



The first week after concussion: Blood flow, brain function and white matter microstructure



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ARTICLE INFO

Article history:

Received 17 November 2016

Received in revised form 25 January 2017

Accepted 18 February 2017

Available online 20 February 2017

Keywords:

Concussion

MRI

Cerebral blood flow

Functional MRI

Diffusion tensor imaging

ABSTRACT

Concussion is a major health concern, associated with short-term deficits in physical function, emotion and cognition, along with negative long-term health outcomes. However, we remain in the early stages of characterizing MRI markers of concussion, particularly during the first week post-injury when symptoms are most severe. In this study, 52 varsity athletes were scanned using Magnetic Resonance Imaging (MRI), including 26 athletes with acute concussion (scanned 1–7 days post-injury) and 26 matched control athletes. A comprehensive set of functional and structural MRI measures were analyzed, including cerebral blood flow (CBF) and global functional connectivity (Gconn) of grey matter, along with fractional anisotropy (FA) and mean diffusivity (MD) of white matter. An analysis comparing acutely concussed athletes and controls showed limited evidence for reliable mean effects of acute concussion, with only MD showing spatially extensive differences between groups. We subsequently demonstrated that the number of days post-injury explained a significant proportion of inter-subject variability in MRI markers of acutely concussed athletes. Athletes scanned at early acute injury (1–3 days) had elevated CBF and Gconn and reduced FA, but those scanned at late acute injury (5–7 days) had the opposite response. In contrast, MD showed a more complex, spatially-dependent relationship with days post-injury. These novel findings highlight the variability of MRI markers during the acute phase of concussion and the critical importance of considering the acute injury time interval, which has significant implications for studies relating acute MRI data to concussion outcomes.

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1. Introduction

Concussion is defined as traumatically-induced injury leading to altered brain function, in the absence of gross structural abnormalities. It is associated with impairments in physical function, cognition and emotion regulation (McCrea et al., 2003), which resolve within 7–10 days for most adults (McCrorey et al., 2013). In the sport context, the medical guidelines for safe return-to-play (RTP) are mainly based on self-reported symptoms in response to physical exertion. However, these symptom-based guidelines only indirectly reflect the underlying

neurobiological changes that occur following a concussion. It is important to establish the acute pathophysiology of concussion, in order to better inform concussion management and safe RTP, as athletes with a history of concussion are at risk of re-injury (Abrahams et al., 2014) and have a higher risk for developing long-term cognitive impairments and depression (Guskiewicz et al., 2005; Guskiewicz et al., 2007; Gavett et al., 2011).

Magnetic Resonance Imaging (MRI) provides a versatile tool for objectively measuring brain function and structure. However, in order for MRI markers to inform clinical assessments and concussion management strategies, it is critical to establish acute post-concussion brain changes, particularly during the initial 1–7 days when most individuals are symptomatic. At present, most of our knowledge of the acute pathophysiology of concussion comes from rodent models (Giza and Hovda, 2001; Hovda et al., 1995; Giza et al., 2013). These studies have shown significant variation in metabolism, neural function and cerebral blood

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flow over the first 7–10 days, indicating that the neurobiological response to concussion is highly dependent on the time interval post-injury. While it is believed that concussion in humans shows a similar response, this has not yet been well established during the early acute phase of injury.

We are currently in the early stages of using MRI to characterize concussion pathophysiology in humans. Arterial spin labelling (ASL) has been used to measure cerebral blood flow (CBF), demonstrating reduced blood flow post-injury, but this modality has seen limited use to date (Hart et al., 2013; Meier et al., 2015). Functional MRI (fMRI) has been used to evaluate the neural activity of athletes with concussion based on fluctuations in cerebral blood-oxygenation, although these studies have mainly focused on the sub-acute interval from one week to one month post-injury (Slobounov et al., 2012; Zhang et al., 2010; Johnson et al., 2012). Despite most athletes being asymptomatic at this time, abnormalities in brain function have been identified, including increased task-related brain activity (Slobounov et al., 2012) and reduced functional connectivity in the resting brain (Johnson et al., 2012). Conversely, Diffusion tensor imaging (DTI) has been mainly used to investigate the long-term effects of concussion on the white matter microstructure in the brain. Most large-scale DTI studies have focused on retired athletes in collision sports, where long-term markers of white matter damage have been identified, including reduced fractional anisotropy (FA) and increased diffusivity within white matter tracts (Chappell et al., 2006; Zhang et al., 2006; Casson et al., 2014; Monaco and Tempel, 2015).

To date, investigations of acute concussion have been more limited, with most studies focusing on a single MRI modality. A study using arterial spin labelling (ASL) found no significant changes in cerebral blood flow (CBF) between 1 and 9 days post-injury (Meier et al., 2015), whereas a preliminary study of resting-state functional MRI (fMRI) found significant within-subject decreases in functional connectivity from 1 to 7 days post-injury (Zhu et al., 2015). In addition, a DTI-based study reported increasing FA and decreasing radial diffusivity in white matter tracts between 2 and 14 days post-injury, potentially indicating recovery from neural injury (Murugavel et al., 2014). These studies have shown significant within-subject brain changes at fixed time intervals during the acute phase of injury.

The present study extends these findings by examining multiple different MRI brain measures within a single group with acute concussion, including ASL, fMRI and DTI. This was investigated in a balanced sample of male and female athletes from a variety of different sports, to ensure that the findings are applicable to the wider sporting community. Given the limited literature on the acute phase of sport concussion, we first examined whether there are reliable differences in MRI markers of acutely concussed athletes relative to a group of control athletes, matched on age, sex and prior concussion history. Subsequently, motivated by the animal modelling literature, we investigated whether regressing MRI measures against the number of days post-injury at which they were acquired shows reliable effects among athletes with concussion and helps to account for inter-subject variability. The results of these analyses have significant implications for future concussion research, as they compare the sensitivity of different MRI markers during the first 7 days of injury, within a single well-characterized cohort.

2. Materials and methods

2.1. Study participants

A total of fifty-two (52) athletes were recruited for the current study, including 26 with acute concussion and 26 matched controls. Study participants were recruited from seven varsity teams at the University of Toronto (volleyball, hockey, soccer, football, rugby, basketball and lacrosse), via the institution's Sport Medicine Clinic. Twenty-six (26) athletes were recruited following a diagnosis of acute concussion by the referring physician, and scanned within 1–7 days post-injury. In

accordance with consensus guidelines (McCrory et al., 2013), athletes diagnosed with concussion were instructed to avoid physical exertion at this time, but were not otherwise restricted from daily activities. Following recruitment, athletes were evaluated using the sport concussion assessment tool 3 (SCAT3) (Guskiewicz et al., 2013), a standardized clinical tool which is used to evaluate symptoms, perform cognitive testing and assesses balance. For this study, we reported the total number of symptoms endorsed and total symptom severity (the sum of all symptom severity scores). We also reported scores from cognitive testing based on the standardized assessment of concussion (SAC) (McCrea et al., 1997), and balance based on the Balance Error Scoring System (BESS) (Guskiewicz, 2011). All SCAT3 scores were compared to pre-season baseline scores, which were collected from all athletes as part of the varsity sport concussion program.

Demographic information was collected, along with relevant clinical history, and each athlete with concussion was matched to a control athlete with no documented concussion in the 6 months prior to scanning. Controls were individually matched on sex and prior number of concussions, as multiple concussions are associated with long-term consequences (McCrory et al., 2013) and differences in brain function (Johnson et al., 2012). They were also matched on age (mean difference \pm standard deviation: 0.1 ± 1.0 yrs.; $p = 0.39$, paired Wilcoxon test) to minimize potential developmental differences. The pre-season SCAT3 scores for controls were also collected, for comparison with the acutely concussed athletes. The study procedures were approved by the University of Toronto and St. Michael's Hospital institutional review boards, and all study participants provided written informed consent.

2.2. Magnetic resonance imaging

Participants were imaged at St. Michael's Hospital using an MRI system operating at 3 Tesla (Magnetom Skyra, Siemens, Erlangen, Germany) and standard 20-channel head receiver coil. In this study, Cerebral blood flow (CBF) was evaluated using Arterial Spin Labelling (ASL), which provided a voxel-wise map of resting CBF, measured in mL/100 g/min. Brain function was evaluated using resting-state fMRI. Global functional connectivity (Gconn) was estimated at each voxel by computing the functional connectivity with all other brain voxels, based on the Pearson correlation between voxel time series. Gconn was measured as the mean of all (positive) connectivity values. This is a well-established measure that quantifies total integrative function (Cole et al., 2012; Rubinov and Sporns, 2010). White matter microstructure was assessed using a DTI sequence followed by calculation of voxel-wise fractional anisotropy (FA), which reflects the degree that water diffusion has a "preferred direction", and mean diffusivity (MD), which reflects the overall rate of water diffusion. These measures are sensitive to local alterations in the cellular environment, including damage to cell membrane integrity and differences in axonal packing density. Structural brain imaging included a T1-weighted Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) sequence. To screen for structural abnormalities, participants were also imaged with fluid attenuated inversion recovery imaging (FLAIR) and susceptibility-weighted imaging (SWI). Structural scans were reviewed in a 2-step procedure: (1) images were visually inspected by an MRI technologist and subsequently reviewed by a neuroradiologist and reported, if abnormalities were identified; (2) statistical testing for was performed, by obtaining global statistics on masked brain images (mean, variance and skew), generating a Z-score per concussed athlete relative to the control distribution, and identifying individuals significantly different at $p < 0.05$. No abnormalities (e.g., white matter hyper-intensities, contusions, micro-hemorrhage, or statistical outliers) were found for concussed athletes in this study.

Details of the MRI sequences and preprocessing steps are provided below.

2.2.1. Structural imaging

T1-weighted MPRAGE was obtained, with field-of-view (FOV) = 24×24 cm, $240 \times 240 \times 192$ acquisition matrix, 0.9 mm isotropic voxels, bandwidth = 250 Hz/px, inversion time (TI)/echo time (TE)/repetition time (TR) = 850/2.63/2000 ms, flip angle (FA) = 8°). FLAIR was obtained with FOV = 22×18.6 cm, 256×196 acquisition matrix, $1.1 \times 0.9 \times 3.0$ mm voxels, TI/TE/TR = 2200/96/9000 ms. SWI was also obtained, with 220×192 FOV, $0.6 \times 0.6 \times 1.2$ mm voxels. TR/TE 28/20 ms, FA = 15° , 384×307 with an encoding gap of 0.2 mm.

2.2.2. Cerebral blood flow

The pulsed ASL PICORE QUIPSS II sequence was used to acquire perfusion-weighted imaging (FOV = 25.6×25.6 cm; 64×64 matrix; 14 slices; $4 \times 4 \times 8$ mm voxels; T1/TI1s/TI2/TE/TR = 700/1600/1800/12/2500 ms, flip angle = 90°). An equilibrium magnetization image M0 was acquired, followed by forty-five (Liang et al., 2013) tag-control image pairs, while patients were instructed to lie still, with eyes closed, and not focus on anything in particular. Data were processed and analyzed via the ASLtbx package (cfp.upenn.edu/~zewang/ASLtbx.php) and custom algorithms developed in the laboratory. This included rigid-body motion correction with tag-control effects regressed out and spatial smoothing with a 6 mm isotropic 3D Gaussian kernel. Voxel-wise CBF estimates were calculated based on the mean difference over all tag-control pairs; see supplementary Text S1 for full details of the kinetic model parameters used to estimate CBF. Co-registration of CBF images was obtained by computing (1) the rigid-body transform of each participant's mean functional volume to their T1 anatomical image, and (2) the 12-parameter affine transformation of their T1 image to the MNI152 template. These transformation matrices were concatenated and the net transform applied to all functional data, resampled at 2 mm^3 resolution. Analyses were restricted to a mask of regions with grey matter likelihood >0.33 (i.e. greater than chance probability of being grey matter tissue), based on the probabilistic segmentation of T1 images using the FMRIB Software Library (FSL) *fast* algorithm (fsl.fmrib.ox.ac.uk).

2.2.3. Brain function

Resting-state fMRI was acquired via multi-slice T2*-weighted echo planar imaging (FOV = 20×20 cm, 64×64 matrix, 32 slices, $3.125 \times 3.125 \times 4.5$ mm voxels, TE/TR = 30/2000 ms, flip angle = 70° , oblique axial interleaved), producing a time-series of 194 images. During acquisition, participants were instructed to lie still with their eyes closed, and not focus on anything in particular. Processing and analysis were performed using the Analysis of Functional Neuroimages (AFNI) package (afni.nimh.nih.gov) and customized algorithms developed in the laboratory. This included rigid-body motion correction (AFNI *3dvolreg*), removal of outlier scan volumes (nitrc.org/projects/spikecor), slice-timing correction (AFNI *3dTshift*), spatial smoothing with a 6 mm Full Width at Half Maximum (FWHM) isotropic 3D Gaussian kernel (AFNI *3dmerge*) and regression of motion parameters and linear-quadratic trends as nuisance covariates. To control for physiological noise, data-driven correction was performed (nitrc.org/projects/physca_plus), along with regression of white matter signal, using the FSL *fast* algorithm to segment the T1 anatomical scan and regress out mean signal in white matter voxels ($p > 0.95$). Co-registration of fMRI data was obtained by computing the rigid-body transform of the mean fMRI volume for each participant to their T1-weighted anatomical image, and the 12-parameter affine transformation of the T1 image for each participant to the MNI152 template. The transformation matrices were concatenated and the net transform applied to fMRI data, resampled at $2 \times 2 \times 2 \text{ mm}^3$ resolution.

2.2.4. White matter microstructure

Diffusion-weighted imaging was based on 30-directions encoding ($b = 700 \text{ s/mm}^2$, FOV = 24×24 cm, 120×120 acquisition matrix, 66 axial slices, 2 mm isotropic voxels, TE/TR = 83/7800 ms). The FSL

eddy_correct protocol was used to perform simultaneous correction of eddy currents and rigid-body motion correction, *bet* was used to mask out non-brain voxels, and *dtifit* was used to calculate voxel-wise FA and MD. Co-registration of Diffusion-weighted brain maps was based on the FSL FDT protocol: (1) masked subject FA maps were eroded by 1 vx width at brain edges, and co-registered to the FMRIB58 template (1 mm^3) via affine transform, using *flirt*; (2) a symmetric, study-specific template was computed by averaging transformed FA maps, then re-averaging with flipped left/right orientations; (3) the average template was used as a reference and non-linear registration of FA maps performed using *fnirt*, which were used to update the study-specific template; (4) the FA maps were registered to the new template via *fnirt* and the mean template was updated again. During the final registration step, images were resampled to 2 mm^3 resolution, and prior to analysis all images were convolved with a 6 mm FWHM 3D Gaussian smoothing kernel to minimize the effects of local variation in white matter structure. All analyses were performed within a mask of regions with a mean FA > 0.25 , in order to restrict analyses to white matter tracts.

2.3. Clinical and demographic data

We tested for significant changes in SCAT3 symptom, SAC and BESS subscales following acute concussion, relative to both baseline SCAT3 scores and the SCAT3 scores of matched controls, by performing non-parametric paired Wilcoxon tests. Significance was reported after correcting for multiple comparisons at a False Discovery Rate of 0.05, along with the mean difference in scores between groups and bootstrapped standard error values (based on 1000 resampling iterations).

2.4. Neuroimaging data: effects of acute concussion

For each MRI measure (CBF, Gconn, FA, MD), we tested for a consistent mean effect of acute concussion. We computed the mean difference in voxel-wise MRI values of acutely concussed athletes relative to their matched controls, in a bootstrapped resampling framework. This non-parametric approach avoids any distributional assumptions about the MRI data being analyzed, and is robust to the influence of outliers. Significance was evaluated by bootstrap resampling on the mean of paired differences (1000 resampling iterations) and reporting brain regions where the bootstrapped 95% CIs did not enclose zero effect. The confidence bounds were adjusted for multiple comparisons as follows: we thresholded voxels for which the 99.5% CIs did not enclose zero (corresponding to $p = 0.005$ 2-tailed significance) and performed cluster-size thresholding at $p = 0.05$, by using AFNI *3dFWHMx* to estimate spatial smoothness of each MRI dataset and using these values as inputs to AFNI *3dClustSim* to estimate the minimum cluster-size threshold. For significant brain voxels, the effect size was reported in terms of the bootstrap ratio, defined as the bootstrapped mean divided by the standard error.

2.5. Neuroimaging data: effects of days post-injury

We then examined whether the inclusion of days post-injury as a predictor significantly accounted for inter-subject variability among the acutely concussed athletes. We defined "days post-injury" as the number of days from the injury date to the MRI scan date for each athlete. Analysis was done using ordinary least squares linear regression, where MRI values were regressed against days post-injury for each brain voxel. We tested for a reliable direction of effect by computing the adjusted 95% CIs on standardized regression coefficients as described in the previous section, and retained voxels where the confidence bounds did not enclose zero effect. Significant brain regions were plotted for each MRI measure, with the effect size reported in terms of bootstrap ratios. In addition, we compared the proportion of variance explained by days post-injury for each MRI measure: for each

subject, we computed the average MRI value over all significant voxels and fit a linear regression to this distribution. We then computed the R^2 coefficient of determination, along with bootstrapped 95% CIs. For comparison with uninjured athletes, we also computed the mean MRI values across significant voxels for controls and reported the normal 90% and 95% CIs.

To further validate the effects of days post-injury, we examined two potential confounds associated with clinical history: acute symptoms and prior concussions. The brain may be affected by post-concussion symptoms, which tend to dissipate over the first week post-injury (McCroory et al., 2013) and prior literature has shown that multiple concussions are associated with greater neurobiological and clinical consequences at early injury (Huh et al., 2007; Guskiewicz et al., 2003). As a test of these potential confounds, we compared regression coefficients of days post-injury, when regressed against (1) MRI data (i.e., as described in the above paragraph) and (2) MRI data, after first regressing out total symptoms and number of prior concussions (i.e., analyzing the residuals). We tested for a significant difference in the effects of days post-injury between (1) and (2) by bootstrap resampling (1000 iterations) and measuring the voxel-wise difference in regression coefficient weights for each resample. We then computed the adjusted 95% CIs on the paired differences, as described in the previous sections, and reported voxels where confidence bounds did not enclose zero effect.

2.6. Neuroimaging data: overlap between MRI measures

We also examined the overlap between different MRI measures, focusing on the identified relationships with days post-injury. We reported areas of spatial overlap between grey matter measures (CBF and Gconn) and between white matter measures (FA and MD) for voxels significantly associated with days post-injury, and quantified overlap based on the Jaccard index (the intersection/union of significant voxels). In addition, we tested whether overlap was significantly greater than chance, based on a permuted null distribution. For the two thresholded images, voxel indices were randomly exchanged and Jaccard overlap measured (5000 iterations). An empirical p -value was then obtained, as the fraction of permuted samples where the overlap value exceeded the un-permuted overlap value.

In addition, we reported concussed individuals where the mean value for at least one MRI measure fell outside the 95% CI range of normal healthy controls. For these individuals, we reported the Z-scored statistics for all 4 MRI measures, relative to the distribution of control values. We subsequently tested whether demographics of this subgroup differed from concussed athletes that were not significantly different from controls. Non-parametric Wilcoxon tests were conducted on all of the demographic and clinical factors: age, sex, prior concussions, total symptoms, symptom severity, adjusted for multiple comparisons at a False Discovery Rate of 0.05.

3. Results

3.1. Clinical and demographic data

Table 1 summarizes demographic and clinical data for athletes with acute concussion (pre- and post-injury) and their matched controls; for a breakdown of individual sport representations, see supplementary Table S1. Only a single athlete self-reported as asymptomatic at acute injury (Total Symptoms = 0), with all other athletes reporting Total Symptoms (and Symptom Severity) of 2 or higher. Relative to their baseline scores, acutely injured athletes showed significantly higher Total Symptoms (mean increase \pm standard error: 4.9 ± 1.4 ; $p = 0.002$) and Symptom Severity scores (mean increase: 13.8 ± 5.3 ; $p = 0.004$). Relative to matched controls, we also observed significantly higher Total Symptoms (mean increase: 6.6 ± 1.5 ; $p < 0.001$) and Symptom Severity scores (mean increase: 16.3 ± 4.9 ; $p < 0.001$). All effects remained significant after correcting for multiple comparisons at a

Table 1

Demographic and clinical data for acute concussed athletes and matched controls. All statistics are reported as the median [min, max]. *** indicates a significant difference in symptom scores post-injury, relative to both baseline data and matched controls.

	Concussed (n = 26)	Controls (n = 26)
Age (mean \pm SD)	20 [17, 24]	20 [18, 23]
Female	14 (54%)	14 (54%)
Prior concussions	1 [0, 4]	1 [0, 3]
Days post-injury (scanned)	4 [1, 7]	–
SCAT3 (baseline)		
Total symptoms	3 [0, 13]	2 [0, 10]
Symptom severity	3 [0, 24]	2 [0, 13]
Orientation	5 [4, 5]	5 [4, 5]
Immediate memory	15 [13, 15]	15 [10, 15]
Concentration	4 [2, 5]	3 [1, 5]
Delayed memory	5 [0, 5]	4 [1, 5]
BESS total errors	3 [0, 11]	2 [0, 10]
SCAT3 (post-injury)		
Total symptoms	7 [0, 22] **	–
Symptom severity	9 [0, 90] **	–
Orientation	5 [4, 5]	–
Immediate memory	15 [13, 15]	–
Concentration	4 [2, 4]	–
Delayed memory	4 [1, 5]	–
BESS total errors	3 [0, 9]	–

False Discovery Rate (FDR) of 0.05. For acute injury, SAC and BESS subscales showed no significant differences relative to baseline or matched controls ($p > 0.23$, all tests). The SCAT3 symptom scores were not significantly correlated with number of days post-injury (Total Symptoms: $\rho = -0.15$, $p = 0.48$; Symptom Severity: $\rho = -0.19$, $p = 0.31$), indicating that they are unlikely to confound analyses of the effects of days post-injury.

3.2. Neuroimaging data: effects of acute concussion

Fig. 1 plots brain regions that are significantly different for acutely concussed athletes relative to controls. For most of the MRI measures, there is limited evidence a reliable mean effect of acute concussion. For CBF, no significant differences were seen, whereas for Gconn, only the left inferior frontal lobe showed significantly elevated global connectivity among concussed athletes (mean difference: 0.038 ± 0.009). Among white matter measures, FA also showed limited effects, with a significant reduction in a single cluster on the superior corona radiata (mean difference: -0.022 ± 0.004). Only MD showed widespread effects of acute concussion in white matter, with increased diffusivity mainly in bilateral longitudinal fasciculi and left-lateralized superior corona radiata (mean difference: $(0.23 \pm 0.06) \times 10^{-4}$ mm/s). Thus, out of the four MRI measures, diffusivity appears to be the most robust marker for the presence of acute concussion.

3.3. Neuroimaging data: effects of days post-injury

Given the lack of consistent mean effects of acute concussion, we subsequently examined whether the number of days post-injury reliably accounted for inter-subject variability of athletes with concussion. Fig. 2 depicts the results for grey matter measures of CBF and Gconn, while Fig. 3 depicts results for white matter measures of FA and MD.

For CBF, Fig. 2A shows widespread frontotemporal effects of days post-injury, with most spatially extensive clusters in bilateral middle frontal and superior frontal lobes, extending ventrally into orbitofrontal and superior temporal regions. Significant effects are also seen in sub-cortical regions, including bilateral middle cingulate, thalamus and hippocampus. For all significant brain regions, the bootstrap ratio values are negative, indicating that CBF reliably decreases as a function of days post-injury. The plot in Fig. 2B shows the linear fit of this trend

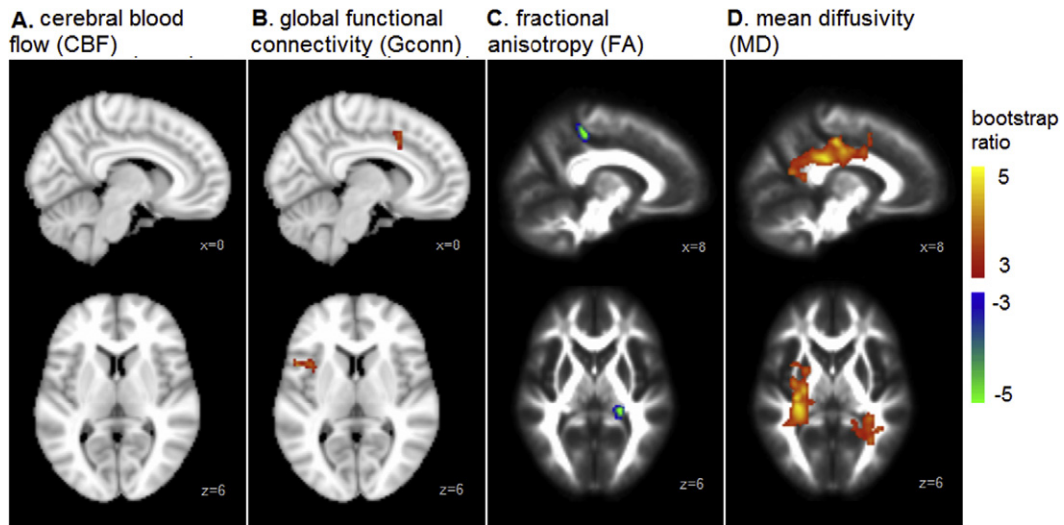


Fig. 1. Brain regions where there is a mean difference in MRI markers between acutely concussed athletes and matched controls. Only mean diffusivity (panel D.) shows widespread, reliable differences for athletes with acute concussion. Significant regions have Bootstrapped 95% CIs on that do not overlap zero (adjusted for multiple comparisons); the effect sizes in significant voxels are reported as bootstrap ratios. Brain maps are shown as maximum intensity projections (MIPs) in each imaging plane, centered on MNI coordinates ($x = 8, z = 6$).

averaged over all significant voxels, where CBF is elevated relative to the mean of the controls (solid grey line) at 1–3 days post-injury, and decreased at 5–7 days post-injury. A relatively high amount of variance

is explained by days post-injury, with mean $R^2 = 0.611$ (95% CI: 0.335 to 0.809). However, for the majority of athletes with concussion, mean CBF falls within 95% CI bounds on controls (outer dashed grey lines),

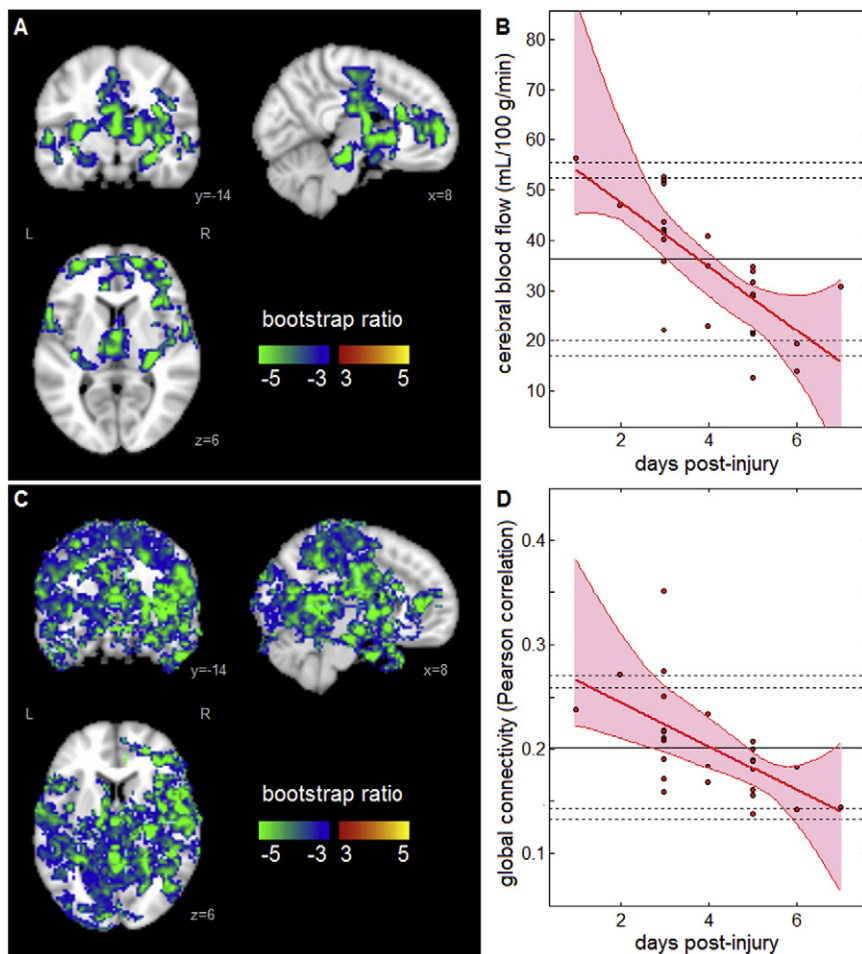


Fig. 2. Brain regions where grey matter measures of CBF (panels A-B) and Gconn (panels C-D) are associated with days post-injury. (A,C) show voxels with significant regression coefficients, with effect sizes reported as bootstrap ratios. Brain maps are displayed as maximum intensity projections (MIPs) in each imaging plane, centered on MNI coordinates ($x = 8, y = -14, z = 6$). Both MRI measures show consistent negative association with days post-injury. (B,D) show values for individual acutely concussed athletes (averaged over significant voxels), with a linear fit (heavy red line) and 95% CIs (pink bands). For comparison purposes, the distribution for control athletes is also shown, including the mean (solid grey line), along with 90% and 95% normal confidence bounds (dashed grey lines).

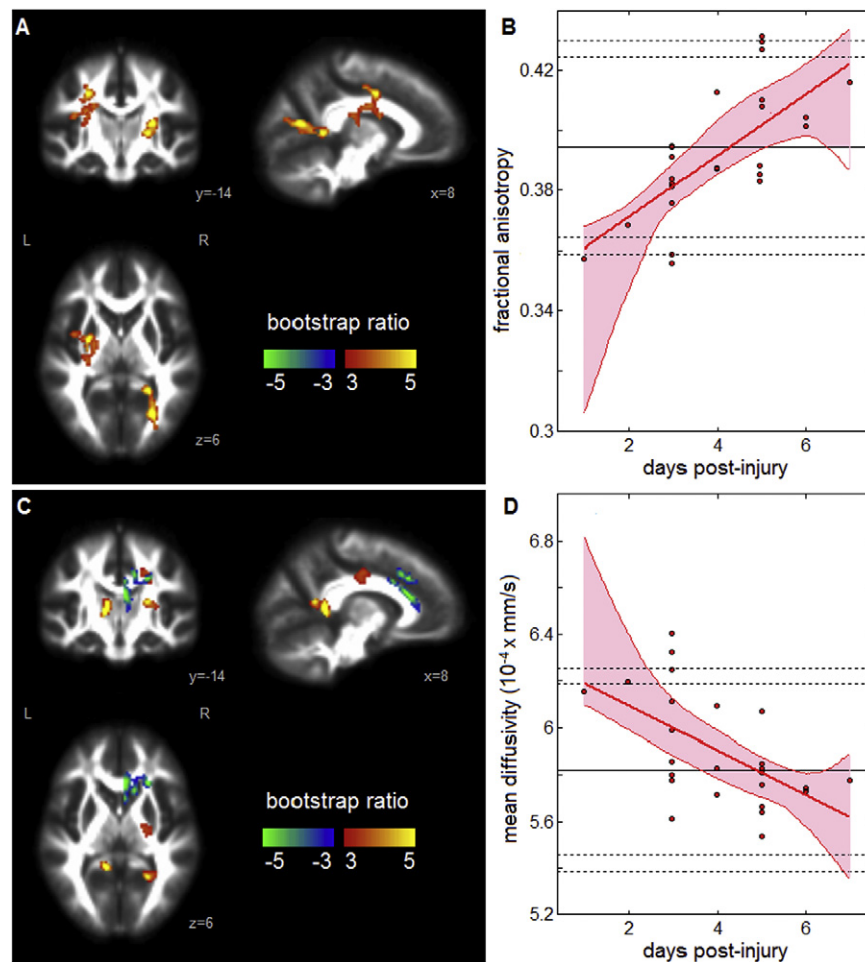


Fig. 3. Brain regions where white matter measures of FA (panels A–B) and MD (panels C–D) are associated with days post-injury. (A,C) show voxels with significant regression coefficients, with effect sizes reported as bootstrap ratios. Brain maps are displayed as maximum intensity projections (MIPs) in each imaging plane, centered on MNI coordinates ($x = 8, y = -14, z = 6$). Both MRI measures show significant association with days post-injury, although FA is uniformly positive while MD shows spatially-dependent effects. (B,D) show values for individual acutely concussed athletes (averaged over significant voxels), with a linear fit (heavy red line) and 95% CIs (pink bands). For comparison purposes, the distribution for control athletes is also shown, including the mean (solid grey line), along with 90% and 95% normal confidence bounds (dashed grey lines). Note that for MD, while panel (C) shows clusters with positive and negative bootstrap ratios, panel (D) only plots mean values of the clusters with negative bootstrap ratio values.

indicating that the effects of acute concussion remain within the range of normal healthy CBF variability.

For Gconn, Fig. 2C also shows significant effects of days post-injury throughout the brain, which are far more spatially extensive than those observed for CBF (Fig. 2A). Similar to CBF, Gconn shows significant frontotemporal clusters, including left middle frontal and bilateral middle temporal lobes. However, clusters are also seen in regions associated with visual attention, including inferior parietal lobes, precuneus and primary visual cortex, along with sensorimotor regions including bilateral postcentral gyri, supplementary motor area and paracentral lobule. Subcortical clusters are also seen, including insula, middle cingulum and hippocampal gyri. For Gconn, the bootstrap ratio values are consistently negative, indicating a uniformly negative association between global functional connectivity and number of days post-injury. The plot in Fig. 2D shows the linear fit of this trend, where Gconn is elevated relative to the mean of the controls at 1–3 days post-injury, and reduced at 5–7 days post-injury. Compared to CBF, a lower proportion of variance is explained by days post-injury, with mean $R^2 = 0.301$ (95% CI: 0.126 to 0.589). Similar to CBF, the Gconn of most athletes with acute concussion falls within the 95% CI on controls (outer dashed grey lines), indicating that this effect is generally within the range of normal healthy Gconn variability.

For FA, Fig. 3A shows significant effects of days post-injury throughout white matter, including clusters in the left superior corona radiata

and longitudinal fasciculus, along with the right-side juncture of the splenium of the corpus callosum and posterior thalamic radiation. Contrary to functional imaging, bootstrap ratio values are consistently positive, indicating that FA increases as a function of days post-injury. The plot in Fig. 3B shows the linear fit of this trend, with FA reduced relative to the mean of the controls at 1–3 days post-injury, and elevated at 5–7 days post-injury. Compared to functional brain measures, the proportion of variance explained by days post-injury is intermediate between CBF and Gconn, with mean $R^2 = 0.462$ (95% CI: 0.216 to 0.672).

For MD, Fig. 3C also shows significant effects of days post-injury. Unlike all other measures, we do not observe a consistent direction of effect (i.e., uniformly positive or negative bootstrap ratios) in significant clusters. This indicates more complex, spatially-dependent effects of days post-injury for MD. A frontal cluster shows negative association with days post-injury in the genu of the corpus callosum and right anterior corona radiata. Conversely, a posterior cluster shows positive association with days post-injury on the right-side splenium of the corpus callosum and juncture with the posterior thalamic radiation, which is spatially overlapped with effects seen with FA. Compared to FA, a lower proportion of variance is explained by days post-injury, with mean $R^2 = 0.356$ (95% CI: 0.117 to 0.586) for regions with negative bootstrap ratio values. As with the functional imaging, the FA and MD of most athletes with acute concussion falls within the 95% CI bounds

on controls, indicating that effects are within the range of normal healthy variability.

We also tested for confounding effects of acute symptoms and number of prior concussions, by examining whether controlling for these covariates significantly altered regression coefficient of days post-injury. After cluster-size correction, this had a non-significant effect for all of the MRI measures. Thus, the effects of day post-injury do not appear to be significantly confounded by these clinical factors.

3.4. Neuroimaging data: overlap between MRI measures

In Fig. 4, overlapping brain regions associated with days post-injury are shown for grey matter measures (CBF and Gconn; Fig. 4A) and white matter measures (FA and MD; Fig. 4B). Both plots show relatively limited overlap, with 4.0% for grey matter and 1.1% for white matter. Nonetheless, these overlap values are significantly greater than chance ($p < 0.001$ for both, permuted null distributions). The regions of spatial overlap between CBF and Gconn include right hippocampal gyrus, insula and middle frontal gyrus, along with left-side middle cingulate and supplementary motor area. For FA and MD, spatial overlap is seen exclusively in the right posterior thalamic radiation.

Table 2 summarizes the z-scored magnitude of individual subject deviations from controls, for concussed athletes outside the 95% confidence intervals of the mean MRI values reported in Fig. 2(B,D) and 3(B,D). All athletes in the table show a significant z-score for at least 1 MRI measure. In total, 8/26 (31%) of subjects were significantly different from controls. However, there is limited agreement between modalities: only 2/8 subjects significantly deviate from controls in >1 MRI measure. Comparing these 8 athletes and the remaining 18 concussed athletes, no significant differences were seen in age, sex, number of prior concussions, total symptoms or symptom severity ($p > 0.176$ uncorrected, for all tests).

4. Discussion

This study examined multiple different MRI-based measures of brain function and structure for athletes with acute concussion, and their individually matched controls. To our knowledge, this is the first study to measure cerebral blood flow, brain function and white matter microstructure within the first week post-injury, for a single athlete group. In addition, this study specifically examined a mixed sample of male and female athletes and multiple different sports, in order to identify brain measures that generalize within the sporting community.

Table 2

Individuals where at least one mean MRI value is significantly different from controls, based on the 95% confidence bounds in Fig. 2(B,D) and Fig. 3(B,D). For each MRI measure, we report the z-scored statistic relative to the control distribution. *** indicates significant difference from controls at $p < 0.05$.

Subject	CBF	Gconn	FA	MD
1	1.60	0.46	-2.09**	2.62**
2	1.66	-0.63	-0.73	2.26**
3	-2.26**	-0.41	0.54	-0.33
4	2.04**	1.04	-2.03**	1.51
5	0.57	4.48**	-1.01	0.17
6	0.61	2.08**	-1.93	1.92
7	-1.47	-0.28	2.03**	-0.81
8	-2.38**	-0.59	-0.84	1.12

4.1. Neuroimaging data: effects of acute concussion

Although concussion is characterized as a disturbance in brain function (McCroary et al., 2013), we observed limited evidence of reliable differences in functional connectivity relative to controls, and no reliable differences in CBF, while the DTI findings depended on the imaging measure of interest. These results reflect the variability of the human brain's response to concussion, particularly during the acute interval when symptoms are most severe. These neuroimaging results are consistent with the heterogeneity of clinical presentation for acutely concussed athletes, who are highly variable in symptom manifestation, and in the severity of cognitive and functional deficits (McCrea et al., 2003; Collie et al., 2006).

Our findings are supported by prior MRI literature showing non-significant or inconsistent variations in function and structure during early injury. For example, significant alterations in functional connectivity have been observed in some studies (Johnson et al., 2012; Zhu et al., 2015) but not in others (Zhang et al., 2012). Similarly, reduced FA and increased mean diffusivity have been reported in acute injury (Murugavel et al., 2014; Chamard et al., 2013), whereas another study reported the opposite trend (Sasaki et al., 2014). This is also consistent with animal modelling studies that found significant variations in the brain following mild traumatic brain injury, depending on the time post-injury that they were assessed (Giza and Hovda, 2001; Giza and Hovda, 2014). As a whole, these results depict the brain response to acute concussion as a highly time- and subject-dependent process,

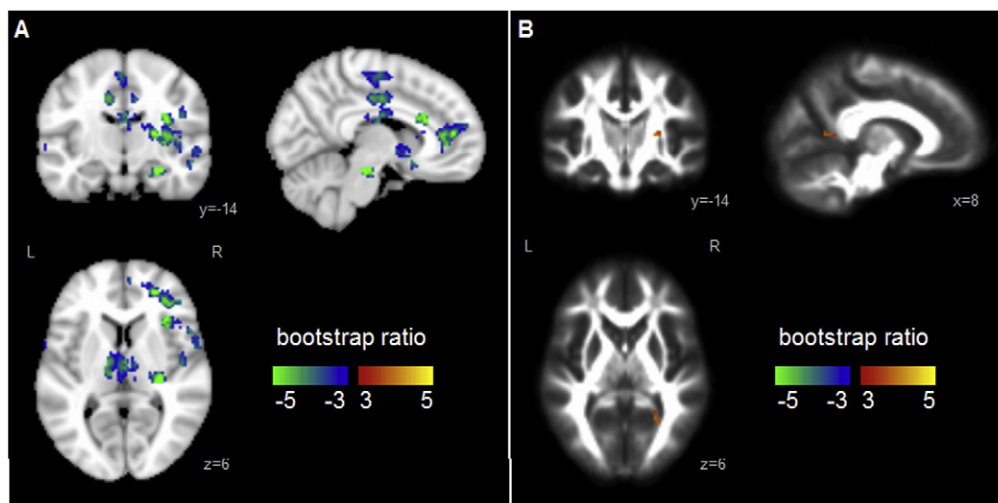


Fig. 4. Spatial overlap between different MRI measures associated with days post-injury. (A) overlapped regions for grey-matter measures CBF and Gconn that are significant in Fig. 2. (B) overlapped regions for white-matter measures FA and MD that are significant in Fig. 3. Effect sizes are reported in terms of mean bootstrap ratio between modalities. The brain maps are displayed as maximum intensity projections (MIPs) in each imaging plane, centered on the MNI coordinates ($x = 8, y = -14, z = 6$).

and emphasize the need to better understand this highly variable cohort.

For fMRI, the sole reliable effect mean effect of concussion was observed in the inferior frontal lobe, indicating that this brain area may be highly sensitive to post-injury changes in brain function. This is of particular interest, given that the inferior frontal lobe plays an important role in aspects of cognitive control, including task switching and response inhibition (Aron et al., 2004; Derrfuss et al., 2005). Impairments in inhibitory motor function have been associated with a history of concussion (De Beaumont et al., 2007), and thus inferior frontal hyper-connectivity may represent an early marker of this process. The elevation in inferior frontal global connectivity is also consistent with models that describe hyper-connectivity as a general marker of neurological insult, e.g., for traumatic brain injury, stroke and age-related decline (Hillary et al., 2015).

In white matter, FA also showed limited group effects of acute injury, with the sole significant region indicating reduced FA in concussed athletes. While FA is generally considered a non-specific marker of altered microstructure, reduced FA is seen in studies of more severe repetitive TBI (Eierud et al., 2014) where it is usually interpreted as a marker of disrupted axonal integrity. The absence of extensive mean decreases in FA suggests limited persistent axonal damage in this cohort of athletes with concussion, as expected. Conversely, MD shows widespread mean effects of acute concussion, and is consistently elevated in the white matter tracts of concussed athletes. Since MD is typically interpreted as a marker of cellular edema, caused by osmotic imbalance and sterile inflammation (Toledo et al., 2012), these findings indicate that this is one of the more reliable sequela of sport concussion, irrespective of time post-injury.

4.2. Neuroimaging data: effects of days post-injury

In a second set of analyses, we examined a potential cause of the lack of reliable differences between concussed athletes and controls. We demonstrated that the number of days post-injury was a predictor that significantly explained inter-subject variability among acutely concussed athletes. Moreover, we found no evidence that the effects were significantly confounded by total acute symptoms or number of prior concussions. This was demonstrated for all examined MRI measures, indicating that these effects should be considered for future analysis of any MRI-based brain biomarker at acute injury. These findings provide encouraging evidence that the limited significance of acute concussion effects may be explained by experimental and demographic factors.

Both of the functional grey matter measures (CBF and Gconn) showed negative associations with the number of days post-injury. Acute concussion was associated with altered CBF and Gconn in the frontal and temporal lobes, which are most vulnerable to impacting the skull during concussion (Graham et al., 2002), with CBF showing the greatest effect sizes (i.e., highest bootstrap ratio values). Thus, vascular function may be highly sensitive to direct impact, as changes in CBF potentially reflect early local inflammatory response, or adaptive auto-regulatory response in order to reduce the risk of micro-hemorrhage (Len and Neary, 2011). Conversely, Gconn showed more widespread effects of acute concussion in the visual and sensorimotor cortices. Because these regions are less vulnerable to skull impact, and show less extensive alterations in CBF, this may reflect reorganization of functional connectivity as adaptation to injury, rather than direct effects of biomechanical impact. Global connectivity in these regions may also be particularly sensitive to functional reorganization because of their intrinsically lower functional integration (Cole et al., 2012). The affected brain regions are consistent with common acute symptom complaints, which often include impaired visual-motor function (Guskiewicz et al., 2001). Thus, the functional connectivity of these regions may be an important biomarker for future study – particularly as proper functioning of these domains is required for athletes to

avoid repeat injury and the negative sequelae associated with multiple concussions (Guskiewicz et al., 2003).

For both CBF and Gconn, we also observed effects in subcortical regions that are not directly exposed to impacts with the skull, particularly within the limbic cortex, including cingulum, insula and hippocampal gyri. This is highly relevant to concussion, given that these brain regions are implicated in autonomic regulation and emotion processing. These domains are common acute symptom complaints (McCrea et al., 2003) and persistent emotional dysregulation is associated with a history of repeated head trauma (Guskiewicz et al., 2007). Hence, there may also be early markers of blood flow and brain function related to these dysfunctions.

For white matter measures, FA showed consistent positive associations between the MRI marker and number of days post-injury, within the first week. This maps onto our understanding of microstructural changes following brain injury, as damage to membrane integrity and cytotoxic edema are thought to reduce FA (Toledo et al., 2012), while brain recovery is expected to restore these values. Moreover, it is consistent with a meta-analysis of TBI studies, which concluded that FA markers of concussion depended on the post-concussion time interval being studied (Eierud et al., 2014). The elevation of FA at late acute injury is also congruent with prior studies of chronic concussion (Sasaki et al., 2014; Churchill et al., 2017), and may reflect the presence of ongoing pathophysiological changes, for example due to persistent neuroinflammatory response or reactive gliosis (Budde et al., 2011).

Interestingly, analyses of MD indicate a more complex relationship with days post-injury, wherein frontal MD is negatively correlated with days post-injury, while posterior MD is positively correlated. Therefore, this measure appears to be a less consistent indicator of the post-concussion time interval. The spatial distribution of different MD may be due to spatial differences in the biomechanics of concussion and their effect on white matter tracts. This is supported by a prior study of sub-concussive head impacts, which found spatially-dependent effects on both axial and radial diffusivity in the absence of a significant change in FA (Koerte et al., 2012). Nonetheless, future research is required to definitively establish this relationship.

4.3. Neuroimaging data: overlap between MRI measures

This section examined the overlap between different MRI measures, further establishing the heterogeneity of functional and structural neuroimaging data. Although spatial overlap was significantly greater than chance for both grey matter and white matter measures, it was relatively low in both cases. Moreover, concussed athletes do not tend to be significantly different from controls across multiple MRI measures – only 2 of the 8 subjects that show significant differences compared to controls do so in more than one measure. The limited overlap between CBF and Gconn is particularly interesting, as the healthy resting brain shows strong spatial correlations between functional connectivity strength and CBF (Liang et al., 2013). The present findings indicate that this relationship may be disrupted during acute concussion. Similarly, during acute concussion, cellular edema is generally thought to both increase MD and decrease FA in affected white matter (Toledo et al., 2012), whereas the present findings suggest that different brain regions are sensitive to FA or MD as a function of days post-injury. While preliminary, these findings emphasize that different MRI parameters reflect distinct aspects of concussion pathophysiology, and that multi-modal MRI may prove important to comprehensively describe the effects of acute concussion.

4.4. Study limitations and implications for future research

Although our findings provide novel information about the pathophysiology of acute concussion, there were some limitations which should be addressed in future research. We examined the mean effects of acute concussion in a mixed group, including male and female

athletes, across different sports. This was done with the goal of identifying the most generalizable effects of concussion across the sporting community. Nonetheless, there is evidence of differences in concussion prevalence and sequelae of male and female athletes (Covassin et al., 2003; Hutchison et al., 2016), and participation in collision sports has been shown to alter brain function (Abbas et al., 2015). Thus, future research should examine sport sub-groups at acute injury, to determine if there are distinct MRI markers of acute injury. In addition, our present findings, regarding the effects of days post-injury on MRI measures, was based on cross-sectional data. Although we may hypothesize that this reflects the trajectory of brain recovery following acute injury, repeated within-subject measures are required to definitively establish this trend, and to control for potential baseline differences between individuals.

Despite the cross-sectional nature of this study, our findings are consistent with post-injury brain changes mapped out in rodent models, and therefore support the presence of similar physiological processes in humans. In both animal models and our current findings, an early period of hyper-metabolic activity and hyper-perfusion is observed, followed by an interval of depressed neural activity and hypo-perfusion (Giza and Hovda, 2001; Giza and Hovda, 2014). This is of particular interest to future studies, as it may reflect the emergence of a delayed period of “metabolic vulnerability” described in (Vagnozzi et al., 2008), which is critical to avoid when establishing safe return-to-play for athletes following concussion. Future studies should investigate these effects in greater detail, with multiple scans at well-defined post-injury intervals.

The results of this study show an effect of days post-injury that is consistent with the neurometabolic cascade seen in rodent models (Giza and Hovda, 2001; Giza and Hovda, 2014). However, we emphasize that in humans this cannot be entirely separated from physical factors such as reduced physical and mental exertion, as this is a part of the consensus concussion management guidelines during the symptomatic phase of injury (McCrory et al., 2013). Athletes in the present study are recommended to avoid strenuous exercise but may otherwise perform daily activities, which may mitigate the detrimental effects of prolonged rest (see, for example, (Silverberg and Iverson, 2013)). Future concussion research should examine this issue, by comparing athletes, who significantly reduce physical exertion following concussion, to non-athlete cohorts.

4.5. Conclusions

In this study, we examined multiple different MRI markers of brain structure and function, for athletes with acute concussion. Our principal finding was the lack of consistent group effects of concussion, which points towards the inherent variability of acute concussion pathophysiology. This presently limits the utility of advanced MRI methods as biomarkers of sport concussion, until the sources of inter-subject variability are better understood. Our second finding was the demonstration that the post-injury interval is a significant covariate for most brain measures. This provides evidence that inter-subject variability in MRI markers at acute concussion may be partly driven by time since injury, and the careful modelling of this effect will lead to more robust acute brain biomarkers. The present findings contribute to our understanding of concussion pathophysiology in humans, and may help to guide future studies regarding acute concussion management.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.nicl.2017.02.015>.

Funding

This research received funding from the Defence Research & Development Canada (DRDC) and the Canadian Institutes of Military and Veterans Health (CIMVHR) grant W7714-145967. This study was approved by the Canadian Forces Surgeon General's Health Research

Program. This study was also partially funded by a Canadian Institutes of Health Research (CIHR) Project Grant RN294001–367456.

References

- Abbas, K., Shenk, T., Poole, V., Breedlove, E., Leverenz, L., Nauman, E., Robinson, M., 2015. Alteration of default mode network in high school football athletes due to repetitive subconcussive mild traumatic brain injury: a resting-state functional magnetic resonance imaging study. *Brain Connectivity* 5, 91–101.
- Abrahams, S., Mc Fie, S., Patricios, J., Posthumus, M., September, A.V., 2014. Risk factors for sports concussion: an evidence-based systematic review. *Br. J. Sports Med.* 48, 91–97.
- Aron, A.R., Robbins, T.W., Poldrack, R.A., 2004. Inhibition and the right inferior frontal cortex. *Trends Cogn. Sci.* 8, 170–177.
- Budde, M.D., Janes, L., Gold, E., Turtzo, L.C., Frank, J.A., 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.
- Casson, I.R., Viano, D.C., Haacke, E.M., Kou, Z., LeStrange, D.G., 2014. Is There Chronic Brain Damage in Retired NFL Players? *Neuroradiology, Neuropsychology, and Neurology Examinations of 45 Retired Players. Sports Health: A Multidisciplinary Approach.* 6 pp. 384–395.
- Chamard, E., Lassonde, M., Henry, L., Tremblay, J., Boulanger, Y., De Beaumont, L., Théoret, H., 2013. Neurometabolic and microstructural alterations following a sports-related concussion in female athletes. *Brain Inj.* 27, 1038–1046.
- Chappell, M., Ulug, A., Zhang, L., Heitger, M., Jordan, B., Zimmerman, R., Watts, R., 2006. Distribution of microstructural damage in the brains of professional boxers: a diffusion MRI study. *J. Magn. Reson. Imag.* 24, 537–542.
- Churchill, N., Hutchison, M., Richards, D., Leung, G., Graham, S., Schweizer, T., 2017. Brain structure and function associated with a history of sport concussion: a multi-modal MRI study. *J. Neurotrauma*. 34 (4), 765–771.
- Cole, M.W., Yarkoni, T., Repovš, G., Anticevic, A., Braver, T.S., 2012. Global connectivity of prefrontal cortex predicts cognitive control and intelligence. *J. Neurosci.* 32, 8988–8999.
- Collie, A., Makdissi, M., Maruff, P., Bennell, K., McCrory, P., 2006. Cognition in the days following concussion: comparison of symptomatic versus asymptomatic athletes. *J. Neurol. Neurosurg. Psychiatry* 77, 241–245.
- Covassin, T., Swanik, C., Sachs, M., 2003. Sex differences and the incidence of concussions among collegiate athletes. *J. Athl. Train.* 38, 238.
- De Beaumont, L., Lassonde, M., Leclerc, S., Théoret, H., 2007. Long-term and cumulative effects of sports concussion on motor cortex inhibition. *Neurosurgery* 61, 329–337.
- Derrfuss, J., Brass, M., Neumann, J., von Cramon, D.Y., 2005. Involvement of the inferior frontal junction in cognitive control: meta-analyses of switching and Stroop studies. *Hum. Brain Mapp.* 25, 22–34.
- Eierud, C., Craddock, R.C., Fletcher, S., Aulakh, M., King-Casas, B., Kuehl, D., LaConte, S.M., 2014. Neuroimaging after mild traumatic brain injury: review and meta-analysis. *NeuroImage Clin.* 4, 283–294.
- Gavett, B., Stern, R., McKee, A., 2011. Chronic traumatic encephalopathy: a potential late effect of sport-related concussive and subconcussive head trauma. *Clin. Sports Med.* 30, 179–188.
- Giza, C., Hovda, D., 2001. The neurometabolic cascade of concussion. *J. Athl. Train.* 36, 228.
- Giza, C., Kutcher, J., Ashwal, S., Barth, J., Getchius, T., Gioia, G., McKeag, D., 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.
- Giza, C.C., Hovda, D.A., 2014. The new neurometabolic cascade of concussion. *Neurosurgery* 75, S24.
- Graham, D., Gennarelli, T., McIntosh, T., 2002. *Trauma. Greenfield's Neuropathology.* New York, Oxford University Press.
- Guskiewicz, K.M., 2011. Balance assessment in the management of sport-related concussion. *Clin. Sports Med.* 30 (1), 89–102.
- Guskiewicz, K., Ross, S., Marshall, S., 2001. Postural stability and neuropsychological deficits after concussion in collegiate athletes. *J. Athl. Train.* 36, 263.
- Guskiewicz, K., McCreary, M., Marshall, S., Cantu, R., Randolph, C., Barr, W., Kelly, J., 2003. Cumulative effects associated with recurrent concussion in collegiate football players: the NCAA concussion study. *JAMA* 290, 2549–2555.
- Guskiewicz, K., Marshall, S., Bailes, J., McCreary, M., Cantu, R., Randolph, C., Jordan, B., 2005. Association between recurrent concussion and late-life cognitive impairment in retired professional football players. *Neurosurgery* 57, 719–726.
- Guskiewicz, K., Marshall, S., Bailes, J., McCreary, M., Harding, H., Matthews, A., Mihalik, J., Cantu, R., 2007. Recurrent concussion and risk of depression in retired professional football players. *Med. Sci. Sports Exerc.* 39, 903–909.
- Guskiewicz, K., Register-Mihalik, J., McCrory, P., McCreary, M., Johnston, K., Makdissi, M., Meeuwisse, W., 2013. Evidence-based approach to revising the SCAT2: introducing the SCAT3. *Br. J. Sports Med.* 47, 289–293.
- Hart, J., Kraut, M.A., Womack, K.B., Strain, J., Didehban, N., Bartz, E., Conover, H., Mansinghani, S., Lu, H., Cullum, C.M., 2013. Neuroimaging of cognitive dysfunction and depression in aging retired National Football League players: a cross-sectional study. *JAMA* 70, 326–335.
- Hillary, F., Roman, C., Venkatesan, U., Rajtmajer, S., Bajo, R., Castellanos, N., 2015. Hyperconnectivity is a fundamental response to neurological disruption. *Neuropsychology* 29, 59.
- Hovda, D., Lee, S., Smith, M., Von Stuck, S., Bergsneider, M., Kelly, D., Shalmon, E., Martin, N., Caron, M., Maziotta, J., 1995. The neurochemical and metabolic cascade following brain injury: moving from animal models to man. *J. Neurotrauma* 12, 903–906.

- Huh, J.W., Widing, A.G., Raghupathi, R., 2007. Repetitive mild non-contusive brain trauma in immature rats exacerbates traumatic axonal injury and axonal calpain activation: a preliminary report. *J. Neurotrauma* 24, 15–27.
- Hutchison, M., Mainwaring, L., Senthinathan, A., Churchill, N., Thomas, S., Richards, D., 2016. Psychological and physiological markers of stress in concussed athletes across recovery milestones. *J. Head Trauma Rehabil.* <http://dx.doi.org/10.1097/HTR.000000000000252> (Epub ahead of print).
- Johnson, B., Zhang, K., Gay, M., Horovitz, S., Hallett, M., Sebastianelli, W., Slobounov, S., 2012. Alteration of brain default network in subacute phase of injury in concussed individuals: resting-state fMRI study. *NeuroImage* 59, 511–518.
- Koerte, I.K., Ertl-Wagner, B., Reiser, M., Zafonte, R., Shenton, M.E., 2012. White matter integrity in the brains of professional soccer players without a symptomatic concussion. *JAMA* 308, 1859–1861.
- Len, T., Neary, J., 2011. Cerebrovascular pathophysiology following mild traumatic brain injury. *Clin. Physiol. Funct. Imaging* 31, 85–93.
- Liang, X., Zou, Q., He, Y., Yang, Y., 2013. Coupling of functional connectivity and regional cerebral blood flow reveals a physiological basis for network hubs of the human brain. *Proc. Natl. Acad. Sci.* 110, 1929–1934.
- McCrea, M., Kelly, J., Kluge, J., Ackley, B., Randolph, C., 1997. Standardized assessment of concussion in football players. *Neurology* 48, 586–588.
- McCrea, M., Guskiewicz, K., Marshall, S., Barr, W., Randolph, C., Cantu, R., Kelly, J., 2003. Acute effects and recovery time following concussion in collegiate football players: the NCAA concussion study. *JAMA* 290, 2556–2563.
- McCrory, P., Meeuwisse, W., Aubry, M., Cantu, B., Dvořák, J., Echemendia, R., Sills, A., 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, T., Bellgowan, P., Singh, R., Kuplicki, R., Polanski, D., Mayer, A., 2015. Recovery of cerebral blood flow following sports-related concussion. *JAMA* 314, 530–538.
- Monaco III, E.A., Tempel, Z., 2015. Concussion related to white matter abnormalities and cognitive dysfunction in aging athletes. *Neurosurgery* 76, N10–N11.
- 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.
- Rubinov, M., Sporns, O., 2010. Complex network measures of brain connectivity: uses and interpretations. *NeuroImage* 52, 1059–1069.
- Sasaki, T., Pasternak, O., Mayinger, M., Muehlmann, M., Savadjiev, P., Bouix, S., Kubicki, M., Fredman, E., Dahlben, B., Helmer, K.G., 2014. Hockey concussion education project, part 3. White matter microstructure in ice hockey players with a history of concussion: a diffusion tensor imaging study: clinical article. *J. Neurosurg.* 120, 882–890.
- Silverberg, N., Iverson, G., 2013. Is rest after concussion “the best medicine?”: recommendations for activity resumption following concussion in athletes, civilians, and military service members. *J. Head Trauma Rehabil.* 28, 250.
- Slobounov, S., Gay, M., Johnson, B., Zhang, K., 2012. Concussion in athletics: ongoing clinical and brain imaging research controversies. *Brain Imag. Behav.* 6, 224–243.
- Toledo, E., Lebel, A., Becerra, L., Minster, A., Linnman, C., Maleki, N., Borsook, D., 2012. The young brain and concussion: imaging as a biomarker for diagnosis and prognosis. *Neurosci. Biobehav. Rev.* 36 (6), 1510–1531.
- Vagnozzi, R., Signoretti, S., Tavazzi, B., Floris, R., Ludovici, A., Marziali, S., Tarascio, G., Amorini, A.M., Di Pietro, V., Delfini, R., 2008. Temporal window of metabolic brain vulnerability to concussion: a pilot 1H-magnetic resonance spectroscopic study in concussed athletes—part III. *Neurosurgery* 62, 1286–1296.
- 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.
- Zhang, K., Johnson, B., Gay, M., Horovitz, S., Hallett, M., Sebastianelli, W., Slobounov, S., 2012. Default mode network in concussed individuals in response to the YMCA physical stress test. *J. Neurotrauma* 29, 756–765.
- Zhang, L., Heier, L., Zimmerman, R., Jordan, B., Uluğ, A., 2006. Diffusion anisotropy changes in the brains of professional boxers. *Am. J. Neuroradiol.* 27, 2000–2004.
- Zhu, D., Covassin, T., Nogle, S., Doyle, S., Russell, D., Pearson, R., Kaufman, D., 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.