Lactate Storm Marks Cerebral Metabolism following Brain Trauma*

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Background: In brain metabolism, neurons are fueled by lactate passed to them by glia in a metabolic coupling.

Results: Following brain trauma, lactate uptake into neurons from glia was impaired, producing a metabolic lactate storm.

Conclusion: Brain trauma results in neuronal-glial metabolic uncoupling, releasing free lactate.

Significance: Inhibition of lactate production or its removal may be an important therapeutic strategy for brain trauma.

Brain metabolism is thought to be maintained by neuronal-glial metabolic coupling. Glia take up glutamate from the synaptic cleft for conversion into glutamine, triggering glial glycolysis and lactate production. This lactate is shuttled into neurons and further metabolized. The origin and role of lactate in severe traumatic brain injury (TBI) remains controversial. Using a modified weight drop model of severe TBI and magnetic resonance (MR) spectroscopy with infusion of 13C-labeled glucose, lactate, and acetate, the present study investigated the possibility that neuronal-glial metabolism is uncoupled following severe TBI. Histopathology of the model showed severe brain injury with subarachnoid and hemorrhage together with glial cell activation and positive staining for Tau at 90 min post-trauma. High resolution MR spectroscopy of brain metabolites revealed significant labeling of lactate at C-3 and C-2 irrespective of the infused substrates. Increased 13C-labeled lactate in all study groups in the absence of ischemia implied activated astrocytic glycolysis and production of lactate with failure of neuronal-glial metabolism. The early increase in extracellular lactate in severe TBI with the injured neurons rendered unable to pick it up probably contributes to a rapid progression toward irreversible injury and pan-necrosis. Hence, a method to detect and scavenge the excess extracellular lactate on site or early following severe TBI may be a potential primary therapeutic measure.

It has been well known that brain lactate levels increase following severe traumatic brain injury (TBI), but its origin remains controversial (1). Lactate serves as an alternative energy substrate to glucose for normal or injured neurons (2–4). Contemporary studies have also suggested its potential neuroprotective or therapeutic role in TBI (5, 6). Indeed, although a recent international clinical trial is aimed at the study of the therapeutic benefit of lactate in TBI, it is important to consider that the findings presented here suggest that this may not be the case.

Using isotopes of carbon, such as 14C and 13C, investigators have studied the effect of TBI on brain metabolism, in particular how it relates to increased brain lactate levels at and around the site of injury as compared with other regions (7–9). This further led to the assumption that increased lactate may be a result of impaired mitochondrial function following TBI with a compensatory increase in anaerobic glycolysis for ionic homeostasis and generation of extracellular fluid lactate (10, 11). This phenomenon has been explained to be a protective mechanism in TBI by which the injured brain is able to uptake and metabolize lactate. The normal human brain already shows striking differences in lactate/pyruvate ratio from plasma, which suggest that lactate is a physiologic fuel for neurons (12). Accordingly, it has been speculated that lactate might be a useful therapy following TBI (13, 14). However, a conundrum arises as a result of the common observation that excessive lactate accumulation following TBI correlates with poor prognosis (15–17). This is in agreement with the common notion that lactate accumulation can be detrimental to neuronal survival, despite its recent demonstration as an alternative fuel for neurons. Lactate is a strong acid with a pKₐ of 3.86, with implications for tissue pH.

The present study is based on the understanding that neuronal-glial metabolism are coupled; the glia take up and metabolize glutamate to glutamine from the neuronal synapse, for maintenance of synaptic glutamate levels below those associated with neurotoxicity (18, 19). The uptake of glutamate is specific to glutamate transporters and requires an ionic gradient. Glutamate is converted to glutamine in an ATP-dependent reaction. The role of astrocytic metabolism can be traced as a decrease in ATP activating astrocytic glycolysis, resulting in production of lactate that is shuttled to neurons, widely described as the astrocytic neuronal lactate shuttle hypothesis.

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‡ The abbreviations used are: TBI, traumatic brain injury; MR, magnetic resonance; SED, spin echo difference.

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model of brain metabolism (20, 21). Accordingly, the present study was designed to enable the dissection of the neuronal and glial metabolic compartments and to investigate how they behave following severe TBI. The study utilizes a modified weight drop impact acceleration model of acute severe TBI, histopathology, and ex vivo high resolution MR spectroscopy with infusion of $^{13}$C-labeled substrates specific to neurons and astrocytes in control and TBI rats.

MR spectroscopy provides precise metabolic mapping of neurons and glia after administration of $^{13}$C labeled glucose, lactate, and acetate to injured and control rats. Glucose is an ambiguous substrate to both neurons and glia, lactate is exclusively neuronal, and acetate is astrocytic. $^{13}$C is a non-radioactive, naturally occurring, safe and stable isotope. Its abundance is $\sim 1.1\%$ of carbon in nature. Due to its half nuclear spin properties, this carbon isotope can be studied with MR spectroscopy (22, 23). The $^{13}$C MR spectroscopy technique provides information on the organic structure of the molecule being studied via incorporation of tissue metabolites, such as glutamate, glutamine, lactate, etc. incorporated with $^{13}$C isotopes following their systemic administration.

MATERIALS AND METHODS

All experiments were approved by the Animal Care Committee of the National Research Council of Canada and of the University of Calgary. Forty-five adult male Sprague-Dawley rats (Charles River Laboratories, Montreal, Canada) weighing 250–300 g (mean 280 ± 70 g) were used. Twelve rats were utilized for histopathology, and the remaining 33 were utilized for $^{13}$C-labeled substrate infusion, the brains being processed for high resolution ex vivo MR spectroscopy.

Anesthesia and Surgical Procedures—Each rat was fasted for 2 h and anesthetized using isoflurane (1–2%, inhalational anesthesia for induction as well as maintenance) in 100% O$_2$. After 10–15 min, the animals were intubated and mechanically ventilated throughout the experiment. An area of inner thigh was shaved, and the femoral artery and vein were cannulated for blood pressure monitoring and labeled substrate infusion. Body temperature was maintained at 37 ± 1°C with the use of a circulating water bath. In TBI groups, head trauma was induced using 500-g/1.4-meter weight/height impact, modified from and based on the model described by Marmarou et al. (24, 25). The pH, pO$_2$, and pCO$_2$ in the blood were determined using an ABL 725 radiometer before and over a 2-h period after trauma and parameters set as follows: pCO$_2$, 35–45 mm Hg; pO$_2$, 120–140 mm Hg; pH, 7.30–7.58; mean arterial pressure, 90–110 mm Hg.

Trauma Device—The trauma device used was composed of a columnar Plexiglas tube and a set of brass weights falling freely by gravity onto a metallic helmet fixed by dental acrylic to the skull vertex of the rat. From a designated height the brass weight fell through the lumen of a transparent Plexiglas tube held in a ring stand. The helmet was a stainless steel disc 10 mm in diameter and 3 mm thick. The contact side of the disc was grooved concentrically to accept acrylic and secure the contact.

Induction of Head Trauma—The scalp of the anesthetized animal was shaved, a midline incision was made, and the peristeum covering the vertex was reflected. The metallic disc/helmet was placed centrally on the skull vault between the coronal and lambdoid sutures. The rat was placed securely in the prone position on a foam bed of known spring constant. The lower end of the Plexiglas tube was positioned directly above the helmet. The injury was delivered by dropping a 400-g weight from the predetermined height of 1.4 m. Rebound impact was prevented by moving the foam bed containing the animal away from the tube immediately following the impact.

Histopathology—At 90 min post-TBI, 12 (10 TBI, 2 controls) rats were euthanized by intraperitoneal administration of pentobarbital (200 mg/kg) and perfusion-fixed with trans-cardiac injection of formaldehyde (0.4%, 60 ml) following a saline flush (0.9%, 60 ml). The animals were decapitated, and the heads were placed in the same fixative for 2 weeks prior to sectioning. The brains were removed and cut coronally into 2.8-mm-thick slices, dehydrated in graded concentrations of ethanol, and embedded in paraffin. Serial sections were cut at 8 µm and stained with hematoxylin and eosin (H&E), Bielschowsky Ag stain, glial fibrillary acidic protein, and Tau immunohistochemistry.

Labeled Substrate Infusion—Thirty-three adult male rats were randomized for substrate infusion and brain metabolite MR spectroscopy. Control animals ($n = 9$, 3 rats/group) received either [1-$^{13}$C]glucose (10%, w/v), sodium [2-$^{13}$C]acetate (7.5%, w/v), or sodium [3-$^{13}$C]lactate (6%, w/v) (Cambridge Isotopes, Andover, MA) for 12 min. Animals receiving TBI ($n = 24$, 4 rats/group) were also randomly allocated to receive one of the three isotopes. In these experiments, the infusion began immediately following TBI in 12 of the animals and at 60 min post-TBI in the other 12 animals. For all isotope infusions, animals received a bolus (0.8 ml/kg) of one of the three solutions, followed immediately by continuous infusion via syringe pump. The rate of infusion was decreased exponentially from an initial 15 ml/kg/h over 4 min to 2.9 ml/kg/h. Following the experiments, the animals were sacrificed by funnel freezing of the heads using liquid N$_2$ while anesthetized and ventilated (26). The brains were stored at −80°C for metabolite extraction and high resolution MR spectroscopy.

Metabolite Extraction for High Resolution MR Spectroscopy—Metabolite extraction from frozen brain specimens was performed as described previously (27). Briefly, the funnel-frozen brain was recovered from −80°C, and neocortical brain samples averaging 18–20 g were weighed in a cold chamber. The samples were homogenized and centrifuged three times in a 2:2:1 chloroform/methanol/H$_2$O mixture at 20,500 rpm for 10 min. The methanol-H$_2$O monolayer was retained following separation from the heavier chloroform-containing organic solvent. Excess methanol was removed using a rotary evaporator and freeze-dried for 48 h. Prior to MR spectroscopy, the sample was reconstituted in 0.6 ml of D$_2$O stock solution (590 µl of D$_2$O, 10 µl of 2,2-dimethyl-2-silapentane sulfonic acid, an internal standard for chemical shift), and the pH was adjusted to 6.9–7.1 using KOD and KCl and placed in 5-mm NMR tubes for analysis.

Metabolism of the labeled substrates was studied by obtaining the spectroscopy data, which allows both identification and position of the compounds of interest based on their known chemical shifts. An inverse detection technique called $^1$H/$^{13}$C
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TABLE 1
Gross injury pattern and locations following TBI based on histopathological assessment (percentage of animals, n = 10).

| Lesions (n = 10) | Traumatic SAH | Traumatic IVH | Parenchymal hemorrhage | Shear injury | Abnormal hippocampal neurons (CA4) | Abnormal neurons (dentate blade) | Brain stem hemorrhage |
|-----------------|---------------|---------------|------------------------|-------------|-----------------------------------|---------------------------------|----------------------|
|                 | %             | %             | %                      | %           | %                                 | %                               | %                    |
| Traumatic SAH   | 100           | 100           | 90                     | 80          | 60                                | 60                              | 30                   |

RESULTS

Histopathology—In histopathological analysis using H&E staining of paraffin-embedded brain sections, all TBI animals showed evidence of severe brain injury (Table 1 and Fig. 1). Subarachnoid hemorrhage and intraventricular hemorrhage was evident in all TBI animals, 89% had neocortical hemorrhage, 80% demonstrated shear injury in the form of dehiscence of the septal nuclei from the inferior aspect of the corpus callosum and with Bielschowsky stain a few early neuroaxonal spheroids. More than 50% showed dark neurons in the CA4 sector and the dentate, and 30% had brain stem hemorrhage. The two control brains were unremarkable.

Brain sections of all TBI animals stained with glial fibrillary acidic protein showed astrocytic activation in the CA3 zone of the hippocampus, more than the CA1 zone (Fig. 2). There was also significant activation in the CA4 zone and the hilus of the dentate gyrus. The dentate gyrus itself showed astrocyte activation throughout the granule cell layer, mostly toward the inner surface that interfaced with the CA4 zone. The neocortex showed both superficial glial activation in layers 1–3 and deep activation in layer 6. Activated astrocytes were not present in layers 4 and 5. The control brains showed minimal glial activation.

Fifty percent of the animals showed positive staining for Tau (Fig. 3). Staining was present in the mossy fibers or axons of the dentate granule cells. The CA3 pyramidal cell band showed positive staining of mossy fibers. Within the neocortex, Tau staining of large neurons was not evident. Lesser staining anatomically restricted to the mossy fibers and subpial/periventricular zones was seen in the two control brains.

MR Spectroscopy—From the acquired 1H[13C] MR spectra, a number of metabolites (lactate, alanine, acetate, N-acetylaspartate, GABA, glutamate, succinate, glutamine, aspartate, inositol, creatine, and taurine) could be compared between groups. Labeling of metabolites following [1-13C]glucose and [3,13C]lactate was similar. The peaks for C-3 lactate and C-4 glutamate show similar chemical shifts and peak height. Following [2-13C] acetate infusion, however, a small amount of labeled lactate and alanine at C-3 position was observed. Representative spectra for each substrate infusion immediately post-TBI and 60 min post-TBI are shown in Fig. 4.

Following severe TBI and immediate infusion of 13C-labeled substrates, C-3 lactate was significantly increased in all three infusion groups (i.e. [1-13C]glucose (p < 0.01), [3-13C]lactate (p < 0.01), and [2-13C]acetate (p < 0.01)) compared with their control counterparts, as shown by percentage enrichment in the subtraction spectra (Fig. 5). It was also observed that percentage enrichment of 3-C alanine was significantly increased immediately following TBI in the [3-13C]lactate infusion group.
Interestingly, 4-C succinate was significantly increased in the [2-13C]acetate infusion group ($p < 0.05$). Glutamate and aspartate showed a decreased labeling pattern and percentage enrichment immediately following severe TBI, reaching a significance level for aspartate only in the [1-13C] glucose infusion group ($p \leq 0.005$).
Following severe TBI and infusion of $^{13}$C labeled substrates 60 min postinjury, again C-3 lactate was significantly increased in all three infusion groups in comparison with the corresponding controls ($p < 0.005$ for $1^{13}$C glucose and $3^{13}$C lactate infusion groups and $p < 0.05$ for $2^{13}$C acetate infusion group) (Fig. 5). A decreased C-4 glutamate was again observed in all three groups 60 min following severe TBI, reaching a significance level only for the $3^{13}$C lactate infusion.
infusion group. A number of metabolites (i.e. alanine, acetate, and GABA) in the [2-13C]acetate infusion group showed a significantly increased labeling pattern \( p < 0.05 \) for all four metabolites), whereas in the lactate and glucose infusion groups, this was not the case.

It was of interest to note that GABA was decreased following [1-13C]glucose infusion both immediately and 60 min following acute severe TBI. However, in the [3-13C]lactate and [2-13C]acetate infusion groups, GABA was increased following severe TBI and infusion of substrates immediately and 60 min after the impact.

The significant increase in C-3 lactate following severe TBI and infusion of 13C-labeled substrates prompted questions as to the origin of this metabolite. Specifically, infusion of [2-13C]acetate showed increased labeling of 3-C lactate, a phenomenon not usually expected. The origin of this was investigated by testing whether lactate was also labeled at the C-2 position, and it was found that regardless of the type of substrate infusion, C-2 lactate was labeled for both the immediate post-TBI and 60-min post-TBI group of animals. Again, the TBI groups showed an increased percentage enrichment for C-2 lactate, but the difference was not statistically significant.

**DISCUSSION**

This study demonstrates that the injured brain continues to produce lactate within minutes following severe TBI, which can be toxic and incompatible with life (16, 30). Furthermore, the injured neurons early following severe TBI are unable to pick up and metabolize the increased lactate, resulting in a lactate storm in the failing metabolic environment. The findings have significant clinical implications because perhaps a mechanism to safely scavenge and/or chelate the excess lactate, as some of the contemporary research has been suggesting (5, 6). Given that uncoupled metabolism occurs rapidly following trauma, such therapeutic measures would best be administered at the accident site, taking advantage of the known blood-brain barrier disruption and understanding of the neurovascular unit (35–37). The efficacy of these pharmacological strategies may be augmented by methods that improve their transport across the blood-brain barrier, such as membrane transporters, receptors, nanosized molecular chaperones, and vectors (38–40).

The present study examined neuronal-glial metabolic coupling following acute severe TBI. Utilizing established techniques of 13C-labeled substrate infusion specific to each of the cell types, the study demonstrates a unique observation: a significantly increased C-3 lactate following severe TBI and infusion of [1-13C]glucose, [3-13C]lactate, and, interestingly, [2-13C]acetate. The metabolic fate of each of the substrates following systemic administration has been well outlined previously. It is important to recognize the divergence that can occur between the C-3 and C-4 carbons in glutamine and glutamate as the metabolites between neuronal and glial pools are exchanged. Specifically, following 1-13C glucose and 3-13C lactate infusion, it is expected that products of glycolysis are labeled at precisely the same carbon skeletons, both being taken up by neurons and metabolized, recognizing that astrocytes also take up glucose. Astrocytic metabolism has been studied by some using [2-13C]acetate, a purely astroglial substrate due to its selective transport activity. Incorporation of 13C label is expected in the glial glutamine and glutamate pool. C-4 glutamate, often observed in acetate metabolism, is attributed to the C-4 glutamine produced by astrocytic oxidative phosphorylation that is picked up by neurons to replenish its C-4 glutamate pool. Although proposing a metabolic modeling is beyond the
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scope of this report, a similar mechanism as outlined by many is a likely proposition.

Production of lactate in the brain following acute severe TBI may be attributed to any of the following key mechanisms. 1) Increased astrocytic glycolysis as a result of increased excitatory amino acid, chiefly glutamate, necessitates rapid uptake from the ECF via Na⁺-dependent co-transport. The resultant intracellular glutamate and Na⁺ increase activates Na⁺/K⁺-ATPase, which stimulates astrocytic glycolysis and production of lactate (20, 41–43). 2) Although less likely in the present study (i.e., controlled for normoxia), in certain cases, when accompanied by tissue hypoxia, increased lactate and H⁺ accumulation occur via accelerated anaerobic glycolysis (44, 45). 3) A less commonly described but suggested mechanism is the role of astrogial cytosolic malic enzyme, which may be activated to an override in its function of degrading excessive Krebs cycle intermediates, such as malate, helping them exit the tricarboxylic acid cycle to generate pyruvate, which ultimately is reduced to form lactate (46–48). This malic enzyme-catalyzed reaction is deemed essential for complete oxidative metabolism of tricarboxylic acid cycle products, substrates, and their many derivatives, such as glutamate, glutamine, GABA, aspartate, etc. 4) A recent understanding of neuronal-astrocytic metabolic coupling emphasizes the role of a newly cloned soluble adenylyl cyclase, an HCO₃⁻ mate, glutamine, GABA, aspartate, etc. A recent understanding of neuronal-astrocytic metabolic coupling emphasizes the role of a newly cloned soluble adenylyl cyclase, an HCO₃⁻ sensor expressed abundantly in astrocytes, that get activated in response to small increases in extracellular K⁺, allowing HCO₃⁻ entry into the astrocytes (49). The increased HCO₃⁻ content triggers the cyclic AMP pathway, which further initiates astrocytic glycolysis and production of lactate that is taken up by neurons. In accordance with this explanation and the known increase in extracellular K⁺ following TBI, it is possible that this pathway is responsible for increased lactate production, which injured neurons are unable to metabolize and clear from the extracellular fluid compartment.

Thus, it is suggested that the latter two mechanisms may be responsible for the astrogial overactivity within the first few minutes to hours following acute severe TBI, resulting in excessive lactate accumulation in the brain. Indeed, this may have already determined the poor outcome (i.e., a seemingly inevitable irreversible brain injury and cellular death). These mechanisms, although not well studied in TBI, could help to explain the possible mechanism of accelerated production of lactate, perhaps initiated as a defense mechanism that fails to serve any useful purpose in severe TBI.

From the present study, it can be inferred that significantly increased lactate with infusion of [2-¹³C]acetate and [1-¹³C]glucose implies ongoing glial metabolism. High lactate levels and increased alanine with [3-¹³C]lactate infusion imply decreased neuronal metabolism with likely continued glial metabolism and production of lactate, which the neurons are unable to pick up and utilize. Increased lactate, alanine, GABA, and succinate following [2-¹³C]acetate infusion further support ongoing glial metabolism, with the latter diverting to an activated malic enzyme pathway. High lactate levels, as seen in [3-¹³C]lactate infusion animals, imply impaired neuronal metabolism that fails to utilize lactate that the glial cells continue to produce. In other words, it appears that following severe TBI, as early as minutes to an hour after injury, the neurons and glial metabolism become uncoupled (i.e., the glial cells are in overdrive to produce lactate, perhaps to feed the injured neurons). However, this phenomenon seems to happen irrespective of glutamate uptake for conversion to glutamine, which in turn activates the astrocytic glycolysis to produce more lactate for neurons, because both glutamate and glutamate labeling are found to be decreased in the post-TBI brain compared with controls. In what appears to be an autonomous astrocytic activity that fails to sense any feedback mechanism for metabolic demand and supply, the excessive lactate that bathes the neurons following severe TBI may be the lethal metabolic blow to the tissue. The combined effects of direct traumatic injury together with such severe metabolic distress could lead to unsalvageable brain.

Severity of injury was established by a graduated weight-height interface with histopathological evidence of severe brain injury yet minimal animal mortality. Upon determination of the weight-height interface, a subgroup of animals receiving TBI sustained the spectrum of intracerebral injury commonly observed in patients of severe TBI (50, 51). Significant activation of astrocytes (glial fibrillary acidic protein), neuronal injury/inflammation with the presence of dark neurons (H&E), and tauopathy (Tau immunohistology) support the observation of neuronal and glial pathology, which in turn would explain perturbed metabolism of the cell population as shown by the MR spectroscopy data. Severe head trauma results in oxidative stress reflected by the rapid accumulation of oxidative stress markers with a decrease in antioxidant defense enzymes (52–54). Oxidative stress is known to induce the formation of hyperphosphorylated Tau, consistent with the results reported here (55, 56).

The pronounced increase in lactate within the first few min to 1 h following acute severe TBI, as demonstrated by labeling at C-3 position and (also) C-2, is indicative of deranged cerebral metabolism in response to injury. The reason remains speculative. Activation of an enzymatic pathway, such as malic enzyme, or an astrocyte-specific HCO₃⁻ sensor is stipulated to be a possible underlying mechanism. With blood gas parameters well controlled and showing minimal variation within predetermined normal limits, hypoxia was ruled out as playing a significant role (i.e., anaerobic lactate production is unlikely). Furthermore, it has been shown that extracellular lactate increase is independent of brain hypoxia-ischemia in severe TBI (57, 58). Although future investigations of the enzymatic pathway and other possible mechanisms may help to dissect this further, it is evident from this study that uncoupled neuronal-glial metabolism leads to excessively increased brain lactate following acute severe TBI. Rather than the well formulated anaerobic mechanism being responsible, metabolic mismatch between neurons and glia and the uncoupling of the symbiotic balance between the two may be the most probable explanation. Complex as this mechanism may appear, it may be as simple as loss of the feedback mechanism for lactate sensing by glia, thereby causing

4. S. Lama, R. N. Auer, R. Tyson, C. N. Gallagher, B. Tomanek, and G. R. Sutherland, unpublished data.
them to become autonomous lactate producers perilous to the neurons, neuropil, and overall tissue survival.

CONCLUSION

The present study emphasizes the uncoupling of metabolism between neurons and glial cells following severe TBI within the first few min to 1 h of sustaining injury. It is of considerable interest to observe significantly high levels of lactate in animals irrespective of substrate infused. Although the study is acute and did not allow for survival as an outcome, the early metabolic injury sustained is comparable with those observed clinically in severe TBI with poor prognosis. Although some investigators have indicated lactate to be of benefit and as the metabolic compartment, inhibiting its production, and not administration of it, could be considered a potential therapeutic strategy for TBI.

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REFERENCES

1. Dienel, G. A. (2012) Brain lactate metabolism: the discoveries and the controversies. J. Cereb. Blood Flow Metab. 32, 1107–1138
2. Chen, T., Qian, Y. Z., Di, X., Zhu, J. P., and Bullock, R. (2000) Evidence for lactate uptake after rat fluid percussion brain injury. Acta Neuropathol. Suppl. 76, 359–364
3. Gallagher, C. N., Carpenter, K. L., Grice, P., Howe, D. J., Mason, A., Timofeev, I., Menon, D. K., Kirkpatrick, P. J., Pickard, J. D., Sutherland, G. R., and Hutchinson, P. J. (2009) The human brain utilizes lactate via the tricarboxylic acid cycle: a 13C-labeled microdialysis and high-resolution nuclear magnetic resonance study. Brain 132, 2839–2849
4. Tyson, R. L., Gallagher, C., and Sutherland, G. R. (2003) 13C-labeled substrates and the cerebral metabolic compartmentalization of acetate and lactate. Brain Res. 992, 43–52
5. Ichai, C., Armando, G., Orban, J. C., Berthier, F., Rami, L., Samat-Long, C., Grimaud, D., and Leverve, X. (2009) Sodium lactate versus mannitol in the treatment of intracranial hypertensive episodes in severe traumatic brain-injured patients. Intensive Care Med. 35, 471–479
6. Alessandri, B., Schwandt, E., Kamada, Y., Nagata, M., Heimann, A., and Kempski, O. (2012) The neuroprotective effect of lactate is not due to improved glutamate uptake after controlled cortical impact in rats. J. Neurotrauma 29, 2181–2191
7. Bartnik, B. L., Hovda, D. A., and Lee, P. W. (2007) Glucose metabolism after traumatic brain injury: estimation of pyruvate carboxylase and pyruvate dehydrogenase flux by mass isotopomer analysis. J. Neurotrauma 24, 181–194
8. Chen, T., Qian, Y. Z., Rice, A., Zhu, J. P., Di, X., and Bullock, R. (2000) Brain lactate uptake increases at the site of impact after traumatic brain injury. Brain Res. 861, 281–287
9. Bartnik, B. L., Lee, S. M., Hovda, D. A., and Sutton, R. L. (2007) The fate of glucose during the period of decreased metabolism after fluid percussion injury: a 13C NMR study. J. Neurotrauma 24, 1079–1092
10. Andersen, B. J., and Marmarou, A. (1992) Post-traumatic Selective Stimulation of Glycolysis. Brain Res. 585, 184–189
11. Andersen, B. J., and Marmarou, A. (1992) Functional compartmentalization of energy production in neural tissue. Brain Res. 585, 190–195
12. Abi-Saab, W. M., Maggs, D. G., Jones, T., Jacob, R., Sridhari, V., Thompson, J., Kerr, D., Leone, P., Krystal, J. H., Spencer, D. D., During, M. J., and Sherwin, R. S. (2002) Striking differences in glucose and lactate levels between brain extracellular fluid and plasma in conscious human subjects: effects of hyperglycemia and hypoglycemia. J. Cereb. Blood Flow Metab. 22, 271–279
13. Rice, A. C., Zsildos, R., Chen, T., Wilson, M. S., Alessandri, B., Hamm, R. J., and Bullock, M. R. (2002) Lactate administration attenuates cognitive deficits following traumatic brain injury. Brain Res. 928, 156–159
14. Holloway, R., Zhou, Z., Harvey, H. B., Levasseur, J. E., Rice, A. C., Sun, D., Hamm, R. J., and Bullock, M. R. (2007) Effect of lactate therapy upon cognitive deficits after traumatic brain injury in the rat. Acta Neurochir. 149, 919–927; discussion 927
15. Makoroff, K. L., Cecil, K. M., Care, M., and Ball, W. S., Jr. (2005) Elevated lactate as an early marker of brain injury in inflicted traumatic brain injury. Pediatr. Radiol. 35, 668–676
16. Timofeev, I., Carpenter, K. L., Nortje, J., Al-Rawi, P. G., O’Connell, M. T., Czosnyka, M., Smielewski, P., Pickard, J. D., Menon, D. K., Kirkpatrick, P. J., Gupta, A. K., and Hutchinson, P. J. (2011) Cerebral extracellular chemistry and outcome following traumatic brain injury: a microdialysis study of 223 patients. Brain 134, 484–494
17. Sanchez, I. J., Bidot, C. J., O’Phelan, K., Gajavelli, S., Yokobori, S., Olevy, S., Jagid, J., Garcia, J. A., Nemeth, Z., and Bullock, R. (2013) Neurmonitoring with microdialysis in severe traumatic brain injury patients. Acta Neurochir. Suppl. 118, 223–227
18. Tascopoulos, M., and Magistretti, P. J. (1996) Metabolic coupling between glia and neurons. J. Neurosci. 16, 877–885
19. Magistretti, P. J. (2009) Role of glutamate in neuron-glia metabolic coupling. Am. J. Clin. Nutr. 87, 875S–880S
20. Pellerin, L., and Magistretti, P. J. (1994) Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. Proc. Natl. Acad. Sci. U.S.A. 91, 10625–10629
21. Pellerin, L., Pellegrin, G., Bitar, P. G., Charnay, Y., Bouras, C., Martin, J. L., Stella, N., and Magistretti, P. J. (1998) Evidence supporting the existence of an activity-dependent astrocyte-neuron lactate shuttle. Dev. Neurosci. 20, 291–299
22. Beckmann, N., Turkalj, I., Seelig, J., and Keller, U. (1991) 13C NMR for the assessment of human brain glucose metabolism in vivo. Biochemistry 30, 6362–6366
23. Gruetter, R. (2002) In vivo 13C NMR studies of compartmentalized cerebral carbohydrate metabolism. Neurochem. Int. 41, 143–154
24. Marmarou, A., Foda, M. A., van den Brink, W., Campbell, J., Kita, H., and Demetriadou, K. (1994) A new model of diffuse brain injury in rats: Part I. Pathophysiology and biomechanics. J. Neurosurg. 80, 291–300
25. Foda, M. A., and Marmarou, A. (1994) A new model of diffuse brain injury in rats: Part II: Morphological characterization. J. Neurosurg. 80, 301–313
26. Pontén, U., Ratcheson, R. A., Salford, L. G., and Siesjö, B. K. (1973) Optimal freezing conditions for cerebral metabolites in rats. J. Neurochem. 21, 1127–1138
27. Tyson, R. L., Perron, J., and Sutherland, G. R. (2000) 6-Aminonicotinamide inhibition of the pentose phosphate pathway in rat neocortex. Neuroreport 11, 1845–1848
28. Peeling, J., and Sutherland, G. (1992) High-resolution 1H NMR spectroscopy studies of extracts of human cerebral neoplasms. Magn. Reson. Med. 24, 123–136
29. Kaibara, T., Sutherland, G. R., Colbourne, F., and Tyson, R. L. (1999) Hypothermia: depression of tricarboxylic acid cycle flux and evidence for pentose phosphate shunt upregulation. J. Neurosurg. 90, 339–347
30. Kraig, R. P., Petitto, C. K., Plum, F., and Pulsinelli, W. A. (1987) Hydrogen ions kill brain at concentrations reached in ischemia. J. Cereb. Blood Flow Metab. 7, 379–386
31. Kishi, K., Warishi, H., Marquez, L., Dunford, H. B., and Gold, M. H. (1994) Mechanism of manganese peroxidase compound II reduction: effect of organic acid chelators and pH. Biochemistry 33, 8694–8701
32. Péront, F., and Aguiani, B. (2006) Lactic acid buffering, nonmetabolic CO2 and exercise hyperventilation: a critical reappraisal. Respir. Physiol. Neurobiol. 150, 4–18
33. Hassel, B., Paulsen, R. E., Johnsen, A., and Fonnun, F. (1992) Selective
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inhibition of glial cell metabolism in vivo by fluorocitrate. *Brain Res.* **576**, 120–124

Hassel, B., Bachelard, H., Jones, P., Fonnun, F., and Sonnewald, U. (1997) Trafficking of amino acids between neurons and glia in vivo. Effects of inhibition of glial metabolism by fluorocacetate. *J. Cereb. Blood Flow Metab.* **17**, 1230–1238

Shlosberg, D., Benifa, M., Kaufer, D., and Friedman, A. (2010) Blood-brain barrier breakdown as a therapeutic target in traumatic brain injury. *Nat. Rev. Neurol.* **6**, 393–403

Chodobski, A., and Zink, B. I., Szymynder-Chodobska, J. (2011) Blood-brain barrier pathophysiology in traumatic brain injury. *Transl. Stroke Res.* **2**, 492–516

Reinert, M., Hoelper, B., Doppenberg, E., Zauner, A., and Bullock, R. (2000) Substrate delivery and ionic balance disturbance after severe traumatic brain injury. *J. Neurotrauma* **17**, 15–35

Brines, M. L., Ghezzi, P., Keenan, S., Agnello, D., de Lanerolle, N. C., Cerami, C., Itri, L. M., and Cerami, A. (2000) Erythropoietin crosses the blood-brain barrier to protect against experimental brain injury. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 10526–10531

Magistretti, P. J., Pellerin, L., Rothman, D. L., and Shulman, R. G. (1999) Energy on demand. *Science* **283**, 496–497

Reinert, M., Hoelper, B., Doppenberg, E., Zauner, A., and Bullock, R. (2000) Substrate delivery and ionic balance disturbance after severe human head injury. *Acta Neurochir. Suppl.* **76**, 439–444

Clausen, T., Kahlid, A., Zauner, A., Reinert, M., Doppenberg, E., Menzel, M., Soukup, J., Alves, O. L., and Bullock, M. R. (2005) Cerebral acid-base homeostasis after severe traumatic brain injury. *J. Neurosurg.* **103**, 597–607

Valadka, A. B., Goodman, J. C., Gopinath, S. P., Uzura, M., and Robertson, C. S. (1998) Comparison of brain tissue oxygen tension to microdialysis-based measures of cerebral ischemia in fatally head-injured humans. *J. Neurotrauma* **15**, 509–519

Zauner, A., Doppenberg, E. M., Woodward, J. J., Choi, S. C., Young, H. F., and Bullock, R. (1997) Continuous monitoring of cerebral substrate delivery and clearance: initial experience in 24 patients with severe acute brain injuries. *Neurosurgery* **41**, 1082–1091; discussion 1091–1093

Vogel, R., Hamprecht, B., and Wiesinger, H. (1998) Malic enzyme isoforms in astrocytes: comparative study on activities in rat brain tissue and astroglia-rich primary cultures. *Neurosci. Lett.* **247**, 123–126

McKenna, M. C., Tildon, J. T., Stevenson, J. H., Huang, X., and Kingwell, K. G. (1995) Regulation of mitochondrial and cytosolic malic enzymes from cultured rat brain astrocytes. *Neurochem. Res.* **20**, 1491–1501

Alves, P. M., McKenna, M. C., and Sonnewald, U. (1995) Lactate metabolism in mouse brain astrocytes studied by [13C]NMR spectroscopy. *Neuroreport* **6**, 2201–2204

Choi, H. B., Gordon, G. R., Zhou, N., Tai, C., Runenga, R. L., and Martinez, J. (2012) Metabolic communication between astrocytes and neurons via bicarbonate-responsive soluble adenyl cyclase. *Neuron* **75**, 1094–1104

Levine, B., Kovacevic, N., Nica, E. I., Cheung, G., Gao, F., Schwartz, M. L., and Black, S. E. (2008) The Toronto traumatic brain injury study: injury severity and quantified MRI. *Neurology* **70**, 771–778

DeKosky, S. T., Blennow, K., Ikonomovic, M. D., and Gandy, S. (2013) Acute and chronic traumatic encephalopathies: pathogenesis and biomarkers. *Nat. Rev. Neurol.* **9**, 192–200

Rodriguez-Rodriguez, A., Egea-Guerrero, J. I., Murillo-Cabezas, F., and Carrillo-Vico, A. (2014) Oxidative stress in traumatic brain injury. *Curr. Med. Chem.* **21**, 1201–1211

Opip, W. O., Nukala, V. N., Sultana, R., Pandya, J. D., Day, K. M., Merchant, M. L., Klein, I. B., Sullivan, P. G., and Butterfield, D. A. (2007) Proteomic identification of oxidized mitochondrial proteins following experimental traumatic brain injury. *J. Neurotrauma* **24**, 772–789

Cornelius, C., Crupi, R., Calabrese, V., Graziano, A., Milone, P., and Pennisi, G. (2013) Traumatic brain injury: oxidative stress and neuroprotection. *Antioxid. Redox Signal.* **19**, 836–853

Melov, S., Adlard, P. A., Morten, K., Johnson, F., Golden, T. R., Hinerfeld, D., Schilling, B., Mavros, C., Masters, C. L., Volitakis, I., Li, Q. X., Laughton, K., Hubbard, A., Cherny, R. A., Gibson, B., and Bush, A. I. (2007) Mitochondrial oxidative stress causes hyperphosphorylation of tau. *PLoS One* **2**, e536

Mondragon-Rodriguez, S., Perry, G., Zha, X., Moreira, P. I., Acevedo-Aquino, M. C., and Williams, S. (2013) Phosphorylation of tau protein as the link between oxidative stress, mitochondrial dysfunction, and connectivity failure: implications for Alzheimer’s disease. *Oxid. Med. Cell Longev.* **2013**, 94063

Sara, N., Suy, S., Zeralth, J. B., Bouzet, P., Messerer, M., Bloch, J., Levier, M., Magistretti, P. J., Meuli, R., and Oddo, M. (2013) Cerebral extracellular lactate increase is predominantly nonischemic in patients with severe traumatic brain injury. *J. Cereb. Blood Flow Metab.* **33**, 1815–1822

Vespa, P., Bergsneider, M., Hattori, N., Wu, H. M., Huang, S. C., and Martin, N. A. (2005) Metabolic crisis without brain ischemia is common after traumatic brain injury: a combined microdialysis and positron emission tomography study. *J. Cereb. Blood Flow Metab.* **25**, 763–774