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Multi-modal MRI of mild traumatic brain injury

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ABSTRACT

Multi-modal magnetic resonance imaging (MRI) that included high resolution structural imaging, diffusion tensor imaging (DTI), magnetization transfer ratio (MTR) imaging, and magnetic resonance spectroscopic imaging (MRSI) were performed in mild traumatic brain injury (mTBI) patients with negative computed tomographic scans and in an orthopedic-injured (OI) group without concomitant injury to the brain. The OI group served as a comparison group for mTBI. MRI scans were performed both in the acute phase of injury (~24 h) and at follow-up (~90 days). DTI data was analyzed using tract based spatial statistics (TBSS). Global and regional atrophies were calculated using tensor-based morphometry (TBM). MTR values were calculated using the standard method. MRSI was analyzed using LC Model. At the initial scan, the mean diffusivity (MD) was significantly higher in the mTBI cohort relative to the comparison group in several white matter (WM) regions that included internal capsule, external capsule, superior corona radiata, anterior corona radiata, posterior corona radiata, inferior fronto-occipital fasciculus, inferior longitudinal fasciculus, forceps major and forceps minor of the corpus callosum, superior longitudinal fasciculus, and corticospinal tract in the right hemisphere. TBSS analysis failed to detect significant differences in any DTI measures between the initial and follow-up scans either in the mTBI or OI group. No significant differences were found in MRSI, MTR or morphometry between the mTBI and OI cohorts either at the initial or follow-up scans with or without family wise error (FWE) correction. Our study suggests that a number of WM tracts are affected in mTBI in the acute phase of injury and that these changes disappear by 90 days. This study also suggests that none of the MRI-modalities used in this study, with the exception of DTI, is sensitive in detecting changes in the acute phase of mTBI.

1. Introduction

Traumatic brain injury (TBI) affects about 1.7 million Americans annually (CDC, 2010), with ~80% of the cases categorized as mild (mTBI) (Faul et al., 2010). Computerized tomography (CT) and conventional magnetic resonance imaging (MRI) often appear normal without anatomical lesions in mTBI (Hughes et al., 2004; Bazarian et al., 2007) and have been considered less sensitive than more advanced forms of MRI to diagnose mTBI or monitor the course of injury. Therefore, mTBI is generally diagnosed on clinical assessment, based upon reported history of loss of or impaired consciousness, post-traumatic amnesia, or post-concussion symptoms (PCS) (e.g., headaches, dizziness, fatigue, irritability), and cognitive/memory complaints. The underlying pathologic mechanisms in mTBI are still unclear although it has been postulated that stretch-induced axonal injuries may be the cause (Blumberg et al., 1995).

Advances in MRI-based neuroimaging techniques, such as diffusion tensor imaging (DTI), magnetization transfer ratio (MTR), magnetic resonance spectroscopic imaging (MRSI), and perfusion imaging, have brought new potential to mTBI diagnosis (Shenton et al., 2012). DTI investigation of mTBI patients has attracted researchers because this imaging modality is particularly sensitive to microscopic white matter (WM) changes and may be able to detect diffuse axonal injury in mTBI (Shenton et al., 2012; Bigler, 2013; Hulkower et al., 2013; Fox et al., 2013; Ling et al., 2013; Kou et al., 2013; Hasan et al., 2014). DTI

Abbreviations: *acr*, anterior region of corona radiata; *alic*, anterior limb of internal capsule; *cc*, corpus callosum; *cs*, centrum semiovale; *cst*, corticospinal tract; *ec*, external capsule; *ic*, internal capsule; *ifo*, inferior fronto-occipital fasciculus; *ilf*, inferior longitudinal fasciculus; *pcr*, posterior region of corona radiata; *plic*, posterior limb of internal capsule; *scr*, superior region of corona radiata; *sfo*, superior fronto-occipital fasciculus; *slf*, superior longitudinal fasciculus; *sfg*, superior frontal gyrus; *mfg*, superior frontal gyrus; *jlc*, juxtapositional lobule cortex; *cg*, cingulate gyrus; *pcg*, paracingulate gyrus.

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is based on diffusion of tissue water molecules and by exploiting the anisotropic nature of diffusion it is possible to gain information about the microstructural organization and integrity of WM fiber tracts that interconnect various brain regions (Mori and Zhang, 2006). The most commonly used DTI-derived measures are the fractional anisotropy (FA) that reflects diffusion anisotropy and mean diffusivity (MD) that represents average diffusivity and axial diffusivity (AD), and radial diffusivity (RD). Together, these DTI measures could serve as markers of tissue integrity (Pierpaoli and Basser, 1996). Previous DTI reports on mTBI have produced somewhat inconsistent results (see for example, the review by Shenton et al., 2012). Reduced FA and elevated MD in various WM regions in the acute and chronic phase of mTBI were reported (see for example, Toth et al., 2013). Increased FA and MD were also reported both in acute and subacute phases (Bazarian et al., 2007; Wilde et al., 2008; Mayer et al., 2010; Lipton et al., 2012). Both increased and decreased FA were reported in the same patients (Kou et al., 2013). A recent study reported changes only in MD in certain WM tracts (Hasan et al., 2014). Finally, no changes in FA were observed in concussed individuals at about 30 days post-injury (Zhang et al., 2010).

Magnetization transfer ratio is sensitive to the macromolecular pool. Since myelin represents major macromolecular pool in brain, it is generally thought that MTR is sensitive to the state of myelin (see for example, McGowan, 1999) and is therefore an indirect measure of myelin integrity (Pike et al., 2000; Mottershead, 2003). Reduction of MTR was reported in some WM regions in mTBI and in some cases significant correlations were found between regional MTR and neuropsychological performance (McGowan et al., 2000). Histogram based analysis showed reduced whole brain MTR in moderate TBI and mTBI subjects with persistent PCS (Hofman et al., 2002).

Brain atrophy is generally thought to represent neurodegeneration. MRI is an excellent modality for estimating global and regional atrophies in mTBI and for following their longitudinal evolution. There are only a few studies that investigated atrophy in mTBI (Ling et al., 2013; Cohen et al., 2007; Lannsjö et al., 2013; Ross et al., 2012; Zhou et al., 2013). These studies suggested measurable atrophy in mTBI around 1 year after the insult (Zhou et al., 2013). Regional atrophy was also observed in the anterior cingulate, cingulate gyrus, and the right precuneal gray matter (GM) (Zhou et al., 2013). These observations are similar to those reported earlier (MacKenzie, 2002). In a 2 year follow-up study, progressive atrophy was also observed in mTBI (Ross et al., 2012). Most of these studies, however, were based on relatively small sample size with variable post-injury scanning times. Also, the earliest time frame at which atrophy could be detected following mTBI is unknown.

Metabolic disturbance is postulated in regions of contused brain (Giza and Hovda, 2001). Magnetic resonance spectroscopy (MRS) measures brain metabolites that reflect local neuronal integrity and cell membrane turnover (Narayana, 2005). Application of MRS to mTBI was recently reviewed (Lin et al., 2012; Gardner et al., 2014). Because mTBI induces a cascade of changes in brain metabolites, including neuronal and axonal loss, MRS has the potential to improve our understanding of the underlying metabolic disturbances in mTBI (Cohen et al., 2007; Lin et al., 2012; Vagnozzi et al., 2008; Henry et al., 2011). The major peaks observed in proton MRS of brain include N-acetylaspartate (NAA) + N-acetyl aspartyl glutamate (NAAG) (it is generally difficult to resolve these resonances and NAA + NAAG is referred to as NAA), creatine (Cr), choline (Cho), and myoinisotol (mI). NAA is believed to be a specific neuronal marker. Creatine resonance has contributions from creatine and phosphocreatine and elevated Cr levels may represent gliosis. Choline resonance has contributions from multiple molecules that include phosphorylcholine, glycerophosphorylcholine, and choline plasmalogen, and a minor contribution from acetylcholine and choline. Choline peak reflects cell membrane metabolism and elevated Cho concentration represents heightened cell membrane turnover as seen in demyelination, remyelination, inflammation, and gliosis. Myoinisitol is thought to be glial specific and is also a precursor of phospholipid membrane constituents and its concentration is affected by the formation and breakdown of myelin (reviewed in Sajja et al., 2009). The results for MRS in mTBI, however, are not always consistent (reviewed by Gardner et al., 2014).

Based on this brief description one may conclude that each of the MRI modalities is sensitive to different aspects of tissue pathology. It is possible to improve the pathologic specificity in mTBI by using multimodal MRI. While there have been many independent studies using any one or two of these modalities (reviewed in Shenton et al., 2012; Lin et al., 2012), we are unaware of the application of all these techniques to the same mTBI cohort.

The main objective of this study was to perform comprehensive multi-modal MRI in mTBI and orthopedic injured (OI) controls. This current study also addresses two shortcomings of many of previously published reports. First, in almost all the previously reported studies scans were acquired days or even weeks post-injury for determining the acute changes in mTBI. It is not clear what changes occurred in the first 24 h after the injury. Second, few published studies included both baseline and follow-up scans.

2. Methods and material

This work was approved by the Institutional Review Boards (IRBs) at participating institutions and the Human Research Protection Official's (HRPO) Review of Research Protocols for the Department of Defense. All procedures were compliant with the Health Insurance Portability and Accountability Act (HIPAA). The project reported here is part of a larger study of mTBI, supported by the Department of Defense, where a consecutive series of civilian patients was recruited prospectively with either mTBI or minor orthopedic/extremity injuries from the Emergency Departments (EDs) of two Level 1 trauma centers and one Level 3 community hospital in a large ethnically-diverse southwestern metropolitan area. Initial and follow-up assessments of the OI subjects provided comparative data from individuals with demographics and risk factors similar to the mTBI subjects. The definition of mTBI used in this study followed the guidelines of the Department of Defense (Assistant Secretary, 2007) and the American Congress of Rehabilitation Medicine (Kay, 1993). Subjects were included irrespective of their gender, race, and ethnicity and were recruited by healthcare professionals (RN, MD, EMT-P) who had clinical experience with brain injury patients, knowledge of research, and excellent interpersonal and problem-solving skills. Screening occurred through review of data in the EDs electronic healthcare system (EHS), consultation with ED staff, and subject interviews. Special permission was obtained from the institutional IRBs to administer the Galveston Orientation and Amnesia Test (GOAT) prior to obtaining informed consent to identify cognitive impairment that would preclude provision of informed consent. Subjects had to score ≥75 on the GOAT (Levin et al., 1979) to provide informed consent; if a subject's score was 74 or lower, the plan was to obtain informed consent from a legally authorized representative (LAR). There have been no scores below 75, so all enrolled subjects have provided written informed consent.

Inclusion criteria for both groups (mTBI and OI) included age 18–50 years, injury occurring within the preceding 24–48 h, and no requirement for hospitalization for the injury for which the participant was enrolled. For mTBI subjects, inclusion criteria also required the presence of a head injury (documented in medical records and/or verified by witnesses), Glasgow Coma Scale (GCS) score between 13 and 15, loss of consciousness for <30 min (including 0 min), post-traumatic amnesia <24 h (including 0 min), and a negative head CT scan. Inclusion criteria for the OI comparison group included an Abbreviated Injury Severity (AIS) score <3 for an extremity or pelvis injury, with no head injury present. Appropriate candidates with orthopedic/extremity injuries that also sustained a head injury were enrolled in the mTBI group. Exclusion criteria for both groups included AIS >3 for any body part, history of significant preexisting disease (e.g., psychotic disorder, bipolar disorder,

PTSD diagnosed by a psychiatrist or psychologist, past treatment for alcohol dependence or substance abuse), blood alcohol level >80 mg/dL at the time of consent, documentation of intoxication, left-handedness, and contraindications for MRI (including claustrophobia and pregnancy). Previous head injury requiring hospitalization or ED treatment was also an exclusion criterion for all subjects.

2.1. MRI protocol

All MRI scans were performed on a Philips 3 T scanner using an eight channel receive-only head coil. The MRI protocol included acquisition of 3D T1-weighted images (T1 images for brevity) using the magnetization prepared rapid acquisition of gradient echoes (MPRAGE) sequence for spatial normalization and morphometric measurements, 3D fluid attenuation by inversion recovery (FLAIR) for visualizing lesions, if present, 2D T2-weighted gradient echo for visualizing hemorrhage, 3D gradient echo sequence for MTR measurements for assessing the state of myelin, and 2D MRSI for metabolite mapping, and a single-shot echo-planar imaging (EPI) for acquiring diffusion weighted imaging (DWI) for calculating the DTI measures. The acquisition parameters for all the sequences are summarized in Table 1. All MRI data were automatically assessed for image quality as described elsewhere (Narayana et al., 2012; Hasan et al., 2014) and scans with inadequate quality were discarded from further analysis.

2.2. Quality assurance of the MRI scanner

Consistent performance of the scanner and its temporal stability are critical for longitudinal studies (Hasan, 2007). The quality assurance (QA) issues are addressed by implementing the American College of Radiology (ACR; http://www.acr.org/quality-safety/accreditation/mri) recommended QA program that measures signal-to-noise ratio, field uniformity, gradient linearity, image distortions and ghosting using the vendor provided phantom every morning. The results of the phantom study were automatically analyzed using custom written software. In addition, as described elsewhere (Hasan et al., 2014), DTI data was acquired monthly on a homogenous spherical water phantom that was kept at a temperature between 18 and 20 °C (temperature in the scanner room), maintained by the central air conditioner. The DTI data was analyzed using the procedure described elsewhere (Hasan et al., 2014). Briefly, DTI data was acquired on a spherical water phantom almost monthly over 4 years (47 time points). The mean FA and MD values and their standard deviations were automatically calculated from an ROI of 21 × 21 voxels at the isocenter of the magnet.

2.3. Image processing

2.3.1. Diffusion tensor imaging

The DWI images in the DICOM format were transferred from the MRI scanner to a local Linux computer (Xeon 2.8 GHz 4-core CPU with 24 GB memory) and were converted to Neuroimaging Informatics Technology Initiative (NIfTI) format using *dcm2nii* file converter from the MRICron software (http://www.cabiatl.com/mricro/mricron/dcm2nii.html). The brain was extracted from the images using the brain extraction tool (BET) software from FSL toolbox (http://www.fmrib.ox.ac.uk/fsl/bet2/index.html). Eddy-current correction was performed in FSL by aligning all the DWI images to the *b*0 volume (images acquired without any diffusion weighting) using affine transformation with 12 degrees of freedom. The DTI measures (FA, MD, and the three eigenvalues, λ_1, λ_2, and λ_3) were then calculated using the FDT software from the FSL toolbox. The DTI data was analyzed using tract based spatial statistics (TBSS) (Smith et al., 2006). The identification of the individual tracts was verified using the MRI atlas of human white matter (Oshi et al., 2010).

Standard TBSS analysis steps as recommended by the developer of TBSS were followed. The FSL-provided FA template (FMRIB58_FA; Smith et al., 2006), which was derived from healthy controls and its derived skeleton in the MNI space were used. A skeleton threshold of 0.2 was used. For group comparison, randomize test on skeletonized DTI images was conducted with Threshold-Free Cluster Enhancement (TFCE) option and 5000 runs. Statistical results were generated both with and without the family-wise error (FWE) correction for multiple comparisons.

2.3.2. Magnetization transfer ratio

Images acquired with (M_{SAT}) and without (M_0) the off- resonance RF pulse were stripped of extrameningeal tissues using BET from the FSL toolbox. Bias field correction was applied using the NT4 module from Advanced Normalization Tools (ANTs) software package (http://sourceforge.net/projects/advants). The corrected M_{SAT} and M_0 images were non-linearly co-registered to the MNI152 1 mm T1 template (included in FSL toolbox) using the symmetric inverse consistent diffeomorphic registration from the ANTs software. The magnetization transfer ratio (MTR) was calculated as:

$$MTR(\%) = (M_0 - M_{SAT})/M_0 \times 100.$$

2.3.3. Morphometry

Tensor based morphometry (TBM) was used for estimating regional and global atrophy. For TBM analysis, the T1 images were non-linearly registered to the MNI152 template using symmetric inverse consistent diffeomorphic registration from the ANTs software. The output of the affine and diffeomorphic transformation files was combined to generate the composed transformation. The Jacobian determinant (JD) maps were constructed from the composed transformation of the subject-to-template registration (Leow et al., 2006). The JDs were normalized to compensate for differences in the brain size and logarithmic transformation was applied (Tao et al., 2009). For group comparison, statistical tests were performed on the JDs using two-sample *t*-test model in SPM8. Paired *t*-tests were also performed on the subjects who had both initial and follow-up scans.

Table 1
Summary of MRI acquisition parameters. The in-plane field of view is 256 × 256. TR: repetition time; TE: echo time; MTR: magnetization transfer ratio; off res frequency: frequency offset for the off resonance pulse.

Sequence	TR/TE (ms)	2D or 3D	MTR		TI (ms)	Voxel dimensions (mm × mm × mm)	Number of gradient directions	Number of averages	Acquisition time (min)
			Off res frequency	Ampl					
MPRAGE	8.1/3.7	3D	N/A	N/A	1071	1 × 1 × 1	N/A	1	5:56.1
FLAIR	8000/337	3D			2400	1 × 1 × 1	N/A	1	8:24.0
Gradient echo (for MTR)	65/5.9	3D	2100 Hz	2.34 uT or 620^0		1.25 × 1.25 × 3	N/A	1	5:01.9
Dual gradient echo (T2-weighted)	510/16/32	2D	N/A	N/A	N/A	1 × 1 × 3	N/A	1	4:33.4
PRESS (for MRSI)	2000/53[a]	2D	N/A	N/A	N/A	10 × 10 × 15	N/A	1	8:26.0
EPI (DWI)	8000/55	2D	N/A	N/A	N/A	2 × 2 × 3	32	1	5:46.9

[a] Minimum echo time allowed on the scanner for the MRSI protocol used in this study.

2.3.4. Magnetic resonance spectroscopy

For 2D CSI a slab of 60 mm × 60 mm × 15 mm was placed in the centrum semiovale region. Great care was taken in placing the CSI slab across all subjects and different time points by using various land marks such as AC–PC plane and location relative the lateral ventricles. Initially all the individual MRS volumes were reconstructed in the subject's native space. For group analysis, each MRSI slab was linearly registered to the T1 scan using rigid body registration with six degrees of freedom. Then the MRS volume in the native space was warped to the MNI space by applying the deformation field obtained from T1-to-MNI152 non-linear registration. In the group analysis only those voxels that were common for all the subjects following transformation to the MNI space, as determined by their spatial coordinates, were included. The resulting common MRS volume contained both cortical and subcortical regions and contained both GM and WM tissues. In order to conduct comparison on homogenous structure and tissue types, the MRS volume was sub-divided into 12 smaller volumes-of-interest (VOI), 6 in each hemisphere, with each region containing only one brain structure and tissue type (*sfg — superior frontal gyrus*, *mfg — superior frontal gyrus*, *jlc — juxtapositional lobule cortex*, *cg — cingulate gyrus*, *pcg — paracingulate gyrus*, *wm — white matter*). Identification of these structures was based on the Harvard–Oxford cortical & sub-cortical parcellation map available from the FSL toolbox. The spectral region between 0.2 and 4.0 ppm was processed. Only the center 6 × 6 voxels (each voxel measured 10 × 10 × 15 mm^3) from the 2D CSI slab were used since they had the highest spectral quality. Metabolite concentration ratios NAA/Cr, Cho/Cr, and mI/Cr were automatically estimated with the LC Model software (http://www.s-provencher.com/pages/lcmodel.shtml). The default basis set for 35 ms was used for the LC Model analysis. Any estimated ratio with greater than 20% SD (Cramer–Rao lower bounds) were considered to be unreliable and deleted from the data analysis.

3. Results

3.1. MRI stability

Based on the daily QA measurements, the performance of the scanner either met or exceeded the ACR recommendations and vendor's specifications. The results of the DTI analysis were reported earlier (Hasan et al., 2014). The temporal changes in FA and MD did not show any systematic drifts. The average ± sd values of MD and FA over the 4 year period were $(1.88 \pm 0.16) \times 10^{-3}$ mm^2/s and (0.015 ± 0.002), respectively. Linear regression of the temporal changes in MD and FA did not show any trend, suggesting the absence of systematic drift with time.

3.2. Subject data

The mechanism of injury in both mTBI and OI subjects is summarized in Table 2. Over 90% of mTBI subjects had visible evidence of head trauma associated with multiple mechanisms, in the form of bruising/contusions/abrasions to the head, scalp, or face (84%) and scalp lacerations (44%), of which 54% (24/44) required sutures. None of the OI subjects had any of these signs of TBI. Subjects in the OI group explicitly stated that to the best of their recollection, they did not hit their head during the event that caused their injury, they had no visible signs of injury to the head (i.e. bruising, abrasions, contusion, tenderness, swelling, or scalp lacerations with or without sutures required), and they reported no LOC or PTA.

Table 2
Injury mechanisms for mTBI and ortho subjects.

Mechanism of injury	mTBI (%)	OI (%)
Assault	16	1
Blow to head	9	0
All falls	21	21
Laceration	0	45
Motor vehicle accidents	51	11
Other[a]	1	1
Sports-related	2	5
Crush injury	0	16

[a] mTBI — only blast injury from exploding tire; ortho — jammed finger in scuffle with brother.

Sixty-two mTBI subjects (30.4 ± 8.8 years age, range 19–50 years; number of males = 43; education = 13.6 ± 2.4 years) and 59 orthopedic controls (age = 29.2 ± 9 years, range 20–51 years; number of males = 45; education = 13.5 ± 2.9 years) were included in this study. All subjects were right handed. All but two subjects had a GCS score in the ED of 15, with the other two having a GCS score of 14. Among the mTBI subjects who lost consciousness after injury, the average LOC duration was 3.95 ± 5 min (range 1–20 min). The initial post-injury scan times for mTBI and OI were 25.5 ± 12.26 h (range 5.8–46 h), and 27.1 ± 13.70 h (range 0.3–56 h), respectively. The corresponding follow-up scan times were 97.9 ± 17.57 days (range 83.3–202 days) and 96.7 ± 9.26 days (range 82.9–126.9 days), respectively. It should be pointed out that the range for the follow-up for mTBI subjects may have been skewed since one subject was scanned 202 days after injury. Not all subjects completed both the initial and follow-up scans. The number of mTBI and OI controls who completed the initial scans was 56 and 54, respectively. The corresponding numbers at the follow-up were 29 (age 29.94 ± 8.22 years, range +18.9–49.7; number of males 18) and 47 (age 29.48 ± 9.03 years; range 20.25–50.75 years, number of males = 36). mTBI subjects had the option to participate in a phase II drug trial (21/65 or 32%) or enroll only in the testing/imaging portion of the study (44/65 or 68%). Those mTBI subjects in the drug trial were not included in the analysis of the follow-up scans, as the investigators are still blinded to which subjects were receiving active drug treatment and which subjects were receiving placebo treatment. This was done to ensure that any observations from this analysis would not be due to or influenced by the study drug. Therefore, the number of mTBI subjects that were included in the final analysis was approximately 50% of the OI subjects.

There were no significant female/male ratio ($p = 0.45$; χ^2 test) and age differences between the mTBI (56 subjects) and OI (54 subjects) control groups ($p = 0.72$; t-test) at the initial study. The follow-up mTBI (29 subjects) and OI (47 subjects) cohorts also did not differ either in the gender ratio ($p = 0.3$) or age ($p = 0.78$).

Two T1, two MTR and one DTI image volumes were excluded due to poor image quality that include poor SNR and motion related artifacts. Fourteen MTR image volumes were excluded due to motion between acquisition of M_{SAT} and M_0 images. MTR is based on subtraction of two images and is therefore more prone to motion artifacts. Four MRSI examinations were excluded due to poor shimming. Three DTI image volumes were excluded due to inconsistent protocol used in the early stage of the study. The final number of mTBI and OI subjects included in the analysis for each MRI modality is summarized in Table 3.

3.3. Diffusion tensor imaging

3.3.1. Comparison between mTBI and OI controls at initial scan

3.3.1.1. Significant differences with FWE correction. Fig. 1 shows the t maps of MD that shows significant differences between the mTBI and OI groups at the initial scan (FWE corrected; $p < 0.05$) superimposed on the WMFA skeleton. As can be seen from this figure, MD was significantly higher in the mTBI relative to OI subjects in several WM regions that include internal capsule (ic), external capsule (ec), superior corona radiata (scr), anterior corona radiata (acr), posterior corona radiata (pcr), inferior fronto-occipital fasciculus (ifo), inferior longitudinal fasciculus (ilf), forceps major and forceps minor of the corpus callosum (cc), superior longitudinal fasciculus (slf), and cortical spinal tract (cst). These significant differences were confined only to the right hemisphere.

Table 3
Summary of subjects included in the final analysis in the initial and follow-up scans for each MRI modality. The numbers in the parentheses indicate the number of rejected scans for reason indicated in the footnotes.

	Initial scan				Follow-up scan			
	Morphometry	DTI	MTR	MRSI	Morphometry	DTI	MTR	MRSI
mTBI	55 (1)[a]	55 (1)[b]	51 (5)[c]	55 (1)[d]	29	29	27 (2)[c]	28 (1)[d]
OI	53 (1)[a]	53 (2)[b]	44 (9)[c]	55 (1)[d]	47	46 (1)[a]	46 (1)[c]	46 (1)[d]

[a] Motion and poor image quality.
[b] Inconsistent protocol.
[c] Motion between M_o and M_{sat} images.
[d] Poor shimming.

3.3.1.2. Differences without correcting for multiple comparisons. A number structures showed differences in the DTI measures at $p < 0.05$ but did not survive the FWE correction. For example, MD was observed to be higher in the mTBI relative to OI in the *ic*, *ec*, *scr*, *acr*, *pcr*, *ifo*, *ilf*, forceps major and forceps minor of the *cc*, *slf*, and *cst* in both hemispheres. Increased MD was also seen in the genu, body and splenium of the *cc*. Increased AD (λ_1) and RD ($(\lambda_2 + \lambda_3) / 2$) were observed in some of the regions where increased MD was also found (*scr*/*pcr*, *plic*, *ifo* and *acr*; $p < 0.05$).

3.3.1.3. Longitudinal comparison (initial vs. follow-up scan). On the TBSS analysis none of the MRI measures on the follow-up scans, either in the mTBI or OI group, showed significant changes from baseline with FWE correction. Without FWE correction in the mTBI group MD decreased at follow-up in the WM regions that included *plic*, *ec*, *slf*, *acr*, *pcr*, *scr*, *ifo* and *cc* ($p < 0.05$). AD also decreased in regions that included *ec, slf, acr, scr* and *cc*) and RD decreased in *plic* and *ifo*($p < 0.05$). As in the case of initial scan, the effects appeared to be significant and more widespread in the right hemisphere.

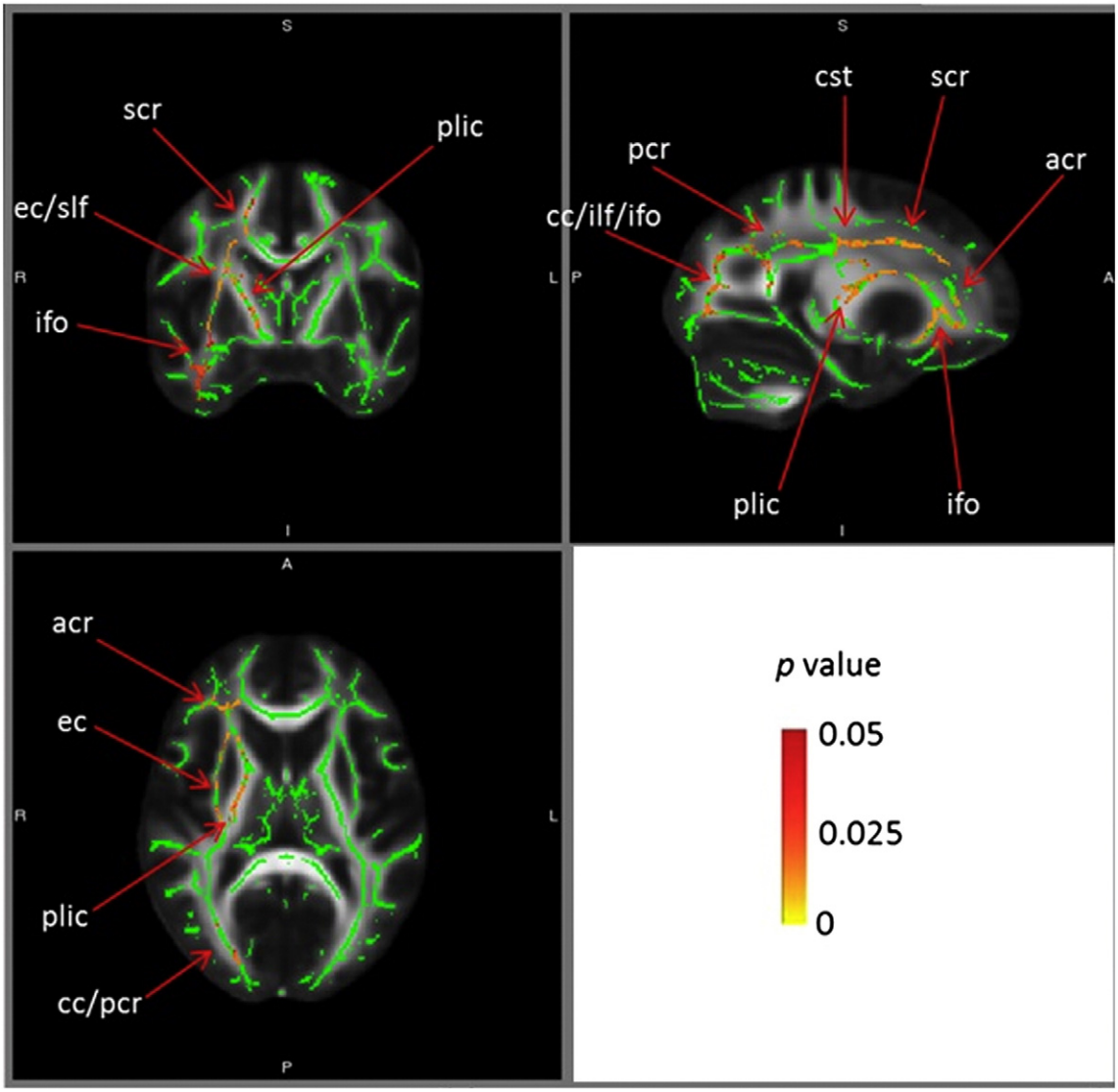

Fig. 1. Color coded t-maps superimposed on the FA skeleton demonstrating increased MD in mTBI relative to OI controls at the initial scan ($p < 0.05$ with FWE correction). The green lines are the skeletonized tracts drawn on the mean FA image obtained by averaging all FA maps registered to the MNI 152 template. *p*-Values are color-coded in the red-yellow scale, with yellow corresponding to the highest confidence level (same in all the following figures).

In the OI controls, TBSS analysis found no significant difference in any of the DTI measures between baseline and follow-up even without the FWE correction.

3.4. Magnetization transfer ratio

After registering all the subjects' MTR image volumes in the MNI space, voxel based analysis was conducted to detect differences between mTBI and controls at the initial scan and also between the initial and follow-up scans in both mTBI and OI controls. No clusters with significant difference were found in either comparison in either group.

3.5. Tensor based morphometry

The Jacobian determinant (JD) maps constructed from the T1 images were used for measuring the regional volumetric changes in the brain. As in other analyses, we investigated the baseline volumetric differences between mTBI and control subjects and volumetric changes in the mTBI and OI subjects between initial and follow-up scans. With FWE correction statistically significant clusters (≥10 voxels) were not observed in any region of the brain.

3.6. Magnetic resonance spectroscopy imaging

The 2DCSI slab was placed about 1 cm above the lateral ventricles, and parallel to the anterior–posterior commissure line (Fig. 2). The ten saturation bands for outer volume suppression are shown in blue in Fig. 2. The individual voxels ($10 \times 10 \times 15$ mm) are shown in red in this figure. The line width for the water peak from the entire spectroscopic VOI was (11 ± 2.4) Hz. The line width of NAA, for example, from individual voxels was (7 ± 1.1) Hz. The SNR in the water suppressed spectrum in each voxel was 45 ± 7. The spectra from the whole slab (average spectrum) along with representative spectra from four different voxels from one patient are also shown in this figure, The minimal lipid contamination and excellent spectral resolution can also be seen on this figure.

Parcellation of various cortical and subcortical structures was based on the Harvard–Oxford atlas as described in the methods. Fig. 3 shows the common MRS voxels in all the subjects (Fig. 3A) along with the parcellation of the common MRS volume into 12 regions (Fig. 3B), with each region containing only one brain structure and tissue type (*sfg — superior frontal gyrus*, *mfg — superior frontal gyrus*, *jlc — juxtapositional lobule cortex*, *cg — cingulate gyrus*, *pcg — paracingulate gyrus*, *wm — white matter*). The results of the metabolite ratios at the baseline and follow-up for both cohorts are summarized in Fig. 4. Cho/Cr ratio did not show significant differences between the mTBI and the control subjects on the initial scan in any of the voxels, nor did this ratio significantly differ between initial and follow-up scans in the mTBI group. mI/Cr ratio, on the other hand, showed lower values in the mTBI than in the comparison subjects on the initial scan in *jlc*, *cg*, and *pcg*, all in the right hemisphere ($p < 0.05$). None of these differences, however, survived FWE correction. In the mTBI cohort NAA/Cr showed lower value on the initial scan compared to follow-up in *jlc* in the right hemisphere ($p < 0.05$). However, this difference also did not survive FWE correction. We also analyzed the longitudinal changes by considering the ratio of the follow-up scans and the corresponding initial scans for each subject (data not shown). This strategy eliminates the inter-subject variation in the metabolite ratios and improves the statistical power. These ratios did not show statistically significant changes. We did not include the analysis of the glutamate + glutamine peaks in this analysis because their CRLB was greater than 20 in majority of the patients.

4. Discussion

To the best of our knowledge, this is the first multi-modal MRI study on mTBI subjects. A novel feature that distinguishes this study from others is that the scans were performed at approximately 24 h after injury in CT-negative mTBI patients. In addition, the same cohort was rescanned at about 3 months post-injury. Furthermore, great care was taken to eliminate confounding factors such as alcohol and drug abuse, history of hospitalization for previous TBI, and pre-injury neuropsychiatric disorders. This assured relatively uncomplicated and homogeneous mTBI cohort.

4.1. MRI stability

Scanner stability is critical in longitudinal studies. The lack of any systematic drift either in the FA or MD values along with the relatively small variances suggests the stability of the scanner over the period of time the data was acquired for this study. As reported elsewhere the possible sources of the observed variance are the temperature fluctuations and scanner table vibrations (Hunsche et al., 2001; Chenevert et al., 2011). Ideally, the FA value of water phantom should be zero. However, the noise and phase fluctuations result in a small nonzero FA value.

4.2. Diffusion tensor imaging

Overall, many of our DTI findings such as increased MD in mTBI in WM structures are in agreement with previous studies (Yuh et al., 2013; Inglese et al., 2005; Lipton et al., 2008; Miles et al., 2008; Rutgers et al., 2008; Kraus et al., 2007; Lo et al., 2009). However, it should be pointed out that strict comparison of our DTI results with other similar studies is somewhat difficult due to differences in the eligibility criteria for subject recruitment, study population, sample size, and choice of post-injury scan periods. Patients with brain lesions on CT performed within 24 h post-injury were excluded in our study, whereas this approach varied across other recent studies (Hulkower et al., 2013; Lo et al., 2009; Kim et al., 2013). In the present study, patients with lesions on MRI that were diagnosed as "incidental" by the senior neuroradiologist who participated in this study were also excluded. In those cases the lesion appearance did not change from the initial to the follow-up MRI. Instead of imaging uninjured subjects for comparison, we recruited patients who suffered orthopedic injury as controls who had similar chronicity of injury and demographic characteristics as the mTBI group. Recruitment of OI subjects accounts, to some extent, for the risk factors that predispose to injury and for the traumatic stress experienced by the mTBI subjects (Levin et al., 2013). Although the criterion for GCS score was 13–15, patients who met all of our eligibility criteria and consented to participate had GCS scores of 15, with the exception of two who had a score of 14, indicating normal consciousness or confusion when the patients were evaluated in the ED. Our mTBI cohort had less severe injuries than in other mTBI studies (see for example, Kou et al., 2013; Mayer et al., 2010).

As described in the results, except for MD in certain structures in the right hemisphere, none of the other MRI-based measures survived multiple comparison correction based on FWE. The FWE correction is known to be conservative and could lead to Type II errors (false negatives). Another commonly used and less conservative approach is the false discovery rate (FDR; Chumbley, 2009). Unfortunately, FDR is currently not an option in the TBSS analysis. Therefore, we presented our results without the FWE correction when the correction led to results that are statistically not significant. We observed significant differences in MD between mTBI and OI at the initial scan that were confined to the right hemisphere. Possible hemispheric asymmetry based on different MRI measures was also indicated by others (Ling et al., 2013; Niogi et al., 2008, 2008a; Koerte et al., 2012, 2012a). One possibility for this asymmetry is that the left hemisphere has more densely packed axon branching (Klingberg et al., 1999), presumably making it more resistant to shock-induced damage. However, caution should be exercised in interpreting the finding of the asymmetric increases in diffusivity in the right hemisphere regions.

4.3. Magnetization transfer ratio

We did not observe any significant differences in the MTR values between mTBI and OI subjects either at the initial or follow-up scans. In one of the earliest MTR studies on mTBI reduced MTR in the splenium of the *cc* was reported (McGowan, 2000). However, that study was based on a small sample size with unspecified post-injury scan periods. In addition, few details about the patient demographics were provided. Therefore it is very difficult to compare their results with ours. Whole brain reduction in MTR was reported earlier (Hofman et al., 2002). However, these authors included both moderate and mTBI subjects in their cohort with the GCS in the range of 9–15. The post injury scan period varied from 1 to 12 years. From their studies it is unclear if mTBI is associated with reduced MTR in the acute or subacute phases.

Quantifying magnetization transfer by a simple measure like MTR limits the potentially useful information about the pathophysiological processes (see for example, Garcia et al., 2012). In contrast, quantitative magnetization transfer (qMT) measures such as concentration of

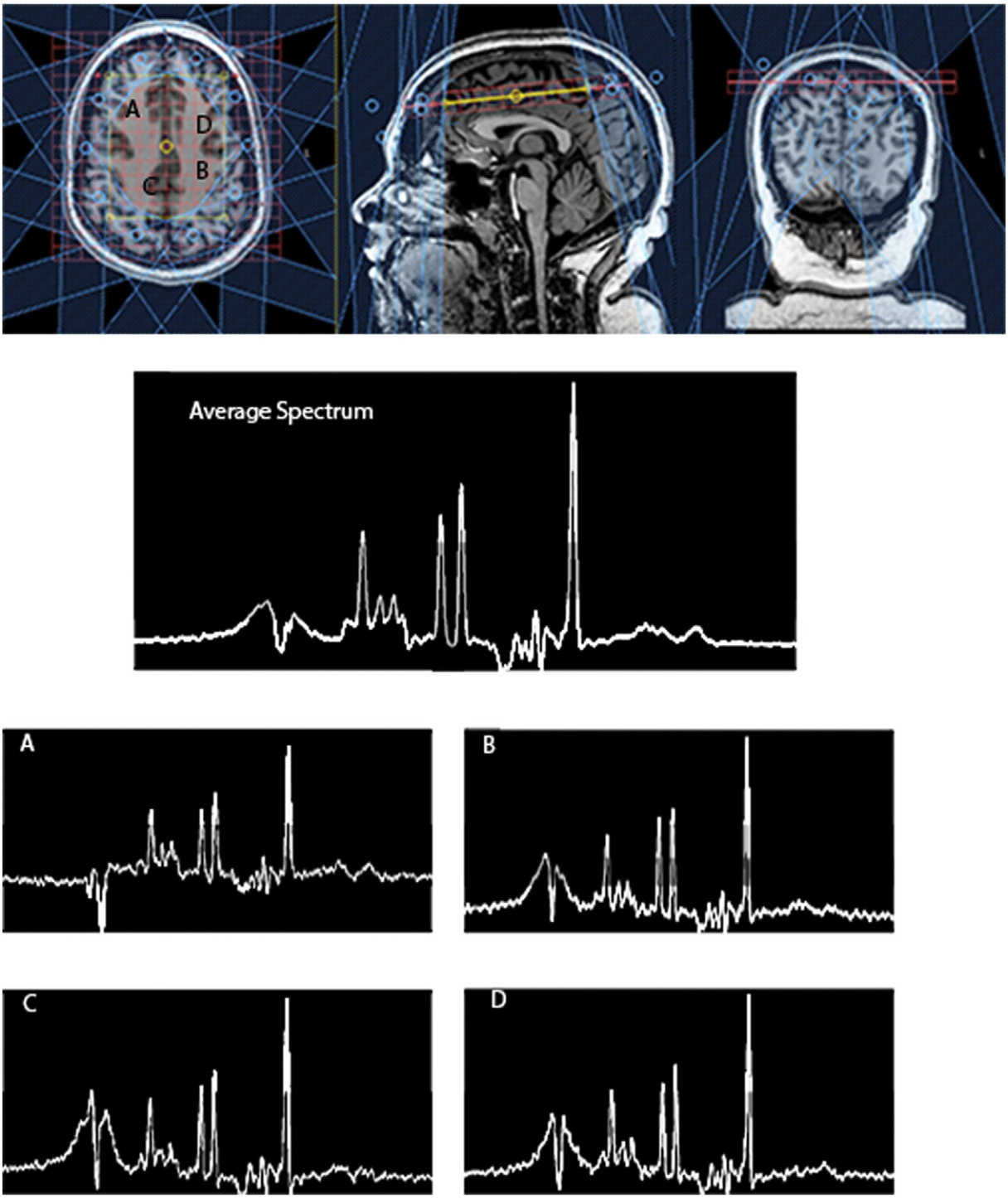

Fig. 2. Placement of the 2D CSI slab in the axial, sagittal and coronal orientations (top). The ten saturation bands for outer volume suppression are shown in blue. The individual voxels are shown in red. The "average spectrum" shown below represents the spectrum from whole slab. The individual spectra (A, B, C, D) shown at the bottom of the figure are from the voxels indicated in the top figure.

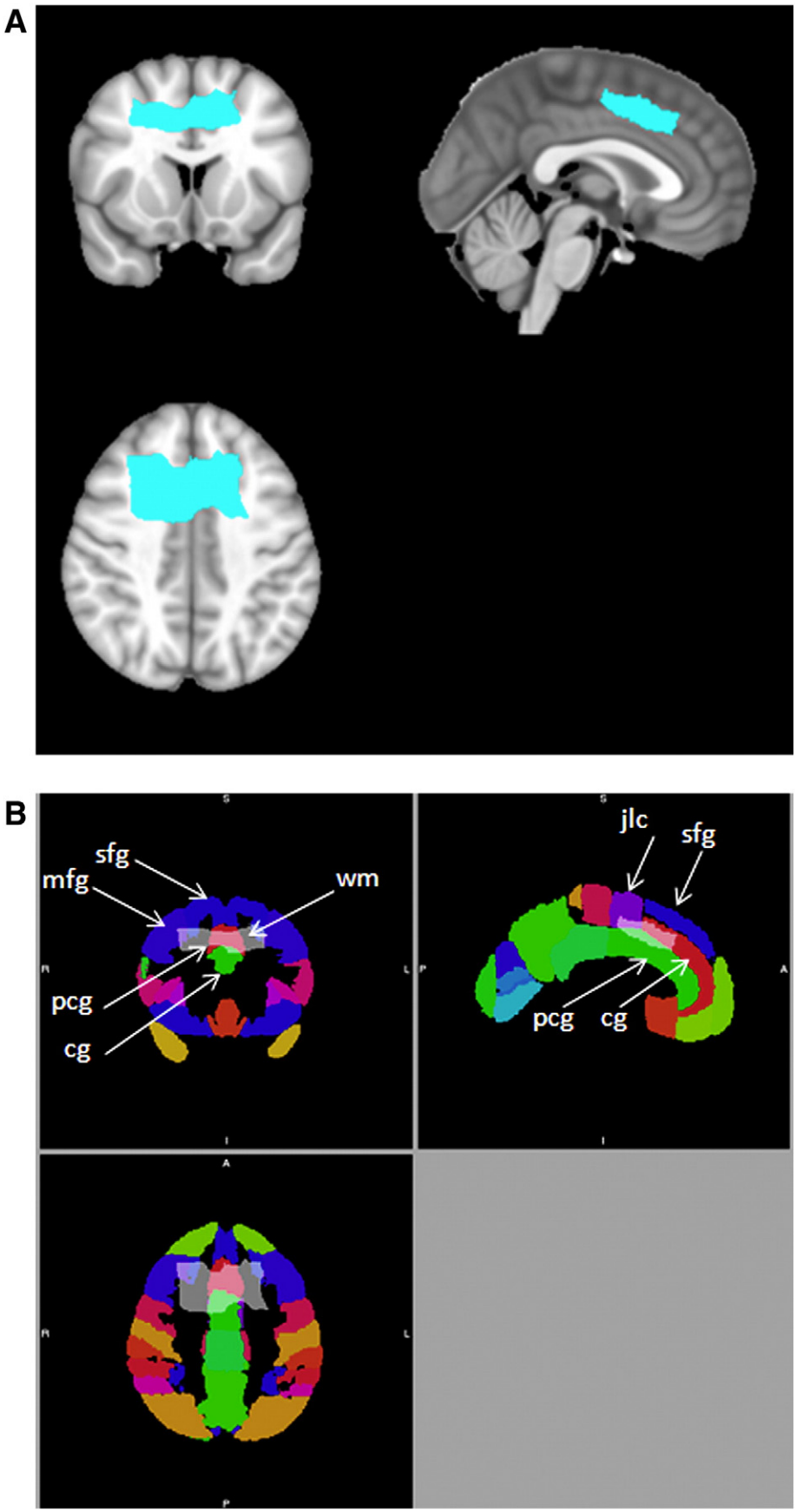

Fig. 3. MRSI and the common MRS voxels in all the subjects. A) The common MRS volume was constructed so that for any given voxel in this volume, at least 80% of all the subjects must have a valid value. B) Parcellation of the common MRS volume into 12 regions, with each region containing only one brain structure and tissue type (*sfg — superior frontal gyrus, mfg — superior frontal gyrus, jlc — juxtapositional lobule cortex, cg — cingulate gyrus, pcg — paracingulate gyrus, wm — white matter*).

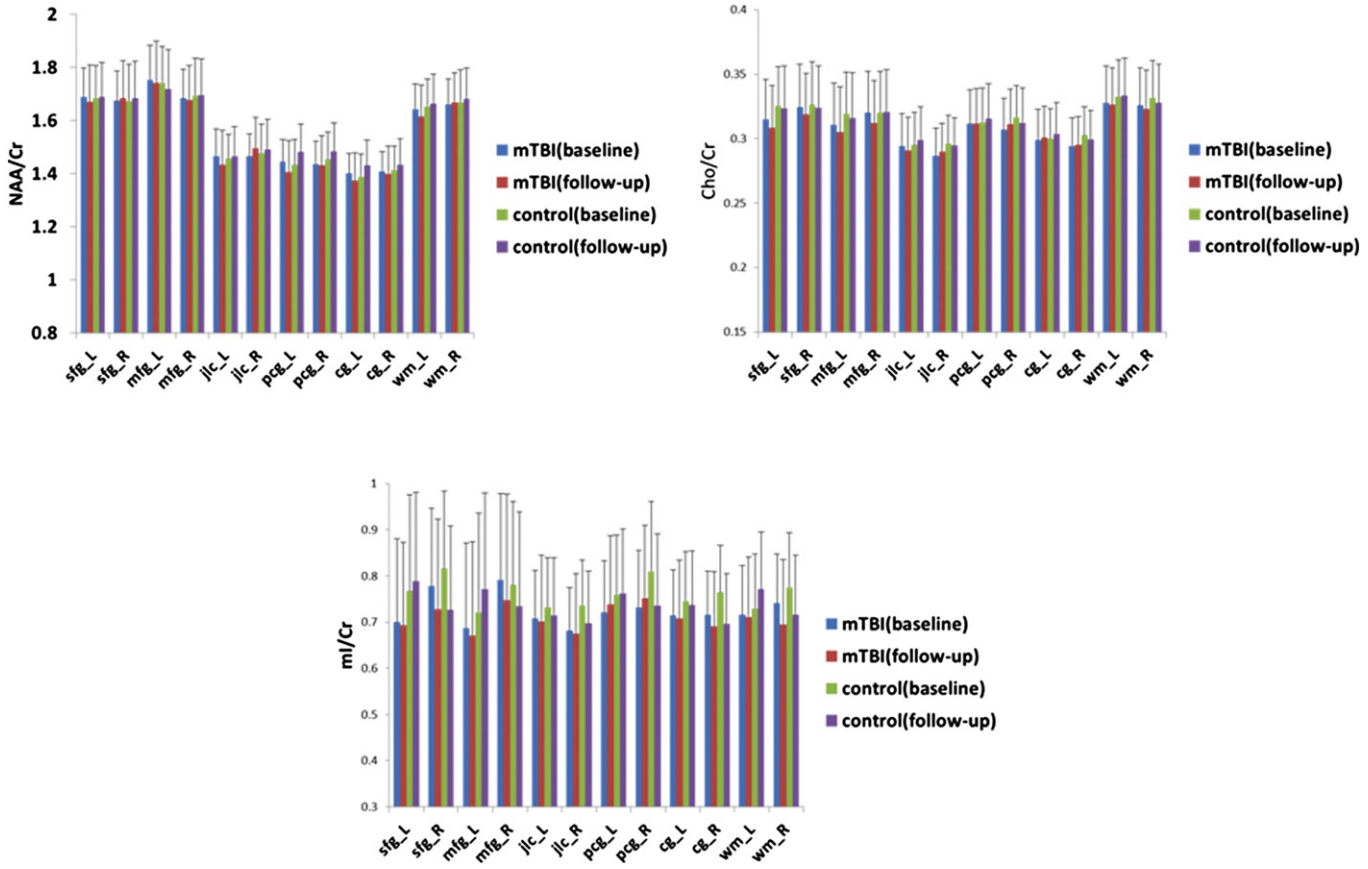

Fig. 4. Metabolite concentration ratios (relative to Cr) in 12 regions parcelled from the common MRS volume in mTBI and OI cohorts at initial and follow-up scans.

macromolecular pool and exchange rates between the pools could provide more robust information about pathology. Another method that appears to hold some promise is the macromolecular proton fraction (MPF) that is based on MTR effect (Yarnykh et al., 2012). In fact, this technique was applied to blast mTBI subjects to probe the white matter integrity with promising results (Petrie et al., 2014). Unfortunately we did not acquire the necessary data for qMT and MPF analyses in the current study.

4.4. Morphometry

Atrophy is thought to represent neurodegeneration that is the result of axonal/myelin loss. Given the mild and uncomplicated nature of injury and relatively short duration of 3 months for follow-up scans, lack of either cross-sectional or longitudinal changes in global and regional volumes in our mTBI cohort is not very surprising. Reduced cerebral volume with time does not necessarily mean atrophy. For example, reduced cerebral volume is expected with the resolution of edema that may be present in the acute phase of injury. The fact that we did not observe any volume changes at the initial or follow-up scan in mTBI relative to OI suggests that significant edema was not present in our cohort. It is difficult to compare our results on atrophy with the published results because of differences in the mTBI and control cohorts, methodology, and post-injury scan periods. For example, atrophy was reported in mTBI after 6 months post-injury (Hofman et al., 2001). In another study on 19 mTBI subjects, atrophy was observed in 4 patients at 3–7 months post-injury (Ross et al., 2012). In a comprehensive study on atrophy in mTBI global and regional atrophy in the anterior cingulate, left cingulate gyrus, istmus, and precuneus were reported at 1 year after the injury (Zhou et al., 2013). It is possible that we would have observed atrophy in our cohort if we scanned them over longer post-injury time period.

4.5. Magnetic resonance spectroscopy

Our MRSI analysis showed differences in NAA/Cr and mI/Cr ratios between the mTBI and OI subjects at the initial scan and between the initial and follow-up scans within the mTBI cohort. However, these differences did not survive multiple comparison correction, indicating that any changes in the metabolite concentrations are very subtle. Most of the published studies on mTBI that reported metabolic changes in the acute phase were performed after post-injury day 3. It is possible that metabolic changes may not be apparent until day 3 post-injury (Vagnozzi et al., 2013). It is also likely that biochemical changes could have disappeared at the 90 day follow-up scan (Vagnozzi et al., 2008, 2013).

The results of the previously published MRS studies in mTBI are not always consistent. While some studies found decreased NAA (Vagnozzi et al., 2008; Henry et al., 2011; Govindaraju et al., 2004; Cimatti, 2006; Vagnozzi et al., 2010; Henry et al., 2010; Johnson et al., 2012) in regions such as parietal lobe, motor cortex, prefrontal cortex, and *cc*, other studies did not report any changes in NAA (Maugans et al., 2012; Yeo et al., 2011). Some of these studies used single voxel MRS and this difference in methods could be a contributing factor for the discrepant results. In one report where similar 2DCSI method was used as in our study, no change was found in NAA concentration, but Cr concentration was found to increase in WM in the mTBI patients (Vagnozzi et al., 2010). Another study using single voxel spectroscopy also detected increased Cr level in white matter (Gasparovic et al., 2009). Since all the above studies that reported decreased NAA were based on the NAA/Cr ratio, including the trends observed in our study, and since increased Cr was found in mTBI, it is not clear that decreased NAA/Cr necessarily implies reduced NAA. Moreover, changes in the metabolite levels vary greatly depending on the brain area (Gardner et al., 2014; Govindaraju et al., 2004). In addition, the differences in the patient cohort, use of healthy

controls, and different post-injury scan times could have contributed to the discrepant results that were reported in the literature.

4.6. Limitations of previous studies

A problem with most of the published MRI studies in mTBI is the lack of standardization in patient selection criteria, post-injury scan times, methodology, and control subjects. These differences make an objective comparison across different studies virtually impossible. Classification of TBI as mild, moderate, or severe, based on GCS score which is global and relatively insensitive to mTBI, fails to incorporate newer insights and findings from neuroimaging (Manley and Maas, 2013). There is a need to implement more precise disease classification.

5. Study limitations

Our study also has a few limitations. It investigated longitudinal changes only at two time points. Acquiring data at multiple points that span longer post-injury periods could have provided a better trajectory of pathologic changes, particularly chronic effects. In this study we did not stratify our results based on the neuropsychological scores or PCS. Another limitation is that the metabolite concentrations were measured relative to Cr. Absolute measurements would have been preferable. However, this requires the acquisition of unsuppressed water data that was not part of our protocol. While the TE used for acquiring MRSI was 53 ms, the default basis set of 35 ms was used for the LC model analysis. While NAA, Cr, Cho, and Ins are expected to be less sensitive to the TE of the basis set, Glx with its relatively short TE and j-coupling coupling is more sensitive to the choice of the basis set. This results in poor fitting and relatively large SD. In most cases, both mTBI and OI subjects had received/been prescribed pain medications during their ED visit and which might be in their system for the initial MRI. We have not considered the effect of medication on the MRI measures. To the best of our knowledge there are no studies that link pain medication and brain MRI measures. Finally, we did not attempt to combine all the MRI measures for improved detection of neural changes. A major problem with combining the multi-modal data is assigning appropriate weight for each measure. We are currently working with our statistician to come up with an algorithm. We plan to address this issue in a future study.

Conflicts of interest

No competing financial interests exist.

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