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Heterogeneous regional and temporal energetic impairment following controlled cortical impact injury in rats

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Objectives: *Following traumatic brain injury metabolic stability is impaired. Duration and reversibility of these changes might be important to guide specific interventions.*
Methods: *To characterize temporal and regional changes in cerebral metabolism, 68 male Sprague–Dawley rats were subjected to a focal cortical contusion. Lesion progression and mitochondrial impairment were determined by magnetic resonance imaging (MRI) and triphenyl tetrazolium chloride (TTC) staining, respectively. Metabolic alterations were determined at hours 6 and 24 and day 7 by measuring extracellular glucose, lactate and hypoxanthine levels with microdialysis catheters placed adjacent and distant to the contusion and by quantifying changes in tissue ATP, lactate and glucose using bioluminescence imaging.*
Results: *The cortical lesion reached its maximal extent at hour 24 and remained confined to the ipsilateral hemisphere. In microdialysate, at hour 6, extracellular hypoxanthine and lactate reached maximal values, thereafter hypoxanthine normalized while lactate remained increased. Extracellular glucose reached the highest values at hour 24 and remained elevated. Bioluminescence imaging revealed heterogeneous changes in areas distant to the contusion. No significant changes were found in ATP content. Slightly elevated tissue glucose until 24 hours in the ipsilateral hemisphere was observed. Following a continuous increase, lactate levels were the highest by 6 hours in the ipsilateral cortex and hippocampus.*
Discussion: *CCI is associated with disturbances in energetic metabolism. Metabolic perturbation is not restricted to the early phase and the contusional region following focal cortical contusion, but also involves hippocampus and primarily uninjured parts of the hemisphere.* [Neurol Res 2007; **29**: 594–603]

Keywords: Traumatic brain injury; microdialysis; magnetic resonance imaging; bioluminescence imaging; energy metabolism

INTRODUCTION

Traumatic brain injury (TBI) is associated with severe structural and functional alterations which reflect underlying damage and contribute to evolving injury. A plethora of different intra- and extracellular changes known to occur simultaneously and in sequence are of pathologic importance. In this context, excessive stimulation of postsynaptic N-methyl D-aspartate (NMDA) glutamate receptors challenges neuronal and glial ionic and energetic homeostasis resulting in sustained glycolysis, lactate production and adenosine triphosphate (ATP) degradation[1–3] which, in turn, can lead to irreversible cell damage if intracellular compensatory mechanisms are exhausted[4]. Released intracellular toxins (e.g. NO, reactive oxygen species, cytokines and calcium overload) contribute to the evolving functional deterioration by impairing functional and structural integrity of cerebral mitochondria[5,6]. Contrary to other organs, e.g. liver and skeletal muscle, the brain's limited energetic reserves are depleted within seconds to minutes and fail to maintain high ATP levels under pathologic conditions[7]. Within the central nervous system, the majority of produced ATP is used by transport pumps, predominantly the neuronal Na^+/K^+-ATPase[8] which is indispensable to maintain ionic

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homeostasis and to prevent uncontrolled neuronal firing and cell death.

Under physiologic conditions, ATP is predominantly generated by the stepwise, enzymatically mediated and metabolically controlled degradation of internalized glucose[7]. ATP can also be generated by degradation of fatty acids and amino acids entering the citric acid cycle via acetyl-CoA or α-ketoglutarate. The complete glycolytic pathway including oxidative phosphorylation involves cytoplasmatic and mitochondrial enzymes and shuttle systems connecting the subcellular compartments. While the degradation of glucose to pyruvate is only partially dependent on oxygen, mitochondrial processing within the citric acid cycle and the respiratory chain are strictly aerobic processes replenishing nicotinamide adenine dinucleotide (NAD^+) which is crucial for several glycolytic steps. Under pathologic conditions, e.g. following TBI, when free oxygen radicals inhibit enzymes of the respiratory chain or insufficient oxygen supply impairs electron transfer within the respiratory chain, glucose is degraded to pyruvate transformed to lactate via lactate dehydrogenase. Subsequent shuttling of lactate to cells with intact mitochondria can attenuate metabolic impairment[9,10]. Inhibition of lactate dehydrogenase by reactive oxygen species leads to a failure of the alternative non-oxidative glycolytic pathway followed by sustained lactate production which, in turn, can lead to tissue acidosis.

Cerebral energetic impairment can be assessed by non-invasive and invasive methods, including magnetic resonance (MR) spectroscopy[11], regional bioluminescence imaging[12], histology [triphenyl tetrazolium chloride (TTC)][13] and cytochrome oxidase (CO) immunohistochemistry[14], *in vitro* autoradiography[15,16], *in vitro* analysis of mitochondrial respiratory coupling[6,17] and analysis of metabolites within the extracellular space by microdialysis[18] or in the cerebrospinal fluid[19].

These experimental *in vitro* and *in vivo* studies revealed partially reversible metabolic changes characterized by a strong regional and temporal heterogeneity. As demonstrated by Kelly and colleagues[15], glucose uptake was dramatically increased within the first 30 minutes after controlled cortical impact (CCI) injury at the site of the impact followed by a long-lasting depressed glucose uptake in the ipsilateral hemisphere. Pharmacologic studies suggest that this sustained glucose uptake and its subsequent metabolisms are caused by glutamate-mediated neuronal excitation which can be blocked by specific NMDA glutamate receptor antagonists[20–22]. However, the employed *in vitro* autoradiography does not allow determining the actual fate of the incorporated ^{14}C-2-deoxy-glucose as this radioactive ligand cannot be processed by downstream enzymes of the glycolytic pathway. Thus, these studies do not reflect extent and duration of glycolytic and subsequent energetic impairment. Sustained glucose uptake with hyperglycolysis leading to increased lactate production does not necessarily reflect uncontrolled and devastating functional impairment as generation of lactate associated with limited ATP production can be utilized to fuel energy-dependent processes[10]. Consequently, assessing energetic stability by measuring tissue ATP and ATP degradation products, e.g. hypoxanthine[23,24], are important approaches to determine if elevated extracellular lactate reflects balanced adaptive energy production.

The aims of the present studies were to determine the temporal and regional profiles of metabolic alterations (ATP, glucose, lactate and hypoxanthine) in conjunction with structural (edema formation and contusion volume) and functional changes (mitochondrial staining) during the first 7 days after inducing a focal cortical contusion in hemodynamically stable rats.

MATERIALS AND METHODS

For the present studies, a total of 68 male Sprague–Dawley rats (300– 400 g; mean: 360 ± 10 g) (Charles River, Germany) were used. Animals were accustomed to the laboratory for ~24 hours. The experimental protocol was approved by the committee for animal research in Berlin, Germany.

Surgery and CCI injury

All anesthetized rats (isoflurane: 1.6–1.8 vol.-%; N_2O: 0. 5 l/min, O_2: 0.3 l/min), spontaneously breathing via a mask, were positioned in a stereotaxic holder and a left parietotemporal cortical contusion was induced with the CCI device as previously described[25–28]. In brief, a pneumatically driven bolt (diameter: 5 mm) accelerated to a velocity of 7 m/s (100 PSI) produced a local compression of the exposed cortex by 1 mm for 300 ms without damaging the dura. After CCI, the scalp was sutured and rats were returned to their cages until further analysis at later time points.

Arterial blood gases and mean arterial blood pressure

Cannulation of the femoral artery allowed continuous measurements of mean arterial blood pressure (MABP) and blood gases during anesthesia before injury, 6 and 24 hours and 7 days after CCI. During anesthesia, body temperature was maintained stable between 37 and 38°C using a homeothermic heating pad.

Microdialysis

Under microscopic guidance to avoid vessel damage and confounding hemorrhage, microdialysis catheters (CMA 12, CMA Microdialysis, Stockholm, Sweden) with a 2 mm tip length (membrane permeability: 20.000 kD molecular weight cut-off) were inserted stereotactically at a depth of 2 mm in the cortex adjacent and distant to the contusion respectively as previously described (*Figure 1*)[25]. To compare the different groups, we used the same coordinates for the areas adjacent and distant to the contusion in non-injured and traumatized rats 6 and 24 hours and 7 days after CCI: adjacent cortex: −6.5 mm from bregma and −4 mm from the sagittal suture; distant cortex: −3 and −4 mm. Owing to the marked tissue loss at the site of the impact, microdialysis catheters were not inserted in the contusion 7 days after CCI. After insertion, catheters were perfused with

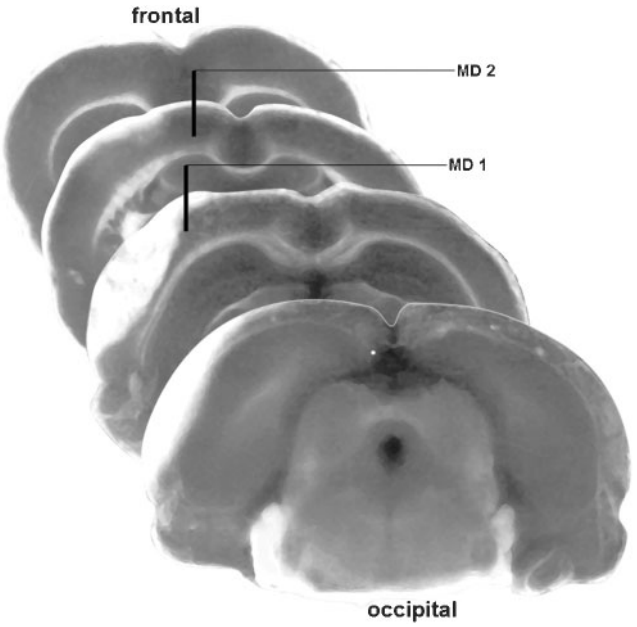

Figure 1: Extent of the induced focal cortical contusion and position of the micordialysis catheters inserted adjacent (MD 1; –6.5 mm from bregma and –4 mm from the sagittal suture) and distant to the contusion (MD 2; –3 and –4 mm). These representative serial brain slices stained with the mitochondrial stain TTC show the cortical contusion at its maximal extent 24 hours after CCI (lack of red staining in the left parietal region)

artificial cerebrospinal fluid (147 mM Na^+, 3 mM K^+, 1.3 mM Ca^{2+}, 1.3 mM Mg^{2+} and 155 mM Cl^-) at a flow rate of 2 µl/min. In microdialysis samples collected in 30 minute intervals, glucose and lactate were measured enzymatically using the CMA 600 bedside analyser as previously described[29,30]. Hypoxanthine was analysed by high performance liquid chromatography (HPLC)[29,19].

Magnetic resonance imaging

Magnetic resonance imaging (MRI) experiments were performed using a 100 MHz Biospec system (Bruker, Karlsruhe, Germany) with a 2.35 T, 40 cm in diameter horizontal magnet using ParaVision software. The system was equipped with an actively shielded gradient coil system (maximum gradient: 50 mT/m) and a custom made coil of 50 mm in diameter. To prevent movement artifacts, rats were placed prone in a custom-made plastic stereotactic frame. One day before CCI, baseline MRI values were determined in all rats. Following CCI, MRI was performed at hours 6 and 24 and day 7. Transversal multi-slice pilot scans [Rare, repetition time (TR): 3300 ms, echo time (TE): 11 ms, Rare factor 16] were acquired for exact positioning of coronal slices for all measurements. T2-weighted images were used to determine the extension of the contusion. Four sequential T1-weighted images were acquired up to 40 minutes following i.v. injection of Gd-DTPA (Schering, Germany) (0.1 mg/kg, b.w.) to assess blood–brain barrier breakdown. During MRI, spontaneously breathing rats were anesthetized with isoflurane. Body temperature was maintained between 37 and 38°C using a feedback controlled heating pad.

Histologic analysis

Following removal and cutting of brains in 1.3 mm slices beginning at the occipital pole with a commercially available matrix for rat brain (Brain Blocker, AgnTho's AB, Lidingö, Sweden), the brain sections were incubated in 2% TTC solution at 37°C for 20 minutes in a dark room as previously described.

Bioluminescence imaging

In non-injured rats and following CCI (1, 6 and 24 hours, 7 days), brains were frozen within the skull with liquid nitrogen to prevent any conflicting enzymatic degradation during a relatively lengthy and tedious removal of the brains. ATP, glucose and lactate were determined by bioluminescence imaging[12,31]. Briefly, a solution was prepared containing all enzymes, coenzymes and cofactors necessary for the substrate-specific bioluminescence reaction, but without the substrate of interest (ATP, glucose or lactate). This solution was frozen in a block and slices of 60 µm thickness were prepared in a cryostat at −20°C. A freeze-dried and heat inactivated coronal section of the brain (20 µm) was then covered with the frozen enzyme block and this open sandwich was placed on a photographic film (Agfapan professional flat film, 100 ASA) for recording of bioluminescent light emitted from the section after warming to room temperature. Exposure durations were 5 minutes for glucose and lactate and 0.5 minutes for ATP. After warming to room temperature, a substrate-specific and concentration-dependent bioluminescence developed which blackened the film. Quantification of bioluminescence- induced blackening of films was performed by computer-assisted densitometry using image analysis (Image Pro Plus, Media Cybernetics, Silver Spring, MD, USA) in the contusional and pericontusional area.

Study design

Study 1

To determine the extent and localization of the induced brain lesion, edema formation and cortical contusion volume were visualized by MRI (n=6) at hours 6 and 24 and day 7. Mitochondrial enzymatic impairment was visualized 6 and 24 hours after trauma using TTC staining (n=6 for each). In these animals, via a right frontal burr hole, an intracranial pressure (ICP) probe (Codman, Johnson & Johnson, Berkshire, UK) was inserted intraparenchymally before killing of the animals 24 hours and 7 days after trauma.

Study 2

In a follow-up study, changes in extracellular hypoxanthine, glucose and lactate were determined in uninjured animals (n=5) as well as in the contused and pericontusional cortex by microdialysis at hours 6 and

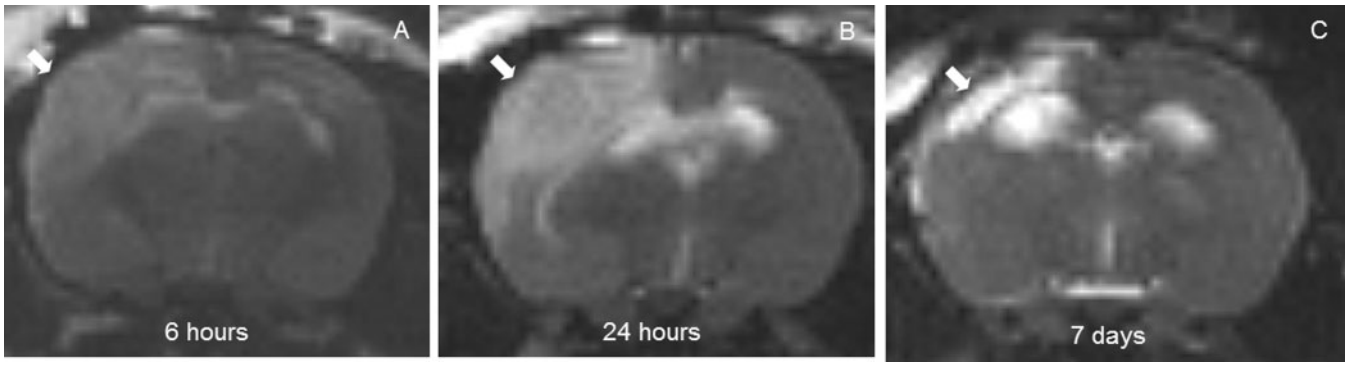

Figure 2: T2-weighted images depicting progressive growth of the hyperintense cortical contusion 6 (**A**) and 24 (**B**) hours, and the cystic, necrotic transformation 7 days (**C**, arrow bars) after CCI

24 and day 7 [n=10 each at hours 6 and 24; n=5 (pericontusion) at day 7].

Study 3

To delineate the regional extent of the energetic changes observed in the extracellular space, cerebral tissue ATP, glucose and lactate content were measured by *in vitro* bioluminescence 1, 6 and 24 hours, and 7 days after CCI (n= 4/time point) and in sham-operated animals (n=4).

Statistical analysis

Results are presented as mean ± standard error of the mean (SEM). Investigated variables were compared for significant differences within and between the groups using analysis of variance on ranks followed by *post hoc* multiple comparisons (SigmaStat 2.0, Jandel Scientific, Chicago, IL, USA). Differences were rated significant at $p<0.05$.

RESULTS

Arterial blood gases and MABP

Blood gases remained within physiologic limits and MABP was stable at all investigated time points (*Table 1*).

Progressive lesion growth

As revealed by MRI, the cortical contusion significantly expanded in the sagittal, coronal and transversal plane during the first hours, reaching its maximal extent by 24 hours (*Figure 2B*), encompassing ~75% of the ipsilateral cortex. The induced lesion remained confined to the ipsilateral cortex as seen in the T2-weighted images (*Figure 2*). Extravasation of gadolinium was only observed around the contusion 6 hours after contusion (*Figure 3*). This lesion growth was also observed in the TTC staining. Here absent staining reflects mitochondrial damage (*Figure 1*).

Changes in energetic/metabolic parameters

Microdialysis

Compared with control rats (2.8 ± 0.1 μM), extracellular hypoxanthine was significantly increased by 6 hours adjacent (12.8 ± 1.6 μM) and distant (8.6 ± 1.0 μM) to the contusion, followed by a gradual decrease over time, reaching normal values by 7 days after CCI (3.4 ± 0.3 μM; *Figure 4A*).

Compared with non-traumatized rats (1.2 ± 0.1 μM), extracellular glucose was significantly increased by 24 hours after CCI within the contusional (1.9 ± 0.2 μM) and pericontusional cortex (2.1 ± 0.2 μM) and remained elevated pericontusionally up to 7 days (1.8 ± 0.1 μM; *Figure 4B*).

Compared with control rats (0.9 ± 0.1 μM), extracellular lactate was significantly increased within the contusional (3.2 ± 0.6 μM) and pericontusional (1.8 ± 0.3 μM) cortex 6 hours after CCI (*Figure 4C*), which was mostly sustained within the contusion. Over time, lactate was significantly decreased within the contusional and pericontusional cortex without, however, reaching normal values (1.6 ± 0.1 μM at day 7).

Compared with non-traumatized rats (0.8 ± 0.1), the extracellular lactate/glucose ratio was significantly increased 6 hours after CCI in the contusional (2.9 ±

Table 1: Changes in arterial blood gases, mean arterial blood pressure (MABP), intracranial pressure (ICP) and cerebral perfusion pressure (CPP) 6 and 24 hours and 7 days following CCI (nd=not determined)

Time point	pH	$PaCO_2$ (mmHg)	PaO_2 (mmHg)	Hb (g/dl)	MABP (mmHg)	ICP (mmHg)	CPP (mmHg)
Before CCI	7.45 ± 0.01	37.5 ± 1.9	174 ± 7	14.2 ± 0.3	90 ± 1	nd	nd
Hour 6	7.46 ± 0.02	36.3 ± 2.1	158 ± 14	13.3 ± 0.8	93 ± 2	nd	nd
Hour 24	7.48 ± 0.03	36.4 ± 3.0	200 ± 10	13.1 ± 0.8	94 ± 4	13 ± 2	83 ± 3
Day 7	7.43 ± 0.01	39.6 ± 3.2	180 ± 8	13.7 ± 0.9	88 ± 5	8 ± 1	80 ± 5

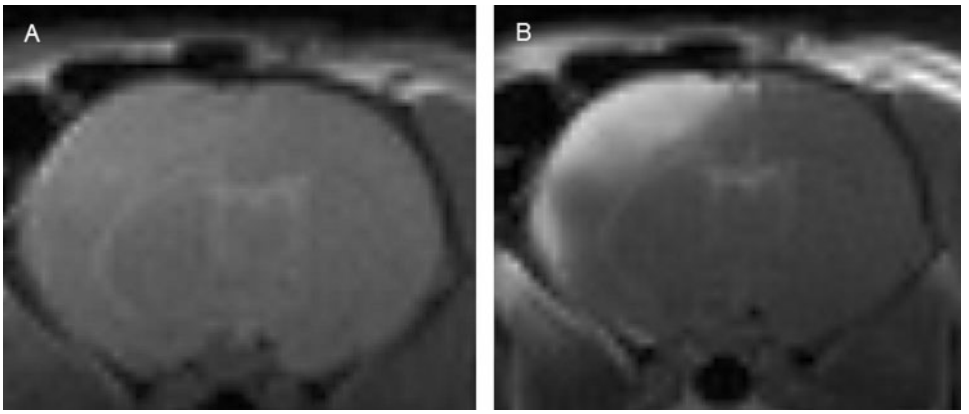

Figure 3: T1-weighted images before (**A**) and 20 minutes after (**B**) injecting gadolinium, allowing assessing the localization and extent of underlying blood–brain barrier damage by contrast enhancement 6 hours after trauma

1.1) and pericontusional (1.8 ± 0.3) cortex (*Figure 4D*), which was mostly sustained in the contused cortex. Over time, lactate-to-glucose ratio decreased, reaching baseline values in the pericontusional cortex 7 days after CCI (0.9 ± 0.1).

Bioluminescence imaging

Compared with uninjured animals, color-coded bioluminescence images visualize mainly focal changes in ATP levels, but heterogeneous lactate and glucose metabolism following CCI injury 6 hours following trauma compared with unaffected brain tissue (*Figure 5*). Descriptive analysis of all images revealed a decrease in optical density within the trauma region. Only marginal changes in ATP signal was observed within the distant brain regions (*Figure 5D*). Glucose metabolism distant to the site of impact was in the ipsi- and contralateral cortex. Close to the contusion, glucose was less elevated, while there was a marked increase within the contra- and ipsilateral hippocampus formation, respectively (*Figure 5E*). Lactate was increased in the ipsilateral cortex distant and adjacent to the contusion as well as the hippocampus formation. In the contralateral hemisphere, lactate showed heterogeneous changes in cortical structures and the hippocampus (*Figure 5F*).

Quantitative analysis of the optical density within the contusional and pericontusional area revealed no significant changes in tissue ATP at all time points (*Figure 6A*). However, 24 hours after trauma (0.43 ± 0.04), a tendency of lower level of ATP was observed in the affected cortex in bioluminescence images compared with baseline (0.49 ± 0.01).

Bioluminescence imaging revealed a tissue glucose level slightly elevated in the early phase until 24 hours after trauma (0.2 ± 0.02) in the ipsilateral cortex. At day 7 (0.17 ± 0.02), baseline level (0.016 ± 0.03) was reached. However, no significant changes between the time points can be evaluated (*Figure 6B*).

Tissue lactate levels showed a continuous increase in the ipsilateral cortex, reaching maximal values at hour 6 (0.1 ± 0.02), which were significantly elevated compared with baseline (0.05 ± 0.01; $p<0.05$). Values at hour 24 (0.06 ± 0.01) and day 7 (0.05 ± 0.01) reached merely baseline levels (*Figure 6C*).

DISCUSSION

Induction of a focal cortical contusion was associated with significant and partially reversible changes in glucose, lactate, ATP and the ATP degradation product hypoxanthine which in part, coincided with the progressive edema formation and cortical contusion growth. Microdialysis studies revealed persisting, significant changes in extracellular glucose and lactate. This was in parts reflected also by bioluminescence imaging. Alterations in hypoxanthine levels in the microdialysate showed a transient increase with its peak in the early period following trauma. However, ATP levels did not show significant changes in bioluminescence imaging. Despite absent signs of structural damage as observed in the MRI and histologic (TTC staining) studies, metabolism depicted in bioluminescence imaging showed also alterations in the contralateral, non-injured hemisphere. Overall, the observed metabolic alterations appeared to be caused by local events not related to systemic influences because the presently investigated rats were not subjected to hypotension or hypoxia known to irreversibly impair mitochondrial function and ATP synthesis[32].

Positioning of the microdialysis catheters in relation to the cortical contusion

Based upon the previously investigated secondary growth of a cortical contusion during the first 48 hours after CCI in rats[33], the stereotactic coordinates used in the present study were chosen to position the microdialysis catheters adjacent and distant to the contusion at 6.5 and 3 mm from bregma, respectively. The distance from the sagittal suture was maintained at 4 mm. With these coordinates, the growing contusion approached the frontal catheter at hour 24 without, however, encompassing this catheter. Simultaneously, the contusion reached the more posterior catheter[25], allowing assessing dynamic alterations within the

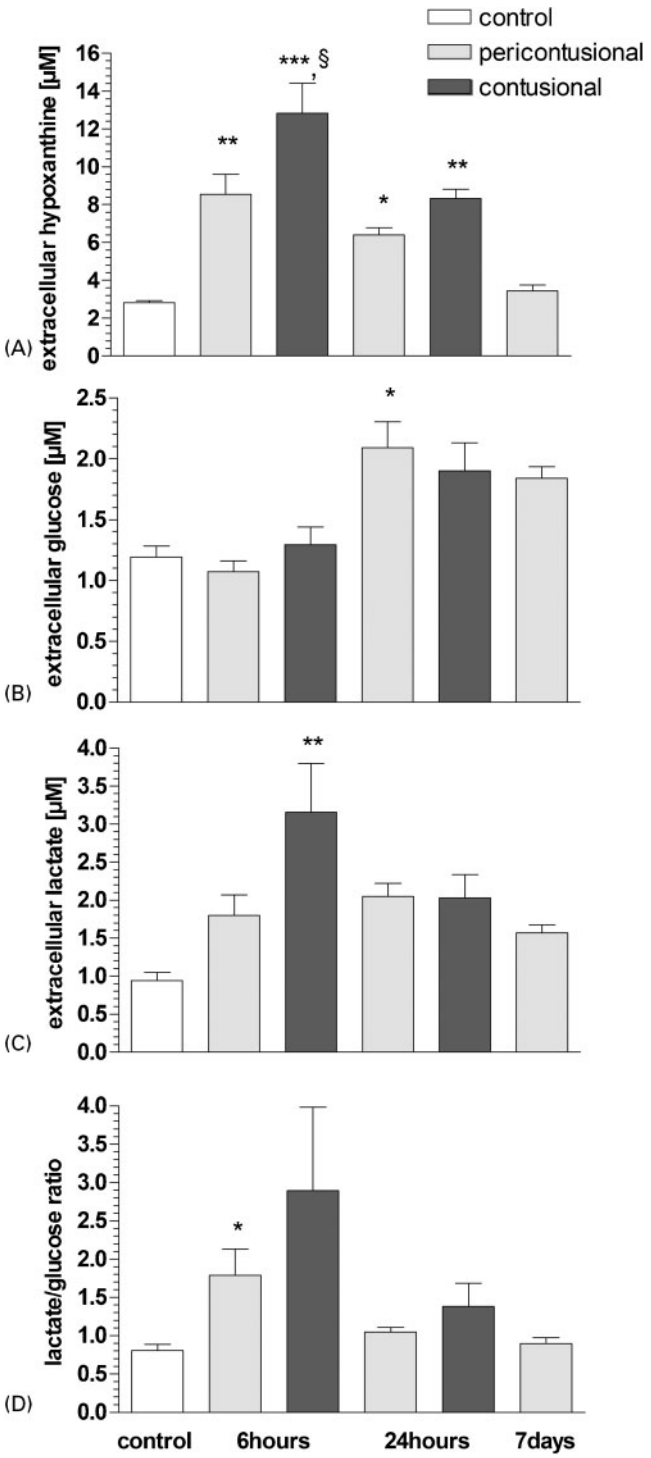

Figure 4: Regional and temporal changes in hypoxanthine, glucose and lactate, determined within the extracellular space by microdialysis 6 and 24 hours, and 7 days after CCI. Following CCI, extracellular hypoxanthine (**A**), glucose (**B**), lactate (**C**) and the calculated lactate-to-glucose ratio (**D**) were significantly increased compared with uninjured control rats. ($p<0.05$ vs control) Changes are significant at *$p<0.05$, **$p<0.01$ and ***$p<0.001$ compared with control rats and $^{§}p<0.05$ compared with the same time point

pericontusional cortex at varying distances from the lesion over time. This approach complements previous investigations as it reveals persistence of pathologic changes exceeding the first hours and reveals a dynamic spatial pattern which cannot be assessed by maintaining the microdialysis catheter at the site of the impact[23,24].

Discrepancy of changes determined within the extracellular space and tissue

Both microdialysis and the employed tissue analysis using bioluminescence imaging revealed significant changes in lactate and glucose over time and within the investigated regions of interest. However, these alterations were only partially congruent. While microdialysis measures alterations of free solutes within the extracellular space, bioluminescence imaging assesses overall changes within the intra- and extracellular compartment. Changes determined in the extracellular space might not reflect intracellular alterations for various reasons: (1) the intracellular compartment exceeds the extracellular space by far; (2) the spatial resolution of the microdialysis catheter is much lower; (3) complex intracellular uptake, metabolism and release processes and mechanisms cannot be differentiated with the presently employed techniques. More sophisticated approaches using radioactive ligands[34] are required to elucidate the actual dynamics behind the 'snap shot view' used in the present study.

In addition, regional differences concerning the nature of brain edema, i.e. cytotoxic and vasogenic edema, make a precise interpretation rather difficult as sustained cell swelling associated with a relative shrinkage of the extracellular space can artificially increase extracellular concentrations while sustained vasogenic edema with its relative expansion of the extracellular space can dilute extracellular levels. These processes are further influenced by the temporal dynamic alterations of development and resolution of vasogenic and cytotoxic edema formation.

Temporal profile of tissue damage

Following induction of a cortical contusion, the lesion and edema formation were significantly increased during the first 24 hours after CCI, followed by a gradual decrease in edema formation[35] as the contusion is transformed to a necrotic cyst by day 7. The maximal extent of the induced lesion is reached at hour 24. This secondary growth is related to continuing cellular perturbation reflected by ionic, energetic and metabolic impairment[36], mitochondrial damage[35,6], increased neuronal activity[30], decreased cortical perfusion[37–39] and disturbed microcirculation[40–42]. The observed progression of the induced lesion determined by MRI correlates well with histologic changes following CCI[43,39,33].

Mitochondrial damage following TBI

TBI involves a multitude of different targets converging in a common pathway shown to destabilize the otherwise well protected mitochondria. In this context, excessive glutamate receptor stimulation with subsequent intracellular alterations, formation of

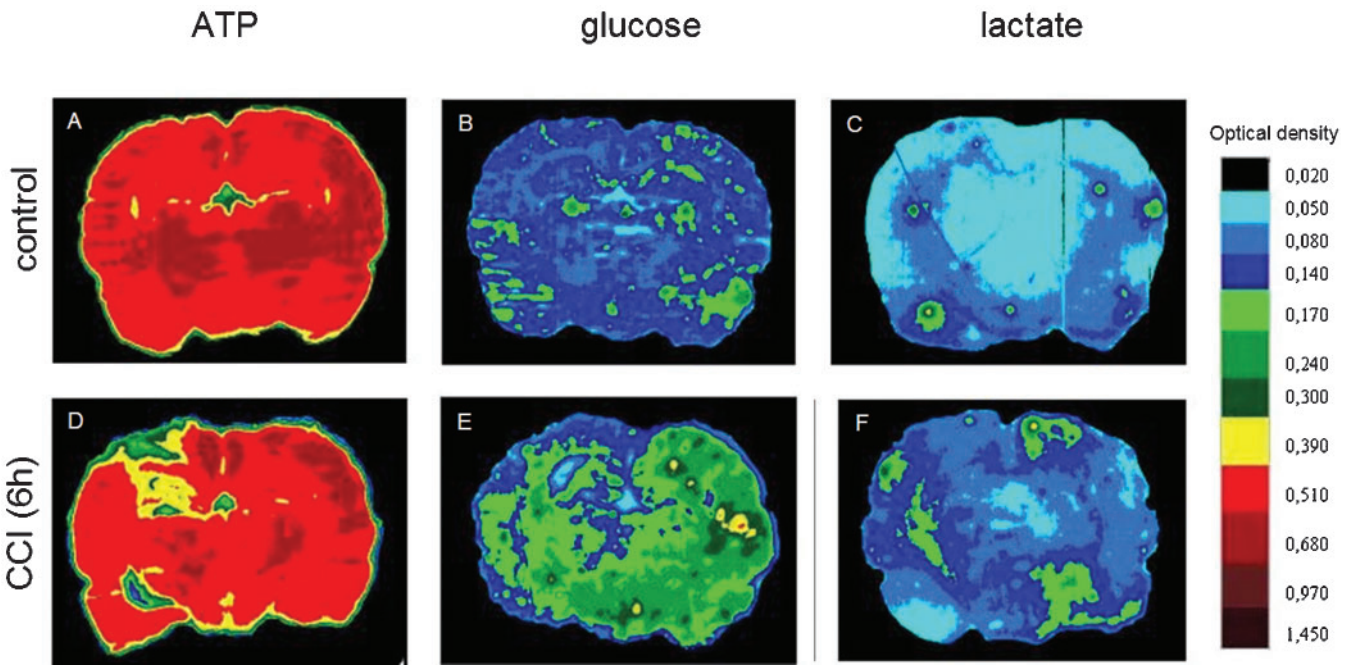

Figure 5: Bioluminescence imaging of sham rats (**A**–**C**) and following CCI 6 hours after trauma (**D**–**F**) showing focal changes in ATP levels and heterogeneous glucose and lactate alterations ipsilateral and contralateral to the contusion in representative cross-sections of the rat brain

reactive oxygen species, production of NO and release of pro-inflammatory cytokines are important mediators[17,1,24,33,5,6]. Apart from its essential role in generating ATP, mitochondria have been shown to execute apoptotic and necrotic reactions by releasing destructive agents, e.g. cytochrome c, Smac/Diablo which amplify activated caspase cascade, and apoptosis inducing factor (AIF) known to fragment DNA and kill cells in a caspase-independent manner[44]. In addition, mitochondria are a major site of production of reactive oxygen species[45,46], thereby deactivating enzymes, inhibiting ATP generation and disturbing protein synthesis. Activation of the mitochondrial permeability transition pore by calcium and reactive oxygen species opens the inner mitochondrial membrane to release pro-apoptotic molecules which form insoluble protein complexes within the cytoplasm and reduce the mitochondrial membrane potential indispensable for the generation of ATP. Functional mitochondrial impairment following TBI is evidenced by a loss in activity of the respiratory enzymes CO[47,14], succinate dehydrogenase[25,48], diminished respiratory function[6], decreased expression of the anti-apoptotic mitochondrial protein Bcl-2, sustained release of cytochrome c[49] and reduced production of N-acetylaspartate[32]. In addition to these functional disturbances, mitochondria show characteristic morphologic alterations in terms of swelling, disrupted cristae and a destroyed outer membrane[17], which reflect a dynamic continuum extending from acute water accumulation to structural disintegration typical for ensuing cell death.

Energetic perturbation following CCI

TBI is associated with significant alterations in energy production which are only partially reversible within 7–14 days after injury[50,15,32]. In this context, perilesional lactate and pyruvate concentrations remained elevated for 7 days following fluid percussion brain injury[50], while glucose uptake remained depressed for ~1 week after CCI[15], and ATP was restored by 120 hours after weight drop injury[32]. Following experimental induction of a traumatic brain lesion by lateral fluid percussion injury[17] and CCI[35,14,33], mitochondrial staining is lost. This reflects mitochondrial death which accounts for irreversible and progressive loss of ATP at the site of impact as revealed by bioluminescence imaging[12,31]. It appears that energetic impairment resulting in elevated extracellular hypoxanthine and lactate levels and increased lactate/glucose ratio proceed the progressive growth of the lesion as their maximal levels are reached by 6 hours while the contusion reaches its maximal extent at hour 24. Pharmacologic studies are warranted to determine if this energetic perturbation in fact causes this progressive lesion growth.

Glycolysis

At all investigated time points, glucose was not depleted despite elevated lactate levels in the regions assessed by tissue analysis and microdialysis. To the contrary, glucose was even significantly increased in the adjacent and contralateral cortical and hippocampal structures. This was verified by bioluminescence imaging; however, changes have not been as obvious compared with microdialysate parameters. This pattern could reflect depressed glucose utilization[36] or sustained cerebral glucose uptake via increased expression of glucose transporters as observed following ischemia and seizures[51–53]. This could also rule out presence of anaerobic glycolysis as observed during irreversible

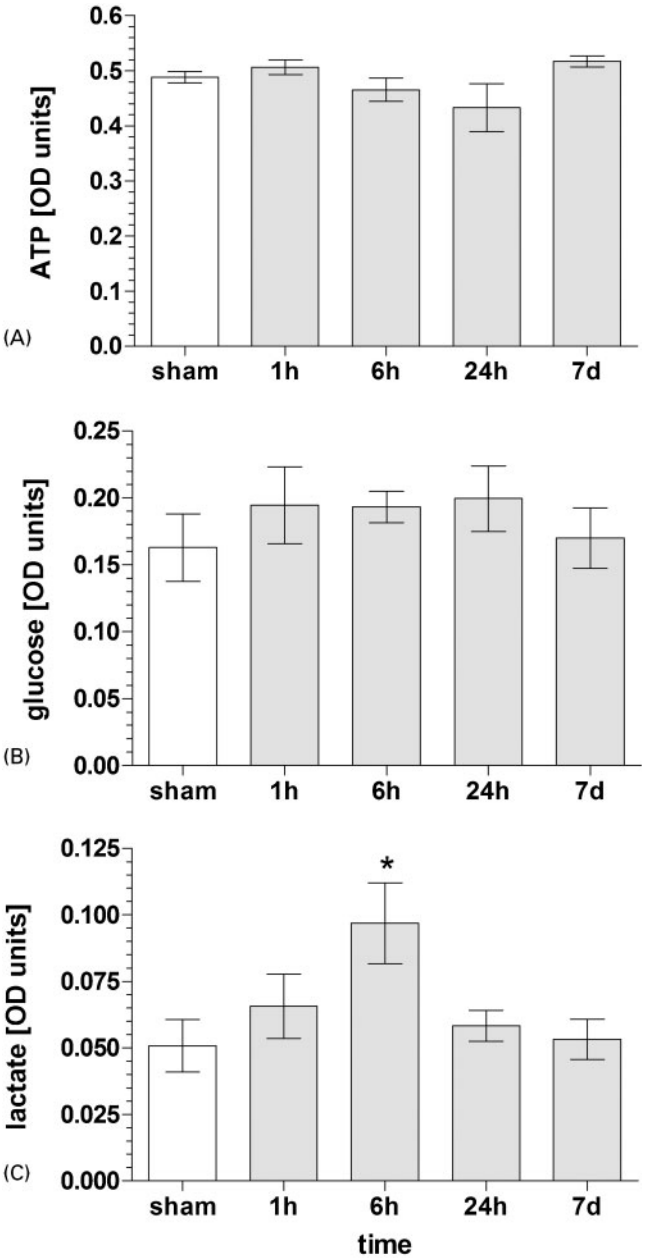

Figure 6: Regional and temporal changes in tissue ATP (**A**), glucose (**B**) and lactate (**C**) levels are determined by bioluminescence imaging. Alterations in optical densities over time are compared with unaffected control animals (gray bars). While ATP is slightly diminished 24 hours after trauma, glucose and lactate levels are increased over time ($p<0.05$ vs control)

ischemia[54]. As shown by Bryan *et al.*[38] and Kochanek *et al.*[39], ischemia is only present within the induced cortical contusion while pericontusional cortical perfusion is reversibly attenuated. Thus, delivery of glucose and oxygen should be maintained by the preserved albeit reduced pericontusional perfusion.

Tissue and extracellular lactate can be elevated owing to sustained production through non-oxidative glycolysis or via increased posttraumatic cerebral uptake[55]. The elevated calculated lactate-to-glucose ratio strongly suggests that non-oxidative glucose degradation resulting in lactate production prevails within the distant pericontusional cortex and the corresponding contralateral region as determined by tissue analysis (bioluminescence imaging). The decreased lactate-to-glucose ratio, however, could also reveal an immediate shuttling with subsequent neuronal and glial metabolism of the produced lactate as suggested by Magistretti[10] which, in turn, could suggest that the increased ratio reflects impaired lactate utilization.

ATP degradation and synthesis

Any increase in extracellular hypoxanthine is accepted to reflect underlying ATP degradation which cannot be balanced by re-synthesis of ATP via hypoxanthine phosphoribosyltransferase. While Bell *et al.*[23] and Nilsson *et al.*[24] focused on the changes at the site of the impact during the initial 2 hours following experimental TBI, we are the first to show that significantly increased extracellular hypoxanthine levels persisted for 24 hours adjacent and distant to the cortical contusion after CCI. As revealed by the TTC stain and the tissue ATP measurement, the cortical contusion as such is characterized by overt mitochondrial damage and absent ATP. The discrepancy between unchanged tissue ATP and increased extracellular hypoxanthine levels in the adjacent and distant pericontusional areas could be related to hypoxanthine diffusing from the core of the contusion to the periphery, thus not necessarily unmasking ATP degradation at the site of the microdialysis catheter. In addition, continuing ATP degradation resulting in elevated hypoxanthine levels could be replenished by increased oxidative and non-oxidative glycolysis. Because the increase in lactate is more pronounced especially in the early phase after trauma, lactate might be also the predominant metabolite to fuel ATP synthesis and the consumption of lactate in this period might explain the stability of tissue ATP content within the investigated cortical and hippocampal areas.

Functional involvement of the contralateral hemisphere

The CCI model employed in the present study induces a structural lesion confined to the ipsilateral hemisphere as revealed by the MRI and histologic studies. Functional analysis using bioluminescence imaging, however, showed that the contralateral hemisphere is also involved in maintaining stable ATP levels as reflected by sustained glucose and increased lactate levels (data not shown). As revealed by the representative brain, there appears to be a diffuse metabolic 'contre-coup' lesions as reflected by the increased signal intensity for glucose and lactate in the contralateral parietal and temporal cortex (*Figure 5E,F*).

CONCLUSIONS

CCI is associated with long-lasting signs of metabolic disturbances and adaptive endogenous regulatory mechanisms to maintain stable ATP levels. These signs of functional derangement could unmask as well as initiate cell damage coinciding with secondary growth

of the cortical contusion and edema formation as determined by MRI. Based on these findings, metabolic perturbation is not restricted to the early phase or the ipsilateral hemisphere following induction of a focal TBI.

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REFERENCES

1 Nicholls DG, Budd SL, Castilho RF, *et al.* Glutamate excitotoxicity and neuronal energy metabolism. *Ann NY Acad Sci* 1999; **893**: 1–12
2 Schuhmann MU, Stiller D, Skardelly M, *et al.* Metabolic changes in the vicinity of brain contusions: A proton magnetic resonance spectroscopy and histology study. *J Neurotrauma* 2003; **20**: 725–743
3 Xiong Y, Peterson PL, Lee CP. Alterations in cerebral energy metabolism induced by traumatic brain injury. *Neurol Res* 2001; **23**: 129–138
4 Calabrese V, Scapagnini G, Giuffrida Stella AM, *et al.* Mitochondrial involvement in brain function and dysfunction: Relevance to aging, neurodegenerative disorders and longevity. *Neurochem Res* 2001; **26**: 739–764
5 Verweij BH, Muizelaar JP, Vinas FC, *et al.* Impaired cerebral mitochondrial function after traumatic brain injury in humans. *J Neurosurg* 2000; **93**: 815–820
6 Xiong Y, Gu Q, Peterson PL, *et al.* Mitochondrial dysfunction and calcium perturbation induced by traumatic brain injury. *J Neurotrauma* 1997; **14**: 23–34
7 Erecinska M, Silver IA. ATP and brain function. *J Cereb Blood Flow Metab* 1989; **9**: 2–19
8 Attwell D, Laughlin SB. An energy budget for signaling in the grey matter of the brain. *J Cereb Blood Flow Metab* 2001; **21**: 1133–1145
9 Deitmer JW. Strategies for metabolic exchange between glial cells and neurons. *Respir Physiol* 2001; **129**: 71–81
10 Magistretti PJ, Pellerin L, Rothman DL, *et al.* Energy on demand. *Science* 1999; **283**: 496–497
11 Moseley ME, Cohen Y, Mintorovitch J, *et al.* Early detection of regional cerebral ischemia in cats: Comparison of diffusion- and T2-weighted MRI and spectroscopy. *Magn Reson Med* 1990; **14**: 330–346
12 Mautes AE, Thome D, Steudel WI, *et al.* Changes in regional energy metabolism after closed head injury in the rat. *J Mol Neurosci* 2001; **16**: 33–39
13 Perri BR, Smith DH, Murai H, *et al.* Metabolic quantification of lesion volume following experimental traumatic brain injury in the rat. *J Neurotrauma* 1997; **14**: 15–22
14 Hovda DA, Yoshino A, Kawamata T, *et al.* Diffuse prolonged depression of cerebral oxidative metabolism following concussive brain injury in the rat: A cytochrome oxidase histochemistry study. *Brain Res* 1991; **567**: 1–10
15 Kelly DF, Kozlowski DA, Haddad E, *et al.* Ethanol reduces metabolic uncoupling following experimental head injury. *J Neurotrauma* 2000; **17**: 261–272
16 Kelly PA, Ford I, McCulloch J. The effect of diazepam upon local cerebral glucose use in the conscious rat. *Neuroscience* 1986; **19**: 257–265
17 Lifshitz J, Friberg H, Neumar RW, *et al.* Structural and functional damage sustained by mitochondria after traumatic brain injury in the rat: Evidence for differentially sensitive populations in the cortex and hippocampus. *J Cereb Blood Flow Metab* 2003; **23**: 219–231
18 Marklund N, Ostman B, Nalmo L, *et al.* Hypoxanthine, uric acid and allantoin as indicators of *in vivo* free radical reactions. Description of a HPLC method and human brain microdialysis data. *Acta Neurochir (Wien)* 2000; **142**: 1135–1141
19 Stover JF, Lowitzsch K, Kempski OS. Cerebrospinal fluid hypoxanthine, xanthine and uric acid levels may reflect glutamate-mediated excitotoxicity in different neurological diseases. *Neurosci Lett* 1997; **238**: 25–28
20 Kawamata T, Katayama Y, Hovda DA, *et al.* Administration of excitatory amino-acid antagonists via microdialysis attenuates the increase in glucose-utilization seen following concussive brain injury. *J Cereb Blood Flow Metab* 1992; **12**: 12–24
21 Kawamata T, Katayama Y, Hovda DA, *et al.* Lactate accumulation following concussive brain injury – the role of ionic fluxes induced by excitatory amino-acids. *Brain Res* 1995; **674**: 196–204
22 Minervini M, Atlante A, Gagliardi S, *et al.* Glutamate stimulates 2-deoxyglucose uptake in rat cerebellar granule cells. *Brain Res* 1997; **768**: 57–62
23 Bell MJ, Kochanek PM, Carcillo JA, *et al.* Interstitial adenosine, inosine, and hypoxanthine are increased after experimental traumatic brain injury in the rat. *J Neurotrauma* 1998; **15**: 163–170
24 Nilsson P, Hillered L, Ponten U, *et al.* Changes in cortical extracellular levels of energy-related metabolites and amino acids following concussive brain injury in rats. *J Cereb Blood Flow Metab* 1990; **10**: 631–637
25 Kroppenstedt SN, Thomale UW, Griebenow M, *et al.* Effects of early and late intravenous norepinephrine infusion on cerebral perfusion, microcirculation, brain-tissue oxygenation, and edema formation in brain-injured rats. *Crit Care Med* 2003; **31**: 2211–2221
26 Stover JF, Sakowitz OW, Kroppenstedt SN, *et al.* Differential effects of prolonged isoflurane anesthesia on plasma, extracellular, and CSF glutamate, neuronal activity, 125I-Mk801 NMDA receptor binding, and brain edema in traumatic brain-injured rats. *Acta Neurochir (Wien)* 2004; **146**: 819–830
27 Thomale UW, Kroppenstedt SN, Beyer TF, *et al.* Temporal profile of cortical perfusion and microcirculation after controlled cortical impact injury in rats. *J Neurotrauma* 2002; **19**: 403–413
28 Thomale UW, Griebenow M, Kroppenstedt SN, *et al.* Small volume resuscitation with HyperHaes improves pericontusional perfusion and reduces lesion volume following controlled cortical impact injury in rats. *J Neurotrauma* 2004; **21**: 1737–1746
29 Kroppenstedt SN, Sakowitz OW, Thomale UW, *et al.* Influence of norepinephrine and dopamine on cortical perfusion, EEG activity, extracellular glutamate, and brain edema in rats after controlled cortical impact injury. *J Neurotrauma* 2002; **19**: 1421–1432
30 Stover JF, Sakowitz OW, Beyer TF, *et al.* Effects of LY379268, a selective group II metabotropic glutamate receptor agonist on EEG activity, cortical perfusion, tissue damage, and cortical glutamate, glucose, and lactate levels in brain-injured rats. *J Neurotrauma* 2003; **20**: 315–326
31 Weinzierl MR, Laurer HL, Fuchs M, *et al.* Changes in regional energy metabolism after cortical cold lesion in the rat brain. *J Mol Neurosci* 2002; **18**: 247–250
32 Signoretti S, Marmarou A, Tavazzi B, *et al.* N-Acetylaspartate reduction as a measure of injury severity and mitochondrial dysfunction following diffuse traumatic brain injury. *J Neurotrauma* 2001; **18**: 977–991
33 Stover JF, Schoning B, Beyer TF, *et al.* Temporal profile of cerebrospinal fluid glutamate, interleukin-6, and tumor necrosis factor-alpha in relation to brain edema and contusion following controlled cortical impact injury in rats. *Neurosci Lett* 2000; **288**: 25–28
34 Waagepetersen HS, Sonnewald U, Larsson OM, *et al.* Multiple compartments with different metabolic characteristics are involved in biosynthesis of intracellular and released glutamine and citrate in astrocytes. *Glia* 2001; **35**: 246–252
35 Baskaya MK, Dogan A, Temiz C, *et al.* Application of 2,3,5-triphenyltetrazolium chloride staining to evaluate injury volume after controlled cortical impact brain injury: Role of brain edema in evolution of injury volume. *J Neurotrauma* 2000; **17**: 93–99
36 Yoshino A, Hovda DA, Kawamata T, *et al.* Dynamic changes in local cerebral glucose utilization following cerebral conclusion in

rats: Evidence of a hyper- and subsequent hypometabolic state. *Brain Res* 1991; **561**: 106–119
37 Zwienenberg M, Muizelaar JP. Cerebral perfusion and blood flow in neurotrauma. *Neurol Res* 2001; **23**: 167–174
38 Bryan RM, Jr, Cherian L, Robertson C. Regional cerebral blood flow after controlled cortical impact injury in rats. *Anesth Analg* 1995; **80**: 687–695
39 Kochanek PM, Marion DW, Zhang W, *et al.* Severe controlled cortical impact in rats: Assessment of cerebral edema, blood flow, and contusion volume. *J Neurotrauma* 1995; **12**: 1015–1025
40 Ginsberg MD, Zhao W, Belayev L, *et al.* Diminution of metabolism/blood flow uncoupling following traumatic brain injury in rats in response to high-dose human albumin treatment. *J Neurosurg* 2001; **94**: 499–509
41 Maeda T, Katayama Y, Kawamata T, *et al.* Hemodynamic depression and microthrombosis in the peripheral areas of cortical contusion in the rat: Role of platelet activating factor. *Acta Neurochir Suppl* 1997; **70**: 102–105
42 Teasdale GM, Graham DI. Craniocerebral trauma: Protection and retrieval of the neuronal population after injury. *Neurosurgery* 1998; **43**: 723–737
43 Albensi BC, Knoblach SM, Chew BG, *et al.* Diffusion and high resolution MRI of traumatic brain injury in rats: Time course and correlation with histology. *Exp Neurol* 2000; **162**: 61–72
44 Rego AC, Oliveira CR. Mitochondrial dysfunction and reactive oxygen species in excitotoxicity and apoptosis: Implications for the pathogenesis of neurodegenerative diseases. *Neurochem Res* 2003; **28**: 1563–1574
45 Sims NR, Anderson MF, Hobbs LM, *et al.* Brain mitochondrial responses to postischemic reperfusion: A role for calcium and hydrogen peroxide? *Dev Neurosci* 2000; **22**: 366–375
46 Sims NR, Anderson MF. Mitochondrial contributions to tissue damage in stroke. *Neurochem Int* 2002; **40**: 511–526
47 Harris LK, Black RT, Golden KM, *et al.* Traumatic brain injury-induced changes in gene expression and functional activity of mitochondrial cytochrome C oxidase. *J Neurotrauma* 2001; **18**: 993–1009
48 Stover JF, Unterberg AW. Increased cerebrospinal fluid glutamate and taurine concentrations are associated with traumatic brain edema formation in rats. *Brain Res* 2000; **875**: 51–55
49 Dong GX, Singh DK, Dendle P, *et al.* Regional expression of Bcl-2 mRNA and mitochondrial cytochrome c release after experimental brain injury in the rat. *Brain Res* 2001; **903**: 45–52
50 Bentzer P, Davidsson H, Grande PO. Microdialysis-based long-term measurements of energy-related metabolites in the rat brain following a fluid percussion trauma. *J Neurotrauma* 2000; **17**: 441–447
51 Gronlund KM, Gerhart DZ, Leino RL, *et al.* Chronic seizures increase glucose transporter abundance in rat brain. *J Neuropathol Exp Neurol* 1996; **55**: 832–840
52 Massa SM, Swanson RA, Sharp FR. The stress gene response in brain. *Cerebrovasc Brain Metab Rev* 1996; **8**: 95–158
53 Suzuki H, Nagashima T, Fujita K, *et al.* Cerebral ischemia alters glucose transporter kinetics across rat brain microvascular endothelium. Quantitative analysis by an *in situ* brain perfusion method. *J Auton Nerv Syst* 1994; **49**: S173–S176
54 Schurr A. Lactate, glucose and energy metabolism in the ischemic brain. *Int J Mol Med* 2002; **10**: 131–136
55 Chen T, Qian YZ, Rice A, *et al.* Brain lactate uptake increases at the site of impact after traumatic brain injury. *Brain Res* 2000; **861**: 281–287