Bachelor of Science in Medicine Degree Program
End of Term Final Report

Student Name: Philip Pries **Date:** 08/06/17

Project Title: Quantitative neuroimaging assessment of cerebrovascular responsiveness in individual sports-related concussion patients

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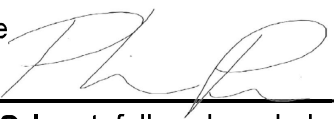
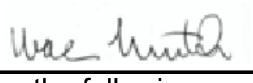
Summary (250 words max single spaced):

Preliminary studies suggest sports-related concussion (SRC) is associated with alterations in cerebral blood flow (CBF) regulation. Here, we use advanced MRI techniques to measure CBF and cerebrovascular responsiveness (CVR) in individual SRC patients and healthy control subjects. Fifteen SRC patients (mean age=16.3, range 14-20 years) and 27 healthy control subjects (mean age=17.6, range 13-21 years) underwent anatomical MRI, pseudo-continuous arterial spin labeling (pCASL) MRI and model-based prospective end-tidal targeting (MPET) of CO2 during blood oxygenation level-dependent (BOLD) MRI. Group differences in global mean resting CBF were examined. Voxel-by-voxel group and individual differences in regional CVR were examined using statistical parametric mapping (SPM). Leave-one-out receiver operating characteristic (ROC) curve analysis was used to evaluate the utility of brain MRI CO2 stress testing biomarkers to correctly discriminate between SRC patients and healthy control subjects. All studies were tolerated with no complications. Traumatic structural findings were identified in one SRC patient. No significant group differences in global mean resting CBF were observed. There were no significant differences in the CO2 stimulus and O2 targeting during BOLD MRI. Significant group and patient-specific differences in CVR were observed with SRC patients demonstrating a predominant pattern of increased CVR. Leave-one-out ROC curve analysis for voxels demonstrating a significant increase in CVR was found to reliably discriminate between SRC patients and healthy control subjects (AUC of 0.879, p=0.0001).

Student Signature  Primary Supervisor Signature 

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Introduction & Background

Expert consensus has historically viewed sports-related concussion (SRC) as an injury resulting in clinical features that arise from transient disturbances in brain functioning occurring in the absence of structural brain injury(McCrory et al., 2013). Clinical studies indicate that the majority of adolescents and adults who sustain a SRC achieve symptomatic and neurocognitive recovery within 1-4 weeks post-injury(McCrea et al., 2013;McCrory et al., 2013;Williams et al., 2015). However, approximately 15-30% of SRC patients will develop persistent post-concussion symptoms that are mediated by heterogeneous pathophysiological processes that remain poorly understood(Ellis et al., 2016a). While advanced neuroimaging studies have provided some insight into these pathophysiological processes, results to date have been largely limited to the detection of differences between groups of SRC patients and healthy control subjects(Yuh et al., 2014;McCrea et al., 2017). As such, there remains an urgent unmet need for novel neuroimaging tools that can provide qualitative and quantitative assessment of these processes in individual SRC patients.

Among the processes implicated in the pathogenesis of acute concussion and traumatic brain injury (TBI) are disturbances in cerebrovascular physiology that result in a mismatch between the metabolic demands of the injured brain and the delivery of cerebral blood flow (CBF)(Bouma et al., 1991;Giza and Hovda, 2001;Coles et al., 2004;Wintermark et al., 2004;Ellis et al., 2016b). One of the means to assess the physiological capacity of the cerebral vasculature to respond appropriately during periods of metabolic stress or injury is cerebrovascular reactivity or responsiveness (CVR). CVR is defined as the change in CBF that occurs in response to a vasoactive stimulus(Bhogal et al., 2015;Leung et al., 2016). Accurate measurement of CVR can be achieved by the administration of a precise, quantifiable, and reproducible vasodilatory stimulus while measuring CBF or its surrogate(Fierstra et al., 2013;Sobczyk et al., 2016). Using model-based prospective end-tidal targeting (MPET) of CO_2 during blood oxygenation leveldependent (BOLD) magnetic resonance imaging (MRI), we have previously demonstrated patient-specific qualitative and quantitative regional alterations in CVR among adolescent post-concussion syndrome patients while global mean resting CBF remained within normal limits(Mutch et al., 2015). Although these results suggest that the cerebrovascular disturbances that characterize acute SRC can persist into the chronic phases of injury, few studies have aimed to quantify alterations in CVR in individual SRC patients with more acute and sub-acute injuries(Len et al., 2011;Len et al., 2013;Militana et al., 2015).

In this study, we examined group differences in global mean resting CBF and CVR in healthy control subjects and adolescent sub-acute SRC patients. Using a leave-one-out receiver operating characteristic (ROC) analysis we examined individual differences in CVR among SRC patients compared to an institutional atlas of healthy adolescent control subjects assessed using the same neuroimaging assessment technique.

Materials & Methods

Research design and clinical assessment

We conducted a case-control study of adolescent SRC patients that were compared to an institutional atlas of healthy control subjects that includes subjects used in previous studies conducted by our group(Mutch et al., 2015;Mutch et al., 2016). Adolescent SRC patients were recruited from the Pan Am Concussion Program, a multidisciplinary pediatric concussion clinic that accepts referrals for children and adolescents with sports- and non sports-related TBI in Winnipeg, Manitoba, Canada. All adolescent SRC patients included in the study underwent a

clinical assessment by a single neurosurgeon. Patient inclusion criteria for this study included: 1) physician diagnosis of SRC according to the definition of the International Consensus on Concussion in Sport guidelines(McCrory et al., 2013); 2) age 13-21 years; 3) patients had to be symptomatic at rest or during exercise at the time of neuroimaging assessment. All healthy control subjects underwent a clinical interview to collect demographic, past medical history, and past concussion history data. Healthy control subject inclusion criteria for the study included: 1) age 13-21 years. Exclusion criteria for control subjects included: 1) the presence of a symptomatic concussion; 2) diagnosis of prior moderate or severe TBI or neurological condition resulting in structural brain abnormality detected on prior neuroimaging; 3) contra-indication to MRI (i.e dental braces, claustrophobia); 4) diagnosis of a neurological condition requiring prescription medication.

In general, SRC patients were deemed clinically recovered when they were asymptomatic at rest, were back to full-time school, had a normal neurological examination and successfully completed the International Consensus on Concussion in Sport return-to-play guidelines(McCrory et al., 2013). Some patients underwent graded aerobic treadmill testing as part of their clinical management to help classify patients into post-traumatic clinical sub-types and inform the design of multi-disciplinary rehabilitation strategies. Patients were diagnosed with autonomic/physiological posttraumatic disorder (PTD) if they demonstrated a symptom-limited threshold on graded aerobic treadmill testing as previously described(Leddy and Willer, 2013;Ellis et al., 2016a).

Institutional research ethics approval was obtained for this study. Informed patient, and where applicable, parental consent was obtained for all participants prior to participating in the study. On the day of neuroimaging all subjects completed the Post-Concussion Symptom Scale (PCSS), a concussion symptom inventory consisting of 22 symptoms that are rated on a 7-point (0-6) Likert scale with a maximum score of (6 × 22) = 132(Kontos et al., 2012).

MRI assessment

Prior to neuroimaging assessment, all study subjects were exposed to a short trial of the breathing sequence to familiarize them with the changes in breathing that would occur while in the MRI scanner. During MRI assessment, subjects underwent noninvasive heart rate, pulse oximetry, and blood pressure (BP) monitoring. Subjects were also given a hand-held call button that allowed them to voluntary terminate the test at any time. Neuroimaging assessment in this study consisted of 1) anatomical imaging; 2) BOLD CVR mapping; and 3) global resting CBF measurement.

Images were acquired using a Siemens Verio 3.0T MR scanner with a 12-channel phased-array head coil. Anatomical imaging was acquired without manipulation of end-tidal gases using a sagittal 3D T1 MPRAGE (whole brain coverage; matrix: 256×256; slice thickness: 2.2 mm; no interslice gap; voxel size 2×2×2 mm) and axial gradient recalled echo planar (GRE) sequences to screen for cerebral microhemorrhages as well as GRE B0-field mapping. CVR was assessed using continuous BOLD MRI during MPET CO_2 targeting. BOLD MRI data was acquired using a T2*-weighted single-shot gradient echo pulse sequence with echoplanar readout (field of view: 24×24 cm; matrix: 64×64; TR: 2000 ms; TE: 30 ms; flip angle: 85°; slice thickness: 5.0 mm; interslice gap: 2.0 mm; voxel size 3.75×3.75×6 mm; number of temporal frames=330; 10 seconds of initial imaging data was discarded to allow for equilibration). Global mean resting CBF was assessed using pseudo-continuous arterial spin labeling (pCASL) that included an initial M0 scan - Siemens ep2d_pCASL – echo planar readout (field of view 24 × 24 cm, TR 8000 ms, TE 12 ms, contrast with a flip angle 900, 20 slices, CASL method – multislice,

label offset 90 mm, post label delay 1200 ms, crusher gradient 0 s/mm2; voxel size 3.8 × 3.8 × 5.0 mm. The formal pCASL sequence consisted of a echo planar readout (field of view 24 × 24 cm, TR 4000 ms, TE 12 ms, contrast with a flip angle 900, 20 slices, slice thickness 5.0 mm, CASL method – multislice, label offset 90 mm, post label delay 1200 ms, crusher gradient 0 s/mm2; voxel size 3.8 × 3.8 × 5.0 mm. Imaging duration was for 3 minutes. The first 2 labeled-nonlabeled pairs were discarded.

The vasodilatory stimulus used for CVR mapping in this study consisted of precise delivery of CO_2 and O_2 via a computer-controlled gas blender (RespirAct, Thornhill Research Inc, Toronto, ON) connected to a re-breathing circuit and mask secured to the subject's face. This device allows MPET targeting of $P_{ET}CO_2$ and under isoxic conditions while providing continuous breath-by-breath measurement of $P_{ET}CO_2$ and $P_{ET}O_2$. Previous work has demonstrated that measurement of $P_{ET}CO_2$ is an accurate surrogate for $PaCO_2$ during MPET CO_2 targeting(Ito et al., 2008). The breathing sequence used for CVR mapping included a triple stimulus square-wave sequence consisting of interval step-changes as follows: baseline $P_{ET}CO_2$ (120 seconds), hypercapnia (5 mmHg above baseline for 120 seconds), baseline $P_{ET}CO_2$ (30 seconds), hypercapnia (5 mmHg above baseline for 120 seconds), baseline $P_{ET}CO_2$ (30 seconds), hypercapnia (5 mmHg above baseline for 120 seconds), baseline $P_{ET}CO_2$ (120 seconds). During this sequence a stable $P_{ET}O_2$ of 115 mmHg was targeted. A representative illustration of the breathing sequence is provided in Figure 1.

Data processing

Prior to data processing, subject head motion artifact and end-tidal CO_2 targeting was examined. Study subjects were excluded if motion over the course of the imaging acquisition was greater than 3 mm in any plane or if inadequate end-tidal CO_2 targeting was identified during BOLD MRI acquisition. Standard pre-processing of MRI EPI output was carried out for the BOLD MRI data using SPM8 software including batch processing by an SPM toolbox and custom written in-house MatLab scripts. The BOLD data were interpolated to the MPRAGE voxel dimensions. The fMRI model specification was a 2-pass process. First, the data was modeled using the finite impulse response (FIR) package in SPM. The MPET triple stimulus square-wave sequence was assessed with a zero offset. An event-related response was calculated by examining the whole brain response to the CO_2 stimulus. The time to maximal response to the CO_2 stimulus was corrected based on a constructed series of time delay block stimulus files generated with delays from 0-30 seconds. Secondly, following determination of the time delay for the CO_2 stimulus to the brain the newly generated time delay-based fMRI model was rerun as above.

Further pre-processing was conducted using a motion correction file generated with realignment as regressors in the model. First level CVR analyses were undertaken using the time corrected analysis and the contrast images generated were then used in the second level CVR analysis. In order to account for BOLD EPI signal inhomogeneities at the skull base and within the choroid plexus and periventricular white matter (Thomas et al., 2013) individualized masks were constructed as previously described (Mutch et al., 2016). Masked images [labeled (gm+wm) – (B0 + dil_ventricles)] were used in the second level CVR analysis to ensure that the abnormal voxels for each individual study were intraparenchymal and not contaminated by these other sources of BOLD EPI signal inhomogeneities. First level CVR analyses were undertaken with the author blinded to the subject's group (healthy control subject versus SRC patient), however second level CVR analyses were not blinded since they were based on the results of the first level analysis. An ASL toolbox was used to undertake pre-processing of the pCASL data (http://www.cfn.upenn.edu/~zewang/ASLtbx.php).

Statistical analysis

Anatomical Imaging: Anatomical imaging was reviewed and reported by a board certified neuro-radiologist.

Global mean resting CBF: CBF was calculated on a voxel-by-voxel basis at rest and used to calculate a global mean resting CBF for all study subjects.

Cerebrovascular responsiveness (first level analysis): First level analyses for the CVR data were undertaken for each study participant. BOLD increases and decreases in response to the MPET triple stimulus square-wave sequence was assessed at the p=0.001 level. The cluster size threshold was 10 voxels.

Cerebrovascular responsiveness (second level analysis): Second level analysis for the CVR data was completed among individual SRC patients and control subjects. Voxel-byvoxel comparisons on an individual basis for the SCR patients was conducted over a series of p-values to identify voxels that responded less than or greater than the mean healthy control group responses to the CO_2 stimulus from the atlas (i.e abnormal voxel counts). For each individual second level comparison the images were masked using the combined individual mask described above versus the atlas output. Voxel counts calculated for each subject were based on the applied individual mask. For the control subjects each of their contrast images were removed in turn (leave one out) from the atlas to determine the 2nd level voxel counts for that control subject. We report on the p = 0.005 values for each patient and subject.

Leave-one-out receiver operating characteristic (ROC) curve analysis: Leave-one-out ROC analyses were undertaken to determine whether abnormal voxel counts derived from the second level CVR analysis could accurately discriminate between individual SRC patients and the leave-one-out results determined for each healthy control subject on a quantitative basis. Second level analysis voxel counts for each individual (leave one out for that individual vs the rest of the control atlas subjects and each individual SRC patient vs the complete control atlas) were determined based on the p=0.005 greater than and less than voxel counts for each individual. The ROC curves were constructed comparing the leave-one-out counts for each control subject (SRC = 0) and the counts calculated for each SRC patient (SRC = 1). The Analyse-It statistical package for Excel 2010 was used to calculate ROC AUC values for these voxel counts.

Results

Participants: A total of 55 subjects were enrolled in the study including 37 healthy control subjects and 18 SRC patients however, subject data for 7 controls and 3 SRC patients were excluded due to excessive motion and data for 3 controls was excluded due to poor end-tidal CO_2 targeting. Therefore, 27 healthy control subjects (13 male, 14 female, mean age=17.6, SD=2.6, range 13-21) and 15 SRC patients (9 male, 6 female, mean age=16.3, SD=1.6, range 14-20 years) were included in the final analysis. Two acute SRC patients included in a prior study of longitudinal CVR imaging were included(Mutch et al., 2016). Normal control subject past medical history was significant for previous concussion in 5/27 (19%) and migraine headaches 1/27 (4%). Past medical history among SRC patients included previous concussion in 12/15 (80%) and pre-injury migraine headaches in 2/15 (13%). The mean PCSS score among healthy controls at the time of neuroimaging assessment was 1 (range 0-7, 1 subject had missing data). The mean PCSS score among SRC patients at the time of neuroimaging

assessment was 42 (range 1-82). The mean time from injury to neuroimaging assessment for the SRC patients was 16 days (range 3-32 days). Additional clinical data are summarized in Table 1.

Study tolerability: All neuroimaging assessments were completed without any serious adverse events. No study subjects voluntarily terminated the test. Three SRC patients developed transient and self-limiting symptoms during neuroimaging assessment including mild headache, lightheadedness and fatigue. None of these patients required any medical attention for these symptoms.

Anatomical imaging: Anatomical imaging studies were normal in 26/27 (96%) of healthy control subjects with 1 patient found to have a right frontal developmental venous anomaly. Anatomical imaging studies were normal in 11/15 (73%) of SRC patients. Abnormalities found among SRC patients included a left frontal developmental venous anomaly (1 patient), pineal cyst (1 patient), non-functioning pituitary microadenoma (1 patient), and a traumatic micro-hemorrhage located in the splenium of the corpus callosum that was identified on imaging obtained following a previous concussion (1 patient).

End-tidal gas targeting and hemodynamics: No differences in baseline $P_{ET}CO_2$ (healthy control subjects 40.6 ± 4.1 mm Hg; SRC patients: 40.7 ± 4.4 mm Hg; p = 0.95) and the change in $P_{ET}CO_2$ during MPET CO_2 targeting (healthy control subjects 4.0 ± 0.9 mm Hg; SRC patients: 4.0 ± 1.1 mm Hg; p = 0.98) were detected between the SRC patients and healthy control subjects. No differences in mean $P_{ET}O_2$ over the period of the CO_2 challenge was seen between groups (healthy control subjects 112.6 ± 3.2 mm Hg; SRC patients: 112.6 ± 2.4 mm Hg; p = 0.94). These results indicate that both groups were exposed to the same magnitude of hypercapnic stimulus under isoxic conditions during neuroimaging assessment.

Global mean resting CBF: There were no significant group differences in global mean resting CBF among SRC patients (36.4 ± 7.0 L/100g/min) and healthy control subjects (41.0 ± 9.7 mL/100g/min; p=0.133).

Cerebrovascular responsiveness (second level analysis): Group comparisons between the SRC patients and normal control subjects revealed significant differences in CVR (higher group CVR in SRC patients in 5.5% of total voxels and lower CVR in SRC patients at 0.0007% of total voxels when compared between groups at the p = 0.01 level respectively; see Figure 2). Second level analysis comparing individual SRC patients to the healthy control atlas demonstrated patient-specific alterations in CVR (see Figure 3). The ROC curve for brain regions manifesting a response less than (AUC=0.468; p = NS) and greater than (AUC=0.879; p<0.0001) mean control group responses at the second level analysis voxel counts at the p=0.005 level is shown in Figure 4. The optimal cut-off for increased CVR declarative for SRC was 1899 voxels resulting in a sensitivity of 0.867 and a specificity of 0.778 for this specific ROC analysis. There was no correlation between abnormal voxel counts and PCSS scores among SRC patients.

Discussion

This study examined global mean resting CBF and global and regional CVR in adolescent SRC patients and healthy controls subjects. In a heterogeneous population of SRC patients who underwent neuroimaging assessment at various stages of acute and sub-acute injury we observed significant group differences in CVR that occurred in the setting of normal global mean resting CBF. When compared to an atlas of healthy control subjects, we found that

SRC patients demonstrated significant patient-specific alterations in CVR with a predominant pattern of increased CVR including those patients that were found to demonstrate exercise intolerance during graded aerobic treadmill testing and were diagnosed with autonomic/physiological PTD. Furthermore, a preliminary leave-one-out ROC analysis utilizing the quantitative biomarker of increased CVR response (voxels demonstrating an increased response to a controlled hypercapnic stimulus) generated from brain MRI CO_2 stress testing was found to reliably discriminate patients with and without SRC with an ROC AUC of 0.879 (p=0.0001). At a cut-point of approximately 1900 voxels for increased CVR derived from our second level analysis at the 0.005 level this biomarker resulted in a sensitivity of 87% and a specificity of 78% for correctly identifying patients with SRC. There was no discrimination seen for a decreased CVR response for these SRC patients (AUC = 0.468). Importantly, all neuroimaging studies were well tolerated with no complications.

At present, the natural history of CVR impairments following SRC remains poorly understood due in large part to the relative paucity of studies that have examined CVR during the acute, sub-acute, and chronic stages of injury. Among available studies, Len et al.(Len et al., 2011) examined CVR in 21 healthy controls and 10 SRC patients within 7 days of injury using transcranial Doppler ultrasonography of the right middle cerebral artery (MCA) and a breath-holding and hyperventilation challenge. No significant differences in percent change in MCA velocity was observed between the SRC and control group during breath-holding, however greater reductions in percent grouped mean MCA velocity were observed among SRC patients during the hyperventilation challenge. Using the same technique, Len et al.(Len et al., 2013) examined CVR in 20 SRC patients at 2, 4 and 8 days post-injury including 18 that underwent baseline pre-season or post-season evaluations. During breath-holding mean MCA velocity was found to be increased on day 2 and decreased on days 4 and 8 with less robust findings observed during hyperventilation. Miltana et al.(Militana et al., 2015) examined CVR in 11 healthy controls and 6 SRC patients 3-6 days post-injury by measuring alterations in BOLD MRI signal during an inhaled CO_2 challenge. In the SRC group, they observed increased CVR for all pre-selected regions of interests including significant increases within the median default mode network and the anterior cingulate.They also found that increased CVR in the hippocampus was positively correlated with increased functional connectivity between the hippocampus and precuneus in the SRC group but not in the healthy control group. The authors also found no group differences in resting mean CBF between groups similar to the results of our present study. Finally, in a previous study our group examined CVR in 17 healthy control subjects and 15 adolescent SRC patients who remained symptomatic during the chronic phase of injury (33-993 days post-injury)(Mutch et al., 2015). Using MPET of CO_2 and BOLD MRI we observed significant group differences in CVR characterized by a predominant pattern of decreased CVR when group means were compared. More importantly when compared to the healthy controls on an individual basis, we found that each SRC patient demonstrated a unique signature of both increased and decreased regional CVR even in the setting of normal global mean resting CBF.

The differences in CVR findings observed across available studies are undoubtedly impacted by the techniques used to measure CVR. To achieve accurate within- and between-subject measures of CVR requires a precise, quantifiable, and reproducible vasodilatory stimulus to be administered to all study participants during assessment of CBF. Although transcranial Doppler and BOLD MRI are commonly used techniques to assess cerebral blood velocity and CBF respectively, the vasoactive stimulus chosen to provide the stress for the CVR study each have their own strengths and limitations. Breath-holding is one of the most widely used methods to induce hypercapnia due to ease of use and lack of requirement for specialized personnel or equipment. Unfortunately, breath-holding fails to result in a linear rise in the arterial partial pressure of carbon dioxide ($PaCO_2$) over time(Stock et al., 2006); the $P_{ET}CO_2$ detected at

the end of breath-holding (the measured stimulus) is not always equal to the $PaCO_2$ (the stimulus acting on the cerebral vasculature); and $P_{ET}CO_2$ and $P_{ET}O_2$ levels cannot be sampled throughout the breath-holding periods(Fierstra et al., 2013). Together, these limitations can lead to significant differences in the vasodilatory stimuli administered across groups and subjects thereby impacting CVR measurement and contributing to experimental noise. This finding is illustrated in the study by Len et al.(Len et al., 2011) discussed above, where mean $P_{ET}CO_2$ levels of 41.2 and 37.9 mm Hg among healthy controls and SRC patients respectively were obtained during breathholding; an important difference between the two groups studied. An alternative method for providing a hypercapnic stimulus is inhaled CO_2. Although this technique relies on the administration of a gas containing a fixed fractional concentration of CO_2, the resultant effect on $PaCO_2$ has also been found to vary considerably between subjects and within the same subject over serial administrations(Hoskins, 1990;Prisman et al., 2008). Importantly, both breath-holding and inhaled CO_2 techniques can also lead to changes in PaO_2 that can result in independent and unaccounted for changes in CBF and CVR, especially with BOLD MRI approaches. In the study by Miltana et al.(Militana et al., 2015) utilizing inhaled CO_2, resting $P_{ET}CO_2$ levels at baseline and during the CO_2 challenge were not reported. In contrast, the MPET CO_2 used here and in our previous studies(Adelson et al., 2011;Mutch et al., 2015;Mutch et al., 2016) has emerged as the only technique that is capable of providing a precise, quantifiable, and reproducible CO_2 stimulus under controlled isoxic conditions(Fierstra et al., 2013). This technique employs end-inspiratory rebreathing that ensures the $P_{ET}CO_2$ is equivalent to the $PaCO_2$(Ito et al., 2008) as well as continuous breath-by-breath sampling(Mark et al., 2010). By combining this vasodilatory stimulus with BOLD MRI and standardized data analysis techniques, brain MRI CO_2 stress testing results in highly reliable within(Spano et al., 2013;Leung et al., 2016) and between subject measures of CVR that can be quantified on an individual subject basis(Sobczyk et al., 2015;Ellis et al., 2016b;Sobczyk et al., 2016). In this study, we observed no significant group differences in baseline $P_{ET}CO_2$ or $P_{ET}CO_2$ and $P_{ET}O_2$ during MPET targeting suggesting the results of brain MRI CO_2 stress testing obtained here are indeed a true reflection of abnormal CVR in these patients and are not due to variations in the vasoactive stimuli applied to these subjects.

Taken together, the available preliminary literature suggests SRC is associated with patient-specific impairments in CVR that are detectable by transcranial Doppler and MRI-based techniques during acute injury but, to date, only by MRI-based techniques during the sub-acute and chronic phases of injury. Results from MRI-based studies suggest that SRC may be characterized by a predominant pattern of increased CVR among acute and sub-acute patients as well as those that develop autonomic/physiological PTD and a predominate pattern of decreased CVR among patients that develop more chronic symptoms. Interestingly, the results of available MRI-based CVR studies do not provide supportive evidence of a correlation between the severity of post-concussion symptoms and the magnitude of CVR alterations detected among SRC patients at different stages of recovery. Most importantly, this study confirms that brain MRI CO_2 stress testing is well tolerated in patients with acute and sub-acute SRC and may be capable of quantifying the extent of CVR impairments in individual SRC patients.

The results of the present study must be considered in light of several important limitations. First, the sample size is relatively small and included adolescent patients that were recruited from a multi-disciplinary pediatric concussion program. Patients evaluated at tertiary concussion programs are likely to have sustained more severe injuries that take longer to recover and therefore may be more likely to exhibit a greater impairment in CVR compared to a more generalized population of adolescent SRC patients. Second, the patient population examined in this study included those with a significant history of previous concussions.

Although approximately 50% of patients that present to our multidisciplinary pediatric concussion program have a history of previous concussion(Ellis et al., 2017), the lifetime burden of concussions among the SRC patients evaluated in this study may have had an impact on the magnitude of CVR alterations detected in this cohort. Indeed, preliminary longitudinal studies suggest that the cerebrovascular disturbances that accompany SRC may persist beyond symptomatic recovery(Maugans et al., 2012;Wang et al., 2015;Mutch et al., 2016). Third, the SRC patients in this study were imaged at non-uniform time-points following injury, thus limiting direct comparisons between individual subjects. As such, future prospective longitudinal studies incorporating larger samples of adolescents and adults without a history of previous concussion who are imaged at uniform time-points are needed to evaluate the impact of individual concussions on CVR alterations during the acute and clinically recovered stages of injury. Fourth, the incorporation of individualized masks during imaging postprocessing removed from analysis some of the prefrontal cortex and periventricular white matter, thus potentially resulting in an under-detection of abnormal voxels within these regions. Fifth, one of the most important limitations of this neuroimaging assessment technique is the effect of subject motion on accurate CBF and CVR measurements. It is well documented that both BOLD EPI and arterial spin labeling techniques are highly susceptible to head motion(Tanenbaum et al., 2015;Turner et al., 2015), which can have a confounding influence on BOLD and pCASL MRI CVR measurements. Although ongoing modifications to our brain MRI CO_2 stress testing technique, including a reduction in the magnitude of the CO_2 stimulus, have helped reduce the work of breathing and head movement during imaging acquisition, this limitation did result in the exclusion of a number of participants from the present study. Future studies should consider the use of head restraints and pre-emptive motion correction techniques(Zun et al., 2014;Zaitsev et al., 2016) as well as ramp CO_2 breathing sequences(Mutch et al., 2012;Duffin et al., 2015) that may be more tolerable than the abrupt changes associated with square-wave sequences as used here. Progressive CO_2 breathing sequences will also allow help facilitate the measurement of dynamic cerebrovascular responses to hypercapnic stimuli(Fisher et al., 2017). Sixth, although the leave-one-out ROC analysis in this study suggests that quantitative increases in CVR may be a promising biomarker to reliably distinguish between SRC patients and normal control subjects, quantitative voxel cut-points will likely vary across future studies as a consequence of institutional and study differences in SRC patients, normal control subjects, and the vasoactive stimuli and neuroimaging platforms utilized. These methodological issues should be carefully considered when comparing results across previous and future CVR studies in this patient population. Lastly, the neuroimaging assessment techniques utilized in this study only allowed for accurate and reliable measurement of global mean resting CBF and CVR and did not assess for other mechanisms that may contribute to the pathophysiology of TBI and patient symptoms including alterations in white matter integrity and resting state functional brain networks(Yuh et al., 2014;McCrea et al., 2017) which may have accounted for the small false positive and false negative rates observed in this study. Consequently, future studies combining brain MRI CO_2 stress testing with other advanced neuroimaging techniques such as quantitative susceptibility-weighted imaging, DTI, and resting state fMRI are needed to provide a more comprehensive assessment of the complex pathophysiological mechanisms mediating SRC and mTBI and examine the potential diagnostic and clinical utility of these multi-modal neuroimaging tools in individual patient diagnosis and management.

In conclusion, this study provides evidence that SRC is associated with patient specific alterations in CVR that occur in the presence of normal global mean resting CBF. This study suggests that with meticulous attention paid to end-tidal gas control and head motion, brain MRI CO_2 stress testing is capable of detecting and quantifying abnormal CVR among individual SRC patients over a wide range of injury acuities and post-injury time-points. The results from our preliminary leave-one-out ROC analysis suggests that alterations in CVR detected by brain MRI

CO_2 stress testing may be a useful qualitative and qualitative biomarker to help reliably identify individual subjects with SRC. Larger prospective studies are needed to evaluate the clinical utility of brain MRI CO_2 stress testing in the diagnosis, prognosis, and management of individual concussion and mild TBI patients.

Tables & Figures

Table 1: Summary of demographic, clinical features, outcomes, and quantitative brain MRI CO_2 stress testing results in sports-related concussion patients.

Subject	Age/ gender	Past medical history	Sport	Time from injury to imaging assessment	PCSS score at time of imaging assess-ment	Abnormal voxel counts (at imaging assessment, p<0.005)	Clinical outcomes
1	16M	3 previous concussions	Football	29	8	Greater: 19973 Less: 370 Total: 20343	- Autonomic/ Physiological PTD - Required medication for post-traumatic headaches - Remained symptomatic at last follow-up (154 days)
2	16M	1 previous concussion	Football	13	82	Greater: 21008 Less: 268 Total: 21276	- Clinically recovered (41 days post-injury)
3	20M	2 previous concussions	Hockey	3	1	Greater: 12885 Less: 176 Total: 13061	- Clinically recovered (9 days post-injury)
4	17F	1 previous concussion	Basketball	27	58	Greater: 1002 Less: 0 Total: 1002	- Autonomic/ Physiological PTD - Remained symptomatic at last follow-up (90 days post-injury)
5	17M	1 previous concussion	Hockey	7	69	Greater: 2091 Less: 210 Total: 2301	- Clinically recovered (34 days post-injury)
6	14M	2 previous concussions, migraine headaches	Hockey	6	46	Greater: 1607 Less: 49 Total: 1656	- Autonomic/ Physiological PTD - Remained symptomatic at last follow-up (40 days post-injury)
7	19F	2 previous concussions	Hockey	7	18	Greater: 35429 Less: 93 Total: 35522	- Autonomic/ Physiological PTD - Clinically recovered (140 days post-injury)
8	16F	None	Rugby	22	56	Greater: 14695 Less: 11 Total: 14706	- Autonomic/ Physiological PTD - Clinically recovered (211 days post-injury)
9	17M	3 previous concussions, depression	Football	4	24	Greater: 12575 Less: 16 Total: 12591	- Remains in treatment - Abnormal formal neuropsychological testing (196 days post-injury)
10	14F	2 previous concussions	Volleyball	12	56	Greater: 16846 Less: 45 Total: 16891	- Autonomic/ Physiological PTD - Remained

							symptomatic at last follow-up (60 days post-injury)
11	15M	1 previous concussion	Hockey	8	48	Greater: 7772 Less: 66 Total: 7838	- Clinically recovered (21 days post-injury)
12	16M	None	Football	27	23	Greater: 4199 Less: 20 Total: 4219	-Autonomic/ Physiological PTD - Clinically recovered (84 days post-injury)
13	15F	None	Soccer	25	42	Greater: 5717 Less: 694 Total: 6411	- Autonomic/ Physiological PTD - Clinically recovered (39 days post-injury)
14	16M	1 previous concussion	Hockey	32	20	Greater: 9890 Less: 0 Total: 9890	- Autonomic/ Physiological PTD - Clinically recovered (101 days post-injury)
15	16F	3 previous concussions, migraine headaches	Ringette	18	71	Greater: 20331 Less: 15 Total: 20346	- Autonomic/ Physiological PTD - BPPV - Clinically recovered (55 days post-injury)

Figure Legend: PCSS=Post-Concussion Symptom Scale; M=male; F=female; PTD=post traumatic disorder; BPPV=benign paroxysmal positional vertigo

Figure 1: Representative diagram of the controlled square-wave triple hypercapnic stimulus under isoxic conditions used during BOLD MRI.

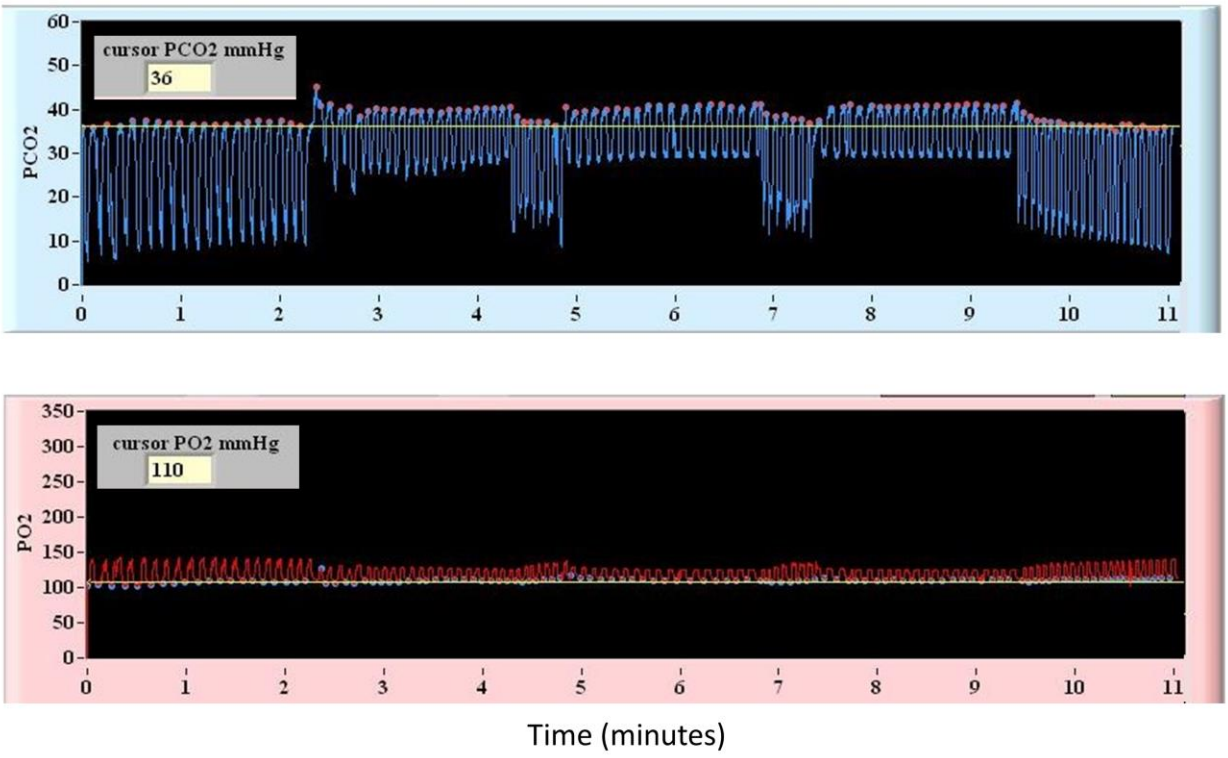


Figure 2: Second level group comparisons of SRC patients against the normal control atlas demonstrates significant differences in global BOLD cerebrovascular responsiveness with a predominant pattern of increased CVR. Examined at the p=0.01 level. Global and regional significant differences in voxel-by-voxel responses that were greater than and less than the mean healthy control atlas response are indicated in color. Hot scale t-statistic level (orange hues) indicate where the SRC group had a greater response to the CO_2 stimulus when compared to the control group; cold scale t-statistic level (blue hues) indicate where the SRC group had a diminished response to the CO_2 stimulus compared to the control group.

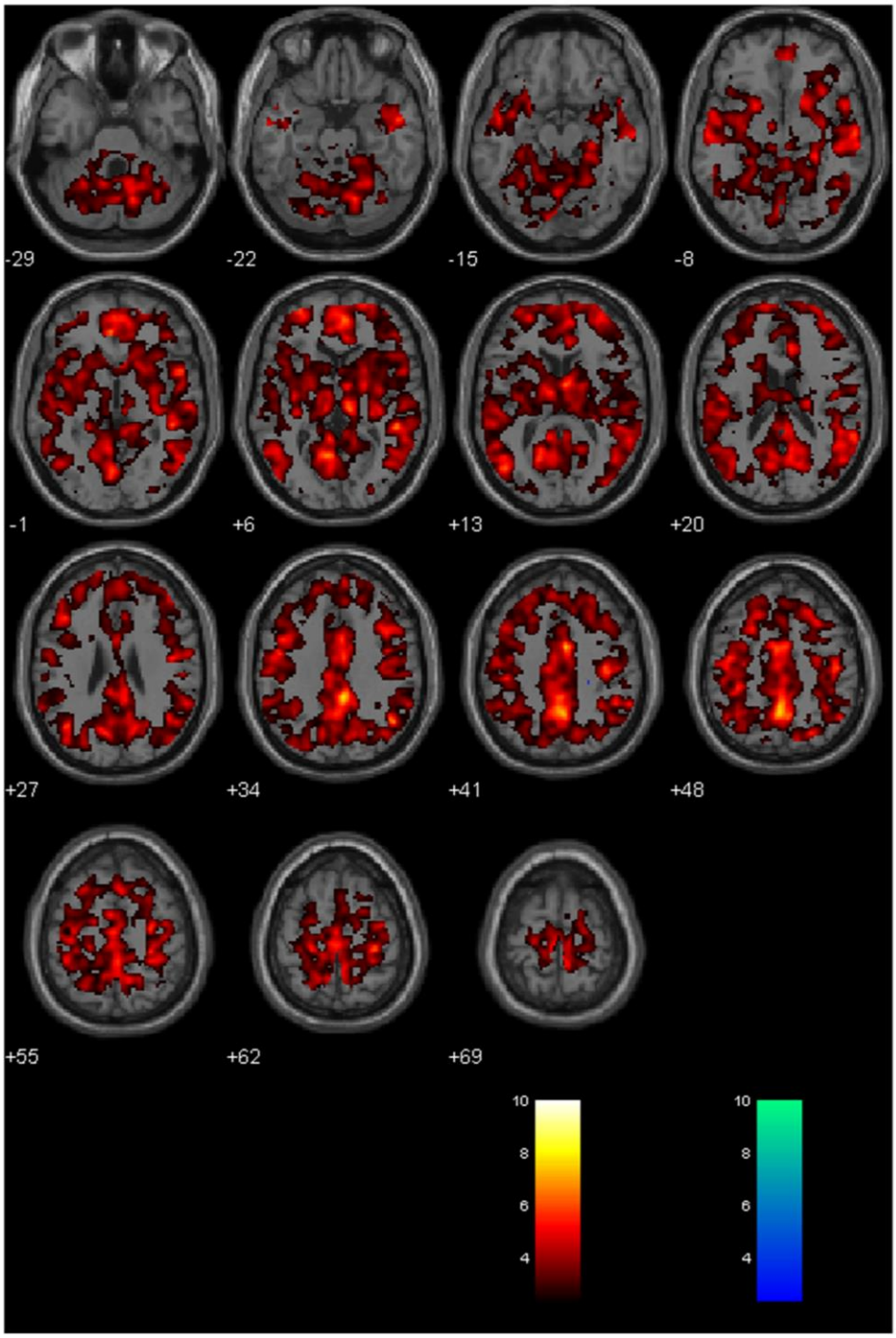


Figure 3: Second level comparisons of one individual control subject (left panel) and 2 SRC patients (middle and right panel) against the normal control atlas. Examined at the p=0.005 level. Global and regional significant differences in voxel-by-voxel responses that were greater than and less than the mean healthy control atlas response are indicated in color. Hot scale t-statistic level (orange hues) indicate where the individual subject had a greater response to the CO_2 stimulus when compared to the control group; cold scale t-statistic level (blue hues) indicate where the individual subject had a diminished response to the CO_2 stimulus compared to the control group.

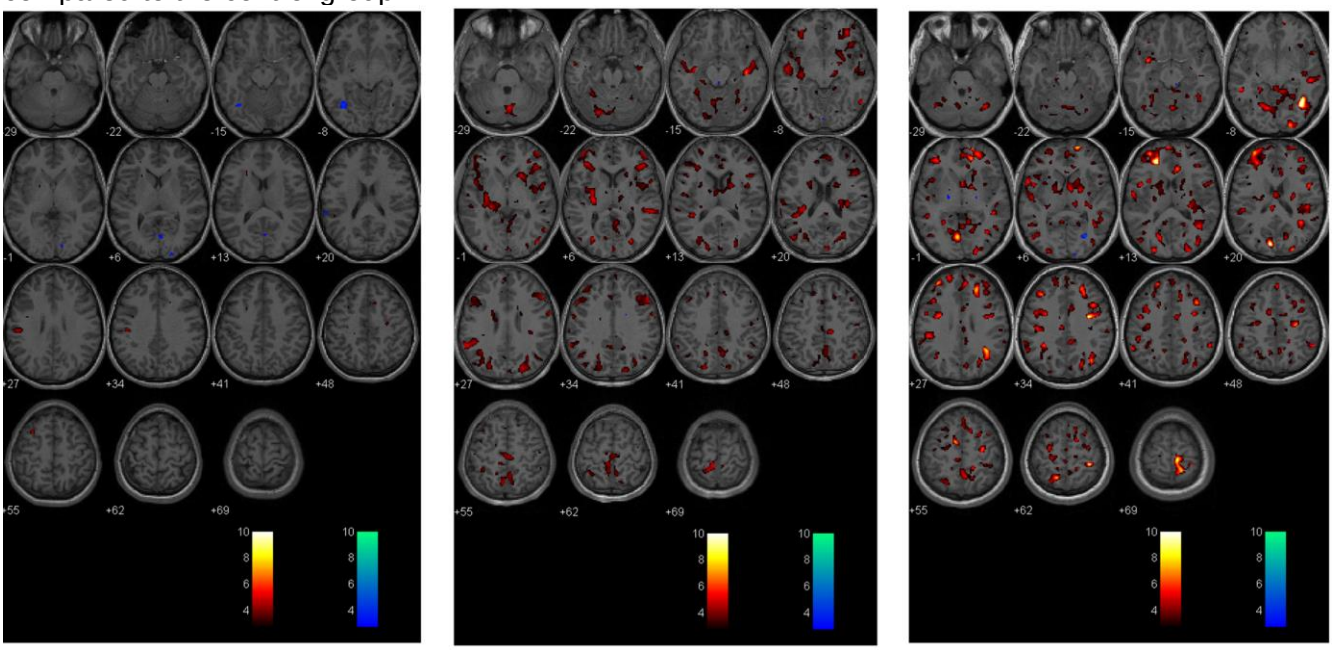
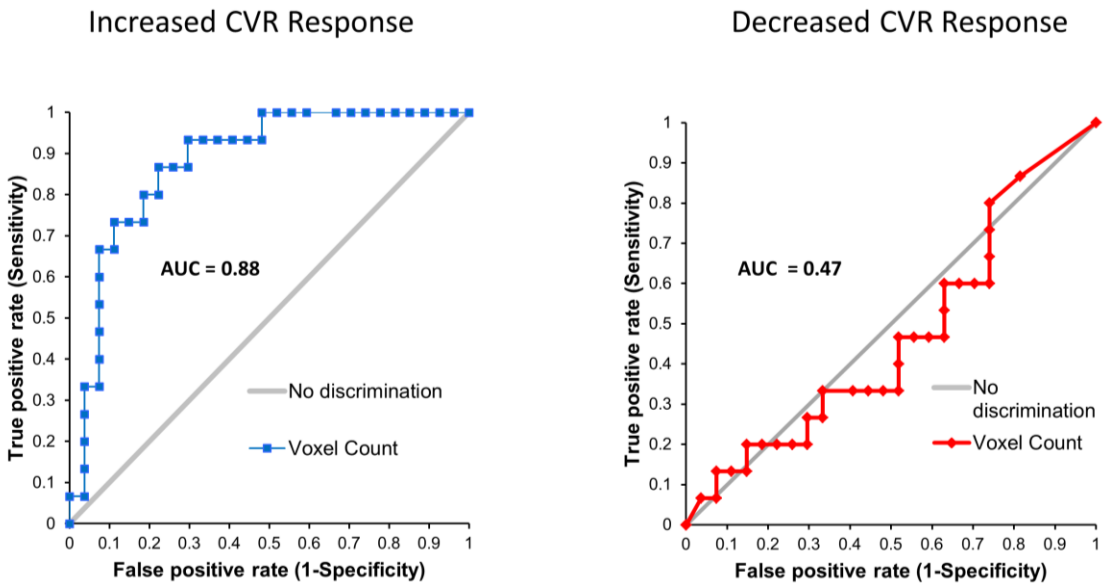


Figure 4: Leave-one-out receiver operating characteristic curves for voxels manifesting a BOLD MRI response to the CO_2 stimulus that was greater than (A) and less than (B) the mean response of the control atlas at the p = 0.005 level.



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