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Late Proton Magnetic Resonance Spectroscopy following Traumatic Brain Injury during Early Childhood: Relationship with Neurobehavioral Outcomes

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Abstract

We sought to extend previous research that demonstrates reduced neurometabolite concentrations during the chronic phase of pediatric traumatic brain injury (TBI) in children injured during early childhood. We hypothesized that young children with TBI in the chronic phase post-injury would have lower *N*-acetyl aspartate (NAA) metabolite concentrations in gray and white matter in comparison to controls. We also hypothesized that metabolite levels would be correlated with acute TBI severity and neurobehavioral skills. Ten children with a history of TBI between the ages of 3 and 6 years were compared to an age, gender, and race-matched group of 10 children with a history of an orthopedic injury (OI). Children completed neurobehavioral testing at 12 months post-injury. Proton magnetic resonance (MR) spectroscopy was completed at least 12 months post-injury when the children were 6–9 years old. Groups were compared on metabolite concentrations in the medial frontal gray matter and left frontal white matter. Metabolite levels were correlated with Glasgow Coma Scale (GCS) scores and neurobehavioral functioning. There was a trend for lower NAA concentrations in the medial frontal gray matter for the TBI group. Late NAA and Cr levels in the medial frontal gray matter and NAA levels in the left frontal white matter were strongly positively correlated with initial GCS score. Metabolite levels were correlated with some neurobehavioral measures differentially for children with TBI or OI. Some neurometabolite levels differed between the TBI and OI groups more than 1 year post-injury and were related to injury severity, as well as some neurobehavioral outcomes following TBI during early childhood.

Keywords

early childhood; neurobehavioral outcomes; proton MR spectroscopy; traumatic brain injury

INTRODUCTION

Traumatic Brain Injury (TBI) in young children is a leading cause of lifelong disability. Approximately 160 per 100,000 children under the age of 5 years suffer a TBI. Recent epidemiological data suggest that children under the age of 5 years are at greater risk for TBI-related Emergency Department visits and hospitalizations in comparison to children aged 5–14 years (CDC, 2003; Yeates, 2000). Survival rates are highest for children between the ages of 2 and 5 years in comparison to older and younger individuals (CDC, 2003; Yeates, 2000). Although research involving young children with TBI is scant, several researchers have demonstrated that children injured at a younger age have worse neurobehavioral outcomes than children injured when they are older (Anderson et al., 2005). Because many neurobehavioral skills are emerging rapidly during early childhood, young children may be particularly susceptible to long-term disruption of neurobehavioral development (Anderson et al., 2005, 2006). Because of high incidence, morbidity, and survival, TBI in early childhood is a serious health concern that affects a large number of children, families, and communities. Meeting the needs of these children, many of whom will have lifelong disabilities, entails understanding and developing better prediction models for long-term outcomes.

Proton MR spectroscopy (MRS) provides a potentially sensitive, non-invasive assessment of select brain metabolites thought to reflect neuronal and axonal viability, glial proliferation, cellular energetics, and cellular membrane integrity (Cecil and Jones, 2001). The metabolites investigated include *N*-acetyl aspartate (NAA), a marker of neuronal and axonal function (for more detailed discussions of potential functions of NAA, see Moffet et al., 2006, 2007); cholines (Cho), such as glycerolphosphocholine and phosphocholine, which are markers for membrane synthesis and repair; and creatine (Cr), composed of creatine and phosphocreatine, which are markers of cellular energy metabolism and mitochondrial functioning (Ashwal et al., 2006a,b). A handful of studies have investigated metabolite levels in children and adolescents following TBI. Most of the research by the same group focuses on the acute (approximately 1 week post-injury) metabolic changes following injury and the possibility that MRS offers early prognostic information regarding outcome (Ashwal et al., 2000; Babikian et al., 2006; Brenner et al., 2003; Holshouser et al., 2005). These investigators have found reductions of NAA/Cr or NAA/Cho and increases of Cho/Cr in normal appearing occipital gray matter and parietal white matter (Ashwal et al., 2000; Babikian et al., 2006; Brenner et al., 2003; Holshouser et al., 2005). Because Cr has been thought to remain constant, Cr has often been used in the denominator in computing metabolite ratios and not studied independently (Ashwal et al., 2006a,b). Researchers have shown correlations between reduced NAA/Cr and NAA/Cho and increased Cho/Cr and severity of injury as assessed by admission GCS score (Ashwal et al., 2000; Brenner et al., 2003), as well as by longer-term neurological (Ashwal et al., 2000; Brenner et al., 2003) and neuropsychological (Babikian et al., 2006; Brenner et al., 2003) outcomes.

Chronic alterations of NAA ratios and Cho/Cr ratios have been demonstrated in adults with TBI using longitudinal magnetic resonance spectroscopic imaging (MRSI; 42 adults at 7 ± 4 days post-injury with repeat measures in 31 patients at 6–12 months post-injury) felt likely to reflect neuronal loss and glial proliferation. Long-term dichotomized outcome (good vs. poor) was predicted with 83% accuracy (Holshouser et al., 2006). A few recent studies have investigated sub-acute to long-term metabolite changes following pediatric TBI and the relationship between long-term disruption of brain metabolism and neuropsychological functioning (Hunter et al., 2005; Parry et al., 2004; Yeo et al., 2006). Yeo et al. (2006) found NAA/Cr reduced and Cho/Cr increased in anterior and posterior compartments during the sub-acute phase (mean 34 days post-injury) using a MRSI technique with 36 children between the ages of 6 and 18 years. GCS scores were positively correlated with NAA/Cr and negatively correlated with Cho/Cr. Additionally, anterior NAA/Cr was positively correlated with total performance, language, and visuomotor domains of functioning; whereas anterior and posterior Cho/Cr was negatively correlated with these same domains. Abnormalities in NAA/Cr and Cho/Cr decreased from 3 to 21 weeks post-injury. Hunter et al. (2005) found persistently lower NAA levels (6 weeks to 3 years post-injury), but no significant group differences in Cho or Cr in seven children with TBI compared to controls (ages 7–12 at time of injury). These researchers noted hemispheric asymmetry with metabolite values greater on the left than right for injured and control children. They also found a relationship between left and right frontal NAA levels and intellectual function and arithmetic scores. Finally, Parry et al. (2004) investigated brain metabolite levels (3 months to 12 years post-injury) in 15 children (ages 10–16 at time of MRS and ages 3–12 at time of injury) with severe TBI and found lower levels of NAA and Cho, but not Cr in the right frontal white matter. They also found a positive correlation between right frontal metabolite levels (NAA and Cho) and reaction time, but not overall intellectual function or memory. In summary, there is some evidence that MRS is a promising tool for investigating acute, sub-acute, and longterm neurometabolic functioning following brain injury in children and adolescents and that spectral metabolite concentrations are related to injury severity, neurological, and neuropsychological outcomes.

Because of the high incidence, morbidity, and survival following TBI in early childhood, the rapid neurological and neurobehavioral development during this developmental period, and the paucity of research on preschoolers, we have been conducting a longitudinal study of child and family outcomes following TBI in young children. Our primary goal for the present sub-study was to characterize long-term brain metabolite levels following TBI in young children. Our first aim was to examine the effects of TBI in young children on their brain metabolite concentrations during the chronic phase post-injury using an appropriate comparison group. We hypothesized that young children with TBI would have lower NAA metabolite levels at long-term follow-up in comparison to children with orthopedic injuries without acute CNS involvement. This control group was chosen to examine the effects of TBI relative to the effects of trauma without acute CNS involvement, as well as to control for demographic and other risk factors associated with traumatic injuries in general. Second, we examined whether brain metabolite levels in the chronic phase post-injury were correlated with acute TBI severity in our TBI sample. We hypothesized that lower brain metabolite levels would be associated with more severe TBI. Finally, we explored the

relationship between long-term brain metabolite concentrations and neurobehavioral outcomes.

METHODS

Participants

Children were recruited from a larger, prospective behavioral study that involved assessment of child and family social and environmental outcomes of TBI and orthopedic injury (OI) shortly after injury, and 6, 12, and 18 months post-injury. Eligibility criteria at time of injury for both groups included the following: (a) age between 3:0 and 6:11 years; (b) overnight stay in the hospital; (c) English as the primary spoken language in the home; (d) no documentation in the medical chart or in parent interview of child abuse as the cause of injury; and (e) no parent report of a history of developmental disability or medical condition associated with neuropsychological deficits (e.g., seizure disorder). Additional eligibility criteria for the TBI group included the following: (a) a GCS score less than 15 at any point since injury or a score of 15 accompanied by neuroimaging evidence of a brain insult on CT or MRI; and (b) TBI due to blunt external injury. Additional eligibility criteria for the OI group included the following: (a) documented bone fracture not involving the head; and (b) no documented loss of consciousness or symptoms of concussion. Children who had reached at least 6 years of age and were 12 months or more post-injury were invited to participate in an imaging sub-study which was conducted at a separate, later time point than the previous neurobehavioral and family assessments.

Magnetic Resonance Imaging and Spectroscopic Methods

Imaging and spectroscopy data were acquired using a clinical 3.0-Tesla MR Scanner (Siemens Trio, Malvern, PA) equipped with a multi-channel phased array head coil. Sagittal T1 MPRAGE, functional magnetic resonance imaging (fMRI), and axial 12-direction diffusion tensor imaging (DTI) were performed prior to MRS. This report describes only the MRS. Single voxel spectra were acquired with a spin-echo localization protocol (repetition time 3000 msec, echo time 144 msec, 96 averages, voxel volume 8 cm³) in the medial frontal gray matter (FGM) and left frontal white matter (LFWM). Water reference spectra were acquired using similar technical parameters except for a reduced number of averages (one average). The participant's head was comfortably secured within the multi-channel phased array head coil to minimize head motion. The child viewed a movie presentation with a video goggle system (Avotec, Stuart, FL) as a distraction technique from the environment of the MR scanner.

Metabolite concentrations for NAA, Cho (cholines including glycerolphosphocholine and phosphocholine), and Cr (creatine and phosphocreatine) were quantitatively determined using LCModel (Stephen Provencher, Guelph, Ontario), a commercially available data software processing package with an automatic, linear least squares frequency domain fitting routine. The method employs a basic set of concentration-calibrated model spectra of individual metabolites to estimate concentrations of similar brain metabolites from *in vivo* spectral data correcting for residual eddy current effects and actual coil loading by using the transmitter reference amplitude and water reference spectra (Provencher, 1993). The

concentration was corrected for T2 relaxation using reported literature values (Traber et al., 2004). Each concentration is reported with a confidence measurement (SD%) reflecting maximum likelihood estimates and their uncertainties (Cramer-Rao lower bounds) (Provencher, 1991). Metabolite concentrations with an SD% of <10 were included in the study. Using this criterion, two of the children with OI and one of the children with TBI had unusable data in the frontal gray matter. All children had useable data in the left frontal white matter.

Neurobehavioral Testing

All children participated in a comprehensive neuropsychological evaluation at approximately 12 months post-injury as part of the larger study. Neurobehavioral data from the standardized measures completed as part of the 12 months post-injury evaluation were used in the analyses. Testing included standardized measures of intelligence (Differential Ability Scales [DAS], Verbal, Nonverbal, Spatial, and General Cognitive Ability) (Elliott, 1990), academic achievement (Bracken School Readiness Composite, Woodcock-Johnson Third Edition [WJ-III], Letter-Word Identification, Applied Problems, and Spelling) (Bracken, 2001; Woodcock et al., 2001) and pragmatic language (Comprehensive Assessment of Spoken Language Pragmatics) (Carrow-Woodfolk, 2000). The primary caregiver completed ratings of the child's behavior problems (Child Behavior Checklist Internalizing and Externalizing) (Achenbach, 1991), executive function (Behavior Rating Inventory of Executive Function (BRIEF), General Executive Composite) (Gioia et al., 2000), and social competence (Social Behavior Scales Social Competence) (Merrell and Caldarella, 2000).

Statistical Analysis

Late brain metabolite levels for the TBI and OI groups (Aim 1) were compared by computing multiple independent *t*-tests. Relationships between brain metabolism and TBI severity (Aim 2) were analyzed using Pearson correlations as were the relationships between brain metabolism and neurobehavioral functioning (Aim 3). Because of the exploratory nature of these preliminary analyses, alpha levels were kept at $p < 0.05$. To further explore Aim 3, we computed the significance of the difference between correlations from our two independent samples (TBI and OI) by following these standard steps: converting correlations to *z*-scores; estimating the standard error of difference between the two correlations ($SE = \sqrt{(1/n_1 - 3) + (1/n_2 - 3)}$); and dividing the difference between the two *z*-score by the standard error. We used the standard *z* value difference cutoff of 1.96 for significance at the 0.05 level and 2.58 at the 0.01 level.

RESULTS

Based on the inclusion and exclusion criteria, 14 children with TBI and 17 children with OI from the larger study were eligible to participate in the imaging substudy. Ten children with TBI and 13 children with OI (74%) agreed to participate. MRS data were obtained for all 10 of the children with TBI and 10 of the 13 children with OI. Descriptive information on these 20 children is detailed in Table 1. There were six males and four females in each group. Eight children in each group were white. The groups did not differ significantly on maternal

education (70% of TBI vs. 60% of OI group completed high school or less), or median census tract income (TBI group mean = \$46,655, SD = \$18,073; OI group mean = \$63,728, SD = \$27,200). The TBI and OI groups did not differ significantly on age at injury (TBI group mean = 5.53 years, SD = 0.92; OI group mean = 5.45 years, SD = 1.03), age at neurobehavioral testing (TBI group mean = 6.67 years, SD = 0.94; OI group mean 6.50 years, SD = 1.04), or age at imaging (TBI group mean 7.78 years, SD = 0.95; OI group mean 7.63 years, SD = 0.97). In the TBI group, three of the children had severe TBI, four had moderate, and three had mild injuries according to their lowest GCS score at the time of injury. For the OI group, three of the children had femur fractures, six had elbow fractures, and one had an ankle fracture.

Late Brain Metabolite Levels (Aim 1)

The TBI and OI groups were compared on mean metabolite concentrations. There was a trend for NAA in the medial FGM to be lower in the TBI group (mean = 6.83, SD = 0.85) in comparison to the OI group (mean = 7.47, SD = .054), $t(1, 15) = 3.28$, $p = 0.09$. All other group comparisons were not statistically significant.

Relationship between Brain Metabolism and TBI Severity (Aim 2)

For the children in the TBI group, GCS recorded at the time of injury was significantly correlated with NAA and Cr levels in the medial FGM during the chronic stage post-injury. There was a trend for a significant relationship between GCS at the time of injury and medial FGM Cho levels in the chronic phase post-injury. Long-term NAA level in the left frontal white matter was significantly correlated with GCS, whereas late Cho and Cr levels in the left frontal white matter were not significantly correlated with GCS at the time of injury (Table 2).

Relationship between Brain Metabolism and Neurobehavioral Functioning (Aim 3)

The OI and TBI groups did not differ significantly on any of the standardized neurobehavioral measures. There were significant group differences on parent report of externalizing behaviors, $t(1, 18) = 10.73$, $p = 0.004$; executive functions, $t(1, 18) = 6.31$, $p = 0.02$; and social competence, $t(1, 18) = 5.46$, $p = 0.03$. (Table 3)

Pearson correlations between long-term metabolite levels and neurobehavioral functioning 12 months post injury were computed for the OI and TBI groups separately (Table 4). For the TBI group, NAA levels were positively correlated with some measures of academic achievement. Specifically, NAA levels in the medial FGM were *positively* correlated with spelling skills and NAA levels in the left FWM were positively correlated with school readiness skills. Cho levels in the medial FGM were positively correlated with spatial skills, some academic skills (word reading, spelling), and pragmatics. Cr levels in the medial FGM were also correlated with some academic skills (school readiness and spelling). In terms of behavioral outcomes, higher Cho and Cr levels in the left FWM were correlated with more internalizing symptomatology and higher Cho and Cr levels in the medial FGM were correlated with better social competence. None of the other correlations were significant. For the OI group, no correlations between cognitive or behavioral functioning and brain metabolism were significant. We computed the significance of the difference between the

correlations for the TBI and OI groups. The correlations between medial FGM Cho levels and spatial skills, spelling skills, and pragmatic language were significantly higher in the TBI than in the OI group. None of the other correlations were significantly different between the two groups.

DISCUSSION

Our overall goal in this study was to examine long term brain metabolite concentrations following TBI in young children. Previous investigators have reported long-term alterations in neurometabolic levels in schoolage children and adults across various times post-injury compared to non-injured controls, particularly with respect to NAA, which is the most commonly reported alteration in brain metabolite concentrations following TBI. In this study, we used a narrower and younger age range at time of injury (3–6 years), a narrower and more clearly defined period post-injury (1–3 years post-injury, rather than, for example, 3 months to 12 years post-injury in Parry et al., and 6 weeks to 3 years in Hunter et al.), a range of injury severity, and an age-, race-, and gender-matched orthopedic injury comparison group. Consistent with previous research, we demonstrated a trend for lower NAA concentrations in the medial frontal gray matter for our cohort. Reductions of NAA within the context of TBI have been attributed to axonal and neuronal loss consistent with varying degrees of hypoxia, ischemia, and toxic cascades, but also potentially reversible processes such as axonal stretching, axonal swelling, changes in myelin organization and other forms of axonal and neuronal dysfunction. Moreover, recent evidence also suggests that reduced NAA synthesis may also be responsible for reduced NAA concentrations (Moffett et al., 2006). In our cohort of children with TBI, only two of the TBI children demonstrated radiological evidence consistent with volume loss. However, as discussed by Holshouser et al. (2005), lower NAA concentrations may reflect neuronal and axonal loss undetected by MR imaging consistent with diffuse axonal injury. Some researchers have suggested a relationship between TBI severity, as measured by the initial GCS score, and brain metabolite values. Again, the most frequently reported finding is a positive correlation between GCS and NAA levels which is thought to reflect less neuronal loss with milder injuries. For example, Yeo et al. (2006) found that initial GCS score correlated positively with sub-acute total brain NAA/Cr and negatively with total brain Cho/Cr. Similarly, Parry et al. (2004) found that NAA concentrations in the chronic stage post-injury were positively correlated with initial GCS. Consistent with this previous research, we also found that NAA levels in the left frontal white matter and medial frontal gray matter were strongly positively correlated with GCS score at the time of injury. Interestingly, we found that long-term Cho and Cr levels in the medial frontal gray matter were also strongly *positively* correlated with initial GCS scores. A relationship between higher Cho levels and milder injuries is consistent with findings of Parry et al. (2004) that showed reduction in Cho levels in children in the chronic stage after severe TBI compared to controls. Moreover, Yeo et al. (2006) found that Cho/Cr levels decreased from 3 to 21 weeks post-injury and NAA/Cr levels increased in children with TBI. Parry et al. (2004) speculated that the chronic reduction of both NAA and Cho in severe pediatric TBI reflects neuronal loss and cerebral atrophy. Taken together, these findings invite speculation that Cho levels continue to decrease in the first year following pediatric TBI and that injury at an early age may result in

ongoing disruption of the normative developmental changes in brain metabolism (Parry et al., 2004; Tzika et al., 1993; van der Knapp et al., 1990). In other words, traumatic brain injury at an earlier age, when choline changes are occurring due to normative maturation/myelination processes, may prolong recovery or perhaps irreversibly alter brain development. Moreover, our findings and the findings of Parry et al. (2004) suggest that absolute concentrations of Cho and Cr are worthy of investigation in studies of pediatric TBI, rather than simple ratio values, because single voxel techniques afford a more robust quantification method with independent measurement of, for example, creatine and phosphocreatine levels, rather than simple ratio values, where it is uncertain if one or both metabolite levels are changing.

Interestingly, Hunter et al. (2005) found hemispheric asymmetry with higher metabolite levels in the left than the right frontoparietal region for NAA, Cho, and Cr. They related this to “age-related increase in left hemisphere specialization for language with associated loss of cortical gray matter and ongoing myelination.” Due to the time requirements for imaging, our MRS protocol was limited to two regions, one gray and one white. Thus, we cannot comment on any hemispheric differences in our populations; however, we found similar relationships between white matter NAA concentrations and measures of academic skills.

Researchers have begun to investigate the relationship between long-term brain metabolism and specific neurobehavioral outcomes following TBI in children. In discussing our findings, we should reiterate that our TBI group was not significantly different from our comparison group on most neurobehavioral measures. This is in contrast to other published studies, which either have a high performing comparison group (Hunter et al., 2005) or a broadly impaired TBI group (Parry et al., 2004). This difference (and others) in study design make comparison with the few published studies difficult. Nevertheless, there does appear to be some overlap in findings. In the current study, the strongest relationships for the TBI group were between higher metabolite concentrations and better academic skills. Similarly, Hunter et al. (2005) found a relationship between left and right frontal NAA levels and arithmetic scores. Moreover, in addition to correlations between specific neurobehavioral skills and NAA levels, we also found *positive* correlations with neuropsychological skills and Cho and Cr levels in the chronic post-injury phase. Similarly, Parry et al. (2004) found a positive correlation between late right frontal Cho levels and reaction time. Unlike most previous studies, we examined the relationship between parent report of behavioral functioning and long-term brain metabolism. In this domain, our strongest relationships were between social competence and higher metabolite levels suggesting that normalization of brain metabolism might not only reflect better intellectual and academic outcomes, but also better behavioral and social outcomes as well. The lack of correlation between brain metabolite values and neurobehavioral functioning in the OI group is likely related to implicit heterogeneity in the small group. That is, although research has supported a link between NAA levels and cognition, particularly on broad measures of functioning such as IQ testing, these relationships appear to be complicated by sampling issues such as region of interest and sex (e.g., Jung et al., 2005).

The primary limitation of this study is the small, cross sectional nature of the sample. That is, the small sample size limited the statistical analyses options available to us. With a

sample size of 10 in each group, we were restricted to correlation analyses. Our use of multiple correlations may have resulted in a few spurious significant correlations. A larger sample size would allow for use of techniques such as multiple regression with which the relationship between multiple predictor variables (e.g., age, gender, race, metabolite levels, injury severity) and neurobehavioral outcomes could be examined. Because this study was cross-sectional with a relatively homogenous age at injury and time post-injury, we were not able to examine interactions between brain metabolism outcomes and age or time since injury. The developmental progression of changes in brain metabolism following TBI in young children would be better studied in a longitudinal fashion rather than a cross-sectional design. Despite the strengths of our comparison group, a couple of the children in the OI group had non-specific imaging findings. However, we ran analyses with and without these children and did not find any differences in our results. Finally, it is possible that use of the lowest GCS score since injury rather than the more stringent criteria of post-resuscitation scores at 6 h after injury may have overestimated TBI severity for some of our participants. However, we do know that, for two of the three participants with GCS scores of 3, the GCS score does accurately reflect their non-sedated, post-resuscitation GCS status more than 6 h after injury.

The results of this study and the limitations delineated above suggest a number of directions for future research. Future studies with larger samples across the TBI severity range will allow for more sophisticated analyses of relationships between variables, as well as group differences. Studies of more homogeneous populations (e.g., all severe TBI) will also be useful in this regard and are likely to find more significant differences than we did in the present study. Determination of the optimal timing post-injury at which MRS would best predict outcome would be useful clinically. Moreover, comprehensive neurobehavioral data available at the time of MRS acquisition and after would be useful in developing outcome prediction models. Researchers should continue to seek appropriate clinical comparison groups rather than using convenience control samples. A more detailed analysis of the relationship between brain metabolism in the chronic post-injury phase and the nature of the injury remains an important area of investigation for future studies. That is, in addition to relating metabolism to GCS scores, researchers might investigate the relationship between MRS and other imaging approaches (diffusion tensor, volumetrics) to further inform our understanding of the relationship between the nature of the brain injury and ongoing brain metabolism. Future use of spectroscopic imaging techniques will allow for investigation of the relationship of metabolism in specific brain regions and theoretically associated neuropsychological skills, provided the MRSI output produces metabolite concentrations. Other metabolites, such as glutamate/glutamine (Glx), myoinositol (mI), and lactate and other brain regions (e.g., corpus callosum), show promise as important metabolite markers and regions of interest in pediatric TBI outcomes research and will be important to include in future studies (Ashwal et al., 2000, 2006; Brenner et al., 2003; Holshouser et al., 2006). Finally, there may be potential utility of MRS to evaluate the effectiveness of medical and rehabilitative interventions. That is, further studies of the potential of interventions to alter metabolic disturbances post-TBI and investigation of the relationship of these alterations in metabolism with neurological and functional recovery may provide insights into mechanisms of recovery and enhance future therapies.

CONCLUSION

This study extends previous reports of long-term lower NAA levels in children with TBI to those who were injured at an early age (3–6 years of age) and were more than 1 year post-injury by comparing them to an age, gender, and race-matched orthopedic injury comparison group. Specifically, during the chronic post-injury phase, we found reduction of NAA levels in the medial frontal gray matter in the TBI group compared to the OI group. Late NAA, Cho, and Cr levels in the medial frontal gray matter and NAA levels in the left frontal white matter were all strongly positively correlated with GCS scores at the time of injury. That is, more severe injury was related to lower brain metabolite concentrations at 1–3 years post-injury. Our data suggest that some neurobehavioral measures are more highly correlated with brain metabolite concentrations than others and that some of these correlations are different for the TBI and OI groups. For the TBI group, NAA, Cho, and Cr levels were most related to various academic outcomes and social competence. The correlations for medial FGM Cho levels with spatial skills, spelling skills, and pragmatic language were significantly different between the two groups. Future work is needed to evaluate the potential to therapeutically alter late metabolic disturbances following pediatric TBI and enhance neuropsychological and social outcomes.

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REFERENCES

- Achenbach, TM. Manual for the Child Behavior Checklist/4-18 and 1991 profile. Department of Psychiatry, University of Vermont; Burlington, VT: 1991.
- Anderson VA, Catroppa C, Morse S, et al. Functional plasticity or vulnerability following early brain injury? *Pediatrics*. 2005; 116:1374–1382. [PubMed: 16322161]
- Anderson VA, Catroppa C, Dudgeon P, et al. Understanding predictors of functional recovery and outcome 30 months following early childhood head injury. *Neuropsychology*. 2006; 20:42–57. [PubMed: 16460221]
- Ashwal S, Holshouser BA, Shu SK, et al. Predictive value of proton magnetic resonance spectroscopy in pediatric closed head injury. *Pediatr. Neurol.* 2000; 23:114–125. [PubMed: 11020636]
- Ashwal S, Babikian T, Gardner-Nichols J, et al. Susceptibility-weighted imaging and proton magnetic resonance spectroscopy in assessment of outcome after pediatric traumatic brain injury. *Arch. Phys. Med. Rehabil.* 2006a; 87:S50–S58. [PubMed: 17140880]
- Ashwal S, Holshouser BA, Tong KA. Use of advanced neuroimaging techniques in the evaluation of pediatric traumatic brain injury. *Dev. Neurosci.* 2006b; 28:309–326. [PubMed: 16943654]
- Babikian T, Freier MC, Ashwal S, et al. MR spectroscopy: predicting long-term neuropsychological outcome following pediatric TBI. *J. Magn. Reson. Imaging*. 2006; 24:801–811. [PubMed: 16941608]
- Bracken, BA. Bracken Basic Concept Scale–Revised. The Psychological Corporation; San Antonio, TX: 1998.
- Brenner T, Freier MC, Holshouser BA, et al. Predicting neuropsychologic outcome after traumatic brain injury in children. *Pediatr. Neurol.* 2003; 28:104–114. [PubMed: 12699860]
- Carrow-Woodfolk, E. Comprehensive Assessment of Spoken Language. American Guidance Services; Circle Pines, MN: 2000.

- CDC (Centers For Disease Control And Prevention). Traumatic Brain Injury in the United States: Assessing Outcomes in Children. National Center for Injury Prevention and Control, CDC; Atlanta, GA: 2003.
- Cecil KM, Jones BV. Magnetic resonance spectroscopy of the pediatric brain. *Topics Magn. Reson. Imaging.* 2001; 12:435–452.
- Elliott, CD. Differential Ability Scales. The Psychological Corporation; San Antonio, TX: 1990.
- Gioia, G.; Isquith, P.; Guy, SC., et al. Behavior Rating Inventory of Executive Function. Psychological Assessment Resources, Inc.; Odessa, FL: 2000.
- Holshouser BA, Tong KA, Ashwal S. Proton MR spectroscopic imaging depicts diffuse axonal injury in children with traumatic brain injury. *Am. J. Neuroradiol.* 2005; 26:1276–1285. [PubMed: 15891197]
- Holshouser BA, Tong KA, Ashwal S, et al. Prospective longitudinal proton magnetic resonance spectroscopic imaging in adult traumatic brain injury. *J. Magn. Reson. Imaging.* 2006; 24:33–40. [PubMed: 16755529]
- Hunter JV, Thornton RJ, Wang ZJ, et al. Late proton MR spectroscopy in children after traumatic brain injury: correlation with cognitive outcomes. *Am. J. Neuroradiol.* 2005; 26:482–488. [PubMed: 15760852]
- Jung RE, Haier RJ, Yeo RA, et al. Sex differences in *N*-acetylaspartate correlates of general intelligence: an ¹H MRS study of normal human brain. *Neuroimage.* 2005; 26:965–972. [PubMed: 15955507]
- Merrell, KW.; Caldarella, P. Home and Community Social Behavior Scales. Assessment-Intervention Resources; Eugene, OR: 2002.
- Moffet, JR.; Tieman, SB.; Weinberger, DR., et al., editors. *N*-Acetylaspartate: A Unique Neuronal Molecule in the Central Nervous System. *Advances in Experimental Medicine and Biology.* Springer; New York, NY: 2006.
- Moffet JR, Ross B, Arun P, et al. *N*-Acetylaspartate in the CNS: from neurodiagnostics to neurobiology. *Prog. Neurobiol.* 2007; 81:89–131. [PubMed: 17275978]
- Parry L, Shores A, RAE C, et al. An investigation of neuronal integrity in severe paediatric traumatic brain injury. *Child Neuropsychol.* 2004; 10:248–261. [PubMed: 15621848]
- Provencher SW. Estimation of metabolite concentrations from localized *in vivo* proton NMR spectra. *Magn. Reson. Med.* 1993; 30:672–679. [PubMed: 8139448]
- Provencher SW. Automatic quantization of localized *in vivo* ¹H spectra with LCModel. *NMR Biomed.* 2001; 14:260–264. [PubMed: 11410943]
- Traber F, Block W, Lamerichs R, Gieseke J, Schild HH. ¹H metabolite relaxation times at 3.0 tesla: measurements of T1 and T2 values in normal brain and determination of regional differences in transverse relaxation. *J. Magn. Reson. Imaging.* 2004; 19:537–545. [PubMed: 15112302]
- Tzika AA, Vigneron DB, Ball WS, et al. Localised proton MR spectroscopy of the brain in children. *J. Magn. Reson. Imaging.* 1993; 3:719–729. [PubMed: 8400557]
- Van der Knapp M, Van der Griend J, Van Rijen P. Age-dependent changes in localized proton and phosphorus MR spectroscopy of the brain. *Radiology.* 1990; 176:509–515. [PubMed: 2164237]
- Woodcock, RW.; McGrew, KS.; Mather, N. Woodcock-Johnson III Tests of Achievement. Riverside Publishing; Itasca, IL: 2001.
- Yeates, KO. Pediatric closed head injury. In: Yeates, KO.; Ris, MD.; Taylor, HG., editors. *Pediatric Neuropsychology: Research, Theory, and Practice.* Guilford Press; New York, pps: 2000. p. 92-116.
- Yeo RA, Phillips JP, Jung RE, et al. Magnetic resonance spectroscopy detects brain injury and predicts cognitive functioning in children with brain injuries. *J. Neurotrauma.* 2006; 23:1427–1435. [PubMed: 17020480]

Table 1
Clinical and Demographic Information for Participants

Group/sex	GCS score	Mechanism of injury	Age at injury	Age at testing	Age at MRS	Late MRI finding
TBI/F	15	Fall	5.7	6.9	9.0	Negative
TBI/M	15	Fall	6.4	7.5	9.1	Negative
TBI/M	14	Fall	4.4	5.4	6.9	Negative
TBI/M	12	Bike crash	5.9	7.1	8.8	Negative
TBI/M	12	Hit by auto	5.7	6.8	7.8	Small cyst bilateral frontal
TBI/M	9	Hit by auto	3.6	4.7	6.7	Negative
TBI/F	9	Fall	6.7	7.7	8.3	Heterotopic left frontal gray matter
TBI/F	3	Fall	5.1	6.5	6.9	Volume loss with areas of mild, moderate, and severe encephalomalacia; small hemorrhage
TBI/M	3	Motor vehicle crash	5.9	7.1	7.2	Prominent perivascular spaces, volume loss
TBI/F	3	Drag by horse into tree	6.0	7.1	7.0	Mildly prominent cerebellar sulci
OI/M	15	Fall	4.4	5.4	7.3	Negative
OI/M	15	Fall	3.9	5.0	6.6	Negative
OI/M	15	Fall	6.1	7.1	8.7	Negative
OI/M	15	Fall	4.6	5.6	7.1	Negative
OI/F	15	Sledding crash	6.8	7.9	9.1	Abnormal signal right parietal white matter; mildly prominent perivascular spaces
OI/M	15	Fall	5.7	6.8	7.7	Supravermian cyst, likely arachnoid
OI/F	15	Fall	6.6	7.7	8.6	Negative
OI/F	15	Fall	4.6	5.6	6.5	Negative
OI/F	15	Fall	6.5	7.5	8.2	Negative
OI/M	15	Hit by auto	5.4	6.4	6.5	Negative

GCS, Glasgow Coma Scale; MRS, magnetic resonance spectroscopy; MRI, magnetic resonance imaging; F, female; M, male; TBI, traumatic brain injury; OI, orthopedic injury.

Table 2
Pearson Correlation Coefficients between Initial GCS and Late Metabolite Concentrations

	Correlation with GCS
<i>N</i> -Acetyl aspartate FGM	0.77*
Choline FGM	NS
Creatine FGM	0.77*
<i>N</i> -Acetyl aspartate LFWM	0.73*
Choline LFWM	NS
Creatine LFWM	NS

GCS, Glasgow Coma Scale; FGM, medial frontal gray matter; LFWM, left frontal white matter; NS, not significant.

*
 $p < 0.05$.

Table 3
Group Differences on Neurobehavioral Measures 12 Months Post-Injury

	TBI, mean (SD)	OI, mean (SD)	p-Value
DAS Verbal	96.0 (14.3)	98.7 (18.9)	NS
DAS Nonverbal	95.8 (15.9)	104.3 (19.0)	NS
DAS Spatial	93.4 (21.8)	101.3 (2.8)	NS
DAS General Cognitive Ability	94.5 (17.6)	101.1 (14.2)	NS
Bracken School Readiness Composite	100.9 (18.6)	108.2 (11.5)	NS
WJ Letter Word Identification	101.7 (15.8)	105.1 (13.5)	NS
WJ Applied Problems	100.2 (19.6)	104.7 (14.3)	NS
WJ Spelling	95.6 (18.3)	101.4 (10.1)	NS
CASL Pragmatics	102.7 (18.1)	104.9 (17.7)	NS
CBC Internalizing	52.1 (10.4)	45.5 (6.5)	NS
CBC Externalizing	57.3 (9.0)	45.1 (7.6)	0.004
BRIEF Global Executive Composite	61.8 (10.1)	49.9 (11.1)	0.02
Social Competence	44.4 (12.4)	54.9 (6.9)	0.03

Cognitive testing completed with the child is presented as standard scores with a mean of 100 and a standard deviation of 15. The CBC, BRIEF, and Social Competence are parent ratings of child behavior presented as *t*-scores with a mean of 50 and a standard deviation of 10.

Boldfaced terms are significant between-groups differences.

TBI, traumatic brain injury; OI, orthopedic injury; DAS, Differential Ability Scales; WJ, Woodcock-Johnson Tests of Academic Achievement—Third Edition; CASL, Comprehensive Assessment of Spoken Language; CBC, Child Behavior Checklist; BRIEF, Behavior Rating Inventory of Executive Function; NS, not significant.

Table 4
Pearson Correlation Coefficients between Brain Metabolic Levels and Neurobehavioral Scores for the TBI Group ($n = 10$)

	<u>Medial frontal gray matter</u>			<u>Left frontal white matter</u>		
	NAA	Cho	Cr	NAA	Cho	Cr
DAS Verbal	NS	NS	NS	NS	NS	NS
DAS Nonverbal	NS	NS	NS	NS	NS	NS
DAS Spatial	NS	0.91 **	NS	NS	NS	NS
DAS General Cognitive Ability	NS	0.71 *	NS	NS	NS	NS
Bracken School Readiness Composite	NS	NS	0.71 *	0.66 *	NS	NS
WJ Letter Word	NS	0.74 *	NS	NS	NS	NS
WJ Applied Problems	NS	NS	NS	NS	NS	NS
WJ Spelling	0.67 *	0.88 **	0.73 *	NS	NS	NS
CASL Pragmatics	NS	0.73 *	NS	NS	NS	NS
CBC Internalizing	NS	NS	NS	NS	0.77 **	0.85 **
CBC Externalizing	NS	NS	NS	NS	NS	NS
BRIEF General Executive Composite	NS	NS	NS	NS	NS	NS
Social Competence	NS	0.74 *	0.71 *	NS	NS	NS

Statistically significant correlations are in bold.

DAS, Differential Ability Scales; WJ, Woodcock-Johnson Tests of Academic Achievement—Third Edition; CASL, Comprehensive Assessment of Spoken Language; CBC, Child Behavior Checklist; BRIEF, Behavior Rating Inventory of Executive Function; NS, not significant.

*
 $p < 0.05$.

**
 $p < 0.001$.