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Alterations in default-mode network connectivity may be influenced by cerebrovascular changes within one week of sports related concussion in college varsity athletes: a pilot study

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Abstract

The goal of this pilot study is to use complementary MRI strategies to quantify and relate cerebrovascular reactivity, resting cerebral blood flow and functional connectivity alterations in the first week following sports concussion in college varsity athletes. Seven college athletes (3F/4M, age = 19.7 ± 1.2 yrs) were imaged 3–6 days following a diagnosed sports related concussion and compared to eleven healthy controls with no history of concussion (5M/6F, 18–23 yrs, 7 athletes). Cerebrovascular reactivity and functional connectivity were measured using functional MRI during a hypercapnia challenge and via resting-state regional partial correlations, respectively. Resting cerebral blood flow was quantified using arterial spin labeling MRI methods. Group comparisons were made within and between 18 regions of interest. Cerebrovascular reactivity was increased after concussion when averaged across all regions of interest (p=0.04), and within some default-mode network regions, the anterior cingulate and the right thalamus (p<0.05) independently. The FC was increased in the concussed athletes within the default-mode network including the left and right hippocampus, precuneus and ventromedial prefrontal cortex (p<0.01), with measures being linearly related to cerebrovascular reactivity in the hippocampus in the concussed athletes. Significant resting cerebral blood flow changes were not detected between the two groups. This study provides evidence for increased cerebrovascular reactivity and functional connectivity in the medial regions of the default-mode network within days of a single sports related concussion in college athletes. Our findings emphasize the utility of complementary

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Compliance with Ethical Standards

Ethics approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the Vanderbilt University and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all participants included in this study.

cerebrovascular measures in the interpretation of alterations in functional connectivity following concussion.

Keywords

concussion; cerebrovascular reactivity; cerebral blood flow; functional MRI; functional connectivity

Introduction

Neuroimaging has traditionally played a limited role in the management of sports-related concussion. This is because the clinical symptoms of concussion in the acute setting likely represent functional manifestations of currently undetectable structural injury (McCrory et al. 2013). Both neurocognitive and physiologic alterations are believed to contribute to the functional impairments following concussion. While the neurocognitive alterations following concussion have been extensively studied and play a significant role in clinical management, objective physiologic measures have not been reliably identified to inform clinical decisions (Grindel et al. 2001).

Using blood oxygenation level dependent (BOLD) functional MRI (fMRI) (Logothetis et al. 2001; Ogawa et al. 1990) images acquired at rest, synchronization in low frequency blood oxygen oscillations can quantify functional connectivity (FC) across brain networks (Biswal et al. 1995; Rogers et al. 2007). This measure has been highly studied after concussion as a potential neurophysiological biomarker of injury. Studies of FC include acquisitions ranging from 10 days (Johnson et al. 2012; Zhang et al. 2012) to years (Palacios et al. 2013) after injury with several reporting general similarities in decreased FC across parts of the default-mode network (Johnson et al. 2012; Mayer et al. 2011; Y. X. Zhou et al. 2012). However, at day 1 after injury increases in some FC regions have been detected (Zhu et al. 2014). Since FC is an indirect probe of neuronal activity mediated by cerebrovascular function, the potential interaction of other cerebrovascular changes such as cerebral blood flow and vascular reactivity to the BOLD measure of FC after concussion should be considered (Lu et al. 2008; Liu et al. 2013).

Alterations in cerebrovascular physiology following concussion have long been suspected to play a role in the symptoms of concussion (Junger et al. 1997; Strebel et al. 1997; McQuire et al. 1998). Cerebral blood flow (CBF) has been found to be decreased immediately following concussion (Maugans et al. 2012) and months to years after injury in some cases (Bonne et al. 2003; Ge et al. 2009; Bartnik-Olson et al. 2014). Cerebrovascular reactivity (CVR), the change in CBF in response to a stimulus, has been less frequently studied (Becelewski and Pierzchala 2003; Len et al. 2011; Len et al. 2013). Len and colleagues have reported abnormal CVR following mild traumatic brain injury in two separate studies using MCA Doppler velocity measurements and breath-hold challenges. Most recently, they describe subject specific differences in CVR at post injury day 2 when compared with baseline and post-injury days 4 and 8 (Becelewski and Pierzchala 2003; Len et al. 2011; Len et al. 2013).

Using multi-modal MRI, measurements of FC, CBF and CVR can be obtained in the same imaging session. Arterial spin labeling (ASL) (Jiang et al. 2010; Chen et al. 2011; Wang et al. 2003; Detre et al. 1992) can be used to quantify resting CBF by comparing images where the in flowing blood is tagged with a radiofrequency pulse to invert the magnetization of blood water to images without the inversion applied. In the fMRI measurement of CVR, hypercapnia is used to elicit blood vessel dilation resulting in blood oxygenation changes detectable with BOLD fMRI (Bright and Murphy 2013; Murphy et al. 2011). Therefore, the goal of this exploratory, pilot study is to use these complementary MRI strategies to quantify and relate the CVR, CBF and FC alterations in the first week following concussion in college athletes. We hypothesize that the concurrent measures will provide a more accurate assessment and interpretation of the cerebrovascular changes early in recovery of sports concussion.

Materials and Methods

Subjects

Seven varsity college athletes were enrolled in this study within one week of experiencing a sports related concussion (3F/4M, age = 19.7 ± 1.2 yrs) (Table 1). All athletes were evaluated on the field by a certified athletic trainer for signs or symptoms of a concussion (McCrory et al. 2013). Athletes with positive findings were referred to a sports medicine physician who confirmed the diagnosis of concussion and entered them into the treatment protocol set by the Vanderbilt Sports Concussion Center. Exclusion criteria were posttraumatic amnesia > 24 hours, loss of consciousness > 30 minutes and prior history of concussion. No structural injury related to concussion was evident on conventional T1 and T2 weighted imaging reviewed by a board certified neuroradiologist. No subjects experienced persistent symptoms longer than 2 weeks.

In addition, eleven healthy college students with no history of concussion were enrolled (6F/5M, age = 20.0 ± 1.6 yrs). Seven of these controls were also varsity college athletes at the time of enrollment. All subjects gave written informed consent per Institutional Review Board guidelines. Symptoms were measured by the Rivermead Post Concussion Symptoms Questionnaire (King et al. 1995) in all subjects at the time of scanning. This assessment asks the subject to rate 16 individual symptoms over the last 24 hours from 0 (not experienced at all) to 4 (a severe problem). The rating for “Headaches” and the total over all symptoms for each subject are included in Table 1.

MRI Imaging

All imaging was performed on a 3T MRI scanner (Philips Healthcare, Best, Netherlands) using a 32-channel head coil. The protocol for each subject included three structural acquisitions: 1) three-dimensional, T1 weighted high-resolution whole brain image for inter-subject normalization ($1 \times 1 \times 1$ mm^3) and identification of structural abnormalities, 2) T2 weighted turbo spin echo, high resolution axial full brain image ($0.5 \times 0.5 \times 2.5$ mm^3) for identification of structural abnormalities, and 3) two-dimensional, T1 weighted high-resolution axial full brain image ($1 \times 1 \times 4$ mm^3) in the same slice locations as the fMRI for functional to 3D data registration. Physiological monitoring of cardiac and respiratory

fluctuations was performed at 500 Hz using the MRI scanner integrated pulse oximeter and the respiratory belt.

For the measurement of FC, a T2* weighted gradient echo, echo-planar fMRI image series was acquired at rest with eyes closed – matrix 80 × 80, FOV = 240 mm, 34 axial slices, TE = 35 ms, TR = 2 sec, slice thickness = 3.5 mm with 0.5 mm gap, 300 volumes (10 minutes). The same T2* weighted sequence was used to acquire images with a hypercapnia challenge for the measurement of CVR, with the only change being in length of scan (270 volumes). The 9 minute hypercapnia challenge consisted of the subject alternating breathing medical air (13 L/min) and medical air with 5% CO_2 (5% CO_2/21% O_2/74% N_2, 13 L/min) in a pattern of 1-2-2-2-2 minute blocks each through a face mask covering the nose and mouth. End-tidal CO_2 was monitored via a nasal cannula and recorded at approximately 20 second intervals. The average change in expired CO_2 (mmHg) between the blocks of medical air and the CO_2 challenge was computed across the entire scan.

For the quantification of resting cerebral blood flow (CBF), a pseudo continuous arterial spin labeling (pCASL) (Chen et al. 2011; Dai et al. 2008) approach was utilized (matrix 80 × 80, FOV = 240 mm × 240 mm, 17 axial slices, 7 mm slice thickness with no gap, echo time = 13.78 ms, SENSE =2.5). A total of 35 pairs of interleaved labeled and control images were acquired. An M_0 image to measure equilibrium magnetization was acquired in the same slice locations with a TR = 20 sec, and echo time = 11.89 msec.

The order of the scans remained the same for all subjects. The session started with the subject wearing the face mask and cannula. The scan order was the following: 1) survey, 2) two-dimensional T1 weighted scan with orientation matching the CVR fMRI, and 3) CVR fMRI scan. The mask and cannula were then removed from the subject. The scanning resumed with 4) survey 5) three-dimensional T1 weighted scan, 6) two-dimensional T1 weighted scan with orientation matching the FC fMRI scan, 7) FC fMRI scan, 8) CBF scan, 9) M_0 scan, and 10) T2 weighted scan. The session concluded with other scans not included in these analyses.

Cerebrovascular Reactivity and Functional Connectivity Analysis

The CVR and the FC fMRI images were corrected for slice timing effects and motion occurring between the fMRI scans using SPM8 software (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). The maximum translation in the x, y, and z directions and the maximum rotation about the x, y, and z axes were computed for each subject. Then images were corrected for physiological noise using a RETROICOR protocol (Glover et al. 2000) using the measured cardiac and respiratory time series. Images were then spatially normalized to the Montreal Neurological Institute (MNI) template and smoothed with a 6 × 6 × 6 mm^3 full width, half maximum kernel. The T1 weighted, three-dimensional, high-resolution MRI image was segmented into its gray matter, white matter and cerebrospinal fluid components using SPM8.

The FC images were temporally low pass filtered at 0.1 Hz (Cordes et al. 2001). Then the average time series within each region of interest described below was calculated. FC was computed between all pairs of regions using the partial correlation between the average time

series in two regions, with the six motion time series and the average white matter time series as confounds.

Using the CVR images, a voxel-wise analysis was performed to determine the average percent signal change between the baseline blocks (medical air) and the stimulus blocks (medical air with 5% CO_2) per voxel. The baseline average was computed from the entire first baseline minute and the last 90 seconds of each of the subsequent two baseline blocks of 2 minutes each. The stimulus signal average was computed from the last 90 seconds of each stimulus block. The first 30 seconds of each block (with the exception of the first minute) was ignored to allow the appropriate gas mixture to reach the subject and respiration to occur. The percent BOLD signal change of each voxel was computed and divided by the average expired mmHg CO_2 described above. This was used as quantification of CVR (%ΔBOLD/ΔmmHg) for comparisons between groups (Fig 1).

Resting Cerebral Blood Flow Analysis

The 35 pairs of pCASL labeled and control images were first realigned using SPM8 software. Then a pairwise subtraction was used to determine perfusion difference images. The voxel-wise CBF (ml/100g tissue/minute) was quantified using the single blood compartment model (Chen et al. 2011; Dai et al. 2008; Buxton et al. 1998):

$$f = \frac{\lambda * \Delta M}{2\alpha * M_0 * T_{1b} * (e^{\frac{-\omega}{T_{1b}}} - e^{-(\tau+\omega)/T_{1b})})} \quad (1)$$

where λ = 0.98 ml/g blood to water partition coefficient, ΔM = the mean of the perfusion difference images, α = 0.85 assumed label efficiency, τ = label duration of 1.65 sec, T_{1b} = 1.7 sec T_1 of blood water at 3T. The ω = the post labeling delay of 1.65 sec corrected for the individual slice acquisition times, and M_0 was taken as the maximum signal in the cerebral spinal fluid in the M_0 image divided by the water density (0.87). The resulting voxel-wise map of CBF was spatially normalized to the MNI template and resampled to 3 × 3 × 3 mm^3 to match the resolution of the CVR maps (Fig 1). Gray matter maps of CBF were determined by masking this map with the individual's gray matter segmented T1 image normalized to MNI space.

Regions of Interest

Eighteen regions of interest were defined across the brain in all subjects for comparison of the cerebrovascular measures. Sixteen of these were chosen from three well-defined networks studied previously with functional MRI – default-mode network (DMN) (Buckner et al. 2008), dorsal attention network (DAN) (Gao and Lin 2012), and frontal parietal control network (FPC) (Gao and Lin 2012). Previous studies have reported changes in functional connectivity in the DMN in mild traumatic brain injury (Johnson et al. 2012; Zhang et al. 2012; Sharp et al. 2011). It is believed that the DAN is anti-correlated with the DMN, and that the balance between these two functional networks is controlled by the FPC (Gao and Lin 2012; Sridharan et al. 2008). By defining widespread regions based on these networks, cerebrovascular characteristics throughout the brain can be investigated, and possibly

interpreted in the context of this balance between networks. The MNI coordinates for each region of interest in the three networks were taken from Table 1 in (Gao and Lin 2012) and identified using the aal atlas in WFU PickAtlas software (Maldjian et al. 2003; Tzourio-Mazoyer et al. 2002). The DMN regions included: hippocampus left, hippocampus right, precuneus (left and right combined), angular left (left posterior inferior parietal lobe), angular right (right posterior inferior parietal lobe), frontal middle orbital (left and right combined as ventromedial prefrontal cortex), and frontal superior medial (left and right combined as dorsomedial prefrontal cortex). The FPC regions included: cingulum anterior (left and right combined), parietal inferior left, parietal inferior right, frontal middle left (dorsolateral prefrontal cortex left), frontal middle right (dorsolateral prefrontal cortex right). The DAN regions included: occipital middle left (middle temporal area left), occipital middle right (middle temporal area right), parietal superior left (intra-parietal sulcus left), parietal superior right (intra-parietal sulcus right). The left and right thalamus were also defined. These were chosen based on findings of disruption of thalamic and thalamic-cortical functional networks in mTBI (Tang et al. 2011; Y. Zhou et al. 2013). Average CBF and CVR in each region of interest were computed.

Statistical Comparisons

The linear correlation between the concussed athletes' Rivermead headache and total scores and days after injury were computed using a Pearson's correlation. A two sample t-test was used to compare the CVR and the CBF values in each region between the concussion group (n=7) and the control group (n=11), and the subset of the control subjects who were athletes (n=7). Where significant differences were found, the effect size was computed as the Cohen's d statistic (Cohen 1988). FC was compared between the control group and the concussion group for each pair of regions using a two sample t-test. The Cohen's d statistic was again computed for any significant difference between groups. Any spatially overlapping findings of significant changes between groups in CVR, CBF and FC were investigated with a Pearson's correlation. This was tested in the control group and the concussion group separately.

Results

Rivermead headache score reported in the concussed athletes was negatively linearly correlated with days after injury (p=0.02), while total Rivermead scores were not. One concussed athlete (pat04) was given 95% O_2 with 5% CO_2 instead of the medical air with 5% CO_2, so was not analyzed for the CVR investigation due to the potential effect of the increased oxygen on the BOLD fMRI signal (Hare et al. 2013), resulting in six patients for the CVR analysis. The CVR averaged across all of the regions of interest was increased in the concussed athletes compared to the healthy controls (p=0.04), and compared to the subset of healthy control athletes (p=0.03). When looking at individual regions, the statistically significant increases between groups were most concentrated in the DMN and the anterior cingulate in the FPC (Fig 2). The CVR averaged over all of the DMN regions was increased in the concussed athletes compared to the healthy controls (p=0.04), and compared to the subset of healthy control athletes (p=0.02). Additionally, regions which did not reach statistical significance demonstrated an average increase in CVR in the concussed

athletes compared to all controls or athlete controls (Fig 3A). The Cohen's d effect size for each statistically significant difference is also given in Fig 2B.

The CBF averaged across all of the regions of interest was not changed in the concussed athletes compared to the healthy controls (p=0.51), nor compared to the healthy control athletes (p=0.11). Fig 3B shows that the average CBF in each region in the concussed athletes tended to be lower than all controls, but did not reach statistical significance. Similarly, when compared to the athlete controls, the concussed athletes tended to have an average decrease in CBF. However, these decreases were statistically significant (p<0.05) only in the left anterior inferior parietal lobe of the FPC and the left middle temporal area of the DAN in the concussed athletes when compared to the healthy control athletes only (Fig 2).

The FC was increased in the concussed athletes compared to the healthy controls across three pairs of regions in the DMN including the left and right hippocampus, precuneus and ventromedial prefrontal cortex (p<0.01) (Fig 4A, solid lines. Two other pairs including the DMN and the anterior cingulate in the FPC were also increased in the concussed athletes (p<0.05) (Fig 4A, dashed lines). Compared to the subset of healthy athletes only, the concussed athletes had increased FC in the DMN between the right hippocampus and the ventromedial prefrontal cortex (p<0.01). Averaged across all DMN pairs of regions there was a trend towards increase FC in concussed athletes compared to healthy controls (p=0.06).

The CVR in the hippocampus was linearly related to the FC between the hippocampus and precuneus across subjects, but this relationship was different between the healthy controls and the concussed athletes. In the right hippocampus, the healthy controls showed a linear decrease in FC between right hippocampus and precuneus with increase in right hippocampus CVR (Fig 4B) (p=0.03, d=1.7), while the concussed athletes had an increase in FC with increase CVR (Fig 4B) (p=0.03, d>2.0). In the left hippocampus, there was an increase in the FC between the left hippocampus and precuneus with an increase CVR in the left hippocampus in concussed athletes (Fig 4C) (p=0.001 d>2.0), but not healthy controls.

Discussion

In this study, we had three main findings. First, we observed increased CVR in response to a hypercapnia challenge in college athletes in the days following a sports-related concussion. Several regions concentrated within the DMN, the anterior cingulate and the right thalamus reached statistical significance independently. While the overall trend of our data suggests that the increase in CVR following concussion is likely a global process, the medial DMN may be particularly sensitive to concussive forces. Second, we found increases in FC between the hippocampus, precuneus and ventromedial prefrontal cortex in the DMN in concussed athletes. There was also a trend towards increase FC across the DMN as a whole in the concussed athletes. Third, we found that the increase in CVR in the hippocampus is positively correlated with the increase in FC between the hippocampus and precuneus in the concussed athletes, but not in healthy controls.

The finding of increased CVR is unusual given that CVR is usually decreased in the majority of pathologic conditions in which cerebrovascular physiology is altered with the exception of migraine headaches (Thomsen et al. 1995). This exception is particularly interesting given that headache is a commonly reported symptom in patients following concussion. We suspect that the mechanism underlying the increases in CVR we observed may be nitric oxide mediated. Upregulation of inducible nitric oxide synthase following traumatic brain injury is well established, and a study in concussed rats following mild traumatic brain injury has shown similar increases in nitric oxide synthase production (Gahm et al. 2000; Petrov et al. 2000; Wada et al. 1998; Tavazzi et al. 2007; Clark et al. 1996). Interestingly, Peebles et al. found increased levels of nitric oxide in cerebral venous samples following hypercapnia indicating that the vasodilatory response to hypercapnia challenge is nitric oxide mediated (Peebles et al. 2008). It remains to be established whether a persistently abnormal CVR has the potential to result in symptom recurrence upon return to physical activity as the partial pressure of carbon dioxide in blood is just one of many factors both systemic and local that influence cerebral blood flow during exercise (Ogoh and Ainslie 2009).

To further investigate the potential link between CVR and headache in our sample, we performed a partial correlation between Rivermead headache score and CVR across the patients in the seven regions shown to be significantly increased in patients vs. all controls in Fig 2. We used the days after injury as a confound. Of those regions, the right hippocampus region of the DMN (p=0.005), the right thalamus (p=0.04), average of ALL regions (p=0.03), and the average across the DMN (p=0.03) showed significant negative correlation with headache after correcting for days after injury. Considering the seven regions tested, only the right hippocampus exceeds the p<0.05 threshold corrected for multiple comparisons (0.05/7=0.007).

While we detected a trend of decreased CBF across most regions of the brain (Fig 3B), we found no statistically significant difference in CBF between concussed athletes and controls. In a pediatric population, CBF was decreased less than 72 hours following concussion, but was not different than matched controls by day 14 (Maugans et al. 2012). Similar findings of focal decreased CBF were reported in college football players (Meier et al. 2015). Other reports of decreased CBF have been detected in the chronic phase after injury when symptoms persist (Bonne et al. 2003; Ge et al. 2009; Bartnik-Olson et al. 2014). Our inability to detect significant decreases in CBF in the concussed athletes may be due in part to our control group. Our data shows a strong trend of increased CBF in athlete controls over non-athlete controls. Also, a few regions showed decreases when comparing concussed athletes to athlete controls only. These suggest that a larger control sample consisting only of athletes may be required to detect these differences (as in (Meier et al. 2015)). In addition, the athletes in both the Maugans et al. (Maugans et al. 2012) and Meier et al. (Meier et al. 2015) studies included concussed football players in which repeated concussive or subconcussive impacts may play a role.

We report a significant and localized finding of increased hippocampal, precuneus and ventromedial prefrontal FC in the concussed athletes over the healthy controls. It is difficult to relate these findings to previous literature. Early studies performed at approximately 30

days post injury (Mayer et al. 2011), 10 days post injury (Johnson et al. 2012), and two months post injury (Y. X. Zhou et al. 2012) detected decreases in resting FC in some DMN regions. However, a longitudinal study found increases in FC compared to controls in the DMN at day 1 followed by significant decreases at day 10 (Zhu et al. 2014), indicating a progression of changing FC in the DMN over the first few weeks after injury. Our findings of increased FC in parts of the DMN in days 3 to 6 may suggest that the decrease in FC occurs after the first week. Our findings are also consistent with those detected immediately following potential subconcussive injury. Johnson et al.(Johnson et al. 2014) looked at differences between pre-game and post-game MRI measures of FC and found that in those players without history of concussion, some increases in FC were detected between the supramarginal gyrus, the orbitofrontal cortex, the retrospenial cortex and the medial prefrontal cortex similar to our findings. Another study found increased DMN connections of FC in football players imaged at multiple sessions throughout a single season (Abbas et al. 2014), presumably within a day after subconcussive impacts. While our data reflects early concussion injury, it is possible that recent (within 24–48 hours) subconcussive impacts may result in similar pathophysiology.

The spatially overlapping, linear relationship of FC between the hippocampus and precuneus and CVR in the hippocampus has not been previously identified. Generally, decreases (not increases) in FC are attributed to network impairment, however, these findings suggest that the increase in FC we detect may be partially related to a pathologic hyper-reactivity of the vasculature across the DMN in the concussed athletes. One possible mechanism for this relationship is that that the increased CVR results in an increase in the power of the resting BOLD oscillations at the same frequency in two regions. This may decrease the contribution of other discrepant frequency oscillations in the FC between the two regions resulting in an overall increase in FC. While the validation of this mechanism is beyond the scope of this study, this relationship underscores the importance of interpretation of functional connectivity results following concussion in the context of concurrent cerebrovascular measurements. This may be particularly important in interpreting progression of FC alterations as previously discussed because longitudinal measures of CVR and CBF may also be required. Task-based fMRI studies where percent BOLD changes in the hippocampus are increased after concussion may be similarly influenced if underlying cerebrovascular pathophysiology is not considered (Slobounov et al. 2010).

The primary limitation of this study is the small sample size. Therefore, we also report the Cohen's *d* effect sizes of our findings. Our analyses of CVR and FC yielded *d* values greater than 1.0, and in many cases greater than 1.5. At d=1.0 and 1.5, the mean of the concussed athletes group is expected to be at the 84% and 93.3% of the control group, respectively, and is considered a large effect size (Cohen 1988). Similarly, it must be noted that due to the exploratory nature of this work with multiple scans, regions and symptoms, our statistics are not corrected for multiple comparisons unless stated. Our intention is that these findings can be used to define more specific hypotheses to be tested in future studies with larger sample sizes.

Another potential limitation is the mix of athletes and non-athletes in our control population. Therefore, we reported the results of the concussed athletes compared to the entire control

population, as well as to the athlete controls subgroup. These controls are used in lieu of baseline measures (before concussion) on the concussed athletes. In addition, our time window of acquisition of 3–6 days post-injury is earlier than several studies discussed, but the variability of recovery or injury within this window is unknown. When interpreting the CVR changes, we considered that a lower baseline CBF level may increase CVR by increasing percent signal change during hypercapnia challenge without increasing peak signal. However, the small decreases in CBF do not account for the magnitude of percent BOLD fMRI signal change we observed in our measurement of CVR. Additionally, there is no overlap in individual regions in which statistically significant increases in CVR and decreased CBF were observed. Finally, we considered that the measurement of resting FC could be artifactually influenced by the hypercapnia challenge used for the CVR measurement. We believe this possibility is accounted for as not only was the scan order the same for all patients (CVR hypercapnia challenge occurring approximately 20 minutes prior to the FC fMRI scan), the BOLD signal returned to baseline following the hypercapnia challenge in all subjects. Furthermore, the facemask was removed following the hypercapnia challenge such that there was no possibility of breathing unaccounted for residual gas within the tube in the interim between the two scans.

In conclusion, this exploratory, pilot study provides preliminary evidence for increased CVR in response to a hypercapnia challenge within days of a single sports related concussion in college athletes, without significant changes in resting CBF. These increases in CVR are accompanied by linearly related increases in FC in medial DMN regions, which suggests that pathologic cerebrovascular hyper-reactivity may play a role in increasing the FC in these regions. Our findings suggest that complementary cerebrovascular measures should be considered in the interpretation of alterations in functional connectivity following concussion.

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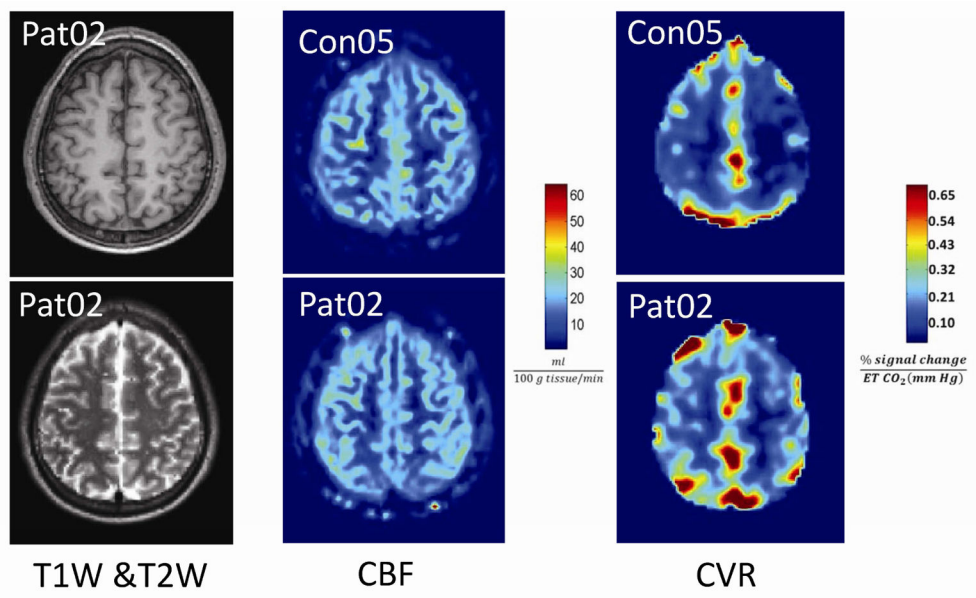
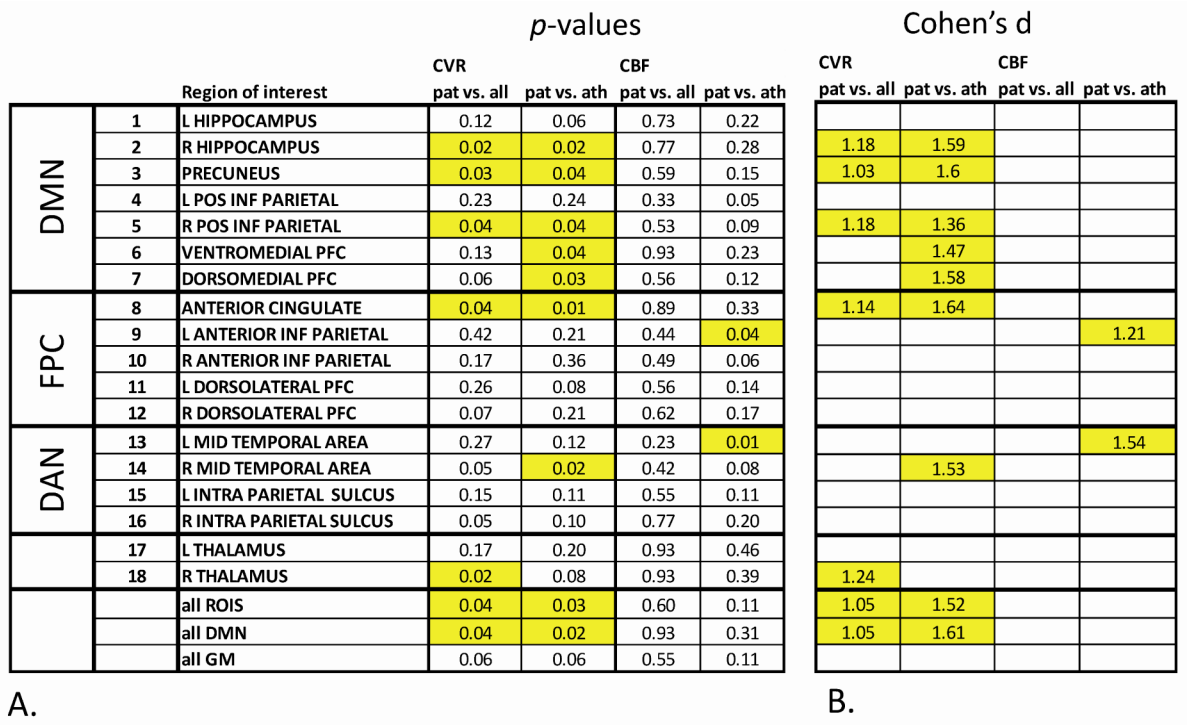


Fig. 1.

Example images and parametric maps of T1-Weighted (T1W), T2-Weighted (T2W), resting cerebral blood flow (CBF) and cerebrovascular reactivity (CVR) in individual subjects Con05 and Pat02. CBF is measured in ml/100g tissue/min. CVR is reported in % signal change/end tidal CO_2 (mmHg).



p-values

		Region of interest	CVR pat vs. all	CVR pat vs. ath	CBF pat vs. all	CBF pat vs. ath
DMN	1	L HIPPOCAMPUS	0.12	0.06	0.73	0.22
	2	R HIPPOCAMPUS	0.02	0.02	0.77	0.28
	3	PRECUNEUS	0.03	0.04	0.59	0.15
	4	L POS INF PARIETAL	0.23	0.24	0.33	0.05
	5	R POS INF PARIETAL	0.04	0.04	0.53	0.09
	6	VENTROMEDIAL PFC	0.13	0.04	0.93	0.23
	7	DORSOMEDIAL PFC	0.06	0.03	0.56	0.12
FPC	8	ANTERIOR CINGULATE	0.04	0.01	0.89	0.33
	9	L ANTERIOR INF PARIETAL	0.42	0.21	0.44	0.04
	10	R ANTERIOR INF PARIETAL	0.17	0.36	0.49	0.06
	11	L DORSOLATERAL PFC	0.26	0.08	0.56	0.14
	12	R DORSOLATERAL PFC	0.07	0.21	0.62	0.17
DAN	13	L MID TEMPORAL AREA	0.27	0.12	0.23	0.01
	14	R MID TEMPORAL AREA	0.05	0.02	0.42	0.08
	15	L INTRA PARIETAL SULCUS	0.15	0.11	0.55	0.11
	16	R INTRA PARIETAL SULCUS	0.05	0.10	0.77	0.20
	17	L THALAMUS	0.17	0.20	0.93	0.46
	18	R THALAMUS	0.02	0.08	0.93	0.39
		all ROIS	0.04	0.03	0.60	0.11
		all DMN	0.04	0.02	0.93	0.31
		all GM	0.06	0.06	0.55	0.11

A.

Cohen's d

CVR pat vs. all	CVR pat vs. ath	CBF pat vs. all	CBF pat vs. ath
1.18	1.59		
1.03	1.6		
1.18	1.36		
	1.47		
	1.58		
1.14	1.64		
			1.21
			1.54
	1.53		
1.24			
1.05	1.52		
1.05	1.61		

B.

Fig. 2.

Regional analysis of CVR and CBF in athletes with concussion. Fig 2A contains the uncorrected *p*-values of group differences. Yellow indicates statistically significant difference at $p<0.05$ (uncorrected). Fig 2B contains the Cohen's d statistic (Cohen 1988) of those comparisons with statistically significant differences. CVR = cerebrovascular reactivity, CBF = resting cerebral blood flow, pat = patients with concussion, all = all controls including athletes and non-athletes, ath = athlete controls only, L = left, R = right, POS = posterior, INF = inferior, DMN = regions in default-mode network, FPC = regions in frontal-parietal control network, DAN = regions in dorsal attention network, GM = all gray matter. Numbers to the left of each ROI correspond to number in x-axes in Fig 3 and in Fig 4.

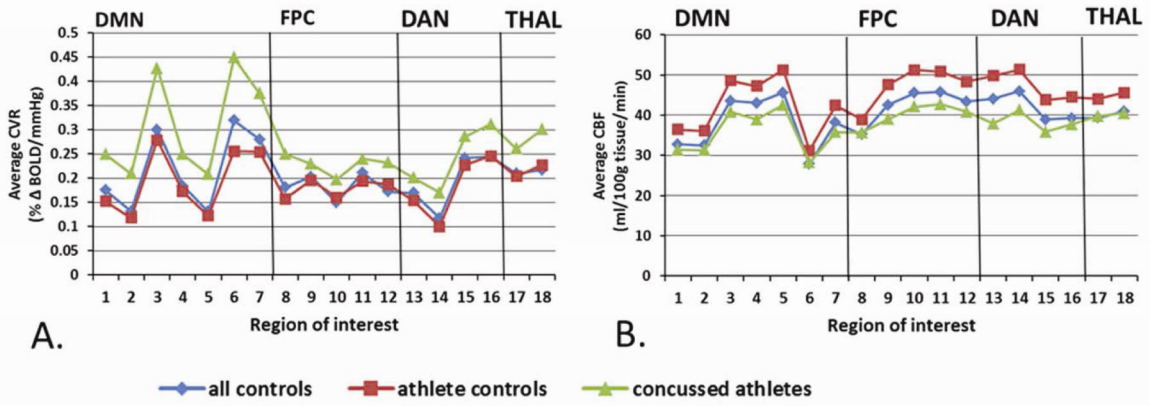


Fig. 3.

Cerebrovascular reactivity (CVR) (A) and resting cerebral blood flow (CBF) (B) in sports concussion. The x-axis represents each region of interest with the numbers corresponding to the numbers in Fig 2. The y-axis is the average across all subjects in the group in the region of interest. DMN = regions in default-mode network, FPC = regions in frontal-parietal control network, DAN = regions in dorsal attention network.

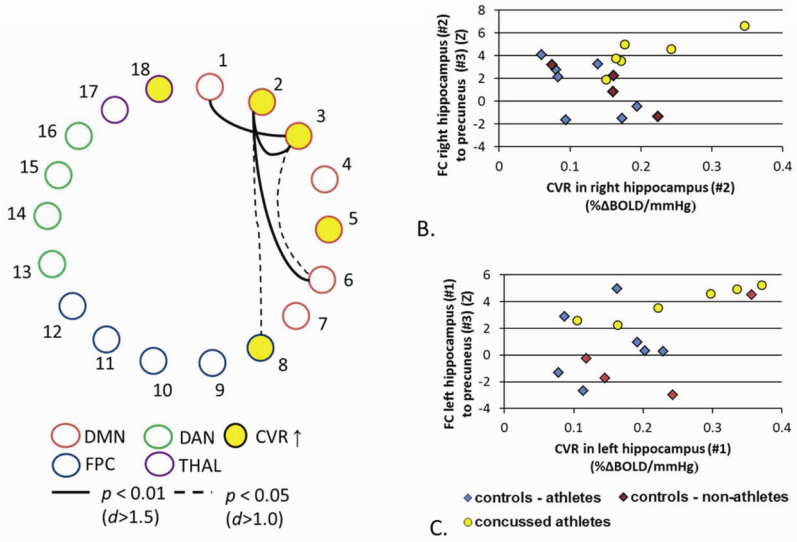


Fig. 4.

Functional connectivity changes in sports concussion. A) FC increases in concussed athletes compared to healthy controls. Nodes of different networks are indicated by different colors as described in the legend. Nodes of increased CVR in concussed athletes are filled in yellow. Numbers of nodes correspond to the numbers in Fig 2. B) Linear relationship between FC in right hippocampus to precuneus and CVR in right hippocampus in controls (p=0.03) and concussed athletes (p=0.03). C) Linear relationship between FC in left hippocampus to precuneus and CVR in left hippocampus in controls (p>0.05) and concussed athletes (p=0.001). Athlete and non-athlete controls are shown in different colors, but regression performed across group as a whole.

Table 1

Subject information

ID	Age (yrs)	M/F	hand	sport	days since injury	Headache score	Rivermead total
Pat01	19	F	R	lacrosse	4	3	25
Pat02	20	M	R	soccer	6	0	1
Pat03	18	F	R	lacrosse	6	1	7
Pat04	19	M	R	baseball	3	2	7
Pat05	22	M	R	soccer	5	2	7
Pat06	20	F	R	soccer	6	1	16
Pat07	20	M	R	baseball	3	3	15
Con01	21	F	R	volleyball	n/a	0	0
Con02	18	F	B	volleyball	n/a	0	0
Con03	20	M	R	none	n/a	0	0
Con04	21	M	R	none	n/a	0	0
Con05	21	F	R	none	n/a	0	0
Con06	18	M	R	none	n/a	0	0
Con07	20	F	L	basketball	n/a	0	0
Con08	21	M	R	cross country	n/a	0	0
Con09	23	M	R	cross country	n/a	0	0
Con10	18	F	R	softball	n/a	0	1
Con11	19	F	L	golf	n/a	0	0

M = Male; F = Female, L = Left; R = Right; B=Bilateral handedness; Headache score (maximum possible = 4) and Rivermead total (maximum possible = 64) come from Rivermead Post-Concussion Symptoms Questionnaire (King et al. 1995).