

# Metabolic and Cognitive Response to Human Traumatic Brain Injury: A Quantitative Proton Magnetic Resonance Study

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## ABSTRACT

Proton magnetic resonance spectroscopy (<sup>1</sup>H-MRS) offers a unique insight into brain cellular metabolism following traumatic brain injury (TBI). The aim of the present study was to assess change in neurometabolite markers of brain injury during the recovery period following TBI. We studied 19 TBI patients at 1.5, 3, and 6 months postinjury and 28 controls. We used <sup>1</sup>H-MRS to quantify *N*-acetylaspartate (NAA), creatine (Cre), choline (Cho), and myoinositol (mIns) in occipitoparietal gray matter (GM) and white matter (WM) remote from the primary injury focus. Neuropsychological testing quantified cognitive impairment and recovery. At 1.5 months, we found cognitive impairment (mean *z* score = -1.36 vs. 0.18, *p* < 0.01), lower NAA (GM: 12.42 mM vs. 13.03, *p* = 0.01; WM: 11.75 vs. 12.81, *p* < 0.01), and elevated Cho (GM: 1.51 vs. 1.25, *p* < 0.01; WM: 1.98 vs. 1.79, *p* < 0.01) in TBI patients compared with controls. GM NAA at 1.5 months predicted cognitive function at outcome (6 months postinjury; *r* = 0.63, *p* = 0.04). GM NAA continued to fall by 0.46 mM between 1.5 and 3 months (*p* = 0.02) indicating continuing neuronal loss, metabolic dysfunction, or both. Between 3 and 6 months, WM NAA increased by 0.55 mM (*p* = 0.06) suggesting metabolic recovery. Patients with poorer outcomes had elevated mean GM Cho at 3 months postinjury, suggesting active inflammation, as compared to patients with better outcomes (*p* = 0.002). <sup>1</sup>H-MRS offers a noninvasive approach to assessing neuronal injury and inflammation following TBI, and may provide unique data for patient management and assessment of therapeutic efficacy.

**Key words:** inflammation; *N*-acetylaspartate; neurochemistry; neuropsychological testing; proton magnetic resonance spectroscopy; traumatic brain injury

## INTRODUCTION

**T**RAUMATIC BRAIN INJURY (TBI) from closed head trauma can induce both immediate primary injury as manifested by contusion, hemorrhage, and necrosis as well as more indolent, but progressive secondary injury, including axotomy, ischemia, edema, metabolic depres-

sion, neurochemical derangements, reactive glial changes, and progressive degeneration of brain parenchyma (Chesnut, 1993). Postmortem studies have shown that this secondary injury continues for as long as 100 days after TBI (Maxwell, 1997; Gentlemen et al., 1999). The nature of these secondary events, although critical to outcome, is not well defined. However, they

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present a potential opportunity for intervention during the resolution phase of head trauma. Indeed, clinical trials of pharmaceutical agents intended to reduce extension of injury following TBI are underway. Unfortunately, these trials are hampered by the inherent heterogeneity of TBI populations and the insensitivity of conventional techniques to distinguish different forms of neuronal injury (Doppenberg, 1997). A marker that might distinguish between irreversible and reversible brain injury would have considerable value in clinical management of patients with TBI and in the evaluation of pharmaceutical agents.

Proton magnetic resonance spectroscopy ( $^1\text{H-MRS}$ ) provides a noninvasive tool for examining brain metabolism *in situ* and is ideally suited for repeated measurements in patients recovering from TBI. Signals arise from such brain metabolites as *N*-acetylaspartate (NAA), an indicator of neuronal/axonal injury and metabolic depression, total choline (Cho), a measure of inflammation, demyelination, and membrane synthesis or repair, and myoinositol (mIns), which is associated with osmotic balance, specific metabolic pathways, and glial proliferation. NAA is reduced following human TBI indicating severe injury or metabolic depression (Cecil et al., 1998; Ross et al., 1998; Holshouser, 1997; Garnett et al., 1999; Friedman et al., 1998, 1999). We have demonstrated that the brain concentration of NAA predicts both cognitive functioning soon after TBI (Friedman et al., 1998) and long-term cognitive outcome (Friedman et al., 1999) indicating the close relationship between diffuse cellular brain injury and cognitive functioning. However, previous neurometabolite studies have focused primarily on the acute and subacute changes following TBI rather than the more indolent long-term changes which require longitudinal study.

We hypothesized that (1) considerable neurometabolite heterogeneity would exist in TBI reflecting both the heterogeneity of injury and individual functional deficits, (2) neurometabolite concentrations would change temporally, representing evolving secondary injury and/or recovery, and (3) neurometabolite measures would be potent predictors of long-term overall functional outcome. We used MRS to determine the temporal evolution of NAA, Cho, creatine (Cre), and mIns following TBI. Also, since cognitive functioning typically improves during the recovery period, we sought to determine whether alterations in neurometabolites that suggested neuronal recovery, were related to resultant behavioral outcome.

## MATERIALS AND METHODS

Nineteen patients with traumatic brain injury (mean age  $\pm$  SD = 32.1  $\pm$  13.2 years, median = 29.0 years,

range 17–65 years, 1 female) and 28 normal controls (mean age  $\pm$  SD = 26.6  $\pm$  11.1 years, median = 20.5 years, range 17–54 years; 11 female) were studied. The average age of patients and controls was not significantly different ( $p = 0.13$ ). Average duration of education was not significantly different (mean  $\pm$  SD for patients = 13.2  $\pm$  2.5 years and for controls = 13.4  $\pm$  1.6 years;  $p = 0.67$ ). Patients were recruited for study if their initial GCS was less than 12, indicating moderate to severe injury, or if significant behavioral/MR findings were demonstrated acutely. One patient whose admission GCS was 14 and who had no subsequent radiological findings was included as he had a field GCS of 8. The mean GCS of patients was 8.4 (SD = 3.4, range = 3–15). The radiological findings of 14 of these subjects have been described in detail (Friedman et al., 1999). In brief, atrophy was noted in four patients, contusion in eight, shearing injury in six, subdural hematoma in five, hemorrhage in five, unidentified hyperintensities on  $T_2$ -weighted scans in three, and fractures in two. Three patients scans revealed no abnormalities. Normal controls were recruited to be demographically similar to TBI patient population prior to injury and to yield a mean cognitive  $z$  score similar to the general population mean, that is, mean  $z$  score = 0.00. To compensate for potential bias due to gender differences, a greater percentage of men was included in the control population because the TBI group was primarily male. To further minimize potential bias, analysis of the control populations demonstrated, not unexpectedly, that neurometabolites between males and females were very similar as previously reported (Komorowski et al., 1999).

The study protocol called for initial examinations in the subacute phase of TBI (approximately 1 month after injury), followed by further examinations at 3 and 6 months. In general, the study group was compliant with this study regimen. However, due to the fragile nature of these convalescing trauma patients, not all participants received all scans and cognitive tests at the precise design date. Accordingly, to provide a design structure to accommodate the clinical needs of the patients, we divided the total of 42 examinations into three time points based on elapsed time following injury—time point 1: scan date  $\leq$  60 days (median = 38 days; range = 13–60 days;  $n = 16$ ); time point 2: 60 days < scan date < 125 days (median = 92 days; range = 66–123 days;  $n = 12$ ); and time point 3: scan date  $\geq$  125 days (median = 184 days; range = 139–320 days;  $n = 14$ ; Table 1).

Normal control subjects were studied only once since literature reports suggest that  $^1\text{H-MRS}$ -visible neurometabolite concentrations, in the age range of the current subjects, do not change appreciably over the time span of the current study (Brooks et al., 1999; Saunders et al., 1999).

TABLE 1. SUMMARY COGNITIVE Z SCORE AND METABOLITE CONCENTRATION DATA FOR TBI SUBJECTS AND NORMAL CONTROLS FOR EACH TIME POINT

	Controls, mean (SD)	TBI (1.5 months)		TBI (3 months)		TBI (6 months)	
		Mean (SD)	p value	Mean (SD)	p value	Mean (SD)	p value
Composite z score	(n = 28) 0.18 (0.48)	(n = 14) -1.36 (1.17)	<0.01	(n = 12) -0.94 (1.22)	<0.01	(n = 14) -0.76 (1.26)	<0.02
Grey matter		(n = 15)		(n = 10)		(n = 12)	
NAA	13.03 (0.79)	12.42 (0.69)	0.01	12.15 (0.57)	<0.01	12.02 (0.94)	<0.01
Cre	8.73 (0.61)	8.64 (0.50)	NS	8.68 (0.58)	NS	8.63 (0.90)	NS
Cho	1.25 (0.21)	1.51 (0.21)	<0.01	1.57 (0.23)	<0.01	1.48 (0.27)	<0.01
mIns	2.15 (0.29)	2.57 (0.99)	0.13	2.33 (0.41)	NS	2.52 (0.36)	<0.01
White matter		(n = 16)		(n = 11)		(n = 14)	
NAA	12.81 (0.74)	11.75 (0.88)	<0.01	11.76 (0.62)	<0.01	11.99 (0.88)	<0.01
Cre	7.34 (0.64)	7.42 (0.87)	NS	7.42 (0.67)	NS	7.41 (0.77)	NS
Cho	1.79 (0.20)	1.98 (0.22)	<0.01	1.84 (0.37)	NS	1.82 (0.31)	NS
mIns	2.14 (0.39)	2.64 (0.59)	<0.01	2.30 (0.41)	NS	2.43 (0.27)	0.01

Metabolite concentrations expressed in mM. *p* values refer to comparisons with normal controls by two-sample *t* tests. A total of 19 TBI patients was studied (number of subjects in each comparison cell is indicated).

NAA, *N*-acetylaspartate; Cre, creatine; Cho, choline; mIns, myoinositol; NS, not significant.

Each examination comprised quantitative magnetic resonance spectroscopy and imaging, and neuropsychological testing.

*Cognitive Testing*

The test battery was designed to probe a wide range of cognitive functions commonly impaired by TBI (Clifton, 1992; Levin, 1990): attention and information processing speed—Paced Auditory Serial Addition Test (PASAT); verbal memory—California Verbal Learning Test (CVLT); visual memory—Benton Visual Retention Test (BVRT); perceptual-motor function—Grooved Pegboard and Symbol Digit Modalities Test (SDMT); frontal “executive” functioning—verbal fluency (FAS), Wisconsin Card Sorting Test (WCST), and Trail Making Tests A and B; pre-morbid intelligence—New Adult Reading Test (NART); and orientation—Galveston Orientation and Amnesia Test (GOAT; Lezak, 1995).

A composite measure of neuropsychological function (composite cognitive *z* score) was calculated as described previously (Friedman et al., 1998). To accomplish this, a representative variable was selected from each test to maximize the assessment of functional impairment: PASAT (number correct on trial four); CVLT (total words recalled over five trials); BVRT (total errors); Grooved Pegboard (speed for both left [Gp-L] and right [Gp-R] hands); SDMT (total correct); FAS (total production over three trials); WCST function (percent perseverative errors); and Trails A and B (time). The *z* scores

were then calculated for each representative variable using normative values by age from literature sources (Spreeen and Strauss, 1991). Composite cognitive *z* scores were subsequently created for each subject by summing representative *z* scores and dividing by the number of measures.

*Magnetic Resonance Imaging and Spectroscopy*

All MR acquisitions were carried out on a 1.5 Tesla clinical MR scanner (GE Medical Systems, Waukesha, WI; software version 5.4). Localizing imaging sequences included a T<sub>1</sub>-weighted fast-SPGR scan (TE = 6.9 msec, TR = 17.7 msec, flip angle = 25°, 3 mm slices) and a conventional proton density/T<sub>2</sub>-weighted series (TE = 30/100 msec, TR = 2800 msec, 4-mm slices). A STEAM pulse sequence, including water suppression, was used to sample 25 × 35 × 21 mm<sup>3</sup> voxels (TE = 30 msec, TR = 2000 msec, 128 averages). The first voxel was carefully positioned within normal-appearing left occipito-parietal tissue to maximize white matter (WM) while minimizing gray matter (GM) and ventricular contributions (Fig. 1). Voxels were prescribed from the first axial image slice of the T<sub>1</sub>-weighted series superior to the lateral ventricles and located to maximize the WM contribution by aligning the medial margin of the voxel with the inter-hemispheric fissure gray-white matter boundary using graphic prescription. A second voxel was placed along the longitudinal fissure to probe occipital GM tissue. This voxel was specifically placed to avoid inclusion of the

sagittal sinus and corpus callosum on inferior slices (Fig. 1). The local magnetic field homogeneity, transmitter pulse power, and water suppression for each voxel were optimized automatically.

Relocalization of spectroscopic voxels in follow-up scans was accomplished using previously published methods (Brooks et al., 1999). In brief, we first recorded the locations of the voxels from the original scans on film. We then identified the specific voxel locations on the thin slice  $T_1$ -weighted images obtained during follow-up examinations, paying particular attention to the pattern of the gray-white boundary. The reproducibility of this approach and the stability of MRS measures so obtained have been established (Brooks et al., 1999).

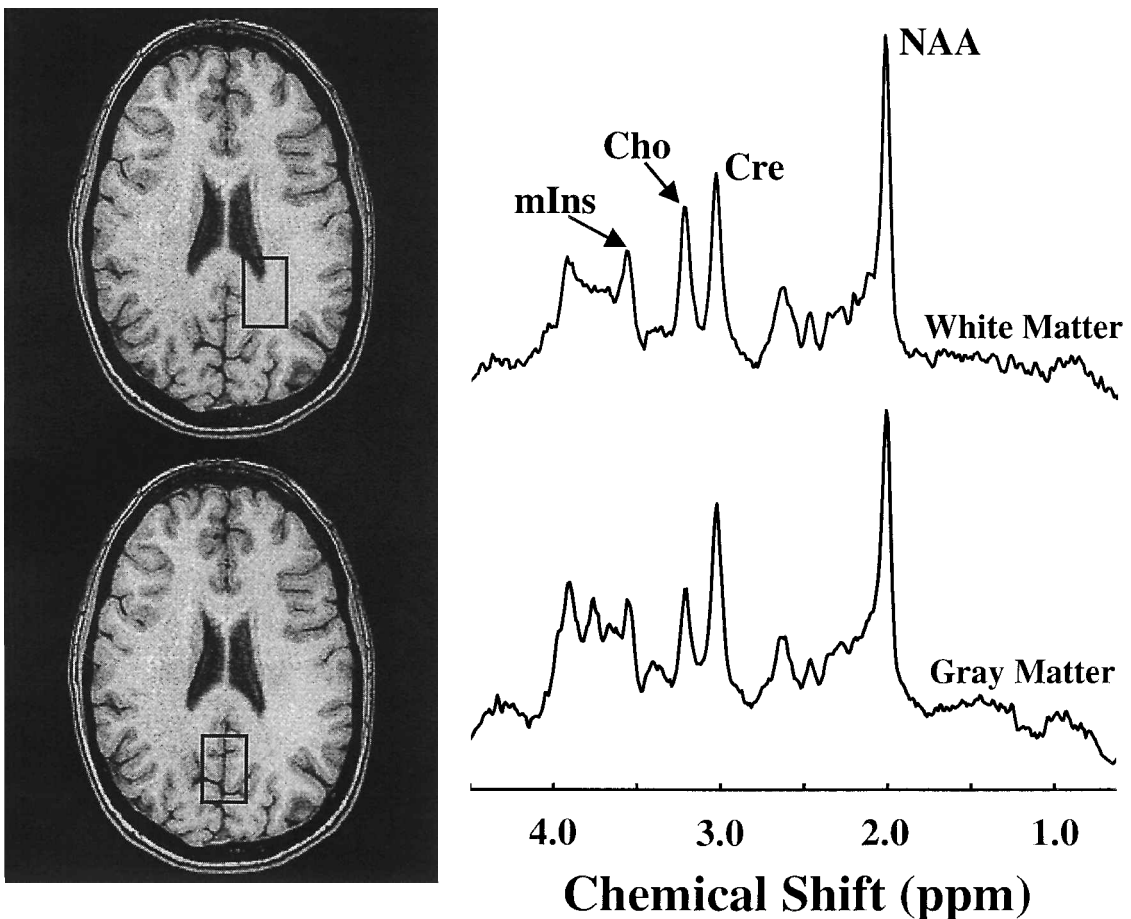
### *Spectroscopic Quantification*

Spectroscopic data were transferred to a Sun Ultra-Sparcstation (SunMicrosystems, Mountain View, CA) for analysis using MRUI (Leuven, Belgium). Residual water resonances were removed using time-domain Han-

kel-Lanczos Single Value Decomposition filtering (Pijnappel et al., 1992). Time-domain fitting of gaussian line-shapes to NAA, Cre, and Cho was carried out using Variable Projection (van den Boogaart et al., 1994) in batch mode and the areas corresponding to NAA, Cre, and Cho recorded. Areas for mIns were obtained by setting the linewidth of the mIns resonance to the mean of the NAA, Cre, and Cho linewidths obtained in each spectrum. The area from the water peak was determined from the unsuppressed water scan using Singular Value Decomposition (Pijnappel et al., 1992). Data were quantified using the internal water signal as a concentration reference and correcting for metabolite and water  $T_1$  and  $T_2$  effects during the pulse sequence using literature values (Barker et al., 1993; Christiansen et al., 1993; Brooks et al., 1999).

### *Atrophy Correction*

Since certain spectroscopic voxels included some CSF, and total voxel water was used to quantify metabolite concentrations, a correction for the fraction of CSF in the



**FIG. 1.** Magnetic resonance images and spectra. A  $T_1$ -weighted axial image showing the location of white matter and gray matter spectroscopic voxels: spectra from gray matter and white matter from a normal control.

voxel was made. Initially each image slice corresponding to the spectroscopic voxel was segmented using  $k$  means clustering optimized to divide image pixels into WM, GM, CSF, and partial volume CSF/gray matter as described previously (Petropoulos et al., 1999). Each metabolite concentration was then divided by the fraction of parenchymal tissue corresponding to that voxel to yield a concentration of metabolite within the actual tissue.

### Statistical Methods

Initial comparisons were made between the complete sample of TBI patients and normal controls at each time point. We describe this analysis as cross-sectional as not all subjects were assessed at consecutive time points. Secondly, since intersubject variability is larger than intrasubject variability across time (Brooks et al., 1999), change within individuals was also determined to probe the temporal evolution of neurometabolite postinjury. Thus, for those subjects who received examinations at time points 1 and 2 or time points 2 and 3, the change in neurometabolite concentrations was also assessed longitudinally.

Differences between TBI patients and normal controls were determined using two sample  $t$  tests and nonparametric Wilcoxon rank-sum tests. Differences between time periods for the TBI patients were examined using paired  $t$  tests and Wilcoxon signed-rank tests. Because many of the subjects were unable to complete the studies at all three time points due to clinical condition, more complex repeated measures models were not appropriate. Rather, spectroscopic findings and neuropsychological testing results were compared initially with both Pearson and Spearman correlation coefficients. This analysis was extended to linear regression models to simultaneously examine the association between multiple variables and the results of neuropsychological testing. However, due to small sample size, only a few important predictor variables were considered in any particular model.

To further assess the value of spectroscopic measures of neuronal injury for predicting cognitive outcome, patients were divided into two groups corresponding to "better" ( $z$  score  $> -0.5$ ) or "poorer" ( $z$  score  $\leq -0.5$ ) outcome as determined from the  $z$  score at 6 months. The mean metabolite concentrations for each subgroup at each time point were then compared using  $t$  tests. Logistic regression models were used to simultaneously examine multiple predictor variables.

In each analysis, we checked plots for potential influential observations, an issue of significant concern because of small sample size. Statistical analyses were performed in SAS (SAS Institute, Cary, NC).

## RESULTS

### Cognitive Performance

TBI subjects were markedly impaired on neuropsychological testing at time point 1 compared with normal controls ( $p < 0.01$ ; Table 1). Although mean performance of the total patient sample improved over time, as shown by an increase in the composite cognitive  $z$  score, at each time point the mean  $z$  score remained significantly reduced from normal controls and also from the expected mean of the normal population of 0.00 (all  $p < 0.02$ ).

In those patients who received cognitive testing at time points 1 and 2, paired analysis showed there was significant improvement in mean cognitive functioning through 3 months postinjury (change = 0.62,  $p = 0.01$ ; Table 2). A similar improvement was recorded between months 3 and 6. Cognitive function at outcome was correlated with composite cognitive  $z$  score at time point 1 ( $r = 0.83$ ;  $p = 0.001$ ) and at time point 2 ( $r = 0.86$ ;  $p = 0.006$ ).

Age was negatively correlated with cognitive outcome ( $r = -0.60$ ,  $p = 0.02$ ) in TBI patients. Similarly, patients with better outcomes were on average younger than patients with poorer outcomes (mean age for better outcomes = 24.2 years, poorer outcomes = 38.5 years;  $p = 0.01$ ).

### Gray Matter Neurochemistry Following Traumatic Brain Injury

In the cross-sectional analysis, the mean GM NAA concentration was reduced at 1.5 months ( $p = 0.01$ ) and continued to fall over the ensuing months ( $p < 0.01$ ; Table 1). GM choline was elevated at each time point compared with controls ( $p < 0.01$ ). Similarly, mIns was elevated compared with normal controls at each time point, although this only reached significance at 6 months postinjury ( $p < 0.01$ ). Figure 2 shows GM spectra from TBI patients recorded at 1.5 months after TBI. The lower spectrum shows reduced NAA and elevated Cho associated with poor outcome. The upper spectrum shows the pattern associated with better cognitive outcome.

Longitudinal changes in metabolite concentration and cognitive function over time are summarized in Table 2. In particular, paired comparisons between time points for those patients who received MRS and cognitive testing at each of two time points (i.e., 1 and 2 or 2 and 3) are presented. These data show a continued and significant reduction of GM NAA over the first three months after injury. In contrast, there was an increase in the mean NAA for those subjects who were examined at time points 2 and 3, although this increase did not reach significance (Fig. 3). Consistent with Table 1, Cho remained elevated compared with control throughout the study pe-

TABLE 2. COMPARISON OF METABOLITE CONCENTRATION CHANGE BETWEEN TIME POINTS

	Time point 1/2		Time point 2/3	
	Change	p value	Change	p value
Composite z score	(n = 8) 0.62 (0.51)	0.01	(n = 8) 0.50 (0.76)	0.11
Grey matter	(n = 8)		(n = 7)	
NAA	-0.46 (0.43)	0.02	0.35 (0.58)	0.17
Cre	-0.11 (0.65)	0.65	0.37 (0.64)	0.18
Cho	-0.03 (0.22)	0.71	-0.07 (0.20)	0.43
mIns	-0.42 (1.20)	0.35	0.48 (0.45)	<0.05
White matter	(n = 8)		(n = 8)	
NAA	-0.14 (0.88)	0.67	0.55 (0.69)	0.06
Cre	-0.36 (0.94)	0.31	0.14 (1.23)	0.75
Cho	-0.14 (0.17)	0.05	0.06 (0.38)	0.65
mIns	-0.22 (0.40)	0.16	0.18 (0.46)	0.33

Since some subjects did not receive examinations at all time points, the number of subjects contributing to each comparison differs in some cells. Data are presented as the change in metabolite between the later and the earlier examination in each case so that a positive value indicates an increase in concentration with time and vice versa (values in parentheses represent standard deviations of change). *p* values represent significance levels for paired *t* tests.

NAA, *N*-acetylaspartate; Cre, creatine; Cho, choline; mIns, myoinositol.

riod; also, there was no significant change in GM Cho between any time points (Fig. 4).

#### White Matter Neurochemistry Following Traumatic Brain Injury

Cross-sectional analysis showed that mean WM NAA was reduced compared with normal controls at each time point ( $p < 0.01$ ; Fig. 3). In contrast, although mean Cho was significantly elevated at 1.5 months postinjury ( $p < 0.01$ ), Cho concentrations were not significantly elevated from normal values at subsequent time points (Table 1 and Fig. 4). The concentration of mIns was higher at each time point than controls, significantly so at 1.5 and 6 months postinjury.

Paired analysis within individuals indicated that NAA concentrations increased between months 3 and 6 ( $p = 0.06$ ). Although initially elevated at 1.5 months, WM Cho fell significantly ( $p = 0.05$ ) between time points 1 and 2 and was not significantly different from normal values for the remainder of the study (Tables 1 and 2). Mean mIns concentrations did not change significantly during the study period.

At no time point in either GM or WM was Cre significantly different from the normal comparison subjects (change from normal  $< 0.1$  mM at any time point).

#### Neurochemistry and Cognitive Functioning

As shown in Table 3, there were generally strong correlations between concurrent measurements of GM NAA

and cognitive function (range  $r = 0.45$ – $0.67$ ). Secondly, GM NAA at 1.5 and 3 months predicted cognitive function at outcome ( $r = 0.63$  and  $0.70$ , respectively).

The correlation between GM Cho and outcome approached significance ( $r = -0.72$ ,  $p = 0.07$ ) at 3 months although not at other time points (Table 3). GM Cre was significantly correlated with cognitive function at 1.5 months postinjury. Correlations between outcome and the metabolites NAA, Cho, or Cre measured in WM were

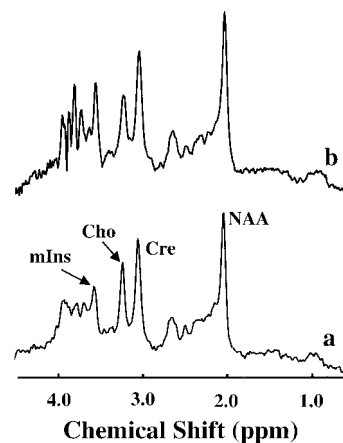
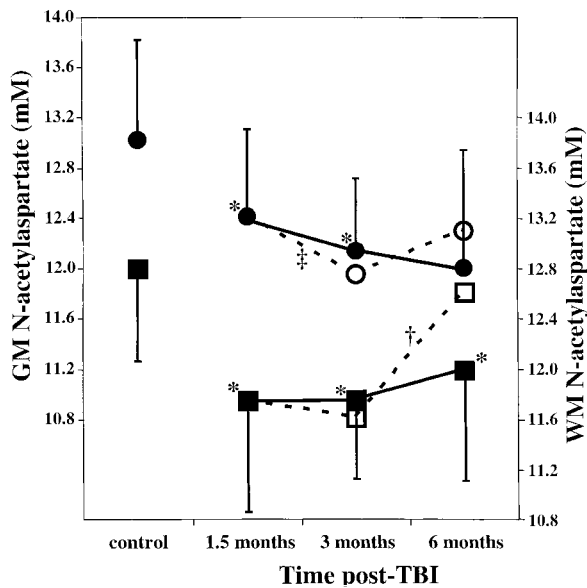


FIG. 2. Gray matter spectra from patients 1.5 months after traumatic brain injury showing low *N*-acetylaspartate (NAA) and high choline (Cho) from a patient with poor outcome (a) and normal NAA and Cho close to normal from a patient with a better outcome (b).

DISCUSSION



**FIG. 3.** N-acetylaspartate (NAA) concentration after traumatic brain injury. The pattern of evolution of NAA concentration following TBI is similar irrespective of whether mean sample concentration or change within subjects is plotted. Solid symbols and solid lines indicate mean NAA concentrations from all subjects measured at each time point. Open symbols and dashed lines indicate change in concentration within subject. Squares (■) indicate white matter and circles (●) indicate gray matter. \*Significant difference from normal controls,  $p < 0.01$ ; ‡significance is  $p = 0.02$ ; †significance of change is  $p = 0.06$ .

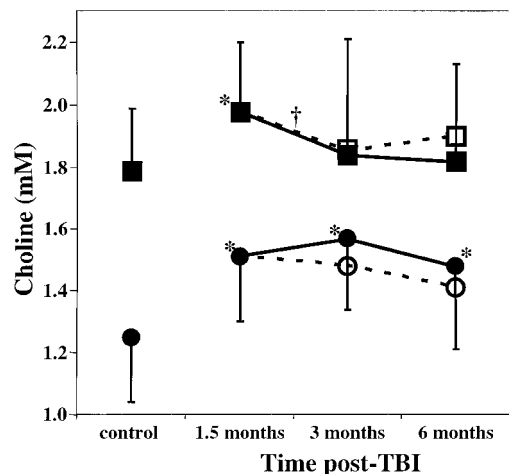
not significant. However, there were strong negative correlations between WM mIns and cognitive function at time points 1 ( $r = -0.89, p = 0.0001$ ) and 3 ( $r = -0.73, p = 0.007$ ).

Using a cut point of  $-0.5$  based on the median cognitive  $z$  score at outcome, we found that mean GM NAA in patients classified as having a “better” outcome ( $z$  score  $> -0.5$ ) was higher at each examination than those with a “poorer” outcome (difference of means ranged from  $0.50$  to  $0.93$  mM), although in no case did this observation reach significance.

Cho in GM was generally lower in patients with better outcome and at the second examination this was highly significant (mean Cho concentrations in better =  $1.36$  mM vs. poorer =  $1.79, p = 0.002$ ; Figs. 5 and 6). Similarly, GM mIns at time point 2 was lower in patients with better outcomes than those with poorer (mean mIns =  $2.02$  vs  $2.49$ , respectively;  $p = 0.14$ ). Although the small number of subjects prevented this difference from reaching statistical significance, the magnitude suggests a substantial effect. At time point 3, Cho remained elevated in patients with poorer outcome ( $p = 0.14$ ).

TBI is a challenging clinical problem with inadequate prognostic indicators. Outcome prediction for individual patients with TBI is further hampered by different mechanisms of TBI in individual patients, varying injury severity, and various confounding nonbrain complications. Objective methods to assess brain injury and prognosis are required.  $^1\text{H-MRS}$  offers a unique noninvasive opportunity to study the human brain in survivors of TBI, although it is limited by certain technical difficulties such as complications associated with metal surgical implants and life support equipment in a magnet environment. This study describes the long-term evolution of brain injury at the cellular level and the relationship to outcome in a group of patients who sustained moderate to severe head injuries.

Following TBI, both animal and human brain studies reveal substantial neuronal damage as well as metabolic depression within and remote from the site of the original injury (Hovda, 1992; Chesnut, 1993; Maxwell, 1997; Yamaki, 1996). Although postmortem studies in fatal cases show extensive damage, in nonfatal cases the extent and severity of injury is difficult to follow noninvasively and available clinical measurements are limited in predicting outcome. We have shown recently that  $^1\text{H-}$

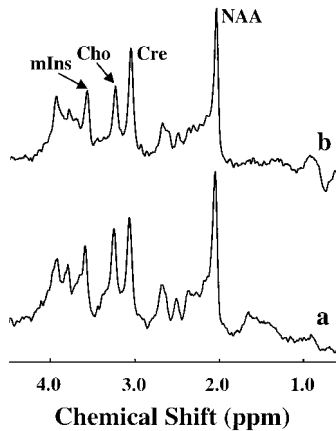


**FIG. 4.** Choline (Cho) concentration after traumatic brain injury. The pattern of evolution of Cho concentration following TBI is similar irrespective of whether mean sample concentration or change within subjects is plotted. Solid symbols and solid lines indicate mean NAA concentrations from all subjects measured at each time point. Open symbols and dashed lines indicate change in concentration within subjects. Squares (■) indicate white matter and circles (●) indicate gray matter. \*Significant difference from normal controls,  $p < 0.01$ ; †significance of change is  $p = 0.05$ .

**TABLE 3. PEARSON CORRELATION COEFFICIENTS BETWEEN COMPOSITE COGNITIVE z SCORES AND METABOLITE CONCENTRATIONS AT EACH TIME POINT**

	Time point	Cognitive z scores		
		Time point 1	Time point 2	Time point 3
Gray matter				
NAA	1	0.45 (0.11) <i>n</i> = 14	0.58 (0.10) <i>n</i> = 9	0.63 (0.04) <i>n</i> = 11
	2		0.67 (0.04) <i>n</i> = 10	0.70 (0.08) <i>n</i> = 7
	3			0.57 (0.03) <i>n</i> = 14
Cre	1	0.70 (0.005) <i>n</i> = 14	0.33 (0.39) <i>n</i> = 9	0.55 (0.08) <i>n</i> = 11
	2		0.33 (0.35) <i>n</i> = 10	0.49 (0.26) <i>n</i> = 7
	3			0.35 (0.23) <i>n</i> = 14
Cho	1	-0.19 (0.52) <i>n</i> = 14	-0.001 (>0.99) <i>n</i> = 9	-0.03 (0.92) <i>n</i> = 11
	2		-0.45 (0.19) <i>n</i> = 10	-0.72 (0.07) <i>n</i> = 7
	3			-0.28 (0.32) <i>n</i> = 14
mIns	1	0.04 (0.90) <i>n</i> = 14	0.19 (0.62) <i>n</i> = 9	0.24 (0.48) <i>n</i> = 11
	2		-0.43 (0.22) <i>n</i> = 10	-0.39 (0.39) <i>n</i> = 7
	3			0.48 (0.12) <i>n</i> = 12
White matter				
NAA	1	0.35 (0.22) <i>n</i> = 14	-0.28 (0.47) <i>n</i> = 9	-0.05 (0.88) <i>n</i> = 12
	2		0.50 (0.12) <i>n</i> = 11	0.38 (0.36) <i>n</i> = 8
	3			0.27 (0.35) <i>n</i> = 14
	1	0.31 (0.28) <i>n</i> = 14	-0.17 (0.65) <i>n</i> = 9	0.27 (0.39) <i>n</i> = 12
	2		0.20 (0.56) <i>n</i> = 11	0.18 (0.67) <i>n</i> = 8
	3			-0.05 (0.87) <i>n</i> = 14
Cho	1	-0.24 (0.41) <i>n</i> = 14	-0.22 (0.57) <i>n</i> = 9	-0.15 (0.65) <i>n</i> = 12
	2		0.31 (0.35) <i>n</i> = 11	0.20 (0.63) <i>n</i> = 8
	3			-0.06 (0.84) <i>n</i> = 14
mIns	1	-0.89 (<0.01) <i>n</i> = 14	-0.09 (0.82) <i>n</i> = 9	-0.73 (<0.01) <i>n</i> = 12
	2		0.11 (.74) <i>n</i> = 11	0.04 (0.93) <i>n</i> = 8
	3			-0.10 (0.78) <i>n</i> = 11

Data expressed as *r* value (*p* value) for each Pearson correlation.  
NAA, *N*-acetylaspartate; Cre, creatine; Cho, choline; mIns, myoinositol.



**FIG. 5.** Gray matter spectra from patients 3 months after traumatic brain injury showing high Cho and high mIns indicating a poor outcome (a) and normal Cho and mIns from a patient with good outcome (b).

MRS measurements of neurometabolite markers of neuronal injury and metabolic depression offer a unique means for predicting function and outcome (Friedman et al., 1998, 1999). The current work extends those preliminary findings by demonstrating differences between TBI patients and normal controls, the temporal evolution of neurochemical markers of injury following TBI, and the relationship between these markers and cognitive outcome.

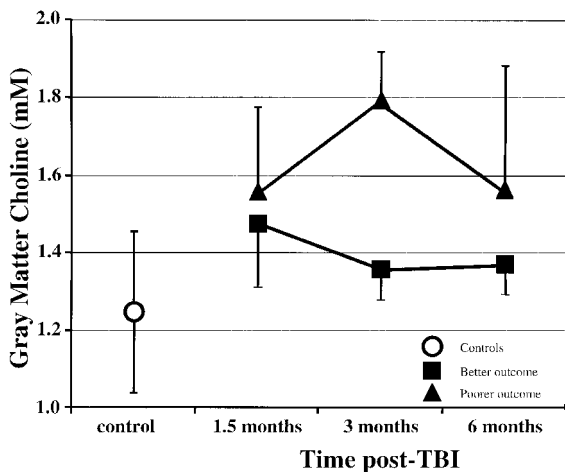
Our overall aim was to determine the importance of neurometabolite change observed remote from the original injury site associated with TBI. We used a reliable method of relocalization to ensure that our spectroscopic

voxels were placed in the original locations in subsequent scans (Brooks et al., 1999). Since cerebral atrophy is a widely recognized sequel to TBI, all spectroscopic measurements were corrected for brain tissue/CSF composition within the specific spectroscopic voxel using an automated segmentation algorithm to determine tissue fraction (Petropoulos et al., 1999).

Increasing evidence suggests that NAA is related to cognitive function in normal individuals (Jung et al., 1999a,b). Thus, NAA concentration following TBI is determined not only by injury related processes, but also by individual premorbid values. Thus, in the context of our aim to determine the relationship between change in neurochemical markers of injury and cognitive function and outcome, we interpreted our results both in terms of group comparisons with uninjured controls, as well as by considering the effects within individuals during recovery. Using this longitudinal approach to compare neurometabolites and cognitive testing results for those patients with paired examinations, we were able to detect intrasubject change during specific intervals with greater sensitivity by reducing the effects of intersubject variability.

Cross-sectional (Table 1) and longitudinal (Table 2) data provide two complementary approaches to understanding neurometabolic change following TBI. Both are consistent with the following interpretation. First, cross-sectional data for WM NAA indicate a rapid fall from normal control values associated with a combination of primary irreversible injury leading to neuronal death (Ross and Michaelis, 1994), possibly reversible metabolic depression (Bates et al., 1996; Feeney, 1982), and continued secondary neuronal loss as seen on histology (Maxwell, 1997). Similar NAA levels persist until 3 months and then demonstrate a small increase at 6 months (Table 1 and Fig. 3). This possible increase is further supported by longitudinal data which also suggest an NAA increase between 3 and 6 months ( $p = 0.06$ ) (Table 2). We suspect this increase represents recovery from metabolic depression as the other determinant of NAA level, axonal death, is irreversible. Cho data (Tables 1 and 2) support this interpretation by first demonstrating an elevation at 1.5 months consistent with a reactive response to neuronal injury and death (i.e., inflammation, glial proliferation, edema; Brenner, 1993; Kugel, 1992; Tedeschi, 1997; Tzika, 1993). Cho then falls to the normal range at 3 and 6 months post-TBI, perhaps reflecting a resolution of these reactive responses (Fig. 4). The pattern and magnitude of the WM mIns elevation also suggest a resolving reactive process.

Results for GM NAA are similar (Fig. 3). As in WM, GM NAA falls rapidly from the presumed preinjury (control) levels over the first 3 months. Although cross-sectional



**FIG. 6.** Plot of gray matter choline measured in those patients who received all three scans. Patients with elevated Cho at 3 months result in a poor outcome, and those with lower Cho have better outcomes.

tional results suggest that this fall continues through 3 and 6 months post-TBI, the longitudinal data suggest a possible recovery in certain individuals, perhaps due to recovery from metabolic depression. Over the same period, GM Cho remains elevated, consistent with previous reports (Garnett et al., 1999; Macmillan et al., 1999). This continued elevation might reflect slowly dying neurons as suggested by studies finding of macrophages in human brain 240 days after TBI (Gentlemen et al., 1999). Although not as striking as the Cho findings, mean GM mIns concentrations were generally higher than control values. This might be another manifestation of the inflammation indicated by elevated Cho. We interpret this elevated mIns as indicating reactive processes such as glial proliferation or cerebral edema as greater mIns may correct for hyperosmolality from blood-brain barrier breakdown (Lien, 1990).

Thus, the longitudinal and cross-sectional data from WM and GM are generally quite consistent. The only exception is for GM NAA in the final interval where the cross-sectional data at 6 months appear to show a fall while longitudinal data show a recovery (Fig. 3). However, we note the increased variance in the GM NAA at 6 months (Table 2). Plots of these individual data points reveal that this increased variance is due to two distinct patient subpopulations, one of which has higher NAA associated with an improvement in cognitive function at this late stage of recovery (analysis not shown). These data suggest a heterogeneous population, with certain individuals demonstrating prolonged and progressive loss of neurons in GM following TBI, while others demonstrate less severe injury and improvement in NAA suggestive of reversible metabolic depression. In contrast, WM appears to primarily undergo metabolic recovery, rather than progressive axonal dropout. Future studies to increase the number of patients who receive all examinations will help to elucidate this apparent inconsistency.

The importance of cellular injury to overall cognitive function is demonstrated by comparisons of neuro-metabolite markers of brain injury with quantitative assessment of cognitive function. GM measurements of NAA predict cognitive outcome in the current sample with a correlation coefficient of greater than 0.6. Indeed, GM NAA at any of the time points employed herein is correlated with cognitive outcome to a similar level. Taken together, these findings support the value of GM NAA as a predictive tool for outcome following TBI.

At 3 months postinjury we found two distinct subsets of patients based on GM Cho. Those patients with elevated GM Cho at 3 months postinjury had substantially poorer outcome than those whose Cho was lower (Fig. 6). In fact, there was impressive separation between the Cho concentrations of those with poor and those with

good outcomes. Thus, at least at this individual time point, our data suggest that elevated Cho is associated with a harmful process. This appears to preclude membrane repair as an explanation for elevated Cho, which we expect would contribute to better rather than poorer function. One patient with high NAA, but also high Cho, had a bad outcome. Thus, Cho might be at least as important as NAA in measuring injury and predicting outcome in TBI. Since reactive changes are seen at histology following TBI (Gentlemen et al., 1999) our results suggest that inflammation might be the cause of elevated Cho and that Cho measurements might prove useful as a noninvasive measure of inflammation or injury severity to be used in patient management. Further studies will be required to decide between these explanations which may take on special clinical importance in the presence of new treatment strategies such as anti-inflammatory or neuro-protective agents which are becoming available.

We found age to be negatively associated with outcome in TBI patients although not with cognitive function in the controls. Thus, our data suggest that well-documented poorer outcome from TBI in older individuals might be related to an age-related neuronal susceptibility or functional depression.

In the absence of histological confirmation, correlation of our findings to specific aspects of cellular injury remains speculative. However, based on animal experiments and autopsy data, we suspect in the early recovery phase, decreased NAA results from the sum of neuronal death and metabolic depression which combine to cause cognitive impairment. As the injury evolves, we suspect continued neuronal loss as described by Maxwell (1997), which would cause continued fall in NAA. However, simultaneous recovery of metabolism of those neurons which are destined to survive may be responsible for improved cognitive function and increasing NAA. This interpretation is consistent with the elevated GM Cho and mIns seen at 3 months in those patients who have poorer outcomes suggesting ongoing inflammation resulting from dead neurons followed by resolution of inflammation. Also the processes affecting neurons and axons may not have identical temporal evolution. In particular, although axonal injury may have an immediate impact on axonal metabolism and NAA, there may be a more delayed effect on the cell body in GM. Thus, this study is important in that it is one of the first to demonstrate possible metabolic uncoupling of GM and WM following injury.

Our study was limited by statistical power, which in turn reflects the substantial practical difficulties of studying severely injured patients. Nonetheless, our results suggest that <sup>1</sup>H-MRS offers a new tool to investigate the

metabolic integrity of the neuron following TBI, and may provide new insight into clinical management, patient heterogeneity, prediction of outcome, and the determination of effectiveness of therapy.

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