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A Longitudinal Diffusion Tensor Imaging Study Assessing White Matter Fiber Tracts after Sports-Related Concussion

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Abstract

The extent of structural injury in sports-related concussion (SRC) is central to the course of recovery, long-term effects, and the decision to return to play. In the present longitudinal study, we used diffusion tensor imaging (DTI) to assess white matter (WM) fiber tract integrity within 2 days, 2 weeks, and 2 months of concussive injury. Participants were right-handed male varsity contact-sport athletes (20.2 ± 1.0 years of age) with a medically diagnosed SRC (no loss of consciousness). They were compared to right-handed male varsity non-contact-sport athletes serving as controls (19.9 ± 1.7 years). We found significantly increased radial diffusivity (RD) in concussed athletes ($n = 12$; paired t-test, tract-based spatial statistics; $p < 0.025$) at 2 days, when compared to the 2-week postinjury time point. The increase was found in a cluster of right hemisphere voxels, spanning the posterior limb of the internal capsule (IC), the retrolenticular part of the IC, the inferior longitudinal fasciculus, the inferior fronto-occipital fasciculus (sagittal stratum), and the anterior thalamic radiation. Post-hoc, univariate, between-group (controls vs. concussed), mixed-effects analysis of the cluster showed significantly higher RD at 2 days ($p = 0.002$), as compared to the controls, with a trend in the same direction at 2 months ($p = 0.11$). Results for fractional anisotropy (FA) in the same cluster showed a similar, but inverted, pattern; FA was decreased at 2 days and at 2 months postinjury, when compared to healthy controls. At 2 weeks postinjury, no statistical differences between concussed and control athletes were found with regard to either RD or FA. These results support the hypothesis of increased RD and reduced FA within 72 h postinjury, followed by recovery that may extend beyond 2 weeks. RD appears to be a sensitive measure of concussive injury.

Key words: diffusion tensor imaging; longitudinal study; mTBI; radial diffusivity; sports-related concussion

Introduction

The diagnosis of mild traumatic brain injury (mTBI) is often hindered by exclusive reliance on neurocognitive and clinical symptoms based on patient self-report. A more promising approach is to exploit radiological evidence from magnetic resonance imaging (MRI) and computed tomography (CT). Conventional clinical imaging techniques used to exclude intracranial hemorrhage or skull fracture do not have the sensitivity to identify alterations in the neural microstructure resulting from mTBI. Advanced neuroimaging techniques, in particular, diffusion tensor imaging (DTI), are therefore worth exploring. The present study reports on the use of DTI to assess white matter (WM) fiber tract integrity in the brains of college athletes who sustained a sports-related concussion (SRC), one source of mTBI.

Sports are indeed a major cause of mTBI (often called ''concussions''). A study by the Centers for Disease Control and Prevention estimates that 300,000 SRCs occur annually in the United States.[1] However, this study only included concussions for which the person reported loss of consciousness (LOC), which is thought to characterize only a fraction of SRCs.[2,3] Given that athletes often do not report their injury, a more accurate approximation may be that 1.6–3.8 million SRCs occur each year, including concussions, for which no medical treatment is sought.[4]

According to the most recent consensus,[5] typical concussive injury results in the rapid onset of short-lived impairment of neurological function that resolves spontaneously. The authors of this

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statement affirm that: ''a concussion may result in neuropathologic injury, but the acute clinical symptoms largely reflect a functional disturbance rather than structural injury.'' This claim is questionable in light of recent neuroimaging research. Although clinical and cognitive symptoms may subside after approximately 2 weeks in most concussed athletes, neurological alterations can persist. For example, magnetic resonance spectroscopy (MRS) studies have demonstrated neurometabolic changes after SRC lasting up to 1 month postinjury.[6–9] Similarly, in a functional MRI study, hyperactivation of the dorsolateral prefrontal cortex was found to persist beyond 2 months postinjury in athletes whose symptoms subsided at 2 weeks after injury.[10] DTI studies demonstrating structural changes from repetitive concussive head impacts have been reported in ice hockey players over the course of a single season,[11] in athletes with prolonged symptoms,[12] as well as in adolescents exhibiting close-to-normal scores on the Sports Concussion Assessment Tool (SCAT2)[13] at 2 months postinjury.[14]

Compared to standard MRI, DTI offers a more sensitive assessment of focal ischemic lesions and diffuse axonal damage.[15] Specifically, DTI provides information about the WM microstructure and fiber tract integrity by measuring the Brownian motion of water molecules in the brain.[16–18] Diffusion properties of water in tissue can be either iso- or anisotropic. In tissues with isotropic diffusion, water molecules diffuse equally in all directions. Isotropic diffusion is typically found in the gray matter of the brain. In the anisotropic case, water has a preferred direction of diffusion. Anisotropic diffusion is typically found in tissue with strong directional organization, such as the deep WM, where axons form tightly packed fiber bundles. In such tissue, diffusion is normally highly restricted along the fiber membranes. Measures of anisotropy thus provide information about the WM microstructure and WM fiber tract integrity,[16–18] which is undetectable by conventional MRI methods.

DTI allows information about multiple diffusion gradients in a given tissue to be combined. A derived measure known as fractional anisotropy (FA) can then be used to quantify the degree of preferred diffusion direction in each voxel.[19] Overall diffusion in a tissue is measured by mean diffusivity (MD), which is calculated as the mean of the three eigenvalues of the diffusion tensor.[20] The eigenvalues of each directional vector can also be examined independently. The eigenvalue of the first eigenvector (also referred to as parallel diffusivity) was selectively altered in the presence of acute axonal damage in retinal ischemia in mice.[21] Similarly, radial diffusivity (RD), the mean of the second and third eigenvalues,[18] may be selectively sensitive to alterations of the myelin sheath, as demonstrated in an animal model[22] and more recently in optic neuritis in humans.[23] These findings lend support to the sensitivity of diffusion measures with regard to specific pathologies.

Decreased FA has been reported in mTBI patients with a Glasgow Coma Scale (GCS) score of 13–15 within 24 h postinjury.[24] WM abnormalities have also been shown[25] in patients with mTBI exhibiting persistent cognitive impairment (8 months to 3 years postinjury). The latter investigators demonstrated decreased FA and increased MD in the corpus callosum (CC), bilateral capsula interna (CI), and other subcortical WM structures. Significant correlations between decreased FA (CC, CI, and centrum semiovale) within 10 days postinjury and neuropsychological (NP) test scores obtained at 6 months postinjury have been reported as well.[26] Abnormalities of WM microstructure in mTBI patients with persistent cognitive impairment have been found[27] in the anterior corona radiata, the uncinate fasciculus (UF), CC, inferior longitudinal fasciculus, and the cingulum bundle. Further, significant correlations between attentional control and FA were found within the left anterior corona radiata as well as memory performance and FA within the UF.[28] A DTI study on patients with mTBI[29] demonstrated increased FA and decreased RD in the subacute phase after injury and subsequent partial normalization of FA values in left corona radiata and splenium. These studies provide evidence that anisotropy measurements cannot only be used to assess alterations in the microstructure of the WM, but also provide a biomarker of cognitive function and dysfunction. Such markers may prove critical in refining the diagnosis, prognosis, and management of mTBI. It should, however, be emphasized that the DTI studies using measurements of anisotropy discussed thus far include diverse individuals with mTBI and a GCS score ranging between 13 and 15; athletes were not targeted for investigation. Although the mechanism of injury in SRC is believed to be comparable to non-sports-related mTBI, SRC represents the mildest form of mTBI. Most individuals with SRC will not score below 15 on the GCS, but will present with rapid onset of short-lived neurological impairment; they typically show no structural changes in traditional MRI and CT scans. It therefore seems prudent to exploit DTI technology to separately examine the case of SRC, especially given the prevalence of this condition (see above).

Only a few studies have assessed structural changes in adult athletes with SRC who do not score below 15 on the GCS. Increased RD and axial diffusivity (AD) after repetitive concussive head impacts in adult ice hockey players over the course of a single season were observed in the right precentral region, corona radiate, and the anterior, posterior limb of the internal capsule.[11] Decreased FA (in temporo-occipital WM) and lower cognitive function (CogState[30]) were found to be associated with high-frequency heading rate (> 885–1800 headings per year) in amateur soccer players.[31] In college athletes exhibiting prolonged symptoms after SRC, increased MD has been reported in parts of the left inferior/superior longitudinal and fronto-occipital fasciculi, the retrolenticular part of the internal capsule, and posterior thalamic and acoustic radiations.[12] Persistent microstructural alterations in deep WM have been shown in female contact sports athletes at 7 months postinjury[6]; all participants were symptom free at this point of their recovery, suggesting that, in female athletes, structural recovery may lag behind behaviorally assessed recovery by up to 7 months postinjury. Finally, changes in WM microstructure were observed in a cohort of contact sports athletes with subconcussive blows to the head (26–399 hits), whereas no such changes were identified in 6 control participants.[32]

There is thus growing evidence suggesting that even in the absence of clinically symptomatic concussions (i.e., subconcussive hits)[31–33] or at a stage of recovery when athletes are symptom free,[6] they are likely to exhibit WM alterations when advanced neuroimaging techniques are used to examine their brains. These findings suggest that DTI may be a useful imaging tool to assess the severity of a concussion and may provide a biomarker for structural injury. DTI examination of the brain may thus serve to monitor the reorganization and reversal of WM injury and to predict recovery. The aim of the present study was to track changes of WM fiber tract integrity during the 2 months after SRC using advanced DTI.

Methods

Participants

All concussed participants were varsity-level college students enrolled in the Princeton University (Princeton, NJ) concussion program for high-risk sports. The program ensures systematic documentation of athletic history, physical exam, and baseline NP

testing, including SCAT2[13] and Immediate Post-Concussion Assessment and Cognitive Testing (ImPACT).[34] Princeton's concussion program also provides acute care and long-term monitoring. All athletes involved in this study were diagnosed with a concussion by team physicians using criteria outlined by the 4th International Consensus Conference on Concussion in Sport.[5] Postinjury testing included SCAT2, traditional paper-and-pencil NP tests, ImPACT, the Patient Health Questionnaire (PHQ-9)[35] and the Generalized Anxiety Disorder (GAD-7) questionnaire.[36] The PHQ-9 and the GAD-7 are assessments for depression and generalized anxiety, respectively. Baseline and postinjury testing protocols were identical to those described in our earlier publication.[10]

Subsequent to their most recent concussion, a certified athletic trainer and team physician at the University Health Services evaluated athletes within 48 h postinjury. None of the athletes experienced an LOC and their overall symptomatology did not warrant further assessment by the GCS or use of a clinical radiological exam. All concussed athletes underwent NP testing within 24–48 h after injury. Abnormal NP performance was determined through comparison of postinjury NP scores to the athlete's baseline scores. Specifically, abnormality of ImPACT clinical composites was based on reliable change indices at the 0.8 confidence interval.[34,37] Similarly, scores on the traditional NP test performance were examined using Princeton-specific normative data. Data from both ImPACT and the NP test were integrated and interpreted by an experienced clinical neuropsychologist.

Athletes were kept out of activity until they were symptom free and their clinical exam, including balance and NP evaluations, was considered to have returned to baseline levels. Return-to-activity decisions were made by the team physician, who supervised a personalized return-to-play progression that exposed athletes to gradual increases in physical exertion as per the 3rd International Consensus Conference on Concussion guidelines.[38] Athletes were cleared to return to full contact play once they were symptom free at rest, had successfully completed the exertional program, and were neurocognitively functioning at baseline levels.

A total of 21 right-handed, male, varsity-level contact sport athletes (mean age, 20.19 years; standard deviation [SD], 1.03; age range, 18–22) who suffered an SRC were enrolled in the study. In addition to having no contraindications to MRI, participants had no self-reported history of medical, genetic, or psychiatric disorder. History of concussion was obtained through self-report after a personal interview with the athlete. The reported count (see Table 1) of previous concussions also includes concussions suffered preceding enrollment into the Princeton University concussion program for high-risk sports. It should be noted that under-reporting of concussion by athletes has been suggested in previous studies.[39] An objective evaluation of the number of previous concussions in contact sport athletes is therefore difficult. Among the pool of 21 concussed athletes, 12 reported no previous history of concussion, 5 reported one previous concussion, 3 reported two previous concussions, and 1 reported three previous concussions. Mean time since the last self-reported concussion for the latter 9 concussed athletes was 2.75 years (SD, 3.02; see Table 1).

Healthy control participants included 16 age-matched, right-handed, male varsity noncontact athletes (mean age, 19.9 years; SD, 1.67; age range, 18–22), with no contraindications to MRI and no self-reported history of previous head trauma, psychiatric, neurological, or developmental disorders. All athletes (concussed and controls) gave written consent to participate in the study, which was approved by the Princeton University's Institutional Review Panel for Human Subjects Research. Concussed athletes were scanned at ~2 days, ~2 weeks, and ~2 months postinjury. Controls were scanned once. All athletes repeated SCAT2, PHQ-9, GAD-7, and NP testing assessments synchronized with the three imaging sessions of the concussed athletes. Concussed athletes participated in additional NP testing in between imaging sessions, as clinically indicated and requested by the team physician. There were eight instances (during a single contiguous time period identified by ''X'' in Table 1) when data collection was not possible on concussed athletes in the time interval required by the experimental design of the present study because of hardware maintenance issues. There was also one instance of a concussed athlete deciding to discontinue participation in the study (identified by ''D'' in Table 1). A strict data quality-assurance protocol (described in the *Data Preprocessing and Quality Assurance* section) resulted in the exclusion of scans for 10 concussed and 2 controls (identified by ''M'' in Table 1). In total, 14 controls, 16 concussed athletes at ~2 days, 17 concussed athletes at 2 weeks, and 13 concussed athletes at 2 months were included in the analyses (identified by ''Y'' in Table 1). From this pool of concussed athletes (see Table 1), only 12 were imaged at both the 2-day time point and at 2 weeks, whereas 11 were imaged at both 2 weeks and 2 months postinjury.

Imaging protocol

Diffusion-weighted images (single-shot spin echo pulse sequence with parameters adapted from our earlier publication[12]) were acquired with a 16-channel, phase-array coil (Siemens, Erlangen, Germany) on a whole-body 3T Siemens Skyra scanner: repetition time (TR) = 12,100 ms; echo time (TE) = 96 ms; 70 axial slices; voxel size, $1.88 \times 1.88\,mm^2$ in plane; slice thickness = 1.9 mm; field of view (FOV) = 256 mm; 64 gradient directions, b-value, $1000\,s/mm^2$; 8 volumes with no diffusion weighting (b = 0); and 2 runs, yielding a total scan time of 26 min 52 sec. In order to facilitate image volume registration to the Montreal Neurological Institute (MNI) space, a high-resolution T1-weighted MPRAGE image was additionally acquired at the start of each imaging session: TR = 1900 ms; TE = 2.13 ms; 192 sagittal slices; $0.90 \times 0.94 \times 0.94\,mm^3$ voxel resolution; flip angle = 9 degrees; FOV = 240 mm; and a total anatomical scan time of 4 min 26 sec. Care was taken to minimize subject motion with prescan instructions and comfortable neck padding. Participants watched a preselected movie of their choice from an online streaming service during the entire scanning session.

Data preprocessing and quality assurance

All data processing was done within the FSL suite (version 4.1.9).[40] The two averages of the acquired diffusion-weighted images of each subject were concatenated in the order of image acquisition and visually inspected for signal dropoffs and other imaging artifacts. All acquired data passed visual inspection. Eddy current correction was done for each subject's concatenated data set, employing the first B0 volume for reference. Each volume's registration parameters from the eddy correction step was then used to implement a strict, quantitative, quality-assurance protocol based on recent findings.[41] Mean motion estimates (translation and rotation in three dimensions) were calculated for each group separately. All individual subject scans with motion estimates greater than 3 SDs from the mean or scans with a net translational motion estimate exceeding two voxels were excluded in their entirety (10 scans of concussed athletes and 2 controls). The concatenated B vectors corresponding to the applied diffusion gradients were then corrected for motion (rotation component[42]) before the FSL function ''dtifit''[43] was applied to fit a diffusion tensor model,[44] generating the three principal eigenvalues $\lambda_1, \lambda_2, \lambda_3$ at each voxel. This step additionally provides scalar diffusion measures of WM microstructure: $FA = \sqrt{\frac{((\lambda_1-\lambda_2)^2+(\lambda_2-\lambda_3)^2+(\lambda_3-\lambda_1)^2)}{2(\lambda_1^2+\lambda_2^2+\lambda_3^2)}}$ and $AD = \lambda_1$. $RD = \frac{[\lambda_2+\lambda_3]}{2}$ and $MD = \frac{[\lambda_1+\lambda_2+\lambda_3]}{3}$ volumes were generated using the radial eigenvalues λ_2, λ_3.

Statistical analyses

Between-group *t*-tests using the function ''randomise'' were performed via tract-based spatial statistics[43] (TBSS; FSL version

TABLE 1. DEMOGRAPHICS OF CONTROLS AND CONCUSSED ATHLETES

Controls				*Concussed*									
											MRI Inclusion		
Subject	*Age (years)*	*Sport*	*MRI Inclusion*	*Subject*	*Age (years)*	*Sport*	*# Prior concussions*	*NP Normal post injury (days)*	*Symptom free (days)*	*return to play (days)*	*2 days scan*	*2 wks. scan*	*2 mon. scan*
1	18	Crew	Y	1	20	Football	1	24	17 (1st), 77 (2nd)	31[a]	Y	Y	Y
2	18	Squash	Y	2	19	Water polo	0	6	6	24	Y	Y	Y
3	21	Crew	Y	3	18	Lacrosse	0	14	11	20	Y	Y	Y
4	18	Crew	Y	4	21	Ice hockey	3	15	162	no return to play	M	Y	Y
5	20	Track + Cross country	Y	5	22	Lacrosse	0	17	10	23	Y	Y	Y
6	18	Crew	Y	6	20	Wrestling	0	11	3	18	Y	Y	M
7	21	Track	Y	7	19	Ice hockey	1	2	4	23	Y	M	M
8	21	Volleyball	Y	8	20	Basketball	0	10	8	15	Y	Y	M
9	22	Track + Cross country	Y	9	20	Rugby	0	23	10	31	Y	Y	Y
10	22	Cross country	Y	10	21	Rugby	0	11	2	12	Y	Y	Y
11	19	Crew	Y	11	21	Rugby	0	6	10	16	Y	Y	M
12	22	Track	Y	12	20	Rugby	1	17	12	no return to play[a]	Y	Y	Y
13	19	Track	Y	13	22	Rugby	2	na[b]	7	na[b]	Y	Y	M
14	18	Swimming	Y	14	20	Basketball	1	na[b]	31	na[b]	M	Y	Y
15	19	Volleyball	M	15	19	Football	0	60[c]	14	no return to play	Y	X	Y
16	22	Cross country	M	16	21	soccer	1	na[d]	11	32	Y	Y	D
				17	21	Ice hockey	0	3	5	12	X	Y	Y
				18	20	Football	0	6	5	15	Y	M	X
				19	20	Basketball	1	9	6	12	Y	X	Y
				20	19	Football	0	18	10	22	X	Y	Y
				21	21	Sprint Football	1	13	7	16	X	Y	M

[a]Returned to play after 1st injury at 31 days, sustained a 2nd concussion and decided not to return to play
[b]NP testing never reached normal range before athlete graduated/season ended
[c]Not normal at 2 weeks, not repeated until 2 months since season over
[d]Subjected discontinued from study and decided not to return to play (although cleared to do so for next, season)
[e]no return to play, graduated
Y: Scan included in analyses, M: Scan excluded due to motion exceeding threshold, X: Period of study during which scanner amplifier failed, D: Subject discontinued

Table 2. Between-Group Mixed-Effects Analysis of FA and RD

FA	*Estimate*	*SE*	t *value*	*One-tailed* p *value*	*Two-tailed* p *value*
(Intercept)	0.619967	0.004693	32.09		
2 days	−0.030114	0.008278	−3.64	0.0004	0.0008*
2 weeks	−0.007985	0.007536	−1.06	0.140	0.28
2 months	−0.017821	0.008550	−2.08	0.022	0.044*
RD	*Estimate*	*SE*	t *value*	*One-tailed* p *value*	*Two-tailed* p *value*
(Intercept)	4.48E-04	4.56E-06	98.15		
2 days	3.06E-05	9.21E-06	3.32	0.001	0.002*
2 weeks	3.90E-06	8.07E-06	0.48	0.320	0.64
2 months	1.53E-05	9.54E-06	1.60	0.059	0.11

FA, fractional anisotropy; RD, radial diffusivity; SE, standard error.
*p value < 0.05.

4.1.9) on the skeletonized WM fiber tracts for all derived scalar diffusion measures of WM microstructure FA, AD, RD and MD. All TBSS processing steps followed recommended guidelines.[43] The ''FMRIB58_FA_1mm'' image volume in MNI space, included in FSL version 4.1.9, served as the target for initial nonlinear registration[45] of subject FA volumes. The mean WM skeleton, based on the included participants FA volumes, was thresholded to only include voxels with FA > 0.25 in order to restrict the analyses to the core WM tracts. The brain stem and the cerebellum were removed (mask included in FSL suite 4.1.9) from all analyses because individual subject variability in brain volumes resulted in omission of inferior parts of these structures in a few cases. The number of randomise permutations was set at 10,000 with the threshold-free cluster enhancement (TFCE) option enabled.[46] Between-sessions comparisons of the concussed athletes were made by paired *t*-tests (TBSS, TFCE, all permutations, and variance smoothing of two voxel sizes) for all four diffusion measures of WM microstructure (FA, AD, RD, and MD).

Two post-hoc tests, a traditional univariate mixed-effects approach[47] and a multi-variate bootstrap method,[48] were selected to test whether regions identified by a whole-brain TBSS analysis differed (in terms of diffusion metrics of WM microstructure) between groups over time. The mixed-effects model incorporates both fixed and random effects and is particularly useful in longitudinal studies because of its ability to deal with repeated measures and missing values (see Appendix A1 for details). The multi-variate bootstrap has the added advantage of accounting for combined responses of identified diffusion measures WM microstructure and is preferred in situations of moderate sample sizes, such as this study (see Appendix A2 for the algorithm). All post-hoc tests were run using the open-source statistical software R (http://www.r-project.org).

Results

No between-group differences were found in TBSS analyses by pooled *t*-tests at $p < 0.05$ (two sided), family-wise error (FWE) corrected with the TFCE option enabled. Significant differences (pointing to structural alterations) were observed in the paired, between-concussed sessions *t*-test (2 days vs. 2 weeks; $p < 0.025$, FWE corrected, TFCE) of the RD measure, with the cluster indicating greater RD values at 2 days, as compared to 2 weeks. The significant RD cluster consisted of 469 contiguous voxels in standard space (MNI, FMRIB58_FA_1mm). The regions implicated are all in the right hemisphere, posterior limb of the internal capsule (IC), retrolenticular part of the IC, sagittal stratum (inferior longitudinal fasciculus and inferior fronto-occipital fasciculus), and anterior thalamic radiation (see Fig. 1). The John Hopkins University (JHU) ICBM-DTI-81 WM and JHU WM tractography atlases[49–51] included in FSL version 4.1.9 were used to determine the anatomical regions referenced. Interestingly, these regions are almost identical to those reported earlier[12] in the contralateral hemisphere. In addition, a trend ($p < 0.05$, one sided, FWE, TFCE, two clusters for a total of 348 voxels in MNI space; FMRIB58_FA_1mm) was observed in the FA measure with both clusters overlaying approximately 58% of the aforementioned significant RD cluster, but with the result trending in the opposite direction. FA values were greater at 2 weeks, as compared to the values at 2 days postinjury. No paired, significant differences in FA, RD, and AD measures were observed between sessions 2 and 3 or sessions 1 and 3.

The RD voxel (paired TBSS, 2 days vs. 2 weeks; $p < 0.025$, FWE corrected, TFCE) mask was used to download individual mean RD and FA values from all eligible subjects' volumes in order to conduct post-hoc between-group statistical tests. Figure 2 illustrates the individual trajectories of the downloaded mean RD values.

The results of the between-group, mixed-effects analyses for RD and FA are presented in Table 2. The results of the mixed-effects model suggest that RD values are, on average, significantly higher 2 days postinjury (two-sided $p = 0.002$), as compared to controls, but the difference at 2 months represents more of a trend (two-sided $p = 0.11$). At 2 weeks postinjury, there is no statistical difference between the groups with regard to the RD measure. The FA results show a similar, but inverted, pattern. FA values are, on average, lower for the injured athletes at all three time points, as compared to controls. At 2 days postinjury, FA values are significantly lower in the concussed, as compared to the controls (two-sided $p = 0.0008$). At 2 months, the differences persist (two-sided $p = 0.044$), but at 2 weeks, the average difference from controls is not statistically significant. The results of the multi-variate (FA and RD) bootstrap analysis are presented in Table 3. These results point to significant differences between groups at 2 days and a trend at 2 months.

Mean RD values from the significant RD cluster and its local vicinity within the WM tract, that is, the inflated RD cluster (see Fig. 1), were correlated with mean RD measures of the remaining deep WM tracts to assess whether the same trend existed globally. The deep WM tracts for each individual volume in MNI space were masked by the JHU ICBM-DTI-81 WM atlas. They were further constrained to include only WM by thresholding the corresponding FA volumes at 0.25. Two-tailed *p* values (testing the null

Table 3. Results of the Between-Group, Multivariate Analysis of Fractional Anisotropy and Radial Diffusivity Measures Using the Bootstrap Method for Hypothesis Testing

Hotelling T^2 statistic for three time points	*2 days*	*2 weeks*	*2 months*
T^2	8.253	3.154	4.315
Boot *p* value	0.0273*	0.189	0.092
Percentiles of null bootstrap distribution of T^2			
95%	6.463	6.076	5.558
97.5%	8.469	7.825	7.038
99%	10.988	9.930	9.569

*p value < 0.05.

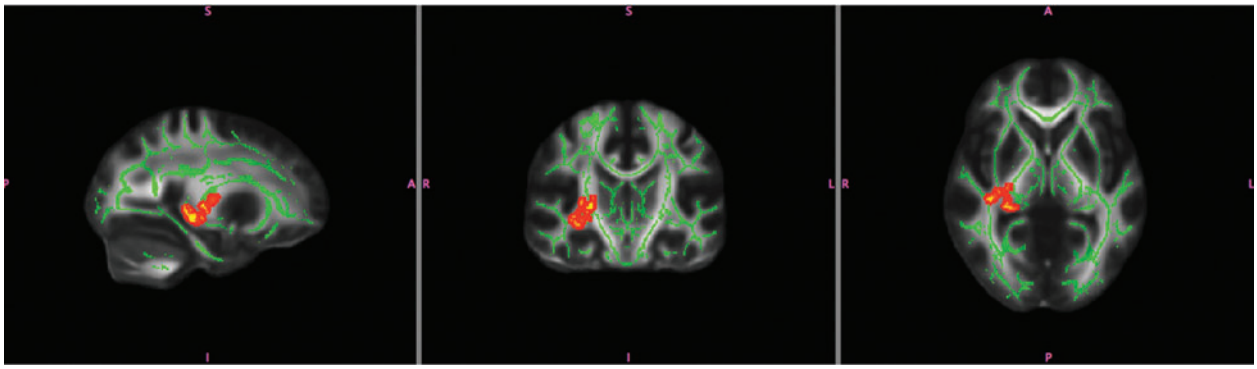

FIG. 1. Results of the paired, between-concussed session (2 days vs. 2 weeks; corrected $p < 0.025$), tract-based spatial statistics *t*-test of the radial diffusivity (RD) values on the white matter (WM) skeleton. Voxels (inflated into adjoining local tracts for visualization) showing significantly higher RD values at 2 days, as compared to 2 weeks, have been highlighted by color mapping (red-yellow). These voxels have been overlaid onto their corresponding WM skeleton (green). The underlay is the ''FMRIB58_FA_1-mm'' image volume (grayscale). Color image is available online at www.liebertpub.com/neu

hypothesis of no correlation) of the control group was 0.14 and less than 0.05 for the concussed across all three imaging sessions. Figure 3 illustrates the individual trajectories of the mean RD values from the aforementioned deep WM region.

Discussion

Results of the current study reveal structural alterations in the deep WM of the brain over the course of the 2 months after injury. Our primary finding is the significant difference observed between sessions 1 and 2 (2 days vs. 2 weeks) within the concussed group in the paired TBSS *t*-test of the RD measure with greater values at 2 days, as compared to 2 weeks. In addition, the same TBSS comparison revealed a reverse trend for the FA measure (within concussed session, paired TBSS *t*-test) with greater values at 2 weeks, as compared to 2 days, postinjury with significant overlap of the FA with the RD cluster. The significant RD cluster spans across parts of the posterior limb, the retrolenticular part of the IC, the inferior

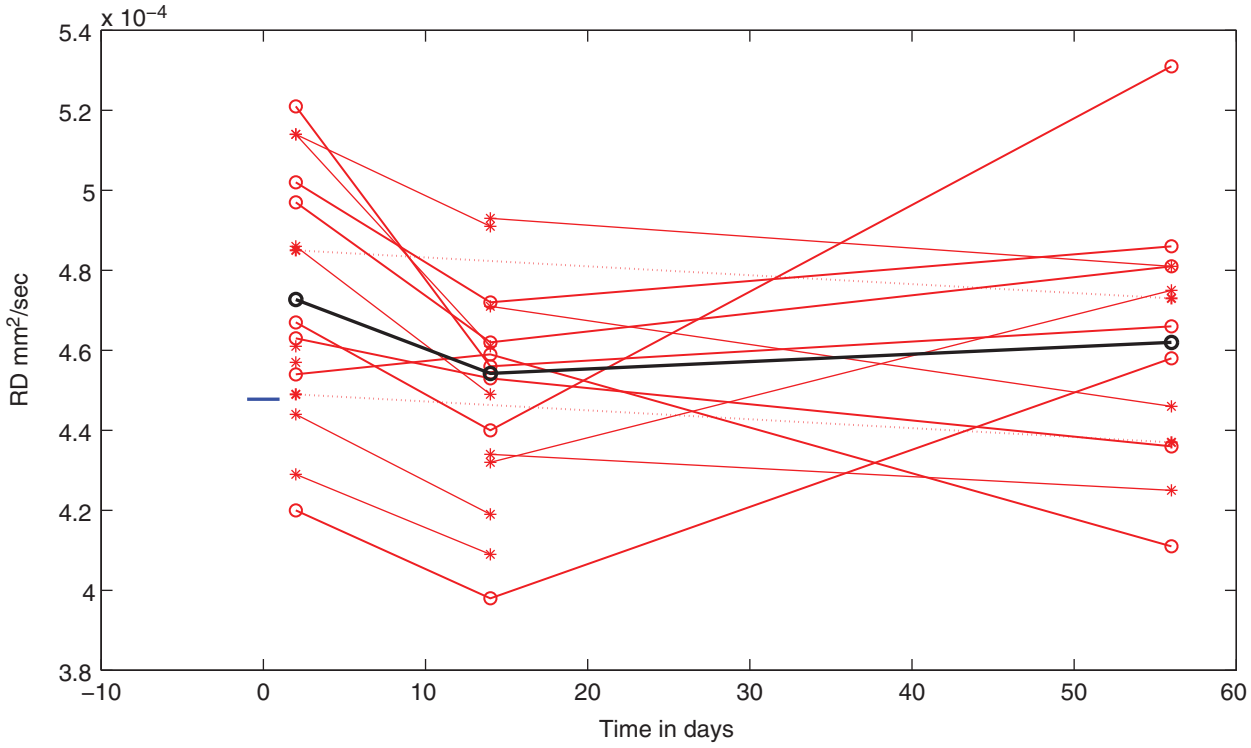

FIG. 2. Individual trajectories of the mean radial diffusivity (RD) values downloaded from the paired tract-based spatial statistics *t*-test RD mask (2 days vs. 2 weeks; $p < 0.025$, corrected) for all concussed athletes across all three sessions. Red circles indicate those concussed athletes who participated at all three sessions. Red stars indicate those athletes who had imaging data at a maximum of two time points. Solid red lines connect athletes with consecutive measurements. Dashed red lines connect nonconsecutive measurements (i.e., sessions 1–3). Blue line marks the mean value of the controls, whereas the solid black line connects the mean of the concussed across the three time points. Color image is available online at www.liebertpub.com/neu

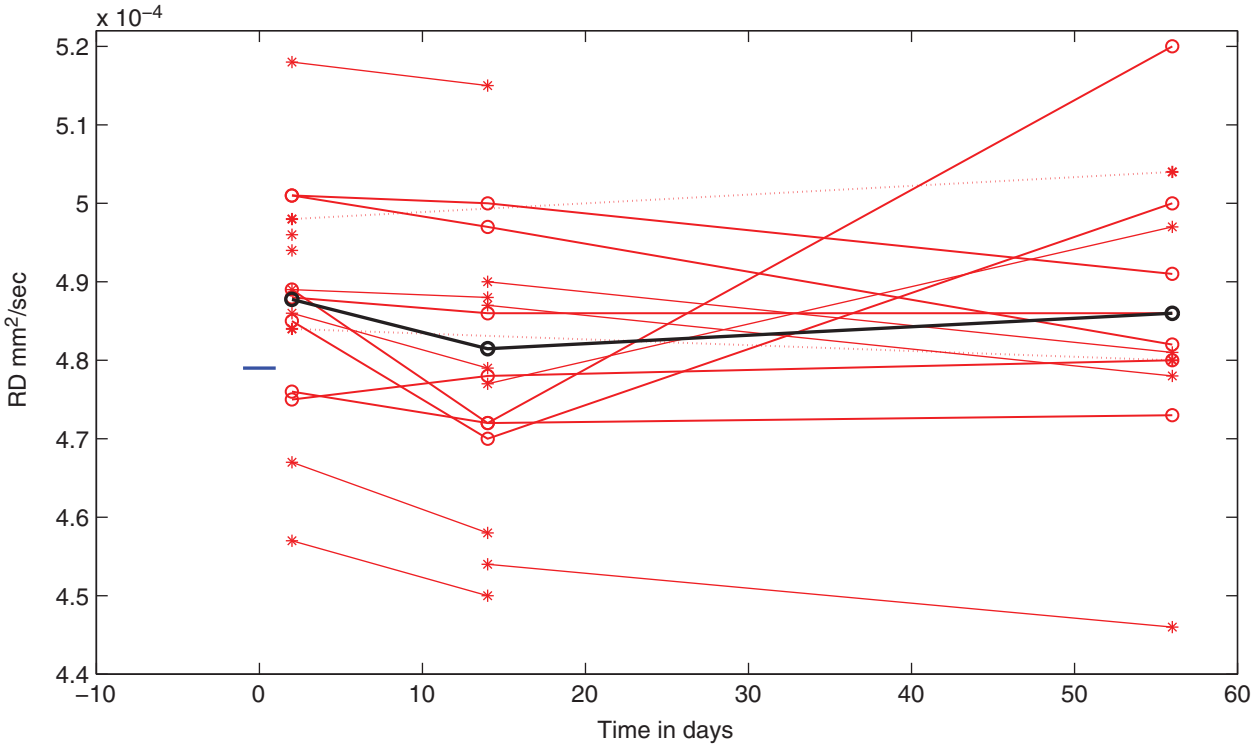

FIG. 3. Individual trajectories of the mean radial diffusivity (RD) values from whole deep white matter region (with the significant RD cluster from the between-session tract-based spatial statistics [TBSS] analysis masked out) for individual concussed athletes at all three sessions. Red circles indicate those concussed athletes who participated at all three sessions. Red stars indicate those athletes who had imaging data at a maximum of two time points. Solid red lines connect athletes with consecutive measurements. Dashed red lines connect nonconsecutive measurements (i.e., sessions 1–3). Blue line marks the mean value of the controls (with the significant RD cluster from the between-session TBSS analysis masked out), whereas the solid black line connects the mean of the concussed across the three time points. Color image is available online at www.liebertpub.com/neu

fronto-occipital fasciculus, inferior longitudinal fasciculus (sagittal stratum), and extends into the anterior thalamic radiation. Of specific note are two recent TBSS studies: a pilot study of veterans with combat related TBI[52] and a comparable study in athletes with prolonged symptoms after SRC,[12] both of which reported nearly the same anatomical region in the contralateral hemisphere, as compared to the significant RD cluster identified in this study. Other research, involving patients with a GCS 13–15, using a broad range of analyses, including TBSS, have reported abnormal diffusion measures in a subset of regions covered by the significant RD cluster reported in the current study. Specifically, such regions were observed in the IC,[24,26,29,32,53–58] in either the inferior fronto-occipital and/or inferior long fasciculus[27,28,59–61] and the anterior thalamic radiation.[60] The current study lends further support to an earlier hypothesis,[12] which suggested that the prevalence of crossing and merging WM fiber tracts in the anatomical region of the RD cluster might make this particular area more vulnerable to the type of forces acting on the brain during the course of a concussion. This hypothesis posits that certain anatomical regions are more vulnerable to trauma than others, independent of the biomechanical load dynamics of the injury. Finite element method (FEM)-based reconstructions[62] of head impacts from the National Football League found early strain ''hot spots'' along the temporal lobe. These strain hot spots then migrated to the fornix, midbrain, and CC and were manifest in 9 of 22 concussion reconstructions. A later FEM study[63] correlating FA and MD values in a different region of interest (ROI; CC) with computer simulations of the impact appear to show strain resulting in hot spots in the temporal lobe as well, although secondary in intensity to the CC. These findings provide additional support for the increased vulnerability of the anatomical regions of the significant RD cluster identified in the current study. Future studies might further elucidate the effect of impact forces by correlating injury mechanism and load dynamics to brain pathology (by postinjury *in vivo* imaging) with retrospective video analyses coupled to a head impact telemetry system.[64]

Given the variability of patient characteristics and concussive injury mechanisms, one may question the validity of searching for common regions of pathology, which is inherent to any between-group, voxel-wise analyses of mTBI.[57,65,66] Instead, mTBI may have a unique spatial pattern of injury in each individual patient's brain. Researchers taking this perspective compare the voxels of individual patients (diffusion measures) in standard space with the corresponding voxel set of a control group. Extreme voxels, deviating either positively or negatively from the control group, are then labeled and clustered (with multiple comparisons correction). The summary statistics of such abnormal loci reported in recent mTBI literature[57,67] reveal significant positive and negative clusters with significant between-group differences.[65] Future approaches to tracking recovery of individual concussions should compare the efficacy of the latter techniques against monitoring of diffusion measures over time, obtained from predetermined regions of vulnerability, such as the mask of the significant clusters arising in the current study.

No previous study has assessed the type of SRC examined here (with no LOC) at three time points (2 days, 2 weeks, and 2 months). Although our permutation tests on the whole-brain WM skeleton

did not reveal any significant between-group differences, the comparisons of the voxels within the RD cluster showed significant between-group difference at 2 days and a trend at 2 months. Closely related studies have demonstrated RD as a useful measure to assess the continuum of the mild end of TBI. RD values have been shown to increase (paired TBSS *t*-tests) over the course of a season in individual contact sport athletes[11] demonstrating significant increases only in Trace, AD, and RD measures when comparing pre- with postseason images. The posterior limb of the IC was reported as a region (among others) with significant differences in structural measures between pre- and post-season, which incidentally is a region implicated in the current study. Further, a significant increase in RD was found in 3 athletes, as compared to the rest of the players in the study, who sustained a medically diagnosed concussion during the course of the season. No significant difference was found in Trace, FA, or AD. Another study[33] compared the WM integrity of swimmers to professional soccer players, with exposure to ''headings'' (without a symptomatic concussion), and found increased RD in several areas, including the inferior fronto-occipital fasciculus (a region implicated in the current study). No significant differences were found in the FA and MD measures. These studies[11,33] suggest that RD might be a potentially sensitive measure to subconcussive hits. A recent DTI study[68] on cerebral WM in 74 boxers and 81 mixed martial arts fighters found that a history of previous knockouts (the ''knockout'' measure includes ''technical knockouts'' with no subsequent LOC) could predict increased RD in the CC, isthmus of the cingulate gyrus, pericalcarine sulcus, the precuneus, and the amygdala in the group of boxers. The same regions had increased MD and decreased FA values. The knockout measure additionally predicted significantly increased RD in the posterior cingulate in the group of mixed martial arts fighters. In addition, they found that the number of previous fights did not predict differences in diffusion measures, suggesting that diffusion measures were sensitive to potential subconcussive hits or concussions, as opposed to time of exposure to the sport. In a longitudinal mTBI study (GCS 13–15) with imaging sessions at 24 h, 1 week, and 1 month postinjury, statistical trends were reported[69] in the paired between-concussed session, based on TBSS *t*-tests of RD (greater at 24 h vs. 1 month postinjury) and FA (lower at 24 h vs. 1 week postinjury). It should, however, be noted that the lack of significant differences might have been a result of random assignment of participants to two different scanners. An ROI study[70] reported increased RD in a sample of mild and moderate TBI patients; imaging occurred an average of 8.9 days postinjury. Despite the fact that these findings appear to lend support to the sensitivity of RD with regard to mTBI, future DTI studies should additionally assess the validity of RD as a diffusion measure for the assessment of mTBI.

The major finding of the current study is the occurrence of significant temporal changes in radial diffusivity between $\sim$2 days and 2 weeks postinjury in a sample of concussed athletes. Multiple cross-sectional mTBI studies with one or more time points[67,69,71] have broadly discussed the coupled, inverse expression of RD/MD and FA measures in the acute and subacute phases postinjury, that is, increased RD/MD and/or decreased FA or decreased RD/MD and/or increased FA with respect to matched controls. The results of the current study support an earlier hypothesis[24] on the role of focal neurofilament misalignment, as an initializing mechanism leading to decreased FA, increased RD, and reduced AD in human mTBI patients (GCS 13–15) imaged approximately 24 h postinjury.[24,71] Such misalignment had been observed to be manifest within 6 h of axonal injury in animal models.[72–75] Though the increased RD/MD and/or decreased FA mode is frequently reported in mTBI as well as moderate-to-severe TBI literature[76] and in studies of subconcussive hits,[32] there is a lack of consensus on the broad directionality of the diffusion measures after a concussive injury.[29,53,77] Earlier findings of increased FA and reduced RD after mTBI[29] have been replicated.[67] The investigators reported a significant reduction in both the count and the volume of positive clusters representing regions of high FA over a 4-month period, with the corresponding reduction in self-reported symptomatology suggesting recovery. Cytotoxic edema[53] was suggested as a potential explanation for the increased FA findings during the recovery interval. A recent longitudinal study[57] assessed individual FA abnormalities in mTBI patients at $\sim$2 weeks, 3 months, and 6 months postinjury. They found that the count of low FA voxels decreases at both 3 and 6 months, but the count of high FA voxels increased at 3 months, followed by a decrease at 6 months, as compared to their initial assessments at 2 weeks postinjury. The investigators note that the continued expression of the positive clusters is inconsistent with cytotoxic edema, which drives ionic edema and signals a premorbid cellular process leading to necrotic cell death.[78] In discussing these findings, other researchers[68] suggest the possibility that contact sport athletes represent a distinct population because of their continued exposure to subconcussive hits leading to constant WM injury and recovery cycles and therefore might present a different recovery profile from the civilian, non-contact-sport population suffering a single mTBI episode. It must be noted that at least one study on SRC[79] showed significantly higher FA, AD, and lower MD (as compared to noncontact controls) values at two time points: $\sim$81 hours (on average) and 6 months postinjury, suggesting no significant recovery in diffusion measures during that time interval. Future work is needed to address these observed differences of diffusion metrics during recovery after SRC.

Animal models of TBI additionally support the findings of the present study. For example, a recent controlled cortical impact (CCI) study on rats[80] showed significantly increased RD and decreased FA in WM. RD may also be selectively sensitive to alterations of the myelin sheath,[81] as shown in the mouse model[22,82] and, more recently, in optic neuritis.[23] Recovery, as observed by histology after CCI-induced TBI in a rat model have correlated with increases in FA[83,84]; this has been attributed to axonal recovery and increased oligodendrocyte generation. A recent histology study scaling biomechanical loads to approximate mTBI in swine found axonal swellings and an accumulation of neurofilament protein.[85] These observations could be expected to increase RD and lower FA according to the focal neurofilament misalignment hypothesis discussed earlier.[24] Further evidence is needed to confirm these findings in humans.

A traditional interpretation of FA increases from 2 days to 2 weeks postinjury and corresponding decreases in RD would indicate that patients are recovering from mTBI. This interpretation has been proposed in more-severe TBI.[86] The fact that no differences (in all four diffusion metrics considered) were identified between 2 weeks and 2 months in the current study might, in part, be because of intersubject variability of these measures. The finding of significant between-group differences of the cluster at 2 days provides support for the view that diffusion measures may offer the required sensitivity to assess injuries as mild as the ones examined in this study. Diffusion measures at the identified anatomical location might have future diagnostic potential as a signature of concussion. Individual subject baselines or a database of normative values in the early phase of concussion might allow for identification of athletes at greater risk of prolonged recovery. There were no significant between-group differences at 2 weeks. Though this could be

interpreted to be indicative of recovery, it should be noted that intersubject variability could potentially mask an ongoing or unresolved recovery process at 2 weeks. A future study should include a baseline MRI scan and a time point at 1 month to further clarify the course of the recovery process, exhibited through diffusion abnormalities.

A majority (80–90%) of concussions resolve between 7 and 10 days postinjury, as measured by behavioral assessments.[13] However, the results of the present study provide evidence of neural recovery extending to at least 2 weeks from a structural perspective. Although these data do not inform us about the absolute maxima and minima of the diffusion metric trajectories because of the absence of measurements between 2 weeks and 2 months postinjury, the statistical trend detected by the between-group analyses at 2 months suggests a minor relapse in the recovery of the structural measures of WM integrity. This finding, taken together with the observed variability in the trajectories of RD and FA between 2 weeks and 2 months, might be reflective of the athletes' exposure to subconcussive hits following return to play (see earlier discussion[11,33,68]). A more recent study,[87] and the first to relate diffusion measures to biomarkers in athletes with subconcussive hits, reported a positive correlation between the percentage change in football post- minus preseason levels of serum autoantibodies of the astrocytic protein, S100B (considered a peripheral marker of blood–brain barrier dysfunction), and the percentage of voxels with changes in MD during the corresponding time period. The same study reported a significant positive trend between the head hit index (a derived measure accounting for both the number and severity of subconcussive hits during a single game) and the increased postgame (as compared to baseline) S100B levels of individual athletes. In addition, these, significant postgame increases in S100B levels were detected only in athletes with subconcussive hits (confirmed by game video analyses). These findings suggest that subject specific exposure to subconcussive hits after return to play may be a potential factor affecting recovery of diffusion measures.[32,87]

Further, variability in the trajectories of the diffusion measures might be affected by differences in number of previous concussions,[68] timing of each athlete's return to play (see Table 1), and individual genetic predisposition.[88,89] Experimental designs of future studies should include the assessment of subconcussive hits, extending at least to the end of season.

Conclusions

This is the first longitudinal study that tracks diffusion measures of contact sport athletes after a single episode of SRC with no LOC at ~2 days, 2 weeks, and 2 months. This study provides support for the hypothesis of increased RD and reduced FA within 72 h postinjury followed by patterns of recovery. It further suggests that neural recovery may extend beyond 2 weeks, as described in other similar imaging studies.[8,10] RD was found to be a sensitive marker of SRC with potential for personalized imaging-based diagnosis.

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Author Disclosure Statement

No competing financial interests exist.

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(Appendix follows →)

APPENDIX

A1. Mixed-effect model

The mixed-effect model was selected based on the following observations unique to this experimental design:

1. ''Participant'' is a random effect and there are repeated measures over the same individuals. The repeated measures are unbalanced as a result of the missing values. This model also accounts for any correlations between the scalar diffusion measures of WM microstructure.
2. Concussed participants cannot be matched (paired) to controls because controls are imaged at only one time point.
3. This model treats time as a fixed effect, with time = 0, denoting the controls. It then allows for comparison of the concussion effect at time = 2 days, 2 weeks, and 2 months in relation to the controls.

The model was implemented using the lme4 library in R, specifically, using the ''lmer'' function.

A2. Multivariate analysis using bootstrap method for hypothesis testing

The following procedure describes the bootstrap method employed for testing the mean effect at 2 days, 2 weeks, and 2 months with respect to the controls. This test preserves repeated measures, the missing value structure, and any correlation structure between the scalar diffusion measures of WM microstructure.

Step 1. Use the data to construct the null distribution of the observed variables by centering the empirical distributions around zero, so that the means of the scalar diffusion measures of WM microstructure for the controls and the three time points of the concussed are all zero. Then, the effect at 2 days, 2 weeks, and 2 months with respect to the control group are exactly zero.

Step 2. Generate a data set with the same variables, groups, and dimensions as the original data, but sampled with replacement from the null distribution defined in step 1, which is also called bootstrap resampling.

Step 3. Calculate the Hotelling T^2 statistic from the data set generated in step 2 and save the value.

Step 4. Repeat steps 2 and 3, 10,000 times and store the 10,000 values of the T^2 statistic. These 10,000 values form the bootstrap distribution of the statistic T^2 under the null hypothesis.

Step 5. Calculate the bootstrap p values by comparing the observed T^2 from the real data to their corresponding bootstrap distribution under the null.