (mean days between sessions $= 1.54 \pm 1.91$ for mTBI patients). Injury severity among subjects in the mTBI group was also rated according to criteria from the American Academy of Neurology, with 17% ($n = 5$) experiencing a grade 1 concussion, 17% ($n = 5$) experiencing a grade 2 concussion, and 66% ($n = 20$) experiencing a grade 3 concussion. One mTBI patient and one HC were not able to complete neuropsychological testing due to scheduling difficulties during their first visit.

Seventeen mTBI patients (12 females; 29.41 ± 10.89 years old; 13.65 ± 2.76 years of education), and 22 HC (15 females; 28.36 ± 9.53 years old; 13.73 ± 2.33 years of education) returned for a follow-up visit approximately 3–5 months (mean days post-injury $= 120.47 \pm 13.30$ for mTBI patients) after their initial screening. Reasons for visit 2 attrition included the participant's inability to schedule a second visit (3 mTBI and 2 HC), inability to contact participants (5 mTBI and 1 HC), and problems with data acquisition (1 HC). Five mTBI and 4 HC were not eligible for follow-up at the time of the current summary.

Neuroimaging and ^{1}H-MRSI acquisition

A subset of mTBI patients (20/30) had CT scans ordered as part of their initial visit to the emergency department. MRI and ^{1}H-MRSI experiments were performed on a Siemens 3T TrioTim scanner. Foam padding and paper tape were used to restrict motion within the scanner. High-resolution T1-weighted anatomical images were acquired with a 5-echo multi-echo MPRAGE sequence [TE (echo time) = 1.64, 3.5, 5.36, 7.22, and 9.08 msec; TR (repetition time) = 2.53 sec; TI (inversion time) = 1.2 sec; 7° flip angle; number of excitations (NEX) = 1; slice thickness = 1 mm; FOV (field of view) = 256 mm; resolution = $256 \times {}^\circ 256$]. T2-weighted images were collected with a fast spin echo sequence [TE = 77.0 msec; TR = 1.55 sec; 152° flip angle; NEX = 1; slice thickness = 1.5 mm; FOV = 220 mm; matrix = 192×192; voxel size = $1.15 \times 1.15 \times 1.5$ mm^3].

^{1}H-MRSI was performed with a phase-encoded version of a point-resolved spectroscopy sequence (PRESS), both with and without water presaturation [TE = 40 msec; TR = 1500 sec; slice thickness = 15 mm; FOV = 220×220 mm; circular k-space

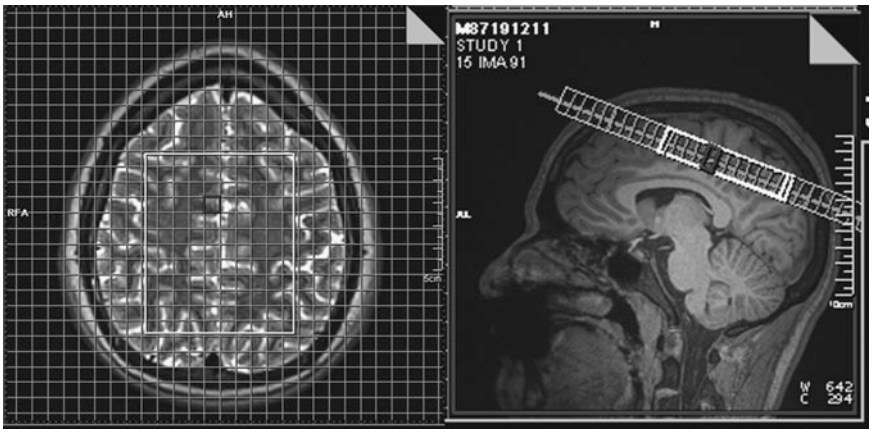

FIG. 1. Location of the supraventricular axial proton magnetic resonance spectroscopy imaging (^{1}H-MRSI) slice in the sagittal and axial planes.

sampling (radius = 24); total scan time = 582 sec]. The nominal voxel size was $6.25\times6.25\times15\,mm^3$ after zero-filling in k-space to 32×32 samples. The ^{1}H-MRSI volume of interest was selected with strong saturation bands to reduce chemical shift artifacts, and was prescribed with the T2 image to lie 1 cm above the lateral ventricles, and parallel to the anterior-posterior commissure axis, and included portions of the anterior cingulate and medial frontal gyri, and the superior longitudinal fasciculus (Fig. 1). To further minimize the chemical shift artifact, the transmitter was set to the frequency of the NAA methyl peak during the acquisition of the metabolite spectra, and to the frequency of the water peak during the acquisition of the unsuppressed water spectra. Additionally, the outermost rows and columns of the volume of interest were excluded from analysis.

^{1}H-MRSI data processing

After zero-filling to 32×32 points in k-space, applying a Hamming filter with a 50% window width, and 2D spatial Fourier transformation, the time domain ^{1}H-MRSI data were analyzed using LCModel (Provencher, 1993) from 4.2–1.8 ppm, using tissue water as a concentration reference. The Cramer-Rao lower bounds of the fit to the peak of interest output by LCModel were used as a criterion to exclude poor-quality data (>20% for a metabolite of interest) from the final analysis. Independent spectroscopic estimates of glutamate and glutamine are less reliable than the combined fit (Glx signal), so we have focused on the latter. In addition, we report the combined fits of N-acetyl aspartate and N-acetyl aspartyl glutamate (abbreviated here as NAA), as well as creatine plus phosphocreatine (abbreviated here as Cre). Subsequent processing of the derived metabolite values has been previously described (Gasparovic et al., 2009). Briefly, concentration values were corrected for partial volume effects using gray matter (GM), white matter (WM), and cerebrospinal fluid maps generated by segmenting the T1-weighted images with SPM5 (Ashburner and Friston, 2005), and corrected for T1 and T2 relaxation effects. Estimates of metabolite concentrations in either GM or WM were generated by linear regression of the metabolite concentration in each, against the normalized GM fraction of the voxel, and extrapolating to a GM fraction of one (pure GM), or zero (pure WM).

Individual distributions of the three main neurometabolites of interest for each group were first evaluated for visit 1 outliers using the general boxplot method in SPSS. A total of seven participants (3.8% of all data) were identified as outliers (one mTBI patient for WM Cre and one mTBI patient for GM Glx; two HC for GM Glx and three HC for WM Glx), and their data were subsequently deleted from the specific metabolite analyses. Individual outlier analyses using an identical method were also performed for both NAA and Cho during the supplementary analyses.

Neuropsychological assessment

Similarly to previous work (Mayer et al., 2009), the participants received a comprehensive battery of neuropsychological tests to provide assessments of attention, working memory, executive function, memory, and processing speed. In addition, all participants also completed several questionnaires regarding current emotional status (Beck Depression Inventory, 2nd Edition [BDI]; State-Trait Anxiety Inventory [STAI]), and levels of cognitive, emotional, and somatic complaints (Neurobehavioral Symptom Inventory [NBI]). The relevant test scores within each cognitive domain were converted to T-scores using published age-specific norms, and averaged to provide overall composite scores (Gasparovic et al., 2009). Likewise, the depression (BDI) and anxiety (STAI) measures were combined to form an "emotional distress" composite. The Wechsler Test of Adult Reading (WTAR) served as an estimate of overall premorbid cognitive functioning. The Test of Memory and Malingering (TOMM) was used to measure participant effort. Finally, handedness was assessed with the Edinburgh Handedness Inventory (Oldfield, 1971).

Results

Table 2 provides descriptive statistics on the mTBI sample ($n = 30$), and the HC sample ($n = 30$), for key demographic and clinical data. As expected given our matching procedure, no group differences were evident for age, sex, or education. Groups were not specifically matched on ethnicity, though a chi square analysis revealed no significant group differences in ethnic composition. In addition, there were no differences

Table 2. Summary of Participant Demographic Characteristics, Neuropsychological Performance, and Symptom Severity at Visit 1

	mTBI		*HC*			
	Mean	*SD*	*Mean*	*SD*	p *Value*	*Cohen's* d
Demographics						
Age	27.30	9.52	26.87	9.24	$p > 0.10$	0.05
Education	12.90	2.37	13.43	2.10	$p > 0.10$	−0.24
HQ	85.16	27.65	72.87	50.32	$p > 0.10$	0.31
Neuropsychology						
TOMM	55.07	4.31	51.48	11.95	$p > 0.10$	0.41
WTAR	50.11	8.68	54.67	7.74	$p < 0.05$	−0.56
Attention[a]	51.78	4.39	53.60	6.26	$p > 0.10$	−0.34
Working memory[a]	51.47	6.16	51.88	7.10	$p > 0.10$	−0.06
Memory[a]	51.72	8.01	51.36	6.36	$p > 0.10$	0.05
Processing speed[a]	46.43	5.82	47.55	6.45	$p > 0.10$	−0.18
Executive functioning[a]	47.79	5.43	48.75	4.95	$p > 0.10$	−0.18
Symptom severity						
NB–somatic[a]	6.95	6.02	1.78	2.31	$p < 0.001$	1.16
NB–cognitive[a]	4.09	3.02	2.05	2.61	$p < 0.05$	0.74
NB–emotional[a]	6.78	4.8	3.11	3.63	$p < 0.01$	0.45
Emotional[a]	48.62	7.63	43.36	5.96	$p < 0.01$	0.48

[a]Means, standard deviations, and effect sizes for neuropsychological indices and self-report measures are corrected for WTAR as covariate at 51.90.

HQ, handedness quotient; TOMM, Test of Memory and Malingering; WTAR, Wechsler Test of Adult Reading; NB, Neurobehavioral Symptom Inventory; mTBI, mild traumatic brain injury; SD, standard deviation; HC, healthy control.

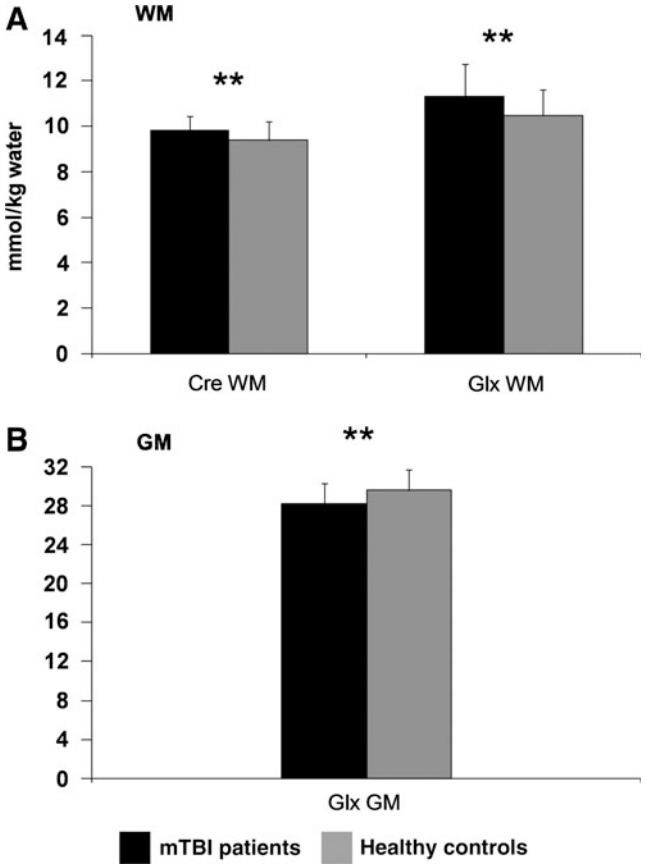

FIG. 2. Graphical depiction of mean metabolite levels at visit 1 for patients with mild traumatic brain injury (mTBI; black bars) compared to healthy controls (gray bars). Error bars are equivalent to the standard deviation, and all values are corrected for premorbid intelligence. (**A**) Graph illustrating creatine (Cre) and glutamate-glutamine (Glx) in white matter (WM). (**B**) Graph depicting Glx in gray matter (GM). Units for the graphs are represented in millimoles per kilogram (mmol/kg) water. Two asterisks (**) denote a significant result ($p < 0.05$).

between the groups on measures of effort (TOMM; $p > 0.10$). However, HC scored significantly higher on an estimate of overall premorbid intellectual ability (WTAR; $t_{56} = 2.07$, $p = 0.043$). Hence, in most subsequent analyses the WTAR score was treated as a covariate.

Two multivariate analyses of covariance (MANCOVAs) with WTAR as a covariate were used to evaluate group differences on both objective measures of cognitive functioning and self-report composite variables (the three NBI variables and the emotional distress composite). As expected, the effect of WTAR was significant ($F_{5,51} = 9.50$, $p < 0.001$) for the MANCOVA examining differences in attention, working memory, executive functioning, memory, and processing speed; however, the multivariate effect of group was not ($F_{5,51} = 0.48$, $p = 0.79$). For the four self-report measures the multivariate effect of group was significant ($F_{4,52} = 5.13$, $p = 0.001$), and significant univariate effects were noted for NBI somatic ($F_{1,55} = 19.00$, $p < 0.001$), cognitive ($F_{1,55} = 7.66$, $p = 0.008$), and emotional ($F_{1.55} = 14.77$, $p < 0.001$) complaints, as well as greater emotional distress as measured by the BDI and STAI ($F_{1,55} = 7.82$, $p = 0.007$).

Structural imaging data

T1- and T2-weighted images were deemed to be free of trauma-related pathology (i.e., non-complicated mTBI patients) by a neuroradiologist blinded to diagnosis. However, 2/20 mTBI patients had positive findings (a right supratentorial subdural hematoma and a small subarachnoid hemorrhage within the right sylvian fissure) on their CT scans during their initial assessment in the emergency department.

^{1}H-MRSI data: Visit 1

Pearson correlations indicated that there was no significant relationship ($p > 0.10$) between the three main metabolites of interest (WM Cre, WM Glx, and GM Glx) for HC at visit 1, although a positive relationship existed between WM Cre and Glx for mTBI subjects ($r_{27} = 0.56$, $p = 0.002$). Three ANCOVAs (Fig. 2) were conducted to evaluate our a priori hypothesis of higher WM Cre and Glx, and lower GM Glx. Our results supported these hypotheses, as metabolite levels were elevated for both WM Cre ($F_{1,56} = 5.23$, $p = 0.026$, Cohen's $d = 0.64$), and Glx ($F_{1,54} = 5.12$, $p = 0.028$, $d = 0.64$), for mTBI patients relative to HC, and GM Glx levels were lower for mTBI patients relative to controls ($F_{1,54} = 5.96$, $p = 0.018$, $d = -0.68$). Supplementary ANCOVAs were performed to

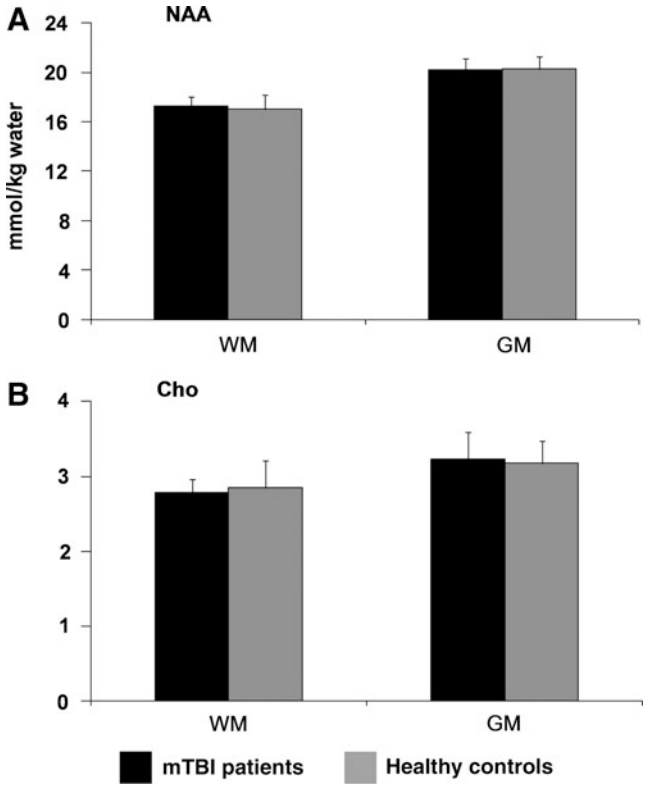

FIG. 3. Graphical illustration of mean white matter (WM) and gray matter (GM) *N*-acetyl aspartate (**A**, NAA) and choline (**B**, Cho) levels at visit 1 for patients with mild traumatic brain injury (mTBI; black bars) compared to healthy controls (gray bars). Error bars are equivalent to the standard deviation and all values are corrected for premorbid intelligence. Units for the graphs are represented in millimoles per kilogram (mmol/kg) water.

examine group-wise differences in NAA and Cho in both WM and GM (Fig. 3), but the main effect of group was not significant ($p > 0.10$) for any of the analyses, and the range of effect sizes was relatively small (all absolute $d \leq 0.24$).

Our next series of analyses examined potential effects of recovery in the cross-sectional sample of mTBI patients (visit 1 data) by correlating metabolite levels with days post-injury. Results indicated non-significant trends for WM Cre ($r_{27} = -0.35$, $p = 0.06$), WM Glx ($r_{28} = -0.34$, $p = 0.06$), and for GM Glx ($r_{27} = 0.34$, $p = 0.07$). Of note, the directions of correlations were consistent with the group differences across all metabolites (i.e., WM Cre was elevated following mTBI and negatively correlated with days post-injury), suggesting a partial normalization as a function of days post-injury. In addition, a significant correlation was observed between GM NAA and days post-injury ($r_{27} = 0.39$, $p = 0.04$), with the direction of correlation also consistent with previous reports of reduced NAA. To evaluate the overall significance of these relationships a regression was conducted to determine how well WM Cre, WM Glx, GM Glx, and GM NAA could predict days post-injury. The overall model was significant ($F_{4,22} = 2.91$, $p = 0.05$), with the four indices accounting for approximately 23% of the total variance (adjusted R^2).

Next, a series of multiple regressions were used to investigate whether the composite neuropsychological indices or the self-report measures (independent variables) predicted metabolite concentration levels (dependent variables) within the mTBI group. Our results indicated no relationship between neuropsychological performance on major cognitive domains and metabolite levels. A non-significant trend was observed between self-report symptoms and WM Glx ($F_{4,24} = 2.47$, $p = 0.071$), with somatic complaints accounting for the majority of the variance in the model ($t = 2.32$, $p = 0.03$; beta = 0.138).

Longitudinal data

Seventeen mTBI patients had returned for their follow-up visit at the time of this report. First, key demographic and clinical variables were compared to ensure that the returning patients were similar to the non-returning patients at the time of their injury (i.e., visit 1 data). Mild TBI patients who returned for their second visit were significantly more educated ($t_{23.54} = -2.29$, $p = 0.032$), reported more cognitive complaints at visit 1 ($t_{26.5} = -2.16$, $p = 0.04$), and trended non-significantly toward being more right-handed ($t_{12.94} = -1.92$, $p = 0.078$). No other significant differences ($p > 0.10$) between the samples in terms of age, estimates of premorbid intelligence, levels of effort, composite neuropsychological measures, or composite self-report variables (NBI, STAI, and BDI) were observed.

Four 2×2 (visit×group) mixed-measures ANCOVAs (with WTAR as covariate) were then performed to determine if there were any changes in the four self-report measures for the 17 mTBI patients and their 17 age-, education-, and gender-matched HC. Our results indicated that the main effect of group was significant for NBI somatic ($F_{1,30} = 18.64$, $p < 0.001$), cognitive ($F_{1,30} = 11.42$, $p = 0.002$), and emotional ($F_{1,30} = 11.50$, $p = 0.002$) complaints, with a non-significant trend being present for main effect of group on the emotional distress composite ($F_{1,30} = 4.04$, $p = 0.053$). In addition, a non-significant trend was also present for the visit by group interaction for somatic complaints ($F_{1,30} = 4.08$, $p = 0.052$), reflecting a greater reduction in somatic complaints for mTBI patients (change in score = −2.89) relative to HC (change in score = 0.18).

Similar to the data at visit 1, a positive correlation between WM Glx and WM Cre was observed in mTBI patients ($r_{15} = 0.74$, $p = 0.001$), with a non-significant trend also being present between GM Glx and WM Glx ($r_{15} = -0.47$, $p = 0.055$). The correlations between metabolites were not significant for HC ($p > 0.10$). Three 2×2 (visit×group) mixed-measures ANCOVAs were performed to determine if there were any changes in WM Cre, WM Glx, and GM Glx, as a function of recovery for the mTBI patients (i.e., a visit×group interaction). Our results indicated that the main effect of group was not significant for any of the metabolites ($p > 0.10$). However, the visit×group interaction term was significant for GM Glx ($F_{1,31} = 4.31$, $p = 0.046$), with a non-significant trend being noted for both WM Cre ($F_{1,31} = 3.14$, $p = 0.086$), and WM Glx ($F_{1,29} = 3.48$, $p = 0.072$). Results comparing change scores (visit 2 – visit 1 data) indicated a significant effect of group for GM Glx ($F_{1,31} = 4.31$, $p = 0.046$, $d = -0.75$), with trends being present for both WM Cre ($F_{1,31} = 3.14$, $p = 0.086$, $d = 0.64$), and WM Glx ($F_{1,29} = 3.48$, $p = 0.072$, $d = 0.70$). All results were

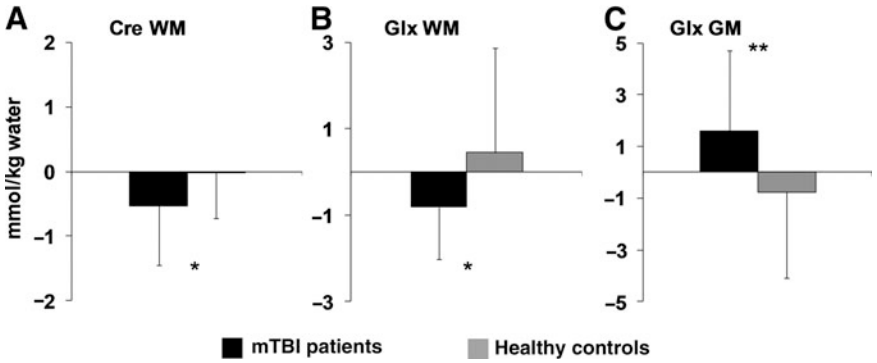

FIG. 4. Graphical representation of mean metabolite change scores (visit 2 – visit 1) for patients with mild traumatic brain injury (mTBI; black bars) compared to healthy controls (gray bars). Error bars are equivalent to the standard deviation and all values are corrected for premorbid intelligence. (**A**) Graph depicting creatine (Cre) in white matter (WM). (**B**) Graph depicting glutamate-glutamine (Glx) in WM. (**C**) Graph depicting Glx in gray matter (GM). Units for the graphs are represented in changes in millimoles per kilogram (mmol/kg) water. A single asterisk (*) denotes a statistical trend, ($.05 \leq p < .10$) whereas two asterisk (**) denote a significant effect ($p < .05$).

consistent with a model of recovery, with mTBI patient WM Cre and Glx change scores decreased, and Glx GM scores increased relative to the control group (Fig. 4).

In addition, these ANCOVAs revealed significant interactions of time×premorbid intelligence (WTAR) for measures of WM Glx ($F_{1,29} = 5.53$, $p = 0.026$), and GM Glx ($F_{1,30} = 10.53$, $p = 0.003$), but not for Cre WM ($F_{1,31} = 2.60$, $p = 0.12$). A series of follow-up analyses correlated estimates of premorbid intelligence with changes in metabolite levels separately for both groups. The results indicated a significant positive relationship between premorbid intelligence and change in GM Glx for mTBI patients ($r_{15} = 0.55$, $p = 0.023$), with a non-significant trend being present for HC ($r_{15} = 0.46$, $p = 0.064$). In contrast, intelligence was negatively correlated with changes in WM Glx ($r_{15} = -0.77$, $p < 0$ .001) only for mTBI patients (HC $p > 0.10$). The WM Glx results suggested a possible moderating effect of group; therefore WM Cre levels were also compared. Similarly to WM Glx, a negative correlation was seen between intelligence and change scores in WM Cre for mTBI patients ($r_{15} = -0.59$, $p = 0.013$), but not HC ($p > 0.10$).

The relationship between metabolite changes and reductions in symptom complaints is an important issue, though we have limited power for such an analysis. To provide preliminary data we obtained partial correlations between changes in concentrations of the three metabolites of interest and total NBI symptom complaints at follow-up, controlling for WTAR score and total NBI symptom score at the initial assessment. These partial correlations were $r_{13} = -0.50$ ($p = 0.058$) for WM Cre change, $r_{13} = -0.42$ ($p > 0.10$) for WM Glx change, and $r_{13} = -0.01$ ($p > 0.10$) for GM Glx change.

Finally, we evaluated the basic statistical properties for the three main neurometabolites of interest. The coefficients of variation were very good for the full sample of HC at visit 1 for WM Cre (.088), WM Glx (.106), and GM Glx (.071). Next, we calculated the intra-class correlation (ICC) coefficients of WM Cre, WM Glx, and GM Glx, for the full cohort of returning healthy controls. Results indicated that WM Cre had very good reliability ($ICC_{21} = 0.51$) relative to both WM Glx ($ICC_{18} = -0.03$) and GM Glx ($ICC_{20} = -0.17$).

Discussion

The current results, augmented by our prior report (Gasparovic et al., 2009), indicate that neurometabolite concentrations are systematically altered by mTBI, and that these changes normalize over a 3- to 5-month interval following injury. The specific nature of the neurometabolic effects of mTBI differed across gray and white matter, with the latter being more affected. Comparison of current results with previous studies of moderate to severe TBI suggests a somewhat different pattern of metabolic abnormalities, one that implicates more subtle alterations in energy metabolism (Cre), and cell signaling (Glx), in mTBI compared to a combination of more pronounced metabolic and structural abnormalities (NAA and Cho) in more severe injuries. Thus, mTBI may not be simply a more subtle version of severe brain injury. The importance of these neuroimaging results is highlighted by the fact that group differences on objective cognitive measures (neuropsychological testing) were not evident at either injury time point. Though other studies have found persisting neuropsychological deficits after mTBI (Konrad et al., 2010), our results suggest that conventional neuropsychological assessment has less-than-optimal sensitivity to mTBI. In an overlapping sample we have previously reported behavioral deficits in disengaging and reorienting auditory attention (Mayer et al., 2009) associated with mTBI, suggesting the potential utility of these types of cognitive measures.

Current results indicated an increase in absolute Cre concentrations in white matter. Govind and colleagues (Govind et al., 2010) is the only other group to investigate absolute Cre concentrations, reporting increased Cre bilaterally for gray and white matter in the temporal lobe, regions we did not interrogate. In addition, their cohort of milder TBI patients (TBI II group) was more severely injured than our mTBI sample, and was studied at a more chronic time point. Current findings of increased white matter Cre are consistent with an upregulation of white matter metabolic activity (Gasparovic et al., 2009), and structural abnormalities of white matter, in a subset of the current participants (Mayer et al., 2010). Greater fractional anisotropy was observed in several

left hemisphere tracts and the corpus callosum as a result of reduced radial diffusivity, which may have resulted from an alteration in the ratio of intracellular and extracellular water (cytotoxic edema). Therefore, increased total Cre (Cre and PCre) in WM may support a larger pool of high-energy phosphates (PCre and ATP), facilitating restoration of ionic balance through upregulation of membrane pumps and other processes necessary for cellular repair. This interpretation is consistent with preliminary evidence that dietary supplementation of Cre can facilitate recovery of function in TBI (Sakellaris et al., 2006, 2008; Sullivan et al., 2000).

Our current and previous (Gasparovic et al., 2009) work is the first to examine alterations in Glx during the semi-acute phase of mTBI. Interpretation of the Glx results is complicated by fact that Glx reflects the sum of glutamate and glutamine, neurometabolites linked not only by the synaptic glutamate cycle, but also in multiple metabolic pathways (McKenna, 2007). In the current combined sample, we estimate based on the glutamate measurements that 76% of the gray matter Glx signal and 88% of the white matter Glx signal represents glutamate, and that gray matter levels of Glx are approximately 2.65 times greater than white matter levels. In gray matter, glutamate serves as an excitatory neurotransmitter at approximately 90% of all synapses, and local glutamate flux is linked to local brain activity (Hyder et al., 2006; Magistretti and Pellerin, 1999). Interestingly, high-frequency transcranial magnetic stimulation, which has an excitatory effect, increases local Glx concentrations (Michael et al., 2003). Thus our observation of reduced gray matter Glx in mTBI suggests reduced neural activity, perhaps also contributing to the reduction in the blood oxygen level response that has been observed in previous studies of attentional disengagement (Mayer et al., 2009).

The role of glutamate in white matter is less well understood. Glutamate receptors are found on oligodendrocytes, and glutamate is released from vesicles on axons during the propagation of action potentials (Kukley et al., 2007). This signaling system may serve to coordinate trophic support (Nave, 2010) and ongoing myelination (Bakiri et al., 2009) from oligodendrocytes, activities that might need to be upregulated following the white matter abnormalities evident in our mTBI sample using DTI (Mayer et al., 2010). These physiological activities represent a significant metabolic burden (Kukley et al., 2007), which would perhaps be aided by upregulation of Cre. In this regard it is interesting to note the high correlation of white matter Cre and white matter Glx in the mTBI sample, a relationship absent in the healthy control sample.

The direct impact of the rather modest metabolite changes observed in this study on neurotransmitter activity or energy utilization is uncertain. In part this reflects ambiguity in interpretation of the spectroscopic signals. The Glx measure does not distinguish between Glu, the brain's major excitatory neurotransmitter, and Gln, which is metabolically coupled to Glu at glutamatergic synapses (Hyder et al., 2006; Magistretti and Pellerin, 1999). Nor does MRS distinguish between separate cellular or subcellular pools of metabolites (e.g., glial, neuronal, cytosolic, synaptic, or vesicular). Finally, the metabolic changes reported here reflect the average change across a large number of gray or white matter voxels within the spectroscopic region of interest. Interpretation of Cre is similarly ambiguous, as the Cre signal measured by ^{1}H-MRS at 3T arises from both Cre and PCre in both glial and neuronal compartments. These results suggest that ^{13}C-enhanced MRS studies of animal models may be needed to more quantitatively elucidate the relationship between neurotransmission (glutamate-glutamine cycling) and energy metabolism following mTBI.

In contrast to previous work involving more moderate to severe TBI populations (Brooks et al., 2000; Friedman et al., 1999), we did not find any evidence for group differences in metabolites that have served as traditional markers of structural abnormalities. Specifically, there were no group differences between measures of NAA and Cho at visit 1, putative measures of neuronal integrity and metabolism (Moffett et al., 2007), and membrane degradation and/or repair (Brooks et al., 2000). Effect sizes were also relatively small, suggesting that null findings were not reflective of power issues. However, NAA in gray matter was positively correlated with days post-injury in the mTBI group, suggesting that this metabolite may more rapidly recover following injury. Collectively, these results suggest that mild injuries may affect energy metabolism (Cre) and cell signaling (Glx) relatively more than the structural abnormalities (NAA and Cho) that are typically found in more severe injuries. However, this interpretation is complicated by the fact that previous studies of semi-acute mTBI (Govindaraju et al., 2004; Govind et al., 2010; Vagnozzi et al., 2008) have also reported alterations in both NAA and Cho.

In comparing our results to those of similar MRS studies of mTBI (Govindaraju et al., 2004; Govind et al., 2010; Vagnozzi et al., 2008), several methodological issues are worth noting. First, as indicated by previous results (Govindaraju et al., 2004), location matters. Our supraventricular slab differs in size and tissue composition from the frontal white matter voxels studied by Vagnozzi and colleagues (Vagnozzi et al., 2008), and the whole-brain analysis conducted by Govind and associates (Govind et al., 2010). The current white matter results come from voxels most similar to regions 22–25 (Govindaraju et al., 2004), regions for which no group differences in neurometabolite ratios involving NAA, Cho, and Cre were found. Second, our mTBI patients had milder injuries than the TBI II group described by Govind and colleagues, and the NAA reduction may have been much more transient in our sample. However, the mTBI patients in other studies (Govindaraju et al., 2004;Vagnozzi et al., 2008) were similar to ours both in terms of severity and chronicity. Third, our control sample was uniquely matched for education through a yoked design, and group differences in premorbid intellectual ability were statistically controlled. NAA has been associated with higher intelligence in some regions (Jung et al., 1999, 2009), such that failing to control for any differences in premorbid intelligence may introduce a confound across populations (i.e., increased NAA as a result of intelligence in controls rather than being reduced following a mild injury). Fourth, previous studies have reported ratio measurements rather than absolute concentrations as a means for reducing metabolite variance. For NAA:Cre ratios, an increase in white matter Cre, such as we observed here, could lead to a reduced NAA:Cre ratio in the absence of an NAA effect.

Finally, the current results also provide evidence of partial normalization in metabolite levels for the mTBI group in both a cross-sectional and longitudinal sample. In the semi-acute injury phase, a cross-sectional regression analysis revealed a

pattern of normalization over days post-injury for gray and white matter Glx, white matter Cre, and gray matter NAA. The significant relationship between NAA concentrations and days post-injury in the mTBI group raises the possibility that very acute assessment of injury (e.g., the first week) might yield evidence of group differences in NAA, providing an alternative explanation for the lack of NAA differences seen in the current study. Indeed, this suggestion is consistent with previous work demonstrating a recovery in NAA within 30 days of injury (Vagnozzi et al., 2008). In addition, comparison across the approximately 4-month follow-up period in the mTBI group also suggested partial normalization that was significant for gray matter Glx, and at a trend level for white matter Cre and Glx. Our preliminary DTI analyses suggested partial normalization of fractional anisotropy and radial diffusivity over a 3- to 5-month time window (Mayer et al., 2010), similar to what was observed with ^{1}H-MRS measurements of Cre and Glx.

Collectively, these multimodal measurements provide evidence of recovery of both white matter and gray matter functioning in human mild traumatic brain injury. The clinical significance of this neurometabolic abnormality and its subsequent normalization is difficult to ascertain, as our mTBI sample did not show significant objective cognitive deficits at either time point. Also, though improvement in self-reported somatic symptoms was noted in the mTBI group as a function of recovery, the group×time interaction was not significant for cognitive complaints. However, an important mediating factor in the recovery of function may be the premorbid level of intellectual ability.

Specifically, the current results indicate that increased premorbid intellectual ability was associated with increased neurometabolic recovery for white matter Cre and Glx, as well as gray matter Glx. Several studies of more severe brain injury have found that cognitive recovery is better in individuals with greater premorbid ability (Dikmen et al., 2009; Grafman et al., 1988), and among mTBI patients higher premorbid intelligence predicts a shorter duration of post-traumatic amnesia (Dawson et al., 2007). However, to our knowledge this is the first study to demonstrate an association of greater premorbid intellectual ability with a neurobiological measure of recovery. Intelligence per se is not affected by mTBI, and neither Cre nor Glx have been linked with intelligence in previous studies. Hence, we suggest that genetic covariation may underlie the observed effect. That is, we speculate that the polygenic, additive genetic factors known to account for 50–70% of variance in intellectual functioning (Plomin and Spinath, 2004) also influence recovery of function after mTBI. This effect is consistent with the well-established finding that greater premorbid intellectual functioning is associated with a reduced incidence of Alzheimer's disease, an effect that also reflects genetic covariation (Yeo et al., in press). One perspective on the genetic sources of variance for intelligence consistent with the observed effect emphasizes the adverse additive effect of overall mutation load on intellectual ability (Furlow et al., 1997; Prokosch et al., 2005).

The apparent normalization of metabolite status cannot be seen as evidence for full recovery, for several reasons. Our MRS data were obtained in a particular part of the brain, one far removed from the most common sites of contusion. Further, functional abnormalities at a very small scale, even single neurons, may have consequences for global activity patterns (Izhikevich and Edelman, 2008). Other neuroimaging techniques may also detect different types of mTBI-induced abnormalities over different time scales (Holli et al., 2010; Metting et al., 2009).

In addition to those mentioned above, other limitations of our methodology should be considered. In contrast to other work (Govind et al., 2010), ^{1}H-MRSI results were obtained only for a very specific slab of tissue comprised of both gray and white matter, selected for its broad relevance to complex attention skills. Regional variation in the neurometabolic response to mTBI is likely, and future investigations with larger sample sizes should examine variation on a subject-to-subject basis, as the physics of brain deformation suggest that variation in impact sites and other clinical variables would affect the location of regional abnormalities (Feng et al., 2010). Second, although this is the largest ^{1}H-MRS prospective study of mTBI done to date, we nonetheless have limited statistical power for follow-up analyses. For this reason we reported effect sizes for all statistical trends, as well as significant findings.

Finally, the reliability of Glx measures over our 4-month interval was relatively low compared to Cre. However, the current Glx results represent a replication of our earlier sample (Gasparovic et al., 2009), and the coefficients of variation for all metabolites were within acceptable ranges based on previous ^{1}H-MRS findings (Govind et al., 2010). Therefore, our observed group differences in Glx suggest that injury-related processes likely outweigh the normal variation that is observed in this metabolite in healthy individuals. Moreover, it is possible that fluctuations in Glx over the 4-month period in our HC may be a result of the dynamic nature of glutamate and glutamine levels under normal physiological conditions. Although vesicular and synaptic glutamate is a small fraction of the total glutamate pool, both gray and white matter Glx may reflect the level of signaling activity, as Glx constituents demonstrate short-term fluctuations due to pain (Gussew et al., 2010; Mullins et al., 2005), and information processing load (Mangia et al., 2007). To further address this issue, additional reliability data were collected on 21 healthy controls over a one-half-hour interval. The results indicated intra-class correlation coefficients of .82 (WM Cre), .45 (WM Glx), and .65 (GM Glx), suggesting that reliability was much improved at this shorter time frame, consistent with the concept of physiological flux in Glx signals over longer time periods.

In summary, our current and previous (Gasparovic et al., 2009) results suggest that the semi-acute stage of mTBI may be associated with a different pattern of alterations in neurometabolism relative to more severe injuries. The alterations appear to be the result of increased signaling (Glx) and energy metabolism (Cre) in white matter, and concurrent decreased signaling in gray matter (Glx). Importantly, convergent evidence of normalization was observed across all three affected metabolites both in the cross-sectional and longitudinal data, suggesting that these disruptions may be temporary in nature.

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Author Disclosure Statement

No competing financial interests exist.

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