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Alterations in cerebral glucose metabolism as measured by ^{18}F-fluorodeoxyglucose-PET in patients with persistent postconcussion syndrome

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Background **Many patients who have traumatic brain injury experience a wide range of psychiatric and neurological symptoms (including impairment in functional status, cognition, and mood), and if persistent are referred to as persistent postconcussion syndrome (PCS). To our knowledge, this is the first study to broadly evaluate metabolic dysregulation in a heterogenous patient population meeting the criteria for PCS.**

Methods **A total of 64 PCS patients and 37 healthy controls underwent ^{18}F-fluorodeoxyglucose-PET (^{18}F-FDG-PET) scanning, and 70 brain structures (including left and right structures where appropriate) were analyzed in each subject.**

Results **Compared to the brains of healthy controls, those of PCS patients demonstrated 15 hypermetabolic and 23 hypometabolic regions. Metabolic changes in the brains of PCS patients were subsequently correlated with various indices of symptom severity, mood, and physical/cognitive function. Among PCS patients, increased metabolism in the right cingulate gyrus correlated with the severity of postconcussion symptoms. Conversely, increased metabolism in the left temporal lobe was associated with both improved mood and measures of adaptability/rehabilitation. Furthermore, increased metabolism in the bilateral orbitofrontal regions correlated with improved working memory.**

Conclusions **Overall, these findings suggest a complex pattern of cerebral metabolism in PCS patients, with a mixture of hypometabolic and hypermetabolic regions that correlate with various symptoms, highlighting both potential pathological and compensatory mechanisms in PCS. The findings also suggest that FDG PET is useful for providing neurophysiological information in the evaluation of patients with PCS and may help guide future targeted therapies.**
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Background

Over 1.5 million Americans suffer from traumatic brain injury (TBI) annually, resulting in approximately 50 000 deaths, 300 000 hospitalizations, and 80 000–90 000 individuals with long term disability. The morbidity and mortality associated with TBI present a substantial public health problem, with the estimated expense for this problem exceeding $60 billion annually [1]. Patients experiencing lasting psychiatric and neurological symptoms (including impairment in functional status, cognition, and mood) are considered to be experiencing chronic TBI, also sometimes referred to as persistent postconcussion syndrome (PCS) [2]. A major obstacle in the management of such patients is finding neurophysiological correlates for their symptoms. Specifically, many patients are told that their brain is structurally normal on MRI and that there is no objective evidence of brain dysfunction to explain their persistent symptoms. The inability to characterize PCS using standard MRI techniques can lead to delayed or even inaccurate diagnosis, which perpetuates a stigma that patients are fabricating their symptoms and thus worsens prognosis [3].

Therefore, obtaining objective data on the neurophysiological effects of PCS and assessing how these effects are associated with symptoms are essential for understanding PCS. Unfortunately, as mentioned above, structural CT or MRI scans are often not helpful for the evaluation

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of patients experiencing PCS symptoms [4]. These neuroimaging modalities are sensitive to structural defects but generally lack the capability to detect more subtle neurophysiological changes in the brain. However, both neurochemical and epidemiological data thus far suggest that TBI can cause long-lasting neuronal damage even in the absence of overt brain lesions, with downstream consequences for cellular and network function that may manifest as persistent cognitive, mood, and functional impairment [5–7]. Indeed, TBI can consist of three phases: an acute phase that represents the immediate damage associated with the mechanism of physical injury, a subacute phase that represents the onset (and sometimes recovery) of initial symptoms, and the following chronic phase experienced by a certain proportion of patients during which clinical symptoms can persist for years (i.e. PCS). The latter phase is thought to be driven by a cycle of inflammation, neuronal and network dysfunction, and cell death [8]. Disruption of physical homeostasis by the initial injury results in an overactive immune response, triggering the accumulation of microglia, inflammatory cytokines, and other acute-phase proteins (e.g. albumin, fibrinogen, and thrombin) within the brain parenchyma [9]. This inflammatory environment places neurons under considerable oxidative stress and has profound consequences for neurotransmission and metabolism. Notably, neuroinflammation can alter the excitability and activity of neural networks in complex and sometimes unpredictable ways. For example, direct injury to a group of neurons may acutely suppress neural activity and thereby reduce local metabolism. However, depending on whether these neurons are excitatory or inhibitory, the initial suppression of neural activity can cause either hypoactivity or hyperactivity in downstream neural networks [10–14]. Neurons can also become hyperexcitable via glial and cytokine signaling [15,16], but this hyperexcitability frequently triggers a subsequent neuromodulatory response that serves to dampen neural activity [17–19].

Given the complexity of neural networks as described above, the present study will utilize PET with ^{18}F-fluorodeoxyglucose (^{18}F-FDG PET) to elucidate patterns of brain glucose metabolism (a proxy for neural network activity) in patients with PCS. FDG PET has been utilized to assess metabolic abnormalities in a variety of neurological and psychiatric disorders over the past 30 years, but only few studies have used this technique to assess neurophysiological changes in patients with PCS [20]. Assessing the metabolic patterns of 70 different brain structures and correlating the neurophysiological signatures of PCS to various symptoms, this study is the first to our knowledge to evaluate PCS on such a broad scale. Moreover, by analyzing metabolic changes in the brain in conjunction with PCS symptomatology, we aim to better characterize the pathophysiology of PCS. Understanding how changes in brain glucose metabolism are linked to various symptoms of PCS may provide key insight into the mechanisms that underlie PCS, both pathological and compensatory. Ultimately, data from this study may be useful for improving the diagnostic workup of PCS patients, removing the stigma associated with PCS, and guiding future targeted therapies for the condition.

Methods

Patients

Patients with PCS were recruited by the Marcus Institute of Integrative Health at Thomas Jefferson University Hospital. Subjects were also recruited from the local community by self-referral and from local neurology offices. Written informed consent, approved by the Institutional Review Board of Thomas Jefferson University, was obtained from all subjects and the study was registered on clinicaltrials.gov with the following identifier: NCT03241732. Subjects had to report a history of one or more prior TBI (one or multiple) meeting the criteria for mild concussion (loss of consciousness <30 min, no significant amnesia, and no structural injury to the brain such as a hematoma, contusion, dural penetration, or brain stem injury). They had to meet International Classification of Diseases-10 criteria for PCS based on symptoms that were the result of the TBI and could include headache, dizziness, irritability, cognitive problems, emotional problems (e.g. depression or anxiety), hypersensitivity to auditory or visual stimuli, balance problems, insomnia, or other subjective complaints specifically associated with the TBI. Patients also had to report that the symptoms lasted for at least 3 months from the last concussion. During the study, subjects were allowed to continue taking medications for symptoms related to the TBI provided that they were on those medications for at least one month. Selecting a heterogeneous group allowed for the observation of changes in cerebral metabolism that affect a diverse array of PCS patients rather than those with one or two specific symptoms. Therefore, this heterogeneous group reflects the more common presentation of PCS patients who frequently report multiple symptoms across a variety of neurological and psychological domains. Cases of moderate-to-severe TBI with positive imaging findings, such as significant lesions, bleeding, or findings that required surgical interventions were excluded. In addition, subjects had to meet the following inclusion criteria: age 18–80 years old, no history of medical, neurological, or psychiatric disorders (other than those that arose as the result of the TBI) that could reasonably be expected to interfere with the assessment of symptoms, or affect any of the study assessments including cerebral metabolic evaluation by FDG PET. Overall, 64 participants with PCS satisfied the inclusion and exclusion criteria for this study. In addition, 37 healthy participants were recruited as part of a control group. The healthy controls who were recruited reported no history of previous TBI, and no history of significant medical, neurological, or psychiatric conditions (see Table 1 for a comparison between the groups). There were no significant differences between the groups in terms of age, ethnicity, and

Table 1 Demographic data for the chronic traumatic brain injury (postconcussion syndrome) patients and healthy controls

	PCS group	Control group
Gender (male/female)	22/42	16/21
Age (mean ± SD)	46 ± 15	44 ± 15
Age range	18–74 years	23–80 years
Ethnicity	58 Caucasian, 3 AA, 3 Asian 2 Hispanic	33 Caucasian 2 AA, 2 Asian 1 Hispanic
Concussions (one or multiple)	23 had one 41 had multiple	0
Time from last concussion (mean ± SD years)	2.4 ± 2.0	
BDI (mean ± SD)	17.0 ± 8.5	
STAI state (mean ± SD)	46.3 ± 13.6	
MPAI-4 (mean ± SD)	37.3 ± 18.6	
Digit span backward (mean ± SD)	7.1 ± 2.4	
RPQ-3 (mean ± SD)	5.5 ± 2.9	
RPQ-13 (mean ± SD)	28.4 ± 10.2	

AA, African American; BDI, Beck Depression Inventory; PCS, postconcussion syndrome.

gender characteristics. No subjects had any pre-existing neuropsychological diagnoses. No subject in the PCS or the control group had any gross structural abnormalities on their associated MRI scan confirming the inclusion criteria of no structural injury in either group of subjects.

Fluorodeoxyglucose PET imaging protocol

The FDG PET imaging was performed utilizing the general standard of care procedures. After subjects arrived at the Marcus Institute of Integrative Health, a signed formed consent form was obtained. For the PET scan, an intravenous catheter was placed in the antecubital vein of the arm and 148–296 MBq of FDG were injected via manual bolus over a period of less than 1 min. The intravenous catheter was removed, and the patient was then asked to lie still in a chair in a dimly lit room with minimal ambient environmental stimuli for approximately 30 min to allow for FDG uptake. PET images, as well as MRI images, were simultaneously obtained on a 3T Siemens mMR PET-MRI scanner (Siemens Medical Solutions USA, Inc., Malvern, Pennsylvania, USA) over the course of approximately 30 min. All PET/MRI acquisitions included the sequence used for the derivation of standard MR attenuation correction maps based on the Dixon sequence which allows for separation of water, fat, and bone signal and automatically applies the calculated attenuation correction. Other standard imaging corrections were applied for detector efficiency, decay, dead time, attenuation, and scatter corrections. Image reconstruction was based on an ordinary Poisson ordered-subsets expectation maximization algorithm with 4 iterations and 21 subsets producing an image with a matrix size of 344 × 344 pixels and a voxel size of 1 × 1 × 2 mm.

The MIMneuro (MIM Software Inc., Cleveland, Ohio, USA) software was then used for PET image analysis and comparisons of FDG uptake in various brain regions. PET data are mapped on a voxel-to-voxel basis to a standard brain template, which is designed to be compared with an integrated anatomical brain atlas with predefined regions of interest. The process uses linear scaling to account for individual brain size and nonlinear warping to minimize differences in brain regions between individual scans and the atlas. This program identifies metabolic activity in 70 named regions that are included in the analysis. Eight of the regions are considered midline structures and thus have only a single value (e.g. the midbrain, pons, medulla, and vermis). Other regions with left and right homologs within the two hemispheres are analyzed as such (e.g. left and right lateral temporal lobe). For each subject, metabolic activity was normalized to their whole brain activity. Normalization to other structures, such as the cerebellum, was not used since most other structures can be affected in PCS patients. The individual values of the PCS patients were compared to a group of healthy control subjects. A comparison with healthy controls rather than against a standardized database avoids additional confounds related to the specific PET-MR scanner and imaging paradigm used for this study, including intensity normalization or identification of specific structures. Additionally, atrophy correction was not used for this analysis since all subjects had structurally normal brains and the population of both PCS and controls were similarly distributed with regard to age and other characteristics.

Clinical measures of mood, cognition, multimodal function, and postconcussion symptoms

These measures were completed in Research Electronic Data Capture (REDCap) by study participants. Study data were collected and managed using REDCap electronic data capture tools hosted at Thomas Jefferson University [21]. REDCap is a secure, web-based application designed to support data capture for research studies, providing: (1) an intuitive interface for validated data entry; (2) audit trails for tracking data manipulation and export procedures; (3) automated export procedures for seamless data downloads to common statistical packages; and (4) procedures for importing data from external sources.

We measured symptoms of depression via scores on the most recent version of the Beck Depression Inventory (BDI), a 21-item, self-report rating inventory that is widely used to evaluate the severity of depressive symptoms, with higher scores indicating more severe depressive symptoms. Both state anxiety and trait anxiety were assessed via a total score on the Speilberger's State and Trait Anxiety Inventory (STAI) [22]. The STAI consists of 40 questions that each employ the four-point Likert scale, with higher total scores indicating higher levels of anxiety. State anxiety includes acute autonomic arousal, such as fear, nervousness, discomfort during the time of a temporary, perceived threat. Trait anxiety also includes symptoms of fear, nervousness, discomfort, but that one experiences on a day to day basis during typical situations. Scores on this questionnaire range from 20 to 80 for both state and trait anxiety. The total score across both types was utilized in our correlations.

Postconcussion symptoms were determined using the Rivermead Post-Concussion Symptoms Questionnaire (RPQ). The RPQ measures both the presence and severity of postconcussion symptoms based on questions that are more related to the acute or chronic phase of concussions, respectively [23]. The RPQ has a total of 16 items; the first three items (RPQ-3) refer to the 'early' symptom cluster and the RPQ-13 refers to the 'late' symptom cluster. Each item is rated on a five-point scale. Hence, the RPQ-3 maintains a range of 0–12 and RPQ-13 maintains a range of 0–52. Higher scores on either questionnaire indicate a more severe problem.

Long-term clinical outcomes in the chronic phase of the TBI were evaluated using the Mayo-Portland Adaptability Inventory (MPAI-4). The MPAI-4 provides an assessment of major obstacles resulting from a TBI that may prevent a subject from integrating back into daily living. Physical abilities, such as mobility, balance, and memory, and psychological attributes, such as anxiety, depression, and fatigue, are rated on a five-point scale ranging from 'none' to 'a severe problem'. Three subscales are produced: ability (sensory, motor, and cognitive abilities), adjustment (mood, interpersonal interactions), and participation (social contacts, money management). The MPAI-4 is scored from 0 to 111, with lower scores indicating greater integration and reduced symptoms.

Both the forward digit-span task and backward digit-span task were used to assess working memory storage capacity. This test is a subtest of both the Wechsler Adult Intelligence Scale and Wechsler Memory Scale. In either test, subjects are read a series of numbers and asked to repeat the sequence back to the examiner in order (digit span forward) or in reverse order (digit span backward, DSB). The forward span test determines attention efficiency and capacity, while the backward span captures executive functioning dependent on working memory. Higher scores on either examination indicate increased working memory capacity.

Statistical analysis

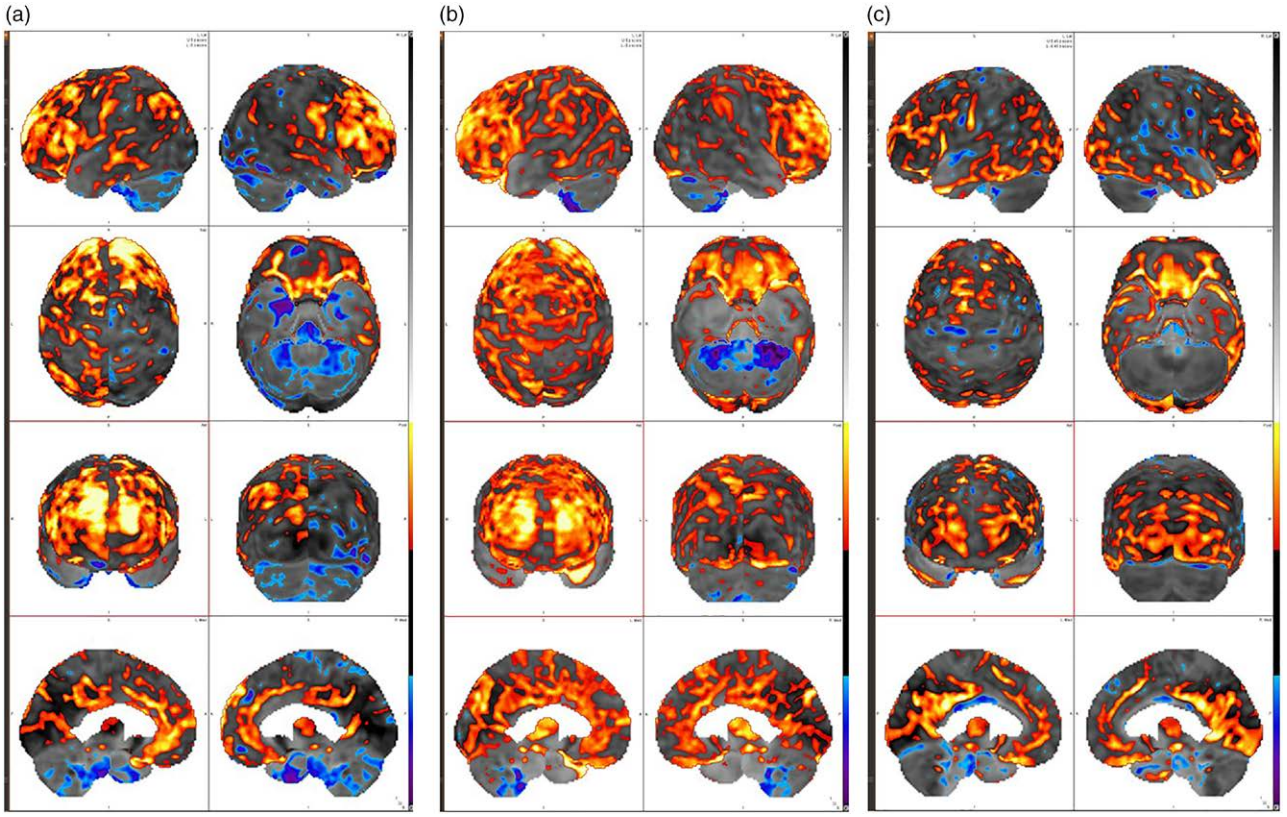
Mean differences between the FDG uptake values of various brain regions in PCS patients versus healthy controls were assessed using multivariate analysis of variance followed by the least significant difference and Tukey's honest significant difference post hoc tests with type I

Table 2 Mean differences in regional ^{18}F-fluorodeoxyglucose uptake between chronic traumatic brain injury patients and healthy controls

Region	Mean difference	Standard error	*P*	95% CI	
L inferior temporal gyrus	0.8333	0.26145	0.002	0.3177	1.3489
L medial occipital gyrus	0.7909	0.23603	0.001	0.3255	1.2564
L temporal lobe	0.7373	0.26116	0.005	0.2222	1.2523
L temporal pole	0.6911	0.24542	0.005	0.2071	1.1750
L orbitofrontal region	0.6297	0.26784	0.020	0.1015	1.1579
L posterior cingulate gyrus	0.6139	0.24857	0.014	0.1237	1.1040
L lateral temporal lobe	0.5637	0.22921	0.015	0.1117	1.0157
L cerebellar hemisphere	0.5260	0.18637	0.005	0.1584	0.8935
R temporal pole	0.5202	0.24542	0.035	0.0363	1.0042
R cerebellar hemisphere	0.4928	0.18637	0.009	0.1253	0.8604
L cingulate gyrus	0.4870	0.23873	0.043	0.0162	0.9578
R nucleus accumbens	0.4826	0.20827	0.022	0.0719	0.8933
L middle temporal gyrus	0.4737	0.20850	0.024	0.0625	0.8848
R temporal operculum	0.4447	0.18696	0.018	0.0761	0.8134
L temporal operculum	0.3804	0.18696	0.043	0.0117	0.7491
R middle occipital gyrus	−0.3796	0.17735	0.034	−0.7294	−0.0299
R paracentral lobule	−0.4304	0.19782	0.031	−0.8205	−0.0403
L superior parietal lobule	−0.4330	0.19580	0.028	−0.8191	−0.0469
L parietal lobe	−0.4384	0.20753	0.036	−0.8476	−0.0292
L occipital lobe	−0.4542	0.22471	0.045	−0.8974	−0.0111
L primary visual cortex	−0.4587	0.21029	0.030	−0.8734	−0.0440
L supplementary motor area	−0.4807	0.17997	0.008	−0.8356	−0.1258
L supramarginal gyrus	−0.5112	0.23599	0.031	−0.9766	−0.0458
R rolandic operculum	−0.5355	0.25368	0.036	−1.0357	−0.0352
R supramarginal gyrus	−0.5724	0.17997	0.002	−0.9273	−0.2175
R superior parietal lobule	−0.6146	0.19580	0.002	−1.0007	−0.2285
R inferior occipital gyrus	−0.6599	0.26437	0.013	−1.1813	−0.1386
L post-central gyrus	−0.6846	0.23049	0.003	−1.1391	−0.2300
R parietal lobe	−0.6853	0.20753	0.001	−1.0945	−0.2760
R cuneus	−0.6942	0.20759	0.001	−1.1036	−0.2848
L inferior occipital gyrus	−0.6974	0.26437	0.009	−1.2187	−0.1760
L superior occipital gyrus	−0.6975	0.23415	0.003	−1.1592	−0.2357
R post-central gyrus	−0.7002	0.23049	0.003	−1.1547	−0.2457
R superior occipital gyrus	−0.7338	0.23415	0.002	−1.1955	−0.2720
R primary visual cortex	−0.8325	0.21029	0.000	−1.2472	−0.4178
L precentral gyrus	−0.8489	0.22546	0.000	−1.2935	−0.4042
R occipital lobe	−0.8729	0.22471	0.000	−1.3160	−0.4298
R precentral gyrus	−0.9146	0.22546	0.000	−1.3592	−0.4700

Brain regions in red type are those that have hypermetabolism, while those in blue type have hypometabolism. L, left. R, right. *P* values and 95% confidence intervals (CI) are also provided. All reported findings are statistically significant with a family-wise error rate controlled at $\alpha=0.05$.

Fig. 1



Surface renderings of patterns of metabolic changes that occur after in patients with chronic traumatic brain injury. (a) There is increased metabolism in a number of frontal lobe structures with an asymmetry in the precentral gyrus region and the parietal region (reduced on the left) and also reductions in the temporal lobes and cerebellum. (b) There are much larger areas of intensely increased metabolism throughout the frontal, parietal, and temporal regions with reductions primarily in the cerebellum. (c) There are only a few areas of mildly increased metabolism in the orbitofrontal and posterior visual regions along with decreases in the temporal and parietal regions and brain stem.

error controlled at $\alpha = 0.05$ (two-sided), which helped to minimize false discovery rate with multiple comparisons.

To correlate FDG uptake in different brain regions with various clinical measures (i.e. symptoms and neuropsychological function), a combination of principle component analysis (PCA) and linear regressions were utilized based on the results from each individual scan of the PCS and the control subjects. PCA is a technique for reducing the dimensionality of large datasets to improve interpretability while minimizing information loss. This procedure clusters brain regions together based on a covariance matrix, creating new variables (i.e. components) that each contains multiple brain regions with similar metabolic signatures, presumably because they operate within coordinated brain networks. Forty regions of interest (selected based on the results of the initial comparison between PCS patients and healthy controls) were included in the PCA analysis. PCAs were performed separately for the left and right hemispheres, and components with eigenvalues greater or equal to 1 were subsequently included in the analysis. This eigenvalue cutoff generates components that cumulatively account for at least 80% of the variation in the overall data. The left hemisphere demonstrated 10 components, while the right hemisphere demonstrated 11 components (see Supplementary Figure, Supplemental digital content 1, *http://links.lww.com/NMC/A188*). Promax rotation with Kaiser normalization was then utilized to generate the most orthogonal components possible prior to pattern matrix analysis, where brain regions were sorted into each component using a minimum loading value of |0.4|. Components were then correlated with various clinical measures using Pearson's R to highlight specific clusters of regions that may be of interest for each clinical outcome. Correlations between clinical measures and variables such as age or other factors affecting the heterogeneity of the population. Thus, we did not stratify the analysis based on these variables and instead focused on the overall findings of this population.

Using PCA first to narrow down relevant clusters of brain regions, linear regressions were then used to identify specific associations between individual brain regions within

Table 3 Correlations between clinical measures of mood, cognition, function, and symptom severity with ^{18}F-fluorodeoxyglucose uptake in various brain regions

Clinical measure	Region	*B*	Standard error	*B′*	*P*	95% CI		α
BDI	L LTL	−4.280	1.175	−0.469	0.000	−6.643	−1.918	0.0167
	L STG	−4.139	1.281	−0.426	0.002	−6.716	−1.561	
	L MTG	−4.534	1.436	−0.418	0.003	−7.423	−1.645	
	R MOG	−3.984	1.545	−0.352	0.013	−7.092	−0.875	
STAI (state anxiety)	L LTL	−5.074	1.707	−0.394	0.005	−8.506	−1.641	0.05
	L MTG	−5.405	1.881	−0.383	0.006	−9.187	−1.623	
	L ITG	−3.451	1.479	−0.319	0.024	−6.425	−0.476	
MPAI–4	L LTL	−9.376	2.181	−0.531	0.000	−13.763	−4.989	0.0167
	L STG	−8.104	2.207	−0.472	0.001	−12.545	−3.664	
	L MTG	−7.557	2.569	−0.394	0.005	−12.724	−2.389	
DSB	R LG	−0.857	0.283	−0.358	0.004	−1.423	−0.290	0.0125
	L OFR	0.601	0.224	0.322	0.010	0.152	1.049	
	R OFR	0.621	0.235	0.318	0.010	0.151	1.090	
	R MOG	0.779	0.297	0.316	0.011	0.184	1.373	
RPQ-3 (acute symptoms)	R CG	1.293	0.352	0.422	0.001	0.588	1.997	0.0167
	L CG	1.168	0.331	0.409	0.001	0.506	1.831	
	R PCG	1.116	0.326	0.399	0.001	0.465	1.768	
	L ACG	0.870	0.329	0.319	0.010	0.213	1.527	
RPQ-13 (chronic symptoms)	R PCG	3.960	1.128	0.407	0.001	1.706	6.214	0.0167
	R CG	3.807	1.260	0.358	0.004	1.287	6.327	
	L IOG	−2.823	1.053	−0.322	0.009	−4.927	−0.719	

All reported findings are statistically significant with unstandardized regression weights (*B*), standardized regression weights (*B′*), *P* values, 95% confidence intervals (CI), and Bonferroni-corrected α levels shown.ACG, anterior cingulate gyrus; BDI, Beck's Depression Inventory; CG, cingulate gyrus; DSB, digit span backward Working Memory Task; IOG, inferior occipital gyrus; L, left; LG, lingual gyrus; LTL, lateral temporal lobe; MPAI-4, Mayo-Portland Adaptability Inventory-4; MTG, middle temporal gyrus; MOG, medial orbital gyrus; OFR, orbitofrontal region; PCG, posterior cingulate gyrus; R, right; RPQ, Rivermead Post-Concussion Symptoms Questionnaire; STAI, Spielberger's State and Trait Anxiety Inventory; STG, superior temporal gyrus.

relevant components and clinical outcomes (i.e. brain regions within components that do not exhibit significant correlations with various neuropsychological indices were excluded from further analysis). Data in regression analyses were modeled by least-squares regression lines, with each line having a slope equivalent to the regression coefficient (*B*), also known as regression weight. A Wald-type 95% confidence interval (CI) for B was then calculated from the standard error of the sampling distribution of data points from the slope of the regression line. The use of PCA prior to performing linear regressions helps to balance the relative risks of committing type I versus type II errors. The reason this balance is important to achieve in the context of brain region analysis is that many brain regions function within interconnected and coordinated neural networks, and thus the metabolism of any particular region is often intercorrelated with the metabolism of other regions [24,25]. Therefore, corrections for multiple comparisons that do not consider the collinearity between brain regions are at an exceptionally high risk of producing false-negative results [26]. PCA helps to remedy this issue by reducing the overall number of comparisons needed to detect significant correlations and providing empiric data for how brain regions are intercorrelated (i.e. the metabolism of brain regions within each component of PCA are highly correlated with one another, while components themselves have minimal collinearity between them). Therefore, even though Bonferroni corrections were used in this study to minimize the false discovery rate, the corrected α levels were based on the number of relevant components involved in the analysis, rather than individual brain regions of interest. All statistical analyses in this study were performed using the SPSS software v25 (IBM, Armonk, New York, USA).

Results

Table 2 shows FDG uptake in the brains of PCS patients compared to that of healthy controls. Overall, there were significant differences in the number of brain regions spread across the cerebrum and cerebellum, with a mix of hypermetabolic (15) and hypometabolic (23) regions. Notably, regions that exhibited increased FDG uptake following TBI were largely within the left hemisphere (11/15 regions, 73.3%) and included many temporal lobe and orbitofrontal structures, as well as the left cingulate gyrus. By contrast, regions that exhibited decreased FDG uptake following TBI were more evenly distributed between the right (13/23 regions, 56.5%) and left (10/23 regions, 43.5%) hemispheres, and included many structures within the Rolandic area, parietal lobe, and occipital lobe. Examples of several different metabolic patterns detected in specific brain regions of PCS patients are shown in Fig. 1.

Given the diverse changes in brain metabolism seen in PCS patients, we then investigated whether these changes could be clinically relevant by correlating the degree of FDG uptake in PCS brains with clinical measures of mood, cognition, overall functional status, and postconcussion symptom severity. Specifically, we correlated FDG uptake with measures of depression via BDI, state anxiety via STAI, multimodal (including physical) function/disability via MPAI-4, working memory via

DSB, acute postconcussion symptoms via RPQ-3, as well as chronic postconcussion symptoms via RPQ-13. The results are shown in Table 3. Importantly, in line with our earlier finding that regions within the left temporal lobe of PCS patients exhibited some of the highest increases in FDG uptake, the metabolism in several left temporal lobe structures demonstrated an inverse correlation with the severity of both depression and anxiety. This finding indicates that increased metabolism within the left temporal lobe is associated with improved mood. Similarly, metabolism in left temporal lobe structures inversely correlated with MPAI-4 scores, signifying both decreased disability and improved function in PCS patients with higher levels of left temporal lobe metabolism. We then found that metabolism in the bilateral orbitofrontal regions and right medial orbital gyrus positively correlated with performance on the DSB. Interestingly, a negative correlation was also noted between metabolism in the right lingual gyrus and DSB performance. Finally, metabolism in the bilateral cingulate gyri positively correlated with acute postconcussion symptom severity (RPQ-3), whereas only metabolism in the right cingulate gyrus positively correlated with chronic postconcussion symptom severity (RPQ-13). These correlations are interesting to consider given that the left cingulate gyrus exhibits overall increased metabolism in PCS patients versus healthy controls, but no significant difference in metabolism was found in the right cingulate gyrus (mean difference, –0.4062; 95% CI, –0.8769 to 0.0646; $P = 0.09$). An inverse correlation between metabolism in the inferior occipital gyrus and RPQ-13 was also demonstrated.

Discussion

Through a combination of FDG PET imaging and neuropsychological testing, we were able to generate a comprehensive profile of metabolic changes in the brain that occurred during the chronic phase of TBI and connect these changes to clinical outcomes. In particular, this study demonstrates an array of hypermetabolic and hypometabolic brain regions that have wide-ranging associations with mood, cognition, overall functional status, and postconcussion symptom severity. These remarkable patterns suggest that the neurophysiological alterations associated with PCS represent a complex mixture of pathological dysregulation and compensatory response, as we will subsequently discuss in detail. To our knowledge, this is the first study to broadly determine the metabolic profile of a heterogenous patient population meeting the criteria for PCS.

Mild nonchronic TBI is generally associated with broad patterns of sustained brain hypometabolism which can be global or focal [27]. Indeed, previous research has demonstrated hypometabolism in the cerebellum, cingulate gyrus, and diffuse areas of the cerebral cortex in mild nonchronic TBI. Similarly, the participants enrolled in our study have experienced only mild TBI and demonstrate hypometabolism in some of these same regions, including the Rolandic area as well as other regions in the parietal and occipital lobes. Important differences between the results of the present study and prior data should also be noted. For example, the cerebellum left cingulate gyrus and left temporal lobe all showed significant hypermetabolism in our study, unlike what had been previously reported. The differences in metabolism may in part reflect the different timeframes of our study compared to past studies. Indeed, an important inclusion criterion for patients enrolled in this study is that they must report symptoms following TBI for at least 3 months, thereby satisfying the requirement for PCS. This criterion was not present in previous FDG PET studies, as they combined data from both chronic and nonchronic TBI (and sometimes even mild and severe TBI) without applying stratification methods to differentiate between the aforementioned groups; this aggregation may confound the results of past analyses. Therefore, it is difficult to compare the results of the present study to that of earlier neuroimaging studies due to differences in acquisition protocols, quantification techniques, patient selection, stage of injury, and normative databases.

Importantly, the results of our analysis suggest that global hypometabolism is not a defining characteristic of PCS, since many brain regions in PCS patients demonstrate elevated metabolism. Notably, the orbitofrontal cortex which is important for working memory in the digit span tasks, showed baseline hypermetabolism. In addition, there were many other areas with significant hypermetabolism including the cingulate gyrus, medial occipital gyrus, and left temporal lobe, all of which are suggested to contribute to emotional processing, perception, and language patterns. A recent study showed similar findings during the investigation of brain volume abnormalities in patients with chronic mild or moderate TBI. In that study, patients had many abnormal areas of structural enlargement and only a few areas of atrophy [28]. Although Ross *et al.* had some differing regions of increased brain volume, the general presence of hypermetabolic regions in PCS remains similar to our study, given that increased brain volume often equates to increased metabolism.

Regarding the overall finding of areas of both increased and decreased metabolism in PCS patients, it is interesting to note that many areas that were significantly abnormal in these patients also have the potential to underlie various common symptoms in this population. For example, some occipital lobe regions were significantly hypermetabolic while others are hypometabolic. This is an interesting finding since many patients with PCS report chronic visual problems including hypersensitivity to light, problems with accommodation, and blurry vision. In our cohort, 51/64 patients reported visual symptoms of some kind (28 blurred vision, 41 photosensitivity, and 13 with double vision) which might help understand the associated abnormalities in the visual cortex and occipital

lobe. The anterior cingulate, putamen, and temporal lobe are involved in emotional regulatory control and thus abnormalities in these regions can be attributed to substantial reports of emotional disturbances including anxiety, depression, and irritability in these patients.

The correlation analysis revealed significant associations between metabolism in the temporal lobe and both anxiety and depressive symptoms [29–31]. Abnormalities in temporal lobe processes, as well as the cingulate gyrus, can contribute to both depression and language and memory-related problems [32–34]. Language and memory abnormalities were reported in 62/64 patients, including slowness in thinking and reduced memory. Such clinical abnormalities will be important to evaluate on a patient-by-patient basis to better understand what types of ongoing symptoms they have and how they might relate to underlying neurophysiological changes on FDG PET.

The correlation analysis in combination with the differences between the PCS and controls reveals important information about the PCS state. PCS patients with lower levels of metabolism in the temporal lobe had higher levels of depression and anxiety. And patients with lower levels of metabolism in the temporal lobes had decreased adaptability based on the MPAI-4. Decreased metabolism in the orbitofrontal gyrus was associated with worse scores on the working memory task DSB. Interestingly, metabolism in these structures is significantly higher in the PCS patients compared to controls. Thus, it appears that increased metabolism might reflect a compensatory response to areas of neuronal injury since higher metabolism results in improved cognitive and emotional functioning. The positive correlation between the cingulate gyrus structures with worse scores on the RPQ-3 and RPQ-13 implies that the cingulate hypermetabolism is part of the PCS process and may reflect persistent inflammation or neuronal excitation. Interestingly, none of the areas of decreased metabolism in the PCS patients correlated with worsening symptoms. Because the correlations are only with PCS patients and do not include control patients, the reduced metabolism in some brain regions may actually be associated with symptoms, but a graded response is not statistically detected. The graded response of neuronal compensatory mechanisms in the chronic phase of mild TBI is consistent with the heterogenous population of PCS patients, as there is considerable variability amongst individuals in their ability to counteract the mechanisms of neuronal injury. However, since every patient included in this study continues to be symptomatic, it seems that compensatory mechanisms only help to a certain extent and do not completely alleviate symptoms. At the same time, the variability in compensatory mechanisms seen amongst PCS patients raises the possibility that these mechanisms could be therapeutically enhanced in the future.

Evidence suggests that neuroinflammation begins in the acute phase of TBI and becomes more evident in the chronic phase [35]. In mouse models, activation of microglia (resident immune sentinels of the central nervous system) gradually disperses throughout the brain and persists for over a year following TBI, with equal densities in the ipsilateral and contralateral regions, suggesting a global response to a localized injury [36]. PET-based neuroimaging using PK11195, a ligand for Translocator Protein, a translocator protein expressed at high levels in activated microglia, also shows global microglial activation in the chronic phases of TBI [37]. Changes in the expression profiles of inflammatory genes following TBI that persist over several months suggest long-term modulation at a cellular level [38]. However, many of these studies were performed *in vitro* and in animal models, which have shown better efficacy in modeling moderate to severe TBI instead of mild TBI. Further direct studies are needed in humans.

Limitations of this study include the heterogeneous group of participants in terms of the types, causes, and number of head injuries. Also, we enrolled subjects with a variety of symptoms without excluding one particular domain. Thus, participants could report emotional, cognitive, sensory, or other types of symptoms, as well as a combination of such symptoms as determined on the RPQ. Although we were able to detect significant patterns of metabolism relevant to clinical outcomes in PCS patients, the sensitivity of our analysis may be limited by patient heterogeneity. However, this population is more representative of the types of patients presenting with PCS symptoms. In addition, although some of the PCS patients reported use of medications to help with their postconcussion symptoms, it is unlikely that the limited medication use would account for the multiple significant findings in the larger cohort of patients, especially since there were a variety of medications that should theoretically dilute findings attributable to PCS. In spite of the heterogeneity of the patient cohort, we still found a combination of significant hypermetabolic and hypometabolic regions that appear to be characteristic of PCS in contrast to healthy controls. Furthermore, this study does not distinguish whether the symptoms measured are primarily caused by the head injury or arise secondarily; however, all symptoms were required to arise after the head injury and thus were not experienced by subjects prior to the injury. Future studies will have to determine if such a mixed pattern of metabolic activity can be used to specifically separate patients with PCS from patients with other neurological or psychiatric disorders, especially when those disorders have symptoms that overlap with those in patients with PCS. These studies could use TBI patients without chronic symptoms as a new control for PCS patients, which allow for robust detection of specific chronic versus nonchronic TBI differences.

We would argue that the characteristic 'pattern' in PCS patients may be a combination of regions with both hypermetabolism and hypometabolism. This combination

of hypermetabolism and hypometabolism in the brain structures reported in this study may be a neurobiological signature of PCS. The mechanism of pathophysiological change underlying this increased metabolic uptake is not known, although chronic-neuroinflammation or excitation in the form of compensatory mechanisms is strong possibility. Causes of hypometabolism are likely related to neuronal dysfunction, but could potentially be associated with heightened inhibitory control from areas that are already in a persistent excitatory state [39]. Regarding the underlying cause of the findings on FDG PET scans in patients with PCS, we propose that PCS presents with a combination of neuroinflammation that potentiates neuro-excitotoxicity/increased glucose metabolism and triggers downstream compensatory mechanisms, coupled with neuronal cell damage associated with decreased metabolic activity. Additional PET imaging studies, including those utilizing a neuroinflammatory radiotracer, are warranted to further investigate these results.

Conclusion

Compared to the brains of healthy controls, those of PCS patients have a 'pattern' that includes multiple areas of both increased and decreased metabolism. These results suggest that FDG PET brain imaging may be highly useful in the evaluation of patients with PCS and chronic symptoms as a way of evaluating their associated brain abnormalities.

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All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional 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 individual participants included in the study.

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of interest

There are no conflicts of interest.

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