Whole-Brain Proton MR Spectroscopic Imaging of Mild-to-Moderate Traumatic Brain Injury and Correlation with Neuropsychological Deficits

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Abstract

Changes in the distribution of the magnetic resonance (MR)-observable brain metabolites N-acetyl aspartate (NAA), total choline (Cho), and total creatine (Cre), following mild-to-moderate closed-head traumatic brain injury (mTBI) were evaluated using volumetric proton MR spectroscopic imaging (MRSI). Studies were carried out during the subacute time period following injury, and associations of metabolite indices with neuropsychological test (NPT) results were evaluated. Twenty-nine subjects with mTBI and Glasgow Coma Scale (GCS) scores of 10–15 were included. Differences in individual metabolite and metabolite ratio distributions relative to those of age-matched control subjects were evaluated, as well as analyses by hemispheric lobes and tissue types. Primary findings included a widespread decrease of NAA and NAA/Cre, and increases of Cho and Cho/NAA, within all lobes of the TBI subject group, and with the largest differences seen in white matter. Examination of the association between all of the metabolite measures and the NPT scores found the strongest negative correlations to occur in the frontal lobe and for Cho/NAA. No significant correlations were found between any of the MRSI or NPT measures and the GCS. These results demonstrate that significant and widespread alterations of brain metabolites occur as a result of mild-to-moderate TBI, and that these measures correlate with measures of cognitive performance.

Key words: brain; magnetic resonance spectroscopic imaging; magnetic resonance spectroscopy; neuropsychological test; traumatic brain injury

Introduction

CLOSED-HEAD TRAUMATIC BRAIN INJURY (TBI) represents one of the most common causes of long-term neurological deficits and is a significant public health problem for which current methods of injury assessment and classification are inadequate. Based on the Glasgow Coma Scale (GCS) score, approximately 80% of brain-injured patients are classified as having had a mild or moderate TBI (collectively termed mTBI) (Cassidy et al., 2004). A large percentage of these subjects show symptoms of neurobehavioral deficits in subacute conditions that subsequently resolve within 3 months of injury; however, approximately 33% of mTBI patients are reported to develop persistent cognitive impairments of memory, attention, and executive function (Belanger et al., 2007; Sterr et al., 2006). Approximately 32–75% of mTBI subjects demonstrate trauma-related parenchymal lesions (Hofman et al., 2001; Hughes et al., 2004; Lee et al., 2008) on

structural neuroimaging (CT and MRI), although it is known that these imaging methods, as well as functional (single-photon emission computed tomography and positron emission tomography) neuroimaging, significantly underestimate the degree of injury (Hofman et al., 2001; Lee et al., 2008; Levin et al., 1992; McAllister et al., 2001; Wilson et al., 1988). It is therefore hypothesized that subtle and diffuse brain injury and/or alteration of cellular physiological processes may have occurred in these subjects that is not manifest as structural damage detectable by conventional neuroimaging methods.

Proton magnetic resonance spectroscopy (MRS) enables evaluation of brain metabolites such as N-acetyl aspartate (NAA; a marker of neuronal functional status), total choline (Cho; an indicator of membrane structural status), and total creatine (Cre; an indicator of cellular energetics). Several studies have demonstrated the value of proton MRS for evaluation of TBI, with findings including significant metabolic alterations in brainstem (Carpentier et al., 2006),

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splenium of the corpus callosum (Cecil et al., 1998a), parietal white matter (WM) (Brooks et al., 2000; Ross et al., 1998; Shutter et al., 2004), occipital gray matter (GM) (Brooks et al., 2000; Friedman et al., 1998; Ross et al., 1998; Shutter et al., 2004), occipital white matter (Friedman et al., 1998), frontal lobe white matter, (Garnett et al., 2000a, 2000b, 2001), and thalamus (Uzan et al., 2003). These reports have primarily studied subjects with more severe degrees of injury, and have used single-voxel spectroscopy acquisition methods to evaluate changes in these metabolites in a few normal-appearing brain locations. To evaluate metabolite changes from a wider extent of the brain, a few studies have used multi-voxel single-slice spectroscopic imaging (MR spectroscopic imaging, or MRSI) (Holshouser et al., 2006; Macmillan et al., 2002; Marino et al., 2007; Signoretti et al., 2002), or volumetric MRSI methods (Govindaraju et al., 2004; Kirov et al., 2007), with analyses based on metabolite ratios from user-selected regions of interest. These studies confirmed that subtle and widespread metabolite changes can occur in subjects with even mild TBI, and that these can occur remote from any MR-observed lesion.

Neurological deficits of memory, attention, and executive functions are commonly observed in patients with mTBI (Kurca et al., 2006; Kwok et al., 2008); however, only limited associations have been found between the location and extent of brain damage as detected by conventional structural neuroimaging techniques and the neurobehavioral outcome (Bigler, 2001a). This finding lends support not only to the presence of brain tissue injury that is not detectable by routine neuroimaging studies, but may also indicate that the neuroimaging methods and neuropsychological tests (NPT) commonly used on patients with TBI may be assessing different processes (i.e., local structural damage versus wider functional connectivity). Brooks and his associates (Brooks et al., 2000; Friedman et al., 1998) have evaluated the relationships between brain metabolite alterations and neurobehavioral changes seen after closed-head injury, and found significant correlations between NAA and Cre values in normal-appearing occipito-parietal white-matter and occipital gray-matter single-voxel regions and composite and individual NPT scores (Brooks et al., 2000; Friedman et al., 1998), suggesting that the observed metabolite changes were indicative of local cellular injury that impacts neuropsychological function.

The aims of this study were to apply a volumetric MRSI acquisition method to examine MR-observable metabolic alterations across the whole brain of subjects with mild-to-moderate TBI, in comparison to age-matched controls, and to test for possible relationships between spatial distributions of these metabolic alterations and the neuropsychological consequences of the injury. In addition to providing information from a much wider extent of the brain, which included cortical surface regions that have been excluded in previous MRS studies of TBI, the volumetric MRSI method has been combined with an automated image processing and analysis approach that provides new analysis methods and potentially offers increased sensitivity for the detection of diffuse metabolic alterations. Together with direct observation of metabolic images and structural MRI, average and tissue-specific brain metabolite concentrations were evaluated in lobar-scale regions and compared with similar measures from the control subject group. To test for potential associations between me-

tabolite alterations and changes of cognitive function, a battery of neuropsychological tests was administered to all TBI subjects to assess memory, attention, and executive functions.

Methods

Subject selection

Thirty-three subjects with closed-head TBI and brief (<20 min) loss of consciousness were recruited from the Ryder Trauma Center, Jackson Memorial Hospital, Miami, Florida, for inclusion in this study. All the subjects were admitted to the trauma center soon after injury. Of these, 29 (25 male, 4 female; mean age: 26 years, range: 18–42 years) completed the MR study, and 26 completed the neuropsychological assessment. Additional studies were carried out for subjects that had never experienced a TBI event or neurologic impairment, and combined with data acquired under another study (Maudsley et al., 2009a) for a total of 52 age-matched control subjects (28 male, 24 female; mean age: 26 years, range: 18–40 years). Based on the GCS score at the time of admission to the trauma center, 20 of the brain-injured subjects were classified with mild TBI (GCS score 13–15), and the remaining nine with moderate TBI (GCS score 10–12). The MR scan was performed at least 1 week post-injury to exclude the hyperacute-to-acute stage of the trauma during which the metabolite levels typically fluctuate. The median time of the study after injury was 20.5 days. Approximately the same percentage of the total number of subjects in each decade of age was included in the control group (18–20 years: 12%; 21–30 years: 77%; 31–40 years: 12%; 41–42 years: 0%) and TBI group (18–20 years: 10%; 21–30 years: 72%; 31–40 years: 14%; 41–42 years: 3%). Standard MRI pre-screening procedures were used to exclude subjects with contraindications to MRI. Additional exclusion criteria included previous brain injury or surgery, or a history of neurological disease, psychiatric illness, and substance abuse. The study protocol was approved by the institutional review board of the University of Miami, and all subjects or their care providers provided signed written consent before participation.

Magnetic resonance imaging and spectroscopy

MR data were acquired at 3 Tesla (Siemens Tim Trio) using eight-channel phased-array detection. An hour-long MR protocol included diagnostically relevant MRI and whole-brain MRSI acquisitions. The MRI sequences included FLAIR (TE/TR: 101 msec/9 sec, 44 slices, 3-mm slice thickness, FOV: 220×220 mm², and ~ 2.3 min), T2-weighted gradient-echo (TE/TR: 18 msec/658 msec, 44 slices, 3-mm slice thickness, FOV: 220×220 mm², and ~ 5.4 min), diffusion-weighted MRI (TE/TR: 85 msec/5 sec, 44 slices, 3-mm slice thickness, FOV: 220×220 mm², and ~ 2.1 min), and T1-weighted (MPRAGE; TE/TR: 4.43 msec/2150 msec, 160 slices, 1-mm slice thickness, FOV: 256×256 mm², and ~ 5 min).

MRSI data were obtained using a volumetric spin-echo EPSI sequence (TR/TE = 1710 msec/70 msec, FOV: 280×280×180 mm³, 100 [read]×50 [phase]×18 [slice] spatial samples over a 135-mm slab, and acquisition time of 26 min). It included frequency-selective water suppression, and inversion-recovery nulling of the lipid signal with TI = 198 msec. This sequence included both a spin-echo excitation for the metabolite signal, and a low flip-angle gradient-echo excitation for

a water reference signal, which was acquired in an interleaved fashion. The MPRAGE MRI and MRSI acquisitions were performed at the same angulation, with the slice or slab orientations of all acquisitions parallel to the anterior commissure-posterior commissure (AC-PC) plane, or angulated at +15° from the AC-PC, which was found to facilitate improved magnetic field homogeneity over a larger volume of the cerebrum.

MRSI data were processed in a fully-automated manner using the MIDAS package (Maudsley et al., 2006). Metabolite image reconstruction included spatial smoothing and interpolation to 64×64×32 voxels, with a resultant voxel volume of approximately 1 mL. The processing steps included calculation of the MRSI voxel tissue content based on tissue segmentation of the high-resolution T1-weighted MRI; signal normalization to institutional units using the water reference data; and spatial registration to the simulated Montréal Neurological Institute MRI template (Collins et al., 1998), which was matched to a brain atlas that delineated the eight hemispheric lobes and the cerebellum (details in Maudsley et al., 2009a).

Neuropsychological tests

An hour-long battery of tests known to be sensitive for assessing cognitive functions in the domains of memory, at-

tention, and executive functions in brain-injured patients was administered to the subjects in the TBI group on the day of the MR scan. This consisted of the Wechsler Memory Scale-III subtests Faces I and II (testing visual memory), Word List I and II (testing verbal memory), and Digit Span (for testing auditory attention; Wechsler 1997); the Trail Making Test Parts A and B (assessing visual scanning and mental flexibility; Reitan and Wolfson 1985); the Wechsler Adult Intelligence Scale-III subtests Matrix Reasoning (testing non-verbal problem solving) and Symbol Search (testing processing speed; Wechsler 2002); and Controlled Oral Word Association (COWA; testing verbal fluency; Benton et al., 1994). The standard scores of these tests were converted to z-scores using the normative population mean and standard deviation values. Out of the 29 TBI subjects, 26 completed the neuropsychological tests (the excluded subjects were numbers 7, 13, and 17, as identified in Table 1).

Data analyses and statistical tests

Average values of the individual metabolites (NAA, Cho, and Cre) and their ratios (NAA/Cre, Cho/Cre, and Cho/NAA) were calculated for gray matter and white matter within each lobar brain region of the cerebrum. For the cerebellum, the metabolite values from both WM and GM were combined, as the cerebellum could not be optimally

TABLE 1. DEMOGRAPHIC AND CLINICAL DATA OF SUBJECTS WITH TRAUMATIC BRAIN INJURY

Subject number	Gender	Age	Cause of injury	GCS score	Time after injury to MR scan (days)	Total lesion volume (mL)	MRI findings
1	Male	42	Assault	14	11	0.14	Contusion
2	Male	39	MVA	14	18	2.01	Contusion, SDH, SAH
3	Male	27	Fall	13	19	9.27	Contusion
4	Male	28	Assault	11	10	6.88	Contusion, TMB
5	Female	21	MVA	15	100	1.36	Hematoma, TMB
6	Female	18	MVA	14	18	0.77	Contusion, SDH, EDH
7	Male	24	MVA	10	18	0.03	TMB
8	Male	21	MVA	15	22	2.58	Contusion, SDH
9	Male	20	MVA	11	16	4.45	Contusion, SAH
10	Male	22	MVA	13	53	0.75	Contusion, SDH, IVH
11	Male	21	MVA	11	30	0.24	Contusion, TMB
12	Male	22	MVA	15	75	0.85	Contusion
13	Male	30	Fall	15	44	1.67	Contusion
14	Male	20	MVA	14	26	20.27	Hema. with edema
15	Male	25	MVA	10	11	0.18	Old contusion
16	Male	24	MVA	15	42	0.75	Contusion
17	Male	26	MVA	15	53	0.08	TMB, Contusion, SDH
18	Male	33	MVA	15	7	0.08	Contusion
19	Female	21	MVA	10	76	0.12	TMB, SDH
20	Male	33	MVA	12	21	0.00	Unremarkable brain
21	Female	25	MVA	10	20	0.00	Unremarkable brain
22	Male	32	Assault	10	11	21.94	Hematoma with mass effect, SDH
23	Male	24	MVA	15	14	0.03	Non-specific lesions, SDH
24	Male	27	MVA	15	22	0.03	Non-specific or trauma-related
25	Male	23	MVA	14	28	1.22	Contusion, EDH, TMB
26	Male	23	MVA	15	13	0.03	Non-specific lesion, unrelated to trauma
27	Male	22	MVA	15	9	0.00	Unremarkable brain
28	Male	25	MVA	15	25	1.32	Contusion, SDH
29	Male	24	MVA	14	31	0.93	Contusion, SDH

MVA, motor vehicle accident; SDH, subdural hematoma; SAH, subarachnoid hemorrhage; IVH, intraventricular hemorrhage; EDH, epidural hematoma; TMB, traumatic micro-bleeds; Hematoma, cortical/subcortical hematoma; GCS, Glasgow Coma Scale; MR, magnetic resonance.

segmented into GM and WM. Values of individual metabolites were first corrected for partial volume signal loss due to CSF contribution at each voxel, and metabolite values corresponding to 100% of each tissue type were obtained using a regression of the metabolite value against the tissue content, for all voxels selected from that region.

For statistical analyses, the following limiting criteria were applied to ensure that the data chosen were based on the quality of spectra. First, a line-width criterion was applied to only include voxels with fitted spectral line-width between 2 and 12 Hz. Next, a fractional tissue volume criterion was applied to only include voxels with underlying tissue volume of $\geq 80\%$. Finally, an outlier exclusion criterion was applied to exclude voxels with metabolite values greater than three times the standard deviation of the corresponding metabolite value over all the voxels within the particular region. This last criterion was applied separately for each metabolite value and ratio. The line-width criterion typically excluded approximately 37% of brain voxels, and a further 0.73% of brain voxels were excluded by using the outlier rejection criterion.

Visible intracranial lesions were identified and classified pathologically based on their appearance on FLAIR, T2-weighted gradient-echo, and diffusion-weighted MRIs. The volume of focal lesions seen in the FLAIR and T2-weighted MRI were quantitated using MRICro (www.sph.sc.edu/comd/rorden/micro.html). Lesion identification and boundary verification were performed once before lesion volume quantitation. A few subjects had traumatic micro-bleeds (TMBs) that were too small for reliable quantitation and were not included in the volume calculation. A secondary analysis was carried out to evaluate the relationship of MRSI findings with the MRI-observed lesion volume, for which the TBI group was sub-categorized according to their whole-brain lesion volume into three groups: TBI-1 (all subjects; $n = 29$), TBI-2 ($n = 16$; whole-brain lesion volume ≤ 0.50 mL, or with TMB or unremarkable brain), and TBI-3 ($n = 13$; whole-brain lesion volume ≥ 0.51 mL). The aim of this sub-categorization was to distinguish a group with minimal injury from those with more extensive damage.

The metabolite measures were compared between groups using a *t*-test (control versus TBI-1) and one-way ANOVA (control versus TBI-2 and TBI-3). To evaluate the associations between NPT scores and the metabolite measures, multiple partial linear regressions were performed. To improve the sensitivity of detection of neurological deficits in each of the functional domains of memory, attention, and executive function, *z*-scores from the tests measuring predominantly the same domain were averaged to obtain mean scores. For this, the tests Faces I and II and Word List I and II were combined for the domain of memory; Trail Making Test A and Digit Span for the domain of attention; and Trail Making Test B, Matrix Reasoning, Symbol Search, and COWA-FAS for the executive domain. In addition, individual test *z*-scores and mean *z*-score of all tests were included in the analyses. The metabolite measures and NPT scores from 26 subjects with TBI were then correlated using Pearson's product moment partial correlation and are reported as coefficients (*r*-values) between the scores or values of the two measures. Age and number of days between injury and scan were used as covariates to account for their possible influences on the correlations performed. Separately, *z*-scores of the NP tests and whole-brain lesion volumes were correlated using Pearson's

product moment partial correlation with age and number of days between injury and scan as covariates. For all the tests, *p*-values ≤ 0.05 were considered significant.

Results

Demographic and injury information and MRI scan findings

Table 1 lists the demographic and injury data, and MRI findings of the 29 TBI subjects that participated in the study. The cause of injury was motor vehicle accident in 24 subjects and fall or assault in five subjects. The MRI findings indicated 22 subjects with contusions and/or hematoma, six with TMBs, and three with no findings. These pathologies were most commonly localized to the left ($n = 14$) and right ($n = 15$) frontal lobe, but were also seen in the left ($n = 8$) and right temporal ($n = 2$), left ($n = 2$) and right parietal ($n = 3$), and occipital ($n = 2$) lobes, and in the corpus callosum ($n = 3$). The total lesion volume within the brain of each of these subjects ranged from 0.03–22 mL, with lesion volumes ≤ 0.50 mL in 13 subjects and >0.51 mL in 16 subjects.

Spectroscopic images and spectra

Representative images from 12 contiguous axial slices for the individual metabolites, metabolite ratios, and MRIs are shown in Figure 1 to demonstrate the quality and spatial extent of the metabolite maps obtained in this study. Data were acquired from a 22-year-old male subject with TBI (subject number 10 in Table 1) with an initial GCS score of 13 who was scanned 53 days after injury. The findings from the clinically-relevant MRIs were contusion, hematoma, and intraventricular hemorrhage, with a total parenchymal lesion volume of 0.75 mL. The T1-weighted images (in Fig. 1a) show the anatomy, and the T2-weighted images (in Fig. 1b) indicate contusions in the left temporal, bifrontal, and splenium of the corpus callosum. Metabolite value or ratio images are shown in Figures 1c through h. The contours surrounding the NAA images (Fig. 1b) represent the outer edge of the brain parenchyma, and were obtained from their corresponding T2-weighted images. The non-black areas within the contours across the NAA images indicate the spatial extent of data of suitable quality for analysis, and previous studies have demonstrated that on average this volumetric MRSI acquisition obtains data from 70% of the brain volume (Maudsley et al., 2009b). Figure 1 also shows that most slices include good sampling of cortical surface gray-matter regions, while limited data quality is obtained from temporal and inferior frontal regions due to high magnetic susceptibility variations within these regions. The bright spots primarily located at the edges of the brain typically indicate voxels with errors in the spectral fitting, commonly caused by residual water or lipid signals, and these results will be excluded by the outlier removal filter applied in the analysis procedure.

In Figure 2 are shown spectra from five contiguous axial slices of a subject with TBI (subject 10 in Table 1), one each from voxels in the right and left parieto-occipital hemispheres (shown in a and b, respectively), and T2-weighted MRIs of the five slices. The T2 MRIs indicate hemorrhagic contusions in the frontal lobe bilaterally and corpus callosum, and intraventricular hemorrhage. For comparison, data from approximately similar axial slices and voxel locations from a control

FIG. 1. Structural and metabolite images from a subject with mild TBI. Data were acquired from a 22-year-old male (subject 10 in Table 1) with an initial GCS score of 13 at 53 days after injury. Images in the rows are: (a) axial T1-weighted MRI (5-mm slice thickness and matched to spectroscopic imaging (SI) slice location); (b) NAA; (c) Cho; (d) Cre; (e) NAA/Cre; (f) Cho/Cre; (g) Cho/NAA (GCS, Glasgow Coma Scale; NAA, N-acetyl aspartate; Cho, choline; Cre, creatine; MRI, magnetic resonance imaging; TBI, traumatic brain injury).

subject (a 23-year-old female) are shown in Figure 2c and d. Upon comparison of spectra a2-b2 from the subject with TBI, it is clear that NAA and Cho in b1 were significantly altered compared to spectra a1, c1, and d1 (from the control subject), which was expected due to the voxel located near the

injury seen on T2 MRI in the left splenium. Comparison of spectra a2-b2, a3-b3, a4-b4, a5-b5, between the pairs and between the corresponding spectra from the control subject (spectra c2 through d5), clearly indicate altered levels of NAA and Cho in normal-appearing tissue on diagnostic MRIs,

FIG. 2. Proton magnetic resonance spectra from a 22-year-old male subject (subject 10 in Table 1) with mild traumatic brain injury (a and b), and a 23-year-old female control subject (c and d). Spectra were obtained from five contiguous axial slices ~ 5.6 mm in thickness, and are shown at a fixed vertical scale. The R on the images indicates the right side of the subjects.

FIG. 3. Proton magnetic resonance spectra from five contiguous axial slices inferior to the first slice shown in Figure 2 (c and d; a 23-year-old female control subject). The spectra are shown at a fixed vertical scale. The R and L on the image indicate the right and left side of the subject, respectively. Spectra R1–R5 and L1–L5 were obtained from the right and left hemispheres, respectively, and the arrows indicate their voxel locations. The spectra were obtained from voxels located in the frontotemporal GM (R1), occipital WM (R2), frontal WM (R3), frontal GM (R4), parietal GM (R5), brainstem (L1), cerebellum (L2), putamen (L3), occipital GM (L4), and parietal WM (L5) regions (GM, gray matter; WM, white matter).

providing evidence for metabolically abnormal regions surrounding and far from the sites of injury. In Figure 3 are shown the quality of spectra typically obtained from the brain region inferior to that shown in Figure 2 (control subject, a 23-year-old female). The spectra shown are from the frontotemporal GM, occipital WM, frontal WM, frontal GM, parietal GM, brainstem, cerebellum, putamen, occipital GM, and parietal WM.

Differences between the TBI and control groups

Average metabolite values (NAA and Cho) and ratios (NAA/Cre, Cho/Cre, and Cho/NAA) over each lobar region for the control group and each of the TBI subject groups for WM and GM are shown in Figures 4 and 5, respectively. Relative to the controls, findings indicate that in the WM: (1) NAA was consistently lower in all lobar regions for all TBI groups, and significant differences were found in all the lobes of the TBI groups except the frontal and temporal lobes in TBI-2; (2) similarly, Cho was consistently higher in all the

FIG. 4. Mean values of the metabolites and ratios in the white matter of control and mild traumatic brain injury (mTBI) groups, by brain region, with error bars for ± 1 standard deviation. Data are shown for control (black bars), TBI-1 (light gray bars), TBI-2 (white bars), and TBI-3 (dark gray bars) groups, with significant differences indicated (single triangle = $p \leq 0.05$; two triangles = $p \leq 0.01$). The y-axis scale for the metabolite values is in institutional units (i.u.). Abbreviations used for the hemispheric lobar regions are: the first letter R/L indicates the right/left side of the brain, and the second letter F/T/P/O indicate frontal/temporal/parietal/occipital lobes, respectively (NAA, N-acetyl aspartate; Cho, choline; Cre, creatine).

lobar regions, with significant differences observed only in the bilateral frontal, temporal, and parietal lobes, and left occipital lobe in TBI-1, left frontal and temporal lobes in TBI-2, and left temporal, parietal, and occipital lobes in TBI-3; (3) there was no clearly observable trend for Cre (data not shown), and although increases were found in the right (TBI-1 and TBI-2) and left (TBI-2) temporal lobes, the p -values were in the conservatively significant range of 0.041 to 0.046; (4) taking ratios of NAA and Cho to Cre resulted in a greater degree of significance in the finding for decreased NAA/Cre, although no clear trend toward increased or decreased significance of the results for Cho/Cre was noted; and finally (5) Cho/NAA showed the most consistent and significant increases, for all TBI groups and in all brain regions.

A summary of findings for the GM measures, relative to the controls, include the following: (1) NAA was slightly decreased in all but the right temporal lobe, reaching significance in the right frontal and bilateral occipital lobes (in TBI-1,

FIG. 5. Mean values of the metabolites and ratios in the gray matter of control and mild traumatic brain injury (mTBI) groups, by brain region, with error bars for ± 1 standard deviation. Data are shown for control (black bars), TBI-1 (light gray bars), TBI-2 (white bars), and TBI-3 (dark gray bars) groups, with significant differences indicated (single triangle = $p \leq 0.05$; two triangles = $p \leq 0.01$). The y-axis scale for the metabolite values is in institutional units (i.u.). Abbreviations used for the hemispheric lobar regions are: the first letter R/L indicates the right/left side of the brain, and the second letter F/T/P/O indicate frontal/temporal/parietal/occipital lobes, respectively (NAA, N-acetyl aspartate; Cho, choline; Cre, creatine).

TBI-2, and TBI-3), the left frontal lobe (TBI-1 and TBI-3), and the left parietal lobe (TBI-3); (2) Cho did not show any consistent trend, although it was found to be significantly increased in the right temporal and left frontal and parietal lobes, but interestingly only in the milder-injury TBI-2 group; (3) there was no trend in Cre (data not shown), although the temporal lobe in TBI-2 showed significant increases bilaterally; (4) NAA/Cre showed consistently reduced ratios in all lobes that were significant in several regions; (5) Cho/Cre was increased in several more regions than indicated by Cho alone, reaching significance in the frontal lobes and right parietal and occipital lobes; and finally (6) Cho/NAA showed an increase in all but the right parietal lobe, reaching significant differences in the TBI groups in the frontal and left parietal and occipital lobes.

The finding of increased significance and a greater number of regions reaching significance for the ratios of NAA and Cho to Cre likely reflects the decreased variance of the ratio measurement relative to the signal-normalized individual me-

tabolite values, as has been observed for both intra- and inter-subject studies (Maudsley et al., 2009a, 2009b), although this does not exclude the possibility of a slight, but non-significant, increase of Cre.

Metabolite values and ratios from the combined WM and GM tissue types of the cerebellum are shown in Figure 6. The observations in the TBI groups as compared to controls are: (1) NAA showed a consistent decrease, and significant differences in the TBI-1 and TBI-3 groups were found; (2) Cho was increased, with no significant differences found in the TBI groups; (3) Cre showed a consistent increase, but no significant difference was found in the TBI groups; (4) NAA/Cre was decreased, and significant differences were found in the TBI-1 and TBI-3 groups; (5) Cho/Cre was increased in the TBI groups, and was significantly different in TBI-1; and (6) Cho/NAA showed significant differences in TBI-1 and TBI-3, and consistent increases in all the TBI groups.

Neuropsychological test scores

The summary z-scores of the neuropsychological tests from 26 subjects with mTBI are listed in Table 2. The mean z-scores of the tests (column 2) ranged from +0.58 (subject 21) to -4.92 (subject 14), and the magnitude of the scores indicates the extent of deviation from the normative data used for deriving the z-scores. Out of 26 subjects with TBI, mean z-scores

FIG. 6. Mean values of the metabolites (A) and ratios (B) in the cerebellum of control and mild traumatic brain injury (mTBI) groups, with error bars for ± 1 standard deviation. Data are shown for control (black bars), TBI-1 (light gray bars), TBI-2 (white bars), and TBI-3 (dark gray bars) groups, with significant differences (single triangle = $p \leq 0.05$; two triangles = $p \leq 0.01$; NAA, N-acetyl aspartate; Cho, choline; Cre, creatine).

TABLE 2. NEUROPSYCHOLOGICAL TEST Z-SCORES OBTAINED FROM 26 SUBJECTS WITH TBI

Subject number	Mean z-scores			
	All tests	Memory	Attention	Executive
1	0.46	0.33	0.73	0.45
2	-0.70	-0.75	-0.60	-0.70
3	-0.07	-0.25	0.97	-0.40
4	-1.27	-1.50	-0.87	-1.23
5	-0.71	-0.25	-0.67	-1.20
6	-1.20	-0.42	-1.08	-2.05
8	-0.41	0.08	-0.50	-0.85
9	-0.46	-0.42	-0.57	-0.45
10	-2.63	-2.50	-2.50	-2.83
11	-0.46	0.42	-1.47	-0.83
12	0.17	0.25	-0.67	0.52
14	-4.92	-6.03	-4.37	-4.09
15	-0.45	0.42	-1.47	-0.82
16	-0.42	-0.50	-0.87	-0.12
18	-0.97	-0.42	-2.40	-0.80
19	-0.21	-0.42	-0.73	0.27
20	-1.15	-1.00	-0.87	-1.45
21	0.58	0.58	0.83	0.45
22	-1.31	-1.17	-1.60	-1.32
23	-0.42	0.00	-0.84	-0.63
24	-0.91	-0.42	-1.50	-1.12
25	-1.74	-1.67	-1.27	-2.05
26	-0.66	-0.59	-0.77	-0.69
27	-1.41	-1.67	-0.63	-1.55
28	-0.13	-0.50	-0.07	0.20
29	0.15	0.50	-0.90	0.32

Standard scores from 10 neuropsychological tests were first converted to z-scores, and then mean z-scores were obtained. The z-scores of memory, attention, and executive domains were obtained by averaging over z-scores of tests within each of the domains, as described in the text.

TBI, traumatic brain injury.

from all but four subjects (subjects 1, 12, 21, and 29) showed negative values, although scores less than -2.0 were found in only two subjects (subjects 10 and 14). Of all the z-scores, 13 subjects showed scores less than -1.00, and five subjects showed z-scores of less than -2.00 (subjects 6, 10, 14, 18, and 25).

Associations between imaging and neuropsychological measures

Significant correlations were found between the NPT z-scores and whole-brain lesion volumes for all but three of the tests, and the individual *r* values are shown in Table 3 (column "MRI-observed lesion volume"). The *r*-values for the significant correlations ranged from -0.46 (for the Matrix Reasoning test) to -0.72 (for Word List II).

A summary of results from the correlation analyses between Cho/NAA ratios in GM and WM by lobar region in the TBI-1 group and NPT z-scores are shown in Table 3. In general, similar findings were observed for the NAA/Cre and Cho/Cre measures (data not shown), with considerable overlap of the brain region and NP test, and therefore only the results for Cho/NAA and the brain regions with significant findings are shown for brevity. The numbers of individual NP test and brain region combinations that reached significance were 15 for Cho/NAA, 13 for NAA/Cr, and 6 for Cho/Cre. Aside from the significant correlations found in the GM and WM of the frontal lobe, WM NAA/Cre showed significant correlations in the left occipital lobe versus Digit Span, and left temporal lobe versus Trail Making Test A, and for Cho/Cre between the left temporal and occipital lobes versus Trail Making Test B.

Significant correlations were observed between the left frontal lobe gray matter Cho/NAA and the z-scores of Faces I, Word List I and II, and Digit Span tests, mean of all test scores, and mean test scores of memory and attention domains. No

TABLE 3. CORRELATION COEFFICIENTS (*r*) OF PARTIAL CORRELATIONS BETWEEN CHO/NAA RATIO IN THE GRAY MATTER AND WHITE MATTER AND NPT SCORES IN FIVE LOBAR REGIONS

Test	Gray matter		White matter			MRI-observed lesion volume
	LF	RF	LF	LT	LO	
Faces I	-0.46*	-0.18	-0.63**	-0.25	-0.31	-0.55**
Faces II	-0.39	-0.19	-0.57**	-0.19	-0.25	-0.47*
Word List I	-0.48*	-0.37	-0.69**	-0.27	-0.31	-0.53**
Word List II	-0.46*	-0.30	-0.69**	-0.15	-0.26	-0.72**
Trail Making A	-0.31	-0.41*	-0.38	-0.44*	-0.33	-0.18
Trail Making B	-0.07	-0.30	-0.27	-0.45*	-0.41*	-0.05
Matrix Reasoning	-0.23	-0.16	-0.47*	-0.06	-0.26	-0.46*
Symbol Search	-0.40	-0.32	-0.64**	-0.23	-0.30	-0.54**
Digit Span	-0.42*	-0.22	-0.65**	-0.20	-0.19	-0.47*
COWA	-0.11	0.06	-0.12	0.03	0.00	-0.19
Mean	-0.44*	-0.31	-0.67**	-0.28	-0.34	-0.56**
Memory	-0.48*	-0.28	-0.70**	-0.23	-0.30	-0.61**
Attention	-0.45*	-0.38	-0.64**	-0.39	-0.32	-0.41*
Executive	-0.29	-0.25	-0.53**	-0.22	-0.32	-0.46*

p* ≤ 0.05; *p* ≤ 0.01.

Age and number of days between injury and scan were used as covariates. Significant correlations are indicated by **p* ≤ 0.05 and ***p* ≤ 0.01. Abbreviations used for the hemispheric lobar regions are: the first letter R/L indicates the right/left side of the brain, and the second letter F/T/O indicate frontal/temporal/occipital lobes, respectively.

COWA, Controlled Oral Word Association.

significant correlations were observed between GM Cho/NAA of other lobar regions and NP test scores, so the results of such correlations were not included in the table. In WM Cho/NAA of the lobar regions, significant correlations ($p \leq 0.05$) were observed only in four regions: right frontal lobe for Trail Making Test A; left frontal lobe for Matrix Reasoning scores; left temporal lobe for Trail Making Test A and B test scores; and left occipital lobe for Trail Making Test B. Significant correlations ($p \leq 0.015$) were noted only for left frontal lobe white matter versus Faces I and II, Word List I and II, Symbol Search, Digit Span, mean test scores, and mean of memory, attention, and executive domain test scores.

When two subjects with NPT z-scores of less than -2.0 (subjects 10 and 14 in Table 1) were excluded from the analyses, the significant correlations between the NPT scores and the MRS measures were no longer present. Similarly, the regression between the lesion volume and NPT scores after excluding the two subjects produced significant correlation only for the Word List II NP test. To examine the relative distributions of both the NPT scores and the metabolite measures, in Figure 7 is plotted the mean NPT score for each subject against the z-score for the whole-brain Cho/NAA value (i.e., the mean value scaled by the standard deviation of the corresponding values from the normal subject group). In addition, the plot separately indicates the subjects from the TBI-2 and TBI-3 groups. Similar analyses performed for the composite NPT scores and individual brain regions showed similar characteristics to those indicated in Figure 7. Observations from this result include that there is no clear association of either NPT score or Cho/NAA measure with lesion volume. Similarly, there was no clear association with GCS (data not shown). When the two outlying points (subjects 10 and 14) are excluded, it can be seen that the range of z-score values for the metabolite measures is approximately twice that of the NPT score, indicating that the range of values across this group of mTBI subjects is greater for the MRSI measurement.

FIG. 7. Comparison of the mean NPT z-score and the mean cerebral white-matter Cho/NAA ratio for all mild traumatic brain injury (mTBI) subjects. Points are coded according to total lesion volume, for the TBI-2 (circle) and TBI-3 (diamond) subject groups (NAA, N-acetyl aspartate; Cho, choline; NPT, neuropsychological test).

Associations with GCS scores

Associations between the GCS and NPT scores were evaluated and no significant correlations were observed. Similarly, tests for associations between the GCS score and metabolite measures in the 29 subjects with TBI showed no significant correlations.

Discussion

In this study we performed a comprehensive evaluation of MR-observed lesions and metabolite distributions in subjects with mild-to-moderate closed-head injury in subacute conditions, and evaluated associations between the imaging measures and NPT assessments of cognitive function. Further, MR metabolite measures were obtained by tissue type and hemispheric lobe, and compared against age-matched control values. The main findings of this study are: (1) more significant and anatomically widespread changes in NAA, Cho, NAA/Cre, Cho/Cre, and Cho/NAA in the TBI group compared to controls; (2) the degree of metabolite alterations varied by brain tissue type (i.e., GM and WM), lobe (e.g., right frontal and right temporal), and hemispheric side (e.g., right and left parietal lobe Cho/NAA in Figure 5); (3) significant metabolite alterations were present in locations remote from, or in the absence of, any MRI-observed traumatic lesions; and (4) significant correlations were found between metabolite measures and individual and functional domain NPT z-scores, mostly for the left frontal lobe.

Group differences in metabolite measures

NAA and NAA/Cre were significantly decreased in white matter throughout the cerebrum and for several gray-matter regions. Given the relatively small MRI-observed lesion volume in most subjects, it is apparent that these changes are observed remote from, and in the absence of, MRI-observed structural injury, and there were no strong correlations between the metabolite measures and the whole-brain lesion volume. Since NAA is localized primarily in neurons and synthesized in neuronal mitochondria, and occurrence of direct neuronal transection is considered unlikely for those with mild injury (Povlishock, 1993), these results may indicate either longer-term axonal degeneration leading to neuroimaging-invisible diffuse white-matter damage, or the presence of a global metabolic depression. The observation that fewer gray-matter regions reached significant reduction in comparison to white-matter could indicate a greater axonal involvement, although this could also reflect the increased uncertainty in the measurement in GM relative to WM (Maudsley et al., 2009a). The finding of decreased NAA and NAA/Cre is in agreement with results of several previous studies (Brooks et al., 2000; Cecil et al., 1998b; Friedman et al., 1998; Garnett et al., 2000a, 2000b; Govindaraju et al., 2004; Macmillan et al., 2002; Marino et al., 2007; Ross et al., 1998), that used single-voxel or MRSI measurements in acute-to-chronic conditions, but this study places greater emphasis on the magnitude of these changes in the sub-acute period following even mild injury, and on the global extent of the observed changes.

The role and functions of NAA are yet to be understood completely; however, it is reported to provide a source of acetate for myelin synthesis in oligodendrocytes (Burri et al.,

1991; Chakraborty et al., 2001), and to play a role in neuronal energetics (Bates et al., 1996). A part of the globally reduced NAA can be attributed to axonal and neuronal damage (via wallerian degeneration) at the sites of focal and TMB lesions seen on CT and MR images. Of the 29 subjects included in this study, 26 showed focal lesions or TMBs, although most were small in size. Additional neuroimaging-invisible injury may also be present, since, unlike hemorrhagic brain injuries, not all non-hemorrhagic and microscopic lesions are visible in CT and MRI scans (Adams et al., 1989; Gennarelli et al., 1982; Mittl et al., 1994). Neuropathological studies (Blumbergs et al., 1994; Jones et al., 1998) have reported that diffuse brain injury was not detected by CT and MRI, and diffusion tensor imaging studies have also shown significant white-matter changes in the corpus callosum (Nakayama et al., 2006; Rutgers et al., 2008), and whole white matter (Benson et al., 2007) after closed head injury with or without macroscopic lesions. Results from these studies indicate that conventional neuroimaging-invisible microscopic brain injury exists, and may be either caused by mechanical (focal) damage to axonal cytoskeleton, or initiated by biochemical changes (Bigler 2003; Povlishock, 1993).

Data from animal studies (Signoretti et al., 2001, 2009) have provided evidence of impairments in neuronal energy metabolism by associating decreased NAA with a decrease in ATP, and the degree of decrease in these compounds correlated with severity of the brain injury (Signoretti et al., 2001). In an animal model of brain injury, Gasparovic and colleagues (Gasparovic et al., 2001) reported an initial NAA/Cr decrease and recovery thereafter in sites distant from injury, with a corresponding decreased/increased uptake of 2-deoxyglucose observed in a separate study. After inducing striatal neurodegeneration in rats and primates using the mitochondrial toxin 3-nitropropionate (3-NP), Dautry and associates (Dautry et al., 2000) showed an early decrease in NAA with no signs of cell loss in both species, and partial reversibility of NAA 4 weeks after 3-NP withdrawal in rats. Therefore, these studies indicate that the observed NAA decrease may reflect early-stage dysfunctional neuronal energy metabolism, with or without cell loss.

Following injury to brain parenchyma, it is hypothesized that NAA in neurons may be transported to oligodendrocytes and hydrolyzed to create a pool of acetate molecules for myelin synthesis (Burri et al., 1991; Chakraborty et al., 2001). Such myelin lipids could be used to repair the damaged or lost myelin sheaths covering the injured axons. Such a process corroborates the initial NAA decrease after injury, and recovery at a later time corresponding to increased NAA hydrolysis (Cecil et al., 1998a) to meet the increased demand for acetate molecules, and the extent of recovery to normal level thereafter, indicating less hydrolysis of NAA, and also severity of injury. In this single-time-point study performed at subacute conditions, another possible reason for the NAA decrease observed in all white-matter regions may be its increased hydrolysis.

Cho and Cho/Cr were increased in all white-matter regions of the TBI groups compared to controls, with a larger increase in the TBI-3 group relative to the milder-injury TBI-2 group. Such a widespread reduction in Cho throughout the cerebrum again indicates that these changes likely reflect an underlying neuroimaging-invisible injury or altered Cho metabolism. However, the limited findings for GM indicate

that this region is relatively less affected than the WM. Our results are consistent with findings from earlier studies (Brooks et al., 2000; Friedman et al., 1998; Garnett et al., 2000a, 2000b; Govindaraju et al., 2004; Marino et al., 2007), while also showing wider involvement of these changes than previously indicated.

The Cho signal observed by proton MRS has contributions from free choline, and mobile components of membrane phosphocholine and glycerophosphocholine (Govindaraju et al., 2000). The observed increase may be due to breakdown products of myelin and cell membranes (Cecil et al., 1998a; Ross et al., 1998), reactive astrogliosis (Garnett et al., 2000a), and increased synthesis of membrane lipids. Other studies have found that the initially elevated Cho resolved towards normal levels with time after injury in patients with good outcomes; however, it remained elevated in patients with poor outcomes, indicative of continued metabolic alterations (Brooks et al., 2000; Shutter et al., 2004). In this subacute study, significant increases of Cho or Cho/Cr were observed in a higher number of WM lobar regions in TBI-3 than in TBI-2 subjects, possibly indicating increased diffuse tissue damage associated with the higher whole-brain lesion volume.

The ratio of Cho/NAA, or NAA/Cho, is commonly used to increase the sensitivity for detecting changes seen after brain injury, as NAA and Cho vary in opposite directions, and this is also clearly indicated in this study, in which significant increases were found in both GM and WM, and in subjects with minimal injury (i.e., those with GCS scores of 14 and 15).

While Cr was found to be increased in right temporal WM and two GM regions, these differences were small. As such, this study does not support the findings of Gasparovic and colleagues (Gasparovic et al., 2009), who found increased Cr in a group of 10 mTBI subjects at a mean time of 11 days after injury.

NPT scores and their association with metabolite measures

In this study, the mean NPT z-scores of nearly half of the subjects ($n = 13$) were below -1.00 (i.e., more than one standard deviation below the mean of normative data), and five subjects were below -2.00 for at least one of the domains, indicating at least subtle cognitive deficits. It is probable that some of the deficits observed are associated with the presence of lesions in the frontal and temporal lobes, as significant correlations were observed between the lesion volumes and scores of 7 of the 10 NP tests. However, the observation of no significant correlations between the lesion volumes and scores of three NP tests in this study and other studies (Bigler, 2001b; Hofman et al., 2001) lead to two possibilities: heterogeneity in lesion type, site, and volume, and its morphological evolution with time, and the possible presence of lesions invisible on conventional structural MRI, and non-specific cellular damage throughout the brain (Bigler, 2000a). Nevertheless, the reduced z-scores observed in this study, though subtle in many subjects, suggested the presence of residual cognitive deficits at subacute conditions.

In this first examination of the association between whole-brain MRSI results and NPT scores, several important points can be deduced from the test results: (1) In WM, despite observing significant Cho/NAA changes throughout the brain

in the TBI groups (see Fig. 4), correlations with NPT scores were found mostly in the left frontal lobe. (2) Several individual test scores in the left frontal lobe white matter correlated with Cho/NAA, whereas only Trail Making Test A correlated with the right frontal lobe WM. (3) Between Cho/NAA versus the NPT scores of the domains, significant correlations were observed only in the left frontal lobe WM. (4) In GM, Cho/NAA in the TBI group increased significantly (see Fig. 5) in all the lobes of the left hemisphere and right frontal and temporal lobes, but significant correlations with NPT scores were only found in the left frontal GM.

It is speculated that such selective correlations in both the WM and GM lobar regions may be due to either the relatively higher number of subjects in this study with lesions in these lobes than in others (20 with frontal lesions, 9 temporal, and 2 parietal), or MRS and NPT may be measuring different processes (localized cellular physiology versus broad functional connectivity). Further, the observation of significant correlations between Cho/NAA and NPT scores only in the left frontal lobe WM and GM, even though more subjects had lesions in the right frontal lobe ($n = 15$) than in the left frontal lobe ($n = 14$), supports the well-known lateralization of frontal lobe functions, and involvement of the left side preferentially for cognitive functions, such as working memory (Goldberg et al., 1994) and language tasks (Frost et al., 1999). In addition, previous studies have reported left hemispheric dominance for language tasks (Stroobant et al., 2009), and differential effect of mass lesions in the left and right frontal lobe on verbal fluency tasks (Baldo et al., 2001). Our results of significant correlations between the metabolite and NPT measures mostly in the left frontal lobe GM and WM are consistent with the above-mentioned previous reports. There are no similar studies in the literature to compare with our results; however, in general terms, our results agree with single-voxel spectroscopy results (Brooks et al., 2000; Friedman et al., 1998, 1999) in moderate-to-severe TBI from the normal-appearing occipital lobe GM and WM metabolites versus NPT scores. In a single, sub-acute time-point study (Friedman et al., 1998), significant correlations were found between the z-scores of several individual NPT tests and metabolites in occipital lobe WM.

Limitations of this study

Limitations of the MRS methods used in this study include that metabolite and water relaxation times (T_1 and T_2) were not accounted for, and therefore the reported MRSI measures include possible contributions from altered relaxation times. This reflects practical difficulty of including the lengthy relaxation time measurement in the study protocol. The use of metabolite ratios to Cre, which is commonly assumed to remain unchanged in pathologies, represents an additional uncertainty, especially in light of findings of altered Cre in other brain injury studies (Gasparovic et al., 2009; Ross et al., 1998). Finally, while the volumetric MRSI method used in this study sampled a much greater fraction of the brain volume than any previous study, the method still leaves approximately 30% of the brain volume unsampled, notably in fronto-temporal regions.

Premorbid intellectual functioning was not assessed in the subjects with TBI, which limits our ability to quantify the true

cognitive deficits that were due to closed-head injury. The NPT battery was not administered to control subjects, and that would have provided better study-population-matched normative values for calculating standard and z-scores, compared to using textbook normative mean and SD values. Long-term functional outcome was not available for many of the brain injury subjects, so the association between the outcome and MRS and NPT measures at subacute conditions was not evaluated in this report.

An additional limitation of this study is that the pool of subjects considered for enrollment in the study were those admitted to the trauma center, but did not include self-reported patients that went to the emergency department and were discharged after evaluation and observation. This may have resulted in a larger proportion of subjects with intracranial lesions, classified as complicated mild TBI.

Conclusions

The results of this study demonstrate that widespread alterations of proton MRS-observed metabolites occur throughout the brain following mild-to-moderate TBI, and that these can be quantified relative to age-matched control values, with the ratio of Cho/NAA showing greatest discrimination. Although some of these alterations were associated with focal lesions and neuroimaging-visible diffuse injury, this study provided evidence for whole-brain metabolic depression indicative of altered cellular metabolism.

Results from neuropsychological tests alone found that majority of the mTBI subjects had either no, or minimal, deficits following injury; however, correlations between the metabolite measures and NPT scores found significant associations of the metabolite measures with the scores of several NP tests, mostly in the left frontal lobe WM and GM. It is, however, likely that these associations were driven by a small number of subjects with the greatest degree of injury that had frontal lobe lesions, and it will be necessary to acquire additional data to further investigate these relationships. Although a greater number of subjects had right frontal lobe lesions than left, our findings may reflect the well-known preferential involvement of left frontal lobe for cognitive functions. The observation of whole-brain lobar level metabolite depressions, minimal or no cognitive deficits in the majority of patients with TBI, and significant correlations of NPT scores with mostly left frontal lobe metabolites following TBI, suggests that the use of whole-brain metabolite measures may provide greater sensitivity for detecting widespread cellular metabolic dysfunction and diffuse injury.

This study has additionally shown that the whole-brain MRSI methodology, with voxel-based analysis of individual-subject whole-brain metabolite data and comparison with normative metabolite data (Maudsley et al., 2009a) can provide quantitative information of great value in the diagnosis and evaluation of mTBI. The MRSI acquisition has been implemented in a routine manner, and although data processing was performed off-line, this could be readily integrated in the clinical setting. The method has also been found to be relatively robust, with loss of 12% of the data due to inadequate quality or subject non-compliance. While this report has presented an analysis based on averaged metabolite values, the altered metabolite information is available in a clinically usable image format.

Acknowledgments

This work was supported by National Institutes of Health grants R01NS055107 and R01EB000822. The authors thank Sulaiman Sheriff for his help in creating the figures.

Author Disclosure Statement

No competing financial interests exist for the authors.

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