Prospective study of myelin water fraction changes after mild traumatic brain injury in collegiate contact sports

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OBJECTIVE Mild traumatic brain injury (mTBI) in athletes, including concussion, is increasingly being found to have long-term sequelae. Current imaging techniques have not been able to identify early damage caused by mTBI that is predictive of long-term symptoms or chronic traumatic encephalopathy. In this preliminary feasibility study, the authors investigated the use of an emerging magnetic resonance imaging (MRI) technique, multicomponent driven equilibrium single pulse observation of T1 and T2 (mcDESPOT), in visualizing acute and chronic white matter changes after mTBI in collegiate football and rugby players.

METHODS This study was a nonrandomized, nonblinded prospective trial designed to quantify changes in the myelin water fraction (MWF), used as a surrogate MRI measure of myelin content, in a group of male collegiate football and rugby players, classified here as a contact sport player (CSP) cohort, at the time of mTBI diagnosis and 3 months after injury when the acute symptoms of the injury had resolved. In addition, differences in the MWF between the CSP cohort and a control cohort of noncontact sport players (NCSPs) were quantified. T-tests and a threshold-free cluster enhancement (TFCE) statistical analysis technique were used to identify brain structures with significant changes in the MWF between the CSP and NCSP cohorts and between immediately postinjury and follow-up images obtained in the CSP cohort.

RESULTS Brain MR images of 12 right-handed male CSPs were analyzed and compared with brain images of 10 right-handed male NCSPs from the same institution. A comparison of CSP and NCSP baseline images using TFCE showed significantly higher MWFs in the bilateral basal ganglia, anterior and posterior corpora calloса, left corticospinal tract, and left anterior and superior temporal lobe (p < 0.05). At the 3-month follow-up examination, images from the CSP cohort still showed significantly higher MWFs than those identified on baseline images from the NCSP cohort in the bilateral basal ganglia, anterior and posterior corpora calloса, and left anterior temporal lobe, and also in the bilateral corticospinal tracts, parahippocampal gyrus, and bilateral juxtapositional (previously known as supplemental motor) areas (p < 0.05). In the CSP cohort, a t-test comparing the MWF at the time of injury and 3 months later showed a significant increase in the overall MWF at follow-up (p < 0.005). These increases were greatest in the bilateral basal ganglia and deep white matter. MWF decreases were seen in more superficial white matter (p < 0.005).

CONCLUSIONS In this preliminary study, MWF was found to be increased in the brains of CSPs compared with the brains of controls, suggesting acute/chronic MWF alterations in CSPs from previous injuries. Increases in the MWF were...
Traumatic brain injury affects an estimated 1.7 million people in the United States annually, with approximately 75% of these injuries categorized as mild. Football is responsible for one of the highest frequencies of mild traumatic brain injury (mTBI) in athletics; approximately 5% of high school and collegiate football players are diagnosed with an mTBI annually. Approximately 20% of these athletes will have disabling symptoms beyond 3 months postinjury. Repeated mTBIs have also been recently linked to chronic traumatic encephalopathy (CTE), a debilitating neurodegenerative condition that develops later in life.

These findings emphasize the need for better diagnostic and prognostic tools for mTBI, especially in populations at risk for repeated mTBIs. The lack of highly sensitive clinical neuroimaging and neuropsychiatric markers of subtle changes due to mTBI makes it difficult to characterize injury severity and to predict outcomes. Researchers are currently investigating new methods for assessing signs of permanent or destructive injuries in young athletes with mTBI. Multiple studies using quantitative MRI analysis have shown decreased cortical volumes after mTBI, from which we can infer decreases in either white or gray matter.

White matter tracts in the brain, both myelinated and nonmyelinated, are susceptible to damage from the impact-acceleration forces experienced during a TBI. Blast impacts cause linear and rotational acceleration of the brain, and the resulting shear strains can severely damage axons (stretch, sever) and their myelin sheaths (separation, decompensate, fragment). It has been reported that myelin is particularly vulnerable to secondary damage (decompaction, demyelination) as a result of secondary chemical cascades and neuroinflammation that occur acutely and chronically after a TBI. Compromised myelin has been shown to cause reciprocal damage to the underlying axon, and further myelin debris can inhibit the differentiation of progenitor cells that are required for remyelination and axon repair. In all, there is evidence that significant white matter injury and myelin loss occur in mTBI, and this damage may be chronic and can negatively affect neural processing speed and cognitive function.

The neuroimaging of white matter is of particular interest, therefore, for assessing mTBI and TBI. Diffusion tensor imaging (DTI) characterizes the underlying microstructure and architecture of white matter through measurements of anisotropy and has thus far shown promise as a tool for approximating injuries to the axon and myelin sheaths. Multiple studies focusing on mTBI have shown decreased fractional anisotropy (FA) values in white matter tracts, suggesting tract damage and, potentially, decreased myelin. Furthermore, researchers have correlated decreased FA values with worsened neuropsychiatric outcomes. Alternatively, multicomponent relaxation (MCR) MRI may be used to assess myelin content in the human brain. In particular, an emerging MCR technique, known as multicomponent driven equilibrium single pulse observation of T1 and T2 (mcDESPOT), may provide improved insights into the study of myelination after mTBI. The basis of mcDESPOT relies on the knowledge that distinct water compartments exist within neural tissue and that the longitudinal (T1) and transverse (T2) relaxation times associated with these water pools can be characterized by modeling the acquired MRI signal into subvoxel contributions. Within the mcDESPOT model, the myelin water fraction (MWF), a surrogate MRI measure of myelin content, is the volume fraction associated with the myelin water pool. Specifically, it provides a voxel-wise estimation ranging from 0 to 1 for myelin content, with higher values providing an indirect marker of greater myelin integrity. The MWF derived from this technique has been shown to correlate strongly with histological findings of myelin density and to provide strong evidence of the technique’s sensitivity to myelination through studies of neurodevelopment and neurological disease. In addition, the reliability of using a MWF control population has been discussed in other reports of mcDESPOT studies in which multiple sclerosis, amyotrophic lateral sclerosis, and mTBI were examined. Unlike DTI, which primarily gives information about myelin–axon bundle interactions, mcDESPOT provides quantitative values with a high sensitivity to myelin on a voxel level.

In this study, our aim was to collect preliminary data on the feasibility of assessing myelin alterations associated with mTBI by using MWF data obtained from the mcDESPOT imaging technique. In a cohort of male collegiate contact sport players (CSPs), we quantified changes in MWF at the time of mTBI diagnosis and again 3 months after injury, when the patients’ acute symptoms had resolved. In addition, images of MWFs in the brains of these CSPs were compared with images of MWFs in the brains of a control population of noncontact sport players (NCSPs) to describe potential differences that may suggest acute/chronic white matter remodeling in the contact sport athletes.

**Methods**

**Study Design**

A total of 31 patients were enrolled in this study: 21 CSPs and 10 NCSPs. Consent was obtained in accordance with the Brown University Institutional Review Board (protocol #262023). Exclusion criteria included a history of psychiatric or other neurological disorder, or a contra-
associated water, and nonexchanging free water. This relaxation times for intra- and extra-axonal water, myelin-3-pool tissue model that estimates fractional volumes and aging time for all sequences for each participant was 60 minutes. From these data, $B_0$ and $B_1$ field calibration maps were calculated. Last, the MWF maps were calculated for each voxel by fitting the SPGR and balanced SSFP data to a 3-pool tissue model that estimates fractional volumes and relaxation times for intra- and extra-axonal water, myelin-associated water, and nonexchanging free water. This 3-pool model was designed for use with pathological conditions in which there may be edema that could affect the calculation of the MWF map. Specifically, the inclusion of a nonexchanging water pool helps adequately control for the effects of slow relaxing water, as would be expected with edema. This model has been shown to provide robust and reproducible findings through numerical simulation and in vivo data.

MWF maps were non-linearly normalized into a common analysis space, allowing for comparisons across patients and controls. A T1-weighted template was used as the reference space to which MWF maps of the current study were registered. The high flip angle SPGR image was normalized to the T1-weighted template using symmetric diffeomorphic normalization to determine the transformations necessary to map an individual’s raw data space to the template space. These resulting transformations were applied to each individual’s MWF map, aligning the MWF map to the reference template. Full details regarding image normalization are described elsewhere.

Final processed images obtained at the 3-month imaging examination show an anatomical white matter mask overlaid with areas of increased (red) or decreased (blue) MWF from baseline.

**Statistical Analysis**

Changes in the MWF were evaluated between each CSP’s immediate postinjury images and the follow-up image obtained 3 months later. In addition, MWF maps obtained from the NCSP control group were compared with MWF maps from the CSP cohort. A voxel-wise t-test was performed using the randomize tool in the FSL software library version 5.0 (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSL). Five thousand permutations with and without threshold-free cluster enhancement (TFCE) were used to identify statistically significant differences in the MWF between the time of injury and 3 months afterward, as well as to evaluate the differences between the CSP and NCSP samples. Statistical significance was defined as p < 0.05 corrected for multiple comparisons using TFCE. In some instances in which analyses did not survive the more rigorous TFCE correction, uncorrected p values at the higher threshold of p < 0.005 are also reported. This higher threshold was used to dissuade possible false-positive findings, as these data were not corrected for multiple comparisons. The locations of significant voxels were identified using a white matter–specific atlas described in detail elsewhere.

**Results**

**Study Cohorts**

Twenty right-handed, male football players and 1 right-handed, male rugby player who sustained mTBIs comprised the CSP cohort. Of the 21 patients who originally underwent imaging studies, 12 (1 rugby and 11 football players) returned for a 3-month follow-up imaging examination. Therefore, the final CSP cohort used for the analysis consisted of 12 players in whom MRI results obtained at the time of injury and 3 months later were both available. The mean age of the CSP cohort was 20.18 ± 1.18 years.
years. One participant acknowledged having sustained a previous concussion, whereas all others had no notable medical history of concussion.

Ten right-handed, male athletes comprised the NCSP cohort. The mean age of the control group was $20.16 \pm 1.27$ years. There was no medical history of concussion in the NCSP cohort.

Comparison of CSP Acute-Phase MRI and NCSP Baseline MRI

A comparison of MR images obtained in the NCSP control population with acute-phase MR images obtained in the CSP cohort (within 72 hours of injury) showed there was a significantly higher MWF signal in the CSP population ($p < 0.05$, familywise error [FWE]-corrected) (Fig. 1A–C). In particular, the MWF in the CSP group was higher in the anterior and posterior corpora callosa, bilateral thalami (particularly the anterior thalamic radiations [left > right]), corticospinal tracts (left > right), and left anterior and superior temporal lobe (Fig. 1D). There were no areas in which the MWF in the CSP cohort was lower than that in the NCSP population.

Comparison of CSP Follow-Up MRI and NCSP Baseline MRI

The comparison between images obtained in the control group and follow-up MR images obtained in the CSP cohort 3 months after injury also showed a globally higher MWF in the CSP cohort. A greater MWF was observed mostly in the anterior and posterior corpora callosa, bilateral corticospinal tracts, and thalami (left > right) (Fig. 2A). These MWF increases were seen in the left anterior temporal and parahippocampal gyri (Fig. 2B) as well as in the bilateral juxtapositional lobes ($p < 0.05$, FWE-corrected). As with the acute-phase MR images, there were no areas in which the MWF in the CSP cohort (12 players) was lower than that in the NCSP population.
Comparison of CSP Acute-Phase MRI and CSP 3-Month Follow-Up MRI

A paired voxel-wise t-test comparing MWF maps obtained during the acute phase of mTBI (within 72 hours) and the 3-month follow-up examination showed a predominant increase in the MWF at the 3-month follow-up (Fig. 3A). The largest MWF increases occurred in the deep white matter. Increases were also seen in the anterior corpus callosum, bilateral corona radiata, superior longitudinal fasciculi, thalami, and left temporal lobe (p < 0.005, uncorrected). Scattered MWF decreases were seen in the more peripheral white matter and bilaterally in the internal capsules (left > right) (p < 0.005, uncorrected) (Fig. 3B). MWF changes in the hemispheres, superior longitudinal fasciculi, temporal lobes, corona radiata, and internal capsules are shown as a box plot in Fig. 4. Notably, despite significant findings at an uncorrected threshold of p < 0.005, the findings from this statistical analysis did not carry through to the more rigorous multiple comparison analysis (TFCE).

Discussion

In this preliminary study, acute and follow-up myelin neuroimaging results for collegiate athletes with mTBI were compared and evaluated against a control population of noninjured athletes. Only one athlete, a member of the CSP cohort, had previously been diagnosed with an mTBI. The most significant and unique findings of this preliminary study were as follows: 1) a significantly higher MWF was identified in acute-phase MR images obtained in the CSPs than that in images collected from the NCSPs; 2) a significantly higher MWF was identified on follow-up images obtained in the CSPs than that in images collected from the NCSPs; and 3) in CSPs who had suffered mTBI there was an increase in the MWF between that measured on acute-phase MR images (within 72 hours of injury) and that found on follow-up images (3 months postinjury).

mTBI and DTI

MRI studies using DTI in patients with mTBI have found decreases in FA values that are thought to correlate with decreases in white matter integrity, which is often interpreted as decreased myelin.29 These post-mTBI changes have been demonstrated most often in the frontal association pathways, which include the anterior corona radiata, uncinate fasciculus, superior longitudinal fasciculus, and anterior corpus callosum.38 These FA value decreases have also been found to correlate with persistent cognitive deficits.34,44 Although the majority of DTI studies of mTBI have found decreased FA values, particularly in the corpus callosum, internal capsule, centrum semiovale, and other major white matter pathways, some studies have shown increased FA values and decreased mean diffusivity values.40 This variability in DTI findings suggests that DTI changes are likely multifactorial and may be due to the timing of imaging versus the time of injury.38

Preclinical Studies of Myelin Remodeling After TBI

Preclinical research has shown that there are white matter changes after a head injury that cannot be seen with conventional neuroimaging.33 In rodent models of mTBI, a pattern of cell death, demyelination, and remyelination has
been demonstrated; however, the remyelination seen in these models does not seem to have the same organization as preinjury myelin. In these models, the myelination is redundant, resulting in increased myelin but fewer and smaller axons. Since these changes are not visible when employing commonly used imaging techniques, the neuropsychiatric, functional, and long-term structural outcomes from these remodeling processes are still unknown. The evidence of disordered remyelination may be part of the explanation for why all CSPs in this study had increased MWFs when compared with the NCSPs.

A similar explanation of disordered myelination may serve to explain DTI studies showing decreased FA values. DTI is not a quantitative myelin imaging technique, and although it provides information about the axon milieu, it does not specifically address the amount of myelin in the myelin sheath. Therefore, decreases in DTI FA values after mTBI are not in conflict with MWF increases. This can be seen in animal models of mTBI that demonstrate vacuolated myelin regions, abnormally thick myelin sheaths, and small axons. If these findings were imaged, this disorganization would likely result in decreased anisotropy (decreased FA) and increased overall myelin due to increased myelin, albeit disorganized. The ability to characterize the differences between beneficial myelin biogenesis and disorderly remyelination with neuroimaging could have significant implications for quantifying TBI severity and prognosis.

In a similar study of the MWF in collegiate hockey players, changes in the MWF were also found. In a study by Wright et al., all participants underwent baseline MRI as well as 72-hour, 2-week, and 2-month post-mTBI follow-up imaging. The authors noted an initial decrease in the MWF followed by a recovery to postinjury myelin levels at the 2-month imaging session, which suggested a similar trajectory of remyelination after injury. No comparison was made to athletes in noncontact sports. Our study showed that contact sport athletes have increases in the MWF 3 months after mTBI, particularly in the major deep white matter tracts. These increases may be a manifestation of exuberant remyelination following an initial decrease in MWF, as was demonstrated by Wright et al. Although our CSP cohort may have had an initial acute decrease in the MWF at the time of injury, compared with their preinjury baseline, we could not quantify this because we did not have baseline images.

Changes in the MWF seen in a comparison of NCSPs and CSPs at the time of mTBI and 3 months after the injury are novel findings of this study. Our analysis showed increased myelination in the CSP cohort at the time of injury.
with CTE. In our study, when the MWF maps of CSPs at were also consistent with autopsy specimens of patients cal and limbic structures on PET scans. These changes changes in the amygdala, dorsal midbrain, and subcortic - in ascribing the clinical significance of these novel find -ings; however, studies of chronic traumatic encephalopa -thies and other possible mTBI-related injuries later in life.6

Limitations
The primary limitation to this study was the lack of preinjury MR images for athletes who sustained an mTBI to compare as a baseline. Additionally, our sample size of 12 CSPs with acute mTBI and 10 NCSPs was small, but this is not uncommon in prospective, traumatic event–re -lated imaging studies of this nature. Because of this small sample size and the test performance variability, the neuropsychiatric data collected were not robust enough to statistically analyze their association with MWF values. Another limitation is the heterogeneity of potential differ -ences in brain injury. Each individual is likely to be af -fected differently, and therefore, group analysis methods are only going to be sensitive where changes are occurring across the group.

Conclusions
In this preliminary investigation, the MWF increased in the brains of CSPs who sustained an mTBI; this was identified both at the 3-month follow-up MRI examination and when compared with a control population of NCSPs. The most significant finding of this study is the increased MWF in the deep white matter tracts in CSPs compared with controls at both time points. These increases may be the result of a reparative process in patients who sustained mTBIs that involves demyelination, remyelination, and even hypermyelination, the significance of which has yet to be defined. This study provides a basis for additional studies aimed at understanding the underlying neuro -pathophysiology of the brain’s recovery from mTBI.

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