Brain magnetic resonance imaging CO₂ stress testing in adolescent postconcussion syndrome

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OBJECTIVE A neuroimaging assessment tool to visualize global and regional impairments in cerebral blood flow (CBF) and cerebrovascular responsiveness in individual patients with concussion remains elusive. Here the authors summarize the safety, feasibility, and results of brain CO₂ stress testing in adolescents with postconcussion syndrome (PCS) and healthy controls.

METHODS This study was approved by the Biomedical Research Ethics Board at the University of Manitoba. Fifteen adolescents with PCS and 17 healthy control subjects underwent anatomical MRI, pseudo-continuous arterial spin labeling MRI, and brain stress testing using controlled CO₂ challenge and blood oxygen level–dependent (BOLD) MRI. Post hoc processing was performed using statistical parametric mapping to determine voxel-by-voxel regional resting CBF and cerebrovascular responsiveness of the brain to the CO₂ stimulus (increase in BOLD signal) or the inverse (decrease in BOLD signal). Receiver operating characteristic (ROC) curves were generated to compare voxel counts categorized by control (0) or PCS (1).

RESULTS Studies were well tolerated without any serious adverse events. Anatomical MRI was normal in all study participants. No differences in CO₂ stimuli were seen between the 2 participant groups. No group differences in global mean CBF were detected between PCS patients and healthy controls. Patient-specific differences in mean regional CBF and CO₂ BOLD responsiveness were observed in all PCS patients. The ROC curve analysis for brain regions manifesting a voxel response greater than and less than the control atlas (that is, abnormal voxel counts) produced an area under the curve of 0.87 ($p < 0.0001$) and 0.80 ($p = 0.0003$), respectively, consistent with a clinically useful predictive model.

CONCLUSIONS Adolescent PCS is associated with patient-specific abnormalities in regional mean CBF and BOLD cerebrovascular responsiveness that occur in the setting of normal global resting CBF. Future prospective studies are warranted to examine the utility of brain MRI CO₂ stress testing in the longitudinal assessment of acute sports-related concussion and PCS.

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KEY WORDS sports-related concussion; adolescent; postconcussion syndrome; magnetic resonance imaging; blood oxygen level–dependent imaging; trauma

ABBREVIATIONS ASL = arterial spin labeling; AUC = area under the curve; BOLD = blood oxygen level–dependent; CBF = cerebral blood flow; CVR = cerebrovascular reactivity; dmlC = dorsal mid–insular cortex; DTI = diffusion tensor imaging; ET = end-tidal; GRE = gradient recalled echo planar; fMRI = functional MRI; MNI = Montreal Neurological Institute; MPET = model-based prospective end-tidal; MPRAGE = magnetization-prepared rapid gradient-echo; PaCO₂ = arterial partial pressure of carbon dioxide; pCASL = pseudo-continuous arterial spin labeling; PCD = postconcussion disorder; PCS = postconcussion syndrome; PCSS = Post-Concussion Symptom Scale; ROC = receiver operating characteristic; SPM = statistical parametric mapping; SRC = sports-related concussion; TBI = traumatic brain injury.

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CHILDREN and adolescents who develop postconcussion syndrome (PCS) following sports-related concussion (SRC) represent a unique population with limited treatment options.^{6,14,52,54} Although animal studies suggest that acute concussion is characterized by temporary alterations in neural activity, cellular metabolism, and cerebral blood flow (CBF),^{8,21,22} the pathophysiological mechanisms mediating PCS remain unclear. Clinical studies suggest that clusters of PCS symptoms may be mediated by alterations in global brain metabolism or dysfunction within the vestibulo-ocular or cervical spine neurological subsystems.^{16,32,34} Unfortunately, to date, few clinical diagnostic tools provide a window into these pathophysiological processes. Clinical neuroimaging studies, including conventional MRI, are normal in the majority of cases.¹⁷ For this reason, authors of recent studies have aimed to apply even more sophisticated neuroimaging techniques to this condition, including magnetoencephalography,⁵³ diffusion tensor imaging (DTI),^{11,25,29,31,44,47} and task-based functional MRI (fMRI).^{7,26,35,49} Despite detecting changes between groups of concussion patients and healthy controls, none of these techniques has emerged as a useful clinical tool that contributes to the management of acute SRC or PCS.^{15,55} Thus, there remains an urgent need for novel neuroimaging tools that can detect alterations in brain physiology in individual concussion patients and generate useful quantitative biomarkers that can aid in the diagnosis, classification, longitudinal assessment, and management of this condition.

Regulation of CBF is mediated by a number of factors including the arterial partial pressure of carbon dioxide (PaCO₂), the most potent physiological stimulus of arterial dilation whereby each mm Hg increase in PaCO₂ is associated with a 2%–15% increase in CBF.^{42,43,45,48} Cerebrovascular responsiveness or reactivity, defined as the change in CBF in response to a vasoactive stimulus, is an important process by which the brain regulates CBF under normal and pathological conditions.⁵⁰ Previous studies using controlled CO₂ challenge and advanced neuroimaging techniques have demonstrated global and regional impairments in cerebrovascular responsiveness in patients with cerebrovascular disorders such as stroke and traumatic brain injury (TBI).^{1,2,9,10,24,36,37,40} To address these issues in concussion, a recent pilot study from our group introduced a novel MRI brain stress test utilizing blood oxygen level-dependent (BOLD) MRI during model-based prospective end-tidal (MPET) CO₂ targeting.⁴¹ Using post hoc statistical parametric mapping (SPM), we demonstrated qualitative global and regional alterations in response to CO₂ challenge among individual adults with PCS that were not present in healthy control volunteers. In addition, quantitative alterations in CO₂ responsiveness expressed as abnormal voxel counts were observed among concussion patients, yielding a potentially novel neuroimaging biomarker unique to this clinical condition.

In the present study, we assessed global and regional resting CBF using pseudo-continuous arterial spin labeling (pCASL) and cerebrovascular responsiveness using controlled CO₂ targeting and BOLD MRI in adolescent PCS patients and healthy controls. These results provide empirical evidence of patient-specific impairments in

cerebrovascular physiology as a mediator of persistent PCS symptoms in adolescents. The results also provide additional evidence to support future studies examining the use of brain MRI CO₂ stress testing as a potential diagnostic clinical assessment tool in individuals with SRC and PCS.

Methods

Research Design

This study was approved by the Biomedical Research Ethics Board at the University of Manitoba. We conducted a prospective cohort study of adolescent PCS patients and healthy control subjects. All PCS patients were recruited from the Pan Am Concussion Program in Winnipeg, Manitoba, Canada. Patient inclusion criteria included 1) physician diagnosis of PCS secondary to an SRC (defined as meeting the ICD-10 criteria of 3 or more concussion symptoms present for at least 1 month)⁵ and 2) age of 13–25 years. Patient exclusion criteria were 1) traumatic abnormalities on prior neuroimaging studies, 2) diagnosis of PCS following a concussion outside of sports, and 3) contraindication to MRI (for example, claustrophobia). Healthy control subjects were recruited through word of mouth and included patient siblings and relatives. The control subject inclusion criterion was an age 25 years or younger. Control subject exclusion criteria were 1) a symptomatic concussion, 2) a history of prior concussion or TBI resulting in structural brain injury on previous neuroimaging, and 3) contraindication to MRI.

Clinical Assessment

All patients underwent a clinical history and physical examination by a single neurosurgeon. At the time of initial medical consultation, all patients completed a standardized data collection form that included demographic data, medical history, past concussion history, and family history. Diagnosis of PCS was made by the neurosurgeon according to the ICD-10 criteria. All healthy control subjects underwent clinical interview to collect demographic data, medical history, and past concussion history. On the day of neuroimaging assessment, all study participants, patients and control subjects, completed the Post-Concussion Symptom Scale (PCSS), a standardized symptom inventory consisting of 22 symptoms rated on a 7-point (0–6) Likert scale with a maximum score of 132 (6 × 22).³⁰ Current medical interventions were summarized including vestibular therapy, cervical spine physiotherapy, and pharmacological treatment of headaches or mood-related symptoms. Some patients underwent standardized graded aerobic treadmill testing as part of their clinical diagnostic workup, as previously described.^{33,34} For the purposes of this study, those patients who achieved a symptom-limiting threshold during treadmill testing were deemed to “fail” this test and were diagnosed with physiological postconcussion disorder (PCD).^{16,32} Patients who were capable of exercising to complete exhaustion without any concussion symptoms were deemed to “pass” this test. At the time of the research study, a certified neuropsychologist was not a member of the multidisciplinary pediatric concussion clinic; therefore, computerized neurocognitive

tools were not used as a supplemental tool to confirm the diagnosis of concussion.

Neuroimaging Assessment

After we obtained informed consent and performed clinical assessments, all participants underwent neuroimaging. Model-based prospective end-tidal (MPET) CO₂ targeting was achieved by precise delivery of CO₂ at a fixed concentration using a rebreathing circuit regulated by a computerized gas-blender (RespirAct, Thornhill Research Inc.). This device allows precise manipulation of end-tidal (ET)CO₂ levels under iso-oxic (target ETO₂ = 115 mm Hg) conditions.^{42,43} Monitoring during the study period included continuous heart rate and pulse oximetry and noninvasive blood pressure at 3-minute intervals.

All images were acquired using a Siemens Verio 3.0-T MR scanner with a 12-channel phased-array head coil. The MRI protocol consisted of baseline anatomical imaging including sagittal 3D T1-weighted magnetization-prepared rapid gradient-echo (MPRAGE; whole brain coverage, matrix 256 × 256, slice thickness 2.2 mm, no interslice gap), axial fluid-attenuated inversion recovery (FLAIR), and axial gradient recalled echo planar (GRE) sequences, as well as pCASL imaging followed by a run of continuous BOLD sequences with MPET targeting. The breathing sequence during BOLD imaging consisted of interval step-changes as follows: baseline ETCO₂ (120 seconds), hypercapnia (5 mm Hg above baseline for 120 seconds), baseline ETCO₂ (30 seconds), hypercapnia (5 mm Hg above baseline for 120 seconds), baseline ETCO₂ (30 seconds), hypercapnia (5 mm Hg above baseline for 120 seconds), baseline ETCO₂ (120 seconds; Fig. 1). For the pCASL imaging, an M0 scan was undertaken first: Siemens ep2d pCASL echo planar readout (FOV 24 × 24 cm, TR 8000 msec, TE 12 msec, contrast with a flip angle 90°, 20 slices; CASL method: multislice, label offset 90

mm, post label delay 1200 msec, crusher gradient 0 sec/mm², voxel size 3.8 × 3.8 × 5.0 mm); the formal pCASL sequence echo planar readout (FOV 24 × 24 cm, TR 4000 msec, TE 12 msec, contrast with a flip angle 90°, 20 slices, slice thickness 5.0 mm; CASL method: multislice, label offset 90 mm, post label delay 1200 msec, crusher gradient 0 sec/mm², voxel size 3.8 × 3.8 × 5.0 mm). The first 2 labeled-nonlabeled pairs were discarded. There were 22 imaging pairs for the baseline CBF determination. The BOLD MRI data were acquired with a T2*-weighted single-shot gradient echo pulse sequence with echo planar readout (FOV 24 × 24 cm, matrix 64 × 64, TR 2000 msec, TE 30 msec, flip angle 85°, slice thickness 5.0 mm, interslice gap 2.0 mm, number of temporal frames 330). The total duration of the MRI assessment was approximately 25 minutes.

Structural Neuroimaging

The structural neuroimaging component of each study, including T1-weighted, FLAIR, and GRE imaging sequences was reviewed by a board-certified neuroradiologist.

Preprocessing of MRI Sequences

Standard preprocessing of MRI echo planar imaging output was accomplished for the BOLD and pCASL sequences with SPM8 software, including batch processing by an SPM toolbox and in-house, custom-written MatLab scripts. An ASL toolbox, developed by Ze Wang, was also used to assist with the preprocessing of pCASL data (<http://www.cfn.upenn.edu/~zewang/ASLtbx.php>). Voxel size reported on for BOLD data was 2 × 2 × 2 mm. Voxel size for pCASL data was 3.8 × 3.8 × 5.0 mm. Preprocessing included realignment of images, slice time correction, coregistration with the MPRAGE images, smoothing, and normalization into Montreal Neurological Institute (MNI)

FIG. 1. End-tidal gas targeting with the MPET device (RespirAct). The triple hypercapnic stimulus is demonstrated (upper). Tight control of the ETO₂ targeting during the BOLD imaging period can also be seen (lower).

space. Motion artifact was examined. Studies were rejected if motion over the conduct of the study period was greater than 3 mm in any plane. First-level analyses were undertaken with the author blinded to the participant's group (healthy control vs PCS patient); however, second-level analyses were not blinded since they were based on the results of the first-level analysis.

Statistical Analysis

Global Resting CBF

The mean global resting CBF was calculated on a voxel-by-voxel basis at rest.

Regional Resting CBF: Second-Level Analysis

A second-level voxel-by-voxel analysis for the pCASL studies at baseline was undertaken by calculating a control atlas of CBF output (data from all healthy control subjects combined, 17 controls). These mean data were then compared with the mean output from the PCS patients (15 patients), and each PCS patient was compared to the control atlas at various *p* values (0.001, 0.005, 0.01, and 0.05). Results were determined for the 2 conditions, that is, CBF values greater than those on the control atlas (greater-than CBF) and less than those on the control atlas (less-than CBF).

BOLD Cerebrovascular Responsiveness: First-Level Analysis

First-level analyses were undertaken for each study participant. The activation response to the hypercapnic breathing stimulus and the inverse response to the stimulus were assessed at the *p* = 0.001 uncorrected level. The cluster size threshold was 10 voxels.

BOLD Cerebrovascular Responsiveness: Second-Level Analysis

A second-level analysis for the BOLD studies was undertaken with data from all 17 control subjects combined into an atlas, allowing data from each study participant to be compared with control mean values. Each study participant underwent voxel-by-voxel comparisons for BOLD signals that were less than and greater than the mean control group response. This second-level analysis was conducted over a series of *p* values (0.001, 0.005, 0.01, and 0.05) to study the behavior and voxel count response that was greater than and less than for each individual versus the mean control atlas output. The voxel counts/whole-brain voxel count ratios for each study participant for the response greater and less than the control atlas values were calculated.

Receiver Operating Characteristic Analysis

Predictive accuracy is defined as the ability of a test to correctly classify patients with or without a specific condition. For receiver operating characteristic (ROC) curve analysis, an area under the curve (AUC) indicates perfect discrimination when equal to 1.0, while an ROC curve ≥ 0.70 is considered a clinically useful predictive model. Therefore, ROC analyses were undertaken to determine to what extent the novel biomarker, abnormal voxel counts, could discriminate between healthy control subjects and PCS patients. Data from the second-level analysis were

used to undertake an ROC curve analysis, where the categorical conditions were defined as no PCS = 0 and PCS = 1. The ROC curves were generated based on voxel counts as determined for each individual at the *p* value levels indicated above (0.001, 0.005, 0.01, and 0.05). At each *p* value a greater-than and less-than ROC curve was generated. A multivariate model was also constructed based on the collation of greater-than and less-than voxel counts at the various *p* values. PROC LOGISTIC of SAS version 9.3 (SAS Institute) was used to calculate AUC values for abnormal voxel counts. Likelihood ratio *p* values were generated due to their superior reliability in small samples as compared with conventional Wald tests.⁴

Results

Participants

A summary of the demographic and clinical features of the PCS patients and healthy controls who participated in this study is presented in Tables 1 and 2. A total of 37 study participants were enrolled. Thirty-two BOLD studies are reported—17 healthy control subjects (8 male and 9 female, mean age 18.3 years, range 13–25 years) and 15 PCS patients (4 male and 11 female, mean age 17.3 years, range 15–22 years). Thirty pCASL studies are reported since only 7 of the 9 female patients from the control group were included in this analysis. Five studies were excluded from BOLD analysis, and 2 additional studies were excluded for excessive motion during pCASL image acquisition, 1 for serious degrading of BOLD images due to field inhomogeneity in a subject with dental braces, 2 for a poor mask seal with an inadequate increase in CO₂ with hypercapnia, and 2 for MPET equipment problems (suction pump failure; subsequently replaced). Medical history for the healthy control subjects was significant for remote concussion in 3 of the 17 participants. No healthy control subject was taking medication at the time of study participation. Medical history among PCS patients was significant for previous concussion in 11 of 15, migraine or headaches in 4 of 15, attention-deficit disorder in 1, and learning disorder in 1 patient. Three of 15 PCS patients reported a loss of consciousness, and 4 of 15 reported retrograde or anterograde amnesia at the time of injury. The mean time from injury to imaging in the PCS patients was 327 days (range 33–993 days). Ten of the 15 PCS patients were being treated with multidisciplinary interventions at the time of the neuroimaging study, while 5 were being managed conservatively.

Study Tolerability

All study participants successfully completed the full imaging study without any serious adverse events. One PCS patient reported transient light-headedness during imaging, while another PCS patient reported a transient increase in headache and dizziness during the imaging.

Structural Neuroimaging

Structural neuroimaging studies were normal in all study participants. No evidence of traumatic abnormalities, including cerebral microhemorrhages, were observed. Normal anatomical variants, including cavum septum pel-

TABLE 1. Summary of demographic and clinical features of 15 adolescent postconcussion patients

Case No.	Age (yrs), Sex	Medical History	Sport	Predominant Symptoms	Exertional Sx	Treadmill Testing	Duration of Sx (days)	Interventions	PCSS Score
1	20, F	Concussion (3)	Soccer	Headache, visual problems, sadness, anxiety	Yes	None	270	Vestibular therapy, cervical spine physiotherapy	73
2	17, F	Concussion	Soccer	Headaches, fatigue, light & sound sensitivity	Yes	Fail	182	Vestibular therapy	32
3	17, F	Concussion (3), idiopathic thrombocytopenia	Hockey	Dizziness, headache, fatigue, drowsiness	Yes	Fail	282	Vestibular therapy	43
5	16, F	Concussion (3)	Soccer	Balance, visual problems, excessive sleep	No	None	279	Vestibular therapy	14
6	15, F	ADHD, concussion	Hockey	Anxiety, headache, fatigue, impaired sleep	Yes	Pass	188	Methylphenidate, fluoxetine, trazodone	8
9	18, F	Headaches	Hockey	Headaches, visual problems, dizziness	Yes	Fail	993	Topiramate, propranolol, vestibular therapy	21
14	16, M	Concussion (3)	Soccer	Headaches, dizziness, fatigue	No	None	33	None	4
15	16, F	Concussion	Football	Headache, visual problems, depression	Yes	Fail	126	None	68
16	22, F	Concussion (6), migraine headaches	Baseball	Headaches, fatigue, drowsiness	Yes	Fail	454	Topiramate, vestibular therapy	31
18	16, M	Concussion (3), learning disorder	Cycling	Headache, light sensitivity, visual problems	Yes	None	832	Gabapentin, amitriptyline, topiramate, vestibular therapy	31
20	18, M	Migraine headaches	Basketball	Headaches, foggy, irritability	Yes	Fail	256	None	18
21	16, F	Concussion	Cycling	Headaches, difficulty concentrating, irritability	Yes	Fail	174	None	28
22	18, F	None	Hockey	Headaches, depression, light-headedness	Yes	Fail	422	Desvenlafaxine	3
23	15, M	Concussion (2)	Football	Headaches, dizziness, light sensitivity	Yes	Fail	33	None	61
25	20, F	Migraine headaches	Hockey	Headaches, fatigue, dizziness, depression	Yes	Fail	388	Topiramate, suboccipital nerve blocks, vestibular therapy	42

ADHD = attention deficit hyperactivity disorder; Sx = symptoms.

lucidum and right frontal developmental venous anomaly, were detected in 2 control subjects.

End-Tidal Gas Targeting and Hemodynamics

No differences in baseline ETCO_2 (38.4 ± 2.9 and 38.9 ± 3.2 mm Hg, respectively) and the change in CO_2 during CO_2 targeting (4.6 ± 0.7 and 4.4 ± 0.7 mm Hg) were detected between the PCS patients and healthy controls, indicating that both groups were exposed to the same magnitude of CO_2 stimulus during BOLD imaging. The ETO_2 was well controlled at the selected tension (115 ± 3 mm Hg in PCS group and 112 ± 3 mm Hg in control group).

Global Resting CBF

There were no differences in the mean global resting CBF between the PCS patients and healthy controls (42.4 ± 7.5 and 42.4 ± 6.7 ml/100 g/min, respectively).

Regional Resting CBF: Second-Level Analysis

Significant differences in the mean regional resting CBF between the controls and PCS patients is shown in Fig. 2. The changes are modest at the level of $p = 0.01$ for the greater-than or less-than regional resting CBF differences between the groups. Second-level analysis of individual PCS patients compared with the healthy control atlas values demonstrated patient-specific alterations in resting CBF that include diffuse areas beyond those identified on group analysis.

BOLD Cerebrovascular Responsiveness: First-Level Analysis

At the $p = 0.001$ level there was no significant difference between the 2 participant groups in the activation response to CO_2 or the inverse response. For the PCS patients the mean activation response to CO_2 was $64\% \pm$

TABLE 2. Summary of demographic and clinical features in 17 adolescent healthy control subjects

Case No.	Age (yrs), Sex	Medical History	PCSS Score
4	17, F	None	0
11	17, F	None	0
12	17, M	Concussion (1)	0
13	19, F	None	0
17	15, F	None	0
19	13, M	None	0
24	15, M	None	0
26	19, F	None	0
28	21, M	None	0
29	19, M	Concussion (1)	4
30	19, F	None	3
31	19, F	None	4
32	21, M	None	0
33	19, F	Concussion (1)	4
34	21, M	None	0
35	20, M	None	0
36	25, F	None	0

22%, and for the control subjects it was $67\% \pm 17\%$ ($p = 0.56$ between groups). The inverse response was $0.37\% \pm 0.26\%$ in the PCS group and $0.24\% \pm 0.22\%$ in controls ($p = 0.35$ between groups). The total number of voxels assessed was $188,000 \pm 7000$ in the PCS group and $185,000 \pm 5500$ in the control group, indicating that warping into the MNI space was consistent between groups.

BOLD Cerebrovascular Responsiveness: Second-Level Analysis

Voxel-by-voxel comparison of the mean values for the control subjects and the PCS patients at the $p = 0.01$ level is shown in Fig. 3. Only 0.15% of voxels in the PCS group had a response significantly greater than that of the control group. In contrast 5.8% of voxels in the PCS group had a response significantly less than that of the control group. Second-level analysis comparing individual healthy control subjects and individual PCS patients to the healthy control atlas demonstrated patient-specific alterations in cerebrovascular responsiveness (Fig. 4). The ROC curve analysis was undertaken at various levels of statistical significance ($p = 0.05, 0.01, 0.005$, and 0.001) to compare the voxel-by-voxel output of a greater than and less than response between each control subject and the atlas of controls and between each PCS patient and the atlas of controls (Table 3). The p values for the respective significance of the AUC

FIG. 2. Second-level group comparisons demonstrate modest but significant regional alterations in mean resting CBF in the group of PCS patients compared with values on the healthy control atlas (A). However, second-order analysis of individual PCS patients compared with the control group atlas demonstrates patient-specific alterations in regional resting CBF that include diffuse areas beyond those identified on group analysis (examples in B, Case No. 1 from Table 1, and C, Case No. 23 from Table 1). All studies were examined at the $p = 0.01$ level. Regional differences in voxel-by-voxel responses that were significantly greater than and less than the healthy control atlas response are indicated in color. Hot scale t-statistic level (orange hues) indicates regions where the PCS group or patient had a resting CBF greater than the control group; cold scale t-statistic level (blue hues) indicates regions where the PCS group or patient had a resting CBF less than the control group. The numbers below each image in this and subsequent figures are the distance below (negative numbers) or above (positive numbers) the anterior commissure–posterior commissure (AC–PC) line. PCSS = PCSS score.

FIG. 3. Second-level group comparisons of healthy controls and PCS patients demonstrate significant differences in global BOLD cerebrovascular responsiveness 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 control atlas response are indicated in color. Hot scale t-statistic level (orange hues) indicates when the mean PCS group had a greater response to the CO_2 stimulus than the control group; cold scale t-statistic level (blue hues) indicates when the mean PCS group had a diminished response to the CO_2 stimulus compared with the control group.

for each condition are shown in the table. The ROC curve for regions manifesting a less-than and greater-than CO_2 responsiveness compared with the control atlas at the $p = 0.001$ level is shown in Fig. 5 left. The AUC is 0.87 for the greater-than response ($p < 0.0001$, likelihood ratio test). The ROC curve for regions manifesting less responsiveness than the control atlas at the $p = 0.001$ level is shown in Fig. 5 right. The AUC is 0.80 in this circumstance ($p = 0.0003$, likelihood ratio test). A multivariate logistic comparison was also undertaken based on the greater-than and less-than output compiled from the various p value voxel outputs. In this composite model the ROC AUC was 0.967. There was no correlation between abnormal voxel counts and PCSS scores among PCS patients.

Discussion

To our knowledge, we are the first to examine altera-

tions in global resting CBF, regional resting CBF, and BOLD cerebrovascular responsiveness in adolescent PCS patients. Using this heterogeneous population, we provide empirical evidence of patient-specific regional impairments in mean resting CBF and cerebrovascular responsiveness that occur in the setting of normal global resting CBF values. These findings suggest that the pathophysiological mechanisms proposed to characterize acute concussion persist in adolescents with PCS and can be safely and reliably detected with the novel brain MRI CO_2 stress testing protocol described here.

To date, very few studies have used neuroimaging techniques to characterize the alterations in cerebrovascular physiology that occur in acute SRC and PCS. In one of these studies, Maugans et al.³⁸ examined neurocognitive profiles and CBF in 12 adolescents during the acute and recovery stages of SRC compared with those in age- and sex-matched controls. Using ASL MRI, these authors detected significant alterations in mean resting CBF in SRC patients compared with levels in normal controls during the acute stage of injury, with the majority of patients demonstrating reduced mean resting CBF. Despite the normalization of computerized neurocognitive scores, only 27% of patients demonstrated a recovery in mean resting CBF within 10% of control group values at 2 weeks postinjury. Bartnik-Olson et al.³ performed DTI, MR spectroscopy, and perfusion-weighted MRI in 15 healthy controls and 15 pediatric SRC patients who remained symptomatic 3–12 months after injury. These investigators observed reductions in mean thalamic CBF and cerebral blood volume among the group of SRC patients compared with levels in healthy controls. Group differences were also observed among DTI and MR spectroscopy indices, suggesting that PCS symptoms may be partially mediated by impairments in neurovascular coupling. More recently, Meier et al.³⁹ completed a prospective longitudinal assessment of mean resting CBF in 17 collegiate football players with SRC and 27 healthy football athletes. Patients underwent clinical, neuropsychiatric, neurocognitive, and neuroimaging assessments with 3D ASL approximately 1 day, 1 week, and 1 month following physician-diagnosed SRC. Using a voxel-wise linear mixed-effects model to assess relative mean CBF within selected regions of interest as a function of recovery time, the authors identified altered CBF within the right dorsal mid-insular cortex (dmIC) and superior temporal sulcus. Specifically, they detected within-subject reductions in CBF in these 2 regions that were lower at 1 day postinjury than the 1-month postinjury values. In group comparisons, mean CBF values within these 2 regions were significantly lower than control values at 1 day postinjury but did not differ from control values at 1 month postinjury, suggestive of recovery. Regional blood flow within the dmIC was inversely related to the magnitude of initial psychiatric symptoms.

The present study makes a number of novel contributions to the existing literature, which has important implications for our understanding of PCS. First, our results indicate that adolescent PCS is not associated with impairments in global resting CBF. The study included a heterogeneous sample of adolescent PCS patients with a wide spectrum of self-reported symptoms and symptom sever-

FIG. 4. Second-level individual comparisons of 1 healthy control (**A**) and 2 PCS patients (**B**, Case No. 1 in Table 1, and **C**, Case No. 25 in Table 1) against the control atlas at the $p = 0.005$ level. No abnormal voxels were detected in this control patient. Both PCS patients demonstrate patient-specific alterations in BOLD cerebrovascular responsiveness. Abnormal voxel counts and PCSS scores are quantified in each case.

ity scores and who underwent neuroimaging at time points that ranged from 1 month to more than 2 years postinjury. The majority of PCS patients demonstrated a symptom-limiting threshold during graded aerobic treadmill testing consistent with a diagnosis of physiological PCD.^{16,32} Despite these clinical findings, there were no differences in global resting CBF values between adolescent PCS patients and healthy control subjects, as evaluated with pCASL imaging. These findings suggest that although acute SRC may be characterized by decreased mean global resting CBF,³⁸ symptoms of SRC and PCS can persist in adolescent patients despite the normalization of global resting CBF levels. While future prospective studies are required to examine the longitudinal trends in global resting CBF among adolescent PCS patients, the findings of this study suggest that mean global resting CBF is not a reliable biomarker that can be used to distinguish between physiologically recovered and unrecovered adolescent SRC patients.

Second, the results of this study indicate that adolescent PCS is associated with regional abnormalities in resting CBF that occur even in the presence of normal global resting CBF. Second-level voxel-by-voxel group comparisons identified several cortical and subcortical brain regions where resting CBF values were significantly higher and lower among PCS patients than among healthy control atlas values. Although these findings are consistent with previous results and provide additional evidence of regional abnormalities in resting CBF following SRC, the clinical significance of these group differences must be interpreted with caution. Meier et al.³⁹ detected within-subject reductions in CBF within 2 specific regions that were significantly lower at 1 day postinjury and returned

to normal control values at 1 month postinjury. The authors concluded that regional resting CBF values may have the potential to serve as objective biomarkers for concussion. Although we detected similar group differences in the present study, it remains unclear how abnormal resting CBF values within 1 or 2 discrete brain regions can reliably serve as objective biomarkers for a condition that presents with such a heterogeneous spectrum of clinical symptoms and may not be associated with quantifiable deficits on neurocognitive and neuropsychiatric screening tools. Indeed, the second-level voxel-by-voxel comparison of individual PCS patients against the control atlas in this study demonstrated a unique, patient-specific signature of impaired resting CBF in all PCS patients that included quantifiable abnormalities within diffuse brain regions that were not limited to those abnormal regions identified on the group comparisons. These findings suggest that resting CBF within limited regions of interest, even when correlated with results from other measures of neurocognitive and neuropsychiatric functioning, may not reflect the global impairments in cerebrovascular physiology that characterize this condition.

Third, we conducted qualitative and quantitative assessments of cerebrovascular responsiveness among adolescent PCS patients and healthy controls using a controlled CO₂ challenge and BOLD MRI. Regional abnormalities in cerebrovascular responsiveness to a controlled vasodilatory stimulus were detected in all PCS patients. Specifically, brain MRI CO₂ stress testing revealed regions with both a diminished and an increased responsiveness to MPET CO₂ challenge during BOLD MRI in individual PCS patients. Overall group comparisons of PCS patients and healthy controls indicated that the predominant cerebrovascular

TABLE 3. Voxel counts from second-level analysis for greater-than or less-than values at stated p values

Level	>0.001	<0.001	>0.005	<0.005	>0.01	<0.01	>0.05	<0.05
Voxel count PCS*	386 (730)	325 (1095)	985 (1717)	1335 (4160)	1498 (2485)	2403 (6893)	4430 (6281)	10,962 (19,544)
Voxel count control*	10 (40)	1 (3)	224 (363)	33 (58)	824 (1202)	161 (233)	6577 (7791)	3173 (3515)
AUC	0.87	0.80	0.69	0.85	0.58	0.79	0.62	0.71
p Value	<0.0001	0.0003	0.039	0.001	0.302	0.007	0.379	0.040

* Values expressed as the mean (standard deviation).

response is an attenuated response to CO₂ challenge, which is consistent with evidence from previous animal model and human neuroimaging studies of concussion and TBI. Even though some patients were imaged years after injury, each PCS patient demonstrated a unique, patient-specific signature of impaired cerebrovascular responsiveness that was not followed in healthy control subjects. Where available, the results of brain MRI CO₂ stress testing were highly correlated with the results of graded aerobic treadmill testing. Specifically, all patients who obtained a symptom-limiting threshold on the graded aerobic treadmill testing (diagnosed with physiological PCD) demonstrated regional abnormalities in cerebrovascular responsiveness. Furthermore, the ROC analysis presented here suggests that future work examining quantitative biomarker thresholds may be helpful to diagnose PCS and define physiological recovery in individual patients. The ROC curve analysis for regions manifesting a voxel response greater than and less than the control atlas values (that is, abnormal voxel counts) produced an AUC of 0.87 ($p < 0.0001$) and 0.80 ($p = 0.0003$), respectively, consistent with a clinically useful predictive model. A multivariate comparison of the PCS and control groups revealed an AUC of 0.967.

In addition to providing evidence for a role of impairment in CBF regulation in the pathophysiology of PCS, this report suggests that future longitudinal studies utilizing brain MRI CO₂ stress testing in patients with acute concussions are warranted. The novel neuroimaging technique described here meets a number of key requirements for assessing this unique population. First, as we have demonstrated here, this assessment tool is safe and well tolerated. There were no serious adverse events associated with this study, and no healthy controls or PCS patients voluntarily withdrew from the study or terminated the test. Two patients experienced mild worsening of concussion symptoms that were self-limiting. In contrast to other imaging methods used to measure cerebrovascular responsiveness, including SPECT, xenon-enhanced CT, and PET, our technique does not require the use of radiation or intravenous radiolabeled tracers or contrast agents.

Second, the stimulus used in this study is reliable and allows qualitative and quantitative assessment of whole-brain cerebrovascular physiology in patients with heterogeneous clinical findings. Unlike task-based fMRI studies that have been completed in broader SRC populations, brain MRI CO₂ stress testing does not rely on results from cognitive or behavioral tasks that interrogate only selected neuroanatomical networks and that often do not distinguish between normal controls and PCS patients. Our technique also does not rely on patient effort but instead utilizes a standardized, controlled breathing sequence such that the result cannot be intentionally influenced, a frequently recognized problem among professional athletes.¹⁸ The vasodilatory stimulus applied does not rely on breath-holding, which has been found to be an imprecise and unreliable stimulus,¹⁹ lacks statistical power for data analysis, and can exacerbate concussion symptoms, in particular headaches, in pediatric SRC and PCS patients. Furthermore, it does not rely on the intravenous administration of acetazolamide, a single dose of which leads to variable changes in CBF^{12,23} and frequent side effects.⁴⁶ Instead, this assess-

FIG. 5. The ROC curves for the greater-than (left) and less-than (right) responses to the control atlas at the $p = 0.001$ level for significant abnormal voxel counts.

ment tool utilizes precise MPET alterations in CO₂ that are commonly experienced by children and adolescents during normal levels of daily physical activity. Given that the optimal goal of concussion neuroimaging assessment tools is to provide standardized within-subject measures of brain physiology over time to track recovery, the reliability of the technique and the reproducibility of the stimulus are also paramount.¹⁹ Because the provocative breathing sequence is patient-specific, the same sequence can be stored and reliably applied in serial studies in the same patient at baseline and at various time points during recovery. Because the MPET device provides breath-by-breath output of ETCO₂, clinicians can confirm that patients are reliably exposed to the same stimulus magnitude during longitudinal studies, an important variable that cannot be controlled through the use of breath-holding and acetazolamide techniques. Indeed, a prospective study in a cohort of healthy controls, utilizing the same MRI technique described here but with different image data analysis, demonstrated excellent within-day measures of gray and white matter cerebrovascular reactivity (CVR), excellent between-day measures of gray matter CVR, and good between-day reproducibility of white matter CVR.²⁷ In comparison with other imaging modalities that can be used to estimate CVR, such as transcranial Doppler ultrasonography,²⁰ our technique provides qualitative and quantitative assessment of whole-brain cerebrovascular physiology that is not limited to only 1 or 2 vascular territories (for example, the middle cerebral artery) and is not dependent on the skill or interpretation of the examiner.

Most notably, unlike DTI and task-based fMRI studies that rely on group comparisons to generate results, brain MRI CO₂ stress testing provides qualitative and quantitative information on an individual patient basis. The results of this study suggest that adolescent PCS is not only characterized by unique clinical manifestations and symptoms, but that the patterns of impaired cerebrovascular responsiveness that potentially mediate these clinical findings

are also heterogeneous and patient specific. These findings reinforce our conceptual understanding of concussion as a condition that results from global alterations in brain physiology and not just dysfunction within 1 selective neuroanatomical network or region of interest. In addition, this assessment tool generates valuable biomarkers that correlate with results from standardized graded aerobic treadmill testing and can be used to compare results from age- and sex-matched controls, individual patient baseline studies, and neuropsychological testing scores.

The findings of this study must be considered in light of several important limitations. First, the sample size is relatively modest and includes PCS patients with long-standing symptoms that may not be reflective of the general population of adolescent SRC and PCS patients. All patients were recruited from a tertiary pediatric concussion program, which may have selected for more severely injured patients. Because 1 of the primary purposes of this study was to evaluate the safety and tolerability of brain MRI CO₂ stress testing in adolescent concussion patients, we chose to evaluate adolescent patients who had more chronic symptoms first before proceeding to patients with more acute injuries. Indeed, studies to investigate the utility of brain MRI CO₂ stress testing in pediatric patients with acute SRC are currently underway at our institution. While the PCS group in the present study was composed of patients with heterogeneous symptoms, the sample size undoubtedly impacted the ROC analysis performed. Thus, the quantitative biomarker thresholds generated in this study must be viewed as preliminary and cannot be used for clinical decision making in broader PCS populations. Indeed, we found no correlation between abnormal voxel counts and symptom severity as measured by the PCSS score. Future longitudinal studies are needed to compare these neuroimaging biomarkers with other measures of concussion severity and recovery, such as results from aerobic treadmill testing and formal neuropsychological testing.

Second, the most important limitation of this study concerns the use of BOLD MRI. To provide a true measure of CVR, the change in CBF per unit of CO₂, neuroimaging assessment tools must incorporate MRI sequences that provide a direct quantitative assessment of CBF. Previous authors have pointed out that the BOLD signal used in fMRI studies and the present study are dependent on a number of factors, including cerebral blood volume, the ratio of deoxyhemoglobin to oxyhemoglobin, and local diffusion effects as well as the CBF within the brain tissue.³⁹ The signal can also be influenced by neuronal activity and the integrity of neurovascular coupling processes within these regions.²⁸ Because BOLD MRI does not provide a true assessment of CBF, we have chosen to use the term “cerebrovascular responsiveness” to define the change in BOLD MRI signal per unit of CO₂ observed in this study. In contrast, several noninvasive MRI-based techniques have been developed to provide quantitative assessment of CBF, including ASL, pulsed ASL, continuous ASL, and pCASL.¹³ Future studies should examine the differences in CO₂ responsiveness between the BOLD and flow techniques.

Third, the methodology of evaluating healthy controls as a comparison group warrants comment. As in our previous study,⁴¹ we compared each control subject to the control group as well as each PCS patient to the same control group atlas. In this way, each study participant, whether control or PCS patient, was compared with the same atlas in an identical manner to allow voxel counts for greater than and less than the mean control group values to be determined (abnormal voxel counts). The ROC curves at various levels of significance were then undertaken based on a categorical assessment of the presence or absence of PCS. While greater statistical restraints reduce the number of abnormal voxels visible on the quantitative maps, this adjustment is significantly offset by a much more robust and discriminative statistical model. To further clarify the extent to which healthy control responses may differ from the control atlas, future studies may be strengthened by the incorporation of control subjects who are not part of the control group atlas.⁵¹ Such methodological subtleties will also be addressed by future studies utilizing a larger atlas of normal controls.

Conclusions

Acknowledging these limitations, we, for the first time, provide a comprehensive examination of cerebrovascular physiology in adolescent PCS patients. Data in this study provide preliminary empirical evidence that adolescent PCS is associated with patient-specific regional impairments in mean resting CBF and cerebrovascular responsiveness that occur in the setting of normal global resting CBF values and can be reliably detected by brain BOLD MRI CO₂ stress testing up to years after injury. Future studies are warranted to examine the use of brain MRI CO₂ stress testing in the diagnosis, longitudinal assessment, and management of acute pediatric SRC and PCS.

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Disclosures

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