An Investigation of Neuronal Integrity in Severe Paediatric Traumatic Brain Injury*

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ABSTRACT

Magnetic Resonance Spectroscopy (MRS) and its association with neuropsychological functioning was examined in the chronic injury phase of paediatric traumatic brain injury (TBI). Fifteen children, aged 10–16 years, with severe TBIs were compared with 15 controls, matched for age and gender. The TBI group was found to have significantly lower levels of N-acetyl aspartate (NAA) and Choline (Cho) in the right frontal lobe and generally displayed reduced performances on neuropsychological tests. A correlation between metabolites and reaction times was also obtained. Findings indicate a role of proton MRS as a measure of neuronal integrity following severe paediatric TBI and suggest a potential association of MRS with specific neuropsychological impairments.

Traumatic brain injury (TBI) is a leading cause of death and a common cause of illness and disability in the community (Ponsford, 1995). Estimates of incidence rates indicate that approximately 300 per 100,000 children sustain traumatic brain injuries in the United States each year (Fletcher, Ewing-Cobbs, Francis, & Levin, 1995).

Delineation of the nature and severity of injury in TBI populations directs decisions regarding management and rehabilitation. To date computerised tomography (CT) and magnetic resonance imaging (MRI) have been among the most common neuroimaging techniques employed for this purpose. Recent histopathological studies have, however, highlighted the limited sensitivity of these methods in detecting the extent of neuropathological involvement following TBI (Jones et al., 1998).

Over the past decade a relatively new neuroimaging technique, Magnetic Resonance Spectroscopy (MRS) has been increasingly applied within a number of clinical populations and has provided enhanced understanding of the pathogenesis of a variety of neurological disorders (Novotny, Ashwal, & Shevell, 1998; Tzika, Vigneron, Ball, Dunn, & Kirks, 1993). On the basis of such research, MRS has been proposed to have increased sensitivity and may therefore improve long term predictions regarding outcomes in TBI.

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populations (Brooks, Friedman, & Gasparovic, 2001; Garnett, Cadoux-Hudson, & Styles, 2001).

MRS is a non-invasive technique that is performed with existing MRI technology combined with software adaptations. Signals, acquired from individual nuclei, assess the intracellular metabolic status of specific cellular compounds. Proton, one of the most commonly studied nuclei to date, typically provides information regarding N-acetylaspartate (NAA), choline containing compounds (Cho) and creatine/phosphocreatine (Cre) (Danielsen & Ross, 1999).

The sensitivity of proton MRS in detecting neuronal injury following TBI has been investigated in a number of studies, predominantly with adult populations. There has, however, been considerable variation in research design. The area of interest has included either normal appearing gray or white matter, or both, and has been placed within a number of different brain regions at varying times post-injury (acute, subacute and chronic).

Nevertheless the most consistent finding reported in the literature has been a significant reduction in the absolute concentration of NAA or in the ratio of NAA/Cre in adult TBI populations compared to age-matched controls (Brooks et al., 2000; Garnett et al., 2001; Garnett, Blamire, Corkill, et al., 2000). The absolute and relative concentration of Cho and Cre in adult TBI patients compared to control populations has been variable with some studies reporting significant elevations in these metabolites whilst other studies have reported non-significant group differences (Cecil et al., 1998; Garnett et al., 2001; Garnett, Blamire, Corkill, et al., 2000; Ross et al., 1998).

Of the two studies to date to specifically investigate metabolic sequelae within paediatric TBI cohorts, variable findings have been obtained. One study of 26 infants and 27 children with subacute closed head injuries reported significant reductions in NAA/Cre and NAA/Cho ratios in addition to significantly increased Cho/Cre ratios in normal appearing occipital gray matter in both the infants and the children who demonstrated worse outcomes on the Paediatric Cerebral Performance Category Scale (PCPCS; Ashwal et al., 2000).

In a similar study, proton MRS data was collected subacutely from the occipital gray matter in 11 infants and 11 children who had sustained closed head injuries and compared with PCPS scores obtained from one to seven years post-injury (Brenner, Freier, Holshouser, Burley, & Ashwal, 2003). A significant reduction in occipital gray matter NAA/Cho was obtained in the infants with worse chronic outcomes while Cho/Cre was reduced in the group of older children with poor outcomes. No other significant metabolite ratio differences were obtained.

The predictive value of proton MRS in providing information regarding long-term outcomes has been investigated in a number of studies. Associations have been found between clinical rating scales, such as the Glasgow Coma Scale (GCS; Jennett & Bond, 1975) and the Glasgow Outcome Scale (GOS; Wilson, Pettigrew, & Teasdale, 1998), and metabolite results collected in adult TBI populations at varying times post-injury and from differing brain regions (Choe et al., 1995; Garnett, Blamire, Rajagopalan, et al., 2000; Sinson et al., 2001). A similar association within a paediatric population has also been reported (Ashwal et al., 2000).

The relationship between proton MRS metabolite concentrations and neuropsychological functioning following TBI has been examined, again primarily with adult cohorts. These studies have generally reported statistically significant associations between neuropsychological test performances, as measured by composite scores, and various metabolites acquired from differing brain regions (Brooks et al., 2000; Friedman et al., 1999; Friedman, Brooks, Jung, Hart, & Yeo, 1998).

In paediatric populations, the association between proton MRS and neuropsychological performance was only recently investigated by Brenner et al. (2003). In this study of 22 infants and children who had sustained a TBI of varying severity, a correlation was obtained between acute occipital gray matter metabolite compounds, including NAA/Cho and Cho/Cre and long-term neuropsychological performance. Cho/Cre and NAA/Cho were also found to be predictive of neuropsychological functioning across a number of cognitive domains.

The aim of the current study was to further investigate the sensitivity of proton MRS within a
paediatric TBI population who were in the chronic recovery phase from a severe TBI. The association between MRS measures and cognitive functioning was also examined to determine whether metabolites were related to specific neuropsychological deficits. A control group, matched for age and gender, was included to account for reported developmental changes in metabolites (Tzika et al., 1993; van der Knapp, van der Grond, & van Rijen, 1990).

METHODS

Participants

A sample of 19 patients who had sustained a severe TBI was referred from the Rehabilitation Department of The Children’s Hospital at Westmead between November 2001 and November 2002. Children were identified during a review clinic appointment and were selected on the basis of the following criteria: aged between 10 and 16 years at the time of the clinic appointment; GCS score of nine or less on hospital admission; evidence of neurological abnormalities on a previous MRI scan; and sufficient language and sensorimotor abilities to perform the selected neuropsychological battery. A group of 19 controls who were siblings and relatives of the TBI group were studied for comparison. Those selected were aged between 10 and 16 years at the time of the study, had no history of a neurological illness, a neurologic insult, such as a TBI, or of a documented learning disorder.

The study conformed to guidelines on research involving human subjects specified by the Australian National Health and Medical Research Council and was approved by the institutional ethics committee. Informed consent was obtained from patients and their families prior to participation. All participants were paid an honorarium of $50.00 (Australian).

Procedures

MRS

The neuroimaging procedure was explained in detail to all subjects and their carers prior to undertaking the scan. The subjects were placed in a dedicated head coil (Philips 1H spectroscopy, Quadrature, send/receive). The head was supported with foam pads on either side, and a band was placed across the forehead and attached to the head support in the coil. A mirror was placed on the coil so that the subjects were able to see outside the tunnel. Music was played to the subjects during imaging and MRS acquisition. If requested, a family member or other support person was in the scan room with the subject to improve confidence and relaxation. Open communication was also available via intercom, and all subjects were monitored visually by closed circuit TV and direct viewing. The procedure lasted for approximately 30 min.

1H MRS spectra were acquired at 1.5 T using a birdcage quadrature head coil and a PRESS pulse sequence (Philips ACS-NT 1.5 T). Proton spectra were obtained from three regions within the brain that are commonly involved following TBI. Firstly spectra were acquired from the right frontal region (8 cc voxel, TR 2.5 s, TE 136 ms, scan time 2 min 45 s) on the basis of control paediatric data that has indicated that there are no significant group differences between the right and left frontal white matter (C. Rae, personal communication, 10 December 2002). Further spectra were acquired from the splenium of the corpus callosum (3 cc voxel, TR 2 s, TE 30 ms, scan time 4 min 20 s) and the left hippocampus gyrus (VOI 30 cm × 10 cm × 10 cm, TR 2 s, TE 30 ms, scan time 4 min 20 s). Spectra without water suppression were also acquired from these regions for use as an internal intensity reference.

Within the right frontal white matter the voxel was placed to fit within the cortical white matter and the anterior horn of the right lateral ventricle (Fig. 1). The voxel for the splenium of the corpus callosum was positioned to fit within the body of the splenium while

![Fig. 1. Placement of the proton MRS volume of interest in the right frontal white matter, on an axial T1-weighted image.](image-url)
the left hippocampal voxel was angled along the length of the hippocampus. Irrespective of subject position in the coil, the voxel was rotated to sample the same region of interest in all subjects. Attempts were made to avoid gray matter in all planes.

Spectra were processed using jMRUI (version 1.0) (Naressi et al., 2001; Vanhamme, van den Boogaart, & Van Huffel, 1997). Quantification of the reconstructed signals was performed in the Time-domain. AMARES (Vanhamme et al., 1997) was used to fit the resonances of NAA, Cho and Cr following removal of the residual water signal with HLSVD (Pijnappel, van den Boogaart, de Beer, & van Ormondt, 1992). In addition, a Lorentzian lineshape was also fitted to the resonance arising from water in the spectrum without water suppression. Results were expressed as peak ratios and also as concentrations relative to the water resonance (in arbitrary units, without correction for relaxation or number of scans). Metabolite concentrations (in arbitrary units) were calculated relative to the water resonance, which was assumed to be at a concentration of 80 M.

Severe metabolic injury to the area of interest and subject movement prevented the collection of adequate spectra in the splenium of the corpus callosum in a large number of the TBI subjects. Technical difficulties also impacted on collection of spectra from the left temporal lobe. Consequently, only data from the right frontal lobe is reported in this study.

Right frontal lobe spectra were not obtained from four of the 19 TBI patients. Consequently these subjects were also excluded from all analyses. The Cho peak was unable to be quantitated in one TBI patient who was included in the analyses.

Clinical Indices
GCS scores on admission, the estimated duration of Post Traumatic Amnesia (PTA) (Marosszeky et al., 1993) and clinical MRI reports were obtained from medical records.

 Neuropsychological Testing
Intelllectual abilities were assessed with a shortened version of the Wechsler Intelligence Scale for Children – Third Edition (WISC-III; Wechsler, 1991). A prorated verbal reasoning index score (VIQ) was based on the Vocabulary and Similarities subtests while a non-verbal reasoning index (PIQ) was prorated from performance on the Picture Completion and Block Design subtests. An overall prorated full scale intelligence quotient (FSIQ) was calculated from the performance on the four subtests. Three children were, however, assessed with the Differential Ability Scales (DAS) (Elliot, 1990). In these cases, DAS Verbal, Non-verbal and Spatial Cluster Scores were converted into VIQ, PIQ and FSIQ scores, on the basis of the correlations between these indices that are reported in the WISC-III manual (Wechsler, 1991).

General memory abilities were investigated with the Children’s Memory Scale (CMS; Cohen, 1997). Index scores examined included the General Memory Index, Immediate Visual Memory Index, the Immediate Verbal Memory Index and the Delayed Verbal Memory Index.

Executive functions and reaction time were examined with selected subtests from the Attention/Executive domain from the Cambridge Neuropsychological Test Automated Battery (CANTAB; CeNes, 1999). The subtests administered included the Intra/Extradimensional set shift, Matching to Sample, Reaction Time (RT), Rapid Visual Processing and the Stockings of Cambridge.

Where practical the full assessment was administered as close to the time of the MRS scan as possible. In some cases, data was taken from assessments recently conducted in the Rehabilitation Department of The Children’s Hospital at Westmead so ongoing rehabilitative management was not compromised.

Four of the control subjects who demonstrated superior range performances on neuropsychological tests were not included in the study as they were considered to be unrepresentative of the normal population. Inclusion would also have distorted subsequent analyses of group differences.

Data Analysis
The sample studied consisted of 15 TBI patients (mean age 14 years, range 10–16) and 15 control subjects (mean age 13 years, range 10–16). Metabolite ratios and neuropsychological test scores of patients and controls were compared with the Mann–Whitney U test. Effect sizes, calculated with Cohen’s d were performed on the raw data of all comparisons (Zakzanis, 2001). A corresponding overlap statistic (OL%), referring to the amount of test measure overlap between the two samples, was also reported.

Spearman correlation analyses were subsequently undertaken to investigate the relation between MRS metabolite ratios and neuropsychological test performance. Initially within group non-parametric correlations were performed followed by pooled non-parametric correlations.

RESULTS

Demographics
Demographic data for the 15 TBI patients are shown in Table 1. The primary cause of injury within this group were motor vehicle accidents (13/15). The remaining two children sustained
TBI from falls. Age at TBI varied from 1 year 9 months to 12 years 9 months (mean = 7.9 ± 3.6). GCS scores recorded on hospital admission were within the moderate to severe range (GCS = 9, mean = 5.6 ± 2.4) for 14 of the children. One child did not have a GCS score recorded. Estimations of the duration of PTA varied from 10 to 67 days (mean = 29.6 ± 16.3). It is, however, noted that three of the children were unable to be formally assessed with the Westmead PTA scale as they were under the age of 4 years at the time of the injury. Furthermore, those who were aged between 5 and 8 years when injured were assessed with a modified version of the Westmead PTA scale. Clinical reports from MRI studies documented widespread injuries, involving the frontal lobe (n = 13), the temporal lobe (n = 6), the parietal lobe (n = 3), the cerebellum (n = 3) and the corpus callosum (n = 5). Evidence of cerebral atrophy was also noted in some reports (n = 5). MRS studies were conducted in the chronic post-injury phase (mean = 5.7 ± 4.7 years).

MRS – Metabolite Concentrations

Metabolite concentrations and ratios acquired from the right frontal lobe, in addition to effect sizes and %OL are shown in Table 2. Both the NAA and Cho concentrations were significantly reduced in the TBI group compared to the controls. The associated effect sizes indicated that approximately 80% of the TBI patients obtained concentrations of NAA unlike those obtained by the controls while approximately 70% of the TBI patients obtained distinct concentrations of Cho. Absolute Cre did not differ significantly between groups.

The NAA/Cre and the Cho/Cre ratios were also significantly reduced in the TBI group. NAA/Cho

| Table 1. Demographic Data for the 15 Paediatric TBI Patients. |
|---|---|---|---|---|---|
| Gender | Cause of TBI | Age at injury (years month) | GCS admission (3–15) | PTA (days) | Time since injury (years month) | GOS-E |
| 1 | F | Fall | 12.4 | 8 | 27 | 1.1 | 3 |
| 2 | F | Fall | 4.1 | 9 | na | 12.0 | 3 |
| 3 | M | MVA | 5.1 | 6 | 21 | 11.1 | 4 |
| 4 | F | MVA | 10.3 | 3 | 49 | 4.1 | 3 |
| 5 | F | MVA | 1.9 | 3 | na | 11.0 | 6 |
| 6 | M | MVA | 7.4 | 6 | 18 | 9.0 | 5 |
| 7 | M | MVA | 11.8 | 5 | 21 | 0.3 | 3 |
| 8 | M | MVA | 6.10 | na | 35 | 9.0 | 5 |
| 9 | M | MVA | 3.10 | 9 | na | 12.0 | 6 |
| 10 | F | MVA | 12.1 | 3 | 23 | 2.0 | 8 |
| 11 | M | MVA | 9.7 | 8 | 10 | 1.0 | 5 |
| 12 | M | MVA | 12.9 | 3 | 14 | 1.0 | 4 |
| 13 | F | MVA | 7.11 | 8 | 42 | 7.9 | 3 |
| 14 | M | MVA | 7.2 | 3 | 28 | 3.1 | 3 |
| 15 | F | MVA | 12.04 | 5 | 67 | 1.0 | 4 |

Note. MVA: motor vehicle accident; GCS 3–15; GOS-E rating scale eight categories, with a score of 1 corresponding to a persistent vegetative state, 2 with a to a lower severe disability and a score of 8 to a upper good recovery; na: not available given the age of the child at time of injury.

Table 2. Right Frontal NAA/Cre, NAA/Cho; Cho/Cre Ratios and Concentrations of NAA, Cho, Cre for the TBI Group and the Controls.

<table>
<thead>
<tr>
<th>TBI group</th>
<th>Controls</th>
<th>Cohen’s d</th>
<th>%OL</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA</td>
<td>6.5 (2.3)**</td>
<td>9.9 (1.1)</td>
<td>2</td>
</tr>
<tr>
<td>Cho</td>
<td>3.3 (1.1)**</td>
<td>5.0 (1.3)</td>
<td>1.5</td>
</tr>
<tr>
<td>Cre</td>
<td>3.6 (1.2)</td>
<td>3.9 (0.9)</td>
<td></td>
</tr>
<tr>
<td>NAA/Cre</td>
<td>2.6 (0.5)**</td>
<td>1.9 (0.7)</td>
<td>1.3</td>
</tr>
<tr>
<td>NAA/Cho</td>
<td>2.3 (0.6)</td>
<td>2.1 (0.5)</td>
<td></td>
</tr>
<tr>
<td>Cho/Cre</td>
<td>0.9 (0.3)**</td>
<td>1.4 (0.5)</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Note. One-tailed tests of significance where, *p < .05, **p < .01. %OL: percent overlap.
ratio did not differ significantly between groups. The effect sizes indicated that approximately 65% of the TBI group obtained distinct NAA/Cre ratios and approximately 60% of the TBI group obtained distinct Cho/Cre ratios.

**Clinical Outcomes**
The relationship between clinical measures of injury severity and right frontal metabolite ratios were investigated within the TBI group. NAA concentrations were found to be positively correlated with admission GCS scores ($r_s = .528$, $p < .05$). PTA was not significantly correlated with any of the right frontal metabolite concentrations or ratios.

**Age Related Factors**
Within the TBI group, age at the time of injury and the time since injury were significantly correlated ($r_s = -.870$, $p < .05$). When these variables were compared to right frontal metabolite ratios and concentrations, NAA was found to be negatively correlated with age at injury ($r_s = -.554$, $p < .05$). No other significant correlations were obtained.

The TBI group was further divided into groups according to age at injury. Those who were less than 7 years of age at the time of injury ($n = 7$) were found to have a significantly higher right frontal mean NAA value than those who were older than 7 years of age at the time of injury ($n = 8$) mean = 8.0 ± 1.5 and mean = 5.2 ± 2.1 respectively, $p = .006$. The mean NAA of the group who were less than 7 years at the time of injury was, however, significantly lower than the control group ($p = .04$).

**Neuropsychological Functioning**

**Intellectual Functioning (WISC-III)**
The pro-rated WISC-III Index Scores, group effect sizes and the %OL are provided in Table 3. Overall, the mean FSIQ, VIQ and PIQ scores of the TBI group were within the borderline range of functioning, significantly lower than the mean average range performance of the control group. The obtained effect sizes indicated distinct population separations of approximately 80% for the FSIQ and VIQ indexes and 65% for the PIQ index.

**Memory Abilities (CMS)**
The mean CMS index scores, effect sizes and the %OL are also reported in Table 3. The mean scores of the TBI group were placed within the borderline to low average range of functioning while the mean performance of the control group fell within the average to high average range. These differences were significant for all indices including the General Memory Index, Immediate Visual Memory Index, Delayed Visual Memory Index, Immediate Verbal Memory Index and the Delayed Verbal Memory Index. The obtained effect sizes indicated distinct population separations of approximately 80% for the General Memory Index, approximately 85% for the

<table>
<thead>
<tr>
<th></th>
<th>TBI patients mean (SD)</th>
<th>Control mean (SD)</th>
<th>Cohen’s d</th>
<th>%OL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WISC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full Scale IQ</td>
<td>77 (20)**</td>
<td>103 (7)</td>
<td>1.9</td>
<td>20.6</td>
</tr>
<tr>
<td>Verbal IQ</td>
<td>79 (17)**</td>
<td>104 (11)</td>
<td>1.8</td>
<td>22.6</td>
</tr>
<tr>
<td>Performance IQ</td>
<td>80 (23)**</td>
<td>102 (11)</td>
<td>1.3</td>
<td>34.7</td>
</tr>
<tr>
<td><strong>CMS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>General Memory Index</td>
<td>75 (22)**</td>
<td>106 (10)</td>
<td>1.9</td>
<td>20.6</td>
</tr>
<tr>
<td>Visual Immediate Index</td>
<td>85 (17)*</td>
<td>98 (10)</td>
<td>1.0</td>
<td>44.6</td>
</tr>
<tr>
<td>Visual Delayed Index</td>
<td>84 (17)*</td>
<td>97 (13)</td>
<td>0.9</td>
<td>48.4</td>
</tr>
<tr>
<td>Verbal Immediate Index</td>
<td>78 (18)**</td>
<td>110 (9)</td>
<td>2.3</td>
<td>13</td>
</tr>
<tr>
<td>Verbal Delayed Index</td>
<td>80 (18)**</td>
<td>109 (14)</td>
<td>2.2</td>
<td>15.7</td>
</tr>
</tbody>
</table>

*Note. One-tailed tests of significance where, *$p < .05$, **$p < .01$. %OL: percent overlap.*
Immediate Verbal Memory Index and the Delayed Verbal Memory Index indexes and approximately 50% for the Immediate Visual Memory Index and the Delayed Visual Memory Index.

**CANTAB – Intra/extra Dimensional Shift Set**
The mean scores of the TBI group and the control group on all components of the Intra/Extradimensional Set Shift test were within the average range and consequently no significant group differences were obtained.

**CANTAB – Matching to Sample**
The mean score obtained by both groups on the main measure of the Matching To Sample task were within normal limits. A significant group discrepancy was not obtained.

**CANTAB – RT**
The mean performance on the main measures from the RT subtest, effect sizes and the %OL are reported in Table 4. Overall, the mean performance of the TBI group was significantly lower than that of the control group on all measures including the Simple Reaction Time, Simple Movement Time, 5-Choice Reaction Time and the 5-Choice Movement time. The obtained effect sizes indicated distinct population separations of approximately 30% for the Simple Reaction Time score, approximately 60% for the Simple Movement Time and 5-Choice Reaction Time scores and 65% for the 5-Choice Movement Time score.

**CANTAB – Rapid Visual Processing**
The mean performance on the main measures of the rapid visual processing subtest, the associated effect sizes and the %OL are also reported in Table 4. The mean score of the TBI on the Total Correct Rejections, Probability of a Hit and Total Hits measures were significantly lower within the TBI group. The obtained effect sizes indicated distinct population separations of approximately 30% for the Total Correct Rejections score and approximately 50% for both the Probability of a Hit and the Total Hits scores.

**CANTAB – Stockings of Cambridge**
The mean performance on the main measure of the Stockings of Cambridge subtest, the associated effect size and the %OL are also reported in Table 4. The overall measure of number of Problems Solved in the Minimum Number of Moves was significantly reduced in the TBI group compared to the controls. The obtained effect size indicated a distinct population separation of approximately 50%.

**Clinical Outcomes**
Clinical measures of injury severity, including GCS and PTA were not significantly correlated with any of the neuropsychological test scores.

### Table 4. Mean Z Scores (SD) of the Control Group and the TBI Patients on CANTAB.

<table>
<thead>
<tr>
<th></th>
<th>TBI mean (SD)</th>
<th>Controls mean (SD)</th>
<th>Cohen’s d</th>
<th>%OL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simple reaction time</td>
<td>−0.6 (1.6)**</td>
<td>0.3 (1.9)</td>
<td>0.5</td>
<td>66.6</td>
</tr>
<tr>
<td>Simple movement time</td>
<td>−0.4 (1.6)**</td>
<td>0.9 (0.8)</td>
<td>1.1</td>
<td>41.1</td>
</tr>
<tr>
<td>5-Choice reaction time</td>
<td>−0.2 (1.3)**</td>
<td>0.9 (0.7)</td>
<td>1.1</td>
<td>41.1</td>
</tr>
<tr>
<td>5-Choice movement time</td>
<td>−0.9 (1.5)**</td>
<td>0.5 (0.8)</td>
<td>1.3</td>
<td>34.7</td>
</tr>
<tr>
<td><strong>RVP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total correct rejections</td>
<td>−2.9 (0.7)**</td>
<td>−2.6 (0.6)</td>
<td>0.5</td>
<td>66.6</td>
</tr>
<tr>
<td>Probability of a hit</td>
<td>−2.0 (1.2)*</td>
<td>−0.9 (1.4)</td>
<td>0.8</td>
<td>52.6</td>
</tr>
<tr>
<td>Total hits</td>
<td>−2.4 (0.5)*</td>
<td>−1.9 (0.6)</td>
<td>0.9</td>
<td>48.4</td>
</tr>
<tr>
<td><strong>Stockings of Cambridge</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Problems solved in min moves</td>
<td>−0.4 (1.1)*</td>
<td>0.5 (1.1)</td>
<td>0.8</td>
<td>52.6</td>
</tr>
</tbody>
</table>

*Note.* RT: reaction time test; RVP: rapid visual processing test; Stockings of Cambridge: stockings of Cambridge test. One-tailed tests of significance where, *p < .05, **p < .01. %OL: percent overlap.
**Age Related Factors**
Age at injury and time since injury were not significantly correlated with any of the neuropsychological test variables.

**MRS and Neuropsychological Functioning**
The metabolite ratios and concentrations were compared with the neuropsychological performance within groups and with the groups pooled.

*Within Group Correlations – TBI Group*
Metabolite concentrations and ratios were not significantly correlated with tests of general intellectual functioning or memory abilities within the TBI group. Analysis of the association between the Executive/Attention subtests from the CANTAB battery and the MRS results, however, revealed positive correlations between NAA and the 5-Choice Movement Time measure ($r_s = .61, p < .01$) and also between NAA/Cre and the 5-Choice Reaction Time measure ($r_s = .58, p < .05$) from the RT subtest (Fig. 2). Two positive correlations were additionally obtained between NAA/Cho and Rapid Visual Processing measures, including Probability of a Hit ($r_s = .64, p < .05$) and Total Misses ($r_s = .64, p < .05$).

*Within Group Correlations – Control Group*
Metabolite concentrations and ratios were not significantly correlated with the control groups performance on tests of general intellectual functioning or memory ability. Some significant associations with CANTAB measures were, however, obtained. Specifically, Cho/Cre was found to be correlated with the 5-Choice Movement Time ($r_s = .80, p < .01$) (Fig. 3) and the Simple Movement Time measures ($r_s = .75, p < .01$) from the RT subtest. NAA/Cho was also correlated with the RT measures, including the 5-Choice Movement Time ($r_s = .84, p < .01$), 5-Choice Reaction Time ($r_s = .53, p < .05$), Simple Movement Time ($r_s = .78, p < .01$) and the Simple Reaction Time measure ($r_s = .57, p < .05$).

*Pooled Correlations*
The groups were combined to investigate the effect of an increased sample size on the association between MRS findings and neuropsychological test performance. Corrections for group mean differences, which can result in spurious correlations, were made. This involved calculating a grand sample mean of the entire sample from which the performance of the individual subjects was subtracted. The association between the correlation coefficients was also examined to ensure that they did not differ significantly from one another.

Significant associations between intellectual and memory indexes were not obtained within the corrected pooled sample. Statistically significant
correlations among MRS results and measures from the RT subtest were, however, found. These are reported in Table 5.

DISCUSSION

TBIs are a major cause of mortality and morbidity within Western industrialised communities. Detailed information regarding the severity and nature of injury facilitates decisions regarding management and rehabilitation. Recent research has, however, revealed the limited predictive and clinical utility of a number of measures commonly used in TBI populations, including CT and MRI studies. Following on from these findings, proton MRS has been proposed to be more sensitive to the presence and extent of neuronal injuries and may therefore improve delineation of TBIs in addition with assisting long-term predictions and rehabilitation programs.

To date the majority of studies have investigated the clinical utility of proton MRS within adult TBI cohorts. Comparison of paediatric and adult TBI populations, however, indicates differences in the both the course of recovery and the pattern of neurobehavioural sequelae, which in paediatric cases, can continue to change for an extended period of time after the initial insult (Ponsford, 1995).

This study is among the first to examine the association between biochemical sequelae, clinical measures of injury severity and neuropsychological outcomes within a severe paediatric TBI population.

Biochemical Sequelae

A significant reduction in white frontal NAA and NAA/Cre concentrations in the paediatric TBI group compared to a group of age-matched controls was found. Given the well established role of NAA as a marker of neuronal density and viability, our findings of NAA reductions within the chronic injury phase were considered to reflect long-term neuronal compromise following severe TBI (Cheng, 2001).

A significant reduction in Cho and Cho/Cre was also found within the paediatric TBI group compared to the controls. This finding contrasts with the literature in adult TBI populations which has found either a significant elevation or no discernable group differences in Cho and Cho/Cre collected from various brain regions at differing post-injury stages (Brooks et al., 2000; Cecil et al., 1998; Condon, Olouoch-Olunya, Hadley, Teasdale, & Wagstaff, 1998; Friedman et al., 1999; Friedman et al., 1998; Garnett, Blamire, Corkill, et al., 2000; Garnett, Blamire, Rajagopalan, et al., 2000; Garnett et al., 2001; Ross et al., 1998).

In adult populations, increases in Cho have been proposed to reflect degenerative or neuronal repair processes that occur following injury (Brooks et al., 2001). In the current severe TBI paediatric group examined at the chronic injury phase, the reduction in Cho may reflect a loss of neuronal cells, as evidenced from the obtained reduction in NAA. This would consistent with cerebral atrophy, which is a common neuropathological outcome in the chronic injury phase in both adult and paediatric TBI populations (Bigler, 1999; Blatter, 1997). Further investigation is to be undertaken from examination of the degree of cerebral atrophy within the current TBI group as compared to the volumes of age-matched controls.

These findings also need to be interpreted within the context of a developing system. A number of studies have revealed age related developmental changes within the major intracellular peaks acquired with proton Magnetic Resonance Spectroscopy. Specifically a continuing
increase in NAA/Cre and NAA/Cho and a reduction in Cho/Cre has been documented from the perinatal period throughout early childhood (Tzika et al., 1993; van der Knapp et al., 1990). The most rapid changes in these metabolite values was reported during the first three years of life, however, further changes continuing to at least 16 years have also been found (van der Knapp et al., 1990). Given the varying ages at injury, with some acquired at very young ages, it is therefore possible that the reduction in Cho in the paediatric population reflects the ongoing disruption to this developmental process.

In the paediatric MRS studies that have been conducted to date, variable Cho and Cho/Cre findings have been reported. One study did not obtain a significant subacute difference in absolute concentrations of occipital Cho when comparing the outcomes of infants and children who had all sustained acquired brain injuries. Cho/Cre was, however, significantly increased in both infants and children with worse outcomes (Ashwal et al., 2000). In contrast a later study, with a similar research design but smaller sample sizes, obtained a significant reduction in subacute occipital Cho/Cre in children with worse outcomes (Brenner et al., 2003). Absolute concentrations were not, however, reported.

There are a number of differences between these two studies and the current investigation which could account for the variable findings. A major factor is the time at which the spectra were obtained. In both the Ashwal et al. (2000) and the Brenner et al. (2003) papers spectra were collected in the subacute injury phase. Longitudinal studies within adult TBI populations have revealed changing patterns of biochemical concentrations over time, as a likely result of the differing neuropathological processes which occur at different stages in response to neurological injuries that disrupt cellular neurometabolism (Brooks et al., 2000; Friedman et al., 1999; Garnett, Blamire, Corkill, et al., 2000). Consequently concentrations of Cho/Cre may vary considerably in the early recovery stage as a result of the number of changes that are occurring in response to trauma. The later acquisition date used in the current study may therefore reflect the long-term impact of injury.

The studies also differed in relation to the neuroanatomical region in which the spectra were acquired. A number of group studies with adults, however, have revealed variations in the distribution of metabolites in gray and white matter (Wang & Li, 1998) and also between different anatomical regions (Komoroski, Heimberg, Cardwell, & Karson, 1999). One may also speculate that regional biochemical differences would be more prominent within the developing brain. It is therefore possible that the biochemical concentrations within the right frontal regions, examined in the current study, may differ from those within the occipital lobe which were sampled in the Ashwal et al. (2000) and Brenner et al. (2003) studies.

Finally, varying research design characteristics were employed in these paediatric studies. The populations examined in the Ashwal et al. (2000) and the Brenner et al. (2003) studies had sustained acquired brain injuries that varied in causal mechanism and injury severity. Comparisons were made between different outcomes within this group (good vs. poor) and were not investigated in relation to an age-matched normal control group.

Such variations in MRS protocols, the time of the study (acute, subacute, chronic) and also in the neuroanatomical region sampled are, however, common among MRS studies within TBI populations and seriously limit comparisons between studies.

**Clinical Measures of Injury Severity**

Analyses of the association between MRS findings and clinical measures of injury severity revealed a positive correlation between NAA and admission GCS scores such that lower concentrations of NAA were associated with lower GCS scores. The relatively small sample in which this relationship was found, suggests that analysis of the concentration of NAA in paediatric TBI may be useful as an additional clinical measure of injury severity.

Associations between age-related factors and metabolic status were inconsistent. Those who were younger at the time of injury were found to have higher concentrations of NAA. There was also a strong association between age at injury
and time since injury. Correlations were not, however, found between NAA and time since injury or chronological age at the time of the study. Overall, the only tentative interpretation that can drawn is that NAA in those who were younger at the time of injury and who had a longer amount of time post injury, was not depressed relative to those who acquired injuries later. Therefore it is possible that there was a continuing increase in NAA in the group who were younger at the time of the injury as a result of recovery and also in relation to the changes that have been reported to occur during development (van der Knapp et al., 1990). This rate of increase is likely to be affected following an acquired injury and in fact those who were injured at a younger age were found to have a significantly lower level of NAA as compared to an age-matched control group.

Clinical Measures of Injury Severity and Neuropsychological Outcomes

Neuropsychological test performance was not associated with clinical measures of injury severity, including GCS or PTA. Overall, there was considerable variability in performances given the severity of the injury. For example, among the 5 TBI patients who received admission GCS scores of 3, FSIQ scores ranged from 45 to 107. Given the complex interaction of the multiple factors reported to influence recovery and outcome in paediatric populations, including age at injury, premorbid psychosocial factors, cumulative processes and latent effects (Anderson et al., 1997; Broman & Michel, 1995; Fletcher et al., 1995), it is not surprising that gross clinical measures such as the GCS are not overly sensitive to the specific cognitive sequelae of TBI.

Biochemical Sequelae and Neuropsychological Outcomes

Consistent with the well documented evidence of neuropsychological sequelae found in severe paediatric TBI samples, the mean performance of the TBI group on neuropsychological measures was generally lower than that of the control group. Of note, however, differences between performances on two of the subtests comprising part of the Attention/Executive battery from CANTAB, Intra/Extradimensional Set Shift and Matching To Sample, did not differ significantly between groups, indicating the reduced clinical validity of these measures in severe paediatric TBI populations.

A significant correlation between right frontal metabolites and the estimated FSIQ, VIQ and PIQ was not obtained within either the current TBI or control sample when examined separately or within a corrected pooled group. Given that the biochemical concentration was only sampled within a small neuroanatomical area it was not expected to be associated with global cognitive functioning. Furthermore recent proton MRS studies have reported a relationship between general intellectual abilities and white matter occipitoparietal regions in healthy adults (Jung et al., 1999, 2000). These studies have not, however, examined the association of general intellectual measures within other neuroanatomical regions.

An association between right frontal metabolites and memory abilities was also not obtained in the current TBI or control group. This is consistent with the well established relationship between memory abilities and medial temporal lobe structures (Kolb & Whishaw, 1996). Future proton MRS studies, sampling temporal brain regions would be necessary to determine whether regional biochemical variations are sensitive to performance on neuropsychological measures of memory functioning.

Reductions in processing speed, reaction time and attentional skills are common behavioural concomitants of TBI (Anderson et al., 1997; Fletcher et al., 1995). Furthermore, a composite executive neuropsychological measure was recently found to be significantly associated with left frontal metabolites within a group of 20 healthy elderly adults (Valenzuela et al., 2000). In the current study, the majority of correlations were obtained between right frontal metabolites and RT measures from CANTAB. These variables were all positively correlated, indicating that lower metabolite ratios and concentrations were associated with reduced performances on RT tests. This association suggests the sensitivity of regional biochemical concentrations to specific neuropsychological dysfunction.
CONCLUSIONS

Our study is limited by small sample sizes. Future studies would benefit from utilising larger populations of children with severe TBI. Consideration of the developmental context is also important and forthcoming studies would benefit from controlling for both age at injury and time since injury. This would necessitate increased numbers within different age groups and also a sufficient number of subjects that could be sectioned into groups according to age at injury and times post-injury so that the interaction could be reliably examined.

Technical difficulties prevented the acquisition of spectra from multiple neuroanatomical locations which would have allowed us to further examine the relationship between regional biochemical variations and discrete neuropsychological processes. Future studies will be able to take advantage of recently developed MRS imaging techniques that simultaneously measure a large array of cerebral voxels. This would allow for a comprehensive study of regional intracerebral metabolite concentrations and also further delineate the potential association of MRS measures with specific neuropsychological functions. Comprehensive normative details regarding variations in brain region metabolite concentrations across the lifespan will, however, be necessary to facilitate this process.

The current results reveal the sensitivity of levels of NAA in distinguishing paediatric TBI patients with severe injuries from normal controls. In contrast with reports from adult populations right frontal Cho and Cho/Cre concentrations were found to be significantly reduced in the severe TBI group, indicating the necessity of considering each group separately and evaluating the changes occurring following a TBI in the context of a developing system wherein acquired injuries may disrupt ongoing biochemical processes.

Associations between right frontal metabolites and RT measures were obtained within individual groups and also within corrected pooled samples whereas general intellectual abilities and memory skills were not significantly associated. Overall, this suggests that regional proton MRS measurements may reflect specific cognitive deficits in TBI. Future examination of the specificity of proton MRS techniques with discrete neuropsychological processes in paediatric TBI samples is therefore warranted.

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