Repeated Mild Traumatic Brain Injury: Mechanisms of Cerebral Vulnerability

Mayumi L. Prins,^{1,4,5} Daya Alexander,^{4,5} Christopher C. Giza,^{1,2} and David A. Hovda^{1,3–5}

Abstract

Among the 3.5 million annual new head injury cases is a subpopulation of children and young adults who experience repeated traumatic brain injury (TBI). The duration of vulnerability after a single TBI remains unknown, and biomarkers have yet to be determined. Decreases in glucose metabolism (cerebral metabolic rate of glucose [CMRglc]) are consistently observed after experimental and human TBI. In the current study, it is hypothesized that the duration of vulnerability is related to the duration of decreased CMRglc and that a single mild TBI (mTBI) increases the brain's vulnerability to a second insult for a period, during which a subsequent mTBI will worsen the outcome. Postnatal day 35 rats were given sham, single mTBI, or two mTBI at 24-h or 120-h intervals. ¹⁴C-2-deoxy-D-glucose autoradiography was conducted at 1 or 3 days post-injury to calculate CMRglc. At 24 h after a single mTBI, CMRglc is decreased by 19% in both the parietal cortex and hippocampus, but approached sham levels by 3 days post-injury. When a second mTBI is introduced during the CMRglc depression of the first injury, the consequent CMRglc is depressed (36.5%) at 24 h and remains depressed (25%) at 3 days. In contrast, when the second mTBI is introduced after the metabolic recovery of the first injury, the consequent CMRglc depression is similar to that seen with a single injury. Results suggest that the duration of metabolic depression reflects the time-course of vulnerability to second injury in the juvenile brain and could serve as a valuable biomarker in establishing window of vulnerability guidelines.

Key words: brain injury; juvenile; metabolism; repeated; vulnerability

Introduction

THE INCREASED INCIDENCE of repeated traumatic brain injury (RTBI) has generated new discussions about the existence of cerebral vulnerability, its duration, and relationship to subsequent injuries. While it is rational to assume that TBI increases the brain's vulnerability to secondary injury, preconditioning effects have been demonstrated.¹ Fluid percussion (FP) injury given 1 h before ischemia exacerbates hippocampal cell loss,² but when given 24 h before ischemia, it is neuroprotective.¹ This evidence suggests a temporal window of cerebral vulnerability in adult rats after mild TBI (mTBI). Age-related differences in the temporal profile of cerebral vulnerability after TBI have yet to be demonstrated.

Mechanisms involved in ischemic preconditioning include prolonged changes in cellular energy glucose metabolism,^{3,4} which could potentially serve as a biomarker of cerebral vulnerability. Changes in cerebral glucose metabolism have been established as a hallmark response after TBI. Immediately after impact, there is a rapid and transient increase in cerebral glucose uptake in the brain, followed by a prolonged period of depression of the glucose metabolism (cerebral metabolic rate of glucose [CMRglc]; mg/100 g/min). This injury-

induced decrease in CMRglc has been observed after FP,^{5,6} controlled cortical impact (CCI),^{7–9} and human TBI.^{10,11}

Alterations in brain glucose metabolism vary with injury severity and age. Glucose metabolic depression was maintained for 5, 10, or 14 days after mild, moderate, or severe FP injury in adults, respectively.¹² Duration of behavioral deficits in the Morris water maze also correlated with injury severity.¹³ Administration of d-amphetamine induced recovery of both the CMRglc depression and behavioral deficits, suggesting a correlation between these outcome measures.^{14,15} The more severe CCI injury also produced a more profound and longer lasting depression than FP injury^{16,17} (Fig. 1). This relationship between CMRglc and injury severity has also been observed in human patients with TBI.¹⁸ TBI in the absence of overt cell death or gross pathology can still produce measurable behavioral dysfunction. Further, mTBI, which may have axonal damage or dysfunction as detected from diffusion tensor imaging (DTI), also shows measurable decreases in CMRglc.^{19,20} More importantly, the decrease in CMRglc in mTBI patients was correlated with persistent post-concussive symptoms.^{20,21}

The magnitude and duration of CMRglc depression after TBI also increases with age^{17,22,23} across all injury models (Fig. 1). For

Departments of ¹Neurosurgery, ²Neurology, ³Molecular and Medical Pharmacology, ⁴The Interdepartmental Program for Neuroscience, ⁵The UCLA Brain Injury Research Center, University of California, Los Angeles, School of Medicine, Los Angeles, California.

FIG. 1. Traumatic brain injury (TBI)-induced cerebral metabolic rate of glucose (CMRglc) depression. This graph shows a comparison of the CMRglc changes with time after various types of TBIs in different aged rats as measured by ^{14}C -2-deoxy-D-glucose autoradiography (data from ^{5,16,17,22,23}). FP, fluid percussion; PND, post-natal day; CCI, controlled cortical impact.

example, FP-injured post-natal day (PND)17 rats showed glucose metabolic recovery to age-matched shams within 3 days²² compared with 10 days in the adult rats.⁵ After CCI injury, PND35 rats showed recovery of metabolic rates of subcortical structures within 3 days compared with 7 days in PND90 rats and better metabolic recovery in the cortex by day 7¹⁷ (Fig. 1).

Despite the unclear mechanisms of induction, the presence of metabolic depression reflects an altered cerebral condition that is associated with functional deficits and has been proposed to reflect a vulnerable cerebral state.^{21,24–26} It is currently unknown how the exposure of a second mTBI during this period of CMRglc depression will affect cerebral metabolism, behavioral function, and histological outcome of the developing brain. The current study will address the central hypothesis that a single mTBI increases the brain's vulnerability to a second insult for a period, during which a subsequent mTBI will worsen the outcome. It is hypothesized that the duration of vulnerability is related to the duration of altered intracellular redox and decreased CMRglc, which may be used as a biomarker for vulnerability (Fig. 2).

FIG. 2. Diagrammatic representation of the central hypothesis. A single mild traumatic brain injury (mTBI) increases the brain's vulnerability to a second insult for a period, during which a subsequent mTBI will worsen outcome. A second injury introduced after this period of vulnerability will result in cerebral metabolic rate of glucose (CMRglc) changes similar to the first injury. ^{14}C -2dg, ^{14}C -2-deoxy-D-glucose.

Methods

^{14}C -2-deoxy-D glucose subjects

PND35 (142.9 ± 3.9 g) male Sprague-Dawley rats were randomly divided into groups that were given sham ($n = 11$), single ($n = 12$), or two injuries at a 24-h interval ($n = 11$) or two injuries at a 120-h interval ($n = 10$). CMRglc were determined using ^{14}C -2-deoxy-D-glucose autoradiographic (2DG) method at 1, 3, or 5 (only single group) days post-injury. All procedures were approved with the UCLA Chancellor's Committee for Animal Research.

The highest incidence of mTBI and RTBI occurs during adolescents. While there are no exact guidelines for determining interspecies age equivalents, we have selected the PND35 age group based on numerous developmental profiles and previously collected data. PND12–13 has been proposed to reflect human newborns,²⁷ sexual maturity is between PND45–60, and glucose metabolism is 90% adult values at PND35.²⁸ The molecular composition of PND34–44 rat synapses was reflective of synaptic changes during the teen years.²⁹ Similar sleep changes, increased risk taking behaviors, and potential for addiction of teenagers is also observed between PND28–45 in the rat.^{30–32} Based on these data, PND35 animals were specifically chosen for this study to reflect the adolescent age group.

Injury model

As previously described and characterized,³³ under isoflurane (2.0%/100% O_2) the head was shaved and the nose placed within the stereotaxic face mask to maintain anesthesia. The animals were placed on a heating pad with the head against a shaped wooden block within the stereotaxic frame, without ear bars. An electronically controlled pneumatic piston cylinder (Hydraulic Control, Inc, Emeryville, CA) was mounted onto a stereotaxic micromanipulator (Kopf Instruments, Tujunga, CA) to allow for precise localization of the impact center. A template was used to mark the center of impact (-3AP , -4ML relative to bregma), and the injury tip was firmly zeroed against the skin. The piston was angled at 23 degrees away from the vertical to allow the impactor to make contact perpendicular to the head's surface. The impactor tip (5-mm diameter) displaced the head 8 mm at 36 psi. The head was free to move in the direction of the injury. This level of impact does not cause skull fractures. It does, however, produce apnea and delays the toe-pinch response and righting time.

2DG autoradiography

Local CMRglc ($\mu\text{mol}/100\text{ g}/\text{min}$) were measured by the 2DG method originally described by Sokoloff and associates.³⁴ Under isoflurane anesthesia (2 L/min), the femoral artery and vein were cannulated with a polyethylene tube (PE-50) on the day of study and restrained on a piece of cardboard for at least 2 h to allow the effects of anesthesia to diminish. Baseline blood-gas measurements were then taken (pH, pO_2 , pCO_2 ; 1304 pH, Blood Gas Analyzer; Instrumentation Laboratory, Stockholm, Sweden), and ^{14}C -2DG (150 mCi/kg) was administered intravenously (IV) over 30 sec. Twelve timed arterial blood samples were collected in polyethylene-heparin lithium fluoride coated microcentrifuge tubes (Beckman) during the 45-min experiment.

At 45 min after 2DG injection, the rats were sacrificed with a lethal dose of sodium pentobarbital (100 mg/kg, IV) and the brains were removed and immediately frozen. Coronal sections (20 μm) were exposed to Kodak Biomax film with ^{14}C -methylmethacrylate standards (Amersham, Arlington Heights, IL). Adjacent sections were processed for cresyl violet staining and examined under light-microscopy to determine the presence of overt histopathology.

Using a computerized image analysis system (NIH Image), the optical densities were measured bilaterally in eight regions. The regions of interest are illustrated in Figure 3 and were selected away

FIG. 3. Three coronal sections indicating the nine autoradiographic regions of interest measured. Fr1 (frontal cortex), Par1 (anterior parietal cortex), Hippo (whole hippocampus), CA1, CA3, Thal (thalamus), PrR (perirhinal cortex), Parietal cortex, Posterior parietal cortical strip. The black bar over parietal indicates the center of the impact site.

from the impact site to avoid regions of potential blood-brain barrier disruption. Optical density values were obtained across at least three sections for each structure and averaged by an investigator blind to group assignment.

The CMRglc was calculated using the equations previously described by Sokoloff and colleagues³⁴ and el Hamdi and co-workers.³⁵ The blood samples collected (range 20–50 μ L) were immediately placed on ice, centrifuged, and the plasma assayed for ¹⁴C-activity using a scintillation counter (LS 5000 CE; Beckman Instruments, Inc, Fullerton, CA). The timed plasma samples were processed for glucose with the YSI 2700 Biochemistry Analyzer for the calculation of CMRglc rates.

Novel object recognition (NOR) behavioral task

A separate group of animals was used to determine the recovery time of NOR deficits previously noted between single and RTBI groups.³¹ PND35 male Sprague Dawley rats ($n = 7$ for each group, 184.2 ± 7.4 g) were given sham, single, or two injuries at a 24-h interval before NOR testing. This method was based on the original Ennaceau and Delacour³⁶ procedure and has been described previously.^{33,37} Briefly, the NOR testing chamber was a white, Plexiglas arena ($52 \times 52 \times 9$ cm). Two objects were secured to the testing chamber floor via Velcro and positioned centrally near the back wall of the chamber. During the habituation phase (post-injury day [PID]2), animals were allowed 10 min to acclimate to the new

environment. On PID3, animals were given 5 min to explore the environment with two identical objects. On PID4, animals were placed in the arena with one familiar object and one novel object and were allowed to explore for 5 min. The duration of time spent with each object and the number of encounters was determined by the SMART tracking system.

Statistical analysis

Measurements for body weights, apnea, toe-pinch, and righting times were analyzed using one-way analysis of variance (ANOVA) for group (sham, single, RTBI 24 h, RTBI 120 h). CMRglc rates were analyzed using one-way ANOVA for groups. *Post hoc* analyses were conducted using the Tukey modestly significant difference. Percent time spent with a novel object in the behavioral tasks was also analyzed with one-way ANOVA for groups with the Tukey *post hoc* analysis.

Results

Physiological changes after TBI

The average weight, apnea, toe pinch, and righting times for 2DG and behavioral animals are shown in Table 1 and Table 2, respectively. There were no significant differences in starting weights between groups for either 2DG animals or behavioral

TABLE 1. PHYSIOLOGICAL DATA FOR 2DG ANIMALS

Groups	Weight at behavioral testing	Injury #1			Injury #2		
		Apnea (s)	Toe pinch (s)	Righting (s)	Apnea (s)	Toe pinch (s)	Righting (s)
Sham	134.4 $\pm$ 8.3	0	—	—	0	—	—
Single	127.7 $\pm$ 4.3	2.7 $\pm$ 0.4	50.7 $\pm$ 6.2	200.2 $\pm$ 19.8	—	—	—
RTBI (24 h)	152.1 $\pm$ 3.4	3.2 $\pm$ 0.7	58.5 $\pm$ 6.6	276.4 $\pm$ 31.2	3.4 $\pm$ 0.6	65.7 $\pm$ 14.3	247.1 $\pm$ 35.9
RTBI (120 h)	162.9 $\pm$ 10.4	2.1 $\pm$ 0.4	51.0 $\pm$ 4.4	225.9 $\pm$ 18.9	2.9 $\pm$ 1.1	46.4 $\pm$ 3.3	183.4 $\pm$ 20.0

RTBI, repeated traumatic brain injury.

TABLE 2. PHYSIOLOGICAL DATA FOR BEHAVIORAL ANIMALS

Groups	Weight at behavioral testing	Injury #1			Injury #2		
		Apnea (s)	Toe pinch (s)	Righting (s)	Apnea (s)	Toe pinch (s)	Righting (s)
Sham	189.2 ± 10.1	0	—	—	0	—	—
Single	176.3 ± 14.8	3.0 ± 0.6	56.1 ± 2.4	268.0 ± 47.1	—	—	—
RTBI (24 h)	186.4 ± 17.0	4.0 ± 1.4	63.8 ± 7.6	310.0 ± 48.1	3.6 ± 0.9	62.4 ± 7.6	264.0 ± 80.1

RTBI. repeated traumatic brain injury.

animals. There were no significant group differences in duration of apnea, toe pinch, or righting times from a single injury in both 2DG and behavioral animals. Duration of apnea, toe pinch, or righting times in RTBI groups did not differ between first and second injuries. These physiological changes are consistent with previously reported findings for this mTBI³³ and suggest that injury severity was the same.

CMRglc changes after single mTBI

While all other structures showed decreasing trends in CMRglc rates at PID1, only the parietal and CA1 attained significance. Single mTBI resulted in a significant 19% decrease in CMRglc in both the ipsilateral parietal cortex and CA1 of the hippocampus at PID1. CMRglc of both ipsilateral structures returned toward sham rates by PID3 and remained at sham levels on PID5 (Fig. 4). Contralateral parietal cortex and CA1 hippocampus also showed 12.9% and 15.2% decreases in CMRglc, respectively, at PID1. CMRglc of the contralateral structures returned to sham levels by PID3.

CMRglc changes after RTBI

Parietal cortex. PND35 sham animals showed average parietal cortex CMRglc rates of 127.1 ± 5.6 (contralateral) and 129.4 ± 7.7 (ipsilateral) $\mu\text{mol}/100\text{g}/\text{min}$, which are consistent with previous studies.¹⁷ When a second mTBI was introduced within 24 h after the first mTBI, the CMRglc rates dropped in the parietal cortex by 17.8% contralaterally and 36.5% ipsilaterally ($p < 0.01$) (Fig. 5). While the decreased rates moved toward sham levels by PID3, they remained significantly depressed ipsilaterally. When the second mTBI was delivered 120 h after the first mTBI, the CMRglc rates on PID1

decreased in the parietal cortex by 8.4% contralaterally and 17.1% ipsilaterally, but these changes did not reach statistical significance. AT PID3, the RTBI-120-h cortex was not different from sham rates.

Hippocampus CA1. The average CMRglc rates for sham PND35 rats were 80.8 ± 4.5 (contralateral) and 83.6 ± 3.1 (ipsilateral) $\mu\text{mol}/100\text{g}/\text{min}$ (Fig. 6). A single mTBI caused a significant bilateral decrease in CMRglc rates for contralateral and ipsilateral CA1 (15.2% and 19.4%, respectively). RTBI with 24-h interval showed significantly greater bilateral decreases in CMRglc rates (33.5% contralateral and 40.1% ipsilateral). These metabolic rates remained significantly depressed in both hemispheres at 3 days. RTBI with 120-h interval showed no significant changes in metabolic rates relative to sham animals at either PID1 or PID3.

NOR task

Animals able to discriminate between the old and new objects should spend more than 50% of their exploration time with the new object. Results from a previous study³¹ examined behavioral differences between sham, single injured, and RTBI groups at PID1. Only in animals who were assigned to the 24-h interval RTBI group were tested with the NOR at different times after injury to follow the time-course of recovery. Both the single (61.3%) and RTBI 24-h (54.3%) groups showed significant decreases in percent time spent with the novel object relative to sham (78.8%). In the current study, the recovery of this deficit was explored at PID3 (Fig. 7). Sham animals presented with a novel object 24 h after familiarization performed as previously expected at 71.7% time with the novel object. Single

FIG. 4. Average ($\pm$ standard error of the means) changes in the cerebral metabolic rate of glucose (CMRglc) with time after single mild traumatic brain injury in both parietal and hippocampal CA1 regions, illustrating the recovery of metabolism by 3 days after injury.

FIG. 5. The graph shows the average ($\pm$ standard error of the mean) changes in the cerebral metabolic rate of glucose (CMRglc) between injury groups at 1 and 3 days post-injury in the contralateral and ipsilateral parietal cortex. * $p < 0.05$; ** $p < 0.01$. RTBI, repeated traumatic brain injury.

FIG. 6. The graph shows the average ($\pm$ standard error of the mean) changes in the cerebral metabolic rate of glucose (CMRglc) between injury groups at 1 and 3 days post-injury in the contralateral and ipsilateral CA1 hippocampus. * $p < 0.05$; ** $p < 0.01$. RTBI, repeated traumatic brain injury.

injured animals (67.0%) showed no significant difference from sham in percent time spent with the novel object. The RTBI 24-h group, however, continued to show significant deficits in recognition of the novel object at PID3 (57.5%) relative to sham.

Discussion

The CMRglc hypometabolic phenomenon

CMRglc hypometabolism has been observed after various types of brain injuries, including freeze lesions, ischemia, TBI, and blast injury. Unilateral freeze lesions in the adult rat produced widespread ipsilateral cortical decrease in CMRglc with less hypometabolism in subcortical structures between 1–3 days post-lesion.³⁸ Similar decreases in CMRglc have been reported in the adult rat within 2 h after ischemia,³⁹ and in numerous models of TBI.^{5,40–42} The magnitude and duration of the hypometabolic phase varies

with injury severity in both animal models of TBI¹² and clinical TBI.^{20,43–45} In adult rats, glucose hypometabolism was maintained for 5, 10, or 14 days after mild, moderate, or severe FP injury, respectively.¹² This relationship between CMRglc and injury severity has also been observed in human patients with TBI, where thalamic, brain stem, and cerebellar regions correlated with the Glasgow Coma Scale.¹⁸ And as mentioned in the Introduction, cerebral development also affects the duration of glucose hypometabolism, with the duration of hypometabolism increasing with age.

The duration of the CMRglc depression has been shown to correlate with the time course of cognitive deficits, suggesting that the metabolic abnormalities have functional consequences. This relationship has been demonstrated after freeze lesions in rats,³⁸ lateral FP injury in rats,¹³ and severe closed head injury in adult patients.⁴⁶ Lesioned adult rats showed significant increase in latency for a somatosensory task of adhesive removal from the forepaws. The recovery profile of this task was temporally correlated with the recovery of average cortical CMRglc.³⁸

Manipulation of CMRglc with drugs, such as d-amphetamine, not only induced recovery of CMRglc in the sensorimotor cortex,¹⁵ but also alleviated the locomotor deficits after cortical lesions.⁴⁷ It is important to note that mTBI or brain injuries that cause no overt cell loss have been shown to produce both CMRglc depression and detectable behavioral deficits.^{48,49} The time course for cognitive recovery of adult rats after FP injury with no cell loss was also shown to correlate temporally with the cortical CMRglc recovery.¹³ Collectively, this growing body of evidence demonstrates the fundamental cerebral response to injury involves alterations in CMRglc regardless of injury type, severity, age, or cell loss and consequently produces functional deficits. It should be noted that very few clinical PET studies have been conducted after TBI in children and, therefore, how the corresponding metabolic time course varies with age is currently unknown.

What causes CMRglc depression?

While CMRglc depression appears to be a universal response to brain injury, what is its etiology? CMRglc determined by PET or ¹⁴C-2DG autoradiography measures cerebral uptake of glucose up to the hexokinase reaction. Brain glucose uptake can decrease under substrate limiting conditions or alterations in glucose transporter expression. The question of whether head-injured patients show changes in their plasma glucose profile as a result of TBI is difficult to determine, given that blood glucose is aggressively managed within a specific range in human patients to prevent hyper-/hypoglycemia.⁵⁰

Results from animal studies show that PND35 rats maintained on standard chow had little change in blood concentrations of glucose or lactate.¹⁷ While rapid increases in the expression of glucose transporter protein, GLUT1, have been reported after hypoxia ischemia,^{51,52} a decrease in GLUT1 immunoreactivity was observed 2–4 h after FP injury in adult rats.⁵³ Clinical evidence, however, suggests that GLUT1 expression along microvessels is patchy, with some regions showing increased expression interspersed with regions of low expression.⁵⁴ Studies examining the kinetics of ¹⁸F-FDG in head-injured patients showed focal decreases in glucose transport at the contusion site and global reduction in hexokinase activity.¹⁸ Interpretation of these findings is difficult, given that the regional differences may not reflect leakage of glucose through the blood-brain barrier acutely after injury. No studies, however, have addressed the changes in GLUT1 vascular

FIG. 7. Time course of novel object task recovery are shown for sham, single, and repeated traumatic brain injury (RTBI) 24 h. The box plot shows the median percent time spent with the novel object at 1 and 3 days post-injury. Behavioral changes after RTBI 120 h were not conducted for this experiment. * $p < 0.05$, ** $p < 0.01$ relative to sham animals.

expression with time after TBI, so its contribution to long-term CMRglc depression is unknown.

If one were to assume that glucose supplies are abundant and transport is sufficiently functional, then the decreases in CMRglc must either reflect decreased need for glucose metabolism or metabolic dysfunction. Decreases in CMRglc have been proposed to reflect changes in neurotransmission,⁵⁵ thereby resulting in decreased neuronal activity and decreased energy demands. Changes in both the serotonergic and adenosine concentrations have been shown to be altered after TBI^{56,57} and affect CMRglc.^{28,58} TBI-induced increases in extracellular adenosine and serotonin, however, are acute changes that return to baseline within 40 min after CCI injury⁵⁹ and 160 min after FP injury.⁵⁶ This acute time course in the rodent is not likely to account for the CMRglc changes that last days. It should be mentioned, however, that severe human TBI has shown elevated cerebrospinal fluid levels of adenosine days after injury, which were correlated with metabolic depression.⁶⁰ Freeze lesions have also been shown to induce CMRglc depression at 3 days, at which time serotonin synthesis was significantly elevated.⁵⁸ While the current body of evidence does not support neurotransmitter-induced metabolic depression, more research in this area is needed, because there remains a possibility that these earlier chemical changes may be the impetus for longer-lasting metabolic cascades.

The mechanism of CMRglc depression may also differ with time after injury. The possibility remains that the acute initiating mechanisms may result in delayed secondary cascades that are reflected by continued CMRglc depression. While these TBI-induced acute neurochemical changes may initiate decreases in CMRglc, delayed effects may be related to diffuse synaptic loss and/or axonal impairments. TBI has been shown to damage axons⁶¹ and result in white matter damage.⁶² After TBI, regions of diffuse axonal injury showing decreased fractional anisotropy were significantly correlated with reduction in CMRglc as measured by PET.⁶³ The axonal injury is often heterogeneous, because myelinated fibers show earlier recovery of action potentials than non-myelinated fibers of the corpus callosum.⁶⁴ In multiple sclerosis, patients who exhibit demyelinated axons also shown remote effects on CMRglc with significant glucose metabolic depression in the thalamus and cerebellum.⁶⁵ This type of denervation-induced CMRglc decrease has also been reported in Alzheimer's disease.⁶⁶ Damage to axons and/or decreases in synaptic connectivity can directly affect cerebral activity levels, CMRglc, and behavioral outcome.⁶⁷

After TBI, axonal changes occur concurrently with ongoing neurometabolic alterations. Our laboratory has proposed that this CMRglc reduction is related to glycolytic dysfunction induced through a cascade of metabolic derangements initiated on impact. This metabolic depression is accompanied by decreased levels of ATP,⁶⁸ increased flux of glucose through the pentose phosphate pathway,⁶⁹ free radical production,^{70,71} and depletion of cytosolic nicotinamide-adenine dinucleotide (NAD⁺) resulting in the glyceraldehyde-3-phosphate dehydrogenase inhibition.⁷² Glycolysis needs a constant supply of NAD⁺, and when it is experimentally depleted, glycolytic inhibition and decreased glucose metabolism is observed.^{72–74} Despite the unclear mechanism, the state of metabolic depression reflects an altered cerebral state that is associated with functional deficits and has been proposed to reflect a vulnerable cerebral state.^{21,24,25}

It is important to also mention that the prolonged changes in CMRglc can also reflect changes in cerebral blood flow (CBF) and therefore autoregulation. Post-traumatic release of adenosine can

initiate changes in CBF, resulting in uncoupling of CBF and metabolism. Impaired autoregulation has been reported after TBI in humans⁷⁵ and rats,⁷⁶ but less is known in younger age groups or after mTBI. While lasting hypoperfusion after mTBI in adults^{77–80} and in the young,^{81–83} future studies addressing the relationship between autoregulation at the neurovascular unit and metabolic changes after concussive injuries in different age groups must be conducted.

Metabolic window of vulnerability

One of the most commonly expressed concerns among physicians regarding concussions in sports or military is the issue of safe “return to play” assessment. When is the brain safe to resume normal activities and can this be objectively quantified independent of the patient? Several repeated TBI studies have demonstrated exacerbation of injury outcomes with subsequent injuries, but not many experimental studies have addressed potential biological indicators for the window of vulnerability. In a two-part study on the effects of repeated mild weight drop on adult rats, investigators have demonstrated that in adult rats given two weight drop injuries at different intervals have the greatest decrease in mitochondrial metabolism (N-acetylaspartate (NAA), adenosine triphosphate) and greatest increase in oxidative injury when the interval between injuries is 3 days.^{24,84} Those animals receiving two mTBIs at 5 days apart showed similar mitochondrial function as controls. A subsequent clinical report with 1H-MRI in 13 concussed athletes showed NAA/Cr ratios decreased by 18.5% at 3 days post-injury with return to control levels by 30 days. Three of these patients who resumed normal activities shortly thereafter sustained a second concussion that resulted in more prolonged deficits in NAA/Cr ratios with delayed recovery at 45 days.²⁶

Results from the current study suggest that the duration of metabolic depression reflects the time-course of cerebral vulnerability to second injury in the juvenile brain and could provide a valuable target in establishing an age-appropriate window of vulnerability guidelines. The fact that injuries of various types within numerous age groups produce a common cerebral response suggests a common underlying mechanism. Further, the correlation between duration of the hypometabolic phase and the duration of the cognitive deficits also suggests that the phenomenon of CMRglc hypometabolism can serve as a biomarker for cerebral dysfunction and vulnerability. While it is not reasonable to imply that mTBI patients should undergo routine or repeated PET imaging, these results provide the physiological data, which can be related to metabolic markers that can be imaged through non-invasive modalities or correlated to behavioral measures of dysfunction.

Author Disclosure Statement

No competing financial interests exist.

References

- Otori, T., Friedland, J.C., Sinson, G., McIntosh, T.K., Raghupathi, R., and Welsh, F.A. (2004). Traumatic brain injury elevates glycogen and induces tolerance to ischemia in rat brain. *J. Neurotrauma* 21:707–718.
- Jenkins, L.W., Moszynski, K., Lyeth, B.G., Lewelt, W., DeWitt, D.S., Allen, A., Dixon, C.E., Povlishock, J.T., Majewski, T.J., and Clifton, G.L. et al. (1989). Increased vulnerability of the mildly traumatized rat brain to cerebral ischemia: the use of controlled secondary ischemia as a research tool to identify common or different mechanisms contributing to mechanical and ischemic brain injury. *Brain Res.* 477:211–224.

3. Mies, G., Paschen, W., and Hossmann, K.A. (1990). Cerebral blood flow, glucose utilization, regional glucose, and ATP content during the maturation period of delayed ischemic injury in gerbil brain. *J. Cereb. Blood Flow Metab.* 10:638–645.
4. Palmer, C., Brucklacher, R.M., Christensen, M.A., and Vannucci, R.C. (1990). Carbohydrate and energy metabolism during the evolution of hypoxic-ischemic brain damage in the immature rat. *J. Cereb. Blood Flow Metab.* 10:227–235.
5. Yoshino, A., Hovda, D., Kawamata, T., Katayama, Y., and Becker D. (1991). Dynamic changes in local cerebral glucose utilization following cerebral concussive in rats: evidence of a hyper- and subsequent hypometabolic state. *Brain Res.* 561:106–119.
6. Kawamata, T., Katayama, Y., Hovda, D.A., Yoshino, A., and Becker DP. (1992). Administration of excitatory amino acid antagonists via microdialysis attenuates the increase in glucose utilization seen following concussive brain injury. *J. Cereb. Blood Flow Metab.* 12:12–24.
7. Andersen, B., and Marmarou, A. (1992). Post-traumatic selective stimulation of glycolysis. *Brain Res.* 585:184–189.
8. Richards, H.K., Simac, S., Piechnik, S., and Pickard JD. (2001). Uncoupling of cerebral blood flow and metabolism after cerebral contusion in the rat. *J. Cereb. Blood Flow Metab.* 21:779–781.
9. Chen, S.F., Richards, H.K., Smielewski, P., Johnström, P., Salvador, R., Pickard, J.D., and Harris NG. (2004). Relationship between flow-metabolism uncoupling and evolving axonal injury after experimental traumatic brain injury. *J. Cereb. Blood Flow Metab.* 24:1025–1036.
10. Bergsneider M., Hovda, D.A., Lee, S.M., Kelly, D.F., McArthur, D.L., Vespa, P.M., Lee, J.H., Huang, S.C., Martin, N.A., Phelps, M.E., and Becker, D.P. (2000). Dissociation of cerebral glucose metabolism and level of consciousness during the period of metabolic depression following human traumatic brain injury. *J. Neurotrauma* 17:389–401.
11. O'Connell, M.T., Seal, A., Nortje, J., Al-Rawi, P.G., Coles, J.P., Fryer, T.D., Menon, D.K., Pickard, J.D., and Hutchinson, P.J. (2005). Glucose metabolism in traumatic brain injury: a combined microdialysis and [18F]-2-fluoro-2-deoxy-D-glucose-positron emission tomography (FDG-PET) study. *Acta Neurochir. Suppl.* 95:165–168.
12. Hovda, D., Le, H., Lifshitz, J., Berry, J., Badie, H., Yoshino, A., and Lee, S. (1994). Long-term changes in metabolic rates for glucose following mild, moderate and severe concussive head injuries in adult rats. *J. Neurosci.* 20:845.
13. Moore, A.H., Osteen, C.L., Chatziioannou, A.F., Hovda, D.A., and Cherry, S.R. (2000). Quantitative assessment of longitudinal metabolic changes in vivo after traumatic brain injury in the adult rat using FDG-microPET. *J. Cereb. Blood Flow Metab.* 20:1492–1501.
14. Hovda, D.A., Narayan, R.K., Wilberger, J.E., and Povlishock, J.T. (1996). *Metabolic Dysfunction in Neurotrauma*. McGraw-Hill:New York, pps. 1459–1478.
15. Queen, S.A., Chen, M.J., and Feeney, D.M. (1997). d-Amphetamine attenuates decreased cerebral glucose utilization after unilateral sensorimotor cortex contusion in rats. *Brain Res.* 777:42–50.
16. Sutton, R.L., Hovda, D.A., Adelson, P.D., Benzel, E.C., and Becker D.P. (1994). Metabolic changes following cortical contusion: relationship to edema and morphological changes. *Acta Neurochir. Suppl.* 60:446–448.
17. Prins, M.L., and Hovda, D.A. (2009). The effects of age and ketogenic diet on local cerebral metabolic rates of glucose after controlled cortical impact injury in rats. *J. Neurotrauma* 26:1083–1093.
18. Hattori, N., Huang, S.C., Wu, H.M., Yeh, E., Glenn, T.C., Vespa, P.M., McArthur, D., Phelps, M.E., Hovda, D.A. and Bergsneider, M. (2003). Correlation of regional metabolic rates of glucose with glasgow coma scale after traumatic brain injury. *J. Nucl. Med.* 44:1709–1716.
19. Humayun, M.S., Presty, S.K., Lafrance, N.D., Holcomb, H.H., Loats, H., Long, D.M., Wagner, H.N., and Gordon, B. (1989). Local cerebral glucose abnormalities in mild closed head injured patients with cognitive impairments. *Nucl. Med. Comm.* 10:335–344.
20. Gross, H., Kling, A., Henry, G., Herndon, C., and Lavretsky, H. (1996). Local cerebral glucose metabolism in patients with long-term behavioral and cognitive deficits following mild traumatic brain injury. *J. Neuropsychiatry Clin. Neurosci.* 8:324–334.
21. Umile, E.M., Sandel, M.E., Alavi, A., Terry, C.M., and Plotkin, R.C. (2002). Dynamic imaging in mild traumatic brain injury: support for the theory of medial temporal vulnerability. *Arch. Phys. Med. Rehabil.* 83:1506–1513.
22. Thomas, S., Prins, M.L., Samii, M., and Hovda, D.A. (2000). Cerebral metabolic response to traumatic brain injury sustained early in development: a 2-deoxy-D-glucose autoradiographic study. *J. Neurotrauma* 17:649–665.
23. Prins, M.L., and Hovda, D.A. (1998). Traumatic brain injury in the developing rat: effects of maturation on Morris water maze acquisition. *J. Neurotrauma* 15:799–811.
24. Vagnozzi, R., Tavazzi, B., Signoretti, S., Amorini, A.M., Belli, A., Cimatti, M., Delfini, R., Di Pietro, V., Finocchiaro, A., and Lazzarino, G. (2007). Temporal window of metabolic brain vulnerability to concussions: mitochondrial-related impairment—part I. *Neurosurgery* 61:379–389.
25. Henry, L.C., Tremblay, S., Boulanger, Y., Ellemberg, D., and Lassonde, M. (2010). Neurometabolic changes in the acute phase after sports concussions correlate with symptom severity. *J. Neurotrauma* 27:65–76.
26. Vagnozzi, R., Signoretti, S., Tavazzi, B., Floris, R., Ludovici, A., Marziali, S., Tarascio, G., Amorini, A.M., Di Pietro, V., Delfini, R., and Lazzarino, G. (2008). Temporal window of metabolic brain vulnerability to concussion: a pilot 1H-magnetic resonance spectroscopic study in concussed athletes—part III. *Neurosurgery* 62:1286–1296.
27. Romijn, H.J., Hofman, M.A., and Gramsbergen, A. (1991) At what age is the developing cerebral cortex of the rat comparable to that of the full-term newborn baby? *Early Hum. Dev.* 26:61–67.
28. Nehlig, A., Pereira de Vasconcelos, A., Dumont, I., and Boyet, S. (1990). Effects of caffeine, L-phenylisopropyladenosine and their combination on local cerebral blood flow in the rat. *Eur. J. Pharmacol.* 179:271–280.
29. Counotte, D.S., Li, K.W., Wortel, J., Gouwenberg, Y., Van Der Schors, R.C., Smit, A.B., Spijker, S. (2010) Changes in molecular composition of rat medial prefrontal cortex synapses during adolescent development. *Eur. J. Neurosci.* 32:1452–1460.
30. Giedd, J.N. (2009). Linking adolescent sleep, brain maturation and behavior. *J. Adolesc. Health* 45:319–320.
31. Spear, L.P. (2004). Adolescent brain development and animal models. *Ann. N.Y. Acad. Sci.* 1021:23–26.
32. Spear, L.P. (2000). The adolescent brain and age-related behavioral manifestations. *Neurosci Biobehav. Rev.* 24:417–463.
33. Prins, M.L., Hales, A., Reger, M., Giza, C.C., and Hovda, D.A. (2010). Repeat traumatic brain injury in the juvenile rat is associated with increased axonal injury and cognitive impairments. *Dev. Neurosci.* 32:510–518.
34. Sokoloff, L., Reivich, M., Kennedy, C., Des Rosiers, M.H., Patlak, C.S., Pettigrew, K.D. Sakurada, O., and Shinohara, M. (1977). The [14C]deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. *J. Neurochem.* 28:897–916.
35. el Hamdi, G., de Vasconcelos, A.P., Vert, P., and Nehlig, A. (1992). An experimental model of generalized seizures for the measurement of local cerebral glucose utilization in the immature rat. I. Behavioral characterization and determination of lumped constant. *Brain Res. Dev. Brain Res.* 69:233–242.
36. Ennaceur, A., and Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behav. Brain Res.* 31:47–59.
37. Reger, M.L., Hovda, D.A., and Giza, C.C. (2009). Ontogeny of Rat Recognition Memory measured by the novel object recognition task. *Dev. Psychobiol.* 51:672–678.
38. Colle, L.M., Holmes, L.J., and Pappius, H.M. (1986). Correlation between behavioral status and cerebral glucose utilization in rats following freezing lesion. *Brain Res.* 397:27–36.
39. Liu, Y., Mituska, S., Hashizume, K., Hosaka, T., and Nukui, H. (1996). The time course of glucose metabolism in rat cerebral ischemia with middle cerebral artery occlusion-reperfusion model and the effect of MK-801. *Neurol. Res.* 18:505–508.
40. Hayes, R.L., Katayama, Y., Jenkins, L.W., Lyeth, B.G., Clifton, G.L., Gunter, J., Povlishock, J.T., and Young, H.F. (1988). Regional rates of glucose utilization in the cat following concussive head injury. *J. Neurotrauma* 5:121–137.
41. Dietrich, W.D., Alonso, O., Busto, R., and Ginsberg, M.D. (1994). Widespread metabolic depression and reduced somatosensory circuit activation following traumatic brain injury in rats. *J. Neurotrauma* 11:629–640.
42. Jiang, X.B., Ohno, K., Qian, L., Tominaga, B., Kuroiwa, T., Nariai, T., and Hirakawa, K. (2000). Changes in local cerebral blood flow,

- glucose utilization, and mitochondrial function following traumatic brain injury in rats. *Neurol. Med. Chir (Tokyo)* 40:16–29.
43. Bergsneider, M., Hovda, D.A., McArthur, D.L., Etchepare, M., Huang, S.C., Sehati, N., Satz, P., Phelps, M.E., and Becker, D.P. (2001). Metabolic recovery following human traumatic brain injury based on FDG-PET: Time course and relationship to neurological disability. *J. Head Trauma Rehabil.* 16:135–148.
 44. Roberts, M.A., Manshadi, F.F., Bushnell, D.L., and Hines, M.E. (1995). Neurobehavioural dysfunction following mild traumatic brain injury in childhood: a case report with positive findings on positron emission tomography (PET). *Brain Inj.* 9:427–436.
 45. Garcia-Panach, J., Lull, N., Lull, J.J., Ferri, J., Martinez, C., Sopena, P., Robles, M., Chirivella, J., Noé, E. (2011). A voxel-based analysis of FDG-PET in traumatic brain injury: regional metabolism and relationship between the thalamus and cortical areas. *J. Neurotrauma* 28:1707–1717.
 46. Lull, N., Noé, E., Lull, J.J., Garcia-Panach, J., Chirivella, J., Ferri, J., Lopez-Aznar, D., Sopena, P., and Robles, M. (2010). Voxel-based statistical analysis of thalamic glucose metabolism in traumatic brain injury: relationship with consciousness and cognition. *Brain Inj* 24:1098–1107.
 47. Feeney, D.M., Boyeson, M.G., Linn, R.T., Murray, H.M., and Dail, W.G. (1981). Responses to cortical injury. I: Methodology and local effects of contusions in the rat. *Brain Res.* 211:67–77.
 48. Prins, M.L., and Hovda, D.A. (2001). Mapping cerebral glucose metabolism during spatial learning: interactions of development and traumatic brain injury. *J. Neurotrauma* 18:31–46.
 49. Lyeth, B.G., Jenkins, L.W., Hamm, R.J., Dixon, C.E., Phillips, L.L., Clifton, G.L., Young, H.F., and Hayes, R.L. (1990). Prolonged memory impairment in the absence of hippocampal cell death following traumatic brain injury in the rat. *Brain Res.* 526:249–258.
 50. Vespa, P., Boonyaputhikul, R., McArthur, D.L., Miller, C., Etchepare, M., Bergsneider, M., Glenn, T., Martin, N., and Hovda, D. (2006). Intensive insulin therapy reduces microdialysis glucose values without altering glucose utilization or improving the lactate/pyruvate ratio after traumatic brain injury. *Crit. Care Med.* 34:850–856.
 51. Vannucci, S.J., Seaman, L.B., Vannucci, R.C. (1996) Effects of hypoxia-ischemia on GLUT1 and GLUT3 glucose transporters in immature rat brain. *J. Cereb. Blood Flow Metab.* 16:77–81.
 52. Bruckner, B.A., Ammini, C.V., Otal, M.P., Raizada, M.K., and Stacpoole, P.W. (1999). Regulation of brain glucose transporters by glucose and oxygen deprivation. *Metabolism* 48:422–431.
 53. Balabanov, R., Goldman, H., Murphy, S., Pellizon, G., Owen, C., Rafols, J., and Dore-Duffy, P. (2001). Endothelial cell activation following moderate traumatic brain injury. *Neurol. Res.* 23:175–182.
 54. Cornford, E.M., and Cornford, M.E. (1986). Nutrient transport and the blood-brain barrier in developing animals. *Fed. Proc.* 45:2065–2072.
 55. Pappius, H.M. (1995). Cortical hypometabolism in injured brain: new correlations with the noradrenergic and serotonergic systems and with behavioral deficits. *Neurochem. Res.* 20:1311–1321.
 56. Nilsson, P., Hillered, L., Ponten, U., and Ungerstedt, U. (1990). Changes in cortical extracellular levels of energy-related metabolites and amino acids following concussive brain injury in rats. *J. Cereb. Blood Flow Metab.* 10:631–637.
 57. Kmiecik-Kolada, K., Felinska, W., Stachura, Z., Majchrzak, H., and Herman, Z.S. (1987). Concentration of biogenic amines and their metabolites in different parts of brain after experimental cerebral concussion. *Pol. J. Pharmacol. Pharm.* 39:47–53.
 58. Tsui, K., Takada, A., Nagahiro, S., Grdisa, M., Diksic, M., and Pappius, H.M. (1995). Synthesis of serotonin in traumatized rat brain. *J. Neurochem.* 64:1319–1325.
 59. Bell, M.J., Kochanek, P.M., Carcillo, J.A., Mi, Z., Schiding, J.K., Wisniewski, S.R., Clark, R.S., Dixon, C.E., Marion, D.W., and Jackson, E. (1998). Interstitial adenosine, inosine, and hypoxanthine are increased after experimental traumatic brain injury in the rat. *J. Neurotrauma* 15:163–170.
 60. Clark, R.S., Carcillo, J.A., Kochanek, P.M., Obrist, W.D., Jackson, E.K., Mi, S., Wisniewski, S.R., Bell, M.J., Marion, D.W. (1997) Cerebrospinal fluid adenosine concentration and uncoupling of cerebral blood flow and oxidative metabolism after severe head injury in humans. *Neurosurgery* 41:1284–1293.
 61. Povlishock, J.T. (1993). Pathobiology of traumatically induced axonal injury in animals and man. *Ann. Emerg. Med.* 22:980–986.
 62. MacDonald, C.L., Dikranian, K., Bayly, P., Holtzman, D., and Brody, D. (2007). Diffusion tensor imaging reliably detects experimental traumatic axonal injury and indicates approximate time of injury. *J. Neurosci.* 27:11869–11876.
 63. Okumura, A., Yasokawa, Y., Nakayama, N., Miwa, K., Shinoda, J., and Iwama, T. (2005). [The clinical utility of MR diffusion tensor imaging and spatially normalized PET to evaluate traumatic brain injury patients with memory and cognitive impairments]. (Jap) *No to Shinkei* 57:115–122.
 64. Reeves, T.M., Phillips, L.L., and Povlishock, J.T. (2005). Myelinated and unmyelinated axons of the corpus callosum differ in vulnerability and functional recovery following traumatic brain injury. *Exp. Neurol.* 196:126–137.
 65. Derache, N., Marie, R.M., Constans, J.M., and Defer, G.L. (2006). Reduced thalamic and cerebellar rest metabolism in relapsing-remitting multiple sclerosis, a positron emission tomography study: correlations to lesion load. *J. Neurol. Sci.* 245:103–109.
 66. Yakushev, I., Gerhard, A., Müller, M.J., Lorscheider, M., Buchholz, H.G., Schemuly, I., Weibrich, C., Hammers, A., Stoeter, P., Schreckenberger, M., and Fellgiebel, A. (2011). Relationships between hippocampal microstructure, metabolism, and function in early Alzheimer's disease. *Brain Struct. Funct.* 216:219–226.
 67. Browne, S.E., Lin, L., Mattsson, A., Georgievska, B., and Isacson, O. (2001). Selective antibody-induced cholinergic cell and synapse loss produce sustained hippocampal and cortical hypometabolism with correlated cognitive deficits. *Exp. Neurol.* 170:36–47.
 68. Lee, S.M., Wong, M.D., Samii, A., and Hovda, D.A. (1999). Evidence for energy failure following irreversible traumatic brain injury. *Ann. N.Y. Acad. Sci.* 893:337–340.
 69. Bartnik, B.L., Sutton, R.L., Fukushima, M., Harris, N.G., Hovda, D.A., and Lee, S.M. (2005). Upregulation of pentose phosphate pathway and preservation of tricarboxylic acid cycle flux after experimental brain injury. *J. Neurotrauma* 22:1052–1065.
 70. Hall, E.D., Andrus, P.K., and Yonkers, P.A. (1993). Brain hydroxyl radical generation in acute experimental head injury. *J. Neurochem.* 60:588–594.
 71. Marklund, N., Clausen, F., Lewander, T., and Hillered, L. (2001). Monitoring of reactive oxygen species production after traumatic brain injury in rats with microdialysis and the 4-hydroxybenzoic acid trapping method. *J. Neurotrauma* 18:1217–1227.
 72. Sheline, C.T., Behrens, M.M., and Choi, D.W. (2000). Zinc-induced cortical neuronal death: contribution of energy failure attributable to loss of NAD⁺ and inhibition of glycolysis. *J. Neurosci.* 20:3139–3146.
 73. Cosi, C., and Marien, M. (1998). Decreases in mouse brain NAD⁺ and ATP induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): prevention by the poly(ADP-ribose) polymerase inhibitor, benzamide. *Brain Res.* 809:58–67.
 74. Ying, W., Germier, P., and Swanson, R. (2003). NAD⁺ repletion prevents PARP-1-induced glycolytic blockade and cell death in cultured mouse astrocytes. *Biochem. Biophys. Res. Commun.* 308:809–813.
 75. Vavilala, M.S., Muangman, S., Tontisirin, N., Fisk, D., Roscigno, C., Mitchell, P., Kirkness, C., Zimmerman, J.J., Chesnut, R., Lam, A.M. (2006). Impaired cerebral autoregulation and 6-month outcome in children with severe traumatic brain injury: preliminary findings. *Dev. Neurosci.* 28:348–353.
 76. Golding, E.M., Steenberg, M.L., Contant, C.F. Jr., Krishnappa, I., Robertson, C.S., Bryan, R.M. Jr. (1999) Cerebrovascular reactivity to CO₂ and hypotension after mild cortical impact injury. *Am. J. Physiol.* 277: H1457–H1466.
 77. Bonne, O., Gilboa, A., Louzoun, Y., Kempf-Sherf, O., Katz, M., Fishman, Y., Ben-Nahum, Z., Krausz, Y., Bocher, M., Lester, H., Chisin, R., Lerer, B. (2003). Cerebral blood flow in chronic symptomatic mild traumatic brain injury. *Psychiatry Res.* 124:141–152.
 78. Junger, E.C., Newell, D.W., Grant, G.A., Avellino, A.M., Ghatan, S., Douville, C.M., Lam, A.M., Aaslid, R., Winn, H.R. (1997). Cerebral autoregulation following minor head injury. *J. Neurosurg.* 86:425–432.
 79. Schneider, G., Fries, P., Wagner-Jochem, D., Thome, D., Laurer, H., Kramann, B., Mautes, A., Hagen, T. (2002). Pathophysiological changes after traumatic brain injury: comparison of two experimental animal models by means of MRI. *MAGMA* 14:233–241.
 80. Yamakami, I., McIntosh, T.K. (1989). Effects of traumatic brain injury on regional cerebral blood flow in rats as measured with radiolabeled microspheres. *J. Cereb. Blood Flow Metab.* 9:117–124.

81. Becelewski, J., Pierzchala, K. (2003). [Cerebrovascular reactivity in patients with mild head injury.] (Pol) Neurol. Neurochir. Pol. 37: 339–350.
82. Maugans, T.A., Farley, C., Altaye, M., Leach, J., Cecil, K.M. (2012). Pediatric sports-related concussion produces cerebral blood flow alterations. *Pediatrics* 129:28–37.
83. Biagas, K.V., Grundl, P.D., Kochanek, P.M., Schinding, J.K., Nemoto, E.M. (1996). Posttraumatic hyperemia in immature, mature and aged rats: autoradiographic determination of cerebral blood flow. *J. Neurotrauma* 13:189–200.
84. Tavazzi, B., Vagnozzi, R., Signoretti, S., Amorini, A.M., Belli, A., Cimatti, M., Delfini, R., Di Pietro, V., Finocchiaro, A., and Lazzarino, G. (2007). Temporal window of metabolic brain vulnerability to concussions: oxidative and nitrosative stresses—part II. *Neurosurgery* 61:390–396.

Address correspondence to:

Mayumi L. Prins, PhD

UCLA Department of Neurosurgery

UCLS School of Medicine

NPI 18-228

Los Angeles, CA 90095

E-mail: mprins@mednet.ucla.edu