Longitudinal Assessment of White Matter Abnormalities Following Sports-Related Concussion

Timothy B. Meier,^{1,2,3} Maurizio Bergamino,³ Patrick S. F. Bellgowan,⁴
T. K. Teague,^{5,6,7} Josef M. Ling,² Andreas Jeromin,⁸ and Andrew R. Mayer^{2,9,10}

¹Department of Neurosurgery, Medical College of Wisconsin, Milwaukee, Wisconsin

²The Mind Research Network/Lovelace Biomedical and Environmental Research Institute,
Albuquerque, New Mexico

³Laureate Institute for Brain Research, Tulsa, Oklahoma

⁴National Institute of Neurological Disorders and Stroke, National Institutes of Health,
North Bethesda, Maryland

⁵Departments of Surgery and Psychiatry, University of Oklahoma College of Medicine, Tulsa,
Oklahoma

⁶Department of Pharmaceutical Sciences, University of Oklahoma College of Pharmacy, Tulsa,
Oklahoma

⁷Department of Biochemistry and Microbiology, Oklahoma State University Center for Health
Sciences, Tulsa, Oklahoma

⁸Quanterix Corporation, Lexington, Massachusetts

⁹Neurology Department, University of New Mexico School of Medicine, Albuquerque,
New Mexico

¹⁰Department of Psychology, University of New Mexico, Albuquerque, New Mexico

Abstract: There is great interest in developing physiological-based biomarkers such as diffusion tensor imaging to aid in the management of concussion, which is currently entirely dependent on clinical judgment. However, the time course for recovery of white matter abnormalities following sports-related concussion (SRC) is unknown. We collected diffusion tensor imaging and behavioral data in forty concussed collegiate athletes on average 1.64 days (T1; $n = 33$), 8.33 days (T2; $n = 30$), and 32.15 days post-concussion (T3; $n = 26$), with healthy collegiate contact-sport athletes (HA) serving as controls ($n = 46$). We hypothesized that fractional anisotropy (FA) would be increased acutely and partially recovered by one month post-concussion. Mood symptoms were assessed using structured interviews. FA differences were assessed using both traditional and subject-specific analyses. An exploratory analysis of tau plasma levels was conducted in a subset of participants. Results indicated that mood symptoms improved over time post-concussion, but remained elevated at T3 relative to HA. Across both group and subject-specific analyses, concussed athletes exhibited increased FA in

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*Correspondence to: Timothy B. Meier, PhD, Neuroscience Research Center, 8701 Watertown Plank Road, Milwaukee, WI 53226. E-mail: tmeier@mcw.edu

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several white matter tracts at each visit post-concussion with no longitudinal evidence of recovery. Increased FA at T1 and T3 was significantly associated with an independent, real-world outcome measure for return-to-play. Finally, we observed a nonsignificant trend for reduced tau in plasma of concussed athletes at T1 relative to HA, with tau significantly increasing by T2. These results suggest white matter abnormalities following SRC may persist beyond one month and have potential as an objective biomarker for concussion outcome. *Hum Brain Mapp* 37:833–845, 2016. © 2015 Wiley Periodicals, Inc.

Key words: diffusion tensor imaging; mTBI; fractional anisotropy; tau

INTRODUCTION

It is estimated that up to 3.8 million sports-related head injuries occur annually, the majority of which are concussions [Langlois et al., 2006]. Due to this high incidence, there is great interest in developing physiological-based biomarkers to aid in clinical management of concussion, which is largely dependent on clinical judgment [Giza et al., 2013]. However, basic knowledge gaps regarding the exact physiological consequences of sports-related concussion (SRC) have hampered biomarker development to date. Thus, there is a pressing need to characterize the acute neurophysiological effects of concussion, the time frame of recovery for these neurophysiological effects, and the relationship between neurophysiological recovery and typical symptom resolution.

One potential biomarker that has shown promise in mild traumatic brain injury (mTBI) is diffusion tensor imaging (DTI). Evidence from work in animals [Mac Donald et al., 2007; Spain et al., 2010] and humans [Dodd et al., 2014; Shenton et al., 2012] demonstrates that white matter abnormalities are a common consequence of mTBI. One commonly assessed DTI metric is fractional anisotropy (FA), which measures the direction of water diffusion along white matter tracts and is thought to reflect axonal microstructure [Beaulieu, 2002]. While much of the work investigating FA abnormalities following mTBI has focused on emergency room cohorts with heterogeneous mechanisms of injuries (e.g., falls, motor-vehicle accidents, assault), interest in white matter abnormalities associated with SRC has increased in recent years [Gardner et al., 2012]. Consistent with observations in other mTBI samples,

increased FA has been reported following SRC at the acute (i.e., days) and subacute phase (one-month) [Borich et al., 2013; Pasternak et al., 2014; Virji-Babul et al., 2013]. For example, increased FA was observed in the corticospinal tract (CST) and corpus callosum (CC) acutely and persisted at 6 months post-concussion relative to controls [Henry et al., 2010]. However, other studies have found no evidence of FA differences in the acute and sub-acute time periods post-concussion [Zhang et al., 2010; Zhu et al., 2015], while others have observed reduced FA [Murugavel et al., 2014]. Large-scale, prospective studies are needed to better capture acute changes and subsequent recovery of white matter abnormalities following SRC.

In addition to neuroimaging markers, there is also great interest in identifying fluid biomarkers for the clinical management of concussion [Zetterberg et al., 2013]. One potential marker that may reflect axonal injury and has recently shown prognostic value is tau, a microtubule-associated protein that is specific to the central nervous system and found predominately in neuronal axons [Trojanowski et al., 1989]. Post-injury increases in tau have been documented in cerebral spinal fluid following severe [Franz et al., 2003] and mild traumatic brain injury [Neselius et al., 2012]. Recent studies have also observed increased tau in blood following mTBI. For example, increased tau has been observed in boxers following a fight compared with controls [Neselius et al., 2013] and following concussion in professional ice hockey players [Shahim et al., 2014], with acute tau levels predicting symptom resolution in the latter study. To our knowledge, the relationship between post-concussion changes in tau concentration and neuroimaging markers such as FA has not been established even though they both putatively measure similar pathology (i.e., axonal injury).

In this study, we investigated the recovery of FA across the acute to sub-acute phase post-concussion, the time period at which large-scale behavioral studies have documented the resolution of typical post-concussion symptoms [McCrea et al., 2003]. Serial DTI was collected from a sample of concussed collegiate athletes with a well-matched group of healthy, contact-sport collegiate athletes serving as controls for potential confounds such as sub-concussive head injuries and other non-specific factors (e.g., stressors, diet, physical fitness levels) associated with

Abbreviations

ACR	anterior corona radiata
CC	corpus callosum
CST	corticospinal tract
DTI	diffusion tensor imaging
FA	fractional anisotropy
LME	linear mixed-effects
mTBI	mild traumatic brain injury
SRC	sports-related concussion
SCR	superior corona radiata

collegiate athletics. Because we have previously reported increased FA in three independent samples of emergency room mTBI patients [Ling et al., 2012b; Mayer et al., 2010; Mayer et al., 2012], presumably reflecting cytotoxic edema [Bazarian et al., 2007; Povlishock and Katz, 2005; Wilde et al., 2008], we hypothesized that concussed athletes would exhibit increased FA relative to healthy athletes with evidence of partial recovery by the sub-acute time period post-concussion. Finally, given a recent report of increased peripheral levels of tau protein following SRC, putatively thought to reflect axonal injury [Shahim et al., 2014], we also conducted an exploratory analysis of plasma tau concentration in a subset of participants.

MATERIALS AND METHODS

Participants

NCAA Division I student-athletes ($N = 86$) provided written informed consent and clinical data for this study following referral by sports medicine professionals. Institutional Review Boards approved all aspects of this study, and all participants self-reported no past or current mood disorders, anxiety disorders, alcohol abuse, or substance abuse. Behavioral data from a subset of athletes included in this study have been reported elsewhere [Meier et al., 2015; Singh et al., in press]. Forty concussed athletes participated in at least one of three visits occurring approximately one day ($T1$: 1.64 ± 0.78 days post-concussion; $N = 33$), one week ($T2$: 8.33 ± 2.23 days post; $N = 30$) and one month ($T3$: 32.15 ± 4.75 days post; $N = 26$) following SRC. Physicians trained in sports medicine diagnosed concussions independently of the study at the time of injury following recommended guidelines [McCrory et al., 2013] based on a clinical exam assessing symptoms, a cranial nerve check, manual muscle testing for strength deficits, the Romberg's test for balance deficits, on-field cognitive testing developed by the UPMC Center for Sports Medicine, and the King-Devick test. Seventeen concussed athletes participated in all three visits and 32 participated in at least two visits. Forty-six healthy, collegiate contact-sport athletes (HA) participated in this study and served as a control group. Thirty-three percent (15 out of 46) of HA had a previous concussion, with the most recent occurring on average 36.3 months prior to study date (range 5–134 months, S.D. = 39 months).

Behavioral Data

Structured interviews for the Hamilton Depression and Anxiety Rating Scales served as the primary behavioral measures. The Automated Neuropsychological Assessment Metrics 4 Sports Medicine Battery (ANAM) was used to collect secondary measures of interest, including computerized assessments of self-reported concussion symptoms, mood scales, and cognitive tests [Cernich et al., 2007]. To

limit the number of comparisons, and because of the high correlation among many of the ANAM measures, composite measures of ANAM scores were calculated by comparing the correlation structure of these measures across healthy athletes. Overall symptom frequency, overall symptom severity, concussion symptom inventory frequency, and concussion symptom inventory severity were strongly correlated (mean $r = 0.91$, range 0.85–0.94; see Supporting Information Table I), thus only the overall symptom frequency was used. ANAM mood scores were not assessed because the primary behavioral measures were structured interviews for mood symptoms. ANAM cognitive tasks include measures of simple processing speed (two simple reaction time tasks), information processing speed (code substitution task, procedural reaction time task), visual-spatial processing (spatial processing task), working memory (matching-to-sample task, memory search task, mathematical processing), memory (delayed code substitution task), and response inhibition (go-no-go task). Throughput, which is the number of correct responses per minute, was used to measure performance on each cognitive task [Thorne, 2006]. Scores on the cognitive battery were converted to standard scores by using a gender and age matched sample of collegiate athletes available in ANAM. Reference group scores for the go-no-go and the mathematical processing tests were not available and these measures were not investigated. All remaining cognitive measures were positively correlated (mean correlation $r = 0.44$, range 0.24–0.82; see Supporting Information Table I), and averaged to create a single cognitive composite measure to reduce the likelihood of Type I error. Analyses of individual ANAM measures can be found in the Supplement to ensure that data reduction did not result in a loss of critical information. The ANAM Validity Indicator Report was used to flag cognitive batteries with questionable effort or scores on individual cognitive modules indicative of misunderstanding subtest instructions [Roebuck-Spencer et al., 2013]. These measures were excluded prior to calculation of composite scores. Likewise, athletes reporting zeros on all self-reported symptoms and mood scale questions (including positive measures) were excluded from analyses. Furthermore, not every participant completed ANAM testing. The final number of athletes included in the behavioral analyses is listed in Table I.

Finally, an independently determined, real-world measure of outcome was defined as the number of days that athletes were withheld from competition (i.e., return-to-play decision) by clinicians trained in sports medicine following recommended guidelines [McCrory et al., 2013].

Blood Processing and Analysis

Non-fasting venous afternoon blood was drawn from a subset of participants (see Table I) in K2 EDTA BD Vacutainer tubes. Blood was centrifuged at 1300g for 10

TABLE I. Sample Characteristics

	HA	CA
Total number	46	40
Gender (M/F)	30/16	30/10
Mean age (years)	20.31 (1.5)	20.12 (1.4)
Mean education (years)	13.33 (1.3)	13.08 (1.0)
Average number of previous concussions*	0.56 (1.1)	0.92 (1.1)
% Athletes with previous concussions	33	58
Median length of return-to-play decision (days)	na	13 (range 4-indefinite)
Sport		
Football	26	27
Soccer	20	4
Basketball	0	6
Volleyball	0	2
Rowing	0	1
Concussion information		
LOC (undefined)	na	1
PTA (<1 min, 5–10 mins, 10–20 minutes, undefined)	na	2, 1, 2, 1
Retrograde (<5 min, 10–20 min, undefined)	na	1, 1, 2
Health/medication for study duration		T1/T2/T3
ADHD	3	2/2/2
Antibiotic, acetaminophen, mucinex	1	0/0/0
Nature-throid	1	0/0/0
Adderall	0	1/1/1
Acetaminophen	0	3/0/0
Ibuprofen	0	1/0/0
Zarah	0	1/0/0
Tramadol	0	0/1/0
Losartan, albuterol, minocycline	0	1/1/0
Advair	0	0/1/0
Final <i>n</i> for analyses		
HAM-A, HAM-D	46	33/30/26
ANAM self-report	39	30/28/22
ANAM cognitive battery	38	27/27/24
DTI	43	28/26/23
Tau	18	19/19/12

*Indicates trend toward significance.

Abbreviations: HA = healthy athletes; CA = concussed athletes; ADHD = attention deficit/hyperactivity disorder, LOC = loss of consciousness, PTA = post-traumatic amnesia, Retrograde = retrograde amnesia, min = minute, undefined = undefined duration.

Medication information not available for 6 HA and medication/concussion severity information not available for 2 CA.

minutes at room temperature and plasma was collected and aliquoted into microfuge tubes and stored at -80°C that day. Frozen plasma samples were shipped overnight on dry ice to Quanterix Corp for quantification of tau using an automated version of a previously described immunoassay [Rissin et al., 2010; Shahim et al., 2014].

Imaging Parameters, Processing, and Analysis

MRI was performed using a General Electric Healthcare Discovery MR750 3-Tesla whole body MRI scanner and brain-dedicated receive-only 32-element coil array optimized for parallel imaging (Nova Medical, Inc.). Diffusion tensor imaging was performed with the following parameters: 30 non-collinear directions, b -value = $1,000 \text{ s/mm}^2$, TR = 8,800 ms, TE = minimum (average 78.5 ms, range 76.8–84.9 ms; see Supporting Information), acquisition matrix = 96×96 interpolated to 256×256 , field of view = $25.6 \times 25.6 \text{ cm}$, slice thickness = 2 mm, inter-slice spacing 0.2 mm, slices = 69, acceleration factor $R = 2$ in the phase encoding direction, original voxel size $2.7 \times 2.7 \times 2.2 \text{ mm}$. For anatomical reference, T1-weighted anatomical images were collected to using a parallelized magnetization-prepared rapid gradient-echo sequence (MPRAGE) with sensitivity encoding (FOV = 240 mm, 130 axial slices, slice thickness = 1.1 mm, image matrix = 256×256 , TR/TE = 5/1.948 ms, acceleration factor $R = 2$ in the phase encoding direction, flip angle = 8 degrees, TI = 725 ms, sampling bandwidth = 31.25 kHz, voxel size = $0.9375 \times 0.9375 \times 1.1 \text{ mm}$). DTI and anatomical imaging data were collected on 46/46 HA, 29/33 T1, 28/30 T2 and 24/26 T3 participants.

A previously described binary classification scheme was used to flag individual gradients with excessive motion or zipper artifacts [Ling et al., 2012a]. Participants that had more than 3 flagged gradients were excluded from DTI analyses (Table I for final *n*). Head motion during DTI scan acquisition was calculated as the Euclidean norm of motion parameters estimated using a 6-parameter affine registration of gradient images to each participant's b_0 image [Jones et al., 2010].

The AFNI analysis suite was used for DTI preprocessing [Cox, 1996]. Gradients images were co-registered to the b_0 volume using a 12-parameter affine alignment to correct for subject motion and eddy currents. The resultant rotational parameters were also applied to the gradient table. A 3.5 mm Gaussian full width at half maximum smoothing kernel was then applied to each diffusion-weighted image to reduce effects of Gibb's ringing artifact [Turken et al., 2009]. A non-linear algorithm was used to calculate diffusion tensors, followed by scalar measures of axial diffusivity (AD), radial diffusivity (RD), and fractional anisotropy (FA). Separate 12-parameter affine alignments were calculated to register each participant's b_0 volume to their anatomical T1 image in native space and the native T1 to the N27 brain template in Talairach space [Talairach and Tournoux, 1988]. These alignment matrices were subsequently concatenated to create one b_0 to standard space normalization matrix for each participant.

A priori region-of-interest (ROI) analyses included the following tracts from the Johns Hopkins University atlas [Mori and van Zijl, 2007]: the genu, body, and splenium of the corpus callosum (CC), and the left and right uncinate fasciculus (UF), superior longitudinal fasciculus (SLF),

anterior corona radiata, (ACR), superior corona radiata (SCR), combined anterior and posterior internal capsule (IC), and the cerebellar peduncles (CP). The inverse of the b0 to standard template normalization matrix was used to transform ROIs to each participant's native b0 image. Analyses were restricted to white matter by applying a binary mask created from the intersection between each ROI and a binary FA map ($FA > 0.25$) individually created for each participant. Voxel-wise analyses were also performed to examine FA in all white matter (restricted to voxels with $FA > 0.25$ from standard template). Non-linear registration was performed to align each participant's FA image to standard template space (FMRIB58_FA) at 1 mm isotropic resolution using FNIRT from the FSL package [Smith et al., 2004].

Finally, a subject-specific analysis was performed to capture the number and volume of clusters with abnormally increased or decreased white matter FA in each individual participant [Mayer et al., 2014]. The mean and standard deviation of FA at each voxel was first calculated across the HA group using FA images normalized to the FMRIB58_FA template. Next, each participant's normalized data were transformed to a z-score on a voxel-wise basis using the mean and standard deviation from the HA group. The z-scores were transformed to a distribution-corrected z-score (DisCo-Z) based on the number of participants included in the control group to control for known bias. Clusters with abnormally increased or decreased FA (volume and total number) were calculated for every HA and concussed athlete at T1, T2, and T3, restricted to white matter (standard FA template > 0.25). A minimum cluster size of 160 μ l was used to reduce false positives based on cluster-level family-wise error correction (see below).

Statistics

Count data (flagged DTI gradients, the number of prior concussions, number and volume of abnormal FA clusters) were square root transformed following addition of a constant prior to statistical analyses. Linear mixed-effects (LME) models with time as a fixed factor were used to investigate changes in time as a function of recovery. The AFNI program 3dLME was used to perform voxel-wise analyses on FA data [Chen et al., 2013]. For ROI analyses, three LME models (corpus callosum, left hemisphere, right hemisphere) were performed with time, ROI, and the interaction between time and ROI as fixed effects. For all LME, a random intercept was used to account for missing data with compound symmetry as the covariance structure. Monte Carlo simulations (10,000 iterations in resample voxel space) determined the necessary correction for family-wise error rate at $P < 0.05$ for all voxel-wise analyses ($P = 0.005$, minimum cluster volume = 160 μ l) based on the average smoothness of (4.15 mm) of the detrended FA data. An identical volume threshold was used for subject-specific analyses. Two-tailed independent sample *t*-tests

characterized cross-sectional differences against HA. Multivariate analyses of variance compared FA in HA and concussed athletes at T1 in left hemisphere, right hemisphere, and corpus callosum ROIs.

RESULTS

Demographics and Behavioral Analyses

Two-tailed independent *t*-tests showed that concussed athletes did not differ from HA in age or education (P 's > 0.10). However, concussed athletes had a trend for more self-reported prior concussions relative to HA ($t(84) = 1.76$, $P = 0.082$, $d = 0.38$).

There was a significant main effect of time for both the HAM-A ($F(2,47) = 37.73$, $P < 0.001$) and HAM-D ($F(2,47) = 33.65$, $P < 0.001$; Fig. 1A), with more severe scores for concussed athletes at T1 (P 's < 0.001) and T2 (P 's < 0.001) relative to T3 for both measures. Scores were significantly higher at T1 versus T2 for the HAM-A ($P < 0.001$), and trended higher for the HAM-D ($P = 0.098$). Results for secondary neurobehavioral measures indicated recovery by one week post-injury (Fig. 1B). A significant main effect of time was observed for symptom frequency ($F(2,40) = 28.82$, $P < 0.001$) and the cognitive composite ($F(2,41) = 14.06$, $P < 0.001$). For each of these measures, scores were worse at T1 relative to T2 (P 's < 0.001) and T3 (P 's < 0.001). Scores at T2 did not significantly differ from scores at T3 (P 's > 0.10).

There was a significant difference between concussed athletes and HA for the HAM-A and HAM-D at T1 ($t(77) = 9.26$, $P < 0.001$, $d = 2.11$ and $t(77) = 9.02$, $P < 0.001$, $d = 2.06$, respectively), T2 ($t(74) = 7.16$, $P < 0.001$, $d = 1.68$ and $t(74) = 8.62$, $P < 0.001$, $d = 2.02$), and at T3 ($t(70) = 2.19$, $P = 0.032$, $d = 0.54$ and $t(70) = 2.69$, $P = 0.009$, $d = 0.66$; Fig. 1A). There were also significant differences between concussed athletes at T1 and healthy contact-sport athletes for symptom frequency ($t(67) = 5.79$, $P < 0.001$, $d = 1.41$) and the cognitive composite ($t(63) = -3.24$, $P = 0.002$, $d = -0.82$; Fig. 1B). There were no differences between concussed athletes at T2 and healthy athletes in symptom frequency ($t(65) = -0.09$, $P = 0.93$, $d = -0.02$) or the composite cognitive measure ($t(63) = -0.86$, $P = 0.39$, $d = -0.22$). Similarly, concussed athletes at T3 and healthy athletes did not significantly differ in the cognitive composite ($t(60) = -0.10$, $P = 0.92$, $d = -0.03$). However, concussed athletes at T3 had significantly lower symptom frequency scores ($t(59) = -3.09$, $P = 0.003$, $d = -0.82$) relative to healthy athletes.

DTI Analyses

There were no differences between concussed athletes at T1 and HA on DTI head motion or the number of flagged DTI gradients, and neither measure significantly differed over time in concussed athletes (P 's > 0.10). There was no

Figure 1.

Primary and secondary behavioral measures: Shown are scores from structured interviews for anxiety (HAM-A) and depression (HAM-D) and the average scores from the cognitive battery and frequency of self-reported symptoms in concussed athletes (CA) at one day (T1), one week (T2), and one month (T3) post-concussion, as well as in healthy athletes (HA). Notches indicate 95% confidence interval of the median and whiskers represent data within 1.5 interquartile range.

significant time $\times$ ROI interaction or main effect of time for FA ROIs (Fig. 2), main effect of time on voxel-wise analyses, or main effect of time for subject-specific analyses (P 's > 0.10).

DTI Analyses-ROI

The multivariate effect of group was significant for left hemisphere ROIs ($\lambda=0.80$, $F(6,64)=2.59$, $P=0.03$), but not for CC or right hemisphere ROIs (P 's > 0.10). Concussed athletes at T1 had significantly increased FA in the SLF than HA ($F(1,69)=10.59$, $P=0.002$, $d=0.79$; Fig. 2). Follow-up analyses indicated that the increase in FA in the left SLF was driven by a decrease in radial diffusivity (RD; $t(69)=-2.45$, $P=0.017$, $d=-0.60$) rather than an increase in axial diffusivity (AD; $P>0.10$). Two-tailed independent sample t -tests confirmed that FA in the left SLF remained significantly increased in concussed athletes at T2 ($t(67)=2.74$, $P=0.008$, $d=0.68$) and T3 ($t(64)=2.60$, $P=0.01$, $d=0.67$) relative to HA.

DTI Analysis-Voxel-Wise

Voxel-wise analyses identified seven clusters of significantly increased FA for concussed athletes relative to HA

at T1 (Fig. 3). These included the right sagittal stratum (476 μ l), the bilateral superior CP (364 μ l), the right retrolenticular IC (299 μ l), the left (276 μ l) and right SLF (262 μ l), the right forceps minor and inferior fronto-occipital fasciculus (201 μ l), and the left posterior corona radiata (176 μ l). Follow-up analyses demonstrated that the voxel-wise FA differences observed between healthy athletes and concussed athletes at T1 were primary driven by decreases in RD (right sagittal stratum $t(69)=-3.62$, $P<0.001$, $d=-0.88$, bilateral superior CP $t(69)=-3.47$, $P=0.001$, $d=-0.84$, right retrolenticular IC $t(69)=-3.44$, $P=0.001$, $d=-0.84$, left SLF $t(69)=-4.2$, $P<0.001$, $d=-1.02$, right SLF $t(69)=-4.10$, $P<0.001$, $d=-1.0$, right forceps minor and inferior fronto-occipital fasciculus $t(69)=-3.68$, $P<0.001$, $d=-0.89$, left posterior corona radiata $t(69)=-2.55$, $P=0.013$, $d=-0.62$). However, AD was also significantly increased in concussed athletes in left SLF ($t(69)=3.6$, $p<0.001$, $d=0.87$), right SLF ($t(69)=2.46$, $p=0.017$, $d=0.60$), and right forceps minor and inferior fronto-occipital fasciculus ($t(69)=4.34$, $p<0.001$, $d=1.05$). Finally, two-tailed independent sample t -tests confirmed that FA in clusters identified in the voxel-wise analysis comparing concussed athletes at T1 and healthy athletes was still significantly increased at T2 (right sagittal stratum $t(67)=2.93$, $P=0.005$, $d=0.73$, bilateral superior CP $t(67)=4.14$, $P<0.001$, $d=1.03$, right retrolenticular IC

Figure 2.

ROI analyses: Shown is the average fractional anisotropy in *a priori* ROI, including the uncinate fasciculus (UF), anterior corona radiata (ACR), superior corona radiata (SCR), superior longitudinal fasciculus (SLF), cerebellar peduncles (CP), corpus callosum (CC), and the internal capsule (IC). * Indicates significant difference

between healthy athletes (HA) and concussed athletes at one day (T1), one week (T2), and one month (T3) post-concussion at $p < 0.05$. Notches indicate 95% confidence interval of the median and whiskers represent data within 1.5 interquartile range.

$t(67) = 2.67$, $P = 0.009$, $d = 0.66$, left SLF $t(67) = 3.69$, $P < 0.001$, $d = 0.92$, right SLF $t(67) = 4.13$, $P < 0.001$, $d = 1.03$, right forceps minor and inferior fronto-occipital

fasciculus $t(67) = 4.88$, $P < 0.001$, $d = 1.21$, and left posterior corona radiata $t(67) = 2.65$, $P = 0.01$, $d = 0.66$. Likewise, FA in these clusters at T3 was still significantly increased

Figure 3.

Cross-sectional voxel-wise differences: Regions where concussed athletes had significantly increased fractional anisotropy relative to healthy athletes are shown in red. The number represents the slice coordinate in the Z direction, L = left, R = right.

compared to healthy athletes (right sagittal stratum $t(64) = 4.54$, $P < 0.001$, $d = 1.17$, bilateral superior CP $t(64) = 3.88$, $P < 0.001$, $d = 1.0$, right retrolenticular IC $t(64) = 2.84$, $P = 0.006$, $d = 0.73$, left SLF $t(64) = 3.58$, $P = 0.001$, $d = 0.92$, right SLF $t(64) = 3.72$, $P < 0.001$, $d = 0.96$, right forceps minor and inferior fronto-occipital fasciculus $t(64) = 4.26$, $P < 0.001$, $d = 1.10$, and left posterior corona radiata $t(64) = 2.91$, $P = 0.005$, $d = 0.75$).

DTI Analysis–Subject-Specific

Concussed athletes at T1 had a significantly greater number of subject-specific clusters with increased FA ($t(69) = 2.22$, $P = 0.03$, $d = 0.54$) and significantly larger volume of increased FA clusters relative to HA ($t(69) = 2.32$, $P = 0.023$, $d = 0.56$; Fig. 4A). The number of clusters with decreased FA and the total volume of decreased FA clusters did not differ between groups (P 's > 0.10). Qualitative inspection of common locations of subject-specific increases in FA at T1 showed high inter-subject overlap of clusters in bilateral SLF, bilateral retrolenticular IC, left anterior limb of IC, right sagittal stratum, and the CP (Fig. 4B). The total volume of clusters with increased FA was

significantly greater at T2 relative to HA ($t(67) = 2.10$, $P = 0.039$, $d = 0.52$), and there was a non-significant trend for a greater number of clusters with increased FA at T2 relative to HA ($t(67) = 1.90$, $P = 0.06$, $d = 0.47$). The number and volume of clusters at T3 with increased FA did not differ from HA (P 's > 0.10).

Exploratory Tau Analyses

The effect of time post-concussion on plasma tau levels trended toward significance ($F(2,26) = 3.18$, $P = 0.058$; Fig. 5). Follow-up tests indicated that T1 tau was significantly lower than T2 ($P = 0.019$), while tau levels at T3 did not differ from T1 or T2 (P 's > 0.10). Cross-sectional analyses indicated a trend for higher tau levels in HA relative to concussed athletes at T1 ($t(35) = 2.01$, $P = 0.052$, $d = 0.66$), while tau levels in concussed athletes at T2 and T3 did not differ from HA (P 's > 0.10).

Relationship Between FA, Behavior, and Outcome

Exploratory Spearman correlations examined whether FA metrics at each time point post-concussion were associated with the primary behavioral measures, concussion outcome, or tau levels. FA in regions identified in the voxel-wise analysis were averaged to reduce the number of comparisons. The number of days that concussed athletes were withheld from competition was positively correlated with FA in the left SLF ROI at T1 ($\rho = 0.44$, $P = 0.02$) and T3 ($\rho = 0.52$, $P = 0.01$). None of the other correlations between behavioral outcomes or tau levels with metrics of increased FA were significant (P 's > 0.10).

DISCUSSION

White matter abnormalities are commonly observed following TBI regardless of severity [Johnson et al., 2013], and represent a potential physiological target for the development of objective biomarkers for the clinical management of SRC. In this study, concussed collegiate athletes had increased FA at one day, one week, and one month post-concussion relative to a demographically matched sample of healthy collegiate athletes. Importantly, we observed no longitudinal evidence of recovery of FA in concussed athletes, suggesting that white matter abnormalities persisted beyond the time frame of typical behavioral symptom recovery commonly reported in the literature [McCrea et al., 2003] and observed in the current sample. Furthermore, increased FA in the left SLF was associated with an independent, real-world outcome measure of concussion severity at both one day and one month post-concussion.

We observed both longitudinal and cross-sectional (i.e., versus healthy athletes) evidence of symptom recovery following SRC in the current sample. The recovery of

Figure 4.

Subject-specific abnormalities: (A) Shown is the square-root transformed volume and number of clusters with increased or decreased fractional anisotropy at the subject-level for healthy athletes (HA) and concussed athletes at one day (T1), one week (T2), and one month (T3) post-concussion. * Indicates significant difference relative to HA at $p < 0.05$, ^ indicates non-

significant trend at $p < 0.10$. Notches indicate 95% confidence interval of the median and whiskers represent data within 1.5 interquartile range. (B) Shown are anatomical regions in which at least three HA or three concussed athletes (CA) had subject-specific clusters with increased FA. The number represents the slice coordinate in the Z direction, L = left; R = right.

cognitive deficits and self-reported symptoms by one week post-concussion is consistent with previous large-scale clinical studies demonstrating that 90% of concussions recover within 7-10 days [McCrea et al., 2003]. In contrast, while scores on the mood symptom metrics showed longitudinal evidence of improvement, scores in concussed ath-

letes were still higher than controls at one month post-concussion. This suggests that the recovery of clinically assessed mood symptoms is prolonged relative to symptoms assessed using common self-report metrics and cognitive batteries, potentially as a result of more accurate assessment of symptoms [Meier et al., 2015]. However,

Figure 5.

Plasma tau levels: Shown are levels of tau in plasma of concussed athletes (CA) at one day (T1), one week (T2), and one month (T3) post-concussion, as well as in healthy athletes (HA). Notches indicate 95% confidence interval of the median and whiskers represent data within 1.5 interquartile range.

structured interviewers were not blinded to concussion diagnoses, and thus potential interviewer bias cannot be ruled out.

Contrary to our hypothesis, we observed no evidence of longitudinal changes (i.e., recovery) in FA for concussed athletes across the acute (i.e., days) to sub-acute post-concussion (i.e., weeks to one month post-injury) phase. Rather, both voxel-wise and ROI analyses indicated that concussed athletes had increased FA at all three time points relative to controls. Importantly, increased FA in the left SLF was positively correlated with the number of days that athletes were withheld from competition at multiple time points, as determined by physicians trained in sports medicine. This supports the assumption that the cross-sectional FA differences reflect pathology associated with SRC and highlights the potential utility of DTI as an objective, physiological-based biomarker.

Previous meta-analyses have demonstrated that the location of white matter abnormalities following concussion varies across studies, though several of the regions observed in the current data are commonly observed, such as the CC, IC, and SLF [Dodd et al., 2014; Eierud et al., 2014; Gardner et al., 2012]. These inconsistencies between studies are likely due to the heterogeneity of mTBI in terms of initial injury conditions (i.e., fall versus motor vehicle accident versus assault), which motivated our use of methods that identify subject-specific abnormalities. Importantly, these analyses do not rely on the assumption of spatial homogeneity of injuries and thus are similar to measures of lesion load [Mayer et al., 2014]. In the current study, concussed athletes had a greater number and larger total volume of clusters with increased FA at one day and one week post-concussion relative to healthy athletes, with common regions of subject-specific clusters including bilat-

eral SLC, IC, right sagittal stratum and CP. Although common regions were observed across athletes, subject-specific analyses may ultimately provide the most useful information for developing individualized treatment plans for concussed athletes given the heterogeneity of SRC.

The increased FA observed in concussed athletes was driven largely by a decrease in RD. Previous studies have hypothesized that this pattern may reflect cytotoxic edema because of the stretching of axons and neuroglia that occurs during mTBI [Bazarian et al., 2007; Ling et al., 2012b; Povlishock and Katz, 2005; Wilde et al., 2008]. This mechanical force potentially alters the function of gated ion channels, causing a decrease in extracellular water and an increase in intracellular water, which would lead to increased FA and decreased RD [Rosenblum, 2007]. Alternatively, gliosis has been associated with increased FA in gray matter following experimental brain injury [Budde et al., 2011; Xu et al., 2011], and could conceivably explain the increased FA in the current study.

Prolonged differences in white matter abnormalities, and specifically increased FA, have been previously reported following mTBI. For example, increased FA in the CST and CC was observed 6 months post-injury [Henry et al., 2010], and we have previously observed evidence of only partial normalization of increased FA in the CC and corona radiata (CR) at 4–5 months post-injury in emergency room samples [Ling et al., 2012b; Mayer et al., 2010]. Furthermore, cross-sectional studies have reported increased whole-brain FA [Virji-Babul et al., 2013] and increased FA in the CC and CR at approximately one-month following SRC relative to controls [Borich et al., 2013]. However, the direction of FA differences following mTBI has been inconsistent [Dodd et al., 2014; Eierud et al., 2014]. The explanation for this inconsistency could be a result of numerous factors including sample characteristics and DTI imaging parameters, such as the number of diffusion weighted images [Dodd et al., 2014].

The fact that some neurophysiological consequences of concussion may persist beyond typical symptom recovery has important implications for the clinical management of concussion, as premature return to play decisions may put athletes at additional risk for subsequent, more severe injuries. For example, there is preclinical evidence that a subsequent temporally proximate injury may result in significantly greater axonal injury and behavioral deficits compared to a single injury alone [Longhi et al., 2005; Prins et al., 2010]. However, recovery of the neurometabolic sequelae of mTBI has been documented in the time frame used in the current study [Vagnozzi et al., 2010]. Similarly, we have previously reported that abnormalities in cerebral blood flow also recover within one month post-injury [Meier et al., 2015]. Therefore, prospective, large-scale multi-modal studies are needed to track the various neurophysiological recovery profiles of the brain following SRC and to document its relationship with common post-concussion symptoms.

An exploratory goal of the current study was to explore plasma levels of tau in a subset of concussed and healthy athletes. Recently, increased peripheral levels of tau, putatively thought to reflect axonal injury, have been observed following an Olympic boxing bout and following concussion in ice hockey players relative to preseason levels [Neselius et al., 2013; Shahim et al., 2014]. In contrast, our data suggest that tau plasma levels were suppressed acutely post-concussion, with return to normal levels at one week post-injury. An initial decrease in both plasma and brain levels of tau has previously been observed at 6 hours following experimental blast TBI, with subsequent increase in tau levels at 24 hours post-injury [Arun et al., 2013]. It is unclear how these observed time-dependencies of tau translate to humans, though the aforementioned increase of plasma tau in hockey players was evident up to 6 days post-concussion relative to pre-season samples [Shahim et al., 2014]. Numerous factors could explain the inconsistency with these earlier reports, including sample characteristics, timing of blood draw, time since injury, and injury severity. Nevertheless, these results highlight the need for additional, well-controlled studies to validate the potential of tau as a biomarker for SRC.

Limitations

Concussed athletes in the current study had more prior concussions than healthy athletes. This could partially explain the cross-sectional differences observed in the current study, as previous work has shown that a history of prior concussions is a risk factor for additional, more severe concussions [Guskiewicz et al., 2003]. Second, although all primary analyses were corrected for multiple comparisons, exploratory analyses were also performed examining relationships between FA and concussion outcome. A strict family-wise error correction would be overly conservative for these exploratory analyses due to the fact that many of the investigated variables were not independent. Nevertheless, we cannot rule out of the possibility of Type I error for these analyses. Furthermore, the primary real-world outcome measure used (return-to-play decision) may be partially dependent on the competitive schedule of the athlete (i.e., temporal relationship between injury date and the next game or end of season). Thus, this measure should be considered a general approximation rather than an exact assessment of outcome. While the current work focused on FA as the main measure of white matter integrity, future studies linking FA with alternative measures of white matter abnormalities, such as FLAIR or SWI abnormalities, are needed to further elucidate the effects of SRC on white matter structure. Finally, although a well-matched sample of healthy contact athletes was included as a control group, baseline scans were not available for athletes that suffered a concussion. Future studies longitudinally tracking athletes pre-injury and at multiple time points post-injury are necessary to fully document

any changes from baseline. In addition, given the complexity and heterogeneity of mTBI, future studies with *a priori* hypotheses regarding associations between specific neuro-behavioral symptoms (e.g., processing speed) with white matter abnormalities are merited.

CONCLUSION

Current results provide cross-sectional and longitudinal evidence of increased FA in the acute and sub-acute phase post-concussion in a large sample of demographically matched collegiate athletes. Increased FA was shown to have real-world validity for predicting concussion outcome, highlighting the potential of DTI as an objective biomarker for SRC. Importantly, there was no evidence of recovery in FA one month post-injury, suggesting that additional studies are required to determine when individuals have truly recovered from concussion.

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REFERENCES

- Arun P, Abu-Taleb R, Oguntayo S, Tanaka M, Wang Y, Valiyaveetil M, Long JB, Zhang Y, Nambiar MP (2013): Distinct patterns of expression of traumatic brain injury biomarkers after blast exposure: Role of compromised cell membrane integrity. *Neurosci Lett* 552:87–91.
- Bazarian JJ, Zhong J, Blyth B, Zhu T, Kavcic V, Peterson D (2007): Diffusion tensor imaging detects clinically important axonal damage after mild traumatic brain injury: A pilot study. *J Neurotrauma* 24:1447–1459.
- Beaulieu C (2002): The basis of anisotropic water diffusion in the nervous system—A technical review. *NMR Biomed* 15:435–455.

- Borich M, Makan N, Boyd L, Virji-Babul N (2013): Combining whole-brain voxel-wise analysis with in vivo tractography of diffusion behavior after sports-related concussion in adolescents: A preliminary report. *J Neurotrauma* 30:1243–1249.
- Budde MD, Janes L, Gold E, Turtzo LC, Frank JA (2011): The contribution of gliosis to diffusion tensor anisotropy and tractography following traumatic brain injury: Validation in the rat using Fourier analysis of stained tissue sections. *Brain* 134: 2248–2260.
- Cernich A, Reeves D, Sun W, Bleiberg J (2007): Automated neuropsychological assessment metrics sports medicine battery. *Arch Clin Neuropsychol* 22: S101–S114.
- Chen G, Saad ZS, Britton JC, Pine DS, Cox RW (2013): Linear mixed-effects modeling approach to fMRI group analysis. *Neuroimage* 73:176–190.
- Cox RW (1996): AFNI: Software for analysis and visualization of functional magnetic resonance neuroimages. *Computers Biomed Res* 29:162–173.
- Dodd AB, Epstein K, Ling JM, Mayer AR (2014): Diffusion tensor imaging findings in semi-acute mild traumatic brain injury. *J Neurotrauma* 31:1235–1248.
- Eierud C, Craddock RC, Fletcher S, Aulakh M, King-Casas B, Kuehl D, LaConte SM (2014): Neuroimaging after mild traumatic brain injury: Review and meta-analysis. *NeuroImage* 4: 283–294.
- Franz G, Beer R, Kampfl A, Engelhardt K, Schmutzhard E, Ulmer H, Deisenhammer F (2003): Amyloid beta 1-42 and tau in cerebrospinal fluid after severe traumatic brain injury. *Neurology* 60:1457–1461.
- Gardner A, Kay-Lambkin F, Stanwell P, Donnelly J, Williams WH, Hiles A, Schofield P, Levi C, Jones DK (2012): A systematic review of diffusion tensor imaging findings in sports-related concussion. *J Neurotrauma* 29:2521–2538.
- Giza CC, Kutcher JS, Ashwal S, Barth J, Getchius TS, Gioia GA, Gronseth GS, Guskiewicz K, Mandel S, Manley G, McKeag DB, Thurman DJ, Zafonte R (2013): Summary of evidence-based guideline update: evaluation and management of concussion in sports: report of the Guideline Development Subcommittee of the American Academy of Neurology. *Neurology* 80:2250–2257.
- Guskiewicz KM, McCrea M, Marshall SW, Cantu RC, Randolph C, Barr W, Onate JA, Kelly JP (2003): Cumulative effects associated with recurrent concussion in collegiate football players: The NCAA Concussion Study. *Jama* 290:2549–2555.
- Henry LC, Tremblay S, Boulanger Y, Ellemberg D, Lassonde M (2010): Neurometabolic changes in the acute phase after sports concussions correlate with symptom severity. *J Neurotrauma* 27:65–76.
- Johnson VE, Stewart W, Smith DH (2013): Axonal pathology in traumatic brain injury. *Exp Neurol* 246:35–43.
- Jones TB, Bandettini PA, Kenworthy L, Case LK, Milleville SC, Martin A, Birn RM (2010): Sources of group differences in functional connectivity: an investigation applied to autism spectrum disorder. *Neuroimage* 49:401–414.
- Langlois JA, Rutland-Brown W, Wald MM (2006): The epidemiology and impact of traumatic brain injury: A brief overview. *J Head Trauma Rehabil* 21:375–378.
- Ling J, Merideth F, Caprihan A, Pena A, Teshiba T, Mayer AR (2012a): Head injury or head motion? Assessment and quantification of motion artifacts in diffusion tensor imaging studies. *Human Brain Map* 33:50–62.
- Ling JM, Pena A, Yeo RA, Merideth FL, Klimaj S, Gasparovic C, Mayer AR (2012b): Biomarkers of increased diffusion anisotropy in semi-acute mild traumatic brain injury: A longitudinal perspective. *Brain* 135:1281–1292.
- Longhi L, Saatman KE, Fujimoto S, Raghupathi R, Meaney DF, Davis J, McMillan BSA, Conte V, Laurer HL, Stein S, Stocchetti N, McIntosh TK (2005): Temporal window of vulnerability to repetitive experimental concussive brain injury. *Neurosurgery* 56:364–374; discussion 364–74.
- Mac Donald CL, Dikranian K, Bayly P, Holtzman D, Brody D (2007): Diffusion tensor imaging reliably detects experimental traumatic axonal injury and indicates approximate time of injury. *J Neurosci* 27:11869–11876.
- Mayer AR, Bedrick EJ, Ling JM, Toulouse T, Dodd A (2014): Methods for identifying subject-specific abnormalities in neuroimaging data. *Human Brain Map* 35:5457–5470.
- Mayer AR, Ling J, Mannell MV, Gasparovic C, Phillips JP, Doezenia D, Reichard R, Yeo RA (2010): A prospective diffusion tensor imaging study in mild traumatic brain injury. *Neurology* 74:643–650.
- Mayer AR, Ling JM, Yang Z, Pena A, Yeo RA, Klimaj S (2012): Diffusion abnormalities in pediatric mild traumatic brain injury. *J Neurosci* 32:17961–17969.
- McCrea M, Guskiewicz KM, Marshall SW, Barr W, Randolph C, Cantu RC, Onate JA, Yang J, Kelly JP (2003): Acute effects and recovery time following concussion in collegiate football players: the NCAA Concussion Study. *Jama* 290:2556–2563.
- McCrary P, Meeuwisse WH, Aubry M, Cantu B, Dvorak J, Echemendia RJ, Engebretsen L, Johnston K, Kutcher JS, Rafferty M, Sills A, Benson BW, Davis GA, Ellenbogen RG, Guskiewicz K, Herring SA, Iverson GL, Jordan BD, Kissick J, McCrea M, McIntosh AS, Maddocks D, Makkissi M, Purcell L, Putukian M, Schneider K, Tator CH, Turner M (2013): Consensus statement on concussion in sport: The 4th International Conference on Concussion in Sport held in Zurich, November 2012. *Br J Sports Med* 47:250–258.
- Meier TB, Bellgowan PS, Singh R, Kuplicki R, Polanski DW, Mayer AR (2015): Recovery of cerebral blood flow following sports-related concussion. *JAMA Neurol* 72:530–538.
- Mori S, van Zijl P (2007): Human white matter atlas. *Am J Psychiatry* 164:1005.
- Murugavel M, Cubon V, Putukian M, Echemendia R, Cabrera J, Osherson D, Dettwiler A (2014): A longitudinal diffusion tensor imaging study assessing white matter fiber tracts after sports-related concussion. *J Neurotrauma* 31:1860–1871.
- Neselius S, Brisby H, Theodorsson A, Blennow K, Zetterberg H, Marcusson J (2012): CSF-biomarkers in Olympic boxing: Diagnosis and effects of repetitive head trauma. *PloS One* 7:e33606.
- Neselius S, Zetterberg H, Blennow K, Randall J, Wilson D, Marcusson J, Brisby H (2013): Olympic boxing is associated with elevated levels of the neuronal protein tau in plasma. *Brain Injury* 27:425–433.
- Pasternak O, Koerte IK, Bouix S, Fredman E, Sasaki T, Mayinger M, Helmer KG, Johnson AM, Holmes JD, Forwell LA, Skopelja EN, Shenton ME, Echlin PS (2014): Hockey concussion education project. Part 2. Microstructural white matter alterations in acutely concussed ice hockey players: A longitudinal free-water MRI study. *J Neurosurgery* 120:873–881.
- Povlishock JT, Katz DI (2005): Update of neuropathology and neurological recovery after traumatic brain injury. *J Head Trauma Rehabil* 20:76–94.
- Prins ML, Hales A, Reger M, Giza CC, Hovda DA (2010): Repeat traumatic brain injury in the juvenile rat is associated with increased axonal injury and cognitive impairments. *Dev Neurosci* 32:510–518.

- Rissin DM, Kan CW, Campbell TG, Howes SC, Fournier DR, Song L, Piech T, Patel PP, Chang L, Rivnak AJ, Ferrell EP, Randall JD, Provuncher GK, Walt DR, Duffy DC (2010): Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations. *Nat Biotechnol* 28:595–599.
- Roebuck-Spencer TM, Vincent AS, Gilliland K, Johnson DR, Cooper DB (2013): Initial clinical validation of an embedded performance validity measure within the automated neuropsychological metrics (ANAM). *Arch Clin Neuropsychol* 28: 700–710.
- Rosenblum WI (2007): Cytotoxic edema: Monitoring its magnitude and contribution to brain swelling. *J Neuropathol Exp Neurol* 66:771–778.
- Shahim P, Tegner Y, Wilson DH, Randall J, Skillback T, Pazooki D, Kallberg B, Blennow K, Zetterberg H (2014): Blood biomarkers for brain injury in concussed professional ice hockey players. *JAMA Neurol* 71:684–692.
- Shenton ME, Hamoda HM, Schneiderman JS, Bouix S, Pasternak O, Rath Y, Vu MA, Purohit MP, Helmer K, Koerte I, Lin AP, Westin CF, Kikinis R, Kubicki M, Stern RA, Zafonte R (2012): A review of magnetic resonance imaging and diffusion tensor imaging findings in mild traumatic brain injury. *Brain Imaging Behav* 6:137–192.
- Singh, R, Savitz, J, Teague, TK, Polanski, DW, Mayer, AR, Bellgowan, PS, Meier, TB (2015): Mood symptoms correlate with kynurenine pathway metabolites following sports-related concussion. *J Neurol Neurosurg Psychiatry*, in press.
- Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TE, Johansen-Berg H, Bannister PR, De Luca M, Drobnjak I, Flitney DE, Niazy RK, Saunders J, Vickers J, Zhang Y, De Stefano N, Brady JM, Matthews PM (2004): Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage* 23:S208–S219.
- Spain A, Dumas S, Lifshitz J, Rhodes J, Andrews PJ, Horsburgh K, Fowler JH (2010): Mild fluid percussion injury in mice produces evolving selective axonal pathology and cognitive deficits relevant to human brain injury. *J Neurotrauma* 27: 1429–1438.
- Talairach, J., Tournoux, P. (1988) *Co-planar Stereotaxic Atlas of the Human Brain*. New York. Thieme.
- Thorne DR (2006): Throughput: A simple performance index with desirable characteristics. *Behav Res Methods* 38:569–573.
- Trojanowski JQ, Schuck T, Schmidt ML, Lee VM (1989): Distribution of tau proteins in the normal human central and peripheral nervous system. *J Histochem Cytochem* 37:209–215.
- Turken AU, Herron TJ, Kang X, O'Connor LE, Sorenson DJ, Baldo JV, Woods DL (2009): Multimodal surface-based morphometry reveals diffuse cortical atrophy in traumatic brain injury. *BMC Medical Imaging*, 9:20
- Vagnozzi R, Signoretti S, Cristofori L, Alessandrini F, Floris R, Isgro E, Ria A, Marziali S, Zoccatelli G, Tavazzi B, Del Bolgia F, Sorge R, Broglio SP, McIntosh TK, Lazzarino G (2010): Assessment of metabolic brain damage and recovery following mild traumatic brain injury: a multicentre, proton magnetic resonance spectroscopic study in concussed patients. *Brain* 133:3232–3242.
- Virji-Babul N, Borich MR, Makan N, Moore T, Frew K, Emery CA, Boyd LA (2013): Diffusion tensor imaging of sports-related concussion in adolescents. *Pediatric Neurol* 48:24–29.
- Wilde EA, McCauley SR, Hunter JV, Bigler ED, Chu Z, Wang ZJ, Hanten GR, Troyanskaya M, Yallampalli R, Li X, Chia J, Levin HS (2008): Diffusion tensor imaging of acute mild traumatic brain injury in adolescents. *Neurology* 70:948–955.
- Xu S, Zhuo J, Racz J, Shi D, Roys S, Fiskum G, Gullapalli R (2011): Early microstructural and metabolic changes following controlled cortical impact injury in rat: a magnetic resonance imaging and spectroscopy study. *J Neurotrauma* 28: 2091–2102.
- Zetterberg H, Smith DH, Blennow K (2013): Biomarkers of mild traumatic brain injury in cerebrospinal fluid and blood. *Nat Rev Neurol* 9:201–210.
- Zhang K, Johnson B, Pennell D, Ray W, Sebastianelli W, Slobounov S (2010): Are functional deficits in concussed individuals consistent with white matter structural alterations: Combined FMRI & DTI study. *Exp Brain Res* 204:57–70.
- Zhu DC, Covassin T, Nogle S, Doyle S, Russell D, Pearson RL, Monroe J, Liszewski CM, DeMarco JK, Kaufman DI (2015): A potential biomarker in sports-related concussion: Brain functional connectivity alteration of the default-mode network measured with longitudinal resting-state fMRI over thirty days. *J Neurotrauma* 32:327–341.