

# Magnetic Resonance Spectroscopy Detects Brain Injury and Predicts Cognitive Functioning in Children with Brain Injuries

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## ABSTRACT

**Proton magnetic resonance spectroscopy (<sup>1</sup>H-MRS) and neuropsychological assessment were utilized in a longitudinal investigation of traumatic brain injury (TBI) in children. A spectroscopic imaging protocol was implemented, and neurometabolite ratios of NAA/Cre and Cho/Cre were calculated for anterior and posterior halves of a supraventricular slab of brain tissue. NAA/Cre was reduced and Cho/Cre increased in TBI patients as compared to controls, for both brain regions. Each ratio predicted aspects of neuropsychological performance, though the specific relationships varied somewhat by region and function. Anterior NAA/Cre increased and anterior Cho/Cre decreased from 3 to 21 weeks post-injury, suggesting neurometabolic recovery.**

**Key words:** cognitive; outcome; pediatric; spectroscopy; traumatic brain injury

## INTRODUCTION

**I**T IS DIFFICULT to overestimate the social importance of traumatic brain injury (TBI) in children, 400,000 of whom are treated in emergency rooms, 30,000 of whom are hospitalized, and 3,000 of whom die every year because of brain injury in the United States alone (Centers for Disease Control and Prevention, 2003). Disabling effects of brain injury in children cut across a variety of cognitive, emotional, and functional domains (Catroppa and Anderson, 2003, 2004; Hawley, 2003; Levin et al., 2004; Poggi et al., 2003; Slomine et al., 2002). Moreover, age does not mitigate the disabling effects of non-penetrating TBI in children (Levin, 2003; Moses and Stiles, 2002). In fact, in such injuries, younger age may

be a risk factor for poorer neuropsychological outcome (Verger et al., 2000). Magnetic resonance spectroscopy (MRS), in particular proton MRS (<sup>1</sup>H-MRS), has emerged as an important tool in both basic research and clinical investigations of TBI. Below we review the nature of MRS, its previous applications to the study of TBI in children, and our own ongoing MRS study of children with TBI.

### *Proton Magnetic Resonance Spectroscopy*

Of the many chemicals of interest in the human brain, only a few occur in sufficient quantity to be visible to <sup>1</sup>H-MRS at the field strengths commonly used in human research and clinical settings. Excluding the water peak,

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the strongest signals are given by N-acetylaspartate (NAA), choline (Cho), and creatine (Cr), although glutamate/glutamine (Glx), myoinositol, and lactate can also render interpretable peaks given sufficient field strength. Understanding the function of these compounds and the meaning of changes in their concentration is fundamental to interpreting  $^1\text{H}$ -MRS findings.

NAA is the most prominent peak on a typical  $^1\text{H}$ -MRS spectrum. Because NAA is present almost exclusively in neurons (Uranjak et al., 1993), declines in NAA have been interpreted as neuronal loss. However, these declines at times underestimate (Demougeot et al., 2001; Sager et al., 2000) or overestimate cell death, because NAA levels can be indicative of changes in cellular function in intact neurons.

Several lines of evidence support this claim. First, reversible changes in NAA occur in clinical conditions in which neuron density itself is not expected to significantly reverse, such as TBI (De Stefano et al., 1995) and contralateral brain regions in temporal lobe epilepsy after surgery (Hugg et al., 1996). Moreover, it has been demonstrated that mitochondrial dysfunction can also cause declines in NAA in the absence of cell death (Dautry et al., 2000; Demougeot et al., 2001). Perhaps most fundamentally, NAA concentration is subject to change because it is part of a dynamic cycle, in which its concentrations are subject to influence at a number of stages. Disruption of production, movement across the neuronal membrane, oligodendrocyte uptake, hydrolysis, or reuptake of aspartate could all potentially alter NAA levels in the brain (Baslow, 2000, 2003).

Baslow (2003) argues that the primary purpose of NAA is to serve as a molecular water pump for neurons, coupling the movement of large quantities of water produced by glucose metabolism to the energetically favorable movement of NAA along its high intracellular-extracellular gradient. The relationship between NAA and cellular metabolism implied in this model is also found in an animal study in which changes in NAA after brain injury closely followed change in ATP in both direction and time course (Signoretti et al., 2001). In addition, because NAA is manufactured only in neurons and catabolized only in oligodendrocytes, it may play a role in signaling between these cells (Baslow, 2000).

Another prominent neurometabolite is choline. Glycero-phosphorylcholine and phosphorylcholine, which are metabolites of phospholipids components of cellular membranes, are the primary constituents of the choline peak (Cho), with free choline also making a minor contribution (Govindaraju et al., 1999). In brain injury, increases in Cho have been associated with increased activity of phospholipase  $A_2$ , an enzyme that catalyzes membrane glycerophospholipid hydrolysis (Boulanger et

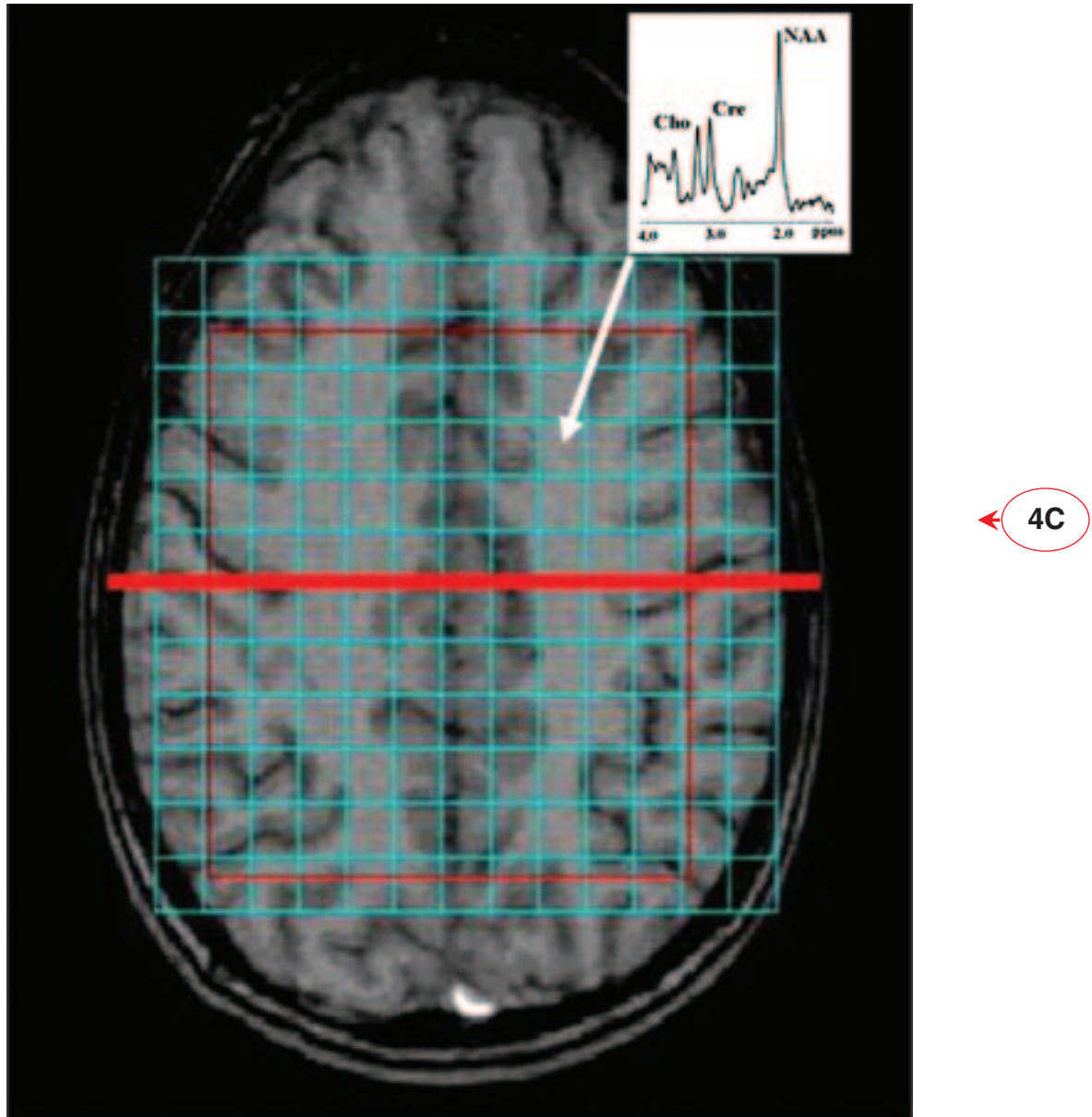
al., 2000). The choline peak has been associated with membrane turnover, inflammatory processes, and changes in myelination. However, in a rat model of brain injury, histological studies linked increased choline signal to reactive astrocytosis (Schuhmann et al., 2003).

A third major neurometabolite is creatine. The creatine signal (Cr) is composed of both creatine and phosphocreatine, which participate in cellular bioenergetics in a number of ways (Brdiczka and Wallimann, 1994; Saks et al., 1996). However, although the ratio of creatine and phosphocreatine varies according to cellular energy demands, in most instances the total Cr signal from both molecules is relatively constant. For this reason, other metabolites are often reported as ratios to creatine. Other neurometabolites, though of interest, are less frequently measured. Glutamate, as detected in the glutamate/glutamine (Glx) peak, is of potential interest because of its role in excitotoxicity after brain trauma. Lactate can be elevated when aerobic metabolism is compromised, such as hypoxic brain injury (Ross et al., 1998). Myo-inositol, which is involved in signal transduction and osmoregulation, is elevated in diabetes, Down's syndrome, Alzheimer disease, and alcoholism (Mason et al., 2005; Shetty and Huang, 2000).

Until recently, almost all MRS studies used a single-voxel approach. Usually, this voxel will sample primarily homogenous gray or white matter regions. Spectroscopic imaging (SI), or chemical shift imaging (CSI), yields metabolite concentrations or ratios from many voxels laid in a grid across a MR slab of tissue. This allows for multiple regional analyses not possible in single-voxel MRS techniques. However, the signal-to-noise ratio is proportional to the size of the voxel, so that the smaller voxels sampled in SI are noisier and difficult to interpret. Commonly, researchers will compromise by averaging some voxels, allowing fewer comparisons, but improving the quality of the chemical data.

### *Utility of $^1\text{H}$ -MRS in Brain Trauma*

$^1\text{H}$ -MRS techniques potentially augment data from structural MRI analyses in predicting outcome in TBI in children. Despite the ability of structural imaging to predict some important outcomes (Grados et al., 2001; Stefano et al., 2000), prediction of long-term outcome in children with TBI is quite difficult (Blackman et al., 2003), and many brain-injured patients show greater deficits than images would predict. After brain injury, NAA has been demonstrated to decrease in anterior white matter voxel, while choline has increased in the same region (Garnett et al., 2001). Decreases in the NAA/Cho ratio and increases in the Cho/Cr detected by  $^1\text{H}$ -MRS correlate with injury severity even when white matter ap-



**FIG. 1.** The anatomic grid for spectroscopic imaging is overlaid on a supraventricular slice. The division of voxels into anterior and posterior halves is revealed by the heavy horizontal mid-line. A representative spectra is shown.

pears normal on the MR image (Garnett et al., 2000). Higher NAA/Cr ratios in normal appearing white matter are associated with better outcome as described on the Glasgow Outcome Scale (GOS) 3 months after injury (Sinson et al., 2001). TBI in adults has been associated with decreased NAA in white matter and increased choline in gray matter (Friedman et al., 1998). Moreover, NAA in gray matter and NAA and creatine in white matter are both correlated with performance on neuropsychological functioning in brain-injured adults but not in healthy controls (Friedman et al., 1998). In another MRS

study of TBI outcome in adults, elevated glutamate/glutamine (Glx) and Cho were related to poorer outcome on the GOS from 6 months to 1 year later (Shutter et al., 2004). Despite these positive results, other researchers failed to find a correlation between metabolite ratios and outcome at 6 months in adults with mild TBI (Govindaraju et al., 2004).

MRS has also been used to study children with TBI. Ashwal and colleagues (2004) examined 38 children aged 1.6–17 years (mean age, 11.0 years) who had sustained a TBI, as well as a control group of 10 children aged

2.3–17.7 years (mean age, 9.6 years), three of whom were completely healthy and seven of whom had normal brain scans in diagnosis of neurological or craniofacial disorders. This study found elevated glutamate/glutamine (Glx) as the sole  $^1\text{H}$ -MRS measure that distinguished brain-injured children from the control group. No differences were found for NAA, creatine, or choline. Moreover, Glx did not distinguish between children with good and poor outcome, as measured by the Pediatric Cerebral Performance Category Scale (PCPCS), an adaptation of the Glasgow Coma Scale (GCS) for use in children. The use of neurology patients as controls, even though their brain scans were normal, may contribute to the negative findings, inasmuch as  $^1\text{H}$ -MRS techniques are sensitive to neurological dysfunction without structural abnormalities. Also, patients were not all scanned in the same time frame after injury, with more severely injured patients typically scanned later. During this additional recovery time, metabolites of the more severely injured patients may have become more similar to those of moderately injured patients. Finally, the PCPCS is a gross measure of outcome that is relatively insensitive to subtle changes in functioning. More recent research by the same group using MRS imaging did find decreased NAA/Cr and increased Cho/Cr in children scanned within 16 days of injury, and a correlation between these metabolites and the PCPCS at 6 months (Holshouser et al., 2005). Studying severely brain-injured children in the chronic stage, other researchers found lower levels of both NAA and Cho compared to controls (Parry et al., 2004). Both of these neurometabolites correlated with reaction time in the injured children.

## METHODS

The New Mexico Pediatric Brain Injury Study is an ongoing investigation of TBI utilizing MRS. All studies were carried out using a 1.5-Tesla GE Signa clinical MR scanner at the University of New Mexico Clinical and Magnetic Resonance Research Center (CMRRC). The protocol included a  $T_1$ -weighted volume axial series (fast-SPGR, TE = 6.9 msec, TR = 17.7 msec, flip =  $25^\circ$ ,  $256 \times 192$  matrix, 1.5-mm slices) and a  $T_2$ -weighted axial series (TE = 30/100, TR = 2800 msec,  $256 \times 192$  matrix, 3-mm slices). In contrast to our prior studies with adults, we have used a SI technique rather than single-voxel spectroscopy. One SI slice was selected above the lateral ventricles to extend from the frontal lobe to the posterior parietal lobes sampling both white and gray matter. Figure 1 shows the supraventricular slice at which a rectangular matrix of data is acquired, as well as a representative spectra. Water-suppressed PRESS localiza-

tion with outer voxel suppression bands was used to excite parenchyma and avoid lipid artifact from the skull. MRS acquisition parameters were as follows: 15-mm slice TE = 62 msec, TR = 1500 msec. The TE value was chosen to obtain a sharp RF pulse excitation profile and efficient crusher gradient durations, which required a minimum TE of 62 msec using the GE Signa sequence. We choose not to extend the TE beyond this minimum in order to maintain a relatively high signal-to-noise ratio (reduced by  $T_2$  relaxation during TE).

To avoid contamination from lipid molecules found toward the periphery of the brain, voxels from the outer rim of the rectangle are discarded. Data from the remaining cells are examined for spectroscopic quality using diagnostic information provided by LC Model (Provencher, 1999). Voxels meeting these technical standards are averaged into two compartments, the anterior and posterior halves of the SI slab, as shown in Figure 1. Previous studies using the same scanner as utilized in present study have demonstrated excellent stability over repeated scanning in both control (Brooks et al., 1999) and patient (Mullins et al., 2003) samples (i.e., coefficients of variation less than 3% for NAA, Cho, and Cre).

We recruit children between the ages of 6 and 18 with TBI (defined as having a GCS score of 13 or less, or having a score of 14 or 15 and a TBI evident on initial CT or MRI scan). Controls are recruited from the families of the TBI children as well as from the local community. Extensive screening ensured that they had no history of TBI or other neurologic disorder, and none were ever diagnosed with learning disorders or other neurodevelopmental problems. The numbers of participants available for the various analyses described below vary for several reasons. Some children were not able to participate in neuropsychological assessment at the time of their initial evaluation. Others were not able to return for follow-up at the desired time points, and some families simply dropped out of the study. Occasionally, MRS data were not of sufficient quality for analysis, usually due to the presence of metal artifacts. We previously reported on the first 15 TBI participants at the time of their initial assessments (Weers et al., 2006).

The test battery (Lezak, 1995) included measures of simple attention and rapid information processing (Wechsler Intelligence Scale for Children, Third Edition [WISC-III], coding subtest; Rapidly Recurring Figures Test; Symbol Digit Modalities Test), short-term memory span (WISC-III Digit Span, Wechsler Memory Scale, 3rd Ed., Spatial Span subtest), short-term/working memory decay (Children's Consonant Trigrams), verbal fluency (Controlled Oral Word Association Test; Animal Naming), verbal memory (Test of Memory and Learning [TOMAL] Story Memory, Verbal Selective Reminding

**TABLE 1. NEUROMETABOLITE RATIOS IN TBI PATIENTS AND CONTROLS**

|                   | <i>TBI patients</i><br>(n = 36) |           | <i>Controls</i><br>(n = 14) |           |
|-------------------|---------------------------------|-----------|-----------------------------|-----------|
|                   | <i>Mean</i>                     | <i>SD</i> | <i>Mean</i>                 | <i>SD</i> |
| Anterior NAA/Cre  | 1.47                            | 0.22      | 1.84                        | 0.13      |
| Posterior NAA/Cre | 1.58                            | 0.20      | 1.96                        | 0.15      |
| Anterior Cho/Cre  | 0.34                            | 0.05      | 0.28                        | 0.03      |
| Posterior Cho/Cre | 0.28                            | 0.04      | 0.23                        | 0.03      |

All differences were significant at  $p < 0.001$  by independent  $t$ -tests.

subtests); nonverbal memory (TOMAL Visual Selective Reminding subtest), executive functioning (Contingency Naming Test; Stroop Test), and motor skill (Grooved Pegboard, strength of grip).

#### Statistical Analysis

**AU1** Data were compared by independent  $t$ -tests and paired-comparison  $t$ -tests as discussed in Results.

## RESULTS

MRS data have now been acquired from 36 participants and 14 controls at the initial time point. For these TBI children, the mean age was 13.62 years (83% male; SD = 3.59 years, range = 6–18 years), versus 15.29 years for controls (28% male; SD = 1.6 years, range = 11–17 years). The mean GCS score for the TBI children was 8.11 (SD = 4.60, range = 3–15), and the mean number of days post-injury was 35.46 (SD = 33.95, range = 4–158). We first analyzed group differences in NAA/Cre and Cho/Cre in the anterior and posterior compartments. Highly significant differences were noted for each ratio in both compartments, with NAA/Cre showing a reduc-

tion and Cho/Cre showing an increase (Table 1). The fact that these ratio scores moved in different directions (i.e., NAA/Cre decreased while Cho/Cre increased) suggests that ratio effects were not primarily due to alterations in Cre concentration. In the TBI group, the correlation between the anterior NAA/Cre and Cho/Cre ratios  $-0.61$  ( $p < 0.001$ ) and the posterior NAA/Cre and Cho/Cre ratios was  $-0.51$  ( $p < 0.001$ ). The corresponding values in the control group were 0.14 and 0.21 (both nonsignificant). GCS scores correlated with total brain NAA/Cre at  $r = 0.45$  ( $p = 0.009$ ) and with total brain Cho/Cre at  $r = -0.34$ ,  $p = 0.053$ . In our prior study of a subsample of the present subjects (Weers et al., 2006) analyses demonstrated that the contribution of voxels characterized by obvious neuroradiological abnormality (e.g., shear or hemorrhage) was quite small. Hence, we interpret our MRS data as detecting significant and widespread neurometabolic dysfunction following TBI. That subsample and the current larger sample were equivalent in terms of clinical injury parameters.

Our next set of analyses examined relationships between the four ratios and neuropsychological performance at the time of the initial assessment. Total scores from each of our neuropsychological tests, were adjusted for age and averaged to produce these composites: total, language, motor, visuomotor, and working memory (Weers et al., 2006). Correlations of composites with the four ratios are presented in Table 2 for TBI patients. None of these was significant in controls. In TBI patients, the only ratio not correlated with several aspects of neuropsychological performance was posterior NAA/Cre. Regression analyses were then conducted to determine the proportion of variance in composite scores that could be predicted by anterior NAA/Cre and Cho/Cre, and posterior Cho/Cre measures. For the overall composite, 38% of the variance was accounted for by these three ratios, as compared to 43% for language and 39% for visuomotor domains ( $p < 0.01$  for each). The models predicting motor and working memory composites were not significant.

**TABLE 2. CORRELATION COEFFICIENTS BETWEEN MRS RATIO SCORES AND NEUROPSYCHOLOGICAL PERFORMANCE DOMAINS IN TBI PATIENTS<sup>a</sup>**

|                   | <i>Anterior</i><br><i>NAA/Cre</i> | <i>Anterior</i><br><i>Cho/Cre</i> | <i>Posterior</i><br><i>NAA/Cre</i> | <i>Posterior</i><br><i>Cho/Cre</i> |
|-------------------|-----------------------------------|-----------------------------------|------------------------------------|------------------------------------|
| Total performance | 0.58***                           | -0.49**                           | 0.22                               | -0.45*                             |
| Language          | 0.45*                             | -0.50**                           | 0.26                               | -0.62***                           |
| Motor             | 0.26                              | -0.02                             | 0.15                               | -0.10                              |
| Visuomotor        | 0.51**                            | -0.55**                           | 0.23                               | -0.51**                            |
| Working memory    | 0.36                              | -0.35                             | 0.37                               | -0.37                              |

<sup>a</sup>The  $n$ 's range between 23 and 28.

\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

T1

T2

Given these relationships of neurometabolites with neuropsychological performance, longitudinal analyses of neurometabolite changes are of great interest. Such analyses are complicated, however, by variability from case to case in the extent of the delay before the first assessment and in the intervals between follow-up assessments. Our prior analyses with adults (Brooks et al., 2000) demonstrated the importance of conducting within subject, rather than cross-sectional analyses. We thus constructed three different within-subject comparisons over progressively longer time frames, each beginning at approximately 3.5 weeks post-injury and extending to 5, 13, and 24 weeks post-injury, respectively. Because some participants were evaluated on multiple occasions, a few were utilized in analyses of more than one time frame. These data are shown in Table 3. No significant changes in neurometabolite changes were evident over the shorter two intervals. However, in the longest time frame, both anterior measures demonstrated significant improvement: anterior NAA/Cre increased and anterior Cho/Cre decreased. The posterior ratios did not differ.

T3

DISCUSSION

A major issue affecting interpretation of the present results is reliance upon ratio scores rather than absolute concentrations. The use of ratios has both advantages and disadvantages. The major limitation is the inherent ambiguity in interpretation of a variable determined by two different values. There is substantial reason to believe, however, that our results reflect the importance of the numerators (NAA, Cho), rather than the denominator (Cre). Two recent studies in children find no effect of TBI on Cre (Parry et al., 2004; Hunter et al., 2005). Further, the NAA/Cre ratio was reduced in TBI, whereas the Cho/Cre ratio was increased; if the observed group differences

were driven by the denominator, effects for each ratio would be expected to be in the same direction. An advantage of ratio scores stems from the fact that both numerators and denominators are equally affected by the CSF fraction in the voxel, which can vary with extent of edema or lesion. Voxel to voxel variations in these common pathologies will have a very limited effect on ratio scores, ensuring that our overall results are not overly influenced by discrete regional pathologies.

In the subsample described in Weers et al. (2006) (comprising 42% of the current sample), 28% of the TBI patients had visible hemorrhagic injury at the level at which we conducted SI, and in these patients, lesions represented only a small proportion of the voxels studied. Neurometabolite ratios from normal-appearing versus hemorrhagic lesions have recently been compared in children with TBI (Holshouser et al., 2005). NAA/Cre did not differ, though Cho/Cre was increased in hemorrhagic voxels. Thus, it is most likely that group differences in NAA/Cre do not stem from the impact of discrete lesions, but instead reflect widespread neurometabolic abnormality in normal-appearing brain tissue.

Two of our observations are quite consistent with prior MRS studies. First, neurometabolite changes in both anterior and posterior halves of the supraventricular MRS slab were noted, reflecting diffuse brain injury. NAA/Cre was reduced and Cho/Cre increased. Second, these neurometabolite concentrations were generally quite predictive of overall neuropsychological performance. Implications of these observations were discussed in our earlier reports (Brooks et al., 2000; Friedman et al., 1998; Weers et al., 2006). However, the present report provides evidence of two additional observations that deserve further attention. We provide greater specificity on the neurometabolite-neuropsychology relationships by examining more discrete neuropsychological component scores and neurometabolite ratios from two distinct brain re-

TABLE 3. LONGITUDINAL COMPARISONS OF CHANGES IN NEUROMETABOLITE RATIOS IN TBI PATIENTS OVER THREE DIFFERENT TIME FRAMES (SHORT, MEDIUM, LONG)

|                 | <i>Weeks to 1st assessment</i> |           | <i>Weeks to 2nd assessment</i> |           | <i>N</i> | <i>Anterior NAA/Cre</i> |           | <i>Anterior Cho/Cre</i> |           | <i>Posterior NAA/Cre</i> |           | <i>Posterior Cho/Cre</i> |           |
|-----------------|--------------------------------|-----------|--------------------------------|-----------|----------|-------------------------|-----------|-------------------------|-----------|--------------------------|-----------|--------------------------|-----------|
|                 | <i>M</i>                       | <i>SD</i> | <i>M</i>                       | <i>SD</i> |          | <i>M</i>                | <i>SD</i> | <i>M</i>                | <i>SD</i> | <i>M</i>                 | <i>SD</i> | <i>M</i>                 | <i>SD</i> |
| Short interval  | 3.50                           | 2.81      | 1.50                           | 2.46      | 13       | 0.02                    | 0.15      | -0.02                   | 0.05      | -0.05                    | 0.13      | 0.01                     | 0.03      |
| Medium interval | 3.48                           | 2.81      | 9.29                           | 2.73      | 14       | -0.02                   | 0.16      | -0.01                   | 0.03      | -0.01                    | 0.07      | -0.01                    | 0.04      |
| Long interval   | 3.28                           | 2.61      | 21.22                          | 6.22      | 9        | 0.13*                   | 0.14      | -0.02*                  | 0.02      | -0.04                    | 0.12      | 0.00                     | 0.04      |

\**p* < 0.05.

Neurometabolite values represent changes from the first time point to the second. Significance values of difference scores were determined by paired-comparison *t*-tests.

gions. Also, we demonstrate recovery of anterior, but not posterior, NAA/Cre and Ch/Cre concentrations over a time interval from 3 to 24 weeks post-injury.

Our data suggest that some neuropsychological measures are more highly correlated with neurometabolite ratios than others. Overall performance, as well as language and visuomotor skills, were positively correlated with NAA/Cre and negatively correlated with Cho/Cre. Our working memory composite showed slightly weaker correlations with the ratios, in the same directions, but these trends did not reach significance. More notably, motor skills were not related to either ratio. The absence of a relationship might reflect the location of data acquisition. It is possible that cognitive functions engage more of the tissue sampled than do the specific motor tasks making up our motor composite (left and right hand performance on both Strength of Grip and Grooved Pegboard Test). In future analyses, we hope to investigate much more localized neurometabolite ratios (e.g., left prefrontal white matter) and lateralized performance measures.

Perhaps the most important observations emerging from the current study concern neurometabolite recovery. Bergsneider and colleagues (2001) reported that metabolic recovery begins at approximately 1 month in TBI patients, while Yamaki et al. (1996) describe maximal changes occurring at 2–6 months. These estimates are quite consistent with the current data. We interpret the change in neurometabolite status at 24 weeks as evidence of clinical recovery, but this interpretation presumes that there is no change in neurometabolite concentrations over the same time interval in normal children. Longitudinal analysis of the control group, which we did not undertake, would have allowed an unambiguous evaluation of this possibility. However, prior studies of developmental changes during childhood reveal very negligible neurometabolite change over this time window (Kreis et al., 1993). Further, in our control sample age was not correlated with any neurometabolite ratio.

Our prior findings (Brooks et al., 2000) emerged from absolute quantitation studies of single posterior voxels (bilateral occipital gray matter, left occipito-parietal white matter) in a small sample of adults. No Cho improvement was noted at 1.5–6 months in either voxel. NAA also did not differ over time in the gray matter voxel, but concentration increased in the white matter voxel. Several factors make direct comparison with the current results difficult, including neuroanatomic issues reflecting the specific regions sampled, the use of ratios versus absolute quantitation, and, importantly, the study of children as opposed to adults. In children, we do not now find any significant posterior changes in either ratio. The NAA/Cre increase in the anterior, predominantly white matter com-

partment is, however, in the same direction as our prior results for occipito-parietal white matter. The recovery of anterior, but not posterior neurometabolites, could potentially reflect greater anterior brain injury, but initial NAA/Cre and Cho/Cre ratios were approximately equally affected in anterior and posterior compartments (Table 1). In future analyses, we will attempt to determine the specific role that age might play in regional variation in recovery. With additional data points, we will also be able to adequately analyze covariance of neuropsychological and neurometabolic changes.

The observed decrease in anterior Cho/Cre over time is to our knowledge the first report of significant change in patients with TBI. Persistent Cho elevations have been associated with poor outcomes following TBI in adults (Brooks et al., 2000). Two different mechanisms may possibly underlie the Cho/Cre decrease: reduced inflammation and reduced myelin turnover. The Cho signal in <sup>1</sup>H-MRS is thought to reflect inflammation and processes of membrane breakdown and repair (Sappey-Marinier et al., 1992). Macrophage activity associated with inflammation, observed as early as 96 h and as long as 240 days after human TBI (Gentleman et al., 1999), contributes to the <sup>1</sup>H-MRS Cho signal. Cho concentration also increases with greater availability of myelin precursors and breakdown products (Davie et al., 1994). Reduction in the Cho/Cre ratio may thus reflect normalization of white matter maintenance processes. In contrast, increased NAA/Cre most likely reflects recovery from metabolic depression, as we suggested earlier (Brooks et al., 2000). Integration of human clinical studies with animal models of brain injury will be critical in the identification of such underlying mechanisms of change.

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← PE1

← AU3

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**YEO**

**AU1**

**We need brief section on “Statistical Analysis”—this one OK?**

**AU2**

**For Bergsneider et al. ref., please clarify article page range.**

**AU3**

**For Weers et al. (2006) ref., please provide update.**

**PE1**

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