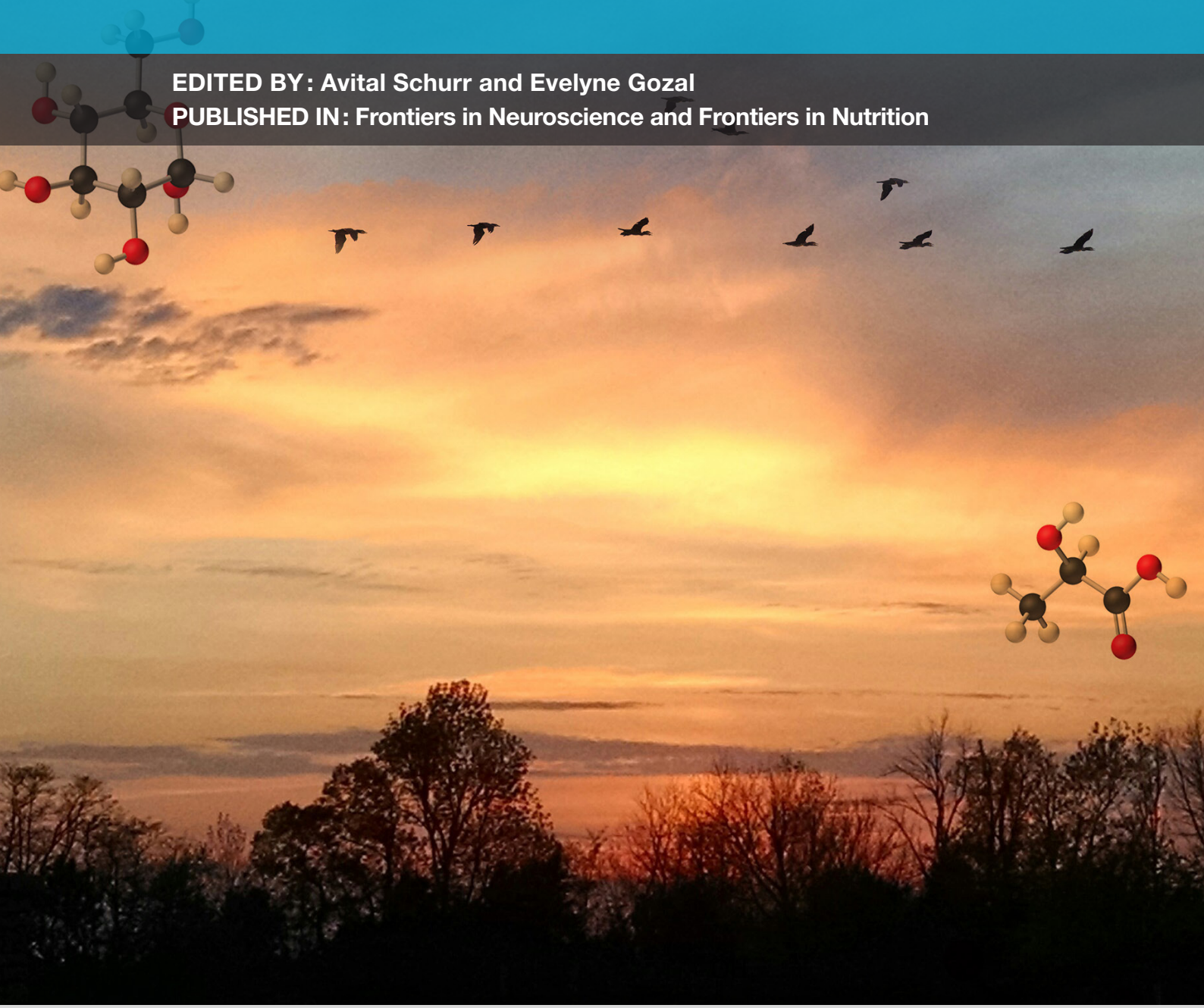
GLYCOLYSIS AT 75: IS IT TIME TO TWEAK THE FIRST ELUCIDATED METABOLIC PATHWAY IN HISTORY?

EDITED BY: Avital Schurr and Evelyne Gozal
PUBLISHED IN: Frontiers in Neuroscience and Frontiers in Nutrition



frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88919-586-2
DOI 10.3389/978-2-88919-586-2

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

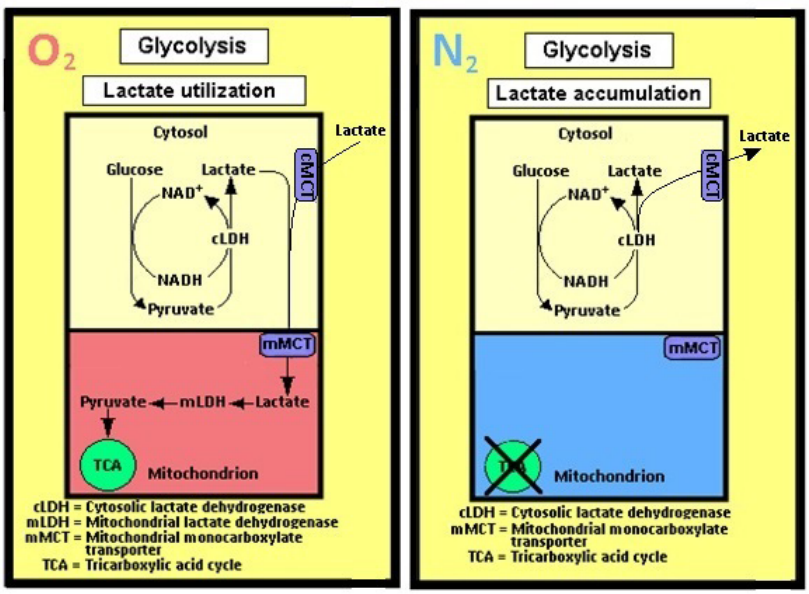
Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view.
By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: **researchtopics@frontiersin.org**

GLYCOLYSIS AT 75: IS IT TIME TO TWEAK THE FIRST ELUCIDATED METABOLIC PATHWAY IN HISTORY?

Topic Editors:
Avital Schurr, University of Louisville School of Medicine, USA
Evelyne Gozal, University of Louisville School of Medicine, USA



Cover image: A sunset with geese flying, a glucose molecule (the starting point of glycolysis) at the left top of the image and a lactic acid molecule (the end-product of glycolysis) at the lower right of the image. The geese symbolize the continuous energy requirement of hard working wing and chest muscles and the high output of their glycolytic system. Image by Avital Schurr

Glycolysis, the pathway of enzymatic reactions responsible for the breakdown of glucose into two trioses and further into pyruvate or lactate, was elucidated in 1940. For more than seven decades, it has been taught precisely the way its sequence was proposed by Embden, Meyerhof and Parnas. Accordingly, two outcomes of this pathway were proposed, an aerobic glycolysis, with pyruvate as its final product, and an anaerobic glycolysis, identical to the aerobic one, except for an additional reaction, where pyruvate is reduced to lactate. Several studies in the 1980s have shown that both muscle and brain tissues can oxidize and utilize lactate as an energy substrate, challenging this monocarboxylate's reputation as a useless end-product of anaerobic glycolysis. These findings were met with great skepticism about the idea that lactate could be playing a role in bioenergetics. In the past quarter of a century

monocarboxylate transporters (MCTs) were identified and localized in both cellular and mitochondrial membranes. A lactate receptor has been identified. Direct and indirect evidence now indicate that the enzyme lactate dehydrogenase (LDH) resides not only in the cytosol, as part of the glycolytic pathway machinery, but also in the mitochondrial outer membrane. The mitochondrial form of the enzyme oxidizes lactate to pyruvate and concomitantly produces the reducing agent NADH. These findings have shed light on a major drawback of the originally proposed aerobic version of the glycolytic pathway i.e., its inability to regenerate NAD+, as opposed to anaerobic glycolysis that features the cyclical ability of regenerating NAD+ upon pyruvate reduction to lactate by the cytosolic form of LDH. The malate-aspartate shuttle (MAS), a major redox shuttle in the brain, was proposed as an alternative pathway for NAD+ generation for aerobic glycolysis. Nonetheless, would MAS really be necessary for that function if glycolysis always proceeds to the end-products, lactate and NAD+? An additional dilemma the originally proposed aerobic glycolysis presents has to do with the glycolytic pathway of erythrocytes, which despite its highly aerobic environment, always produces lactate as its end-product. It is time to reexamine the original, dogmatic separation of glycolysis into two distinct pathways and put to test the hypothesis of a unified, singular.pathway, the end-product of which is lactate, the real substrate of the mitochondrial TCA cycle.

Citation: Schurr, A., Gozal, E., eds. (2015). Glycolysis at 75: Is it Time to Tweak the First Elucidated Metabolic Pathway in History? Lausanne: Frontiers Media. doi: 10.3389/978-2-88919-586-2

Table of Contents

frontiers in Neuroscience

EDITORIAL
published: 15 May 2015
doi: 10.3389/fnins.2015.00170

Glycolysis at 75: is it time to tweak the first elucidated metabolic pathway in history?

Avital Schurr[1]* *and Evelyne Gozal*[2,3]

[1] Department of Anesthesiology and Perioperative Medicine, School of Medicine, University of Louisville, Louisville, KY, USA, [2] Department of Pediatrics, School of Medicine, University of Louisville, Louisville, KY, USA, [3] Department of Pharmacology and Toxicology, School of Medicine, University of Louisville, Louisville, KY, USA

Keywords: energy metabolism, glycolysis, lactate, lactate dehydrogenase, cancer, traumatic brain injury, pyruvate, monocarboxylate transporters

Edited by:
Juan P. Bolanos, University of Salamanca - Consejo Superior de Investigaciones Científicas, Spain

Reviewed by:
Anne-Karine Bouzier-Sore, Université Bordeaux Segalen-Centre National de la Recherche Scientifique, France

***Correspondence:**
Avital Schurr, avital.schurr@gmail.com

Specialty section:
This article was submitted to Neuroenergetics, Nutrition and Brain Health, a section of the journal Frontiers in Neuroscience

Received: *30 March 2015*
Accepted: *27 April 2015*
Published: *15 May 2015*

Citation:
Schurr A and Gozal E (2015) Glycolysis at 75: is it time to tweak the first elucidated metabolic pathway in history? Front. Neurosci. 9:170. doi: 10.3389/fnins.2015.00170

The sequence of the glycolytic enzymatic reactions responsible for the breakdown of glucose into two trioses and further into pyruvate or lactate was elucidated in 1940. For over seven decades it has been taught precisely the way that sequence was first described by Embden, Meyerhof and Parnas. Accordingly, two different glycolytic outcomes were proposed; the aerobic one with pyruvate as the final product, and the anaerobic one, identical to aerobic glycolysis except for one additional step, the reduction of pyruvate to lactate. While pyruvate has been presented as the mitochondrial substrate of the tricarboxylic acid cycle, lactate has regularly been vilified as a useless and frequently toxic end product of anaerobic glycolysis. Several studies in the 1980s have shown that both muscle and brain tissues are capable of oxidizing and utilizing lactate as an energy substrate, thus challenging the monocarboxylate's ill reputation. Expectedly, these findings were met with great skepticism and doubts that lactate could play any role in bioenergetics. Despite many studies from the 1920s and 1930s that have clearly showed the ability of brain tissue to oxidize lactate, even the investigators who published them assumed that lactate oxidation was simply a clearing mechanism of this end-product of anaerobic glycolysis (Schurr, 2006, 2014). Nevertheless, in the past two decades monocarboxylate transporters (MCTs) were identified and localized in both cellular and mitochondrial membranes and the existence of a lactate receptor in the brain has also been suggested (Bergersen and Gjedde, 2012). Both direct and indirect evidence now indicate that the enzyme lactate dehydrogenase (LDH) resides not only in the cytosol, as part of the glycolytic pathway machinery, but also in the mitochondrial outer membrane. The mitochondrial form of the enzyme oxidizes lactate to pyruvate and concomitantly produces the reducing agent NADH (Passarella et al., 2014; Rogatzki et al., 2015). These findings have shed light on a major drawback of the originally proposed aerobic version of the glycolytic pathway i.e., its inability to regenerate NAD^+, in contrast to the anaerobic glycolysis that features a cyclical ability to regenerate NAD^+upon pyruvate reduction to lactate by the cytosolic LDH. The malate-aspartate shuttle (MAS), a major redox shuttle in the brain was proposed as an alternative pathway for NAD^+ generation for aerobic glycolysis. However, the necessity for MAS to fulfill such function could be questioned if the glycolytic pathway always proceeded to its end-products, lactate and NAD^+, or when the suggested alternative, a lactate-malate-aspartate shuttle (Kane, 2014) is considered. An additional dilemma the originally proposed aerobic glycolysis presents, has to do with the glycolytic pathway of erythrocytes, which despite its highly aerobic environment always produces lactate as its end-product. Meanwhile, several other functions have been suggested for lactate (Chambers et al., 2014; Galow et al., 2014; Hertz et al., 2014; Brooks and Martin, 2015; Goodwin et al., 2015). For decades, pyruvate has always been the substrate of choice when measuring the rate of mitochondrial State III respiration employing an oxygen electrode.However, only recently it has been shown that lactate can be used as a substrate, too (de Bari et al., 2008; Passarella et al., 2008, 2014).

Additional experiments could be designed to provide further insight into the role of lactate in energy metabolism. For example, would the addition of mitochondria to ruptured red blood cells switch their glycolytic pathway to produce pyruvate instead of lactate?

This Research Topic, now published as an E-book, includes 14 contributions from investigators conducting research in this field who review, opine, comment, or publish original data. In their perspective, Mariga et al. (2014) elaborate on the role of lactate in cerebral malaria (CM). Based on the data available, the authors suggest that monocarboxylate transporters (MCTs) and the lactate receptor GPR81 could become novel therapeutic targets in CM. Hertz et al. (2014), in their article, review the different roles lactate plays in brain tissue, including the modulation of glucose utilization rate, diagnosis of brain-injured patients and mediation of redox and receptor signaling, memory, and gene transcription. While these authors are listing multiple roles for lactate, mainly involving gap junction-coupled astrocytes, lactate's direct role as an oxidative energy substrate is not on the list. In his contribution, Schurr (2014) has reviewed a great number of papers on muscle and brain energy metabolism that werepublished in the waning years of the 19th century and the first four decades or so of the 20th century, providing a historical perspective to narrate the evolution of thinking and research in this field. These studies were crucial in the formation of the dogma according to which lactate, the end-product of anaerobic glycolysis, is useless and at times, poisonous. Using Margolis's (1993) concept that "habits of mind" govern scientific beliefs, Schurr argues that habits of mind still influence many scientists' beliefs today pertaining to lactate uselessness and the dissociation of glycolysis into aerobic and anaerobic pathways. These "habits of mind" persist, despite data already available in the 1920s and 1930s demonstrating that the brain oxidizes lactate very efficiently. The contribution of glycogen in supporting axon conduction both in peripheral and central nervous systems, and the role lactate plays in it, is reviewed by Chambers et al. (2014). The authors suggest that the presence of significant steady concentration of lactate in the periphery of both central white matter and peripheral nerve under unstimulated baseline conditions indicates a continuous efflux of lactate to the interstitium. They argue for the reexamination of the "on demand" shuttling of lactate between cellular elements based on the existence of those lactate pools, and thus, offer a continuous lactate efflux surplus available for immediate neural requirements. In a perspective on lactate oxidation at the mitochondria, Kane (2014) put forward the idea that shuttled lactate from the cytosol to the mitochondrion operates in a manner very similar to the malate-aspartate shuttle, the purpose of which has been proposed to be the oxidation of lactate and a mitochondrial electron shuttle. Moreover, he proposes that the two shuttles, the lactate one and the malate-aspartate one, are necessarily interconnected. Passarella et al. (2014) opine on the continuous debate among scientists as to whether or not L-lactatedehydrogenase (L-LDH) is localized in mitochondria. The debate goes on despite the overwhelming evidence supporting the localization of mitochondrial L-lactatedehydrogenase (m-L-LDH) inside these organelles. The authors argue that m-L-LDH can be detected in the mitochondrion when isolation and purification procedures are carried out carefully such that mitochondria stay coupled. Several measurements and assays can be easily and persuasively be performed to ascertain the metabolism of lactate in mitochondria. Goodwin et al. (2015), in an opinion article, present a concise overview of some recent developments in the field of lactate metabolism and cancer. Summarizing their overview, the authors conclude that lactate is both a potent fuel oxidatively and a signaling molecule involved in angiogenesis, and that it can be generated and exported or imported by tumors. They propose that lactate-protected hypoglycemia may be a viable strategy in tumors that exhibit high lactate production despite adequate tissue oxygen tension, while monocarboxylate transporter inhibitors could be useful against tumors whose angiogenesis is driven by lactate. In another opinion article, Deitmer et al. (2015) provide a perspective on the role of lactate as a signaling molecule and a modulator of metabolic processes due to its cotransport with H^+ via monocarboxylate transporters (MCTs). Accordingly, lactate transporters form a "transport metabolon" with carbonic anhydrases. Allaman et al. (2015) review the existing knowledge on the cerebral glyoxalase system in both astrocytes and neurons. These two cerebral cell types with their high glycolytic activity presumably produce the highly reactive dicarbonyl compound, methylglyoxal, as a by-product of glycolysis. Methylglyoxal is associated with pathologies such as diabetes and aging, although its neurotoxicity in the brain is not well characterized. The authors advance a concept attributing astrocytes and neurons differential adaptive glyoxalase defense mechanisms against methylglyoxal-induced cellular damage. Brooks and Martin (2015) review theirs and others' studies on the post traumatic brain injury (TBI) treatment. In their studies, the authors compared dextrose + insulin treatment to exogenous lactate infusion in TBI patients with intact hepatic and renal functions, demonstrating that the latter results in normal glycemia. Carpenter et al. (2015) also review the role of lactate in TBI and the possible beneficial effect of intravenous exogenous lactate supplementation to TBI patients. Zilberter et al. (2015) opine in their paper on the potential of pyruvate, the other glycolytic monocarboxylate, as the treatment for signature characteristics of many neurological diseases i.e., hypometabolism, oxidative stress, and neuroinflamation. The authors believe that pyruvate is an ideal candidate for the treatment of these pathologies due to its unique combination of neuroprotective properties.

In light of the wealth of information already available, as can be gleaned from the present compilation of papers about lactate and its central role in energy metabolism in most, if not in all, tissues and specifically in the brain, it is clearly the time to reexamine theoriginal dogma of glycolysis.The separation of glycolysis into two distinct pathways, aerobic and anaerobic, is outdated and misleading. The first metabolic pathway to be elucidated 75 years ago should be redrawn as a singular pathway, its substrate being glucose and its end-product being lactate, the ultimate substrate of the mitochondrial TCA cycle.

References

Allaman, I., Bélanger, M., and Magistretti, P. J. (2015). Methylglyoxal, the dark side of glycolysis. *Front. Neurosci*. 9:23. doi: 10.3389/fnins.2015.00023

Bergersen, L. H., and Gjedde, A. (2012). Is lactate a volume transmitter of metabolic states of the brain? *Front. Neuroenergetics* 4:5. doi: 10.3389/fnene.2012.00005

Brooks, G. A., and Martin, N. A. (2015). Cerebral metabolism following traumatic brain injury: new discoveries with implications for treatment. *Front. Neurosci*. 8:408. doi: 10.3389/fnins.2014.00408

Carpenter, K. L., Jalloh, I., and Hutchinson, P. J. (2015). Glycolysis and the significance of lactate in traumatic brain injury. *Front. Neurosci.* 9:112. doi: 10.3389/fnins.2015.00112

Chambers, T. W., Daly, T. P., Hockley, A., and Brown, A. M. (2014). Contribution of glycogen in supporting axon conduction in the peripheral and central nervous systems: the role of lactate. *Front. Neurosci*. 8:378. doi: 10.3389/fnins.2014.00378

de Bari, L., Chieppa, G., Paventi, G., Pizzuto, R., Valenti, D., Atlante, A., et al. (2008). "The lactate-mitochondria affair," in *Proceedings of Bari International Symposium on "Mitochondrial Physiology and Pathology" IUBMB S1/2008,* Bari.

Deitmer, J. W., Theparambil, S. M., Ruminot, I., and Becker, H. M. (2015). The role of membrane acid/base transporters and carbonic anhydrases for cellular pH and metabolic processes. *Front. Neurosci*. 8:430. doi: 10.3389/fnins.2014.00430

Galow, L. V., Schneider, J., Lewen, A., Ta, T.-T., Papageorgiou, I. E., and Kann, O. (2014). Energy substrates that fuel fast neuronal network oscillations. *Front. Neurosci*. 8:398. doi: 10.3389/fnins.2014.00398

Goodwin, M. L., Gladden, L. B., Nijsten, M. W. N., and Jones, K. B. (2015). Lactate and cancer: revisiting the Warburg effect in an era of lactate shuttling. *Front. Nutr*. 1:27. doi: 10.3389/fnut.2014.00027

Hertz, L., Gibbs, M. E., and Dienel, G. A. (2014). Fluxes of lactate into, from, and among gap junction-coupled astrocytes and their interaction with noradrenaline. *Front. Neurosci*. 8:261. doi: 10.3389/fnins.2014.00261

Kane, D. A. (2014). Lactate oxidation at the mitochondria: a lactate-malate-aspartate shuttle at work. *Front. Neurosci*. 8:366. doi: 10.3389/fnins.2014.00366

Margolis, H. (1993). *Paradigms and Barriers: How Habits of Mind Govern Scientific Beliefs*. London: The University of Chicago Press, Ltd.

Mariga, S. T., Kolko, M., Gjedde, A., and Bergersen, L. H. (2014). Lactate transport and receptor actions in cerebral malaria. *Front. Neurosci*. 8:125. doi: 10.3389/fnins.2014.00125

Passarella, S., de Bari, L., Valenti, D., Pizzuto, R., Paventi, G., and Atlante, A. (2008). Mitochondria and L-lactate metabolism. *FEBS Lett*. 582, 3569–3576. doi: 10.1016/j.febslet.2008.09.042

Passarella, S., Paventi, G., and Pizzuto, R. (2014). The mitochondrial L-lactate dehydrogenase affair. *Front. Neurosci*. 8:407. doi: 10.3389/fnins.2014.00407

Rogatzki, M. J., Ferguson, B. S., Goodwin, M. L., and Gladden, L. B. (2015). Lactate is always the end product of glycolysis. *Front. Neurosci*. 9:22. doi: 10.3389/fnins.2015.00022

Schurr, A. (2014). Cerebral glycolysis: a century of persistent misunderstanding and misconception. *Front. Neurosci*. 8:360. doi: 10.3389/fnins.2014.00360

Schurr, A. (2006). Lactate: the ultimate cerebral oxidative energy substrate? *J. Cereb. Blood Flow Metab*. 26, 142–152. doi: 10.1038/sj.jcbfm.9600174

Zilberter, Y., Gubkina, O., and Ivanov, A. I. (2015). A unique array of neuroprotective effects of pyruvate in neuropathology. *Front. Neurosci*. 9:17. doi: 10.3389/fnins.2015.00017

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in
NEUROSCIENCE

PERSPECTIVE ARTICLE
published: 19 May 2014
doi: 10.3389/fnins.2014.00125

Lactate transport and receptor actions in cerebral malaria

Shelton T. Mariga[1], Miriam Kolko[1,2], Albert Gjedde[1] and Linda H. Bergersen[1,3]*

[1] Department of Neuroscience and Pharmacology, University of Copenhagen, Copenhagen, Denmark
[2] Department of Ophthalmology, Roskilde Hospital, Roskilde, Denmark
[3] The Brain and Muscle Energy Group and SN-Lab, Department of Anatomy and Department of Oral Biology, Institute of Basic Medical Sciences and Centre for Molecular Biology and Neuroscience/SERTA Healthy Brain Aging Centre, University of Oslo, Oslo, Norway

Edited by:
Avital Schurr, University of Louisville, USA

Reviewed by:
Oliver Kann, University of Heidelberg, Germany
Juan P. Bolanos, University of Salamanca, Spain
Leif Hertz, China Medical University, China

***Correspondence:**
Linda H. Bergersen, The Brain and Muscle Energy Group and SN-Lab, Department of Anatomy and Department of Oral Biology, Institute of Basic Medical Sciences and Centre for Molecular Biology and Neuroscience/SERTA Healthy Brain Aging Centre, University of Oslo, N-0317 Oslo, Norway
e-mail: l.h.bergersen@medisin.uio.no

Cerebral malaria (CM), caused by *Plasmodium falciparum* infection, is a prevalent neurological disorder in the tropics. Most of the patients are children, typically with intractable seizures and high mortality. Current treatment is unsatisfactory. Understanding the pathogenesis of CM is required in order to identify therapeutic targets. Here, we argue that cerebral energy metabolic defects are probable etiological factors in CM pathogenesis, because malaria parasites consume large amounts of glucose metabolized mostly to lactate. Monocarboxylate transporters (MCTs) mediate facilitated transfer, which serves to equalize lactate concentrations across cell membranes in the direction of the concentration gradient. The equalizing action of MCTs is the basis for lactate's role as a volume transmitter of metabolic signals in the brain. Lactate binds to the lactate receptor GPR81, recently discovered on brain cells and cerebral blood vessels, causing inhibition of adenylyl cyclase. High levels of lactate delivered by the parasite at the vascular endothelium may damage the blood–brain barrier, disrupt lactate homeostasis in the brain, and imply MCTs and the lactate receptor as novel therapeutic targets in CM.

Keywords: energy metabolism, cerebral malaria, lactate transport, lactate receptor, volume transmitter

CEREBRAL MALARIA

Malaria is caused by the protozoan parasite *Plasmodium*. About two million children die each year world-wide (Breman, 2001), mainly due to severe complications, especially from cerebral malaria (CM), of *Plasmodium falciparum* infection, the majority in sub-Saharan Africa, but also in Southeast Asia and South America. In developed countries, CM affects mainly returning travelers. Brain edema, lactate accumulation, and intracranial hypertension are important characteristics of CM, and untreated CM often leads to coma and death within 24 h (Newton and Krishna, 1998). CM survivors sometimes suffer from general disorders such as acidosis, as well as more specific neurological disorders such as ataxia, epilepsy, and blindness. However, the pathogenesis of CM remains unclear. Despite intense research, no effective vaccine is yet available, and the problem of drug-resistant malaria is increasing (Mariga et al., 2004).

CM FROM CIRCULATING ASEXUAL *Plasmodium falciparum*

The *Plasmodium* parasite invades erythrocytes as part of its asexual life cycle within the human host. It matures within these blood cells (*Plasmodium*-invaded red blood cells, PRBC) to the trophozoite and schizont stages. It then disappears from the peripheral circulation and is sequestrated in the vascular beds of critical organs such as the brain (Kristensson et al., 2013). Progression to CM is closely linked to erythrocytes containing mature stages of *P. falciparum* confined in the brain microvasculature (Dorovini-Zis et al., 2011) and host immunocompentent cells targeting the malaria parasite (Idro et al., 2005). Mechanisms by wish an intraerythrocytic parasite lodged in the vascular space of the brain can elicit such dramatic neurological effects necessarily involve local and systemic metabolic intermediates, as well as the transport systems for water and metabolites that link the membranes of the PRBC to the blood–brain barrier (BBB) and the end-feet of astrocytes.

DYNAMICS OF BLOOD AND BRAIN LACTATE IN CM

Several factors contribute to the accumulation of lactate in brain tissue in CM. The lack of a functional citric acid (TCA) cycle in human and murine *Plasmodium* parasites makes them largely reliant on glycolysis to fulfill their very substantial energy requirements (Sherman, 1998). Erythrocytes infected with mature parasites at the trophozoite stage consume glucose two orders of magnitude faster than normal, uninfected erythrocytes (Scheibel et al., 1979; Roth et al., 1982) with commensurate generation of lactic acid (Pfaller et al., 1982) i.e., at 5–100 times the rates of uninfected erythrocytes (Zolg et al., 1984). The resulting quantities of lactate leave the cells as directed by the concentration gradient. At rest, lactate in brain also is transported from brain tissue to plasma, but given the high rate of release of lactate from *Plasmodium* infected erythrocytes sequestered on the endothelial cells, lactate generated by parasites moves from the plasma to brain tissue down a concentration gradient.

CEREBRAL LACTATE CONTENT AND TRANSPORT

The quantities of lactate produced by the parasites compete with the lactate generated in the host's own brain metabolism for

transport to or from the brain tissue. A system of transporters of lactate is present at the parasite surface as H^{++}-coupled monocarboxylate transporters (MCTs) with features in common with members of the MCT family of higher eukaryotes (Kanaani and Ginsburg, 1991, 1992; Elliot et al., 2001). The MCT family members are well characterized in human brain (Bergersen et al., 1999, 2001; Pierre and Pellerin, 2005; Bergersen, 2007; Lauritzen et al., 2013a,b). In general, MCT1 is at the vascular endothelium, MCT4 in astrocytes, and MCT2 on neurons. Microglia expresses MCT1 and MCT2 after cerebral ischemia (Moreira et al., 2009). Both the MCTs and the lactate receptor GPR81 display affinities for L-lactate in the low mM range (Cai et al., 2008) that are consistent with the range of extracellular lactate concentrations measured by microdialysis in the brain tissue *in vivo* in human subjects (Abi-Saab et al., 2002; Jalloh et al., 2013).

LACTATE FLUX RECEPTOR ACTION AT THE BLOOD–BRAIN BARRIER

The BBB is constituted by the tight junctions of the endothelium of cerebral microvessels and capillaries. Interaction between lactate and lactate transport systems is likely to be perturbed at the BBB that is an important site of CM pathogenesis according to data from human autopsies, animal models, and *in vitro* cell cultures (Tripathi et al., 2007). At the BBB interfaces between the brain parenchyma and the parasite infected erythrocytes, any impairment of the integrity of endothelial cells of the cerebral microvasculature will lead to cerebral edema and lactic acidosis by the disruption of ion and solute homeostasis that follows increased membrane permeability. Normally, lactate enters the brain by first being transported through the endothelial cells and then diffusing extracellularly in the brain interstitial space and intracellularly after transport by the MCTs. Lactate downregulates cAMP by interacting with GRP81, which is exposed on the luminal as well as the abluminal membranes of the endothelial cells and to a lesser extent on the membranes of astrocytes (Lauritzen et al., 2013a,b).

CM ASSOCIATION WITH ELEVATED LACTATE

Association of CM with increased CSF (cerebral spine fluid) lactate is now well established. As a signaling molecule as well as a substrate for energy metabolism, brain lactate is an important diagnostic marker in clinical settings. Lactate concentrations can be used to monitor disease progress and during trials of drug effects in CM. Results from clinical settings and animal models provide convincing evidence of increased lactate concentrations both in extracellular fluid and CSF in CM. Several investigators confirmed lactate as the single best indicator of prognosis (White et al., 1985; Krishna et al., 1994) when measured in CSF of patients infected with *P. falciparum* (Tosti et al., 2007). In the patients, CSF concentrations correlated with the state of the disease (White et al., 1985), with death observed at levels higher than 6–9 mM lactate. Survivors always had less than 3.4 mM.

More than 80% of children from Malawi, Kenya, and Gambia with CM (Waller et al., 1991) with elevated CSF pressure revealed 40% higher lactate concentrations compared to normal (Newton et al., 1994; Potchen et al., 2012). The combination of elevated pressure and elevated lactate often is fatal (Newton et al., 1994, 1997), with most autopsies revealing high brain lactate concentrations (SenGupta and Naraqi, 1992; Walker et al., 1992). In adult Thai patients, CNS lactate in CSF at twice the normal value was associated with 100% mortality, suggestive of metabolic disturbances in the brain, indicative of a fundamental pathological process (Dorovini-Zis et al., 2011). Declining lactate during CM is associated with recovery, confirming that severe lactacidosis is a reliable indicator of brain damage, (Newton, 2005). Similar lactate profiles are observed in murine CM models, when brain lactate increases a few days post-infection (Sanni et al., 2001).

ASSOCIATION WITH MALARIA RETINOPATHY AND LACTATE

Malaria retinopathy occurs in approximately two-thirds of pediatric patients with CM (Beare et al., 2004, 2006). It is characterized by retinal whitening, vessel changes and retinal hemorrhages. Correlation between malaria retinopathy and the severity of clinical CM symptoms has consistently been shown, and examination of the retina by ophthalmoscopy is useful for diagnosing CM (Beare et al., 2011). Increased plasma lactate levels are associated with the severity of retinopathy and CM, suggesting that the microvascular obstruction observed in the retina reflects systemic and cerebral microvascular obstruction and focal ischemia (Maude et al., 2009a). The eye is an easily examined, and the retina is developmentally a part of the brain and its vasculature a continuation of the cerebral vasculature. Consequently there is increasing attention on retinal pathology in malaria, with the perspective of diagnosis as well as of understanding mechanisms in CM (Maude et al., 2009b; White et al., 2009; Beare et al., 2011). Yet, the cascade of reactions involving lactate and its relation to malaria retinopathy is not fully understood.

MOUSE MODELS OF CM

The present perspective focuses on the critical role of lactate in the etiology and development of CM, although CM likely has a multifactorial pathogenesis. Murine models are critical to the study of mechanisms of underlying symptoms observed in CM (Hunt and Grau, 2003; Rénia et al., 2010; Riley et al., 2010). In humans, the signs typically encountered with CM include lactacidosis, seizures, brain edema (Polder et al., 1992; Potchen et al., 2012), and coma, followed by death (Rest, 1982; Hunt and Grau, 2003; Medana and Turner, 2006). Lactate accumulation is associated with decreased brain perfusion and brain hypoxia in a mouse model (Promeneur et al., 2013). These observations indicate intravascular obstruction and reduced perfusion of brain microvessels in CM.

To resolve the mechanisms of these changes, it is necessary to determine the cellular expression, localization and potential redistribution of the brain MCTs (MCT1, MCT2, MCT4), as well as the lactate receptor (GPR81), to reveal the dynamics of lactate's role in CM. This is possible with the mouse models of CM, established by infection with the rodent malaria parasite *P. berghei* ANKA in susceptible strains, C57BL/6 (Rest, 1982) or CBA/J (Penet et al., 2005). *In vivo* studies of the latter

model demonstrated massive brain edema coupled to lactacidosis associated with compression of cerebral arteries, ultimately causing coma and death, but no attempt was made to study the brain lactate transport systems (Penet et al., 2005). Using the CM C57BL/6 mouse model, Promeneur and collaborators (Promeneur et al., 2013) found that the water channel aquaporine 4 (AQP4) was downregulated in CM. Also, knock-out C57BL/6 mice lacking AQP4 succumbed earlier to *P. berghei* ANKA infection and were more severely affected than wild type mice, indicating that the water channel is protective in CM. It is particularly important to repeat these experiments in GPR81 knock-out mice to determine whether volume transmission followed by remote action at the lactate receptor is an important factor in CM.

MCT AND GPR81 IN CM

In the brain and retina, MCTs, like AQP4, are highly expressed in ependymal cells and astrocytes with polarized distribution in end-feet membranes facing cerebral capillaries and pia mater (Bergersen, 2007). GPR81 has a similar distribution (Lauritzen et al., 2013a,b). MCTs have also been identified in retinal sections (Bergersen et al., 1999) and unpublished results reveal GPR81 staining in human retinal sections (Kolko and Bergersen, in preparation). In addition MCT1 and GPR81 are highly expressed in the endothelial cells. The strategic locations at the blood–brain and cerebrospinal fluid–brain interfaces indicate that MCTs and GPR81 are major factors in the regulation of lactate movement into and out of the brain. Such movements were suggested already by early *in vivo* work (Gjedde et al., 1975) and are now firmly established (van Hall et al., 2009). Lactate receptor actions have been demonstrated in brain, reducing cAMP (apparent IC_{50} 29 mM; Lauritzen et al., 2013a,b) and neuronal calcium spiking (apparent IC_{50} 4.2 mM; Bozzo et al., 2013) through GPR81 activation. In addition, lactate stimulates locus coeruleus neurons (apparent IC_{50} 0.7 mM; Tang et al., 2014), ascribed to a receptor other than GPR81. These observations and the volume transmitter role of lactate (Bergersen and Gjedde, 2012), whereby lactate diffuses through large volumes of brain tissue to produce distant receptor effects, therefore imply actions of lactate produced by malaria parasites as important factors in CM.

Epileptic seizures occur in more than 70% of CM patients on admission (Newton et al., 2000; Idro et al., 2010). Elevated lactate concentrations in the brain are highly correlated with the severity of repeated seizures commonly observed in CM. Similarly, seizures in temporal lobe epilepsy (TLE) with hippocampal sclerosis, known as mesial temporal lobe epilepsy (MTLE), are correlated with high lactate levels as observed by microdialysis in epileptic patients (During et al., 1994). Brain MCTs expressions and distributions might be influenced by multiple forms of brain pathology including MTLE, stroke, meningitis, neuromyelitis optica, and brain tumors (Bergersen, 2007; Lauritzen et al., 2011, 2012, 2013a,b). Observations in MTLE and other neuropathological disorders resemble in some respects, the CM-related clinical and pathological status (i.e., seizures, lactacidosis). However, the role of lactate and the receptor action and transport in CM and other disease states need to be clarified.

HYPOTHESIS OF A NEW FACTOR IN CM

Based on the extant evidence, we claim that the lactate transporters and the lactate receptor GPR81 together are an essential factor in the pathophysiology of CM. The most obvious scenario (**Figure 1**) under this hypothesis is that lactate, profusely released from *Plasmodium* infected erythrocytes sequestered at the capillary endothelium, excessively down-regulates cAMP via GPR81. High concentrations of lactate will reach receptors both at the luminal and abluminal surface of the endothelial cells, because of the presence of MCT1 (Lauritzen et al., 2011) as well as GPR81 (Lauritzen et al., 2013a,b) at both sides of the endothelium. Normally as well as in disease, cAMP is perhaps the most potent signaling molecule to stabilize the endothelial barrier, and also regulates inflammation response and vascular tone (Roberts and Dart, 2014; Schlegel and Waschke, 2014). It acts on three classes of proteins: protein kinase A (PKA), cyclic-nucleotide-gated ion channels, and Epacs

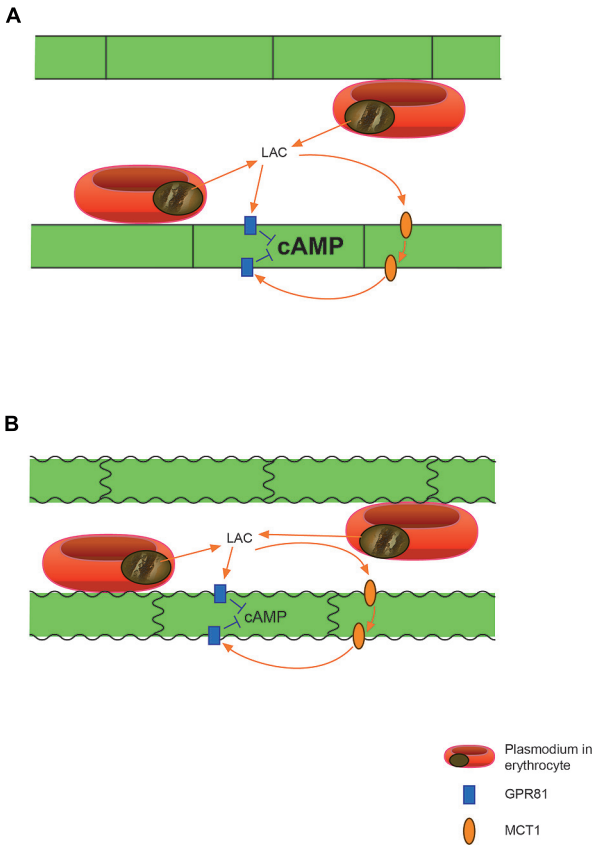


FIGURE 1 | Proposed action of extreme quantities of *Plasmodium* derived lactic acid on brain vascular endothelium. (A) Lactate penetrating the endothelial cells through MCT1 activates the lactate receptor GPR81, which is situated on both sides of the endothelium, to cause down-regulation of cAMP by inhibiting adenylyl cyclase activity. **(B)** Reduced levels of cAMP can lead to vasoconstriction and endothelial dysfunction with weakening of cell–cell junctions ultimately causing break-down of the blood-brain-barrier. Not shown: the GPR81 mediated effects may act in concert with pH effects of lactic acid and with other deleterious agents from the protozoon. Lactate penetrating the endothelium may also act on GPR81 expressed on neurons and to a lesser degree on astrocytic perivascular end-feet.

(exchange proteins directly activated by cAMP). The Epacs constitute a family of guanine-nucleotide-exchange factors for the small Ras-related GTPases Rap1 and Rap2. Both PKA and Rap1 can cause Rac1-mediated strengthening of adherens junctions and of the actin cytoskeleton (Schlegel and Waschke, 2014).

Extreme down-regulation of cAMP can therefore be expected to cause deterioration of the endothelium, including break-down of regulated trans-endothelial transport and of the junctions between endothelial cells, i.e., break-down of the BBB (**Figure 1**). In addition, increased vascular tone (Roberts and Dart, 2014) may aggravate the hypoperfusion caused by clogging of microvessels by sequestered erythrocytes. The effects via GPR81, and possibly other lactate receptors (cf. Tang et al., 2014), may converge with effects of acidification caused by proton from the parasite produced lactic acid, as well as with other deleterious compounds originating in the *Plasmodium* infected erythrocytes (Aird et al., 2014).

Within the brain parenchyma, excessive lactate concentrations delivered by the parasites must be expected to perturb the volume transmitter function of lactate (Bergersen and Gjedde, 2012), although the concentration of lactate will decrease with the distance from the parasites, which are confined to the vasculature. The vascular end-feet processes of astrocytes have relatively low GPR81 concentrations, while the synaptic membranes of excitatory synapses have the highest concentrations of GPR81 (Lauritzen et al., 2013a,b), but are at a greater distance from the parasites. The effects of excessive lactate in the parenchyma are harder to predict than those on the BBB, but they are expected to be exacerbated in CM, because lactate induced down-regulation of cAMP will upregulate MCT1 at the endothelial plasma membrane (Smith et al., 2012).

Inflammatory responses through the innate immune system may serve to confine the infection or cause serious tissue damage, the relative weight of these effects depending on genetic characteristics of the *Plasmodium* and of the host (Wu et al., 2014). Careful dissection of the mechanisms may reveal ways to augment antiparasite mechanisms but suppress tissue damage. A recent paper (Hoque et al., 2014) found that lactate by activating GPR81 reduced tissue injury in disease models. The fact that this effect was mediated down stream through arrestin beta-2 rather than cAMP predicts the possibility of separately modifying deleterious and beneficial effects of GPR81 activation.

Our hypothesis may be tested in mouse models of CM. The role of GPR81 can be ascertained by inducing CM in GPR81 knock-out mice. This will reveal whether the lack of the lactate receptor confers resistance to CM and/or ameliorates the pathology and outcome, in which case GPR81 antagonists would offer novel treatment for CM. GPR81 antagonists are not available at present, but may soon be. Further, MCT1 blockers, which are presently introduced in cancer therapy (Parks et al., 2013; Draoui et al., 2014) may slow the release of lactate from the infected erythrocytes and its transfer across the endothelium. If so, they would offer another novel therapy against CM. The existence of MCT blockers that selectively inhibit lactate influx but not efflux (Draoui et al., 2014) would allow the action to be restricted to the luminal side of the endothelium, avoiding possibly deleterious actions of the drugs on the brain parenchyma, which may also be avoided by using GPR81 and MCT antagonists that are hydrophilic and do not penetrate the BBB. The hypothesis can be further tested by analysis of MCT and GPR81 mRNAs and protein expressions and by immunohistochemistry by light and electron microscopy of the brain in CM mice and non-CM controls. It is particularly important to examine the hippocampal formation, in view of its central role in epileptogenesis and in view of the demonstrated roles of MCTs in human epilepsy and animal models of TLE (Lauritzen et al., 2013a,b). In addition, retinal sections must be examined to identify the expression pattern of MCTs and GPR81 in CM mice. The possible impact of antimalarial drugs on lactate transport and receptor activity should be tested.

CONCLUSION

Studies on lactate transport and receptor action in CM are expected to provide important new insights into the pathogenesis of CM, and to identify novel therapeutic targets.

ACKNOWLEDGMENTS

This study has been supported by grants from the University of Copenhangen and the Lundbeck Foundation, Denmark, and from the University of Oslo and the Research Council of Norway.

REFERENCES

Abi-Saab, W. M., Maggs, D. G., Jones, T., Jacob, R., Srihari, V., Thompson, J., et al. (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. doi: 10.1097/00004647-200203000-00004

Aird, W. C., Mosnier, L. O., and Fairhurst R. M. (2014). *Plasmodium falciparum* picks (on) EPCR. *Blood* 123, 163–167. doi: 10.1182/blood-2013-09-521005

Beare, N. A. V., Lewallen, S., Taylor, T. E., and Molyneux, M. E. (2011). Redefining cerebral malaria by including malaria retinopathy. *Future Microbiol.* 6, 349–355. doi: 10.2217/fmb.11.3

Beare, N. A. V., Southern, C., Chalira, C., and Taylor, T. E. (2004). Prognostic significance and course of retinopathy in children with severe malaria. *Arch. Ophthalmol.* 122, 1141–1147. doi: 10.1001/archopht.122.8.1141

Beare, N. A. V., Taylor, T. E., Harding, S. P., Lewallen, S., and Molyneux, M. E. (2006). Malarial retinopathy: a newly established diagnostic sign in severe malaria. *Am. J. Trop. Med. Hyg.* 75, 790–797.

Bergersen, L. H. (2007). Is lactate food for neurons? Comparison of monocarboxylate transporter subtypes in brain and muscle. *Neuroscience* 145, 11–19. doi: 10.1016/j.neuroscience.2006.11.062

Bergersen, L. H., and Gjedde, A. (2012). Is lactate a volume transmitter of metabolic states of the brain? *Front. Neuroenergetics* 4:5. doi: 10.3389/fnene.2012.00005

Bergersen, L., Jóhannsson, E., Veruki, M. L., Nagelhus, E. A., Halestrap, A., Sejersted, O. M., et al. (1999). Cellular and subcellular expression of monocarboxylate transporters in the pigment epithelium and retina of the rat. *Neuroscience* 90, 319–331. doi: 10.1016/S0306-4522(98)00427-8

Bergersen, L., Wærhaug, O., Helm, J., Thomas, M., Laake, P., Davies, A. J., et al. (2001). A novel postsynaptic density protein: the monocarboxylate transporter MCT2 is co-localized with delta-glutamate receptors in postsynaptic densities of parallel fiber-Purkinje cell synapses. *Exp. Brain Res.* 136, 523–534. doi: 10.1007/s002210000600

Bozzo, L., Puyal, J., and Chatton, J. Y. (2013). Lactate modulates the activity of primary cortical neurons through a receptor-mediated pathway. *PLoS ONE* 8:e71721. doi: 10.1371/journal.pone.0071721

Breman, J. G. (2001). The ears of the hippopotamus: manifestations, determinants, and estimates of the malaria burden. *Am. J. Trop. Med. Hyg.* 64(Suppl. 12), 1–11.

Cai, T. Q., Ren, N., Jin, L., Cheng, K., Kash, S., Chen, R., et al. (2008). Role of GPR81 in lactate-mediated reduction of adipose lipolysis. *Biochem. Biophys. Res. Commun.* 377, 987–991. doi: 10.1016/j.bbrc.2008.10.088

Dorovini-Zis, K., Schmidt, K., Huynh, H., Fu, W., Whitten, R. O., Milner, D., et al. (2011). The neuropathology of fatal cerebral malaria in Malawian children. *Am. J. Pathol.* 178, 2146–2158. doi: 10.1016/j.ajpath.2011.01.016

Draoui, N., Schicke, O., Seront, E., Bouzin, C., Sonveaux, P., Riant, O., et al. (2014). Antitumor activity of 7-aminocarboxycoumarin derivatives, a new class of potent inhibitors of lactate influx but not efflux. *Mol. Cancer Ther.* doi: 10.1158/1535-7163.MCT-13-0653 [Epub ahead of print].

During, M. J., Fried, I., Leone, P., Katz, A., and Spencer, D. D. (1994). Direct measurement of extracellular lactate in the human hippocampus during spontaneous seizures. *J. Neurochem.* 62, 2356–2361. doi: 10.1046/j.1471-4159.1994.62062356.x

Elliot, J. L., Saliba, K. J., and Kirk, K. (2001). Transport of lactate and pyruvate in the intraerythrocytic malaria parasite, *Plasmodium falciparum*. *Biochem. J.* 355, 733–739.

Gjedde, A., Andersson, J., and Eklöf, B. (1975). Brain uptake of lactate, antipyrine, water and ethanol. *Acta Physiol. Scand.* 93, 145–149. doi: 10.1111/j.1748-1716.1975.tb05802.x

Hoque, R., Farooq, A., Ghani, A., Gorelick, F., and Mehal, W. Z. (2014). Lactate reduces organ injury in toll-like receptor- and inflammasome-mediated inflammation via GPR81-mediated suppression of innate immunity. *Gastroenterology* doi: 10.1053/j.gastro.2014.03.014 [Epub ahead of print].

Hunt, N. H., and Grau, G. E. (2003). Cytokines: accelerators and brakes in the pathogenesis of cerebral malaria. *Trends Immunol.* 24, 491–499. doi: 10.1016/S1471-4906(03)00229-1

Idro, R., Jenkins, N. E., and Newton, C. R. (2005). Pathogenesis, clinical features, and neurological outcome of cerebral malaria. *Lancet Neurol.* 4, 827–840. doi: 10.1016/S1474-4422(05)70247-7

Idro, R., Marsh, K., John, C. C., and Newton, C. R. J. (2010). Cerebral malaria; mechanisms of brain injury and strategies for improved neuro-cognitive outcome. *Pediatr. Res.* 68, 267–274. doi: 10.1203/PDR.0b013e3181eee738

Jalloh, I., Helmy, A., Shannon, R. J., Gallagher, C. N., Menon, D. K., Carpenter, K. L., et al. (2013). Lactate uptake by the injured human brain: evidence from anarteriovenous gradient and cerebral microdialysis study. *J. Neurotrauma* 30, 2031–2037. doi: 10.1089/neu.2013.2947

Kanaani, J., and Ginsburg, H. (1991). Transport of lactate in *Plasmodium falciparum*-infected human erythrocytes. *J. Cell. Physiol.* 149, 469–476. doi: 10.1002/jcp.1041490316

Kanaani, J., and Ginsburg, H. (1992). Effects of cinnamic acid derivatives on in vitro growth of *Plasmodium falciparum* and on the permeability of the membrane of malaria-infected erythrocytes. *Antimicrob. Agents Chemother.* 36, 1102–1108. doi: 10.1128/AAC.36.5.1102

Krishna, S., Waller, D. W., ter Kuile, F., Kwiatkowski, D., Crawley, J., Craddock, C. F., et al. (1994). Lactic acidosis and hypoglycaemia in children with severe malaria: pathophysiological and prognostic significance. *Trans. R. Soc. Trop. Med. Hyg.* 88, 67–73. doi: 10.1016/0035-9203(94)90504-5

Kristensson, K., Masocha, W., and Bentivoglio, M. (2013). Mechanisms of CNS invasion and damage by parasites. *Handb. Clin. Neurol.* 114, 11–22. doi: 10.1016/B978-0-444-53490-3.00002-9

Lauritzen, F., de Lanerolle, N. C., Lee, T. S., Spencer, D. D., Kim, J. H., Bergersen, L. H., et al. (2011). Monocarboxylate transporter 1 is deficient on microvessels in the human epileptogenic hippocampus. *Neurobiol. Dis.* 41, 577–584. doi: 10.1016/j.nbd.2010.11.005

Lauritzen, F., Heuser, K., de Lanerolle, N. C., Lee, T. W., Spencer, D. D., Kim, J. H., et al. (2012). Redistribution of monocarboxylate transporter 2 on the surface of astrocytes in the human epileptogenic hippocampus. *Glia* 60, 0894–1491.

Lauritzen, F., Eid, T., and Bergersen, L. H. (2013a). Monocarboxylate transporters in temporal lobe epilepsy: roles of lactate and ketogenic diet. *Brain Struct. Funct.* doi: 10.1007/s00429-013-0672-x [Epub ahead of print].

Lauritzen, K. H., Morland, C., Puchades, M., Holm-Hansen, S., Hagelin, E. M., Lauritzen F., et al. (2013b). Lactate receptor sites link neurotransmission, neurovascular coupling, and brain energy metabolism. *Cereb. Cortex* doi: 10.1093/cercor/bht136 [Epub ahead of print].

Mariga, S. T., Gil, J. P., Sisowath, C., Wernsdorfer, W. H., and Björkman, A. (2004). Synergism between amodiaquine and Its major metabolite, desethylamodiaquine, against *Plasmodium falciparum* in vitro. *Antimicrob. Agents Chemother.* 48, 4089–4096. doi: 10.1128/AAC.48.11.4089-4096.2004

Maude, R. J., Beare, N. A. V., Abu, S. A., Chang, C. C., Charunwatthana, P., Faiz, M. A., et al. (2009a). The spectrum of retinopathy in adults with Plasodium falciparum malaria. *Trans. R. Soc. Trop. Med. Hyg.* 103, 665–671. doi: 10.1016/j.trstmh.2009.03.001

Maude, R. J., Dondorp, A. M., Abu, S. A., Day, N. P. J., White, N. J., and Beare, N. A. V. (2009b). The eye in cerebral malaria: what can it teach us? *Trans. R. Soc. Trop. Med. Hyg.* 103, 661–664. doi: 10.1016/j.trstmh.2008.11.003

Medana, I. M., and Turner, G. D. (2006). Human cerebral malaria and the blood-brain barrier. *Int. J. Parasitol.* 36, 555–568. doi: 10.1016/j.ijpara.2006.02.004

Moreira, T. J., Pierre, K., Maekawa, F., Repond, C., Cebere, A., Liljequist, S., et al. (2009). Enhanced cerebral expression of MCT1 and MCT2 in a rat ischemia model occurs in activated microglial cells. *J. Cereb. Blood Flow Metab.* 29, 1273–1283. doi: 10.1038/jcbfm.2009.50

Newton, C. R. (2005). Interaction between *Plasmodium falciparum* and human immunodeficiency virus type 1 on the central nervous system of African children. *J. Neurovirol.* 3, 45–51. doi: 10.1080/13550280500511881

Newton, C. R., Crawley, J., Sowumni, A., Waruiru, C., Mwangi, I., English, M., et al. (1997). Intracranial hypertension in Africans with cerebral malaria. *Arch. Dis. Child.* 76, 219–226. doi: 10.1136/adc.76.3.219

Newton, C. R. J., Hien, T. T., and White, N. (2000). Neurological aspects of tropical disease Cerebral malaria *J. Neurol. Neurosurg. Psychiatry* 69, 433–441. doi: 10.1136/jnnp.69.4.433

Newton, C. R., and Krishna, S. (1998). Severe falciparum malaria in children: current understanding of pathophysiology and supportive treatment. *Pharmacol. Ther.* 79, 1–53. doi: 10.1016/S0163-7258(98)00008-4

Newton, C. R., Peshu, N., Kendall, B., Kirkham, F. J., Sowunmi, A., Waruiru, C., et al. (1994). Brain swelling and ischaemia in Kenyans with cerebral malaria. *Arch. Dis. Child.* 70, 281–287. doi: 10.1136/adc.70.4.281

Parks, S. K., Chiche, J., and Pouysségur, J. (2013). Disrupting proton dynamics and energy metabolism for cancer therapy. *Nat. Rev. Cancer* 13, 611–623. doi: 10.1038/nrc3579

Pierre, K., and Pellerin, L. (2005). Monocarboxylate transporters in the central nervous system: distribution, regulation and function. *J. Neurochem.* 94, 1–14. doi: 10.1111/j.1471-4159.2005.03168.x

Penet, M. F., Viola, A., Confort-Gouny, S., Le Fur, Y., Duhamel, G., Kober, F., et al. (2005). Imaging experimental cerebral malaria in vivo: significant role of ischemic brain edema. *J. Neurosci.* 25, 7352–7358. doi: 10.1523/JNEUROSCI.1002-05.2005

Pfaller, M. A., Krogstad, D. J., Parquette, A. R., and Nguyen-Dinh, P. (1982). *Plasmodium falciparum*: stage-specific lactate production in synchronized cultures. *Exp. Parasitol.* 54, 391–396. doi: 10.1016/0014-4894(82)90048-0

Polder, T. W., Eling, W. M., Curfs, J. H., Jerusalem, C. R., and Wijers-Rouw, M. (1992). Ultrastructural changes in the blood-brain barrier of mice infected with *Plasmodium berghei*. *Acta Leiden* 60, 31–46.

Potchen, M. J., Kampondeni, S. D., Seydel, K. B., Birbeck, G. L., Hammond, C. A., Bradley, W. G., et al. (2012). Acute brain MRI findings in 120 malawian children with cerebral malaria: new insights into an ancient disease. *AJNR Am. J. Neuroradiol.* 33, 1740–1746. doi: 10.3174/ajnr.A3035

Promeneur, D., Lunde, L. K., Amiry-Moghaddam, M., and Agre, P. (2013). Protective role of brain water channel AQP4 in murine cerebral malaria. *Proc. Natl. Acad. Sci. U.S.A.* 110, 1035–1040. doi: 10.1073/pnas.1220566110

Rénia, L., Grüner, A. C., and Snounou, G. (2010). Cerebral malaria: In praise of epistemes. *Trends Parasitol.* 26, 275–277. doi: 10.1016/j.pt.2010.03.005

Rest, J. R. (1982). Cerebral malaria in inbred mice. a new model and its pathology. *Trans. R. Soc. Trop. Med. Hyg.* 76, 410–415. doi: 10.1016/0035-9203(82)90203-6

Riley, E. M., Couper, K. N., Helmby, H., Julius, C. R., Hafalla, J. C. R., de Souza, J. B., et al. (2010). Neuropathogenesis of human and murine malaria. *Trends Parasitol.* 26, 277–278. doi: 10.1016/j.pt.2010.03.002

Roth, E. F. Jr., Raventos-Suarez, C., Perkins, M., and Nagel, R. L. (1982). Glutathione stability and oxidative stress in P. falciparum infection in vitro: responses of normal and G6PD deficient cells. *Biochem. Biophys. Res. Commun.* 109, 355–362. doi: 10.1016/0006-291X(82)91728-4

Roberts, O. L., and Dart, C. (2014). cAMP signalling in the vasculature: the role of Epac (exchange protein directly activated by cAMP). *Biochem. Soc. Trans.* 42, 89–97. doi: 10.1042/BST20130253

Sanni, L. A., Rae, C., Maitland, A., Stocker, R., and Hunt, N. H. (2001). Is ischemia involved in the pathogenesis of murine cerebral malaria? *Am. J. Pathol.* 159, 1105–1112. doi: 10.1016/S0002-9440(10)61786-5

Scheibel, L. W., Adler, A., and Trager, W. (1979). Tetraethylthiuram disulfide (Antabuse) inhibits the human malaria parasite *Plasmodium falciparum*. *Proc. Natl. Acad. Sci. U.S.A.* 76, 5303–5307. doi: 10.1073/pnas.76.10.5303

Schlegel, N., and Waschke, J. (2014). cAMP with other signaling cues converges on Rac1 to stabilize the endothelial barrier- a signaling pathway compromised in inflammation. *Cell Tissue Res.* 355, 587–596. doi: 10.1007/s00441-013-1755-y

SenGupta, S. K., and Naraqi, S. (1992). The brain in cerebral malaria: a pathological study of 24 fatal cases in Papua New Guinea. *P. N. G. Med. J.* 35, 270–274.

Sherman, I. W. (1998). *Malaria: Parasite Biology, Pathogenesis, and Protection.* Washington, DC: ASM Press.

Smith, J. P., Uhernik, A. L., Li, L., Liu, Z., and Drewes, L. R. (2012). Regulation of Mct1 by cAMP-dependent internalization in rat brain endothelial cells. *Brain Res.* 1480, 1–11. doi: 10.1016/j.brainres.2012.08.026

Tang, F., Lane, S., Korsak, A., Paton, J. F., Gourine, A. V., Kasparov, S., et al. (2014). Lactate-mediated glia-neuronal signalling in the mammalian brain. *Nat. Commun.* 5:3284. doi: 10.1038/ncomms4284

Tosti, C. L., Petersen, E. T., Laothamatas, J., Golay, X., Swaminathan, S. V., Van Cauteren, M., et al. (2007). Cerebrospinal fluid lactate in P. falciparum malaria: measurement by chemical shift imaging at 3 Tesla. *Proc. Intl. Soc. Mag. Reson. Med.* 15:2262.

Tripathi, A. K., Sullivan, D. J., and Stins, M. F. (2007). *Plasmodium falciparum*–infected erythrocytes decrease the integrity of human blood-brain barrier endothelial cell monolayers. *J. Infect. Dis.* 195, 942–950. doi: 10.1086/512083

van Hall, G., Strømstad, M., Rasmussen, P., Jans, Ø., Zaar, M., Gam, C., et al. (2009). Blood lactate is an important energy source for the human brain. *J. Cereb. Blood Flow Metab.* 29, 1121–1129. doi: 10.1038/jcbfm.2009.35

Waller, D., Waller, D., Crawley, J., Nosten, F., Chapman, D., Krishna, S., et al. (1991). Intracranial pressure in childhood cerebral malaria. *Trans. R. Soc. Trop. Med. Hyg.* 85, 362–364. doi: 10.1016/0035-9203(91)90291-6

Walker, O., Salako, L. A., Sowunmi, A., Thomas, J. O., Sodeine, O., and Bondi, F. S. (1992). Prognostic risk factors and post mortem findings in cerebral malaria in children. *Trans. R. Soc. Trop. Med. Hyg.* 86, 491–493. doi: 10.1016/0035-9203(92)90082-N

White, N. J., Looareesuwan, S., Phillips, R. E., Warrell, D. A., Chanthavanich, P., and Pongpaew, P. (1985). Pathophysiological and prognostic significance of cerebrospinal fluid lactate in cerebral malaria. *Lancet* 325, 776–778. doi: 10.1016/S0140-6736(85)91445-X

White, V. A., Lewallen, S., Beare, N. A. V., Molyneux, M. E., and Taylor, T. E. (2009). Retinal pathology of pediatric cerebral malaria in Malawi. *PLoS ONE* 4:e4317. doi: 10.1371/journal.pone.0004317

Wu, J., Tian, L., Yu, X., Pattaradilokrat, S., Li, J., Wang, M., et al. (2014). Strain-specific innate immune signaling pathways determine malaria parasitemia dynamics and host mortality. *Proc. Natl. Acad. Sci. U.S.A.* 111, E511–E520. doi: 10.1073/pnas.1316467111

Zolg, J. W., Macleod, A. J., Scaife, J. G., and Beaudoin, R. L. (1984). The accumulation of lactic acid and its influence on the growth of *Plasmodium falciparum* in synchronized cultures. *In Vitro* 3, 205–215. doi: 10.1007/BF02618189

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 13 March 2014; accepted: 22 April 2014; published online: 19 May 2014.
Citation: Mariga ST, Kolko M, Gjedde A and Bergersen LH (2014) Lactate transport and receptor actions in cerebral malaria. Front. Neurosci. ***8****:2. doi: 10.3389/fnins.2014.00125*
This article was submitted to Nutrition and Brain Health, a section of the journal Frontiers in Neuroscience.

frontiers in
NEUROSCIENCE

MINI REVIEW ARTICLE
published: 09 September 2014
doi: 10.3389/fnins.2014.00261

Fluxes of lactate into, from, and among gap junction-coupled astrocytes and their interaction with noradrenaline

Leif Hertz[1], Marie E. Gibbs[2] and Gerald A. Dienel[3]*

[1] *Laboratory of Brain Metabolic Diseases, Institute of Metabolic Disease Research and Drug Development, China Medical University, Shenyang, China*
[2] *Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University, Clayton, VIC, Australia*
[3] *Department of Neurology, University of Arkansas for Medical Sciences, Little Rock, AR, USA*

Edited by:
Avital Schurr, University of Louisville, USA

Reviewed by:
Linda H. Bergersen, University of Oslo, Norway
Johannes Hirrlinger, University of Leipzig, Germany

***Correspondence:**
Gerald A. Dienel, Department of Neurology, University of Arkansas for Medical Sciences, Slot 500, 4301 W. Markham St. Little Rock, AR 72205, USA
e-mail: gadienel@uams.edu

Lactate is a versatile metabolite with important roles in modulation of brain glucose utilization rate (CMR_{glc}), diagnosis of brain-injured patients, redox- and receptor-mediated signaling, memory, and alteration of gene transcription. Neurons and astrocytes release and accumulate lactate using equilibrative monocarboxylate transporters that carry out net transmembrane transport of lactate only until intra- and extracellular levels reach equilibrium. Astrocytes have much faster lactate uptake than neurons and shuttle more lactate among gap junction-coupled astrocytes than to nearby neurons. Lactate diffusion within syncytia can provide precursors for oxidative metabolism and glutamate synthesis and facilitate its release from endfeet to perivascular space to stimulate blood flow. Lactate efflux from brain during activation underlies the large underestimation of CMR_{glc} with labeled glucose and fall in CMR_{O2}/CMR_{glc} ratio. Receptor-mediated effects of lactate on locus coeruleus neurons include noradrenaline release in cerebral cortex and c-AMP-mediated stimulation of astrocytic gap junctional coupling, thereby enhancing its dispersal and release from brain. Lactate transport is essential for its multifunctional roles.

Keywords: astrocyte, acetate, lactate, locus coeruleus, neuron, monocarboxylic acid transporter, memory

METABOLIC, DIAGNOSTIC, AND SIGNALING ROLES OF LACTATE

Lactate has well-known and intriguing roles in brain function. Its resting concentration (~0.5–1 mmol/L) doubles during brain activation, and increases ~10–20-fold during abnormal states (Siesjö, 1978; Mangia et al., 2007). Lactate is generated from pyruvate when (i) glycolytic flux exceeds the rates of the TCA cycle and the malate-aspartate shuttle (MAS) that transfers reducing equivalents from cytoplasmic NADH into mitochondria, or (ii) when oxygen levels are insufficient to sustain oxidative metabolism. Thus, lactate formation is a "safety valve" to quickly regenerate NAD^+ from NADH, thereby allowing rapid up-regulation and maintenance of high glycolytic flux. Lactate and pyruvate readily move down their concentration gradients to extracellular fluid, and the lactate/pyruvate concentration ratio in microdialysate is an important diagnostic tool predictive of clinical outcome of patients with traumatic brain injury; the higher the ratio the worse outcome (Nordström et al., 2013). Increased lactate production to sustain high glycolytic rate is associated with greater lactate release to blood because the brain concentration then exceeds that in blood. High cerebral blood flow maintains this gradient and "pulls" lactate from brain. Lactate in perivascular fluid, presumably mainly released from astrocytic endfeet (Gandhi et al., 2009), stimulates blood flow to activated regions (Laptook et al., 1988; Hein et al., 2006; Lombard, 2006; Yamanishi et al., 2006; Gordon et al., 2008), increasing nutrient delivery and by-product removal.

Conversely, increasing blood lactate concentration by intense physical activity drives lactate down its concentration gradient into all brain cells. Lactate oxidation supplements brain glucose metabolism to an increasing extent with rising blood level (Dalsgaard et al., 2004; Van Hall et al., 2009), and it does not accumulate in brain above resting levels (Dalsgaard et al., 2004). Metabolism of lactate requires its conversion back to pyruvate that, in turn, can have different metabolic fates (conversion to alanine, oxaloacetate, or acetyl CoA), which vary with cell type and metabolic state. Continued net uptake of lactate depends on its oxidation to pyruvate plus NADH and may cause the intracellular redox state to become more reduced, although cytosolic NAD^+/NADH ratio is relatively stable in cell lines (Sun et al., 2012). Lactate is co-transported with a proton via equilibrative monocarboxylic acid transporters (MCTs) (Poole and Halestrap, 1993), and lactate influx accordingly causes intracellular acidification (Nedergaard and Goldman, 1993). Lactate uptake can, therefore, inhibit glycolysis by reducing availability of NAD^+ for glycolysis and by acidification that can inhibit phosphofructokinase, which has a steep pH-activity profile (Dienel, 2012). Widespread lactate signaling, especially to neurons, via the receptor GPR81 decreases cAMP (IC_{50} ~29 mmol/L), which can decrease glycolysis at high

extracellular lactate concentrations; a significant effect on cAMP requires ≥10 mmol/L lactate (Lauritzen et al., 2013). Thus, "pushing" lactate into all brain cells from blood provides supplementary fuel and evokes regulatory mechanisms that reduce brain glucose utilization when muscular lactate production is high.

Lactate can also influence astrocytic and neuronal activities by redox-mediated signaling. Astrocyte calcium signals are regulated by NAD^+/NADH redox state (Requardt et al., 2012; Wilhelm and Hirrlinger, 2012), and changes in intracellular NAD^+ and NADH levels arising from lactate fluxes may affect their binding to transcription factors and influence gene expression (Nakamura et al., 2012). For example, the transcription co-repressor, C-terminal binding protein (CtBP), is a dehydrogenase that undergoes conformational change with binding of NAD^+ and NADH; NADH has a much higher affinity for CtBP, allowing it to serve as a redox sensor that destabilizes interactions with CtBP and transcription factors (Kumar et al., 2002; Fjeld et al., 2003). Increased NADH levels are thought to underlie seizure-induced increased expression of brain-derived neurotrophic factor (BDNF) and its receptor TrkB (Garriga-Canut et al., 2006). NAD^+ is required for the action of sirtuins, a family of deacetylases that regulate activities of transcription factors and metabolic cofactors, and important roles for sirtuins in brain development, aging, and neurodegenerative diseases have been identified (Harting and Knoll, 2010; Bonda et al., 2011).

To summarize, lactate serves vital functions that include metabolic regulation (sustaining glycolysis by regenerating NAD^+ or inhibiting glycolysis by intracellular acidification, NAD^+ depletion and signaling), blood flow stimulation, influence on gene transcription via redox state, and signaling via receptor binding. During intense exercise muscle-derived lactate serves as an important metabolite for brain. Movement of lactate to and from cells via MCTs seems to be a central element in its multifunctional roles.

MCT TRANSPORTER FUNCTION

Lactate is bi-directionally transported across cell membranes by MCT-mediated diffusional, saturable co-transport with H^+. In the absence of a transcellular H^+ gradient, extracellular lactate can increase its intracellular concentration up to, but not beyond the extracellular level and vice versa (Poole and Halestrap, 1993; Juel and Halestrap, 1999). Transporter-mediated diffusional uptake is equilibrative and energy-independent. However, continuing inwardly-directed diffusional *net* transport (influx) can be achieved by intracellular metabolism that reduces the intracellular level of the non-metabolized lactate and maintains a concentration gradient between extra- and intracellular concentrations of the non-metabolized compound (metabolism-driven uptake). This cannot increase the intracellular concentration of lactate itself. Analogously, continued removal of extracellular lactate by diffusion or uptake into other cells can increase net outward transport of lactate (efflux), but not its extracellular concentration. If extra- and intracellular pH differ, the equilibrium level is determined by the gradients of both lactate anions and H^+, and it is reached when the product of intracellular lactate and H^+ concentrations equals that of extracellular lactate and H^+ concentrations. Extracellular pH in brain is normally 7.3, but it is lower in brain slices (∼7.1) incubated at pH 7.3–7.4 (Chesler, 2003). Most results for intracellular pH have been obtained in brain slices or cultured cells and it is generally lower than in extracellular fluid although only by 0.2–0.3 pH units, indicating that the H^+ concentration is at most two-fold higher intracellularly than extracellularly (e.g., Roos and Boron, 1981). Thus, the H^+ gradient only moderately enhances diffusional lactate efflux and reduces its diffusional influx.

Diffusional uptake is only measurable during very short incubation times and contribution of metabolism-driven uptake will distort its kinetics (Hertz and Dienel, 2005). **Figure 1A** illustrates lactate uptake into cerebellar neurons at 1 mmol/L extracellular lactate. The initial diffusional uptake is very brief (<∼30 s; **Figure 1A** inset), rapid (∼10 nmol/mg protein or 1 μmol/g wet wt.), and only occurs in cells containing <1 mmol/L lactate. Thereafter, metabolism-driven net uptake takes over and is sustained for ≥1 h at 0.5 nmol lactate/mg protein per min, corresponding to 0.25 nmol glucose equivalent/mg protein per min. Lactate metabolism is lower than measured rates of non-stimulated and stimulated glucose oxidation (1.0 and 2.23 nmol/mg protein per min, respectively) in cerebellar neurons (Peng et al., 1994). The above glucose oxidation rates are minimal values because the assays were based on $^{14}CO_2$ production, and exchange reactions cause label dilution in amino acid pools, slowing $^{14}CO_2$ release and causing underestimation of oxidation rate. Thus, the *potential* contribution of any lactate to total CO_2 formation in the neurons under activated conditions would be <10% of that from glucose. In cultured astrocytes, diffusional uptake is faster than in neurons (suggesting higher V_{max}), but the rate of metabolism-driven uptake is similar (Dienel and Hertz, 2001).

Neurons and astrocytes express different MCTs. MCT2 has a K_m for lactate of ∼0.7 mmol/L and is predominantly neuronal, whereas MCT1 (K_m 3–5 mmol/L) and MCT4 (K_m 15–30 mmol/L) are mainly astrocytic (for references see Hertz and Dienel, 2005). These MCT's do not determine net lactate fluxes, which are mainly metabolism-driven for influx or concentration gradient-driven for efflux (although potentially increased by lactate removal by extracellular diffusion or cellular re-uptake), but they may be rate-limiting when concentration gradients develop rapidly. Lactate transport is governed by lactate concentration, K_m, and transporter number, and it is enhanced by "transacceleration" (Juel, 1991; Juel et al., 1994). Lactate exit is stimulated by extracellular pyruvate (San Martin et al., 2013), perhaps stimulating a heteroexchange. The lower affinity MCTs in astrocytes may promote astrocytic release and re-uptake even at high concentrations. MCTs are inhibited by several drugs, including 4-CIN, and lactate transport is competitively inhibited by D-lactate. These toxins have repeatedly been used to allegedly show the importance of MCT-mediated intercellular transport. However, it has never been demonstrated that these drugs at the same concentrations do not also inhibit pyruvate uptake into mitochondria, as shown by McKenna et al. (2001), who demonstrated that incubation with 0.25 mmol/L 4-CIN decreased oxidation of glucose to ∼50% of control values in both astrocytes and neurons in primary cultures, although cellular glucose uptake was not inhibited by 4-CIN.

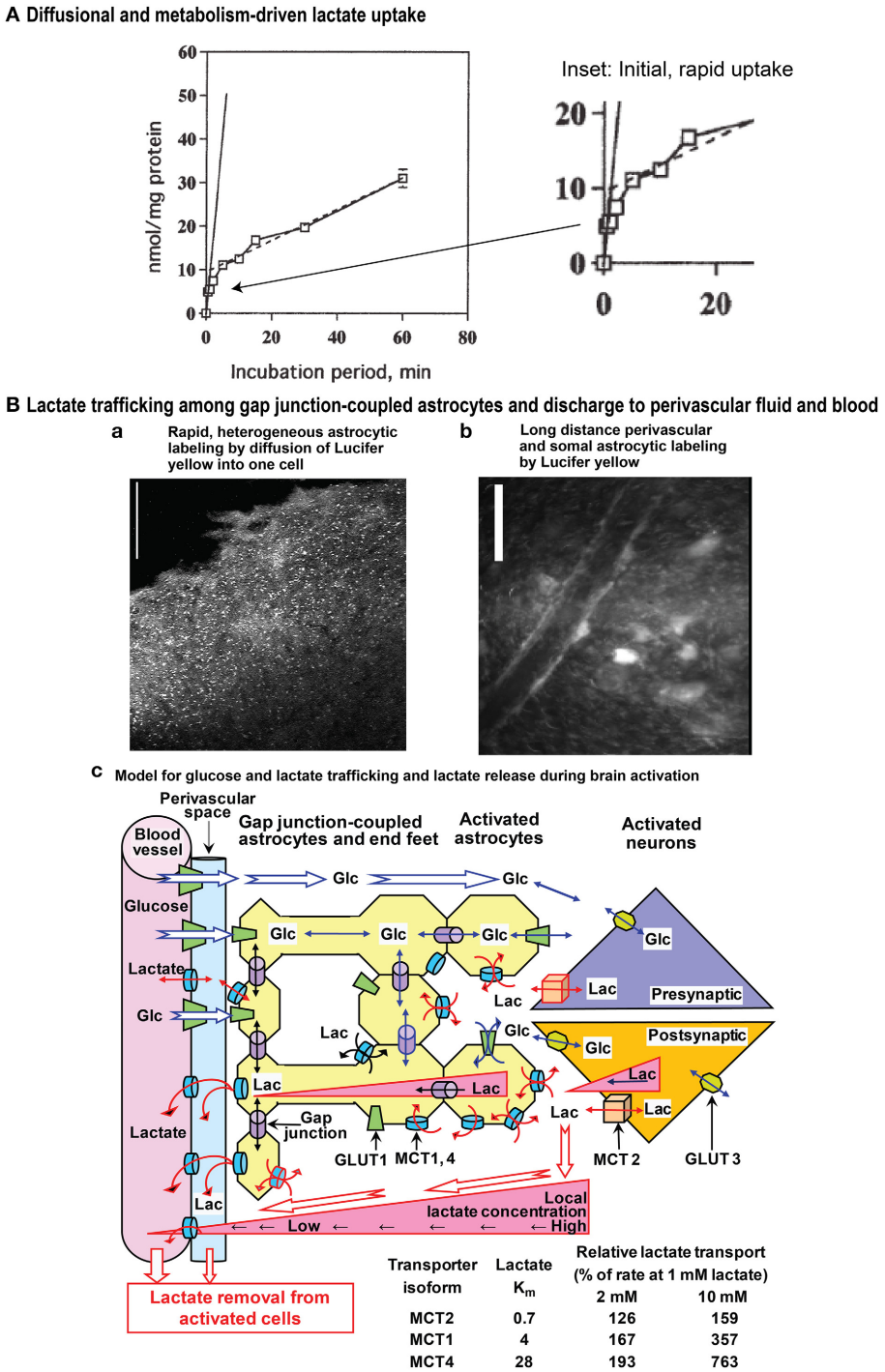


FIGURE 1 | Influx and gap junction-mediated trafficking of lactate. (A) Diffusional and metabolism-driven lactate uptake. Accumulation of [U-^{14}C]lactate into primary cultures of cerebellar granule cell neurons in primary cultures incubated in tissue culture medium of approximately similar pH as the intracellular water phase, shown as a function of time of exposure to 1 mmol/L [U-^{14}C]lactate. The solid line is an extrapolation of the initial, rapid uptake by facilitated diffusion during the first few seconds at ~10 nmol/mg protein. The inset (right panel) emphasizes the early component of lactate uptake. The continued slower uptake of label after the initial rapid phase represents metabolism-driven uptake, and its rate, indicated by the stippled line, is sustained for at least an hour at 0.5 nmol lactate/mg protein per min. Slightly modified from Dienel and Hertz (2001), ©2001 Wiley-Liss, Inc., with permission of John Wiley and Sons, Inc. **(B)** Lactate trafficking among astrocytes. Gap junction-coupled astrocytes in slices of adult rat brain inferior colliculus were visualized by 5 min diffusion of Lucifer yellow from a micropipette inserted into a single astrocyte. Lucifer yellow labeled the soma (light spots) of as many as 10,000 astrocytes located up to about 1 mm from the impaled cell **(a)**, and diffusion of dye into astrocytic endfeet surrounding blood vessels caused high perivasculature labeling **(b)**. Scale bars in a and b denote 100 and 25 μm, respectively. Note that Lucifer yellow is retained within the coupled cells and it reveals the

(Continued)

FIGURE 1 | Continued
size of the syncytium coupled to a single astrocyte. Lactate can enter and leave cells via MCT transporters, and its direct diffusion (i.e., without exit and re-entry) throughout the extent of the entire Lucifer yellow-labeled syncytium is probably less than that of Lucifer yellow. Lactate was directly shown to diffuse through gap junctions to coupled cells located ∼50 μm from the impaled cell (longer distances were not tested; Gandhi et al., 2009). Lactate exit plus re-entry into the same syncytium or to separate nearby syncytia would lead to extensive diffusion of lactate from the point source of the impaled cell. The schematic model for metabolite trafficking **(c)** illustrates uptake of glucose from blood into interstitial fluid and astrocytic endfeet, followed by diffusion of glucose down its concentration gradient from blood through extracellular fluid and the astrocytic syncytium, ultimately to the cells that are actively metabolizing glucose and creating a local sink toward which unmetabolized glucose diffuses. Detailed studies of (i) rates and capacities for lactate uptake from extracellular fluid into astrocytes and neurons and (ii) shuttling of lactate among gap junction-coupled astrocytes (yellow) compared with shuttling from astrocytes to neurons revealed that astrocytes have faster and greater capacity for lactate uptake and for lactate shuttling within the syncytium compared with neuronal uptake and transfer of lactate to neurons; glucose can also diffuse from an impaled astrocyte to neurons (Gandhi et al., 2009). Thus, astrocytic lactate uptake from interstitial fluid prevails, regardless of the cellular origin of the lactate. Once inside the syncytium (yellow) diffusion of lactate down its concentration gradient through gap junctions (purple cylinders) to other coupled astrocytes and their endfeet facilitates lactate discharge to perivascular fluid (blue) where it can be removed from brain by perivascular-lymphatic flow and by discharge into cerebral venous blood. The perivascular fluid space is color coded only to emphasize its location; there is no physical boundary between interstitial fluid and perivascular fluid, although diffusion between these locations is influenced by tortuosity. Isoforms of monocarboxylic acid transporters (MCTs) have different K_m values for lactate, and relative rates of lactate transport by these isoforms when lactate concentration rises are illustrated in the table for K_m values within the ranges given in the text (i.e., 0.7, 3–5, and 15–30 mmol/L for MCT2, 1, and 4, respectively). The low K_m MCT2 in neurons restricts lactate influx and efflux compared with the higher Km isoforms in astrocytes. During brain activation in sedentary subjects, brain lactate level in activated structures is higher than that in blood. Triangles denote outward lactate gradients from intracellular to extracellular fluid, from extracellular fluid to blood, and from intracellular fluid of astrocytes located near cells with high glycolytic activity to endfeet and blood. During strenuous physical exercise that greatly increases blood lactate concentration, these gradients would be reversed, driving lactate into all brain cells (not shown). Glc, glucose; Lac, lactate; GLUT, glucose transporter. Modified from Gandhi et al. (2009) ©2009, the authors. Journal compilation ©2009 International Society for Neurochemistry, with permission from John Wiley and Sons, Inc and the authors.

Acetate is a preferential substrate for astrocytic, but not neuronal, MCTs, and it is also metabolized by astrocytes (Muir et al., 1986; Waniewski and Martin, 1998). Acetate may, accordingly, serve as an indicator of astrocyte-specific lactate transport. Inhibition of learning in day-old chicks by the non-metabolizable D-lactate can be prevented by administration of acetate at two different time periods, immediately after training and 20 min later (Gibbs and Hertz, 2008). Immediately after training, rescue by acetate requires co-administration of aspartate, which alone has no effect. Twenty min after training acetate by itself rescues learning; this is a time at which astrocytic metabolism is known to be activated, a further indication that acetate rescues energy metabolism. These observations identify the affected cells as astrocytes, and the aspartate requirement shows that the rescue immediately after training is due to formation of glutamate, which is normally formed in astrocytes from lactate/pyruvate by a combination of pyruvate carboxylation to oxaloacetate (which is astrocyte-specific) and pyruvate metabolism via the pyruvate dehydrogenase. No pyruvate carboxylation is possible with acetate as sole substrate, but co-administration of aspartate abolishes this requirement, because aspartate is an alternative oxaloacetate precursor. Thus, at both times, the rescue by acetate is due to support of *astrocytic* metabolism impaired by D-lactate, not to MCT-mediated inhibition of *neuronal* lactate uptake.

BRAIN LACTATE FLUXES

Because lactate transport is concentration-gradient driven, knowledge of both transport and metabolism is needed to evaluate net fluxes and ultimate fate of transported lactate. Microdialysis and microelectrode studies have shown that extracellular lactate levels rise quickly to about twice the resting value of ∼0.5–1 mmol/L during an activating stimulus, then return to normal; up-and-down cycling of extracellular and total lactate concentrations occurs with repeated transient stimuli (e.g., Korf and De Boer, 1990; Mangia et al., 2007). Changes in lactate concentration reflect net input and output fluxes to the lactate pool and are not indicators of lactate *flux through* the lactate pools. Most extracellular lactate produced during brain activation may come from astrocytes (Elekes et al., 1996), but modeling supports a neuronal origin and shuttling to astrocytes (Mangia et al., 2009).

Small amounts of lactate, equivalent to ∼5% of the glucose entering brain, are released to blood under resting conditions (Quistorff et al., 2008; Dienel, 2012), whereas during activation considerable quantities of lactate are released from brain to blood, both directly (22% during spreading depression; Cruz et al., 1999) and via the perivascular-lymphatic drainage system (Ball et al., 2010). Lactate efflux causes (i) a large (∼50%) underestimation of the calculated rate of glucose utilization (CMR_{glc}) when assayed with labeled glucose, in contrast to labeled deoxyglucose that is quantitatively trapped after its initial phosphorylation and (ii) a fall in the CMR_{O2}/CMR_{glc} ratio due to greater rise in glucose utilization than oxygen consumption (Dienel, 2012). These two events reflect lactate release and occur under various conditions, e.g., sensory stimulation (Fox et al., 1988) and mental testing (Madsen et al., 1995) of humans and spreading depression (Adachi et al., 1995; Cruz et al., 1999) and sensory stimulation (Madsen et al., 1999; Schmalbruch et al., 2002) of rats. The CMR_{O2}/CMR_{glc} ratio also falls with increased lactate uptake into brain during vigorous exercise (Quistorff et al., 2008). A common factor in all these situations may be an increase in extracellular lactate concentration.

CELLULAR LACTATE UPTAKE SHUTTLING

To compare astrocytic and neuronal rates and capacities for uptake of lactate from extracellular fluid and for its transcellular shuttling, Gandhi et al. (2009) devised a real-time, selective, sensitive assay to measure lactate concentration in single cells in adult rat brain slices. At 2 mmol/L extracellular lactate, the approximate concentration during brain activation, initial rates of lactate uptake into astrocytes were twice those of neurons, and over the range 2–40 mmol/L the initial rate of diffusional

lactate uptake into astrocytes was four-fold greater than that into neurons. The capacity for lactate uptake into astrocytes was also double that of neurons over this range. Because as many as ten thousand astrocytes are coupled via gap junctions (Ball et al., 2007) (**Figures 1Ba,b**), lactate can diffuse down its concentration gradient to other astrocytes within the large syncytium, as shown directly for coupled cells located ~50 μm apart (Gandhi et al., 2009). The initial rate of transfer among coupled astrocytes increased with lactate concentration from 0 to 5 mmol/L, whereas there was no concentration dependence of lactate transfer to neurons; net lactate transfer to another astrocyte was about five-fold greater than transfer to an equidistant neuron.

Together, these findings demonstrate that astrocytes avidly take up extracellular lactate, and quickly distribute the lactate to other astrocytes within the syncytium. There is a small, slower uptake of extracellular lactate by neurons and low transfer rate from astrocytes to neurons. Astrocytic endfeet surround capillaries and are also connected together via gap junctions (**Figure 1Bb**). Some of the lactate diffuses via its concentration gradient within the syncytium to endfeet where it can be released to perivascular fluid and ultimately to cerebral venous blood (**Figure 1Bc**) (Gandhi et al., 2009; Dienel, 2012), where it can stimulate blood flow that also washes out lactate from perivascular space fluid. Because more glucose is delivered to brain than is phosphorylated, release of a portion of excess fuel as lactate is not an energetic waste when viewed from a whole-body perspective. Other organs oxidize the released lactate.

INFLUENCE OF NORADRENALINE ON LACTATE TRAFFICKING

The reduced CMR_{O2}/CMR_{glc} ratio during activation is prevented by propranolol, an inhibitor of β-adrenergic signaling. In control rats, the CMR_{O2}/CMR_{glc} ratio fell from 6.1 to 4.0 after stimulation of brain activity by release from their shelter boxes, and it rose back to 5.8 after the animals re-entered the box. After

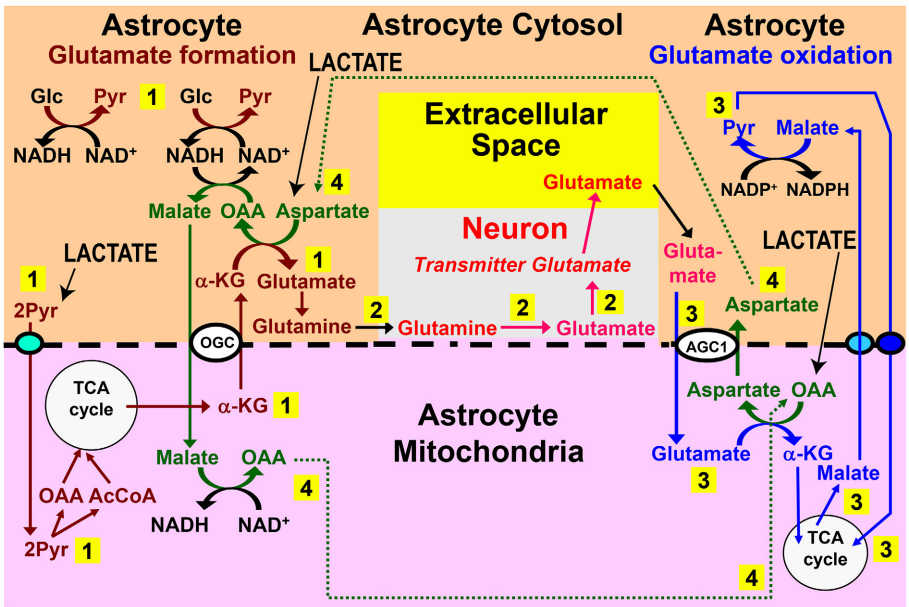


FIGURE 2 | Role for trans-astrocytic lactate trafficking in glutamate turnover. Why would the brain want a lactate transport from one astrocyte to different neighboring astrocytes? One possibility is that lactate-pyruvate interconversions could be of importance for proposed pathways linking glutamate formation, which is astrocyte-specific, with its oxidative degradation, which may also be mainly or exclusively astrocytic (see papers cited in Hertz and Rodrigues, 2014). The proposed pathways linking glutamate synthesis, excitatory neurotransmission, and glutamate oxidation are illustrated in this figure. Pathway 1 (numbered in yellow rectangle) shows the proposed cytosolic-mitochondrial metabolite trafficking associated with astrocytic production of glutamine. Pathway 2 shows glutamine transfer from astrocytes to glutamatergic neurons and extracellular release of transmitter glutamate. Pathway 3 illustrates subsequent re-uptake of glutamate and its oxidative metabolism in astrocytes. Pathway 4 provides the necessary aspartate- and oxaloacetate-dependent connections between pathways 1 and 3, with all pathways located in the same cell. A major problem with this model is that glutamate formation and oxidation may not occur in the same astrocyte, but, instead, in spatially-separated astrocytes. Trans-astrocytic lactate transport and its subsequent conversion to pyruvate and carboxylation would allow rapid synthesis of oxaloacetate (OAA) and aspartate that are needed for oxidation and synthesis of glutamate, respectively, according to this model (pathway 4) (lower right corner for OAA and upper left corner for aspartate). Lactate influx (shown in capital letters and with black arrows) could compensate for a lack of trafficking of these two compounds (pathway 4) between *spatially separated* glutamate-synthesizing and glutamate-oxidizing astrocytes. In addition, provision of lactate-derived pyruvate to astrocytes would provide a faster source than glucose for provision of the precursor carbon skeleton, and if only one of the two glucose molecules is replaced with pyruvate, malate would still be able to enter the mitochondria during glutamate synthesis. Biosynthesis of glutamine is shown in brown, and metabolic degradation of glutamate in blue. Redox shuttling and astrocytic release of glutamine and uptake of glutamate are shown in black, and neuronal hydrolysis of glutamine to glutamate and its release is shown in red. Reactions involving or resulting from transamination between aspartate and oxaloacetate are shown in green. Lactate could provide pyruvate for many of the reactions in these pathways in many astrocytes. AGC1, aspartate-glutamate exchanger, aralar; α-KG, α-ketoglutarate; Glc, glucose; Pyr, pyruvate; OGC, malate/α-KG exchanger. Slightly modified from Hertz (2011), with permission of the author. ©2011 International Society for Cerebral Blood Flow and Metabolism, Inc.

propranolol administration, the CMR_{O2}/CMR_{glc} ratio remained unaltered during rest, stimulation, and recovery (6.2, 6.3, 6.4) (Schmalbruch et al., 2002). Thus, (i) stimulation activates glycolysis in stimulated region(s) with much less effect on oxidative metabolism, (ii) this effect is dependent on β-adrenergic stimulation, and (iii) there must be efflux of a glucose metabolite, e.g., lactate, from the stimulated area. Part of the reduction in CMR_{O2}/CMR_{glc} ratio during brain activation may also reflect retention of some glucose in tissue by (i) an increase in lactate, (ii) use of glucose for glycogen synthesis, and (iii) increased pyruvate carboxylation (Öz et al., 2004) leading to enhanced glutamate formation (Gibbs et al., 2007; Mangia et al., 2012). The reduced CMR_{O2}/CMR_{glc} ratio during exercise is also inhibited by propranolol (Quistorff et al., 2008; Gam et al., 2009).

Inhibition by propranolol of an activation-induced fall in CMR_{O2}/CMR_{glc} ratio is consistent with a recent demonstration that specifically locus coeruleus (LC) neurons (the principal source of noradrenaline to brain cortex (Moore and Bloom, 1979), including astrocytes (Bekar et al., 2008), are stimulated by L-lactate, independent of its caloric value (Tang et al., 2014). Release of L-lactate from cultured astrocytes excites LC neurons and triggers release of noradrenaline, and physiologically-relevant concentrations of exogenous L-lactate (EC_{50} ~0.5 mmol/L) mimics these effects (Tang et al., 2014). The effects of L-lactate were stereo-selective, independent of its uptake into neurons, and involved a cAMP-mediated step. *In vivo* injections of L-lactate in the LC evoked arousal similar to the excitatory transmitter, L-glutamate. Because (i) lactate release is associated with activation-induced decreases in CMR_{O2}/CMR_{glc} ratio (inhibited by propranolol) and (ii) astrocytic gap junction conductivity is up-regulated by cAMP, an intermediate in β-adrenergic signaling (Enkvist and McCarthy, 1994) blockade by propranolol may reduce gap junction-mediated lactate transport and release from brain.

There might be additional beneficial effects of an adrenergically-stimulated, gap junction-mediated astrocyte-to-astrocyte lactate trafficking. Subsequent conversion of lactate to pyruvate would boost synthesis of oxaloacetate since pyruvate carboxylation in liver (and probably also in astrocytes) is stimulated by α-adrenergic activity (Garrison and Borland, 1979). Oxaloacetate is rapidly converted to aspartate which causes a 50% increase of astrocytic glutamate production (Pardo et al., 2011), consistent with increased mitochondrial glutamate formation by aspartate addition (Von Korff et al., 1971). Based on this aspartate dependence of glutamate formation and consistent with rapid astrocytic oxidative degradation of glutamate (McKenna, 2013; Whitelaw and Robinson, 2013), an interaction between glutamate synthesis and degradation has been suggested (Hertz, 2011). This interaction, illustrated in **Figure 2** and described in its legend, would make the aspartate formed during glutamate oxidation available during glutamate synthesis. Moreover, use of aspartate transaminase, rather than of glutamate dehydrogenase in the inter-conversion between α-ketoglutarate and glutamate is consistent with predominant transamination-dependent glutamate degradation in brain mitochondria (Balazs, 1965; Dennis et al., 1977) vs. extensive use of glutamate dehydrogenase by cultured, isolated astrocytes (Yu et al., 1982; McKenna et al., 1996). However, the **Figure 2** schematic shows cycling of aspartate and oxaloacetate within one astrocyte, and a problem with this model is that glutamate synthesis and its subsequent oxidation may occur in different astrocytes. Lactate transport between synthesizing and degrading astrocytes could rectify this problem by providing a substrate for rapid synthesis of both oxaloacetate and aspartate in the cells receiving lactate (**Figure 2**, black arrows), which could also partly replace glucose in α-ketoglutarate/glutamate synthesis. The huge flux in this cycle (Sibson et al., 1998; Rothman et al., 2011) and high rates of glutamate neosynthesis, accounting for 15–30% of the flux are consistent with the major trans-astrocytic lactate fluxes indicated by the large difference between glucose oxidation and total glucose utilization rates determined with glucose and deoxyglucose, which was described above.

CONCLUDING REMARKS

Lactate transport between brain cells is mainly among astrocytes and occurs both via gap junctions and release to extracellular space. The latter mechanism is important for LC-adrenergic signaling, and it also leads to a significant exit of lactate from the brain via peri-capillary flux and the lymphatic system. Adrenergic signaling plays a role in regulating lactate fluxes, and inter-astrocytic lactate flux may assist glutamate production and degradation in the glutamate-glutamine cycle.

REFERENCES

Adachi, K., Cruz, N. F., Sokoloff, L., and Dienel, G. A. (1995). Labeling of metabolic pools by [6-^{14}C]glucose during K(+)-induced stimulation of glucose utilization in rat brain. *J. Cereb. Blood Flow Metab.* 15, 97–110. doi: 10.1038/jcbfm.1995.11

Balazs, R. (1965). Control of glutamate oxidation in brain and liver mitochondrial systems. *Biochem. J.* 95, 497–508.

Ball, K. K., Cruz, N. F., Mrak, R. E., and Dienel, G. A. (2010). Trafficking of glucose, lactate, and amyloid-beta from the inferior colliculus through perivascular routes. *J. Cereb. Blood Flow Metab.* 30, 162–176. doi: 10.1038/jcbfm.2009.206

Ball, K. K., Gandhi, G. K., Thrash, J., Cruz, N. F., and Dienel, G. A. (2007). Astrocytic connexin distributions and rapid, extensive dye transfer via gap junctions in the inferior colliculus: implications for [(14)C]glucose metabolite trafficking. *J. Neurosci. Res.* 85, 3267–3283. doi: 10.1002/jnr.21376

Bekar, L. K., He, W., and Nedergaard, M. (2008). Locus coeruleus alpha-adrenergic-mediated activation of cortical astrocytes *in vivo*. *Cereb. Cortex* 18, 2789–2795. doi: 10.1093/cercor/bhn040

Bonda, D. J., Lee, H. G., Camins, A., Pallas, M., Casadesus, G., Smith, M. A., et al. (2011). The sirtuin pathway in ageing and Alzheimer disease: mechanistic and therapeutic considerations. *Lancet Neurol.* 10, 275–279. doi: 10.1016/s1474-4422(11)70013-8

Chesler, M. (2003). Regulation and modulation of pH in the brain. *Physiol. Rev.* 83, 1183–1221. doi: 10.1152/physrev.00010.2003

Cruz, N. F., Adachi, K., and Dienel, G. A. (1999). Rapid efflux of lactate from cerebral cortex during K+ -induced spreading cortical depression. *J. Cereb. Blood Flow Metab.* 19, 380–392. doi: 10.1097/00004647-199904000-00004

Dalsgaard, M. K., Quistorff, B., Danielsen, E. R., Selmer, C., Vogelsang, T., and Secher, N. H. (2004). A reduced cerebral metabolic ratio in exercise reflects metabolism and not accumulation of lactate within the human brain. *J. Physiol.* 554, 571–578. doi: 10.1113/jphysiol.2003.055053

Dennis, S. C., Lai, J. C., and Clark, J. B. (1977). Comparative studies on glutamate metabolism in synpatic and non-synaptic rat brain mitochondria. *Biochem. J.* 164, 727–736.

Dienel, G. A. (2012). Fueling and imaging brain activation. *ASN Neuro* 4:e00093. doi: 10.1042/AN20120021

Dienel, G. A., and Hertz, L. (2001). Glucose and lactate metabolism during brain activation. *J. Neurosci. Res.* 66, 824–838. doi: 10.1002/jnr.10079

Elekes, O., Venema, K., Postema, F., Dringen, R., Hamprecht, B., and Korf, J. (1996). Evidence that stress activates glial lactate formation *in vivo* assessed with rat hippocampus lactography. *Neurosci. Lett.* 208, 69–72. doi: 10.1016/0304-3940(96)12553-2

Enkvist, M. O., and McCarthy, K. D. (1994). Astroglial gap junction communication is increased by treatment with either glutamate or high K+ concentration. *J. Neurochem.* 62, 489–495. doi: 10.1046/j.1471-4159.1994.62020489.x

Fjeld, C. C., Birdsong, W. T., and Goodman, R. H. (2003). Differential binding of NAD+ and NADH allows the transcriptional corepressor carboxyl-terminal binding protein to serve as a metabolic sensor. *Proc. Natl. Acad. Sci. U.S.A.* 100, 9202–9207. doi: 10.1073/pnas.1633591100

Fox, P. T., Raichle, M. E., Mintun, M. A., and Dence, C. (1988). Nonoxidative glucose consumption during focal physiologic neural activity. *Science* 241, 462–464. doi: 10.1126/science.3260686

Gam, C. M., Rasmussen, P., Secher, N. H., Seifert, T., Larsen, F. S., and Nielsen, H. B. (2009). Maintained cerebral metabolic ratio during exercise in patients with beta-adrenergic blockade. *Clin. Physiol. Funct. Imaging* 29, 420–426. doi: 10.1111/j.1475-097X.2009.00889.x

Gandhi, G. K., Cruz, N. F., Ball, K. K., and Dienel, G. A. (2009). Astrocytes are poised for lactate trafficking and release from activated brain and for supply of glucose to neurons. *J. Neurochem.* 111, 522–536. doi: 10.1111/j.1471-4159.2009.06333.x

Garriga-Canut, M., Schoenike, B., Qazi, R., Bergendahl, K., Daley, T. J., Pfender, R. M., et al. (2006). 2-Deoxy-D-glucose reduces epilepsy progression by NRSF-CtBP-dependent metabolic regulation of chromatin structure. *Nat. Neurosci.* 9, 1382–1387. doi: 10.1038/nn1791

Garrison, J. C., and Borland, M. K. (1979). Regulation of mitochondrial pyruvate carboxylation and gluconeogenesis in rat hepatocytes via an alpha-adrenergic, adenosine 3′:5′-monophosphate-independent mechanism. *J. Biol. chem.* 254, 1129–1133.

Gibbs, M. E., and Hertz, L. (2008). Inhibition of astrocytic energy metabolism by D-lactate exposure impairs memory. *Neurochem. Int.* 52, 1012–1018. doi: 10.1016/j.neuint.2007.10.014

Gibbs, M. E., Lloyd, H. G., Santa, T., and Hertz, L. (2007). Glycogen is a preferred glutamate precursor during learning in 1-day-old chick: biochemical and behavioral evidence. *J. Neurosci. Res.* 85, 3326–3333. doi: 10.1002/jnr.21307

Gordon, G. R., Choi, H. B., Rungta, R. L., Ellis-Davies, G. C., and Macvicar, B. A. (2008). Brain metabolism dictates the polarity of astrocyte control over arterioles. *Nature* 456, 745–749. doi: 10.1038/nature07525

Harting, K., and Knoll, B. (2010). SIRT2-mediated protein deacetylation: an emerging key regulator in brain physiology and pathology. *Eur. J. Cell. Biol.* 89, 262–269. doi: 10.1016/j.ejcb.2009.11.006

Hein, T. W., Xu, W., and Kuo, L. (2006). Dilation of retinal arterioles in response to lactate: role of nitric oxide, guanylyl cyclase, and ATP-sensitive potassium channels. *Invest. Ophthalmol. Vis. Sci.* 47, 693–699. doi: 10.1167/iovs.05-1224

Hertz, L. (2011). Brain glutamine synthesis requires neuronal aspartate: a commentary. *J. Cereb. Blood Flow Metab.* 31, 384–387. doi: 10.1038/jcbfm.2010.199

Hertz, L., and Dienel, G. A. (2005). Lactate transport and transporters: general principles and functional roles in brain cells. *J. Neurosci. Res.* 79, 11–18. doi: 10.1002/jnr.20294

Hertz, L., and Rodrigues, T. B. (eds.). (2014). Astrocytic-neuronal-astrocytic pathway selection for formation and degradation of glutamate/GABA. *e-Book Frontiers in Endocrinology*.

Juel, C. (1991). Muscle lactate transport studied in sarcolemmal giant vesicles. *Biochim. Biophys. Acta* 1065, 15–20. doi: 10.1016/0005-2736(91)90004-R

Juel, C., and Halestrap, A. P. (1999). Lactate transport in skeletal muscle—role and regulation of the monocarboxylate transporter. *J. Physiol.* 517 (Pt 3), 633–642. doi: 10.1111/j.1469-7793.1999.0633s.x

Juel, C., Kristiansen, S., Pilegaard, H., Wojtaszewski, J., and Richter, E. A. (1994). Kinetics of lactate transport in sarcolemmal giant vesicles obtained from human skeletal muscle. *J. Appl. Physiol.* 76, 1031–1036.

Korf, J., and De Boer, J. (1990). Lactography as an approach to monitor glucose metabolism on-line in brain and muscle. *Int. J. Biochem.* 22, 1371–1378. doi: 10.1016/0020-711X(90)90225-R

Kumar, V., Carlson, J. E., Ohgi, K. A., Edwards, T. A., Rose, D. W., Escalante, C. R., et al. (2002). Transcription corepressor CtBP is an NAD(+)-regulated dehydrogenase. *Mol. Cell* 10, 857–869. doi: 10.1016/S1097-2765(02)00650-0

Laptook, A. R., Peterson, J., and Porter, A. M. (1988). Effects of lactic acid infusions and pH on cerebral blood flow and metabolism. *J. Cereb. Blood Flow Metab.* 8, 193–200. doi: 10.1038/jcbfm.1988.49

Lauritzen, K. H., Morland, C., Puchades, M., Holm-Hansen, S., Hagelin, E. M., Lauritzen, F., et al. (2013). Lactate receptor sites link neurotransmission, neurovascular coupling, and brain energy metabolism. *Cereb. Cortex* 24, 2784–2795. doi: 10.1093/cercor/bht13

Lombard, J. H. (2006). A novel mechanism for regulation of retinal blood flow by lactate: gap junctions, hypoxia, and pericytes. *Am. J. Physiol. Heart Circ. Physiol.* 290, H921–H922. doi: 10.1152/ajpheart.01268.2005

Madsen, P. L., Cruz, N. F., Sokoloff, L., and Dienel, G. A. (1999). Cerebral oxygen/glucose ratio is low during sensory stimulation and rises above normal during recovery: excess glucose consumption during stimulation is not accounted for by lactate efflux from or accumulation in brain tissue. *J. Cereb. Blood Flow Metab.* 19, 393–400. doi: 10.1097/00004647-199904000-00005

Madsen, P. L., Hasselbalch, S. G., Hagemann, L. P., Olsen, K. S., Bulow, J., Holm, S., et al. (1995). Persistent resetting of the cerebral oxygen/glucose uptake ratio by brain activation: evidence obtained with the Kety-Schmidt technique. *J. Cereb. Blood Flow Metab.* 15, 485–491. doi: 10.1038/jcbfm.1995.60

Mangia, S., Giove, F., and Dinuzzo, M. (2012). Metabolic Pathways and Activity-Dependent Modulation of Glutamate Concentration in the Human Brain. *Neurochem. Res.* 37, 2554–2561. doi: 10.1007/s11064-012-0848-4

Mangia, S., Simpson, I. A., Vannucci, S. J., and Carruthers, A. (2009). The *in vivo* neuron-to-astrocyte lactate shuttle in human brain: evidence from modeling of measured lactate levels during visual stimulation. *J. Neurochem.* 109 (Suppl. 1), 55–62. doi: 10.1111/j.1471-4159.2009.06003.x

Mangia, S., Tkac, I., Logothetis, N. K., Gruetter, R., Van De Moortele, P. F., and Ugurbil, K. (2007). Dynamics of lactate concentration and blood oxygen level-dependent effect in the human visual cortex during repeated identical stimuli. *J. Neurosci. Res.* 85, 3340–3346. doi: 10.1002/jnr.21371

McKenna, M. C. (2013). Glutamate pays its own way in astrocytes. *Front. Endocrinol.* 4:191. doi: 10.3389/fendo.2013.00191

McKenna, M. C., Hopkins, I. B., and Carey, A. (2001). Alpha-cyano-4-hydroxycinnamate decreases both glucose and lactate metabolism in neurons and astrocytes: implications for lactate as an energy substrate for neurons. *J. Neurosci. Res.* 66, 747–754. doi: 10.1002/jnr.10084

McKenna, M. C., Sonnewald, U., Huang, X., Stevenson, J., and Zielke, H. R. (1996). Exogenous glutamate concentration regulates the metabolic fate of glutamate in astrocytes. *J. Neurochem.* 66, 386–393. doi: 10.1046/j.1471-4159.1996.66010386.x

Moore, R. Y., and Bloom, F. E. (1979). Central catecholamine neuron systems: anatomy and physiology of the norepinephrine and epinephrine systems. *Annu. Rev. Neurosci.* 2, 113–168. doi: 10.1146/annurev.ne.02.030179.000553

Muir, D., Berl, S., and Clarke, D. D. (1986). Acetate and fluoroacetate as possible markers for glial metabolism *in vivo*. *Brain Res.* 380, 336–340. doi: 10.1016/0006-8993(86)90231-3

Nakamura, M., Bhatnagar, A., and Sadoshima, J. (2012). Overview of pyridine nucleotides review series. *Circ. Res.* 111, 604–610. doi: 10.1161/circresaha.111.247924

Nedergaard, M., and Goldman, S. A. (1993). Carrier-mediated transport of lactic acid in cultured neurons and astrocytes. *Am. J. Physiol.* 265, R282–R289.

Nordström, C. H., Nielsen, T. H., and Jacobsen, A. (2013). Techniques and strategies in neurocritical care originating from southern Scandinavia. *J. Rehabil. Med.* 45, 710–717. doi: 10.2340/16501977-1157

Öz, G., Berkich, D. A., Henry, P. G., Xu, Y., Lanoue, K., Hutson, S. M., et al. (2004). Neuroglial metabolism in the awake rat brain: CO2 fixation increases with brain activity. *J. Neurosci.* 24, 11273–11279. doi: 10.1523/JNEUROSCI.3564-04.2004

Pardo, B., Rodrigues, T. B., Contreras, L., Garzon, M., Llorente-Folch, I., Kobayashi, K., et al. (2011). Brain glutamine synthesis requires neuronal-born aspartate as amino donor for glial glutamate formation. *J. Cereb. Blood Flow Metab.* 31, 90–101. doi: 10.1038/jcbfm.2010.146

Peng, L., Zhang, X., and Hertz, L. (1994). High extracellular potassium concentrations stimulate oxidative metabolism in a glutamatergic neuronal culture and glycolysis in cultured astrocytes but have no stimulatory effect in a GABAergic neuronal culture. *Brain Res.* 663, 168–172. doi: 10.1016/0006-8993(94)90475-8

Poole, R. C., and Halestrap, A. P. (1993). Transport of lactate and other monocarboxylates across mammalian plasma membranes. *Am. J. Physiol.* 264, C761–C782.

Quistorff, B., Secher, N. H., and Van Lieshout, J. J. (2008). Lactate fuels the human brain during exercise. *FASEB J.* 22, 3443–3449. doi: 10.1096/fj.08-106104

Requardt, R. P., Hirrlinger, P. G., Wilhelm, F., Winkler, U., Besser, S., and Hirrlinger, J. (2012). Ca2+ signals of astrocytes are modulated by the NAD+/NADH redox state. *J. Neurochem.* 120, 1014–1025. doi: 10.1111/j.1471-4159.2012.07645.x

Roos, A., and Boron, W. F. (1981). Intracellular pH. *Physiol. Rev.* 61, 296–434.

Rothman, D. L., De Feyter, H. M., De Graaf, R. A., Mason, G. F., and Behar, K. L. (2011). 13C MRS studies of neuroenergetics and neurotransmitter cycling in humans. *NMR Biomed.* 24, 943–957. doi: 10.1002/nbm.1772

San Martin, A., Ceballo, S., Ruminot, I., Lerchundi, R., Frommer, W. B., and Barros, L. F. (2013). A genetically encoded FRET lactate sensor and its use to detect the Warburg effect in single cancer cells. *PLoS ONE* 8:e57712. doi: 10.1371/journal.pone.0057712

Schmalbruch, I. K., Linde, R., Paulson, O. B., and Madsen, P. L. (2002). Activation-induced resetting of cerebral metabolism and flow is abolished by beta-adrenergic blockade with propranolol. *Stroke* 33, 251–255. doi: 10.1161/hs0102.101233

Sibson, N. R., Dhankhar, A., Mason, G. F., Rothman, D. L., Behar, K. L., and Shulman, R. G. (1998). Stoichiometric coupling of brain glucose metabolism and glutamatergic neuronal activity. *Proc. Natl. Acad. Sci. U.S.A.* 95, 316–321. doi: 10.1073/pnas.95.1.316

Siesjö, B. K. (1978). *Brain Energy Metabolism.* Chichester: John Wiley and Sons.

Sun, F., Dai, C., Xie, J., and Hu, X. (2012). Biochemical issues in estimation of cytosolic free NAD/NADH ratio. *PLoS ONE* 7:e34525. doi: 10.1371/journal.pone.0034525

Tang, F., Lane, S., Korsak, A., Paton, J. F., Gourine, A. V., Kasparov, S., et al. (2014). Lactate-mediated glia-neuronal signalling in the mammalian brain. *Nat. Commun.* 5:3284. doi: 10.1038/ncomms4284

Van Hall, G., Stromstad, M., Rasmussen, P., Jans, O., Zaar, M., Gam, C., et al. (2009). Blood lactate is an important energy source for the human brain. *J. Cereb. Blood Flow Metab.* 29, 1121–1129. doi: 10.1038/jcbfm.2009.35

Von Korff, R. W., Steinman, S., and Welch, A. S. (1971). Metabolic characteristics of mitochondria isolated from rabbit brain. *J. Neurochem.* 18, 1577–1587. doi: 10.1111/j.1471-4159.1971.tb00019.x

Waniewski, R. A., and Martin, D. L. (1998). Preferential utilization of acetate by astrocytes is attributable to transport. *J. Neurosci.* 18, 5225–5233.

Whitelaw, B. S., and Robinson, M. B. (2013). Inhibitors of glutamate dehydrogenase block sodium-dependent glutamate uptake in rat brain membranes. *Front. Endocrinol. (Lausanne)* 4:123. doi: 10.3389/fendo.2013.00123

Wilhelm, F., and Hirrlinger, J. (2012). Multifunctional Roles of NAD(+) and NADH in Astrocytes. *Neurochem. Res.* 37, 2317–2325. doi: 10.1007/s11064-012-0760-y

Yamanishi, S., Katsumura, K., Kobayashi, T., and Puro, D. G. (2006). Extracellular lactate as a dynamic vasoactive signal in the rat retinal microvasculature. *Am. J. Physiol. Heart. Circ. Physiol.* 290, H925–H934. doi: 10.1152/ajpheart.01012.2005

Yu, A. C., Schousboe, A., and Hertz, L. (1982). Metabolic fate of 14C-labeled glutamate in astrocytes in primary cultures. *J. Neurochem.* 39, 954–960. doi: 10.1111/j.1471-4159.1982.tb11482.x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 09 July 2014; accepted: 04 August 2014; published online: 09 September 2014.
Citation: Hertz L, Gibbs ME and Dienel GA (2014) Fluxes of lactate into, from, and among gap junction-coupled astrocytes and their interaction with noradrenaline. Front. Neurosci. ***8****:261. doi: 10.3389/fnins.2014.00261*
This article was submitted to Neuroenergetics, Nutrition and Brain Health, a section of the journal Frontiers in Neuroscience.

frontiers in
NEUROSCIENCE

REVIEW ARTICLE
published: 19 November 2014
doi: 10.3389/fnins.2014.00360

Cerebral glycolysis: a century of persistent misunderstanding and misconception

Avital Schurr*

Department of Anesthesiology and Perioperative Medicine, University of Louisville School of Medicine, Louisville, KY, USA

Edited by:
Pierre J. Magistretti, École Polytechnique Fédérale de Lausanne, Switzerland

Reviewed by:
Juan P. Bolanos, University of Salamanca-Consejo Superior de Investigaciones Científicas, Spain
Igor Allaman, École Polytechnique Fédérale de Lausanne, Switzerland (in collaboration with Pierre J. Magistretti)

***Correspondence:**
Avital Schurr, Department of Anesthesiology and Perioperative Medicine, University of Louisville School of Medicine, Louisville, KY 40202, USA
e-mail: avital.schurr@gmail.com

Since its discovery in 1780, lactate (lactic acid) has been blamed for almost any illness outcome in which its levels are elevated. Beginning in the mid-1980s, studies on both muscle and brain tissues, have suggested that lactate plays a role in bioenergetics. However, great skepticism and, at times, outright antagonism has been exhibited by many to any perceived role for this monocarboxylate in energy metabolism. The present review attempts to trace the negative attitudes about lactate to the first four or five decades of research on carbohydrate metabolism and its dogma according to which lactate is a useless anaerobic end-product of glycolysis. The main thrust here is the review of dozens of scientific publications, many by the leading scientists of their times, through the first half of the twentieth century. Consequently, it is concluded that there exists a barrier, described by Howard Margolis as "habit of mind," that many scientists find impossible to cross. The term suggests *"entrenched responses that ordinarily occur without conscious attention and that, even if noticed, are hard to change."* Habit of mind has undoubtedly played a major role in the above mentioned negative attitudes toward lactate. As early as the 1920s, scientists investigating brain carbohydrate metabolism had discovered that lactate can be oxidized by brain tissue preparations, yet their own habit of mind redirected them to believe that such an oxidation is simply a disposal mechanism of this "poisonous" compound. The last section of the review invites the reader to consider a postulated alternative glycolytic pathway in cerebral and, possibly, in most other tissues, where no distinction is being made between aerobic and anaerobic glycolysis; lactate is always the glycolytic end product. Aerobically, lactate is readily shuttled and transported into the mitochondrion, where it is converted to pyruvate via a mitochondrial lactate dehydrogenase (mLDH) and then is entered the tricarboxylic acid (TCA) cycle.

Keywords: cerebral energy metabolism, glycolysis, lactate, mitochondrial LDH, NAD-NADH recycling, habit of mind

INTRODUCTION

More than 70 years ago, the identity and sequence of the reactions of glycolysis, also known as the *Embden-Meyerhof pathway*, were elucidated. Nevertheless, for the past 25 years investigators in the field of brain energy metabolism have been hotly debating the details of that sequence. A somewhat similar debate first took place among exercise physiologists and biochemists when Brooks (1985) published results showing that lactic acid (lactate) is the glycolytic product and the oxidative substrate during sustained exercise. Soon thereafter, a few studies by neuroscientists questioned the status quo in our understanding of how the brain handles increased energy requirements during stimulation. First, Fox and Raichle (1986) demonstrated a focal physiological uncoupling between cerebral blood flow and oxidative metabolism upon somatosensory stimulation in humans. Two years later Fox et al. (1988) showed that during focal physiologic neural activity the consumption of glucose is non-oxidative. Simultaneously, Schurr et al. (1988) demonstrated the ability of brain (hippocampal) slices to maintain normal synaptic function with lactate as the sole oxidative energy substrate. Many scientists in the field were surprised by these findings, while others discounted them (Chih et al., 2001; Dienel and Hertz, 2001; Chih and Roberts, 2003; Dienel and Cruz, 2004; Hertz, 2004; Fillenz, 2005). Despite the allowance of time necessary for new findings to overcome "habits of mind" (Margolis, 1993) or the incommensurability of "new" and "old" paradigms (Kuhn, 1996), the great debate has not subsided. Hence, lines have been drawn between two camps; one, still a majority, which discounts any key role for lactate in brain (and muscle) energy metabolism and another, a growing minority, which holds lactate as an important, and at times, crucial, oxidative substrate for energy production in the brain (and other tissues).

The unusual longevity of this debate is somewhat surprising. Being on the minority side of it, I have been intrigued by both its persistence and its emotional flair. The drive to settle the unresolved issues that continue to sustain this debate has prompted the following review of the recorded research on energy metabolism through the formative years of the field of biochemistry during the first half of the twentieth century. The aim of this review has been to uncover the basis and reasoning for lactate's long-lasting negative reputation among scientists and clinicians that has prevented its "rehabilitation" and thus its consideration as an integral

part of oxidative energy metabolism. Suspicions that lactate's ill reputation has contributed greatly to its dismissal as anything, but useless end-product of anaerobic energy metabolism, led me to search for recorded hints to discount any such suspicions. Upon reading through the troves of research papers of the past, it is clear that one cannot separate the science from the scientists who practice it. Disagreements among investigators in the fields of muscle and brain energy metabolism had already existed in the early decades of the twentieth century. Lactate, by the majority of interpretations of research results, had been considered for a long time to be a product that must be disposed of in order to achieve normalization of tissue functioning. This is despite findings by several investigators of brain energy metabolism in the 1920 and 1930s, who demonstrated the ability, especially of brain gray matter, to oxidize lactate. With an emphasis on glycolysis, this paper attempts to sort out as many as possible conceptions and misconceptions about (brain) energy metabolism in the formative years of modern biochemistry. A plausible explanation is proposed for how that great leap in knowledge, which occurred over seven decades ago, and the research that led to it, have shaped minds and beliefs both then and now. Guidance from the wisdom of three philosophers, Barber (1961), Kuhn (1996) and Margolis (1993) has been instrumental in this attempt to understand how scientific concepts and beliefs have determined both the direction and the pace of scientific progress in the field of energy metabolism.

DESPITE ITS DEFICIENCIES, THE DOGMA OF MUSCULAR GLYCOLYSIS, CIRCA 1900–1940, HAS REMAINED UNCHANGED AND ALMOST UNCHALLENGED, EVEN TODAY

As to the discovery of lactic acid, first in milk and then in muscles, the reader is directed to other sources including a recent review by Gladden (2008). However, before focusing on early brain energy metabolism, a close attention must be given to the pioneering research on muscle respiration and metabolism to comprehend its significant influence on how the former has been conducted and understood. The first strike against lactic acid was, of course, its association with sour (spoiled) milk. Upon its discovery in muscle, lactic acid was quickly blamed for muscle fatigue and rigor. Experiments were carried out specifically to test lactic acid effect on muscle respiration and rigidity. For instance, Fletcher (1898) refers in his research to preliminary reports showing "*that weak solutions of lactic acid (0.1–0.25%), upon injection through the blood vessels of a frog caused immediate rigidity of the muscles*" and to the suggestion that the development of lactic acid during survival (post excision) respiration (CO_2 discharge) periods was the cause of natural rigor mortis. Fletcher found out that any concentration of lactic acid he used (0.05–5.0%) produced rigor mortis in an excised frog *Gastrocnemius* muscle immersed in it. The higher the lactic acid concentration the quicker rigor mortis set in. Fletcher was a thorough investigator who published his studies in great detail. He has shown that the presence of oxygen prolonged the survival of excised muscle and measured the effect of oxygen on the rate of disposal of lactic acid from it as a way to bring the muscle back to a state of irritability (Fletcher and Hopkins, 1907). The opening paragraph of the authors' paper is very revealing as to how lactic acid was perceived then: "*For a generation it has been recognized that there are means available within the body by which the acid products of muscular activity may be disposed of, and there is already a large body of well-known evidence which indicates that this disposal of acid products—whatever the site of it may be—is most efficient when the conditions for oxidative processes are most favorable, and that it is incomplete when these conditions are unfavorable.*" These investigators set out to investigate the muscle's own means for an oxidative control of lactic acid formation and for the alteration or destruction of lactic acid, which has already been formed.

Locke and Rosenheim (1907) investigated the consumption of dextrose (glucose) by the isolated rabbit heart in an atmosphere of oxygen. They found that cardiac muscle when supplied with both dextrose and oxygen did not produce any lactic acid, similar to the findings in skeletal muscle. These investigators recognized that "*the oxygen supply of the heart in our experiments, although by no means so great as that in the intact organism, was doubtless sufficient to prevent the formation of a detectable amount of lactic acid.*" It should already be clear from the above few examples that prevention of lactate formation and/or its disappearance is simply a means to keep both skeletal and cardiac muscles functioning.

Understandably, with the negative reputation of lactic acid, no one would consider it to be anything but an anaerobic poisonous product that must be disposed of to assure the survival of healthy, respiring tissue or organ. Under such circumstances, the idea that a muscle could utilize lactate aerobically for the production of energy could not have any chance to emerge without direct scientific evidence to support it. And thus, the concept of "lactic acid as the culprit" in muscle fatigue and rigor mortis continued to be forwarded (Burridge, 1910), although research by others at the time (Barcroft and Orbeli, 1910) have pointed out that lactic acid is not all "bad news." The latter authors found lactic acid to be a valuable accessory in tissue respiration as carbonic acid is, i.e., "*when oxygen reaches the capillaries at a low tension, the lactic acid tends to turn the oxygen out of the blood.*" Nevertheless, Feldman and Hill (1911) investigated human oxygen inhalation during hard work and concluded "*that the increased production of lactic acid by the muscles is due to oxygen want, and that oxygen inhalation has a favorable influence, at any rate in part, by lessening the rise of acid concentration.*" Even Hill, who then had just begun his impressive work on the heat production of muscle contracture (Hill, 1910), explained his findings in a following study (Hill, 1911) thusly: "*the presence of O_2 diminishes the duration of the reaction which gives out heat,*

$$A + B + C \rightarrow ABC + Heat.$$

Hence we should expect O_2 to be one of the bodies participating in the reaction: for in that case the velocity would be, among other things, proportional to the concentration of free O_2 in the tissue. Thus, by increasing the O_2 tension in the tissue an atmosphere of O_2 would decrease, and similarly an atmosphere of H_2 would increase, the duration of the heat production. In this connexion, the experiments of Fletcher and Hopkins (1907) on the oxidative removal of lactic acid are very suggestive. They found that the presence of O_2 removed lactic acid, and presumably replaced it in its former position in the tissues." Hill attempted to explain muscle contraction using physical principles: "*On stimulation therefore certain*

molecules are thrown into solution, which before stimulation were lightly connected in some physical or chemical way with other bodies, so as to be inactive. The presence of these chemical molecules sets up a tension, possibly at certain colloidal membranes in the fiber: the tension falls again, owing to the diffusion of these chemical molecules into the general free space in the fiber, away from the sensitive membranes; the molecules are then oxidized, or replaced in their original positions, under the action of O_2, with an evolution of heat proportional to the amount of those bodies present."

In another study, Fletcher (1911) went after conflicting "evidence" regarding the chemical action involved in the formation of lactic acid in muscle and in other cells. He also justified his efforts since "*it has been urged by several observers in recent years that considerable and continued production of d-lactic acid* (the old nomenclature of L-lactic acid) *maybe found during autolysis (aseptic or antiseptic) of minced or crushed muscle long after the extinction of irritability and destruction of structure.*" Not surprising, "observers" were making a connection between muscle damage, its death and the production of lactic acid. Fletcher concluded from his studies that "*the evidence hitherto produced of an autolytic production of lactic acid by muscle cannot be accepted.*" Somewhat surprising conclusion in Fletcher's study is that "*no glycolytic enzyme leading to lactic acid formation appears to exist in muscle. After the addition of dextrose to intact surviving muscle, or to preparations of disintegrated muscle, no increase of lactic acid is found in the absence of bacteria.*" Peters (1913), using Hill's calorimeter for heat production measurements combined with those of lactic acid production confirmed both Hill's (1911) and Fletcher and Hopkins's (1907) findings, concluding that "*heat production and lactic acid liberation in fatiguing amphibian muscle are extremely intimately connected.*" Later, Fletcher (1913) repeated his amphibian muscle studies with several mammalian muscles, essentially concluding that muscles from both of these sources are similar in their survival respiration and lactic acid production. That very year, Hill (1913) published results on muscle heat production using a newly designed and constructed "*thermoelectric apparatus with which it was possible to estimate very rapidly the rise of temperature of muscle, if necessary to within a millionth of a degree.*" In the summary of his paper Hill suggested "*that the processes of muscular contraction are due to liberation of lactic acid from some precursor, and that the lactic acid increases the tension in some colloidal structure of the tissue: that the lactic acid precursor is rebuilt after the contraction is over in the presence of, and by the use of oxygen, with the evolution of heat: and finally that the heat liberated by the muscle excited in the complete absence of oxygen is due simply to the breakdown of the lactic acid precursor, and is the same in nature as the heat-production of rigor.*"

Again, the conclusion was that lactic acid induces muscle contraction via a physico-chemical process and, if not disposed of, would result in fatigue and rigor mortis. Roaf (1914) employed an electro-chemical method that recorded increases in acidity when muscle contracts. He, too, concluded "*that the increase in acidity is the cause of the shortening of muscle.*" Moreover, using his heat production measurements of muscle contraction, Hill presented calculations and arguments in the Proceedings of the Physiological Society on February 14, 1914 (Hill, 1914) in support of the hypothesis that lactic acid formed in the muscle after activity is not removed by the process of oxidation, but rather by a process of replacement into its previous position (sugar). He thus argued as follows: "*The production of 1 grm of lactic acid is accompanied by the evolution of about 450 calories. Now I have shown that during the recovery processes of muscles in oxygen there is a 'recovery heat-production' of about the same order of size as the heat-production occurring in the initial processes of contraction. In the oxidative removal of 1 grm of lactic acid therefore there is a heat-production of about 450 calories. Now, the oxidation of 1 grm of lactic acid leads to heat-production of about 3700 calories, which is about eight times as large as the quantity observed… Therefore, apparently the lactic acid is not oxidized but replaced in its previous position under the influence and with the energy of the oxidation, either (a) of a small part of the lactic acid itself, or (b) of some other body. Evidence given elsewhere shows that it must be some other body. The lactic acid therefore is part of the machine and not part of the fuel.*" Hill voiced his position and, eventually, the position of the majority of his colleagues, that lactic acid is not a fuel, since the expected heat-production of its oxidation was much lower than the calculated value of its complete combustion. It is surprising that Hill would argue that if lactate were a fuel, all the energy of its oxidation would be released as heat. In essence, Hill's own measurements that lactic acid oxidation produces only 12% of the expected heat-production should have indicated to him and others that the majority of the energy released from this oxidation, not measured as heat, could indicate controlled utilization and/or possibly a conversion to some other forms of energy. Nevertheless, Hill's and others' prevailing position on "lactic acid is not a fuel" has endured to the present day.

Fletcher and Brown (1914) also looked into the Inogen theory, according to which, the discharge of energy by the muscle cell—and by inference its discharge by any other cell—depends upon the dissociative breakdown of some labile molecule (Inogen). For the breakdown to occur, oxygen takes Inogen's place beforehand in such a manner that upon the dissociation of the molecule the energy yielded is due to combustion, and the final products, carbonic acid and water, represent the result of that combustion. Furthermore, lactic acid, which supposedly also arises from Inogen, was considered to be either another final product or an intermediate product destined to be used in future reconstruction of the Inogen complex. Based on their experimental results Fletcher and Brown concluded that CO_2 and lactic acid do not originate from a common source. They emphatically asserted "*that in the muscle the respiratory oxidative process yielding CO_2 as an immediate product has its chief end in the supply of energy for replacing the lactic acid in the molecular position from which stimulation of some kind has displaced it, and there appears to be no reason at present for supposing that the material which is oxidized in the respiratory process is the same as, or related to, the material from which lactic acid appears and into which, as it seems, it may again disappear.*" Hence, the leading investigators in the field held that lactic acid is a separate entity from the one that is oxidized during muscle respiration and which yields energy and CO_2.

Meanwhile, other investigators were attempting to explain cocaine's racking effects on its users or the devastation of diabetes through the increased tissue production of lactate. Underhill and

Black (1912) studied the influence of cocaine on the metabolism of dogs and rabbits receiving daily injections of the drug. With daily doses of cocaine (20 mg/kg), lactic acid excretion in the urine was markedly increased in well-fed animals. The investigators concluded that the increase in lactic acid elimination in the urine is unlikely associated with increased muscular activity induced by the drug. They also stated that "*lactic acid and carbohydrate metabolism are presumably intimately associated although there are indications that lactic acid may at times arise from more than a single antecedent*." This statement appears to be an attempt to tie lactic acid to another process besides carbohydrate metabolism, one that might be responsible for the effect of cocaine. Where diabetes was concerned, the famous Ringer (1914) stated that "*parallelism exists between the degree of acidosis and the degree of disturbance in the carbohydrate metabolism*." Considering the fact that at that time the role of insulin was unknown and glycolysis was yet to be elucidated, the repeated use of this kind of statements about lactic acid and acidosis was part of an accepted and, almost expected, vernacular. Interestingly, Ringer theorized that diabetic mechanism involves the inability to form the glucoside bond of glycogen. Marriott (1914) in his quantitative study of blood acidosis in diabetes cited a discussion of diabetic acidosis by Magnus-Levy in John Hopkins Hospital Bulletin (Magnus-Levy, 1911) who expressed the view that the "*acid poisoned animal and the diabetic patient do not die from the acid which has been eliminated in the neutralized state, but from the acid which remains in the body*."

By 1916, the general consensus had been "*that sugar is utilized by muscle as a source of energy and the main product of its activity is carbon dioxide*" (Tsuji, 1916). Consensus had not yet achieved "*with regard to the origin of lactic acid formed in the tissues. Some authors ascribe it to the disintegration of carbohydrates (glucose), while others suggest that deaminization of amino acids (alanine) is its source*." Tsuji (1916), a researcher from Kyoto, Japan, working at the Institute of Physiology, University College, England, employed a heart lung preparation in his studies and summarized his findings as followed: "*1. Lactic acid is produced in the circulating blood of the heart lung preparation under conditions approaching normal. 2. These results may indicate that lactic acid is one of the normal metabolites of muscular activity. 3. The formation of lactic acid is increased in poisoning of chloroform and in the presence of deficient supply of oxygen in a heart lung preparation. 4. When the heart beat is accelerated by adding adrenalin or amino-acid (alanine, glycine or ereptone) to the circulating blood, or the heart work is increased by alterations in the blood-pressure, lactic acid is not only not produced, but the lactic acid previously contained in the circulating blood disappears*." Finding number 4 of Tusji might be the first time that aerobic lactate disappearance by the heart was mentioned, although the author could not, of course, have had any inclination to consider the possibility of oxidative utilization lacking supportive evidence.

As scientists appear to form an understanding of lactate intermediary role in energy metabolism, efforts to assign other "roles" did not subside. Ito (1916) confirmed the accidental finding by his countryman, Tatsukichi Irisawa, of the presence of lactic acid in pus, and went ahead to determine that *d*-lactic acid (an old nomenclature analogous to today's L-lactic acid) is a constant constituent of pus and is distinctly increased by the autolysis of pus.

Although investigations into the possible enzymatic (ferments) nature of glycolysis were pursued as early as the dawn of the twentieth century, doubters and supporters of a glycolytic enzyme system being an integral part of muscle and other tissues questioned each other's findings as late as 1917. Ransom (1910) was able to prepare ferments from frozen plasma capable of converting glucose or glycogen into lactic acid, CO_2 and alcohol. Moreover, he was able to precipitate the plasma with alcohol-ether and to obtain a powder which was similarly active, although not with the same velocity. Most interestingly was Ransom's statement that "*there is reason for thinking that the production of lactic acid precedes that of carbon dioxide in the process of fermentation in muscle plasma*." Of course, lactate production that is followed by CO_2 production could mean lactate oxidation. However, such a language in those days could not be spoken. By 1917, Hoagland and Mansfield reaffirmed the glycolytic properties of muscular tissue, also demonstrating that dead tissue, while capable of glycolysis and lactic acid production, did not produce CO_2. The prevailing understanding had been that most of the CO_2 produced during muscle work is due to lactic acid production, which brings about CO_2 release from bicarbonate in muscle and blood. Moreover, it was believed that the CO_2 thus produced stayed within the muscle upon and immediately after the muscle work, assuming that this CO_2, together with the lactic acid, must necessarily remain inside the muscle fiber itself.

Adam (1921), working on oxygen consumption in muscle and nerve, also rejected the "inogen" idea and speculated that "*at the moment of contraction, the muscle fiber must work by drawing on stores of potential energy with the tissue, and it appears that the function of the oxidations is to restore to its normal resting level. The muscle fiber is further so constructed that the demand for replenishment of these stores of potential energy, available for future activity, is automatically supplied: for the activity of the cell leaves behind a condition leading immediately to an accelerated oxidation*." Adam also speculated that the muscle's "*resting respiration is an index of an anabolic process, compensating, and proceeding at an equal rate with, some such catabolic process as the survival formation of lactic acid, observed to occur in resting tissues at a constant rate*." Clearly, the prevailing notion was, and still is in some circles today, that the working muscle does it anaerobically, utilizing energy stores (carbohydrates) to contract, while producing lactic acid. Any oxidative process that takes place comes after the initial non-oxidative one, where its main purpose is to replenish the energy stores, thus the repeated efforts that were made to show carbohydrate production from lactate under aerobic conditions. Foster and Moyle (1921) also attempted to find answers to "*the fate, during recovery in oxygen, of the lactic acid formed in the muscle during fatigue or survival*." They stated the known facts thusly: "*The contraction of muscle is a strictly anaerobic process, and is accompanies by the production of lactic acid. The recovery process is dependent on the presence of oxygen, and is accompanied by the removal of lactic acid*." These investigators showed carbohydrate production (mainly as glycogen) upon muscle recovery in oxygen and a corresponding decline in lactic acid content.

Hartree and Hill (1922, 1923) were interested in investigating how the lactic acid produced in the working muscle is accommodated within the muscle in addition to the CO_2 already there, without raising the hydrogen ion concentration that is likely to destroy the muscle colloidal structure. The authors concluded from their experiments that in muscle, as was known for blood, there is a buffer mechanism, which is much more effective than a bicarbonate solution. They, along with Otto Meyerhof, assumed this buffer mechanism to be an alkali-protein salt capable of neutralizing acid. The concept that working muscle produces lactic acid aerobically and that the CO_2 released in the process is all due to the acid action on bicarbonate in the tissue, still holds today. Holden (1924) who investigated the "respiration substance" (Meyerhof's term for the enzymes responsible for the glycolytic process) of mammalian muscle, showed that this substance is heat labile and in reality is a collection of irreversibly oxidizable substances, although lactic acid is not one of them.

A complicating issue in glycolysis is the relationship between lactate and glycogen in muscle and, eventually, in other tissues, including brain. Otto Meyerhof and Archibald Hill were co-awarded the Nobel Prize in Physiology or Medicine in 1923 for their discovery of the fixed relationship between the consumption of oxygen and the metabolism of lactic acid in the muscle. Although the importance of the conversion of glycogen to lactate in muscle is still under debate today (Shulman and Rothman, 2001), both Meyerhof and Hill, the two most dominating scientists of their time in the field of muscle energy metabolism, had a long-lasting influence on the direction and progress of that field. The next section of this monograph deals with their influence on both the research and the researchers of brain energy metabolism.

By the mid-1920s, "the lactic acid as a trouble maker" had become a "habit of mind" (Margolis, 1993) and the tendency to look for lactate as the culprit in any disorder or abnormal condition was almost a given. Ronzoni et al. (1924) measured lactic acid production during ether anesthesia, since acidosis had been reported to be one of its consequences. These investigators concluded that "*1. Accumulation of lactic acid accounts in a large part for the acidosis of ether anesthesia. 2. Its increase is independent of CO_2 tension and produces the changes in pH rather than being itself controlled by pH… 3. Decreased oxygen supply to tissues does not account for its production. 4. The source of lactic acid seems to be the muscle tissue. 5. Production of lactic acid in the muscle, together with loss of phosphate from the muscle, during anesthesia, points to a breakdown of some hexose phosphate, such as the Embden's 'lactacidogen'.*" These findings disagreed with those of Koehler (1924) who demonstrated that the acidosis during ether anesthesia "*is the summation effect of CO_2 excess and alkali deficit. The CO_2 excess is the result of inefficient respiration probably caused by decreased sensitiveness of the respiratory center.*" Koehler et al. (1925) expanded their studies to measure the production of acidosis by anoxemia and concluded that "*anoxemia is fundamentally of an acidotic nature as far as disturbances in the acid-base balance are concerned.*" In essence, the authors continue to argue that, although lactic acid production continues to rise during axonemia, the amounts are relatively small and thus, they "*…do not presume to state what is the nature of the acidity.*" Evans (1925) investigated the role of lactic acid in resting striated muscle. Here are some of his findings: "*Lactic acid rapidly accumulates in plain muscle when this is kept under anaerobic conditions, but scarcely at all when kept in oxygen*" and "*The oxygen usage of resting plain muscle indicates that the recovery process in oxygen is of much the same nature as that in skeletal muscle; actually the fraction oxidized under experimental conditions was about one third. Owing, it is thought, to the errors incidental to the determination of small amounts of glycogen, it has not been possible, up to the present, to demonstrate that lactic acid arises from glycogen, or indeed, from any carbohydrate. In any case the glycogen content of the tissue is small, though, when allowance is made for the possible errors of experiment, perhaps large enough to make it possible that glycogen is the parent substance from which lactic acid is formed.*"

Clearly, despite the recognition by the Nobel committee given to Meyerhof for his work on glycogen and lactate, doubts persisted about this polycarbohydrate or any carbohydrate as a source of lactate. Riegel (1927a) demonstrated that severe blood hemorrhage in dogs caused an increase in blood lactic acid concentration and that the total increase and its duration were dependent upon the extent of the hemorrhage. Riegel also experimented with injecting sodium lactate to dogs and followed its disappearance (Riegel, 1927b). She summarized her findings thusly:

A. "*Sodium lactate injected into dogs in large amounts is readily removed from the blood. The removal may be divided into two phases*:
1. *A rapid decrease in concentration of lactic acid in the blood due to diffusion of lactic acid from the blood to other body fluids.*
2. *A slower decrease in concentration due to utilization of lactic acid by the tissues.*

B. *Injection of sodium lactate causes an immediate decrease in inorganic phosphate in the blood and a delayed rise in the sugar of blood.*

C. *The conclusion is drawn that lactic acid injected into the blood is synthesized to lactacidogen and glycogen by a process analogous to removal of lactic acid formed in muscle exercise*."

Although the author indicated in her summary that part of the decrease in blood lactate after an injection of sodium lactate is due to lactate utilization by tissues, she did not mean to indicate oxidative utilization for the production of energy, but rather to indicate utilization in the synthesis of glycogen. The postulated synthesis of lactacidogen had meant to indicate the formation of a hexose diphosphate from lactate and inorganic phosphate as was suggested at the time by Embden himself.

While the above list of cited papers is just but a part of a much longer list, it does convey the general gist of the principles by which scientists of the day were guided in their attempts to elucidate the chemical reactions of aerobic and anaerobic glycolysis. Central to all these studies is muscle tissue and its glycolytic formation of lactate, always anaerobically and mainly through the breakdown of glycogen and, when aerobic oxidation occurred, only after muscle contraction, its main purpose is to remove the accumulated lactate and the accompanied acidosis, and hence, the lactate's reputation as the "black sheep" of energy metabolism. Scientists who were involved in muscle

glycolytic research vastly outnumbered those who researched brain glycolysis. Naturally, most of the published findings on muscle energy metabolism greatly influenced not only how muscle researchers related to the sometimes "outlying" findings of brain researchers, but even more striking is how brain researchers had related to their own findings, always examining and measuring them with a "muscular" yardstick. This was the "affliction" of the small scientific community that investigated cerebral glycolysis in the early years of the twentieth century. That community considered lactate to be a useless end-product that must be rid of via oxidation. This habit of mind (Margolis, 1993) would become abundantly clear as the work of these scientists is reviewed and analyzed in the following section.

THE STUDY OF CEREBRAL GLYCOLYSIS, CIRCA 1900–1940, WAS GREATLY INFLUENCED BY THE MUSCULAR DOGMA AND IS BEING LARGELY IGNORED AND FORGOTTEN TODAY

In a very early paper Hill and Nabarro (1895) compared the exchange of blood-gasses in brain and muscle during and after tonic and clonic epileptic episodes induced by intravenously injecting essential oil of absinthe to the animal (presumably a dog). From those experiments and based on the results comparing oxygen and carbonic acid content in arteries and veins of muscle and brain, the investigators concluded that "*the brain is not a seat of active combustion, and considering the very small increase in CO_2 in the torcular blood it seems to us very improbable that the temperature of the brain should be perceptibly greater than that of the blood.*"

According to Holmes (1932), who authored the very first review paper on brain and nerve energy metabolism, "*Tashiro was the first worker to show that nerve produced CO_2 and ammonia during its metabolism*" in 1913. Later, others "*investigated the gaseous metabolism of nerve, and all of these workers are agreed that nerve uses oxygen and produces CO_2 during rest, and that these processes are intensified during activity.*" By 1921, Adam had shown that not like resting muscle, the sciatic nerve exhibited a very small effect of stimulation on its respiration rate. "*Even tetanising currents of one minute's to half-an-hour's duration gave a very small total effect, if any…*" Nevertheless, work in Hill's laboratory (Gerard et al., 1927) had shown that nerve (the frog sciatic nerve) produces a measurable amount of heat, which increased during activity (electric stimulation) and of a magnitude that agreed with the magnitude of oxygen consumption. Holmes (1932) in his review indicated the fundamental importance of the above findings as a conclusive proof that "*nervous impulse is a chemical affair*."

Eric G. Holmes had established himself as a leading investigator of brain energy metabolism beginning with a paper he and his wife, Barbara E. Holmes, published in 1925 (Holmes and Holmes, 1925a). That preliminary publication followed "[T]*he work of Warburg, Posener and Negelein in 1924 who showed that brain tissue is capable of converting large amounts of glucose into lactic acid.*" For that preliminary investigation the Holmes compared glucose metabolism of the brain in a normal animal (rabbit) and in an animal suffering from the effects of a convulsive dose of insulin. They summarized their findings as follows: "*…there is no marked change in the amount of reducing substance as a result of insulin administration.*" By "reducing substance" the authors meant carbohydrates. "*The reducing substance of brain is not capable of giving rise to the formation of lactic acid, although in similar conditions, abundance of lactic acid is formed by the brain from added glucose. Determinations of "resting" lactic acid on the brains of normal and of "insulin" rabbits show a greatly reduced lactic acid formation in the latter case. Neither in "normal" nor in "insulin" brains is there an increase in lactic acid formation over the "resting" value after standing or incubation at body p_H.*" In a follow-up study, Holmes and Holmes (1925b) determined that a fall in brain lactic acid levels of insulin-treated rabbits "*does not occur until the blood-sugar has reached a fairly low level.* They concluded *that the fall in the resting lactic acid content of brain after insulin injection is not due to a direct effect of insulin in promoting increased oxidation of lactic acid, nor to any direct effect of insulin or an accompanying impurity in depressing the production of lactic acid by the brain cells, but is rather caused by the fall in the blood-sugar level, and the resulting shortage of glucose in the brain.*" By 1926, the Holmes published a detailed study in which they measured the levels of both glycogen and lactate in rabbit brains. They found the content of the former to be "*small, and very variable,*" a finding that they speculated could be the outcome of the procedure of brain tissue preparation through which there might be a rapid breakdown of glycogen. "*The lactic acid content of rabbits' brains shows no appreciable rise, nor does the glycogen content show any significant fall, when the chopped tissue is kept at room temperature, or incubated under anaerobic conditions at alkaline p_H. Under aerobic conditions, lactic acid rapidly disappears from chopped brain, but the glycogen suffers no significant change. It is suggested that the brain depends upon blood sugar, rather than on any other substance which it stores itself, for lactic acid precursor.*" These investigators thus established that glucose is the precursor of lactic acid in the brain and that under aerobic conditions lactic acid content decreases. Further, the Holmes team (1927) also showed that brain's "*lactic acid formed from glucose supplied by the blood and that the values of lactic acid in the brain fall and rise with the blood sugar, both in hypo- and hyperglycaemic condition*." In addition, they found that "*the brain tissue of diabetic, like that of normal animals, is capable of converting glucose to lactic acid, and of removing lactic acid under aerobic conditions.*"

By 1929, Ashford and Holmes had delved into investigating the part played by inorganic phosphate in the production of lactic acid from carbohydrate in brain tissue. This followed the studies on muscle and yeast metabolism that had shown the prominent role phosphate plays in carbohydrate metabolism. The investigators were somewhat surprised that their findings did not line up with the role of phosphate in muscle and yeast carbohydrate metabolism. They summarized their study thusly:

"*1. Inorganic phosphate is liberated from brain tissue both anaerobically and aerobically, and in the presence as well as in the absence of glucose. No evidence of hexosephosphate synthesis has been found at any stage in the process of formation of lactic acid, although the tissue is capable to a small extent of performing this synthesis.*

2. Both phosphate liberation and lactic acid production from glucose by brain tissue are inhibited by sodium fluoride, but, whilst the former is affected only by a high fluoride concentration, the latter is sensitive to very high dilutions of the salt. No quantitative relationship can be traced between the amounts of phosphate and lactic acid which are prevented from appearing by fluoride.

3. Lactic acid is freely formed from glucose, even when all available phosphate is immobilized. The velocity of lactic acid formation from glucose is not increased by the replacement of phosphate.

4. Much less lactic acid is formed from glycogen than from glucose; the process is inhibited by fluoride and by immobilizing phosphate. It can be restored by replacing phosphate.

5. It is concluded that brain tissue possesses two mechanisms of lactic acid formation: one, involving glucose, is quantitatively the more important, and is independent of phosphate; the other is much smaller, involves glycogen, and depends on the availability of phosphate." Ashford and Holmes (1929) and Holmes in a followed up study (1930) have thus demonstrated for the first time a correlation between lactic acid disappearance and oxygen consumption i.e., an aerobic utilization of lactate in brain tissue. Moreover, they show the ability of sodium fluoride (NaF) to inhibit the conversion of glucose to lactate and concomitantly to inhibit oxygen consumption, making use of the first known glycolytic inhibitor. Furthermore, Holmes (1930) found out that NaF completely blocked oxygen consumption in the presence of glucose in brain gray matter preparation. However, if glucose was replaced by lactate, no inhibition of oxygen consumption was observed. And thus, Holmes concluded "*that glucose must be converted into lactic acid before it can be oxidized by the gray matter*." This straight forward conclusion, as will be discussed later, has been ignored now for more than 80 years. Holmes and Ashford (1930) and Ashford and Holmes (1931) have also related to a ratio known as the "Meyerhof quotient," which was established by Meyerhof in muscle as: *Total lactic acid disappearing/Lactic acid oxidized*. This ratio was determined to have a value of approximately 3, and was used by Meyerhof and colleagues to support a proposal known as the "Meyerhof cycle." Accordingly, when lactic acid is added to an oxygenated muscle tissue, the amount of lactic acid disappearing is approximately three times greater than the amount of oxygen consumed in the process. That finding led Meyerhof to propose that the extra lactic acid disappearing beyond what could be accounted for by oxygen consumption must be recycled to a carbohydrate. Expecting to confirm the existence of a similar "Meyerhof quotient" in brain to that of muscle, Ashford and Holmes were unable to demonstrate a "Meyerhof quotient" greater than 1 in oxygenated brain tissue, which prompted them to state that "*there is no synthesis of carbohydrate from that portion of lactic acid which disappears but is not accounted for by O_2 uptake*." Moreover, they believed that their *"experiments throw doubt on the reality of the alleged 'Meyerhof cycle' in the case of cells in which the actual synthesis of carbohydrate has not been demonstrated by chemical estimation*." In addition, Holmes and Ashford found that the O_2 uptake in oxygenated brain tissue shaken with lactate in the presence of bicarbonate buffer in an O_2/CO_2 atmosphere is greater than in the presence of phosphate buffer and that such uptake increases with increased oxygen tension in both cases. They also related to the "Meyerhof quotient" as the "respiratory quotient" and found its value, both of brain tissue alone and of tissue oxygenated with extra oxygen, to be close to unity, including in the case of brain from animals rendered hypoglycemic by insulin injection. They concluded that lactate oxidation is unlikely to spare the utilization of another substrate.

In as much as these investigators clearly demonstrated the ability of brain tissue to oxidize lactate, it never occurred to them that the monocarboxylate could be an energy substrate in the brain. The influence of the "muscle school" prevented them from considering lactate to be more than just a substance that the brain is able to get rid of via oxidation. The fact that they could not observe a sparing effect of lactate on other substrates such as glucose also prevented them from thinking of lactate as a substrate. Thus, despite the significant differences they observed between muscle and brain tissues, where lactate was concerned, the scientific community in those days did not change its consideration of lactate as a useless by-product of carbohydrate metabolism, if not worse. Nevertheless, in a paper published in 1933, Holmes hinted at the possibility that lactate oxidation could support brain activity. And yet, a year earlier Quastel and Wheatley (1932), published their studies where they measured oxidations of different substrates by different brains using the Barcroft differential manometer. To increase the accuracy of their measurements, they allowed the brain preparation to become greatly depleted of its oxidizable materials before substrates were added. First they found that *"the rate of oxidation of added substrate to the brain varies inversely to the size of the animal*," a generalization that does not apply to the muscle. More importantly, for the purpose of the present paper, is their finding that "*glucose, sodium lactate and sodium pyruvate at equivalent concentrations are oxidized at approximately the same rate by brain tissue*." Also, by their estimates, lactate was completely oxidized by brain tissue. The investigators also found the toxin, iodoacetic acid (IAA), to inhibit the oxidation of glucose by brain. Although they could not categorically state that the inhibition of glucose glycolysis by IAA (and by NaF) is "*evidence that glucose necessarily passes through lactic acid for its oxidation to take place*," they had clearly considered it as a strong possibility, unlike Holmes (1930), who all but concluded just that. Interestingly, Quastel and Wheatley mentioned that oxalate inhibits glucose oxidation, but unlike IAA and NaF, also inhibits the oxidation of lactate. Clearly, these investigators were not privy then to the existence of lactate dehydrogenase (LDH), which is known to be inhibited by oxalate and by its derivative, oxamate (Schurr and Payne, 2007). Dixon (1935) had reproduced the results of Holmes & Ashford and Quastel & Wheatley, detecting no formation of lactic acid from glucose by brain tissue in oxygen. Dixon surmised that if there is any lactate produced under those conditions, it is produced at a rate slow enough to be removed by complete oxidation, and thus, concluded *"that oxygen exerts its sparing effect on glycolysis at some point in the system prior to the formation of lactic acid*." Again, despite the clear observation that lactic acid is oxidized completely in oxygenated brain tissue, the dogma that such oxidation has only one purpose, i.e., rid the tissue of its presence, has always prevailed. And since very little or no lactic

acid formation from glucose was detected under oxygen atmosphere, the interpretation of that outcome has been that oxygen spares the tissue from forming lactic acid glycolytically. Hence, the "habit of mind" (Margolis, 1993) regarding lactate as a useless by-product of anaerobiosis has entrenched itself also in the minds of the scientists who worked with brain tissue, where lactate oxidation was established and where several of them specifically voiced, based on data from their own studies, that for glucose to be oxidized it must be first converted to lactate. Consequently, and against their own observations, these investigators never considered that the ability of the tissue to oxidized lactate could have any other purpose, besides being a mechanism aimed at the removal of lactic acid from the tissue.

HAS HABIT OF MIND PLAYED A CONTINUOUS ROLE IN MISCONSTRUING THE GLYCOLYTIC PATHWAY?

Has there been a chance that lactate oxidation would imply anything else, but the purging mechanism of the monocarboxylate from the tissue? Could any of the investigators working on the glycolytic breakdown of glucose during the first 40 years of the twentieth century, and especially those who worked with brain tissue, had a chance to interpret these reactions differently? Reviewing the history of the research that had led to the elucidation of the sequence of the glycolytic pathway and considering the possible mindsets of the scientists working in the field, then and today, I believe that the answer to these questions would be "no." By the late 1930s and early 1940s the cumulative work of some of the leading researchers in the field of bioenergetics, including Meyerhof, Embden, Zimmerman, Fisk, and Subbarow, Lohmann, Kiessling, Cori and Cori, Warburg and many others, had already determined that there are two separate types of glycolysis, aerobic and anaerobic. Accordingly, the former ends up with pyruvate, while the latter ends up with lactate. Eventually, the demonstration that brain tissue is able to completely oxidize lactate did not sway Holmes, Ashford, Quastel and other investigators of brain carbohydrate metabolism to consider other purpose(s) of such a reaction, despite their own speculation that for glucose to be fully oxidized this process must proceed via the formation of lactate. They were all guided (misguided) at the time by the dominant habit of mind and fully accepted the dogma held by the investigators who worked on muscle carbohydrate metabolism, according to which, lactate is a useless end-product of anaerobic glycolysis that the tissue must rid itself of by any means possible. Moreover, the publication of the possible sequence of the citric acid cycle, known today as the tricarboxylic acid (TCA) cycle (Krebs and Johnson, 1937a,b,c; Krebs et al., 1938), 3 years prior to the final elucidation of the glycolytic pathway, had probably strengthened and deepened the hold of that dogma. Krebs and Johnson (1937a,c) proposed, alas with a question mark (see below), that the carbohydrate derivative that interacts with oxaloacetate to form citrate in the TCA cycle is pyruvate. Krebs et al. (1938) opened their paper with the following paragraph: "*From experiments reported in a previous paper (Krebs and Johnson, 1937a) we concluded that carbohydrate is oxidized in animal tissues through the following series of reactions:*

Considering the importance of Krebs and Johnson's work, for which the former was awarded the 1953 Nobel Prize in Physiology or Medicine, the suggestion that pyruvate is the glycolytic product entering the TCA cycle had undoubtedly been of great influence on the elucidators of the sequence of the glycolytic pathway. However, in those days, the role of mitochondria in respiration and the fact that the enzymes of the TCA cycle are located in these organelles were still unknown. Moreover, none of these scientists could have known that mitochondria also contain in their membrane the enzyme lactate dehydrogenase (LDH) (Brandt et al., 1987; Brooks et al., 1999b; Hashimoto et al., 2006; Atlante et al., 2007; Schurr and Payne, 2007; Lemire et al., 2008; Passarella et al., 2008; Gallagher et al., 2009; Elustondo et al., 2013; Jacobs et al., 2013), an enzyme that can easily convert lactate to pyruvate.

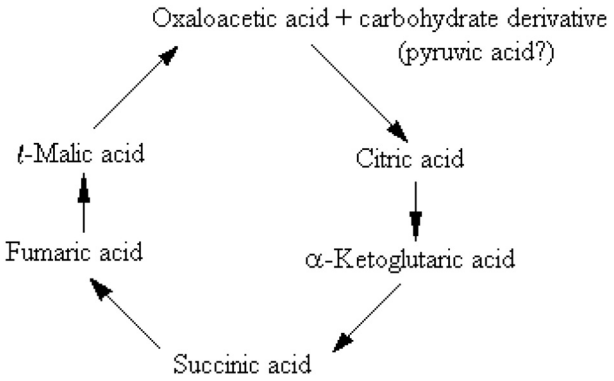


Table 1 lists the references cited and quoted from circa 1900–1940 and summarized their principal findings and interpretations.

Nevertheless, even today, more than seven decades after the puzzle of the glycolytic pathway sequence has been resolved, including the identity of its enzymes, substrates and products, if one were to open any of the hundreds of biochemistry textbooks that were published since 1940, glycolysis is described as a process of two separate biochemical pathways. These are described as an aerobic and an anaerobic glycolysis, similar to each other in every enzyme, substrate and product, except for the terminal reaction of the anaerobic one, in which pyruvate is converted to lactate, a conversion catalyzed by lactate dehydrogenase (LDH). Here's a typical description of glycolysis in the fourth edition of *Biochemistry* by Stryer (1995): "*Glycolysis is the sequence of reactions that convert glucose into pyruvate with the concomitant production of a relatively small amount of ATP. In aerobic organisms, glycolysis is the prelude to the citric acid cycle and the electron transport chain, which together harvest most of the energy contained in glucose. Under aerobic conditions, pyruvate enters mitochondria, where it is completely oxidized to* CO_2 *and* H_2O*. If the supply of oxygen is insufficient, as in actively contracting muscle, pyruvate is converted to lactate.*" This is the dogma that has survived unchanged and mostly unchallenged for all these years. Even in its most recent, seventh edition, *Biochemistry* (Berg et al., 2012) is a textbook that describes glycolysis in somewhat more detail, but unchanged in principles from its 1995 edition. And although one can accept and understand why and how this dogma was developed and formulated with the knowledge that was available in the first half of the twentieth century, the knowledge available today presents several dilemmas that many scientists have chosen to ignore or circumvent

Table 1 | Circa 1900–1940 cited articles on muscular and cerebral glycolysis: The main findings and their interpretations.

Hill and Nabarro, 1895—Compared the exchange of blood gasses in brain and muscle during and after epileptic episodes induced by essential oil of absinthe in the dog. Conclusion: The brain is not a seat of active combustion.
Fletcher, 1898—Lactic acid produced rigor mortis in excised frog *Gastrocnemius* muscle when immersed in it. The higher the lactic acid concentration, the faster rigor mortis sets in.
Fletcher and Hopkins, 1907—The body has means to dispose of lactic acid. The most favorable conditions for such a disposal are those that support oxidative processes.
Locke and Rosenheim, 1907—Dextrose and oxygen supply to an isolated rabbit heart are sufficient to prevent any formation of lactic acid, hence better functioning of the heart muscle.
Burridge, 1910—Lactic acid causes muscle fatigue and rigor mortis.
Barcroft and Orbeli, 1910—Lactic acid tends to turn oxygen out of blood capillaries at low oxygen tension, as carbonic acid does, hence it does have some beneficial value.
Feldman and Hill, 1911—The increased lactic acid concentration in the working muscle is due to oxygen want. Oxygen inhalation lessens the rise in acid concentration.
Hill, 1911—The presence of O_2 in the tissue (muscle) diminishes the duration of heat release of muscle contracture. Thus, by increasing O_2 tension in the tissue an atmosphere of O_2 would decrease, and an atmosphere of H_2 would increase the duration of heat production, suggestive of Fletcher and Hopkins (1907) experiments on the oxidative removal of lactic acid.
Fletcher, 1911—Contrary to observations that there is a connection between muscle damage, its death and production of lactic acid, the author concluded that no glycolytic enzyme leading to lactic acid formation appears to exist in muscle. Addition of dextrose to intact surviving muscle or to preparation of disintegrated muscle did not result in increase lactic acid production in the absence of bacteria.
Underhill and Black, 1912—The increase in lactic acid secretion in the urine of cocaine-treated dogs is unlikely associated with increase muscular activity induced by the drug. Although lactic acid and carbohydrate metabolism are presumably intimately associated, there are indications that lactic acid may arise from more than one source, and thus, possibly be still associated with the effects of cocaine.
Peters, 1913—Agrees with both Hill (1911) and Fletcher and Hopkins (1907) that heat production and lactic acid liberation in fatiguing muscle are extremely intimately connected.
Hill, 1913—The processes of muscular contraction are involved the liberation of lactic acid from some precursor. Lactic acid increases the tension in some colloidal structure of the tissue. The lactic acid precursor is rebuilt and the end of the contraction in the presence of and by the use of O_2, with the evolution of heat. The heat liberated by excited muscle in the complete absence of O_2 is due to the breakdown of the lactic acid precursor and is of the same nature of heat production of rigor.
Tashiro, 1913—Was the first investigator to show that nerve produced CO_2 and ammonia during metabolism.
Roaf, 1914—The increase in acidity is the cause of the shortening of muscle.
Hill, 1914—Argues in support of the hypothesis that lactic acid formed in the muscle after activity is not removed by oxidation, but rather replaced into its previous position, a sugar. Hence, lactic acid is part of the machine, not part of the fuel. A position that endured to present day.
Fletcher and Brown, 1914—Concluded that CO_2 and lactic acid do not originate from a common source.
Ringer, 1914—About diabetes: Parallelism exists between the degree of acidosis and the degree of disturbance in the carbohydrate metabolism.
Tsuji, 1916—Lactic acid is one of the normal metabolites of muscular activity. By using heart-lung preparation, Tsuji showed that accelerating heart beat via injection of adrenaline or elevated blood pressure the blood concentration of lactic acid declined. That had probably been the first demonstration of heart consumption of lactate an energy substrate, unbeknown to the author.
Ito, 1916—confirmed the accidental finding by his countryman, Tatsukichi Irisawa, of the presence of lactic acid in pus and determined that lactic acid is a constant constituent of pus and is distinctly increased by the autolysis of pus.
Adam, 1921—Concluded that at the moment of contraction, the muscle fiber work by drawing on stores of potential energy within the tissue, and it appears that the function of the oxidations is to restore to its normal resting level. Adam also speculated that the muscle's resting respiration is an index of an anabolic process, compensating, and proceeding at an equal rate with, some such catabolic process as the survival formation of lactic acid, observed to occur in resting tissues at a constant rate.

(Continued)

Table 1 | Continued

Foster and Moyle, 1921—Showed that carbohydrate production (mainly as glycogen) occurred upon muscle recovery in oxygen with a corresponding decline in lactic acid content.

Adam, 1921—Showed that not like resting muscle, the sciatic nerve exhibited a very small effect of stimulation on its respiration rate.

Hartree and Hill, 1922, 1923—Concluded from their experiments that in muscle, as was known for blood, there is a buffer mechanism, an alkali-protein salt capable of neutralizing acid, which is much more effective than a bicarbonate solution.

Holden, 1924—Showed that the "respiration substance" (Meyerhof's term for the enzymes responsible for the glycolytic process) of mammalian muscle is heat labile and in reality is a collection of irreversibly oxidizable substances, although lactic acid is not one of them.

Koehler, 1924—Suggested that acidosis during ether anesthesia is the summation effect of CO_2 excess and alkali deficit. The CO_2 excess is the result of inefficient respiration probably caused by decreased sensitiveness of the respiratory center.

Ronzoni et al., 1924—Showed that accumulation of lactic acid accounts in a large part for the acidosis of ether anesthesia; that its increase is independent of CO_2 tension and produces the changes in pH rather than being itself controlled by pH; decreased oxygen supply to tissues does not account for its production; the source of lactic acid seems to be the muscle tissue; production of lactic acid in the muscle, together with loss of phosphate from the muscle, during anesthesia, points to a breakdown of some hexose phosphate.

Koehler et al., 1925—Measured the production of acidosis by anoxemia and concluded that "anoxemia is fundamentally of an acidotic nature as far as disturbances in the acid-base balance are concerned.

Evans, 1925—Showed that lactic acid rapidly accumulates in plain muscle under anaerobic conditions, but scarcely at all when kept in oxygen; that the recovery process in oxygen is of much the same nature as that in skeletal muscle. Suggested that glycogen could be the parent substance from which lactic acid is formed.

Holmes and Holmes, 1925a—Showed that under convulsive dose of insulin there was no marked change in the level of brain reducing substance (carbohydrate) and that this substance in not capable of giving rise to the formation of lactic acid, although and abundance of lactic acid is formed under these conditions from added glucose. Basal levels of lactic acid in "insulin" brains were greatly reduced.

Holmes and Holmes, 1925b—Showed that the fall in brain lactic acid levels of insulin-treated animals does not occur until the blood sugar has reached a fairly low level. Hence, lactic acid levels fall not due to a direct insulin effect, rather to the shortage of glucose.

Holmes and Holmes, 1926—Showed that under aerobic conditions, lactic acid rapidly disappears from chopped brain. Investigator suggested that the brain depends on blood sugar, rather than any other substance which it stores itself, as lactic acid precursor.

Holmes and Holmes, 1927—Showed that the values of brain lactic acid fall and rise with blood sugar, in hypoglycemic and hyperglycemic conditions, respectively.

Riegel, 1927a—Demonstrated that severe blood hemorrhage in dogs caused an increase in blood lactic acid concentration and that the total increase and its duration were dependent upon the extent of the hemorrhage.

Riegel, 1927b—Experimented with injecting sodium lactate to dogs and followed its disappearance. Followed sodium lactate injection blood lactic acid concentration rapidly decreased due to diffusion to other tissues and a slower decrease due to lactic acid utilization by those tissues. She concluded that lactic acid injected into the blood is synthesized to lactacidogen and glycogen by a process analogous to removal of lactic acid formed in muscle exercise.

Gerard et al., 1927—Showed that the frog sciatic nerve produces a measurable amount of heat, which increased during activity (electric stimulation) and of a magnitude that agreed with the magnitude of oxygen consumption, a "conclusive proof that nervous impulse is a chemical affair" (Holmes, 1932).

Ashford and Holmes, 1929; Holmes, 1930—Showed that inorganic phosphate is liberated by brain tissue both anaerobically and aerobically; that phosphate liberation and lactic acid production from glucose by brain tissue are inhibited by NaF, although lactic acid production is much more sensitive to the fluoride; that lactic acid is freely formed from glucose even when all available phosphate is immobilized; that much less lactate is formed from glycogen that from glucose. It was concluded that the brain possesses two mechanisms of lactic acid production: One involves glucose, which is quantitatively more important and is independent of phosphate; the other is much smaller, involves glycogen and depends on phosphate availability. It was also demonstrated, for the first time, that a correlation exists between lactic acid disappearance and oxygen consumption i.e., an aerobic utilization of lactate in brain tissue; the ability of sodium fluoride (NaF) to inhibit the conversion of glucose to lactate and concomitantly to inhibit oxygen consumption, using a glycolytic inhibitor for the first time; in the presence of NaF, oxygen consumption in the presence of glucose was completely blocked in brain gray matter preparation, but by replacing glucose with lactate, fluoride did not inhibit oxygen consumption. Conclusion: Glucose must be converted into lactic acid before it can be oxidized by the gray matter.

(Continued)

Table 1 | Continued

Holmes and Ashford, 1930; Ashford and Holmes, 1931—Measured the "Meyerhof quotient," the [total lactate disappearing]/[lactate oxidize], which in muscle was determined to be ∼3, and found it in brain to be 1; found that the O_2 uptake in oxygenated brain tissue shaken with lactate in the presence of bicarbonate buffer in an O_2/CO_2 atmosphere is greater than in the presence of phosphate buffer and that such uptake increases with increased oxygen tension in both cases; termed "Meyerhof quotient" the "respiratory quotient" and found its value, both of brain tissue alone and of tissue oxygenated with extra oxygen, to be close to unity, including in the case of brain from animals rendered hypoglycemic by insulin injection; concluded that lactate oxidation is unlikely to spare the utilization of another substrate.
Quastel and Wheatley, 1932—Found that the rate of oxidation of an added substrate to brain tissue varies inversely with the size of the animal, a generalization that does not apply to muscle; that glucose, lactate and pyruvate at equivalent concentrations are oxidized at the same rate by brain tissue; that lactate is completely oxidized by brain tissue; that iodoacetic acid (IAA) inhibits glucose oxidation and stated the possibility that glucose necessarily passes through lactic acid for its oxidation to take place; found that oxalate, unlike IAA and NaF, also inhibits the oxidation of lactate.
Holmes, 1933—Hinted at the possibility that lactate oxidation could support brain activity.
Dixon, 1935—Confirmed the fact that in oxygenated brain tissue lactic acid formation cannot be detected, but concluded that the purpose of the complete oxidation of lactate is simply to remove it from the tissue.
Krebs and Johnson, 1937a,b,c; Krebs et al., 1938—Suggested the carbohydrate derivative to enter the Krebs cycle (tricarboxylic acid cycle, TCA) is pyruvate, alas with a question mark.

due, most probably, to habit of mind (Margolis, 1993). If there is any need for one to realize how strong an influence habit of mind can have, one needs only to recall how successful the, now defunct, lactic acidosis hypothesis of ischemic brain damage had been throughout the 1980s and 1990s (Kalimo et al., 1981; Rehncrona et al., 1981; Siesjö, 1981). Even four decades after the elucidation of the glycolytic pathway it was very easy to persuade a large contingency of scientists who studied possible mechanisms of hypoxic and ischemic brain damage that the culprit behind such damage is no other than the "usual suspect" i.e., lactate.

As has already been mentioned at the beginning of this monograph, Brooks (1985) has demonstrated that lactate is the glycolytic product and the oxidative substrate during sustained exercise. Later, Fox and Raichle (1986) have demonstrated a focal physiological uncoupling between cerebral blood flow and oxidative metabolism upon somatosensory stimulation in humans, and Fox et al. (1988) showed that during focal physiologic neural activity the consumption of glucose is non-oxidative. Simultaneously, Schurr et al. (1988) demonstrated the ability of brain tissue to maintain normal neuronal function with lactate as the sole oxidative energy substrate. With more publications adding support to the possible role of lactate in oxidative energy metabolism, both in muscle (Brooks, 1998, 2000, 2002a,b; Brooks et al., 1999a,b) and especially in brain (Izumi et al., 1994; Pellerin and Magistretti, 1994, 2003; Larrabee, 1995, 1996; Tsacopoulos and Magistretti, 1996; Hu and Wilson, 1997a,b; Schurr et al., 1997a, 1999a,b; Schurr and Rigor, 1998; Magistretti and Pellerin, 1999; Magistretti et al., 1999; Magistretti, 2000; Qu et al., 2000; Van Hall, 2000; Bliss and Sapolsky, 2001; Bouzier-Sore et al., 2003; Mangia et al., 2003; Smith et al., 2003; Dalsgaard et al., 2004; Kasischke et al., 2004; Schurr, 2006; Schurr and Payne, 2007; Herrero-Mendez et al., 2009; Zielke et al., 2009; Schurr and Gozal, 2011), a hot debate has ensued, focusing, unfortunately, on the premise that lactate is somehow an alternative oxidative substrate to glucose in tissue energy metabolism (Chih et al., 2001; Dienel and Hertz, 2001, 2005; Chih and Roberts, 2003; Hertz, 2004; Hertz et al., 2007; Dienel, 2012a,b). Consequently, rather than viewing the oxidative utilization of lactate as an integral part of the oxidative energy metabolic pathway, which begins with glucose and glycolysis and ends with CO_2, H_2O and the mitochondrial electron transport chain, many have portrayed lactate as a competitor of glucose. Hence, several studies have aimed at showing that glucose is the obligatory energy substrate for maintenance of various neuronal functions (Dienel and Cruz, 2004; Fillenz, 2005; Bak et al., 2006; Cruz et al., 2007; Gandhi et al., 2009). However, this very role of glucose has never been questioned or challenged by those who unraveled the oxidative utilization of lactate, either by muscle or brain tissue. After all, the principal source of tissue lactate is glucose, a fact that has never been in dispute. The utilization of lactate via its oxidation should have been understood simply as the most plausible and expected progression of glucose breakdown via the glycolytic pathway where lactate, not pyruvate, is the real first step in the mitochondrial TCA cycle. Nonetheless, a concerted effort has been mounted by many established investigators to minimize or marginalize lactate's role in energy metabolism. The following quote from the chapter by Clarke and Sokoloff (1994) on Circulation and Energy Metabolism in the Brain in the fifth edition of Basic Neurochemistry (1994) is most telling: "*Lactate, pyruvate, fructose-1,6-biphosphate, acetate, β-hydroxybutyrate, and acetoacetate can all be utilized by brain slices, homogenates, or cell-free fractions… but the substrate is not available to the brain because of inadequate blood levels or restricted transport through the BBB (blood brain barrier).*" Clarke and Sokoloff, who were leading scientists in the field of brain energy metabolism at the time, felt compelled to emphasize the limitations lactate faces as an energy substrate specifically in response to the findings of Fox and Raichle (1986), Fox et al. (1988) and Schurr et al. (1988), as if the role of glucose in the process was somehow being diminished by lactate. Hence, Clarke and Sokoloff reemphasize that "*… the nervous system function in the intact animal depends on substrates supplied by the blood and no satisfactory, normal, endogenous substitute for glucose has been found. Glucose must*

therefore be considered essential for the normal physiological behavior of the central nervous system." Therefore, if one requires a proof that habit of mind is long-lived, one could simply follow the heated debate over the role of lactate in oxidative energy metabolism, as exemplified by the large number of con and pro publications on the topic. At least in part, the strong rejection of lactate as an "alternative" oxidative substrate to glucose in energy metabolism, despite the fact that by now this monocarboxylate is recognized as an oxidative substrate, has to do with the old dogma of "lactate is a useless end-product of glycolysis." Moreover, if lactate, as being offered here, is the real end product of glycolysis, both under aerobic and anaerobic conditions,

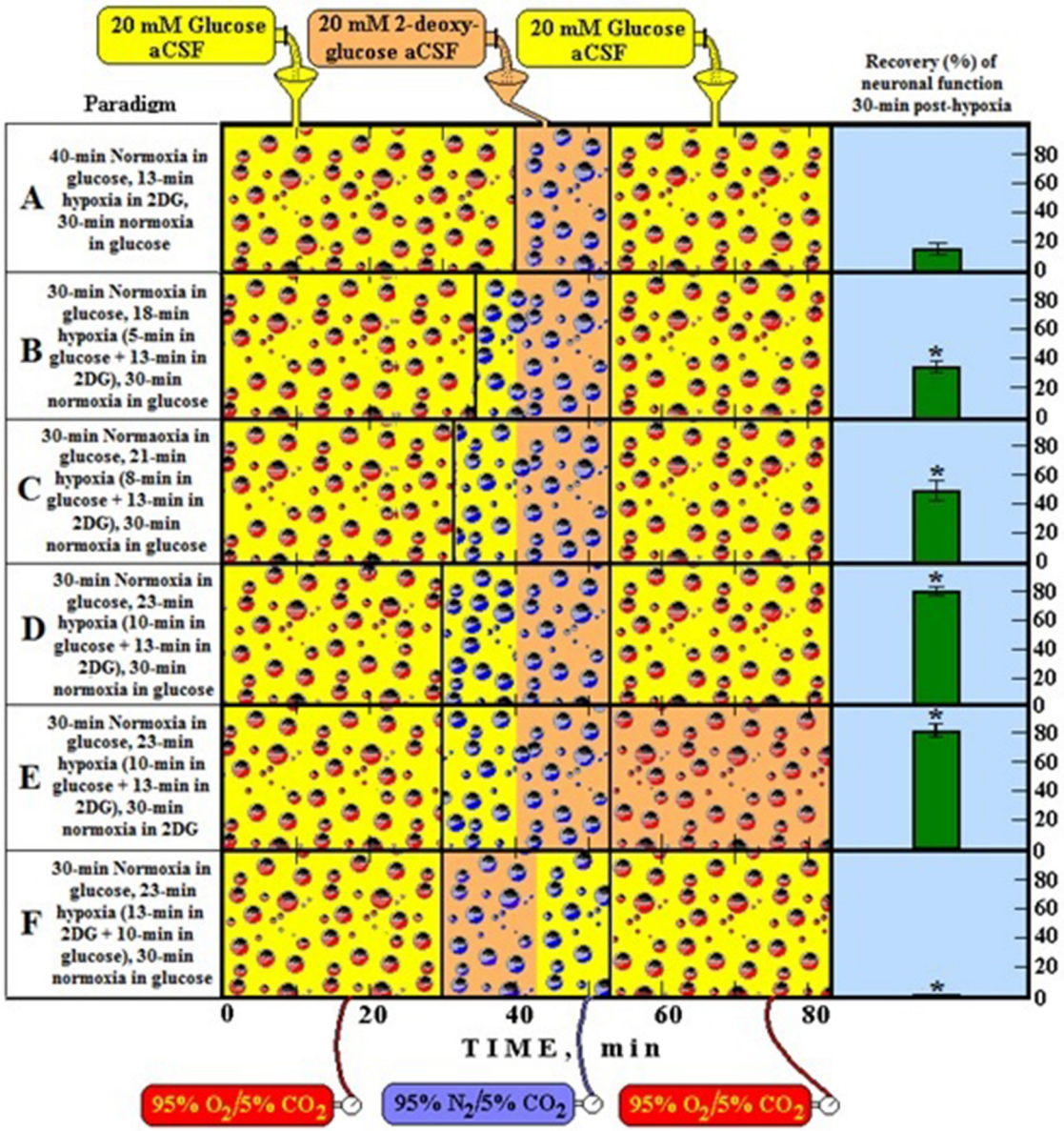


FIGURE 1 | A schematic representation of six different experimental paradigms using rat hippocampal slices and electrophysiological recording of CA1 evoked population spikes (neuronal function). In each experimental paradigm, slices were supplied either with 20 mM glucose (yellow bottle) or 20 mM 2-deoxy glucose (2DG, a glycolytic inhibitor) (orange bottle) and the gas mixture bubbled through the incubation chamber of the slices was either 95% O_2/5% CO_2 (normoxia, red bubbles) or 95% N_2/5% CO_2 (hypoxia, blue bubbles). At the end of each experimental paradigm, all slices in each compartment of the dual chamber were tested for the presence of neuronal function. Functional slices are shown as percentage of the total number of slices present (green histograms on the right). Accordingly, by following the timeline from left to right, paradigm A is a protocol in which slices were incubated under normoxic conditions for 40 min, followed by 13-min hypoxia in the presence of 2DG and then re-oxygenated for 30 min in the presence of glucose. Under these conditions less than 20% of the slices recovered their neuronal function at the end of the 80-min protocol. Similarly, each of the remaining paradigms (B–F) describes its corresponding protocol and its outcome in terms of percentage of slices exhibiting neuronal function. *Significantly different from paradigm A ($P < 0.05$).

the established, dogmatic concept of two types of glycolysis would be shaken, rendering the most celebrated pioneering elucidation of a biochemical pathway somewhat misconstrued. Nonetheless, such strong objections, as exhibited by the majority of scientists working in the fields of brain and muscle energy metabolism, to the idea that lactate plays any role and especially a major role in this metabolism, undoubtedly projects the fundamentals of habit of mind (Margolis, 1993), a barrier many find very difficult to cross. This is especially true when some of the most celebrated names in the field are behind the challenged paradigm. Obviously, the aim of this monograph is not to elaborate on the psychology behind habits of mind however, almost all scientists and laymen have accepted the sequence of the glycolytic pathway unchallenged from the time of its inception in 1940. The reasons for such a wide, unchallenged acceptance are beyond the scientific data themselves, reasons that emerged every time a challenge to a long accepted scientific paradigm arose throughout the history of science. I expect that the challenge being put forward here to the dogmatic paradigm depicting glycolysis as two pathways, aerobic and anaerobic, will face as much antagonism as has already being seen in the case of the proposition that lactate plays a role in energy metabolism.

Nonetheless, arguments for a unified, single format of glycolysis, which always ends up with lactate as its final product, are presented below along with arguments against the accepted depiction of two separate types of glycolysis, an aerobic one, with pyruvate as its end product, and an anaerobic one, where lactate is its end product.

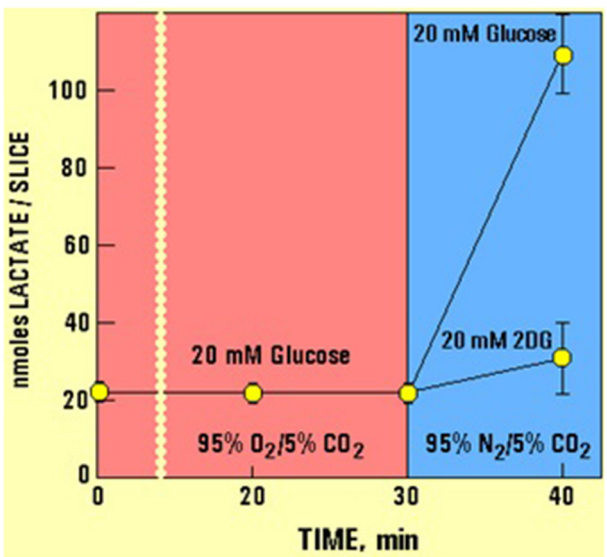


FIGURE 2 | The levels of lactate and glucose in hippocampal slices (nmoles/slice), as determined using enzymatic kits (Schurr et al., 1997a), during the experimental paradigms D and F detailed in Figure 1. Allowing slices to utilize glucose anaerobically during the first 10 min of a 23-min hypoxia resulted in an over 5-fold increase in tissue lactate content. Changing the supply of glucose to 2DG at the very beginning of a 23-min hypoxia blocked the ability of hippocampal slices to produce lactate via anaerobic glycolysis.

ONE GLYCOLYTIC PATHWAY, ONE STARTING SUBSTRATE (GLUCOSE), ONE END PRODUCT (LACTATE)

The preceding sections have detailed how and why the pioneers who drew the glycolytic pathway decided to branch it into two types, aerobic and anaerobic. Upon reviewing many of the studies that had led to the formulation of the glycolytic pathway, it becomes clear that the pioneers of that research had faced several hurdles as they put together the available data of their time, including some conflicting results and, understandably, several unknowns. However, glycolysis, as it was drawn then, has remained unchanged today, despite the major dilemmas that the original formulation has created for future generations of investigators in the field of energy metabolism. Tweaking biochemical pathways with the advancement of research is a regular occurrence and yet, the one pathway that has never been tweaked throughout its 70 odd year history is glycolysis. For whatever reason, scientists have been reluctant to suggest any correction or reconsideration of its original formulation. Surely, many would argue that there is absolutely no need for any correction, yet the mere fact that today lactate is an accepted oxidative energy substrate suggests that reconsideration of the original formulation is necessary. Most importantly, maintaining the pathway in its original formulation forces one to circumvent the more straightforward and logical correction i.e., a glycolytic pathway that always ends with the production of lactate, by offering awkward solutions to a major shortcoming of the dogmatic aerobic glycolysis, that is, the need for regeneration of NAD^+, which assures the maintenance of the cyclical nature of that portion of the pathway. Although this requirement is met in anaerobic glycolysis upon the conversion of pyruvate to lactate and NADH to NAD^+, this last step does not take place in the formulated aerobic glycolysis, and therefore, aerobic glycolysis, as held today is not capable of regenerating NAD^+ (the cyclical portion of the pathway that requires lactate production). Although no one knows how or has offered a mechanism by which oxygen converts anaerobic, lactate-producing glycolysis, into an aerobic, pyruvate-producing glycolysis, this process, somehow, has been accepted as an axiom. This is, despite the fact that glycolysis of red blood cells, one of the richest tissues in oxygen concentration, proceeds to produce lactate from glucose in an almost complete stoichiometry, while producing only minute amounts of pyruvate (Bartlett, 1959). No difference is known between the glycolytic apparatus of red blood cells and that of any other tissue and yet, erythrocyte glycolysis produces lactate in the presence of oxygen (or its absence), while glycolysis of any other oxygenated tissue supposedly produces mainly pyruvate. If one could add, in a test tube, mitochondria to oxygenated red blood cells, would glycolysis in these cells produced mainly pyruvate instead of lactate? One could accept for this paradox to remain unresolved until the late waning years of the twentieth century, however, the data accumulated hence forth should be sufficient to explain why this paradox is no more than a misconception. Nevertheless, the scientific community appears content to accept an unresolved paradox, rather than to disturb an imperfect formula of a biochemical pathway.

Since the main problem aerobic glycolysis presents at its original formulation is its inability to reproduce NAD^+, as

pyruvate conversion to lactate via the LDH reaction is supposedly inactive, alternative pathways for NAD^+ production have been explored.

One such alternative has been offered to be the malate-aspartate shuttle (MAS), a major redox shuttle in brain that supposedly regenerates NAD^+ when aerobic glycolysis is operational (McKenna et al., 2006; Pardo et al., 2006). Dienel (2012a), in a robust review article, assigns a major role for the MAS in the supply of NAD^+ to the NAD^+-less aerobic glycolysis in the brain. The above mentioned review is one of many written by Dienel and colleagues over the years, where they adamantly reject the "suggestion" that lactate could be an alternative oxidative substrate for glucose and hence that glucose is an obligatory energy substrate in the brain. Interestingly, in the above mentioned review Dienel suggests that aerobic utilization of lactate requires a stoichiometric MAS activity to oxidize the NADH in cytoplasm by LDH, and thus, is completely ignoring the possibility that lactate may be oxidized by the mitochondrial LDH and that the NADH thus formed is not in the cytoplasm, but in the mitochondria. The localization of LDH in the mitochondrial membrane and the ability of mitochondria to utilize lactate as a substrate for the TCA cycle have been demonstrated repeatedly (Brandt et al., 1987; Brooks et al., 1999a,b; Brooks, 2002a,b; Hashimoto et al., 2006; Atlante et al., 2007; Schurr and Payne, 2007; Lemire et al., 2008; Passarella et al., 2008; Gallagher et al., 2009; Elustondo et al., 2013; Jacobs et al., 2013). Therefore, the existence of a functional LDH in the mitochondrial membrane negates the need for the MAS as the mechanism responsible for transporting NAD^+ into the mitochondrion from the cytosol. Acceptance of LDH as a mitochondrial membranous enzyme would, in essence, forces one to question its role there, which is unlikely for the conversion of pyruvate to lactate. Thus, the aggressive push against Brooks et al. (1999a,b) findings, demonstrating the presence of LDH in mitochondrial preparations and its possible role in lactate oxidation was expected (Rasmussen et al., 2002; Sahlin et al., 2002; Ponsot et al., 2005; Yoshida et al., 2007).

Figure 1 illustrates an *in vitro* experiment (Schurr et al., 1997a) performed using rat hippocampal slices where the cerebral tissue was exposed to hypoxic conditions under which lactate is produced from glucose. Upon reoxygenation, the tissue utilized the hypoxically-produced lactate as the preferred energy substrate over glucose. The more lactate produced during hypoxia prior to 2DG (glycolytic inhibitor) supplementation, the higher the recovery rate of neuronal function posthypoxia (paradigms A–D). When lactate production is blocked during hypoxia, no recovery of neuronal function was observed despite the ample supply of glucose posthypoxia (paradigm F). Even when glucose was not supplied posthypoxia, neuronal function recovered and sustained on hypoxia-produced lactate alone (paradigm E) at least for the duration of the experiment.

In a separate experiment (**Figure 2**, but see also Schurr et al., 1999b) it has been shown that 10 min of hypoxia increased the lactate concentration over 5-fold in hippocampal slices, while 2DG completely blocked that increase.

The idea that lactate is shuttled not only intracellularly from the cytosol to the mitochondria, but also intercellularly, as has been shown by many investigations cited above, is illustrated in **Figure 3**.

Nevertheless, despite the multiple studies that have demonstrated the existence and function of mitochondrial LDH (see above), none of the most recent biochemistry textbooks (Devlin, 2011; Berg et al., 2012) dare mention the presence of LDH in the mitochondrial membrane.

With the abundance of published studies over the past 39 years, all pointing, in one way or another, at a simpler, straight forward, singular glycolytic pathway, it is of utmost importance to redefine "glycolysis" as the cytosolic biochemical pathway that under both, aerobic and anaerobic conditions, constantly utilizes glucose as its

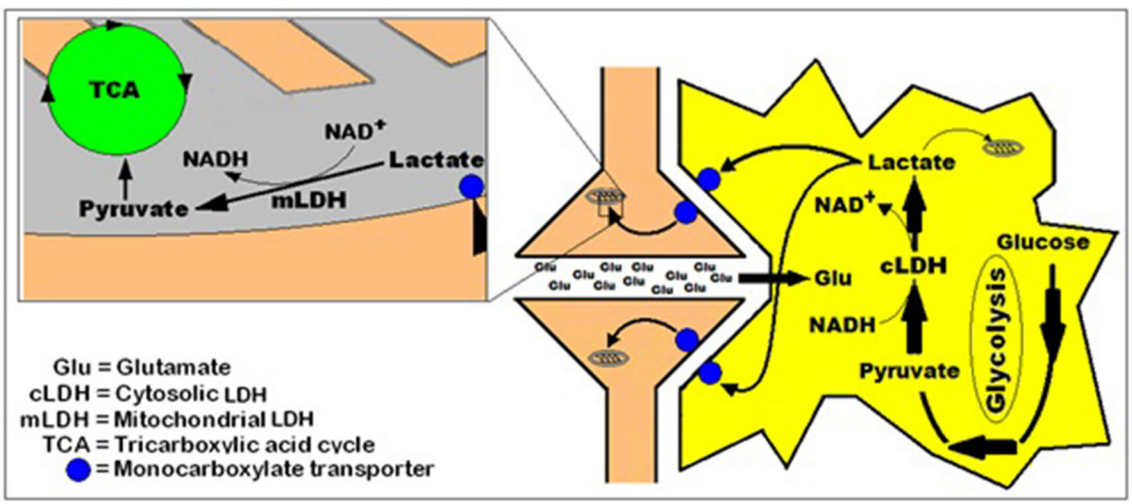


FIGURE 3 | A simplified schematic representation of the hypothesis of intracellular lactate shuttle between the cytosol and the mitochondria (Brooks, 2000, 2002a,b) and the intercellularly, astrocytic-neuronal lactate shuttle hypothesis (ANLSH, Pellerin and Magistretti, 1994; Herrero-Mendez et al., 2009) combined with the hypothesis that postulates lactate as the end-product of aerobic glycolysis (Schurr, 2006; Schurr and Gozal, 2011).

initial substrate to produce lactate as its end product in cerebral tissue and in almost any other tissue. The NAD^+ that is reduced to NADH during the formation of pyruvate is being reformed by the glycolytic LDH during the conversion of pyruvate to lactate, affording this portion of the pathway its cyclical nature. Under aerobic conditions lactate is utilized as the main substrate of the mitochondrial tricarboxylic acid (TCA) cycle, and thus, is defined as the major coupler between the two pathways, each localized in a separate cellular compartment, the cytosol and the mitochondrion, respectively. In the mitochondrion, lactate, transported from the cytosol via a monocarboxylate transporter (MCT) (Mowbray, 1975; Brooks et al., 1999a), is oxidized to pyruvate by the mitochondrial LDH (mLDH), which also provides the mitochondrion with NADH, circumventing the need for the proposed function of the malate-aspartate shuttle (MAS). Under anaerobic conditions, glycolysis continues to function unabated, resulting in lactate accumulation, as the TCA cycle is non-functional (**Figure 4**). When lactate is accumulating, under anaerobic conditions, it becomes, upon return to aerobic conditions, the principal energy substrate until its levels are falling back to their minimal normal levels (**Figure 1**, Schurr et al., 1997a,b, 1999a).

SUMMARY

Lactate is a metabolite that has earned a negative reputation ever since its discovery 234 years ago. Therefore, with the advent of biochemistry and the elucidation of the different pathways involved in carbohydrate metabolism and bioenergetics, any appearance of lactate seemed to signal harmful or damaging consequence, such that any reaction or treatment that could minimize lactate concentration was considered beneficial. The large scientific community that worked on muscular carbohydrate metabolism had determined the tone and direction of their research and influenced how the research of carbohydrate metabolism in other tissues, especially brain, was interpreted. Hence, when studies in the mid-1980s have appeared to challenge the prevailing dogma, assigning a possible role, perhaps even a crucial role, for lactate in energy metabolism, the habit of mind of the majority within the scientific community then, and even

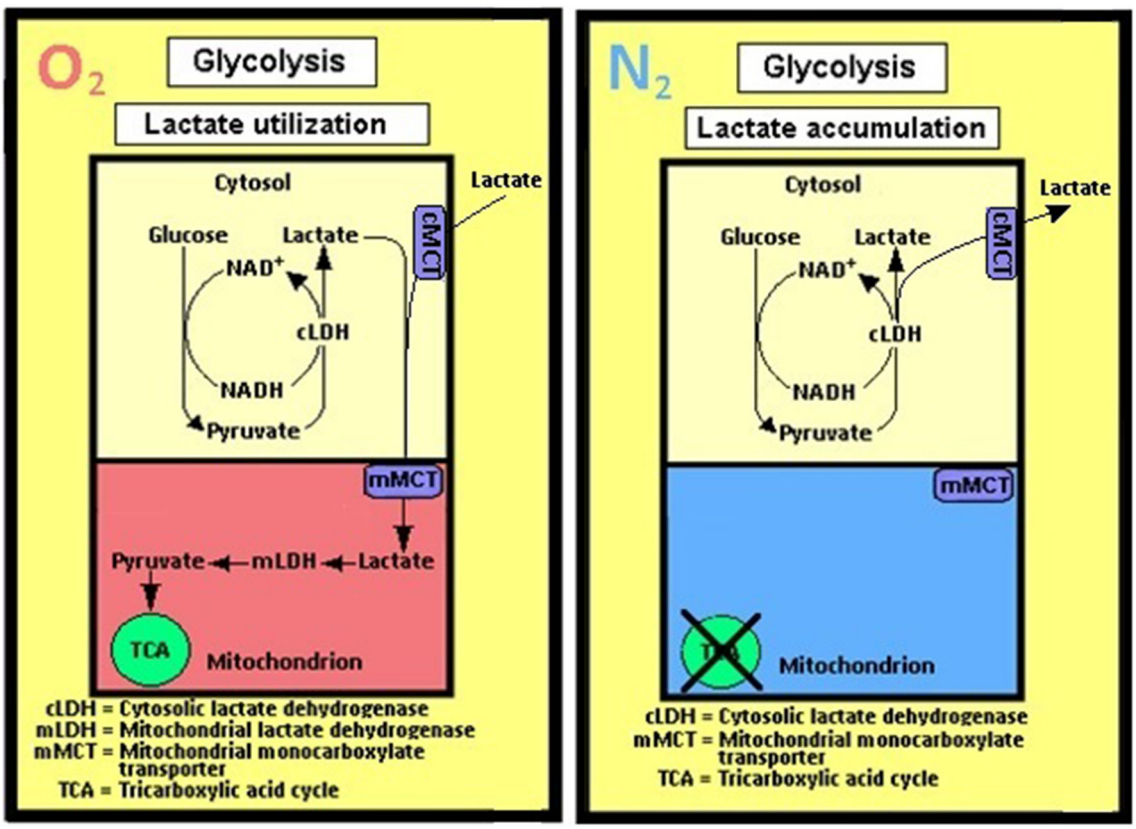


FIGURE 4 | A schematic representation of the cerebral (and most probably all other tissues) glycolytic pathway as perceived by the author. Accordingly, no separation is being made between aerobic and anaerobic glycolyses; the singular pathway's first step is the entry of glucose via its phosphorylation to glucose-6-phosphate by hexokinase and the last step is the conversion of pyruvate to lactate by the cytosolic lactate dehydrogenase (cLDH). When mitochondria are functional, in the presence of oxygen (O_2), lactate is being shuttled from the cytosol to the mitochondrion via the mitochondrial monocarboxylate transporter (mMCT) and, when available, from the extracellular space (Pellerin and Magistretti, 1994; Herrero-Mendez et al., 2009), via the cell membrane monocarboxylate transporter (cMCT). There lactate is oxidized by the mitochondrial lactate dehydrogenase (mLDH) to pyruvate, which enters the tricarboxylic acid (TCA) cycle, hence lactate utilization. The only difference between glycolysis under oxygen (O_2) atmosphere and glycolysis under nitrogen (N_2) atmosphere is lactate accumulation and release into the extracellular space under the latter, as it cannot be oxidized in the mitochondria.

today, erected a barrier that prevents the acceptance of such role, despite the continuously growing number of publications in support of it. This monograph attempted to review the publications of the first four or five decades of muscle and brain carbohydrate metabolism in an effort to persuade its readers to open their minds to the possibility that glycolysis, in cerebral and other tissues, is a singular pathway, in the presence or absence of oxygen, which begins with glucose as its substrate and terminates with the production of lactate as its main end product.

ACKNOWLEDGMENTS

The author greatly appreciates Prof. Roald Hoffmann, Cornell University, for his helpful comments and encouragement.

REFERENCES

Adam, N. K. (1921). Note of the oxygen consumption of amphibian muscle and nerve. *Biochem. J.* 15, 358–362.

Ashford, C. A., and Holmes, E. G. (1929). Contributions to the study of brain metabolism. V. Role of phosphates in lactic acid production. *Biochem. J.* 23, 748–759.

Ashford, C. A., and Holmes, E. G. (1931). Further observations on the oxidation of lactic acid by brain tissue. *Biochem. J.* 25, 2028–2049.

Atlante, A., de Bari, L., Bobba, A., Marra, E., and Passarella, S. (2007). Transport and metabolism of l-lactate occur in mitochondria from cerebellar granule cells and are modified in cells undergoing low potassium dependent apoptosis. *Biochim. Biophys. Acta* 1767, 1285–1299. doi: 10.1016/j.bbabio.2007.08.003

Bak, L. K., Schousboe, A., Sonnewald, U., and Waagepetersen, H. S. (2006). Glucose is necessary to maintain neurotransmitter homeostasis during synaptic activity in cultured glutamatergic neurons. *J. Cereb. Blood Flow Metab.* 26, 1285–1297. doi: 10.1038/sj.jcbfm.9600281

Barber, B. (1961). Resistance by scientists to scientific discovery. *Science* 134, 596–602. doi: 10.1126/science.134.3479.596

Barcroft, J., and Orbeli, L. (1910). The influence of lactic acid upon the dissociation curve of blood. *J. Physiol.* 41, 355–367.

Bartlett, G. R. (1959). Human red blood cell glycolytic intermediates. *J. Biol. Chem.* 234, 449–458.

Berg, J. M., Tymoczko, J. L., Stryer, L., and Gatto, G. J. Jr. (2012). *Biochemistry, 7th Edn*. New York, NY: W. H. Freeman and Company.

Bliss, T. M., and Sapolsky, R. M. (2001). Interactions among glucose, lactate and adenosine regulate energy substrate utilization in hippocampal cultures. *Brain Res.* 899, 134–141. doi: 10.1016/S0006-8993(01)02218-1

Bouzier-Sore, A.-K., Voisin, P., Canioni, P., Magistretti, P. J., and Pellerin, L. (2003). Lactate is the preferential energy substrate over glucose for neurons in culture. *J. Cereb. Blood Flow Metab.* 23, 1298–1306. doi: 10.1097/01.WCB.0000091761.61714.25

Brandt, R. B., Laux, J. E., Spainhour, S. E., and Kline, E. S. (1987). Lactate dehydrogenase in rat mitochondria. *Arch. Biochem. Biophys.* 259, 412–422. doi: 10.1016/0003-9861(87)90507-8

Brooks, G. A. (1985). "Lactate: glycolytic product and oxidative substrate during sustained exercise in mammals – 'the lactate shuttle'" in *Comparative Physiology and Biochemistry – Currecnt Topics and Trends Vol A. Respiration-Metabolism-Circulation*, ed R. Gilles (Berlin: Springer-Verlag), 208–218.

Brooks, G. A. (1998). Mammalian fuel utilization during sustained exercise. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 120, 89–107. doi: 10.1016/S0305-0491(98)00025-X

Brooks, G. A. (2000). Intra- and extra-cellular lactate shuttles. *Med. Sci. Sports. Exerc.* 32, 790–799. doi: 10.1097/00005768-200004000-00011

Brooks, G. A. (2002a). Lactate shuttle – between but not within cells? *J. Physiol.* 541, 333. doi: 10.1113/jphysiol.2002.023705

Brooks, G. A. (2002b). Lactate shuttles in nature. *Biochem. Soc. Trans.* 30, 258–264. doi: 10.1042/BST0300258

Brooks, G. A., Brown, M. A., Butz, C. E., Sicurello, J. P., and Dubouchaud, H. (1999a). Cardiac and skeletal muscle mitochondria have a monocarboxylate transporter MCT1. *J. Appl. Physiol.* 87, 1713–1718.

Brooks, G. A., Dubouchaud, H., Brown, M., Sicurello, J. P., and Butz, C. E. (1999b). Role of mitochondrial lactate dehydrogenase and lactate oxidation in the intracellular lactate shuttle. *Proc. Natl. Acad. Sci. U.S.A.* 96, 1129–1134. doi: 10.1073/pnas.96.3.1129

Burridge, W. (1910). An inquiry into some chemical factors of fatigue. *J. Physiol.* 41, 285–307.

Chih, C.-P., Lipton, P., and Roberts, E. L. Jr. (2001). Do active cerebral neurons really use lactate than glucose? *Trends Neurosci.* 24, 573–578. doi: 10.1016/S0166-2236(00)01920-2

Chih, C.-P., and Roberts, E. L. Jr. (2003). Energy substrates for neurons during neural activity: a critical review of the astrocyte-neuron lactate shuttle hypothesis. *J. Cereb. Blood Flow Metab.* 23, 1263–1281. doi: 10.1097/01.WCB.0000081369.51727.6F

Clarke, D. D., and Sokoloff, L. (1994). "Circulation and energy metabolism of the brain (Chap. 31)," in *Basic neurochemistry, 5th Edn*, eds G. J. Siegle, B. W. Agranoff, R. W. Albers, and P. B. Molinoff (New York, NY: Raven Press), 645–680.

Cruz, N. F., Ball, K. K., and Dienel, G. A. (2007). Functional imaging of focal brain activation in conscious rats: impact of [(14)C]glucose metabolite spreading and release. *J. Neurosci. Res.* 85, 3254–3266. doi: 10.1002/jnr.21193

Dalsgaard, M. K., Quistorff, B., Danielsen, E. R., Selmer, C., Vogelsang, T., and Secher, N. H. (2004). A reduced cerebral metabolic ratio in exercise reflects metabolism and not accumulation of lactate within the human brain. *J. Physiol.* 554, 571–578. doi: 10.1113/jphysiol.2003.055053

Devlin, T. M. (2011). *Textbook of Biochemistry with Clinical Correlations, 7th Edn*. Hoboken, NJ: John Wiley & Sons, Inc.

Dienel, G. A. (2012a). Brain lactate metabolism: the discoveries and the controversies. *J. Cereb. Blood Flow Metab.* 32, 1107–1138. doi: 10.1038/jcbfm.2011.175

Dienel, G. A. (2012b). Fueling and imaging brain activation. *ASN Neuro* 4, e00093. doi: 10.1042/AN20120021

Dienel, G. A., and Cruz, N. F. (2004). Nutrition during brain activation: does cell-to-cell lactate shuttling contribute significantly to sweet and sour food for thought? *Neurochem. Int.* 45, 321–351. doi: 10.1016/j.neuint.2003.10.011

Dienel, G. A., and Hertz, L. (2001). Glucose and lactate metabolism during brain activation. *J. Neurosci. Res.* 66, 824–838. doi: 10.1002/jnr.10079

Dienel, G. A., and Hertz, L. (2005). Astrocytic contributions to bioenergetics of cerebral ischemia. *Glia* 50, 362–388. doi: 10.1002/glia.20157

Dixon, K. C. (1935). Theoxidative disappearance of lactic acid from brain and the pasture effect reaction. *Bichem. J.* 29, 973–977.

Elustondo, P. A., White, A. E., Hughes, M. E., Brebner, K., Pavlov, E., and Kane, D. A. (2013). Physical and functional association of lactate dehydrogenase (LDH) with skeletal muscle mitochondria. *J. Biol Chem.* 288, 25309–25317. doi: 10.1074/jbc.M113.476648

Evans, C. L. (1925). Studies on the physiology of plain muscle. IV. The lactate acid content of plain muscle under various conditions. *Biochem. J.* 19, 1115–1127.

Feldman, I., and Hill, L. (1911). The influence of oxygen inhalation on the lactic acid produced during hard work. *J. Physiol.* 42, 439–443.

Fillenz, M. (2005). The role of lactate in brain metabolism. *Neurochem. Int.* 47, 413–417. doi: 10.1016/j.neuint.2005.05.011

Fletcher, W. M. (1898). The survival respiration of muscle. *J. Physiol.* 23, 10–99.

Fletcher, W. M. (1911). On the alleged formation of lactic acid in muscle during autolysis and post-survival periods. *J. Physiol.* 43, 286–312.

Fletcher, W. M. (1913). Lactic acid formation, survival respiration and rigor mortis in mammalian muscle. *J. Physiol.* 47, 361–380.

Fletcher, W. M., and Brown, G. M. (1914). The carbon dioxide production and hear rigor in muscle, and the theory of intra-molecular oxygen. *J. Physiol.* 48, 177–204.

Fletcher, W. M., and Hopkins, F. G. (1907). Lactic acid in amphibian muscle. *J. Physiol.* 35, 247–309.

Foster, D. L., and Moyle, D. M. (1921). A contribution to the study of the interconversion of carbohydrate and lactic acid in muscle. *Biochem. J.* 15, 672–680.

Fox, P. T., and Raichle, M. E. (1986). Focal physiological uncoupling of cerebral blood flow and oxidative metabolism during somatosensory stimulation in human subjects. *Proc. Natl. Acad. Sci. U.S.A.* 83, 1140–1144. doi: 10.1073/pnas.83.4.1140

Fox, P. T., Raichle, M. E., Mintun, M. A., and Dence, C. (1988). Nonoxidative glucose consumption during focal physiologic neural activity. *Science* 241, 462–464. doi: 10.1126/science.3260686

Gallagher, C. N., Carpenter, K. L. H., Grice, P., Howe, D. J., Mason, A., Timofeev, I., et al. (2009). The human brain utilizes lactate via the tricarboxylic acid cycle: a ^{13}C-labelled microdialysis and high-resolution nuclear magnetic resonance study. *Brain* 132, 2839–2849. doi: 10.1093/brain/awp202

Gandhi, G. K., Cruz, N. F., Ball, K. K., and Dienel, G. A. (2009). Astrocytes are poised for lactate trafficking and release from activated brain and for supply of glucose to neurons. *J. Neurochem.* 111, 522–536. doi: 10.1111/j.1471-4159.2009.06333.x

Gerard, R. A., Hill, A. V., and Zotterman, Y. (1927). The effect of frequency of stimulation on the heat production of nerve. *J. Physiol.* 63, 130–143.

Gladden, L. B. (2008). 200th Anniversary of lactate research in muscle. *Exerc. Sport Sci. Rev.* 36, 109–115. doi: 10.1097/JES.0b013e31817c0038

Hartree, W., and Hill, A. V. (1922). The recovery heat production in muscle. *J. Physiol.* 56, 367–381.

Hartree, W., and Hill, A. V. (1923). The anaerobic processs involved in muscular activity. *J. Physiol.* 58, 127–137.

Hashimoto, T., Hussien, R., and Brooks, G. A. (2006). Colocalization of MCT1, CD147, and LDH in mitochondrial inner membrane of L6 muscle cells: evidence of a mitochondrial lactate oxidation complex. *Am. J. Physiol. Endocrinol. Metab.* 290, E1237–E1244. doi: 10.1152/ajpendo.00594.2005

Herrero-Mendez, A., Almeida, A., Fernandez, E., Maestre, C., Moncada, S., and Bolanos, J. P. (2009). The bioenergetic and antioxidant status of neurons is controlled by continuous degradation of a key glycolytic enzyme by APC/C-Cdh1. *Nature Cell Biol.* 11, 747–752. doi: 10.1038/ncb1881

Hertz, L. (2004). The astrocyte-neuron lactate shuttle: a challenge of a challenge. *J. Cereb Blood Flow Metab.* 24, 1241–1248. doi: 10.1097/00004647-200411000-00008

Hertz, L., Peng, L., and Dienel, G. A. (2007). Energy metabolism in astrocytes: high rate of oxidative metabolism and spatiotemporal dependence on glycolysis/glycogenolysis. *J. Neurosci. Res.* 27, 219–249. doi: 10.1038/sj.jcbfm.9600343

Hill, A. V. (1910). The heat produced in contracture and muscular tone. *J. Physiol.* 40, 389–403.

Hill, A. V. (1911). The position occupied by the production of heat, in the chain of processes constituting a muscular contraction. *J. Physiol.* 42, 1–43.

Hill, A. V. (1913). The energy degraded in the recovery processes of stimulated muscles. *J. Physiol.* 46, 28–80.

Hill, A. V. (1914). The oxidative removal of lactic acid. *J. Physiol.* 48(Suppl.), 10–11.

Hill, L., and Nabarro, D. N. (1895). On the exchange of blood-gases in brain and muscle during states of rest and activity. *J. Physiol.* 18, 218–229.

Holden, H. F. (1924). Experiemnts on respiration and fermentation. *Biochem. J.* 18, 535–542.

Holmes, B. E., and Holmes, E. G. (1925a). Contributions to the study of brain metabolism. I. Carbohydrate metabolism. Preliminary paper. *Biochem. J.* 19, 492–499.

Holmes, E. G. (1930). Oxidations in central and peripheral nervous tissue. *Biochem. J.* 24, 914–925.

Holmes, E. G. (1932). The metabolism of brain and nerve. *Ann. Rev. Biochem.* 1, 487–506. doi: 10.1146/annurev.bi.01.070132.002415

Holmes, E. G. (1933). The relation between carbohydrate metabolism and the function of the grey matter of the central nervous system. *Biochem. J.* 27, 523–536.

Holmes, E. G., and Ashford, C. A. (1930). Lactic acid oxidation in brain with reference to the "Meyerfof cycle." *Biochem. J.* 24, 1119–1127.

Holmes, E. G., and Holmes, B. E. (1925b). Contributions to the study of brain metabolism. II. Carbohydrate metabolism. *Biochem. J.* 19, 836–839.

Holmes, E. G., and Holmes, B. E. (1926). Contributions to the study of brain metabolism. III. Carbohydrate metabolism relationship of glycogen and lactic acid. *Biochem. J.* 20, 1196–1203.

Holmes, E. G., and Holmes, B. E. (1927). Contributions to the study of brain metabolism. IV. Carbohydrate metabolism of the brain tissue of depancreatised cats. *Biochem. J.* 21, 412–418.

Hu, Y., and Wilson, G. S. (1997a). Rapid changes in local extracellular rat brain glucose observed with an *in vivo* glucose sensor. *J. Neurochem.* 68, 1745–1752. doi: 10.1046/j.1471-4159.1997.68041745.x

Hu, Y., and Wilson, G. S. (1997b). A temporary local energy pool coupled to neuronal activity: fluctuations of extracellular lactate levels in rat brain monitored with rapid-response enzyme-based sensor. *J. Neurochem.* 69, 1484–1490. doi: 10.1046/j.1471-4159.1997.69041484.x

Ito, H. (1916). The formation of d-lactic acid by the autolysis of pus. *J. Biol. Chem.* 26, 173–176.

Izumi, Y., Benz, A. M., Zorumski, C. F., and Olney, J. W. (1994). Effect of lactate and pyruvate on glucose utilization in rat hippocampal slices. *Neuroreport* 5, 617–620. doi: 10.1097/00001756-199401000-00021

Jacobs, R. A., Meinild, A.-K., Nordsborg, N. B., and Lundby, C. (2013). Lactate oxidation in human skeletal muscle mitochondria. *Endocrin. Metabol.* 304, E686–E694. doi: 10.1152/ajpendo.00476.2012

Kalimo, H., Rehncrona, S., Soderfeldt, B., Olsson, Y., and Siejö, B. K. (1981). Brain lactic acidosis and ischemic cell-damage. 2. Histopathology. *J. Cereb. Blood Flow Metab.* 1, 313–327. doi: 10.1038/jcbfm.1981.35

Kasischke, K. A., Vishwasrao, H. D., Fisher, P. J., Zipfel, W. R., and Webb, W. W. (2004). Neural activity triggers neuronal oxidative metabolism followed by astrocytic glycolysis. *Science* 305, 99–103. doi: 10.1126/science.1096485

Koehler, A. E. (1924). The acidosis of operative anesthesia. *J. Biol. Chem.* 62, 435–451.

Koehler, A. E., Brunquist, E. H., and Loevenhart, A. S. (1925). The production of acidosis by anoxemia. *J. Biol. Chem.* 64, 313–323.

Krebs, H. A., and Johnson, W. A. (1937a). The role of citric acid in intermediary metabolism in animal tissue. *Enzymologia* 4, 148–156.

Krebs, H. A., and Johnson, W. A. (1937b). Metabolism of ketonic acids in animal tissues. *Biochem. J.* 31, 64–660.

Krebs, H. A., and Johnson, W. A. (1937c). Acetopyruvic acid (αydiketovaleric acid) as an intermediate metabolite in animal tissues. *Bichem. J.* 31, 772–779.

Krebs, H. A., Salvin, E., and Johnson, W. A. (1938). The formation of citric and α-ketoglutaric acids in the mammalian body. *Bichem. J.* 32, 113–117.

Kuhn, T. S. (1996). *The Structure of Scientific Revolutions, 3rd Edn.* London: The University of Chicago Press, Ltd. doi: 10.7208/chicago/9780226458106.001.0001

Larrabee, M. G. (1995). Lactate metabolism and its effects on glucose metabolism in the excised neural tissue. *J. Neurochem.* 64, 1734–1741. doi: 10.1046/j.1471-4159.1995.64041734.x

Larrabee, M. G. (1996). Partitioning of CO_2 production between glucose and lactate in excised sympathetic ganglia, with implications for brain. *J. Neurochem.* 67, 1726–1734. doi: 10.1046/j.1471-4159.1996.67041726.x

Lemire, J., Mailloux, R. J., and Appanna, V. D. (2008). Mitochondrial lactate dehydrogenase is involved in oxidative-energy metabolism in human astrocytoma cells (CCF-STTG1). *PLoS ONE* 3:e1550. doi: 10.1371/journal.pone.0001550

Locke, F. S., and Rosenheim, O. (1907). Contributions to the physiology of the isolated heart. The consumption of dextrose by mammalian cardiac muscle. *J. Physiol.* 36, 205–220.

Magistretti, P. J. (2000). Cellular bases of functional brain imaging: insights from neuron-glia metabolic coupling. *Brain Res.* 886, 108–112. doi: 10.1016/S0006-8993(00)02945-0

Magistretti, P. J., and Pellerin, L. (1999). Cellular mechanisms of brain energy metabolism and their relevance to functional brain imaging. *Philos. Tarns. R. Soc. Lond. B Biol. Sci.* 354, 1155–1163. doi: 10.1098/rstb.1999.0471

Magistretti, P. J., Pellerin, L., Rothman, D. L., and Shulamn, R. G. (1999). Energy on demand. *Science* 283, 496–497. doi: 10.1126/science.283.5401.496

Magnus-Levy, A. (1911). *John Hopkins Hospital Bulletin.* Baltimore, MD: John Hopkins Hospital Press.

Mangia, S., Garreffa, G., Bianciardi, M., Giove, F., Di Salle, F., and Maraviglia, B. (2003). The aerobic brain: lactate decrease at the onset of neural activity. *Neuroscience* 118, 7–10. doi: 10.1016/S0306-4522(02)00792-3

Margolis, H. (1993). *Paradigms and Barriers: How Habits of Mind Govern Scientific Beliefs.* London: The University of Chicago Press, Ltd.

Marriott, W. M. (1914). The blood in acidosis from the quantitative standpoint. *J Biol. Chem.* 18, 507–517.

McKenna, M. C., Waagepetersen, H. S., Schousboe, A., and Sonnewald, U. (2006). Neuronal and astrocytic shuttle mechanisms for cytosolic-mitochondrial transfer of reducing equivalents: current evidence and pharmacological tools. *Biochem. Pharmacol.* 71, 399–407. doi: 10.1016/j.bcp.2005.10.011

Mowbray, J. (1975). A mitochondrial monocarboxylate transporter in rat liver and heart and its possible function in cell control. *Biochem. J.* 148, 41–47.

Pardo, B., Contreras, L., Serrano, A., Ramos, M., Kobayashi, K., Iijima, M., et al. (2006). Essential role of aralar in the transduction of small Ca2+ signals to neuronal mitochondria. *J. Biol. Chem.* 281, 1039–1047. doi: 10.1074/jbc.M507270200

Passarella, S., de Bari, L., Valenti, D., Pizzuto, R., Paventi, G., and Altane, A. (2008). Miochondria and l-lactate metabolism. *FEBS Lett*. 582, 3569–3576. doi: 10.1016/j.febslet.2008.09.042

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. doi: 10.1073/pnas.91.22.10625

Pellerin, L., and Magistretti, P. J. (2003). Food for thought: challenging the dogmas. *J. Cereb. Blood Flow Metab*. 23, 1282–1286. doi: 10.1097/01.WCB.0000096064.12129.3D

Peters, R. A. (1913). The heat production of fatigue and its relation to the production of lactic acid in amphibian muscle. *J. Physiol*. 47, 243–271.

Ponsot, E., Zoll, J., N'Guessan, B., Ribera, F., Lampert, E., Richard, R., et al. (2005). Mitochondrial tissue specificity of substrates utilization in rat cardiac and skeletal muscles. *J. Cell Physiol*. 203, 479–486. doi: 10.1002/jcp.20245

Qu, H., Haberg, A., Haraldseth, O., Unsgard, G., and Sonnewald, U. (2000). (13)CMR spectroscopy study of lactate as substrate for rat brain. *Dev. Neurosci*. 22, 429–436. doi: 10.1159/000017472

Quastel, J. H., and Wheatley, A. H. M. (1932). Oxidations by the brain. *Biochem. J*. 26, 725–744.

Ransom, F. (1910). A contribution to the study of muscle-enzymes. *J. Physiol*. 40, 1–16.

Rasmussen, H. N., van Hall, G., and Rasmussen, U. F. (2002). Lactate dehydrogenase is not a mitochondrial enzyme in human and mouse vastus lateralis muscle. *J. Physiol*. 541, 575–580. doi: 10.1113/jphysiol.2002.019216

Rehncrona, S., Rosen, I., and Seisö, B. K. (1981). Brain lactic acidosis and ischemic cell-damage. 1. Biochemistry and neurophysiology. *J. Cereb. Blood Flow Metab*. 1, 297–311. doi: 10.1038/jcbfm.1981.34

Riegel, C. (1927a). Formation of lactic acid in the body after severe hemorrhage. *J. Biol. Chem*. 74, 123–134.

Riegel, C. (1927b). The rate of disappearance of sodium lactate injected intravenously and its effect upon sugar and inorganic phosphate of the blood. *J. Biol. Chem*. 74, 135–148.

Ringer, A. I. (1914). Studies in diabetes. I. Theory of diabetes, with consideration of the probable mechanism of antiketogenesis and the cause of acidosis. *J. Biol Chem*. 17, 107–119.

Roaf, H. E. (1914). The time relations of acid production in muscle during contraction. *J. Physiol*. 48, 380–391.

Ronzoni, E., Koechig, I., and Eaton, E. (1924). Ether Anesthesia. III. Role of lactic acid in the acidosis of ether anesthesia. *J. Biol. Chem*. 61, 465–492.

Sahlin, K., Fernstrom, M., and Tonkonogi, M. (2002). No evidence of an intracellular lactate shuttle in rat skeletal muscle. *J. Physiol*. 541, 569–574 doi: 10.1113/jphysiol.2002.016683

Schurr, A. (2006). Lactate: the ultimate cerebral oxidative energy substrate? *J. Cereb. Blood Flow Metab*. 26, 142–152. doi: 10.1038/sj.jcbfm.9600174

Schurr, A., and Gozal, E. (2011). Aerobic production and utilization of lactate satisfy increased energy demands upon neuronal activation in hippocampal slices and provide neuroprotection against oxidative stress. *Front. Pharmacol*. 2:96. doi: 10.3389/fphar.2011.00096

Schurr, A., Miller, J. J., Payne, R. S., and Rigor, B. M. (1999a). An increase in lactate output by brain tissue serves to meet the energy needs of glutamate-activated neurons. *J. Neurosci*. 19, 34–39.

Schurr, A., and Payne, R. S. (2007). Lactate, not pyruvate, is neuronal aerobic glycolysis end product: an *in vitro* electrophysiological study. *Neuroscience* 147, 613–619. doi: 10.1016/j.neuroscience.2007.05.002

Schurr, A., Payne, R. S., Miller, J. J., and Rigor, B. M. (1997a). Brain lactate, not glucose, fuels the recovery of synaptic function from hypoxia upon reoxygenation: an *in vitro* study. *Brain Res*. 744, 105–111. doi: 10.1016/S0006-8993(96)01106-7

Schurr, A., Payne, R. S., Miller, J. J., and Rigor, B. M. (1997b). Brain lactate is an obligatory aerobic energy substrate for functional recovery after hypoxia: further *in vitro* validation. *J. Neurochem*. 69, 423–426. doi: 10.1046/j.1471-4159.1997.69010423.x

Schurr, A., Payne, R. S., Miller, J. J., and Rigor, B. M. (1999b). Study of cerebral energy metabolism using the rat hippocampal slice preparation. *Methods* 18, 117–126.

Schurr, A., and Rigor, B. M. (1998). Brain anaerobic lactate production: a suicide note or a survival kit? *Dev. Neurosci*. 20, 348–357. doi: 10.1159/000017330

Schurr, A., West, C. A., and Rigor, B. M. (1988). Lactate-supported synaptic function in the rat hippocampal slice preparation. *Science* 240, 1326–1328. doi: 10.1126/science.3375817

Shulman, R. G., and Rothman, D. L. (2001). The "glycogen shunt" in exercising muscle: a role for glycogen in muscle energetic and fatigue. *Proc. Natl. Acad. Sci. U.S.A.* 98, 457–461. doi: 10.1073/pnas.98.2.457

Siesjö, B. K. (1981). Cell-damage in the brain – a speculative synthesis. *J. Cereb. Blood Flow Metab.* 1, 155–185. doi: 10.1038/jcbfm.1981.18

Smith, D., Pernet, A., Hallett, W. A., Bingham, E., Marsden, P. K., and Amiel, S. A. (2003). Lactate: a preferred fuel for human brain metabolism *in vivo*. *J. Cereb. Blood Flow Metab*. 23, 658–664. doi: 10.1097/01.WCB.0000063991.19746.11

Stryer, L. (1995). *Biochemistry, 4th Edn, Chapter 19*. New York, NY: W. H. Freeman and Company.

Tashiro, S. (1913). Carbon dioxide production from nerve fibres when resting and when stimulated; a contribution to the chemical basis of irritability. *Am. J. Physiol*. 32, 107–136.

Tsacopoulos, M., and Magistretti, P. J. (1996). Metabolic coupling between glia and neurons. *J. Neurosci*. 16, 877–885

Tsuji, K. (1916). Lactic acid metabolism in the isolated heart (heart lung preparation). *J. Physiol*. 50, 312–321.

Underhill, F. P., and Black, C. L. (1912). The influence of cocaine upon metabolism with special reference to the elimination of lactic acid. *J. Biol. Chem*. 11, 235–252.

Van Hall, G. (2000). Lactate as a fuel for mitochondrial respiration. *Acta Physiol. Scand*. 168, 643–656. doi: 10.1046/j.1365-201x.2000.00716.x

Yoshida, Y., Holloway, G. P., Ljubicic, V., Hatta, H., Spriet, L. L., Hood, D. A., et al. (2007). Negligible direct lactate oxidation in subsarcolemmal and intermyofibrillar mitochondria obtained from red and white rat skeletal muscle. *J. Physiol*. 582, 1317–1335. doi: 10.1113/jphysiol.2007.135095

Zielke, H. R., Zielke, C. L., and Baab, P. J. (2009). Direct measurement of oxidative metabolism in the living brain by microdialysis: a review. *J. Neurochem*. 109, 24–29. doi: 10.1111/j.1471-4159.2009.05941.x

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 11 September 2014; accepted: 21 October 2014; published online: 19 November 2014.

*Citation: Schurr A (2014) Cerebral glycolysis: a century of persistent misunderstanding and misconception. Front. Neurosci. **8**:360. doi: 10.3389/fnins.2014.00360*

This article was submitted to Neuroenergetics, Nutrition and Brain Health, a section of the journal Frontiers in Neuroscience.

frontiers in
NEUROSCIENCE

PERSPECTIVE ARTICLE
published: 25 November 2014
doi: 10.3389/fnins.2014.00366

Lactate oxidation at the mitochondria: a lactate-malate-aspartate shuttle at work

Daniel A. Kane*

Department of Human Kinetics, St. Francis Xavier University, Antigonish, NS, Canada

Edited by:
Avital Schurr, University of Louisville, USA
Evelyne Gozal, University of Louisville, USA

Reviewed by:
Tibor Kristian, University of Maryland School of Medicine, USA
Daniela Calvetti, Case Western Reserve University, USA

****Correspondence:***
Daniel A. Kane, Department of Human Kinetics, St. Francis Xavier University, 1 West Street, PO Box 5000, Antigonish, NS B2G 2W5, Canada
e-mail: dkane@stfx.ca

Lactate, the conjugate base of lactic acid occurring in aqueous biological fluids, has been derided as a "dead-end" waste product of anaerobic metabolism. Catalyzed by the near-equilibrium enzyme lactate dehydrogenase (LDH), the reduction of pyruvate to lactate is thought to serve to regenerate the NAD^+ necessary for continued glycolytic flux. Reaction kinetics for LDH imply that lactate oxidation is rarely favored in the tissues of its own production. However, a substantial body of research directly contradicts any notion that LDH invariably operates unidirectionally *in vivo*. In the current Perspective, a model is forwarded in which the continuous formation and oxidation of lactate serves as a mitochondrial electron shuttle, whereby lactate generated in the cytosol of the cell is oxidized at the mitochondria of the same cell. From this perspective, an intracellular lactate shuttle operates much like the malate-aspartate shuttle (MAS); it is also proposed that the two shuttles are necessarily interconnected in a lactate-MAS. Among the requisite features of such a model, significant compartmentalization of LDH, much like the creatine kinase of the phosphocreatine shuttle, would facilitate net cellular lactate oxidation in a variety of cell types.

Keywords: lactate, lactate dehydrogenases, mitochondria, pyruvates, malate aspartate

INTRODUCTION: LACTATE DEHYDROGENASE REACTION

The reduction of pyruvate to lactate, catalyzed by lactate dehydrogenase (LDH; Pyruvate + NADH + $H^+ \rightleftharpoons$ Lactate + NAD^+) in the cytosol of many cells, has been regarded as a metabolic "dead-end" (Luft, 2001; Quistorff and Grunnet, 2011a), or perhaps more aptly, a metabolic *cul de sac* (Barros, 2013), because lactate can only rejoin the metabolic network via pyruvate. In mammals, the LDH reaction is also considered to be "near-equilibrium" (Spriet et al., 2000; Quistorff and Grunnet, 2011a,b), meaning that the reaction is regulated chiefly by the concentrations of its reactants and products, rather than by more sophisticated means of allosteric regulation or covalent modification (Crabtree and Newsholme, 1978). Because the equilibrium for the LDH reaction lies far to the right (i.e., lactate formation favored) (Williamson et al., 1967), regardless of LDH isoform (Quistorff and Grunnet, 2011a,b), the implication might be that LDH rarely favors the reverse reaction (i.e., lactate oxidation) *in vivo*. Indeed, the mass action ratio ([lactate][NAD^+]/[pyruvate][NADH][H^+]) necessary for appreciable lactate oxidation would need to exceed the equilibrium constant for LDH. However, experimental evidence increasingly belies any notion that LDH operates unidirectionally *in vivo*, and supports that lactate serves as an important metabolic fuel for many tissues, including skeletal (Brooks et al., 1991; Bergman et al., 1999; Donovan and Pagliassotti, 2000) and cardiac muscle (Gertz et al., 1988; Chatham et al., 2001), liver (Skilleter and Kun, 1972; Kline et al., 1986), and brain (Schurr et al., 1988; Bouzier-Sore et al., 2006; Wyss et al., 2011; Funfschilling et al., 2012; reviewed in Barros, 2013). The purpose of the current Perspective is to forward a model in which lactate is central to the shuttling of energetic substrate between the cytosol (glycolysis) and the mitochondria (oxidative phosphorylation). Components of such a concept have been demonstrated in heart (Safer et al., 1971) and skeletal muscle (Schantz, 1986), were later expanded to a *lactate shuttle* perspective (Stainsby and Brooks, 1990; Brooks et al., 1999) and comprehensively reviewed (Gladden, 2004) and again commented upon (Gladden, 2007). The concept is particularly supported by recent research in neuronal cells (Gellerich et al., 2012, 2013; Rueda et al., 2014). While the concept outlined in the current Perspective is not new, *per se* (Safer et al., 1971), an apparent lack of conventional recognition or acceptance of its theoretical underpinnings, warrants further attention.

THE MALATE-ASPARTATE SHUTTLE

Due to the impermeability of the inner mitochondrial membrane to NAD^+ and NADH, NADH generated by glycolysis under aerobic conditions depends on the indirect transfer of reducing equivalents into the mitochondria via the malate-aspartate shuttle (MAS) and glycerol-phosphate shuttle. These shuttles are also thought to regenerate cytosolic NAD^+ necessary to support glycolytic flux at the NAD^+-requiring glyceraldehyde 3-phosphate dehydrogenase reaction. The MAS has been demonstrated to be the predominant means by which this occurs in most oxidative tissues, and appears to constitute the principal NADH shuttle in mature neurons (Kauppinen et al., 1987; Ramos et al., 2003; Contreras and Satrustegui, 2009; Gellerich et al., 2012). It is also well established that during conditions of increased cellular energy demand and/or increased glycolytic flux (e.g.,

during strenuous exercise), as well as hypoxia, that the concentration of lactate will increase as the LDH reaction facilitates increased rates of cytosolic NAD^+ regeneration (Robergs et al., 2004). In the brain, however, increasing the concentration of lactate in circulation (e.g., as during exercise) results in an increase in lactate disposal in the brain (Quistorff et al., 2008; van Hall et al., 2009; Boumezbeur et al., 2010; Dienel, 2012). It has also been suggested that the increased LDH activity (and, in turn, lactate production) simply compensates for the inability of the MAS to keep pace with cytosolic NAD^+ demand (Schantz, 1986). In neurons, Ca^{+2} activation of the MAS and TCA cycle are competitive, such that lower levels of Ca^{+2} stimulates MAS activity by activating the glutamate/aspartate carrier (Contreras and Satrustegui, 2009), while higher concentrations of Ca^{+2} activate α-ketoglutarate dehydrogenase in the mitochondrial matrix, limiting the α-ketoglutarate available for the MAS (Contreras and Satrustegui, 2009). It is also possible that lactate is formed continuously in the cytosol, regardless of metabolic state, and that lactate oxidized at the mitochondria is coupled to the MAS. In isolated cardiac mitochondria, for example, the MAS exhibits an excess capacity, suggesting that the MAS activity alone is sufficient to maintain cytosolic NAD^+ regeneration (Digerness and Reddy, 1976). Why, then, at rest and under fully aerobic conditions, would lactate be produced during glycolysis, if all the pyruvate *should* be going to the mitochondria for oxidative phosphorylation, and the MAS should be regenerating sufficient NAD^+?

CONVENTIONAL (AN)AEROBIC GLYCOLYSIS

The appearance and disappearance of lactate during varying metabolic states has been a topic of much historical conjecture, controversy, and intrigue. There have been many reviews of the literature examining lactate metabolism, to which readers may be directed. Some of the more recent include (Cruz et al., 2012; Dienel, 2012; Kitaoka et al., 2012; Doherty and Cleveland, 2013; Newington et al., 2013; Brooks, 2014; Schurr, 2014; Todd, 2014). Unfortunately, many contemporary textbooks still use the metabolic fate of pyruvate to distinguish two types of glycolysis: aerobic (i.e., requiring oxygen) and anaerobic (i.e., without oxygen). In the presence of oxygen, it has been said, pyruvate will proceed to the mitochondria to meet its metabolic demise via oxidative phosphorylation, the net result of which is mitochondrial ATP resynthesis and oxygen consumption (i.e., respiration) (Voet et al., 2011). Conversely, when oxygen is limiting, the pyruvate is reduced to lactate in the cytosol by LDH, oxidizing its cofactor NADH in the process (Voet et al., 2011). A problem with this traditional construct is that it does not reconcile well with some recurring scientific observations. For example, it is well established that lactate is produced, and consumed, under fully aerobic conditions. Indeed, in healthy, normoxic individuals at rest in the postabsorptive state, it can be expected that approximately $50\,\mu mol \cdot min^{-1}$ of lactate are released from the brain alone (van Hall et al., 2009; van Hall, 2010). Clearly, lactate is more than a dead-end waste metabolite of anaerobic glycolysis; rather, shuttling of lactate throughout the organism provides useful perspective in which to interpret experimental observation.

THE LACTATE SHUTTLE CONCEPT

Two lactate shuttle concepts have been forwarded which describe the movement and utilization of lactate within and between cells (Brooks, 1998). The *intra*cellular lactate shuttle hypothesis posits that lactate formed during glycolysis can be continuously used as an energy source within the same cell (Brooks, 1998). The *inter*cellular, or cell-cell lactate shuttle involves lactate generated and exported from a cell to be taken up and utilized by another cell (Brooks, 1998). The cell-cell lactate shuttle has gained general acceptance; the finer details of the intracellular lactate shuttle continue to be investigated, however. Recently, we demonstrated both a physical, as well as functional association of LDH with mitochondria in skeletal muscle (Elustondo et al., 2013). Using laser-scanning confocal microscopy, we confirmed the colocalization of LDH with mitochondrial membrane proteins in rat skeletal muscle. We found that mitochondria in saponin-permeabilized skeletal muscle fibers from rats oxidized lactate in the presence of NAD^+, malate, and ADP (Elustondo et al., 2013); this was found similarly by another group in human fibers (Jacobs et al., 2013). The pyruvate was then transported into the mitochondria where it was further oxidized by pyruvate dehydrogenase (PDH), then the TCA cycle, with reducing equivalents stimulating respiration (Elustondo et al., 2013; Jacobs et al., 2013). We were able to inhibit respiration with just $5\,\mu M$ alpha-cyano-hydroxycinnamate, an inhibitor of mitochondrial pyruvate transport, further supporting that pyruvate, but not lactate is transported into the mitochondrial matrix. These findings support that LDH is strategically positioned to functionally interact with mitochondria, and suggest that lactate oxidation occurs near the outer surface of the inner mitochondrial membrane. How might an intracellular lactate shuttle operate in an intact cell? Let us return to the MAS.

In vivo, cytosolic NAD^+ could, in theory, be regenerated by malate dehydrogenase outside of the mitochondrial matrix, as part of the MAS. The literature gives some insight into different tissues and their mitochondrial shuttling activities. In the brain, the MAS has been considered the most important shuttle system for getting cytosolic NADH into the mitochondria (McKenna et al., 2006, and references therein); conversely, the glycerol-phosphate shuttle appears to be of lesser importance (Nguyen et al., 2003). Indeed, the intimate association of the MAS and the biosynthesis of neurotransmitter glutamate has been reported (Palaiologos et al., 1988). The published activities of the MAS measured in mitochondria isolated from rat brain are in the order of $26.7\,nmol \cdot min^{-1} \cdot mg^{-1}$ (Pardo et al., 2006). In synaptosomes, pharmacological inhibition of the MAS results in a pronounced (i.e. 50%) decrease in lactate oxidation (McKenna et al., 1993), supporting the model illustrated in **Figure 1**. Intracerebral production of lactate from ^{13}C labeled glucose further supports the notion that lactate is an important fuel for neurons (Sampol et al., 2013).

It should be noted that the MAS may have its limits. At high cardiac workloads, it has been shown that the α-ketoglutarate/malate transporter of the inner mitochondrial membrane cannot compete with matrix α-ketoglutarate dehydrogenase for their shared substrate, α-ketoglutarate (O'Donnell

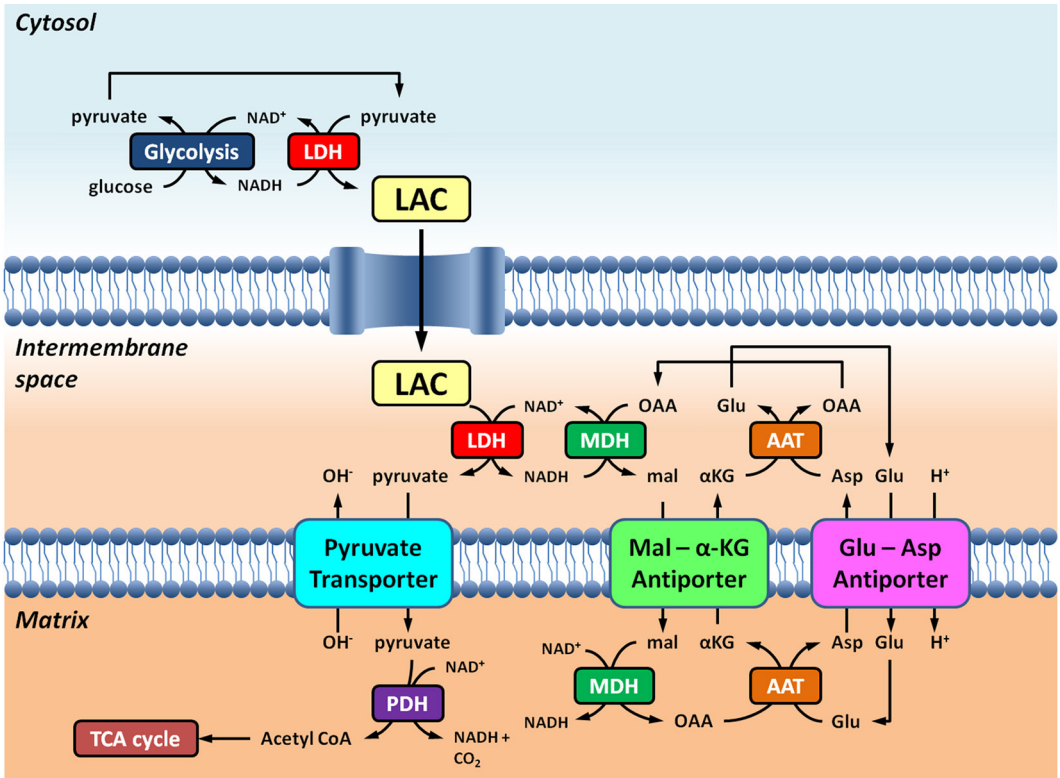


FIGURE 1 | Schematic representation of the link between glycolysis and lactate oxidation at the mitochondria outlined in this Perspective. Overall, glycolysis yields pyruvate and NADH, in addition to ATP. NAD^+ can be regenerated for glycolysis by the reduction of pyruvate to lactate (LAC) by lactate dehydrogenase (LDH). LAC can diffuse to the mitochondria where it is oxidized to pyruvate by LDH. NAD^+ is regenerated by extra-matrix malate dehydrogenase (MDH) of the malate-aspartate shuttle. Pyruvate is subsequently transported across the inner mitochondrial membrane into the matrix, where it is then oxidized by pyruvate dehydrogenase (PDH) to acetyl CoA. Abbreviations: α-KG, alpha-ketoglutarate; Glu, glutamate; AAT, aspartate-aminotransferase; OAA, oxaloacetate; Mal, malate.

et al., 2004). This results in a limiting effect to the MAS and its shuttling of NADH into the mitochondria. The net effect of this limitation to the MAS would be a rise in cytosolic lactate concentration as NAD^+ regeneration via the LDH reaction would help to preserve homeostatic NAD^+/NADH, even in the presence of adequate oxygen. Indeed, this is the classic phenomenon observed during especially strenuous exercise where lactate can accumulate in the blood, despite adequate oxygen availability.

INTRACELLULAR COMPARTMENTALIZATION OF LDH: LESSONS FROM THE PHOSPHOCREATINE SHUTTLE

The notion of shuttling compounds between the mitochondria and cytosol to meet the energetic demands of the cell using near-equilibrium enzymes is certainly not new. The phosphocreatine (PCr) shuttle involves distinct mitochondrial and cytosolic creatine kinase (CK) isoforms to essentially shuttle "high energy" phosphate from the mitochondria to the cytosol. Like the LDH reaction, the CK reaction (phosphocreatine + ADP + $H^+ \rightleftharpoons$ creatine + ATP), is considered to be "near-equilibrium," favoring ATP resynthesis. However, experimental evidence demonstrates that in myocardial cells, only *cytosolic* CK is actually at, or near, equilibrium (Reviewed in Joubert et al., 2004). Mitochondrial CK, on the other hand, localized to the intermembrane space, is displaced from equilibrium, favoring net PCr resynthesis. By way of analogy, two distinct LDH populations are thought to be involved in the intracellular lactate shuttle: cytosolic and mitochondrial. The cytosolic LDH would be at or near equilibrium, whereas the mitochondrial LDH would be displaced from equilibrium. The cytosolic LDH would favor net lactate production, while the mitochondrial LDH would favor lactate oxidation. And much like the adenine nucleotide translocase (ANT), which transports ADP into the matrix across the inner mitochondrial membrane in exchange for ATP, facilitates the displacement from equilibrium for mitochondrial CK in the intermembrane space, so too would the pyruvate transporter continuously transport pyruvate, displacing the mitochondrial LDH reaction from equilibrium (**Figure 1**). Such a lactate shuttle would benefit from LDH localization in the intermembrane space near the inner mitochondrial membrane, bound to the outside of the outer mitochondrial membrane at contact sites of the outer and inner mitochondrial membrane, or both. If intracellular lactate oxidation is to occur at the mitochondria via compartmentalization, as with the PCr shuttle, the cellular localization of LDH in, at, or about the mitochondria would be a salient feature.

INTERCELLULAR COMPARTMENTALIZATION OF LACTATE METABOLISM: THE ASTROCYTE-NEURON LACTATE SHUTTLE

A rich and growing body of neuroenergetic research also supports the existence of compartmentalized lactate metabolism among neighboring brain cells—namely, astrocytes and neurons (Reviewed in Pellerin and Magistretti, 2012). A variant of the intercellular lactate shuttle generally (Brooks, 2009), the astrocyte-neuron lactate shuttle (Pellerin and Magistretti, 2012) is based upon the idea that astrocytes are predominantly glycolytic, whereas neurons are more oxidative (Bouzier-Sore and Pellerin, 2013 and references therein). Using a metabolic modeling approach, it was argued that greater metabolic flux through PDH and the mitochondrial NADH shuttles in neurons compared to astrocytes necessarily dictates net lactate release by astrocytes and oxidation by neurons (Neves et al., 2012), supporting many experimental observations (reviewed in Bouzier-Sore and Pellerin, 2013). As mentioned previously, the MAS constitutes the major mitochondrial NADH shuttle in mature neurons (Kauppinen et al., 1987; Ramos et al., 2003; Contreras and Satrustegui, 2009; Gellerich et al., 2012). Hence, an important feature of the lactate-consuming neuron may well be its high MAS activity (Neves et al., 2012). Is it time for a *lactate-malate-aspartate shuttle*? Is there additional theoretical support for such a model in which lactate serves as a reducing equivalent?

REGENERATION OF CYTOSOLIC NAD^+

Lactate oxidation at the mitochondrion further makes sense of aerobic glycolysis by permitting cytosolic NAD^+ regeneration by cytosolic LDH. Indeed, evidence in cultured cells points to a highly labile lactate/pyruvate ratio which varies to preserve homeostatic NAD^+/NADH (Sun et al., 2012). This would be advantageous for the cell for a number of reasons. Firstly, it would provide an immediate means by which to regenerate NAD^+ locally (i.e., in the cytosol, where glycolysis occurs); the greater relative diffusability of lactate (molecular weight = 89.07 g/mol) vs. NAD^+ (molecular weight = 663.43 g/mol) means lactate can readily diffuse from the cell under conditions of increased glycolytic flux (e.g., intense exercise, hypoxic stress), while also being directed toward the mitochondria. During times of reduced cellular energy demand, continued lactate production during much lower rates of glycolytic flux would still be used to maintain homeostatic NAD^+/NADH within the cell, as well as for continued coupling of intracellular lactate shuttling to the MAS.

PROTON SHUTTLING AND MITOCHONDRIAL SUBSTRATE TRANSPORT

Lactate oxidation at the mitochondria makes sense of aerobic glycolysis because lactate production in the cytosol effectively consumes a proton (Robergs et al., 2004), which is thought to help mitigate the metabolic acidosis associated with increased ATP turnover and high rates of glycolysis (Robergs et al., 2004). The cytosolic concentration of lactate typically exceeds that of pyruvate by at least 10-fold, meaning that lactate, and not pyruvate is the predominant monocarboxylate entering the mitochondria intermembrane space (Brooks et al., 1999). By oxidizing lactate in the mitochondrial intermembrane space, protons would be released where they could contribute to the ΔpH component of the mitochondrial proton motive force across the inner membrane (Santo-Domingo and Demaurex, 2012), and/or be transported indirectly into the mitochondria by the MAS. As with the transport of inorganic phosphate and some other substrates and ions (Santo-Domingo and Demaurex, 2012), carrier-mediated transport of pyruvate across the inner mitochondrial membrane in rat liver mitochondria appears to be directly coupled to proton symport (or OH^- antiport) (Papa et al., 1971; Halestrap, 1975). Oxidation of lactate near the outer surface of the inner mitochondrial membrane, which releases a proton, would contribute to the ΔpH, and in turn, pyruvate transport into the matrix. Adjacent to the mitochondrial inner membrane, the LDH mass action ratio (i.e., concentrations of products/concentrations of reactions) could be largely facilitated by the "bleeding off" of pyruvate as it is continuously transported across the lactate-impermeable mitochondrial inner membrane, as well as a generous regeneration of NAD^+ by the extra-matrix malate dehydrogenase of the MAS. In this model, the transport of pyruvate across in the inner mitochondrial membrane would directly influence the rate of lactate oxidation just outside the matrix. Lactate oxidation at the mitochondria would therefore be expected to be regulated indirectly at the PDH reaction in the matrix. This would be advantageous because unlike LDH, PDH is highly regulated via allostery and covalent modification. As mentioned, modeling predicts high PDH activity to dictate neuronal lactate consumption *in vivo* (Neves et al., 2012); and high PDH activity also characterizes lactate-consuming neurons in culture (Halim et al., 2010).

METHODOLOGICAL CONSIDERATIONS

If mitochondrial lactate oxidation is functionally linked to the activity of the MAS, then it would be important to include components of the MAS in *in vitro* analyses of mitochondrial lactate oxidation, such as malate itself or oxaloacetate. Malate is the likely choice, as it is routinely included to stimulate respiration *in vitro*, where it is transported into the matrix and oxidized by mitochondrial malate dehydrogenase to oxaloacetate. This oxaloacetate can then condense with acetyl coA formed, for example, when pyruvate is added. Including glutamate in addition to malate, allows full operation of the MAS at the level of mitochondrial respiration. An important, but sometimes overlooked aspect of appropriate mitochondrial lactate oxidation assessment is the inclusion of NAD^+ as the requisite cofactor for the LDH reaction, and ADP as the phosphate acceptor to stimulate oxidative phosphorylation (i.e., state 3 respiration). Also, the extra-matrix component of the MAS involves the malate dehydrogenase reaction: oxaloacetate + NADH + H^+ $\rightleftharpoons$ malate + NAD^+. Experimental protocols examining respiratory oxygen consumption in isolated mitochondria from muscle using high malate concentrations (e.g., 4 mM; Rasmussen et al., 2002), may favor the malate dehydrogenase reaction in the reverse direction (i.e., malate oxidation and NADH + H^+ production) when added to the mitochondrial sample in combination with NAD^+. Indeed, reversibility of the MAS has been observed in isolated hepatocytes (Berry, 1971) and mitochondria with reconstituted MAS (Kunz and Davis, 1991). The net effect of this MAS reversal on respiration would

be to reduce malate from entering the mitochondria, forming oxaloacetate. More importantly, the reversal would prevent lactate oxidation to pyruvate, and subsequent transport and oxidation of the pyruvate in the matrix. Solutions to these methodological barriers to observing mitochondrial lactate oxidation *in vitro* involve including at least one component of the MAS. If adding malate, the appropriate concentration should be determined experimentally. Including ADP and NAD^+ or NADH (recall, the MAS will generate NAD^+ for the LDH reaction) is necessary also to observe appreciable mitochondrial lactate oxidation.

SUMMARY

A lactate-MAS is the interaction between the lactate and malate-aspartate shuttles to translocate reducing power to the mitochondria, particularly within oxidative, metabolically active cells.

ACKNOWLEDGMENTS

Many thanks to Dr. Erich Gnaiger (Medical University of Innsbruck), and Dr. George Brooks (University of California, Berkeley) for each critiquing earlier drafts of this work.

REFERENCES

Barros, L. F. (2013). Metabolic signaling by lactate in the brain. *Trends Neurosci.* 36, 396–404. doi: 10.1016/j.tins.2013.04.002

Bergman, B. C., Wolfel, E. E., Butterfield, G. E., Lopaschuk, G. D., Casazza, G. A., Horning, M. A., et al. (1999). Active muscle and whole body lactate kinetics after endurance training in men. *J. Appl. Physiol. (1985)* 87, 1684–1696.

Berry, M. N. (1971). Energy-dependent reduction of pyruvate to lactate by intact isolated parenchymal cells from rat liver. *Biochem. Biophys. Res. Commun.* 44, 1449–1456. doi: 10.1016/S0006-291X(71)80248-6

Boumezbeur, F., Petersen, K. F., Cline, G. W., Mason, G. F., Behar, K. L., Shulman, G. I., et al. (2010). The contribution of blood lactate to brain energy metabolism in humans measured by dynamic 13C nuclear magnetic resonance spectroscopy. *J. Neurosci.* 30, 13983–13991. doi: 10.1523/JNEUROSCI.2040-10.2010

Bouzier-Sore, A. K., and Pellerin, L. (2013). Unraveling the complex metabolic nature of astrocytes. *Front. Cell. Neurosci.* 7:179. doi: 10.3389/fncel.2013.00179

Bouzier-Sore, A. K., Voisin, P., Bouchaud, V., Bezancon, E., Franconi, J. M., and Pellerin, L. (2006). Competition between glucose and lactate as oxidative energy substrates in both neurons and astrocytes: a comparative NMR study. *Eur. J. Neurosci.* 24, 1687–1694. doi: 10.1111/j.1460-9568.2006.05056.x

Brooks, G. A. (1998). Mammalian fuel utilization during sustained exercise. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 120, 89–107. doi: 10.1016/S0305-0491(98)00025-X

Brooks, G. A. (2009). Cell-cell and intracellular lactate shuttles. *J. Physiol.* 587, 5591–5600. doi: 10.1113/jphysiol.2009.178350

Brooks, G. A. (2014). "Metabolic systems: the formation and utilization of lactate," in *History of Exercise Physiology. Human Kinetics,* ed C. M. Tipton (Champaign, IL: Human Kinetics), 447–475.

Brooks, G. A., Butterfield, G. E., Wolfe, R. R., Groves, B. M., Mazzeo, R. S., Sutton, J. R., et al. (1991). Decreased reliance on lactate during exercise after acclimatization to 4,300 m. *J. Appl. Physiol. (1985)*. 71, 333–341.

Brooks, G. A., Dubouchaud, H., Brown, M., Sicurello, J. P., and Butz, C. E. (1999). Role of mitochondrial lactate dehydrogenase and lactate oxidation in the intracellular lactate shuttle. *Proc. Natl. Acad. Sci. U.S.A.* 96, 1129–1134. doi: 10.1073/pnas.96.3.1129

Chatham, J. C., Des Rosiers, C., and Forder, J. R. (2001). Evidence of separate pathways for lactate uptake and release by the perfused rat heart. *Am. J. Physiol. Endocrinol. Metab.* 281, E794–E802.

Contreras, L., and Satrustegui, J. (2009). Calcium signaling in brain mitochondria: interplay of malate aspartate NADH shuttle and calcium uniporter/mitochondrial dehydrogenase pathways. *J. Biol. Chem.* 284, 7091–7099. doi: 10.1074/jbc.M808066200

Crabtree, B., and Newsholme, E. A. (1978). Sensitivity of a near-equilibrium reaction in a metabolic pathway to changes in substrate concentration. *Eur. J. Biochem.* 89, 19–22. doi: 10.1111/j.1432-1033.1978.tb20891.x

Cruz, R. S., de Aguiar, R. A., Turnes, T., Penteado Dos Santos, R., de Oliveira, M. F., and Caputo, F. (2012). Intracellular shuttle: the lactate aerobic metabolism. *ScientificWorldJournal* 2012:420984. doi: 10.1100/2012/420984

Dienel, G. A. (2012). Brain lactate metabolism: the discoveries and the controversies. *J. Cereb. Blood Flow Metab.* 32, 1107–1138. doi: 10.1038/jcbfm.2011.175

Digerness, S. B., and Reddy, W. J. (1976). The malate-aspartate shuttle in heart mitochondria. *J. Mol. Cell. Cardiol.* 8, 779–785. doi: 10.1016/0022-2828(76)90084-5

Doherty, J. R., and Cleveland, J. L. (2013). Targeting lactate metabolism for cancer therapeutics. *J. Clin. Invest.* 123, 3685–3692. doi: 10.1172/JCI69741

Donovan, C. M., and Pagliassotti, M. J. (2000). Quantitative assessment of pathways for lactate disposal in skeletal muscle fiber types. *Med. Sci. Sports Exerc.* 32, 772–777. doi: 10.1097/00005768-200004000-00009

Elustondo, P. A., White, A. E., Hughes, M. E., Brebner, K., Pavlov, E., and Kane, D. A. (2013). Physical and functional association of lactate dehydrogenase (LDH) with skeletal muscle mitochondria. *J. Biol. Chem.* 288, 25309–25317. doi: 10.1074/jbc.M113.476648

Funfschilling, U., Supplie, L. M., Mahad, D., Boretius, S., Saab, A. S., Edgar, J., et al. (2012). Glycolytic oligodendrocytes maintain myelin and long-term axonal integrity. *Nature* 485, 517–521. doi: 10.1038/nature11007

Gellerich, F. N., Gizatullina, Z., Gainutdinov, T., Muth, K., Seppet, E., Orynbayeva, Z., et al. (2013). The control of brain mitochondrial energization by cytosolic calcium: the mitochondrial gas pedal. *IUBMB Life* 65, 180–190. doi: 10.1002/iub.1131

Gellerich, F. N., Gizatullina, Z., Trumbekaite, S., Korzeniewski, B., Gaynutdinov, T., Seppet, E., et al. (2012). Cytosolic Ca2+ regulates the energization of isolated brain mitochondria by formation of pyruvate through the malate-aspartate shuttle. *Biochem. J.* 443, 747–755. doi: 10.1042/BJ20110765

Gertz, E. W., Wisneski, J. A., Stanley, W. C., and Neese, R. A. (1988). Myocardial substrate utilization during exercise in humans. Dual carbon-labeled carbohydrate isotope experiments. *J. Clin. Invest.* 82, 2017–2025. doi: 10.1172/JCI113822

Gladden, L. B. (2004). Lactate metabolism: a new paradigm for the third millennium. *J. Physiol.* 558, 5–30. doi: 10.1113/jphysiol.2003.058701

Gladden, L. B. (2007). Is there an intracellular lactate shuttle in skeletal muscle? *J. Physiol.* 582, 899. doi: 10.1113/jphysiol.2007.138487

Halestrap, A. P. (1975). The mitochondrial pyruvate carrier. Kinetics and specificity for substrates and inhibitors. *Biochem. J.* 148, 85–96.

Halim, N. D., McFate, T., Mohyeldin, A., Okagaki, P., Korotchkina, L. G., Patel, M. S., et al. (2010). Phosphorylation status of pyruvate dehydrogenase distinguishes metabolic phenotypes of cultured rat brain astrocytes and neurons. *Glia* 58, 1168–1176. doi: 10.1002/glia.20996

Jacobs, R. A., Meinild, A. K., Nordsborg, N. B., and Lundby, C. (2013). Lactate oxidation in human skeletal muscle mitochondria. *Am. J. Physiol. Endocrinol. Metab.* 304, E686–E689. doi: 10.1152/ajpendo.00476.2012

Joubert, F., Mateo, P., Gillet, B., Beloeil, J. C., Mazet, J. L., and Hoerter, J. A. (2004). CK flux or direct ATP transfer: versatility of energy transfer pathways evidenced by NMR in the perfused heart. *Mol. Cell. Biochem.* 256–257, 43–58. doi: 10.1023/B:MCBI.0000009858.41434.fc

Kauppinen, R. A., Sihra, T. S., and Nicholls, D. G. (1987). Aminooxyacetic acid inhibits the malate-aspartate shuttle in isolated nerve terminals and prevents the mitochondria from utilizing glycolytic substrates. *Biochim. Biophys. Acta* 930, 173–178. doi: 10.1016/0167-4889(87)90029-2

Kitaoka, Y., Hoshino, D., and Hatta, H. (2012). Monocarboxylate transporter and lactate metabolism. *J. Phys. Fitness Sports Med.* 1, 247–252. doi: 10.7600/jpfsm.1.247

Kline, E. S., Brandt, R. B., Laux, J. E., Spainhour, S. E., Higgins, E. S., Rogers, K. S., et al. (1986). Localization of L-lactate dehydrogenase in mitochondria. *Arch. Biochem. Biophys.* 246, 673–680. doi: 10.1016/0003-9861(86)90323-1

Kunz, W. S., and Davis, E. J. (1991). Control of reversible intracellular transfer of reducing potential. *Arch. Biochem. Biophys.* 284, 40–46. doi: 10.1016/0003-9861(91)90260-P

Luft, F. C. (2001). Lactic acidosis update for critical care clinicians. *J. Am. Soc. Nephrol.* 12 Suppl 17: S15–S19.

McKenna, M. C., Tildon, J. T., Stevenson, J. H., Boatright, R., and Huang, S. (1993). Regulation of energy metabolism in synaptic terminals and cultured rat

brain astrocytes: differences revealed using aminooxyacetate. *Dev. Neurosci.* 15, 320–329. doi: 10.1159/000111351

McKenna, M. C., Waagepetersen, H. S., Schousboe, A., and Sonnewald, U. (2006). Neuronal and astrocytic shuttle mechanisms for cytosolic-mitochondrial transfer of reducing equivalents: current evidence and pharmacological tools. *Biochem. Pharmacol.* 71, 399–407. doi: 10.1016/j.bcp.2005.10.011

Neves, A., Costalat, R., and Pellerin, L. (2012). Determinants of brain cell metabolic phenotypes and energy substrate utilization unraveled with a modeling approach. *PLoS Comput. Biol.* 8:e1002686. doi: 10.1371/journal.pcbi.1002686

Newington, J. T., Harris, R. A., and Cumming, R. C. (2013). Reevaluating metabolism in alzheimer's disease from the perspective of the astrocyte-neuron lactate shuttle model. *J. Neurodegener. Dis.* 2013, 13. doi: 10.1155/2013/234572

Nguyen, N. H., Brathe, A., and Hassel, B. (2003). Neuronal uptake and metabolism of glycerol and the neuronal expression of mitochondrial glycerol-3-phosphate dehydrogenase. *J. Neurochem.* 85, 831–842. doi: 10.1046/j.1471-4159.2003.01762.x

O'Donnell, J. M., Kudej, R. K., LaNoue, K. F., Vatner, S. F., and Lewandowski, E. D. (2004). Limited transfer of cytosolic NADH into mitochondria at high cardiac workload. *Am. J. Physiol. Heart Circ. Physiol.* 286, H2237–H2242. doi: 10.1152/ajpheart.01113.2003

Palaiologos, G., Hertz, L., and Schousboe, A. (1988). Evidence that aspartate aminotransferase activity and ketodicarboxylate carrier function are essential for biosynthesis of transmitter glutamate. *J. Neurochem.* 51, 317–320. doi: 10.1111/j.1471-4159.1988.tb04872.x

Papa, S., Francavilla, A., Paradies, G., and Meduri, B. (1971). The transport of pyruvate in rat liver mitochondria. *FEBS Lett.* 12, 285–288. doi: 10.1016/0014-5793(71)80200-4

Pardo, B., Contreras, L., Serrano, A., Ramos, M., Kobayashi, K., Iijima, M., et al. (2006). Essential role of aralar in the transduction of small Ca2+ signals to neuronal mitochondria. *J. Biol. Chem.* 281, 1039–1047. doi: 10.1074/jbc.M507270200

Pellerin, L., and Magistretti, P. J. (2012). Sweet sixteen for ANLS. *J. Cereb. Blood Flow Metab.* 32, 1152–1166. doi: 10.1038/jcbfm.2011.149

Quistorff, B., and Grunnet, N. (2011a). High brain lactate is not caused by a shift in the lactate dehydrogenase A/B ratio. *Proc. Natl. Acad. Sci. U.S.A.* 108, E21. author reply: E22. doi: 10.1073/pnas.1017750108

Quistorff, B., and Grunnet, N. (2011b). The isoenzyme pattern of LDH does not play a physiological role; except perhaps during fast transitions in energy metabolism. *Aging (Albany, NY)* 3, 457–460.

Quistorff, B., Secher, N. H., and Van Lieshout, J. J. (2008). Lactate fuels the human brain during exercise. *FASEB J.* 22, 3443–3449. doi: 10.1096/fj.08-106104

Ramos, M., del Arco, A., Pardo, B., Martinez-Serrano, A., Martinez-Morales, J. R., Kobayashi, K., et al. (2003). Developmental changes in the Ca2+-regulated mitochondrial aspartate-glutamate carrier aralar1 in brain and prominent expression in the spinal cord. *Brain Res. Dev. Brain Res.* 143, 33–46. doi: 10.1016/S0165-3806(03)00097-X

Rasmussen, H. N., van Hall, G., and Rasmussen, U. F. (2002). Lactate dehydrogenase is not a mitochondrial enzyme in human and mouse vastus lateralis muscle. *J. Physiol.* 541, 575–580. doi: 10.1113/jphysiol.2002.019216

Robergs, R. A., Ghiasvand, F., and Parker, D. (2004). Biochemistry of exercise-induced metabolic acidosis. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 287, R502–R516. doi: 10.1152/ajpregu.00114.2004

Rueda, C. B., Llorente-Folch, I., Amigo, I., Contreras, L., Gonzalez-Sanchez, P., Martinez-Valero, P., et al. (2014). Ca^{2+} regulation of mitochondrial function in neurons. *Biochim. Biophys. Acta* 1837, 1617–1624. doi: 10.1016/j.bbabio.2014.04.010

Safer, B., Smith, C. M., and Williamson, J. R. (1971). Control of the transport of reducing equivalents across the mitochondrial membrane in perfused rat heart. *J. Mol. Cell. Cardiol.* 2, 111–124. doi: 10.1016/0022-2828(71)90065-4

Sampol, D., Ostrofet, E., Jobin, M. L., Raffard, G., Sanchez, S., Bouchaud, V., et al. (2013). Glucose and lactate metabolism in the awake and stimulated rat: a (13)C-NMR study. *Front. Neuroenergetics* 5:5. doi: 10.3389/fnene.2013.00005

Santo-Domingo, J., and Demaurex, N. (2012). Perspectives on: SGP symposium on mitochondrial physiology and medicine: the renaissance of mitochondrial pH. *J. Gen. Physiol.* 139, 415–423. doi: 10.1085/jgp.201110767

Schantz, P. G. (1986). Plasticity of human skeletal muscle with special reference to effects of physical training on enzyme levels of the NADH shuttles and phenotypic expression of slow and fast myofibrillar proteins. *Acta Physiol. Scand. Suppl.* 558, 1–62.

Schurr, A. (2014). Cerebral glycolysis: a century of persistent misunderstanding and misconception. *Front. Neurosci.* 8:360. doi: 10.3389/fnins.2014.00360

Schurr, A., West, C. A., and Rigor, B. M. (1988). Lactate-supported synaptic function in the rat hippocampal slice preparation. *Science* 240, 1326–1328. doi: 10.1126/science.3375817

Skilleter, D. N., and Kun, E. (1972). The oxidation of L-lactate by liver mitochondria. *Arch. Biochem. Biophys.* 152, 92–104. doi: 10.1016/0003-9861(72)90197-X

Spriet, L. L., Howlett, R. A., and Heigenhauser, G. J. (2000). An enzymatic approach to lactate production in human skeletal muscle during exercise. *Med. Sci. Sports Exerc.* 32, 756–763. doi: 10.1097/00005768-200004000-00007

Stainsby, W. N., and Brooks, G. A. (1990). Control of lactic acid metabolism in contracting muscles and during exercise. *Exerc. Sport Sci. Rev.* 18, 29–63. doi: 10.1249/00003677-199001000-00005

Sun, F., Dai, C., Xie, J., and Hu, X. (2012). Biochemical issues in estimation of cytosolic free NAD/NADH ratio. *PLoS ONE* 7:e34525. doi: 10.1371/journal.pone.0034525

Todd, J. J. (2014). Lactate: valuable for physical performance and maintenance of brain function during exercise. *Biosci. Horiz.* 7, 1–7. doi: 10.1093/biohorizons/hzu001

van Hall, G. (2010). Lactate kinetics in human tissues at rest and during exercise. *Acta Physiol. (Oxf.)* 199, 499–508. doi: 10.1111/j.1748-1716.2010.02122.x

van Hall, G., Stromstad, M., Rasmussen, P., Jans, O., Zaar, M., Gam, C., et al. (2009). Blood lactate is an important energy source for the human brain. *J. Cereb. Blood Flow Metab.* 29, 1121–1129. doi: 10.1038/jcbfm.2009.35

Voet, D., Voet, J. G., and Pratt, C. W. (2011). *Fundamentals of Biochemistry: Life at the Molecular Level.* Hoboken, NJ: Wiley.

Williamson, D. H., Lund, P., and Krebs, H. A. (1967). The redox state of free nicotinamide-adenine dinucleotide in the cytoplasm and mitochondria of rat liver. *Biochem. J.* 103, 514–527.

Wyss, M. T., Jolivet, R., Buck, A., Magistretti, P. J., and Weber, B. (2011). *In vivo* evidence for lactate as a neuronal energy source. *J. Neurosci.* 31, 7477–7485. doi: 10.1523/JNEUROSCI.0415-11.2011

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 11 September 2014; accepted: 25 October 2014; published online: 25 November 2014.

Citation: Kane DA (2014) Lactate oxidation at the mitochondria: a lactate-malate-aspartate shuttle at work. Front. Neurosci. ***8****:366. doi: 10.3389/fnins.2014.00366*

This article was submitted to Neuroenergetics, Nutrition and Brain Health, a section of the journal Frontiers in Neuroscience.

frontiers in
NEUROSCIENCE

REVIEW ARTICLE
published: 25 November 2014
doi: 10.3389/fnins.2014.00378

Contribution of glycogen in supporting axon conduction in the peripheral and central nervous systems: the role of lactate

Tom W. Chambers[1], Timothy P. Daly[1], Adam Hockley[1] and Angus M. Brown[1,2]*

[1] School of Life Sciences, Queen's Medical Centre, University of Nottingham, Nottingham, UK
[2] Department of Neurology, University of Washington, Seattle, WA, USA

Edited by:
Avital Schurr, University of Louisville, USA

Reviewed by:
L. Felipe Barros, Centro de Estudios Científicos, Chile
Daniela Calvetti, Case Western Reserve University, USA

***Correspondence:**
Angus M. Brown, School of Life Sciences, University of Nottingham, Nottingham, NG7 2UH, UK
e-mail: ambrown@nottingham.ac.uk

The role of glycogen in the central nervous system is intimately linked with the glycolytic pathway. Glycogen is synthesized from glucose, the primary substrate for glycolysis, and degraded to glucose-6-phosphate. The metabolic cost of shunting glucose via glycogen exceeds that of simple phosphorylation of glucose to glucose-6-phosphate by hexokinase; thus, there must be a metabolic advantage in utilizing this shunt pathway. The dogmatic view of glycogen as a storage depot persists, based on initial descriptions of glycogen supporting neural function in the face of aglycemia. The variable latency to conduction failure, dependent upon tissue glycogen levels, provided convincing evidence of the role played by glycogen in supporting neural function. Glycogen is located predominantly in astrocytes in the central nervous system, thus for glycogen to benefit neural elements, intercellular metabolic communication must exist in the form of astrocyte to neuron substrate transfer. Experimental evidence supports a model where glycogen is metabolized to lactate in astrocytes, with cellular expression of monocarboxylate transporters and enzymes appropriately located for lactate shuttling between astrocytes and neural elements, where lactate acts as a substrate for oxidative metabolism. Biosensor recordings have demonstrated a significant steady concentration of lactate present on the periphery of both central white matter and peripheral nerve under unstimulated baseline conditions, indicating continuous cellular efflux of lactate to the interstitium. The existence of this lactate pool argues we must reexamine the "on demand" shuttling of lactate between cellular elements, and suggests continuous lactate efflux surplus to immediate neural requirements.

Keywords: glycogen, white matter, axon, lactate, biosensor

INTRODUCTION

In the last 20 years interest in brain energy metabolism, and in particular specific cellular substrate utilization and trafficking of metabolites between neural elements and glia has witnessed a rapid growth, which shows no sign of abating. This is due to several factors which may be identified as follows: the realization that certain neurological conditions e.g., Alzheimer's disease, may in part be due to metabolic disturbance in the brain (Cai et al., 2012), improved or novel technologies revealing previously unavailable information regarding metabolism (Brown et al., 2012; Barros et al., 2013), and an increased awareness of the importance of the role of lactate (Dienel and Hertz, 2001; Dienel, 2004). It is this final topic that is the subject of this review article. The dogmatic view of lactate is as a waste product, the result of incomplete metabolism of glucose, where glucose is glycolytically converted to pyruvate / lactate but not oxidatively metabolized (Stryer, 1995). The role of lactate in the periphery must not be confused with its role in the CNS. In the periphery, and in particular muscle, lactate is the result of incomplete oxidative metabolism of glucose (Stryer, 1995), and has entered the common vernacular in relation to sport e.g., lactate threshold (Messonnier et al., 2013). The lactate accumulation is due to the bodies' inability to take in sufficient oxygen during intense exercise to completely metabolize available glucose, with the shortfall between oxygen uptake and glucose availability being manifest as an accumulation of lactate. As an aside, it has recently been shown that the lactate generated in the periphery as a result of maximal exercise is not a waste product after all, and crosses the blood brain barrier where it is oxidatively metabolized by the brain (Dalsgaard, 2006), although glucose is still the main energy substrate. The relationship shows a degree of metabolic co-operation between the muscles and the brain, whereby the muscle takes up all available glucose, the only substrate muscle can efficiently metabolize, for instantaneous energy production, and the brain extracts lactate from the systemic circulation, which can exceed 5 mmol l^{-1} during maximal exercise (Dalsgaard, 2006). Brain energy metabolism is more complex than that which occurs in muscle due to the variety of different cell types in the brain, each of which has individual requirements dependent upon function. Given the absolute requirements of glucose and oxygen as

the main energy support of the brain, and the extremely rapid (6–8 s) loss of consciousness that occurs when blood supply to the brain is interrupted for even the shortest period of time (Rossen et al., 1943), glucose and oxygen are clearly vital commodities to the brain. Thus, it is surprising that not all glucose is oxidatively metabolized in the brain. If such were the case the respiratory quotient (RQ; the ratio of oxygen molecules consumed per glucose molecule metabolized) for the brain would be 6 (as 6 oxygen molecules are required in the metabolism of one glucose molecule, which contains 6 carbon molecules); the RQ for resting brain is in fact nearer 5.5 indicating a significant proportion of the glucose is not oxidatively metabolized. In addition this ratio decreases with increased activity, falling to values as low as 3 (Dienel, 2009). This provokes several questions: what is the benefit to the brain of incomplete glucose oxidation? Is there cellular compartmentalization with regard to glucose metabolism, and if so how does this change during increased tissue energy demand? What role does glycogen play in this scheme?

THE RODENT OPTIC NERVE MODEL

In our studies of brain energy metabolism we have chosen the rodent optic nerve as a model of central white matter (Ransom et al., 1997). In the adult human brain white matter occupies 50% of brain volume whereas the equivalent is only about 15% in the rodent, a ratio that clearly illustrates the high degree of inter-regional connectivity (Zhang and Sejnowski, 2000; Karbowski, 2003). Thus, any metabolic disturbances within the brain are likely to affect both gray and white matter areas. The optic nerve offers the following advantage over other white matter tissue, such as the corpus callosum and the ventral column of the spinal cord, in that it is easy to remove without damaging the tissue. An additional advantage is that the stimulus-evoked response recorded from the optic nerve is a stereotypical triple peaked profile that offers a stable baseline against which the compound action potential (CAP) resulting from experimental interventions can be compared (Stys et al., 1991), whereas corpus callosum and spinal cord white matter tracts are far more prone to variability in stimulus evoked potentials due to the plane in which the section is cut and degree of injury incurred by the tissue during slicing (Baltan, 2006; Velumian et al., 2011). The optic nerve comprises myelinated axons (almost all axons are myelinated in the rodent adult) (Ransom et al., 1997), oligodendrocytes and astrocytes, and as such it is a simple model system without the complications of synapses or neuronal cell bodies. In our experimental set-up the acutely isolated optic nerve is maintained in an oxygenated chamber at 37°C and superfused with bicarbonate buffered artificial cerebrospinal fluid (aCSF) containing 10 mM glucose. Under these circumstances a stable CAP can be recorded for many hours (Brown et al., 2003b). *In vitro* experimental studies have demonstrated that both glucose and lactate (20 mM lactate is the carbon equivalent of 10 mM glucose) can fully support the CAP for extended periods of time (Brown et al., 2003b). When these experiments commenced over a decade ago the role of oligodendrocytes was ignored with axons and astrocytes of primary interest, thus only these cells shall be addressed. That glucose can support function is to be expected, and given the intimate regulatory roles that astrocytes play in supporting neural elements, significantly their role in buffering $[K^+]_o$ (Hoppe et al., 1991), we conclude that both astrocytes and axons can take up glucose, and metabolize it efficiently such as to promote the continued maintenance of the cell i.e., maintain their trans-membrane ion gradients. In the absence of sufficient glucose-derived ATP the Na pump would cease to function and the trans-membrane ion gradients would be lost, leading to a decrease and eventual loss of the CAP (Leppanen and Stys, 1997; Stys, 1998). Substituting glucose with lactate had no material impact on the CAP suggesting that lactate can be taken up and utilized by both axons and astrocytes for extended periods of time. These experiments do not, however, cover the role of substrate transfer between axons and astrocytes. For example supplying the nerve with glucose could result in astrocytes taking up glucose and converting it to lactate, which is then shuttled to the axons (this is in part what the astrocyte neuron lactate shuttle hypothesis proposes); in such a condition although supplying exogenous glucose supported axon function the axons did not take up glucose directly, but survived on astrocyte-derived lactate. To circumvent such arguments we used the compounds D-lactate and cinnemate, which block lactate transport, D-lactate as a competitive inhibitor and cinnemate as a conventional blocker. Neither compound affected the ability of glucose to support the CAP (Brown et al., 2003a), thus we feel we can safely assume that axons and astrocytes can directly take up glucose. These assumptions are supported by the presence of the glut-1 glucose transporter on astrocyte membranes (Morgello et al., 1995). The argument for lactate being used independently by both axons and astrocytes is that it is unlikely a cellular compartment would take up lactate and subsequently release it, given that the uni-directional transport of lactate and H^+ is controlled by their respective trans-membrane ion gradients (Halestrap and Price, 1999).

The dogma of cellular compartmentalization of metabolism is that neural elements are oxidative and astrocytic elements are glycolytic, thus astrocytes produce lactate, which is consumed by neurons. There is a considerable body of evidence to support this simplistic assumption, although much of it derives from tissue culture studies, which must be viewed with caution, as a very important aspect, namely the intercommunication between cell types, is absent in these types of study. There are many studies showing that astrocytes in culture do release lactate when bathed in a medium containing glucose (Dringen and Hamprecht, 1992; Dringen et al., 1995). Similar studies of cultured neurons tend to show that neurons take up, rather than release lactate (Schurr et al., 1988, 1997a,b). Indeed lactate fuels both neuronal recovery after hypoxia (Schurr et al., 1997a), and is consumed by neurons in culture at rest, in the presence of normoglycemic glucose (Bouzier-Sore et al., 2006). In our rodent optic nerve model we investigated the trafficking of lactate between astrocytes and axons (neural elements), by investigating the role of glycogen in supporting axon conduction. It is recognized that in adult mammalian brain the glycogen resides primarily in astrocytes (Cataldo and Broadwell, 1986), a serendipitous location as it allows us to pinpoint the cellular location of glycogen-derived lactate. Our initial study localized glycogen to astrocytes, and biochemical assay revealed significant depots of glycogen in the tissue. The glycogen content was labile, increasing commensurately with elevations

in bathing glucose concentration. Under conditions designed to simulate *in vivo* aglycemia, when aCSF lacking glucose was superfused, the CAP was lost, but only after a significant latency of 20 min in mouse (Brown et al., 2003a), and 30 min in rat (Wender et al., 2000). The latencies could be altered by experimentally manipulating the tissue glycogen content prior to aglycemia, such that elevated glycogen content led to increased latencies and vice versa (Brown et al., 2003a). Biochemical assay revealed that at the onset of aglycemia glycogen content fell and continued to fall until it reached its nadir, which coincided with the loss of the CAP (Brown et al., 2003a) (Wender et al., 2000). These results showed that tissue glycogen was metabolized and supported axon conduction in the absence of exogenously applied glucose, but that once the glycogen was exhausted the CAP failed. The nature of the support afforded by glycogen was investigated, based on assumptions drawn from previously published data, namely that astrocytes produce lactate and neural elements consume lactate. For this to be the case several conditions must be met, which can be tested experimentally. First, the astrocytes and axons must express the means to transport lactate i.e., monocarboxylate transporters (MCTs). There are four isoforms of this transporter (Halestrap, 2012), but we focused on the two isoforms most likely to be involved, MCT1, which is expressed in tissue that releases lactate, and MCT2 that is expressed on tissue that takes up lactate. Immunohistochemical data using specific antibodies that recognize MCT1 and MCT2 in combination with cell specific markers, namely GFAP for astrocytes and neurofilament for axons, showed expression patterns supportive of astrocyte to axon lactate transport, namely axonal MCT2 expression and astrocytic MCT1 expression. In addition the expression pattern of the enzyme lactate dehydrogenase demonstrated neuronal expression of the LDH1 isoform, whereas astrocytes expressed both LDH1 and LDH5 isoform indicating that both cell types are capable of inter-converting lactate and pyruvate (Tekkok et al., 2005).

These experiments convincingly demonstrate glycogen-derived lactate transport from astrocytes to axons under conditions of aglycemia. What is of more interest is to determine the role of glycogen under more physiological conditions, such as hypoglycaemia, which is a common side effect experienced by type 1 diabetic patients who use exogenous insulin to manage their symptoms (Bittar et al., 1996), and under increased tissue energy demand. Mouse optic nerves superperfused with 10 mM glucose can sustain conduction for many hours. Switching to 2 mM glucose aCSF had no effect on the CAP (Frier and Fisher, 2007). However, adding cinnemate, which blocks lactate transport at the MCT causes a decrease in CAP area (Brown et al., 2003a). In a separate experiment superfusing nerves with 2 mM glucose after previously depleting glycogen with 20 min of 0 glucose superfusion caused the CAP to fall (Brown et al., 2003a). Our conclusion from these experiments is that 2 mM glucose is insufficient to fully support the CAP and that glycogen is metabolized in the astrocyte to lactate, which is then shuttled to the axon to maintain axon conduction. Interrupting lactate transport or decreasing glycogen content results in CAP failure. The mouse optic nerve is able to sustain firing when stimulated at frequencies of up to 50 Hz i.e., the CAP remains above baseline during sustained stimulation. At 100 Hz the CAP area initially increased above baseline due to the CAP spreading out (Brown et al., 2003a) as a result of increased extracellular K^+ accumulation (Brown et al., 2003a), followed by a slow decrease below baseline after about 5 min. Addition of isofagomine, an inhibitor of glycogen phosphorylase (Ransom et al., 1985), or D-lactate, or cinnemate (Brown et al., 2005), caused a decrease in the CAP, indicative of glycogen metabolism sustaining the CAP during periods of increased energy demand. Thus, the increased tissue energy demand invoked by imposing high frequency stimulus on the nerve, is not met by ambient glucose concentrations, and glycogen derived lactate provides supplementary energy substrate. An alternative view has been proposed whereby astrocytes metabolize glycogen thus maintaining high levels of glucose-6-phosphate, diverting glucose for neuronal oxidation (Dienel, 2012).

Thus, in the rodent optic nerve any lactate present in the extracellular space is likely to be due to glycogen metabolism under conditions of increased metabolic need by axons. However, we are interested in whether glucose contributes to the putative extracellular lactate pool. That astrocytes release lactate, which is taken up by neural elements has a firm foundation backed up by experimental evidence as described above. However, in the mid 1990's the astrocyte neuron lactate shuttle hypothesis (ANLSH) proposed that a significant proportion of glucose delivered to the brain was shuttled into astrocytes where it was converted to lactate and subsequently released into the extracellular space and taken up by neurons (Dienel and Cruz, 2014). However, this scheme proposed an "on demand" aspect, such that the initiating factor for the sequence was release of glutamate into synapses, with the glutamate subsequently taken up by astrocytes, which provoked glucose uptake. As such the scheme would appear to propose that under resting conditions there was little lactate trafficking between astrocytes and neurons, and that this was only initiated by metabolic need by neurons, and under conditions of increased energy demand both glucose and glycogen are proposed to be sources of lactate (Pellerin and Magistretti, 1994). Recent data has suggested that the brain does not use lactate as an obligate substrate to the extent that the brain can release lactate into the systemic circulation, and lactate is only used when present in high non-physiological concentration (Magistretti et al., 1999).

LACTATE BIOSENSORS

The development of lactate biosensors offered an ideal opportunity to investigate the dynamics of extracellular lactate. The rodent optic nerve is an ideal preparation to use as the biosensor can be placed alongside the nerve, i.e., just outside the meninges, without damaging the tissue (Yang et al., 2014). It is worthwhile examining this configuration in detail as illustrated in **Figure 1**. In such a configuration the sensor does not record lactate in the interstitium but rather at the periphery of the nerve. Thus, any lactate recorded has been released by the nerve and cannot be used as a substrate i.e., it can be considered surplus to current energy requirements. Given the superfusion system, there is a constant flow of aCSF over the sensor, thus the sensor would record any lactate released by the tissue into the aCSF. What this means is

that a stable reading of lactate implies there is a constant efflux of lactate from the nerve, a decreased level means a decreased release and an increase means an increased release. Compare this to a static enclosed system, where an acute release of lactate would be recorded as a continuously elevated level. With this in mind it is timely to discuss the implications of recent recordings using lactate biosensors in mouse optic nerve. It is immediately apparent that in nerves perfused with control concentrations of glucose the lactate concentration was about 500 μM (the concentration may not be an accurate reflection of the interstitial [lactate] due to continuous superfusion and incomplete contact of the sensor with the nerve etc.). That such a large concentration is recorded may be viewed as surprising, but is in agreement with *in vivo* data using dialysis techniques that have recorded equivalent concentrations (Hu and Wilson, 1997). However, the *in vivo* situation is different as the ECS is a stable compartment, and dependent upon the rate of release or uptake of lactate, may not reflect a vibrant flux of lactate, but rather a stationary lactate pool. Alternatively, the conditions present in these experiments in which aCSF lacking lactate superfuses the tissue may exaggerate any true release of lactate from the tissue due to the extracellular pool lacking lactate.

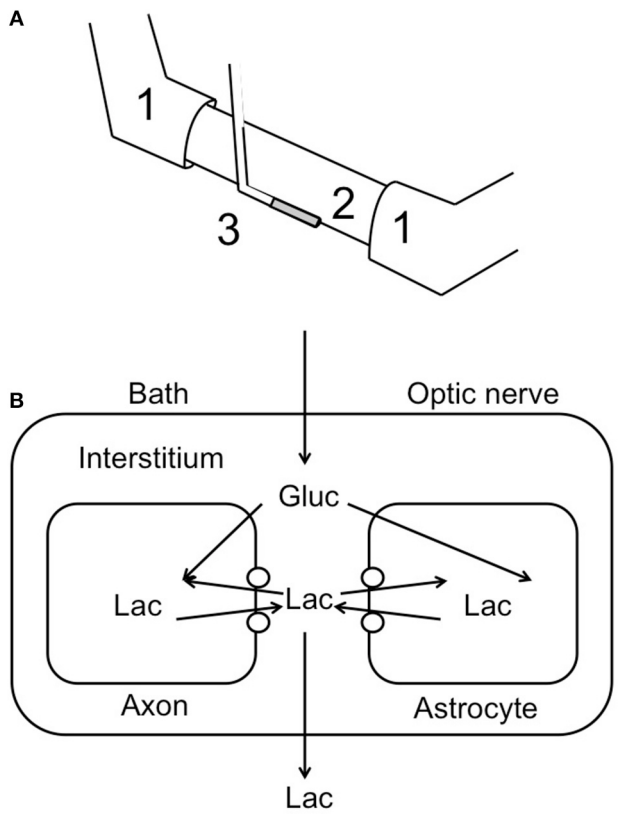


FIGURE 1 | Recording lactate release from optic nerve. (A) Schematic illustrating the use of a lactate biosensor (3) to record lactate release from optic nerve (2). The CAP is recorded via the suction electrodes (1). **(B)** A model of possible lactate movements in the continually superfused optic nerve. The open circles indicate monocarboxylate transporters; Gluc, glucose; Lac, lactate.

In addition it has recently been shown that extracellular lactate inhibits astrocytic glycolysis (Sotelo-Hitschfeld et al., 2012). Another intriguing possibility, although we present no evidence to support it, is that white matter is a lactate producer and gray matter is a lactate consumer. Such a high level of recorded lactate release warrants comment, and strongly suggests that the tissue creates and exports lactate to the interstitium irrespective of metabolic need. This contradicts the ANLSH, where lactate is exported by astrocytes on demand by neurons (Magistretti et al., 1999). The compartmentalization of lactate production has not been elucidated in this system, but it is highly likely that the glial (astrocytic) component produces most of the lactate, and thus exports lactate as a continuous process. That the lactate is released and unutilized is initially surprising, but appears logical on closer inspection. An axon in an *in vivo* situation that suddenly increases its energy demand would be fed by an increase in localized blood flow that is initially via Ca^{2+} waves in astrocytes, and as such is not an instantaneous process taking between tens of seconds and minutes. In the latency between this occurring the axons may be bereft of instantly available glucose if relying in ambient glucose. However, an additional pool of extracellular lactate may be an ideal reserve from which axons feed prior to hyperemia. Introduction of aglycemia to mouse optic nerves led to a rapid fall in lactate to zero after about 10 min. However, this preceded the fall in the CAP suggesting that lactate is taken up by the axons during aglycemia, but that once it has been used there is no energy substrate for the axons to use, and hence CAP failure ensues. On reperfusion of glucose the lactate rapidly increases, overshoots, then returns to baseline (**Figure 2**). The mechanism behind this overshoot is unknown but may be in part due to the aglycemia-induced elevated extracellular K^+ concentration (Brown et al., 2001), which is supported by recent data showing K^+ stimulated glycogen degradation in astrocytes mediated by soluble adenyl

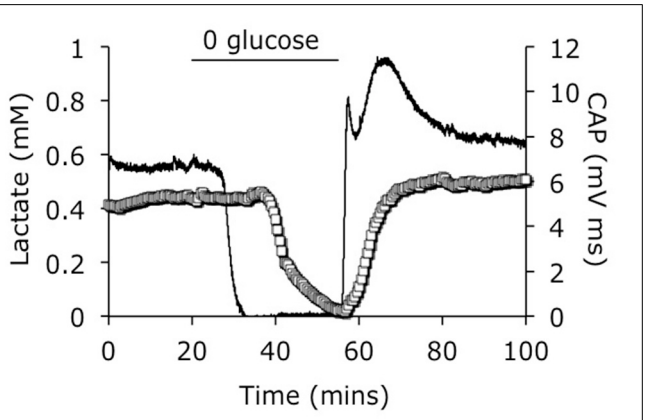


FIGURE 2 | Lactate concentration in response to aglycemia. A baseline period of superfusion with aCSF containing 10 mM glucose produced a stable CAP area and a robust release of lactate from the tissue. Introduction of aglycaemia caused a rapid fall in lactate to zero, prior to the fall in the CAP area. On reperfusion of glucose containing aCSF the CAP rapidly recovered, but the lactate level rapidly overshot before returning to the baseline levels. The CAP (open squares) is represented on the left y-axis and the lactate signal (line) is represented on the right y-axes. Adapted from Figure 4 Yang et al. (2014).

cyclase (Choi et al., 2012), and astrocyte specific bicarbonate-sensitive NBCe1-mediated stimulation of glycolysis (Bittner et al., 2011; Ruminot et al., 2011).

In conclusion, glycogen supports axon conduction in both peripheral and central axons. The glycogen is located in the glial compartment in each tissue, and is metabolized to the transportable conduit lactate, which is shuttled to the axons to support conduction. Such metabolic cell-to-cell communication relies on signaling between the cells such that reliable information is relayed to the glial cells concerning the current metabolic requirements of the neural elements. Our knowledge of the mechanism(s) of this communication is incomplete, but the use of reduced models of brain tissue in combination with emerging technologies will surely advance our understanding of the fascinating and complex interaction between neurons and glia.

REFERENCES

Baltan, S. (2006). Surviving anoxia: a tale of two white matter tracts. *Crit. Rev. Neurobiol.* 18, 95–103. doi: 10.1615/CritRevNeurobiol.v18.i1-2.100

Barros, L. F., San Martin, A., Sotelo-Hitschfeld, T., Lerchundi, R., Fernandez-Moncada, I., Ruminot, I., et al. (2013). Small is fast: astrocytic glucose and lactate metabolism at cellular resolution. *Front. Cell. Neurosci.* 7:27. doi: 10.3389/fncel.2013.00027

Bittar, P. G., Charnay, Y., Pellerin, L., Bouras, C., and Magistretti, P. J. (1996). Selective distribution of lactate dehydrogenase isoenzymes in neurons and astrocytes of human brain. *J. Cereb. Blood Flow Metab.* 16, 1079–1089. doi: 10.1097/00004647-199611000-00001

Bittner, C. X., Valdebenito, R., Ruminot, I., Loaiza, A., Larenas, V., Sotelo-Hitschfeld, T., et al. (2011). Fast and reversible stimulation of astrocytic glycolysis by K+ and a delayed and persistent effect of glutamate. *J. Neurosci.* 31, 4709–4713. doi: 10.1523/JNEUROSCI.5311-10.2011

Bouzier-Sore, A. K., Voisin, P., Bouchaud, V., Bezancon, E., Franconi, J. M., and Pellerin, L. (2006). Competition between glucose and lactate as oxidative energy substrates in both neurons and astrocytes: a comparative NMR study. *Eur. J. Neurosci.* 24, 1687–1694. doi: 10.1111/j.1460-9568.2006.05056.x

Brown, A. M., Baltan Tekkök, S., and Ransom, B. R. (2003a). Glycogen regulation and functional role in mouse white matter. *J. Physiol.* 549, 501–512. doi: 10.1113/jphysiol.2003.042416

Brown, A. M., Evans, R. D., Black, J., and Ransom, B. R. (2012). Schwann cell glycogen selectively supports myelinated axon function. *Ann. Neurol.* 72, 406–418. doi: 10.1002/ana.23607

Brown, A. M., Sickmann, H. M., Fosgerau, K., Lund, T. M., Schousboe, A., Waagepetersen, H. S., et al. (2005). Astrocyte glycogen metabolism is required for neural activity during aglycemia or intense stimulation in mouse white matter. *J. Neurosci. Res.* 79, 74–80. doi: 10.1002/jnr.20335

Brown, A. M., Tekkok, S. B., and Ransom, B. R. (2003b). Glycogen regulation and functional role in mouse white matter. *J. Physiol.* 549, 501–512. doi: 10.1113/jphysiol.2003.042416

Brown, A. M., Wender, R., and Ransom, B. R. (2001). Ionic mechanisms of aglycemic axon injury in mammalian central white matter. *J. Cereb. Blood Flow Metab.* 21, 385–395. doi: 10.1097/00004647-200104000-00007

Cai, H., Cong, W. N., Ji, S., Rothman, S., Maudsley, S., and Martin, B. (2012). Metabolic dysfunction in Alzheimer's disease and related neurodegenerative disorders. *Curr. Alzheimer Res.* 9, 5–17. doi: 10.2174/156720512799015064

Cataldo, A. M., and Broadwell, R. D. (1986). Cytochemical identification of cerebral glycogen and glucose-6-phosphatase activity under normal and experimental conditions. I. Neurons and glia. *J. Elec. Micro. Tech* 3, 413–437. doi: 10.1002/jemt.1060030406

Choi, H. B., Gordon, G. R., Zhou, N., Tai, C., Rungta, R. L., Martinez, J., et al. (2012). Metabolic communication between astrocytes and neurons via bicarbonate-responsive soluble adenylyl cyclase. *Neuron* 75, 1094–1104. doi: 10.1016/j.neuron.2012.08.032

Dalsgaard, M. K. (2006). Fuelling cerebral activity in exercising man. *J. Cereb. Blood Flow Metab.* 26, 731–750. doi: 10.1038/sj.jcbfm.9600256

Dienel, G. A. (2004). Lactate muscles its way into consciousness: fueling brain activation. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 287, R519–R521. doi: 10.1152/ajpregu.00377.2004

Dienel, G. A. (2009). "Energy metabolism in the brain," in *From Molecules to Networks: an Introduction to Cellular and Molecular Neuroscience, 2nd Edn*., eds J. H. Byrne and J. L. Roberts (Burlington, MA: Academic Press), 49–110.

Dienel, G. A. (2012). Brain lactate metabolism: the discoveries and the controversies. *J. Cereb. Blood Flow Metab.* 32, 1107–1138. doi: 10.1038/jcbfm.2011.175

Dienel, G. A., and Cruz, N. F. (2014). Contributions of glycogen to astrocytic energetics during brain activation. *Metab. Brain Dis*. doi: 10.1007/s11011-014-9493-8. [Epub ahead of print].

Dienel, G. A., and Hertz, L. (2001). Glucose and lactate metabolism during brain activation. *J. Neurosci. Res.* 66, 824–838. doi: 10.1002/jnr.10079

Dringen, R., and Hamprecht, B. (1992). Glucose, insulin, and insulin-like growth factor I regulate the glycogen content of astroglia-rich primary cultures. *J. Neurochem.* 58, 511–517. doi: 10.1111/j.1471-4159.1992.tb09750.x

Dringen, R., Peters, H., Wiesinger, H., and Hamprecht, B. (1995). Lactate transport in cultured glial cells. *Dev. Neurosci.* 17, 63–69. doi: 10.1159/000111275

Frier, B. M., and Fisher, B. M. (2007). *Hypoglycaemia in Clinical Diabetes*. New York, NY: John Wiley and Sons Ltd. doi: 10.1002/9780470516270

Halestrap, A. P. (2012). The monocarboxylate transporter family-Structure and functional characterization. *IUBMB Life* 64, 1–9. doi: 10.1002/iub.573

Halestrap, A. P., and Price, N. T. (1999). The proton-linked monocarboxylate transporter (MCT) family: structure, function and regulation. *Biochem. J.* 343, 281–299. doi: 10.1042/0264-6021:3430281

Hoppe, D., Chvatal, A., Kettenmann, H., Orkand, R. K., and Ransom, B. R. (1991). Characteristics of activity-dependent potassium accumulation in mammalian peripheral nerve *in vitro*. *Brain Res.* 552, 106–112. doi: 10.1016/0006-8993(91)90666-J

Hu, Y., and Wilson, G. S. (1997). A temporary local energy pool coupled to neuronal activity: fluctuations of extracellular lactate levels in rat brain monitored with rapid-response enzyme-based sensor. *J. Neurochem.* 69, 1484–1490. doi: 10.1046/j.1471-4159.1997.69041484.x

Karbowski, J. (2003). How does connectivity between cortical areas depend on brain size? Implications for efficient computation. *J. Comput. Neurosci.* 15, 347–356. doi: 10.1023/A:1027467911225

Leppanen, L., and Stys, P. K. (1997). Ion transport and membrane potential in CNS myelinated axons. II. Effects of metabolic inhibition. *J. Neurophysiol.* 78, 2095–2107.

Magistretti, P. J., Pellerin, L., Rothman, D. L., and Shulman, R. G. (1999). Energy on demand. *Science* 283, 496–497. doi: 10.1126/science.283.5401.496

Messonnier, L. A., Emhoff, C. A., Fattor, J. A., Horning, M. A., Carlson, T. J., and Brooks, G. A. (2013). Lactate kinetics at the lactate threshold in trained and untrained men. *J. Appl. Physiol.* 114, 1593–1602. doi: 10.1152/japplphysiol.00043.2013

Morgello, S., Uson, R. R., Schwartz, E. J., and Haber, R. S. (1995). The human blood-brain barrier glucose transporter (GLUT1) is a glucose transporter of gray matter astrocytes. *Glia* 14, 43–54. doi: 10.1002/glia.440140107

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. doi: 10.1073/pnas.91.22.10625

Ransom, B. R., Waxman, S. G., and Fern, R. (1997). "Pathophysiology of white matter anoxic injury," in *Cerebrovascular Disease*, ed H. H. Bajter (Philadelphia, PA: Lippincott Raven), 309–318.

Ransom, B. R., Yamate, C. L., and Connors, B. W. (1985). "Developmental studies on brain extracellualr space: activity-dependent K^+ accumulation and shrinkage," in *Ion Measurements in Physiology and Medicine*, ed M. Kessler (Berlin: Springer-Verlag), 206–213. doi: 10.1007/978-3-642-70518-2_32

Rossen, R., Kabat, H., and Anderson, J. P. (1943). Acute arrest of cerebral circulation in man. *Arch. Neurol. Psych.* 50, 510–528. doi: 10.1001/archneurpsyc.1943.02290230022002

Ruminot, I., Gutierrez, R., Pena-Munzenmayer, G., Anazco, C., Sotelo-Hitschfeld, T., Lerchundi, R., et al. (2011). NBCe1 mediates the acute stimulation of astrocytic glycolysis by extracellular K+. *J. Neurosci.* 31, 14264–14271. doi: 10.1523/JNEUROSCI.2310-11.2011

Schurr, A., Payne, R. S., Miller, J. J., and Rigor, B. M. (1997a). Brain lactate is an obligatory aerobic energy substrate for functional recovery after hypoxia:

further *in vitro* validation. *J. Neurochem.* 69, 423–426. doi: 10.1046/j.1471-4159.1997.69010423.x

Schurr, A., Payne, R. S., Miller, J. J., and Rigor, B. M. (1997b). Brain lactate, not glucose, fuels the recovery of synaptic function from hypoxia upon reoxygenation: an *in vitro* study. *Brain Res.* 744, 105–111. doi: 10.1016/S0006-8993(96)01106-7

Schurr, A., West, C. A., and Rigor, B. M. (1988). Lactate-supported synaptic function in the rat hippocampal slice preparation. *Science* 240, 1326–1328. doi: 10.1126/science.3375817

Sotelo-Hitschfeld, T., Fernandez-Moncada, I., and Barros, L. F. (2012). Acute feedback control of astrocytic glycolysis by lactate. *Glia* 60, 674–680. doi: 10.1002/glia.22304

Stryer, L. (1995). *Biochemistry*. New York, NY: W. H. Freeman and Co.

Stys, P. K. (1998). Anoxic and ischemic injury of myelinated axons in CNS white matter: from mechanistic concepts to therapeutics. *J. Cereb. Blood. Flow. Metab.* 18, 2–25. doi: 10.1097/00004647-199801000-00002

Stys, P. K., Ransom, B. R., and Waxman, S. G. (1991). Compound action potential of nerve recorded by suction electrode: a theoretical and experimental analysis. *Brain Res.* 546, 18–32. doi: 10.1016/0006-8993(91)91154-S

Tekkok, S. B., Brown, A. M., Westenbroek, R., Pellerin, L., and Ransom, B. R. (2005). Transfer of glycogen-derived lactate from astrocytes to axons via specific monocarboxylate transporters supports mouse optic nerve activity. *J. Neurosci. Res.* 81, 644–652. doi: 10.1002/jnr.20573

Velumian, A. A., Wan, Y., Samoilova, M., and Fehlings, M. G. (2011). Contribution of fast and slow conducting myelinated axons to single-peak compound action potentials in rat spinal cord white matter preparations. *J. Neurophysiol.* 105, 929–941. doi: 10.1152/jn.00435.2010

Wender, R., Brown, A. M., Fern, R., Swanson, R. A., Farrell, K., and Ransom, B. R. (2000). Astrocytic glycogen influences axon function and survival during glucose deprivation in central white matter. *J. Neurosci.* 20, 6804–6810.

Yang, X., Hamner, M. A., Brown, A. M., Evans, R. D., Ye, Z., Chen, S. D., et al. (2014). Novel hypoglycemic injury mechanism: N-Methyl-D-Aspartate receptor–mediated white matter damage. *Ann. Neurol.* 75, 492–507. doi: 10.1002/ana.24050

Zhang, K., and Sejnowski, T. J. (2000). A universal scaling law between gray matter and white matter of cerebral cortex. *Proc. Natl. Acad. Sci. U.S.A.* 97, 5621–5626. doi: 10.1073/pnas.090504197

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 25 September 2014; paper pending published: 17 October 2014; accepted: 05 November 2014; published online: 25 November 2014.

Citation: Chambers TW, Daly TP, Hockley A and Brown AM (2014) Contribution of glycogen in supporting axon conduction in the peripheral and central nervous systems: the role of lactate. Front. Neurosci. ***8****:378. doi: 10.3389/fnins.2014.00378*

This article was submitted to Neuroenergetics, Nutrition and Brain Health, a section of the journal Frontiers in Neuroscience.

frontiers in
NEUROSCIENCE

ORIGINAL RESEARCH ARTICLE
published: 05 December 2014
doi: 10.3389/fnins.2014.00398

Energy substrates that fuel fast neuronal network oscillations

Lukas V. Galow, Justus Schneider, Andrea Lewen, Thuy-Truc Ta, Ismini E. Papageorgiou† and Oliver Kann*

Institute of Physiology and Pathophysiology and Interdisciplinary Center for Neurosciences (IZN), University of Heidelberg, Heidelberg, Germany

Edited by:
Avital Schurr, University of Louisville, USA

Reviewed by:
Neil Sims, Flinders University, Australia
David Mazzocchi-Jones, Keele University, UK

****Correspondence:***
Oliver Kann, Institute of Physiology and Pathophysiology, University of Heidelberg, Im Neuenheimer Feld 326, D-69120 Heidelberg, Germany
e-mail: oliver.kann@physiologie.uni-heidelberg.de

†Present address:
Ismini E. Papageorgiou, Institute for Diagnostic and Interventional Neuroradiology, University of Göttingen, Göttingen, Germany

Fast neuronal network oscillations in the gamma-frequency band (30–100 Hz) provide a fundamental mechanism of complex neuronal information processing in the hippocampus and neocortex of mammals. Gamma oscillations have been implicated in higher brain functions such as sensory perception, motor activity, and memory formation. The oscillations emerge from precise synapse interactions between excitatory principal neurons such as pyramidal cells and inhibitory GABAergic interneurons, and they are associated with high energy expenditure. However, both energy substrates and metabolic pathways that are capable to power cortical gamma oscillations have been less defined. Here, we investigated the energy sources fueling persistent gamma oscillations in the CA3 subfield of organotypic hippocampal slice cultures of the rat. This preparation permits superior oxygen supply as well as fast application of glucose, glycolytic metabolites or drugs such as glycogen phosphorylase inhibitor during extracellular recordings of the local field potential. Our findings are: (i) gamma oscillations persist in the presence of glucose (10 mmol/L) for greater than 60 min in slice cultures while (ii) lowering glucose levels (2.5 mmol/L) significantly reduces the amplitude of the oscillation. (iii) Gamma oscillations are absent at low concentration of lactate (2 mmol/L). (iv) Gamma oscillations persist at high concentration (20 mmol/L) of either lactate or pyruvate, albeit showing significant reductions in the amplitude. (v) The breakdown of glycogen significantly delays the decay of gamma oscillations during glucose deprivation. However, when glucose is present, the turnover of glycogen is not essential to sustain gamma oscillations. Our study shows that fast neuronal network oscillations can be fueled by different energy-rich substrates, with glucose being most effective.

Keywords: brain energy metabolism, electrophysiology, glycogen phosphorylase, information processing, lactate, mitochondria, monocarboxylate transporter, synaptic transmission

INTRODUCTION

The mammalian brain is a highly oxidative organ owing to the disproportionately large fraction of oxygen consumption compared with the small fraction of the total body mass (in humans about 20% and 2%, respectively) (Rolfe and Brown, 1997; Erecińska and Silver, 2001). This suggests that complex neuronal information processing is associated with high energy expenditure and requires continuous delivery of glucose from the blood (Shulman et al., 2001; Attwell et al., 2010; Kann, 2012). Glucose enters the extracellular space of the brain parenchyma based on a large concentration gradient (5–7 mmol/L in the blood and 1–2 mmol/L in the extracellular space) via glucose transporters (GLUTs) that are located on endothelial cells of the blood-brain-barrier as well as astrocytes (Roberts, 2007; Hertz et al., 2014). For normal conditions, glucose has been considered to be the dominant exogenous energy substrate in the adult brain (Chih and Roberts, 2003; Dienel, 2012). Having a role in different biochemical pathways, glucose metabolism has important functions related to bioenergetics, neurotransmission, and oxidation–reduction (redox) reactions in the brain parenchyma (Bak et al., 2006; Kann and Kovács, 2007; Dienel, 2012). However, neurons are able to utilize exogenous and endogenous energy substrates other than glucose in certain physiological and pathophysiological conditions (Roberts, 2007).

A prominent example is lactate that is generated during neuronal activity through glycogenolysis and/or anaerobic glycolysis (1–2 mmol/L in the extracellular space) or may even enter the brain parenchyma from the blood (Ide et al., 2000; Roberts, 2007; Overgaard et al., 2012; Hertz et al., 2014). Lactate metabolism may also involve complex interactions between neurons and astrocytes (Suzuki et al., 2011; Pellerin and Magistretti, 2012). Many studies on lactate and neuronal activity were made in slice preparations of the hippocampus (Schurr et al., 1988, 1999; Stittsworth and Lanthorn, 1993; Galeffi et al., 2007; Schurr and Payne, 2007; Ivanov et al., 2011, 2014; Hall et al., 2012; Schurr and Gozal, 2012). In all of these studies, neuronal activation was induced by repetitive electrical stimulation, application of neurotransmitters such as glutamate, or Mg^{2+}-free recording

Abbreviations: Ampl, amplitude; AUC, area under curve; DIV, days *in vitro*; Freq, peak frequency; FWHM, full width at half maximum; LFP, local field potential; PSD, peak power spectral density; Var, variance of the amplitude.

solution. Notably, either of these experimental tools evokes quite robust neuronal activation, with widely undefined activity states in the local neuronal network, or even spreading (epileptic) depolarization. Thus, detailed information about the capability of different energy substrates to fuel specific, naturally occurring network activity states such as fast neuronal network oscillations in the gamma-frequency band (30–100 Hz) is lacking in the literature.

Gamma oscillations (30–100 Hz) have been observed in many mammalian brain regions, including the hippocampus and the neocortex (Uhlhaas and Singer, 2010). Gamma oscillations reflect synchronous rhythmic fluctuations of the membrane potential in many neurons of a local neuronal network. In the hippocampus, these subthreshold fluctuations are generated by complex and precise synaptic interactions of excitatory pyramidal cells and inhibitory GABAergic interneurons (Whittington and Traub, 2003; Hájos and Paulsen, 2009; Kann, 2012). The synchronizing effect of gamma oscillations permits the coordinated activation of defined sets of neurons that carry and process information (Hájos and Paulsen, 2009; Kann et al., 2014). Therefore, gamma oscillations provide a temporal matrix for complex neuronal information processing during higher brain functions such as sensory perception, motor activity, and memory formation (Paulsen and Moser, 1998; Uhlhaas and Singer, 2010; van Vugt et al., 2010).

Notably, excitatory pyramidal cells and certain subtypes of inhibitory GABAergic interneurons might differ in the energy demands during gamma oscillations and in their capability to utilize energy substrates. Parvalbumin-positive basket cells, for example, generate action potentials at much higher frequency ("fast-spiking") compared with pyramidal cells during gamma oscillations (30–100 Hz and 1–3 Hz, respectively). Moreover, parvalbumin-positive basket cells synchronize the activity of numerous pyramidal cells by rhythmic inhibition. As prerequisites, theses interneurons have unique electrophysiological properties that are likely associated with extraordinary high energy expenditure (Gulyás et al., 2006; Hu and Jonas, 2014; Kann et al., 2014).

The present study was designed to identify energy substrates that are capable to power gamma oscillation *in vitro*. Gamma oscillations can be reliably induced in hippocampal slices by bath application of low micromolar concentrations of cholinergic receptor agonists such as acetylcholine that mimics input from the septum (Fisahn et al., 1998; Kann et al., 2011). These oscillations share many features with hippocampal gamma oscillations observed *in vivo* (Kann, 2012). We used organotypic hippocampal slice cultures that were maintained on Biopore™ membranes in an interface recording chamber. This experimental approach permits the induction of persistent gamma oscillations, with superior oxygen supply as well as rapid exchange of energy substrates and drugs (Huchzermeyer et al., 2013).

MATERIALS AND METHODS

SLICE CULTURES AND RECORDING CHAMBER

Animals were purchased from Charles-River (Sulzfeld, Germany) and housed, cared, and killed in accordance with the recommendations of the European Commission and the authorities of Baden-Württemberg (T56/11). Organotypic hippocampal slice cultures were prepared as described (Kann et al., 2003a,b, 2011). In brief, hippocampal slices (400 μm) were cut with a McIlwain tissue chopper (Mickle Laboratory Engineering Company Ltd., Guildford, UK) from 7 to 9 days-old Wistar rats under sterile conditions. Slices were maintained on Biopore™ membranes (Millicell standing inserts, Merck Millipore, Schwalbach, Germany) between culture medium, which consisted of 50% minimal essential medium, 25% Hank's balanced salt solution (Sigma-Aldrich, Taufkirchen, Germany), 25% horse serum (Life Technologies, Darmstadt, Germany), and 2 mM L-glutamine (Life Technologies) at pH 7.3, and humidified normal atmosphere (5% CO_2, 36.5°C) in an incubator (Heracell, Thermoscientific, Dreieich, Germany). Biopore™ membranes provide high viability and excellent trans-membrane oxygen transport. The culture medium (1 ml) was replaced three times per week. Slice cultures were used after 7–21 days *in vitro* (DIV) (residual thickness of about 200 μm), when the tissue had recovered from the slice preparation and damaged cut surfaces were re-organized (Kann and Kovács, 2007).

For recordings, the intact Biopore™ membrane carrying slice cultures was inserted into the recording chamber. Slice cultures were maintained at the interface between recording solution and ambient gas mixture. Intact Biopore™ membrane inserts ensure constant supply of oxygen and energy substrates from the recording solution (rate 1.8 ml/min) that flows underneath the Biopore™ membrane; the interface condition permits constant oxygen supply from the ambient gas mixture (95% O_2 and 5% CO_2, rate 1.5 l/min).

RECORDING SOLUTIONS AND DRUGS

Slice cultures were constantly supplied with pre-warmed (34 ± 1°C) recording solution, i.e., artificial cerebrospinal fluid that contained: 129 mM NaCl, 3 mM KCl, 1.25 mM NaH_2PO_4, 1.8 mM $MgSO_4$, 1.6 mM $CaCl_2$, 26 mM $NaHCO_3$, and 10 mM glucose (Sigma-Aldrich). The pH was 7.3 when the recording solution was saturated with the gas mixture (95% O_2 and 5% CO_2).

Gamma oscillations were induced by bath application of low concentrations of cholinergic receptor agonist, acetylcholine (2 μmol/L) and acetylcholine-esterase inhibitor, physostigmine (400 nmol/L) (Huchzermeyer et al., 2013). The absence of action potentials (spiking) was induced by bath application of tetrodotoxin, which blocks fast voltage-gated Na^+-channels. Acetylcholine was from Sigma-Aldrich, physostigmine was from Tocris and tetrodotoxin from Biotrend (Köln, Germany).

For further specific experiments, Na-pyruvate (Sigma-Aldrich), Na-L-lactate (Alfa Aesar, Karlsruhe, Germany), L-glutamine, CP-316819 (5-Chloro-*N*-[(1*S*,2*R*)-2-hydroxy-3-(methoxymethylamino)-3-oxo-1-(phenylmethyl)propyl]-1*H*-indole-2-carboxamide; Tocris, R&D Systems GmbH, Wiesbaden-Nordenstadt, Germany) and DAB (1,4-dideoxy-1,4-imino-d-arabinitol; Sigma-Aldrich) were used. Stock solution of DAB was made in double distilled H_2O and CP-316819 was dissolved in DMSO, with a final solvent fraction of less than 0.001% in the recording solution.

RECORDINGS OF LOCAL FIELD POTENTIALS

The local field potential (LFP) was recorded with glass electrodes (tip diameter 3–5 μm) that were pulled from GB150F-8P borosilicate filaments (Science Products GmbH, Hofheim, Germany) with a PC-10 vertical micropipette puller (Narishige International Ltd., London, UK) and backfilled with recording solution. The glass electrode was positioned in stratum pyramidale of the CA3 subfield with a mechanical micromanipulator (MM 33, Märzhäuser, Wetzlar). LFPs were recorded with an EXT 10-2F amplifier in EPMS-07 housing (npi electronic GmbH, Tamm, Germany), low-pass filtered at 3 kHz, and digitized at 10 kHz using CED 1401 interface and Spike2 software (Cambridge Electronic Design, Cambridge, UK) for offline analysis.

TOLUIDINE BLUE STAINING

Slice cultures were fixed in paraformaldehyde (4%, 0.1 M phosphate buffer; Applichem, Darmstadt, Germany) and rinsed in 0.1 M phosphate-buffered salt solution (PBS). Thereafter, slice cultures were exposed for 20 min to toluidine-blue working solution, which was a mixture of 5 ml stock solution (1 g of Toluidine Blue O in 100 ml of 70% ethanol; Sigma-Aldrich) and 45 ml of 1% NaCl solution (pH 2.0–2.5). Thereafter, 96% ethanol (100 ml of 96% ethanol and 4 drops of acetic acid) was used for color-differentiation of the staining. The differentiation step with strong acid removes unspecific staining of weak acidic structures and, thus, increases the contrast between background and stained cells. The process was stopped using 0.1 M PBS, once the differentiation was clearly visible. After brief rinsing with double distilled water, slice cultures were placed on object plates and dried overnight. The slices were then exposed to xylol (Sigma-Aldrich) for 10 min and embedded with Entellan Neu (Merck Millipore, Schwalbach, Germany).

DATA ANALYSIS

Offline signal analysis of gamma oscillations was performed in MatLab 11.0 (MathWorks). Data segments of 100 s were low-pass filtered with a digital Butterworth algorithm at 200 Hz corner frequency and processed with Welch's algorithm and Fast Fourier Transformation with a Hamming window size of 4096 points for calculation of the power spectral density and the power spectrum, respectively (bin size = 2.441 Hz). Gamma oscillations were analyzed for various parameters, i.e., peak frequency (Freq), area under curve (AUC), full width at half maximum (FWHM), peak power spectral density (PSD), amplitude (Ampl), and variance of the amplitude (Var).

Data are presented as mean ± SD derived from (n) slice cultures and (N) preparations of rat pups. Statistical significance ($P < 0.05$) was determined using SigmaPlot 12.5 (Systat Software, Inc., San Jose, CA, USA). Data distribution was tested for normality with Shapiro-Wilk test. Comparisons between paired data were made with paired *t*-test or Wilcoxon signed rank test. For multiple variance comparison, One-Way ANOVA or Kruskal-Wallis One-Way ANOVA on ranks with Dunn's *post-hoc* test was used for unpaired data and One-Way repeated measures ANOVA with Holm-Sidak *post-hoc* test or Friedman repeated measures ANOVA on ranks with Tukey *post-hoc* test was used for paired data. Figures were generated using Excel (Microsoft Corporation, Redmond, USA) and CorelDRAW (Corel Corporation, Ottawa, Ontario, Canada).

RESULTS

GAMMA OSCILLATIONS IN THE PRESENCE OF GLUCOSE

We induced gamma oscillations by bath application of acetylcholine in organotypic hippocampal slice cultures and performed extracellular recordings of the local field potential (LFP) in stratum pyramidale of the CA3 subfield (Kann et al., 2011). In standard recording solution, i.e., in the presence of glucose (10 mmol/L) and high oxygen fraction (95%) in the ambient atmosphere (Kann and Kovács, 2007), gamma oscillations were fully established after about 15 min of acetylcholine application (**Figures 1A–C**). The oscillations were characterized by a frequency of around 40 Hz in the power spectrum and persisted for 60 min (**Figure 1C**) and longer (data not shown). This experiment demonstrates that the reduced composition of recording solution as well as the supply of oxygen in excess for 1 h does not result in evident functional disturbances in the local neuronal network of the CA3 subfield.

We next tested whether the properties of persistent gamma oscillations changed with maturation of slice cultures (Bahr et al., 1995; De Simoni et al., 2003). The characteristics of gamma oscillations in standard recording condition (10 mmol/L glucose, 95% oxygen fraction) did not significantly change after 21 DIV (**Figure 2**), albeit the well-known decrease of slice thickness over time in culture (Bahr et al., 1995; Kann and Kovács, 2007). For further experiments, we used slice cultures after 7 DIV and up to 14 DIV.

Bath application of tetrodotoxin (1 μmol/l), which blocks fast voltage-gated Na^+-channels and thus action potentials, suppressed gamma oscillations after about 5 min (data not shown). This experiment reveals that maintenance of slice cultures on Biopore™ membranes in the interface recording chamber permits rapid drug application and tissue saturation.

We further addressed whether application of glucose in a concentration closer to physiological conditions (2.5 mmol/L) would affect gamma oscillations (Roberts, 2007; Schurr and Payne, 2007). In this condition, gamma oscillations were still present. However, there were significant disturbances in the characteristics of gamma oscillations such as lower amplitude (AUC, PSD, Ampl) and widening of FWHM, reflecting less numbers and less synchrony of activated synapses, respectively (**Figure 3**). This experiment shows that even in optimized recording condition, i.e., utilization of Biopore™ membranes and interface recording chamber, a larger concentration gradient of glucose is required to fuel gamma oscillations *in vitro*.

LACTATE AND PYRUVATE AS ENERGY SUBSTRATES

We next tested whether lactate in a concentration of 2 mmol/L was capable to fuel gamma oscillations, similar as reported for neuronal population responses evoked by electrical stimulation (Schurr et al., 1988). To exclude that breakdown of the glycogen reserve in slice cultures (Cater et al., 2001) affected the outcome of this experiment (see below), we first depleted the glycogen stores by glucose deprivation (recording solution with 0 mmol/L

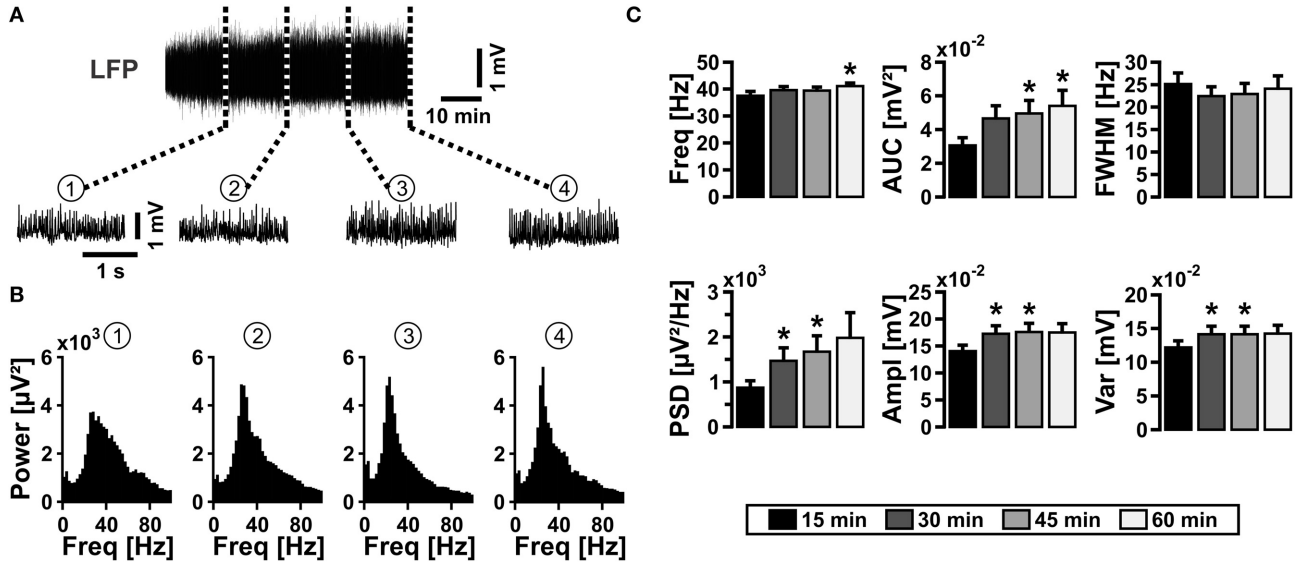


FIGURE 1 | Gamma oscillations in slice cultures. (A) Gamma oscillations were induced by bath application of acetylcholine (2 μmol/L) and physostigmine (400 nmol/L) (upper sample trace), and they persisted for more than 60 min ($n = 13$, $N = 6$). Gamma oscillations are shown with a higher temporal resolution after 15 min (1), 30 min (2), 45 min (3), and 60 min (4) (lower sample traces). Local field potentials (LFP) were recorded in stratum pyramidale of the CA3 subfield in organotypic hippocampal slice cultures of the rat. **(B)** Corresponding power spectra of sample traces shown in **(A)** were calculated from 100 s taken at the end of each data segment. **(C)** Gamma oscillations were analyzed for various parameters, i.e., peak frequency (Freq), area under curve (AUC), full width at half maximum (FWHM), peak power spectral density (PSD), amplitude (Ampl), and variance of the amplitude (Var). Friedman repeated-measures ANOVA on ranks and Tukey *post-hoc* test. Statistical significance is marked by asterisks ($P < 0.05$).

glucose) for 15 min, which widely resulted in suppression of gamma oscillations (**Figures 4A–B**). Subsequent bath application of lactate (2 mmol/L) for 20 min did not rescue gamma oscillations. By contrast, re-application of glucose (10 mmol/L) resulted in almost full recovery of the oscillations.

Subsequently, we determined the capability of equicaloric concentrations of lactate (20 mmol/L) or pyruvate (20 mmol/L) to fuel gamma oscillations (Cater et al., 2003; Galeffi et al., 2007; Gandhi et al., 2009). Either of these substrates could indeed sustain gamma oscillations for 30 min (**Figures 4C–F**) and longer (data not shown). However, in the presence of lactate or pyruvate gamma oscillations showed significantly lower amplitudes (AUC, PSD, Ampl) compared with controls (10 mmol/L glucose); lactate significantly increased the frequency of the oscillations.

Supporting lactate (20 mmol/L) with glutamine (2 mmol/L), which is an important precursor for neurotransmitters, glutamate, and GABA (Waagepetersen et al., 1998; Hertz et al., 2014), did not rescue the amplitude of gamma oscillations (**Figure 5**).

These experiments show that a high concentration of either lactate or pyruvate can basically power gamma oscillations but alters their characteristics.

GLYCOGEN STORES AND INHIBITION OF GLYCOGEN PHOSPHORYLASE

We further explored whether glycogen breakdown is capable to fuel gamma oscillations. Glycogen stores have been described in astrocytes and, more recently, also in neurons (Choi et al., 2012; Dienel and Cruz, 2014; Saez et al., 2014). At first, we determined the time course of suppression of gamma oscillations during glucose deprivation in the presence of 95% oxygen fraction (**Figures 6A–C**). Activity was completely suppressed after 29 ± 1 min ($n = 10$) in recording solution with 0 mmol/L glucose (data not shown). This time course reflects utilization of various energy reserves such as glycogen for ATP generation in different pathways (Roberts, 2007; Dienel and Cruz, 2014) and, presumably, a considerable glycogen reserve in slice cultures (Cater et al., 2001).

We next pharmacologically blocked glycogen phosphorylase, which is a crucial enzyme for glycogen breakdown and thus serves in the initiation of glycogen metabolism (Dienel and Cruz, 2014). We applied two different inhibitors, i.e., DAB and CP-316819, at various concentrations (Gibbs et al., 2006; Dienel et al., 2007; Suh et al., 2007; Walls et al., 2008; Sickmann et al., 2009). Blockade of glycogen phosphorylase resulted in significantly faster suppression of gamma oscillations of 8 ± 1 min ($n > 10$) during glucose deprivation, indicating that glycogen breakdown can indeed support the maintenance of fast neuronal-network oscillations and thus higher brain functions in situations when glucose supply becomes limited (Wender et al., 2000; Abdelmalik et al., 2007).

We finally tested whether the turnover of glycogen was essential for sustainment of gamma oscillations. After gamma oscillations had been fully established, bath application of DAB (100 μmol/L) or CP-316819 (20 μmol/L) for 20 min left gamma oscillations widely intact (**Figures 7A–C**). We even partially observed increases in power and frequency of the oscillations.

These data show that glycogen is an important fuel reserve to sustain gamma oscillations for a transient period under pathological conditions such as hypoglycemia. However, the turnover of glycogen does not significantly contribute to sustainment of gamma oscillations when glucose is present.

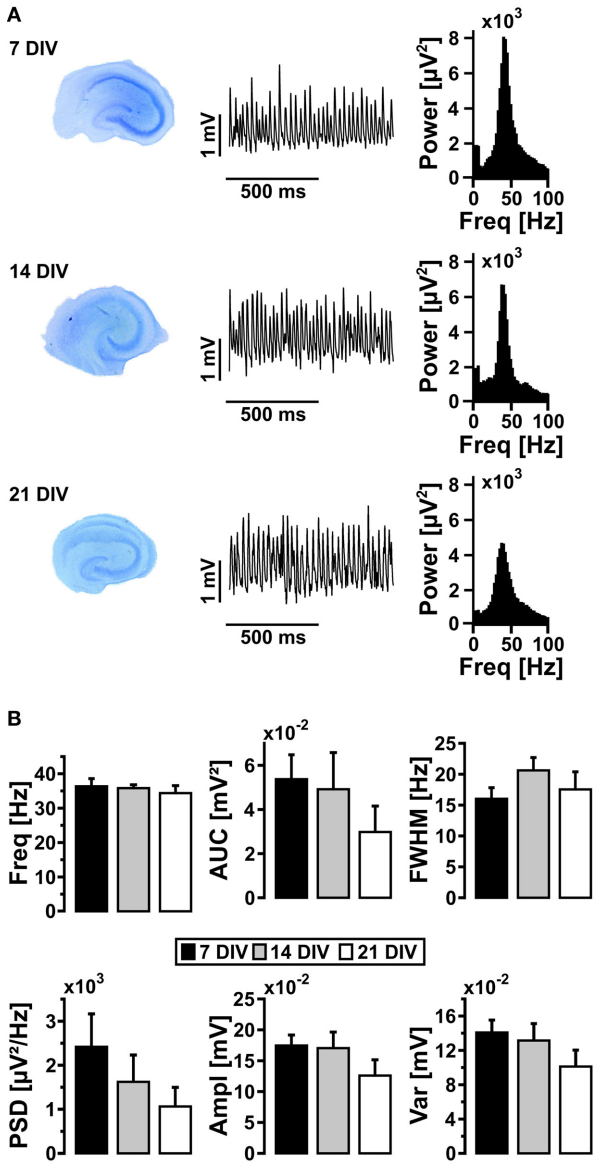


FIGURE 2 | Gamma oscillations and maturation of slice cultures. (A) Slice cultures were stained with toluidine blue after 7, 14, and 21 DIV (left). Gamma oscillations were induced by bath application of acetylcholine (2 μmol/L) and physostigmine (400 nmol/L) and local field potentials (LFP) were recorded in stratum pyramidale of the CA3 subfield (middle). Corresponding power spectra of the sample traces were calculated from data segments of 100 s (right). **(B)** Gamma oscillations were analyzed for various parameters, i.e., peak frequency (Freq), area under curve (AUC), full width at half maximum (FWHM), peak power spectral density (PSD), amplitude (Ampl) and variance of the amplitude (Var) (7 DIV, $n = 12$, $N = 4$; 14 DIV, $n = 7$, $N = 3$; 21 DIV, $n = 6$, $N = 3$). One-Way ANOVA and Kruskal-Wallis ANOVA on ranks.

DISCUSSION

GAMMA OSCILLATIONS IN ORGANOTYPIC HIPPOCAMPAL SLICE CULTURES

Gamma oscillations provide a temporal matrix for complex neuronal information processing during higher brain functions such as sensory perception, motor activity, and memory formation (Paulsen and Moser, 1998; Uhlhaas and Singer, 2010; Kann et al., 2014).

Persistent gamma oscillations can be reliably induced in acute slices and slice cultures of the hippocampus by bath application of cholinergic receptor agonists such as acetylcholine, which mimics cholinergic neuronal input from the septum *in vivo* (Bartos et al., 2007). Acetylcholine activates primarily muscarinic receptors in pyramidal cells and interneurons that interact via complex synaptic mechanisms (Bartos et al., 2007; Hájos and Paulsen, 2009). Cholinergic receptor activation finally leads to subthreshold membrane potential fluctuations in neurons and concomitant rhythmic network oscillations as detected by extracellular recordings of the LFP (Hájos et al., 1998; Fisahn et al., 2002; Kann et al., 2014). Notably, fast rhythmic GABAergic inhibition by parvalbumin-positive fast-spiking interneurons is crucial for the generation of gamma oscillations (Bartos et al., 2007; Gulyás et al., 2010; Oren et al., 2010). Cholinergically induced hippocampal gamma oscillations *in vitro* are most prominent in the CA3 subfield (Fisahn et al., 1998; Kann et al., 2011), which is similar to the pattern of gamma oscillations *in vivo* (Penttonen et al., 1998; Montgomery and Buzsáki, 2007). Gamma oscillations *in vivo* occur transiently on the 100 ms time scale upon sensory input or during specific cognitive tasks. In the human brain, for example, gamma oscillations can last in the range of minutes, dependent on the task (Lehmann et al., 2001; Lutz et al., 2004). This aspect is important for the validation of *in vitro* models, most of which feature persistent gamma oscillations (Bartos et al., 2007; Hájos and Paulsen, 2009).

Recent *in vitro* and *in vivo* studies demonstrated that gamma oscillations were associated with high energy expenditure (Niessing et al., 2005; Nishida et al., 2008; Kann et al., 2011; Huchzermeyer et al., 2013), which is most likely caused by increased rates of action potentials and synaptic interactions. In particular, the significant increase in excitatory and inhibitory postsynaptic potentials during gamma oscillations elicits strong ion fluxes across the neuronal membrane of pyramidal cells and inhibitory interneurons that finally need to be restored by ATP-dependent ion pumps such as Na^+/K^+-ATPase (Kann et al., 2014).

Taken together, persistent gamma oscillations in organotypic hippocampal slice cultures are a useful model for a specific neuronal network activity state with high energy expenditure that naturally occurs *in vivo*.

GLUCOSE, LACTATE AND PYRUVATE AS ENERGY SUBSTRATES

Here we show that gamma oscillations reliably persist for more than 1 h in standard recording condition, i.e., in the presence of 10 mmol/L glucose and 95% oxygen fraction. Traditionally, brain slices are maintained in the presence of 10 mmol/L glucose for two main reasons: (i) improved recovery from the preparation procedure and (ii) heterogeneity in glucose and oxygen availability owing to the use of interface or submerged conditions, which also includes different application rates of recording solution (Li and McIlwain, 1957; Kann and Kovács, 2007). Our optimized recording condition, i.e., the combination of slice cultures, Biopore™ membranes, and interface recording chamber,

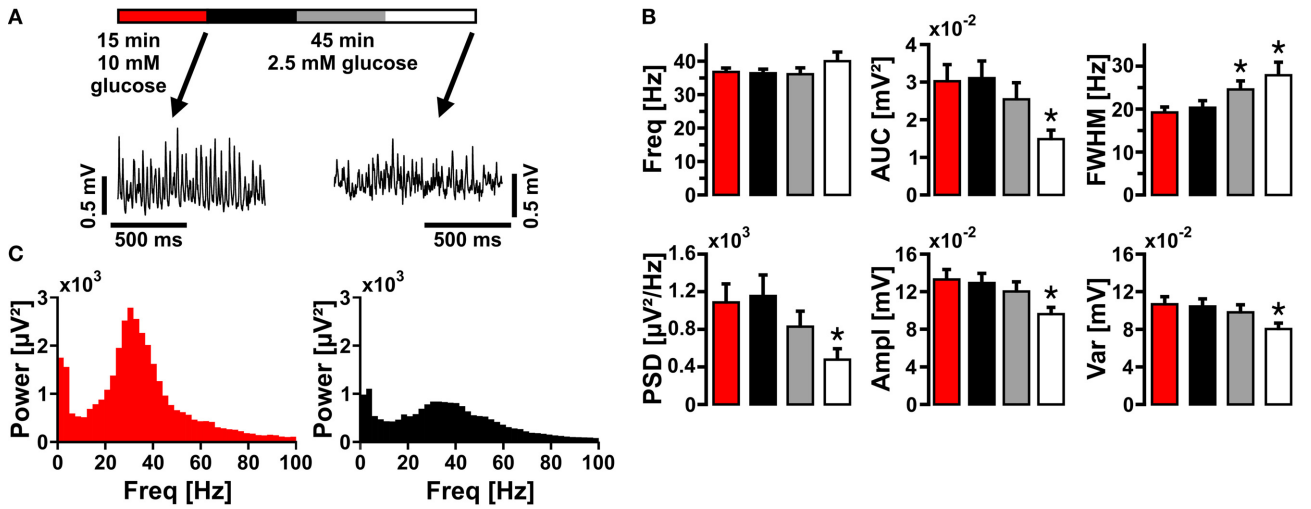


FIGURE 3 | Gamma oscillations at low glucose concentration. (A) Gamma oscillations were induced by bath application of acetylcholine (2 µmol/L) and physostigmine (400 nmol/L) in the presence of 10 mmol/L glucose (red). Then, glucose was lowered to 2.5 mmol/L in the recording solution and the properties of gamma oscillations were analyzed after 15 min (black), 30 min (gray), and 45 min (white). Local field potentials (LFP) were recorded in stratum pyramidale of the CA3 subfield (sample traces). **(B)** Corresponding power spectra of sample traces shown in **(A)** were calculated from 100 s taken at the end of each data segment. **(C)** Gamma oscillations were analyzed for various parameters, i.e., peak frequency (Freq), area under curve (AUC), full width at half maximum (FWHM), peak power spectral density (PSD), amplitude (Ampl), and variance of the amplitude (Var) ($n = 14$, $N = 3$). Note the decrease in amplitude at low glucose concentration. Repeated-measures ANOVA and Holm-Sidak *post-hoc* test or Friedman repeated-measures ANOVA on ranks and Tukey *post-hoc* test. Statistical significance is marked by asterisks ($P < 0.05$).

features superior supply of energy substrates and oxygen as well as fast drug application (Huchzermeyer et al., 2013).

However, gamma oscillations showed clear disturbances in the presence of 2.5 mmol/L glucose, even in the optimized recording condition. This finding differs from experiments in acute hippocampal slices, in which 2.5 mmol/L glucose were sufficient to sustain neuronal population responses as evoked by electrical stimulation (Schurr and Payne, 2007). Our experiments with energy substrates other than glucose showed that a high concentration (20 mmol/L) of lactate or pyruvate could indeed sustain gamma oscillations, but these oscillations were of significantly lower amplitude. This is also in contrast with other studies showing that lactate at 2 mmol/L or higher could maintain synaptic function in hippocampal tissue *in vitro* as well as, or better than, glucose (Schurr et al., 1988; Schurr and Payne, 2007; Ivanov et al., 2011) and/or proposing that lactate is the preferred energy substrate of neurons (Bouzier-Sore et al., 2003).

These different findings on the capability of low glucose or lactate to sustain neuronal activity are most likely due to (i) the respective activity state that is induced in the local neuronal network and/or (ii) intracellular acidification of neurons in the presence of lactate or pyruvate.

(i) In previous studies, electrical stimulation with either single (Schurr et al., 1988; Schurr and Payne, 2007) or repetitive pulses (at 10 Hz, for 10 or 30 s) (Ivanov et al., 2011) was used in acute hippocampal slices. Such electrical stimulation is quite robust and brief, and it enforces all neurons in the local network to generate action potentials. The final individual action potential frequency may considerably vary depending on neuronal subtypes and accommodation characteristics (Kann et al., 2014). By contrast, hippocampal gamma oscillations are a specific, naturally occurring network activity state that is based on complex and precise synaptic interactions between excitatory pyramidal cells and inhibitory GABAergic interneurons (Whittington and Traub, 2003; Bartos et al., 2007; Hájos and Paulsen, 2009). During gamma oscillations, fast-spiking interneurons generate action potentials at 30–100 Hz while pyramidal cells spike at 1–3 Hz *in vitro* and *in vivo* (Csicsvari et al., 1999; Traub et al., 2000; Hájos et al., 2004; Gloveli et al., 2005). In particular, parvalbumin-positive fast-spiking interneurons are crucial for the generation of gamma oscillations and have very special characteristics such as formation of a basket cell network (Ribak et al., 1993; Traub et al., 2001), perisomatic control of pyramidal cells (Hájos et al., 2004; Gloveli et al., 2005), rapid action potential kinetics and high sodium entry ratio (Carter and Bean, 2009; Hu and Jonas, 2014). Moreover, parvalbumin-positive fast-spiking interneurons contain large numbers of mitochondria (Kageyama and Wong-Riley, 1982; Gulyás et al., 2006; Takács et al., 2014), which likely reflects the extraordinary high energy expenditure of this neuronal subtype during fast network oscillations (Kann et al., 2014). In agreement with these biophysical and biochemical characteristics, fast-spiking interneurons and gamma oscillations are exquisitely sensitive to metabolic stress (Huchzermeyer et al., 2008; Kann et al., 2011; Whittaker et al., 2011). The inability of 2 mmol/L glucose and 20 mmol/L lactate or pyruvate to (fully) sustain gamma oscillations might thus be related to properties of fast-spiking interneurons such as high energy expenditure, limitations in the uptake of substrates via glucose transporter GLUT-3 and

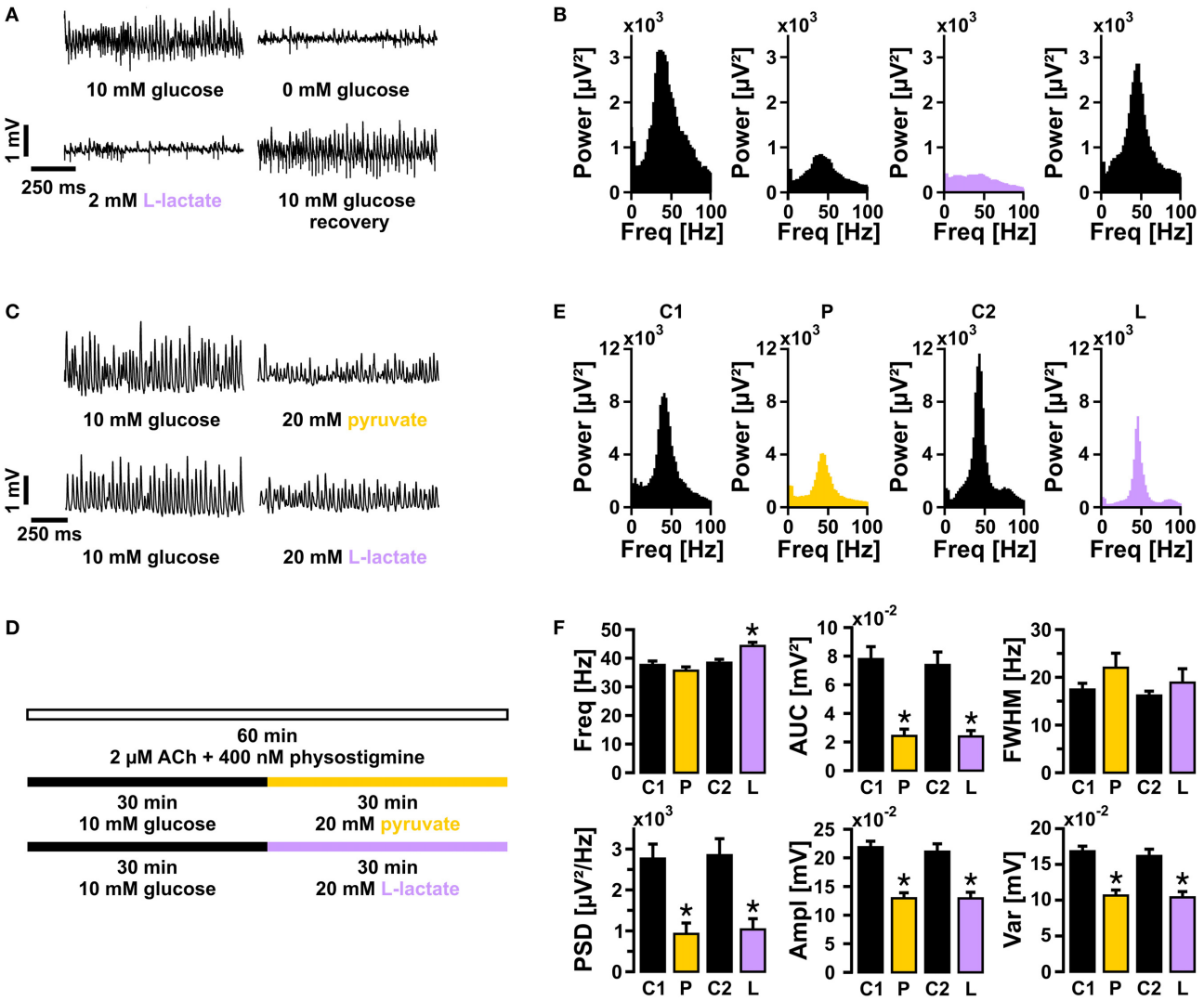


FIGURE 4 | Gamma oscillations in the presence of either lactate or pyruvate. (A) Gamma oscillations were induced by bath application of acetylcholine (2 μmol/L) and physostigmine (400 nmol/L). Initially, the recording solution contained 10 mmol/L glucose. Subsequently, recording solutions containing 0 mmol/L glucose (15 min), 2 mmol/L lactate (20 min), and again 10 mmol/L glucose were applied ($n = 15$, $N = 3$), while local field potentials (LFP) were recorded in stratum pyramidale of the CA3 subfield (sample traces). **(B)** Corresponding power spectra of sample traces shown in **(A)** were calculated from 100 s taken at the end of each data segment. **(C)** Sample traces of gamma oscillations in the presence of 10 mmol/L glucose vs. 20 mmol/L pyruvate (upper traces) or 20 mmol/L lactate (lower traces), according to the protocol given in **(D)**. **(E)** Corresponding power spectra of sample traces shown in **(C)** were calculated from data segments of 100 s. **(F)** Gamma oscillations were analyzed for various parameters, i.e., peak frequency (Freq), area under curve (AUC), full width at half maximum (FWHM), peak power spectral density (PSD), amplitude (Ampl), and variance of the amplitude (Var) (control 1 and pyruvate, $n = 12$, $N = 4$; control 2, and lactate, $n = 13$, $N = 4$). Note the significant decrease in amplitude even at the high concentration of lactate or pyruvate. Paired *t*-test or Wilcoxon signed rank test. Statistical significance is marked by asterisks ($P < 0.05$).

monocarboxylate transporter MCT-2 and/or limitations by rate-limiting enzymes in cytoplasmic and mitochondrial pathways related to energy and neurotransmitter metabolism (Waagepetersen et al., 1998; Bak et al., 2006; Simpson et al., 2007; Barros, 2013). However, the properties of transporters and enzymes in parvalbumin-positive fast-spiking interneurons are widely unknown (Kann et al., 2014).

(ii) A complementary or alternative explanation for the disturbances in gamma oscillations might be intracellular neuronal acidification in the presence of high concentration of lactate or pyruvate. Both substrates are taken up by neurons via MCT-2, which is a proton-linked monocarboxylate transporter (Roberts, 2007; Halestrap, 2013). Previous studies indeed showed decreases in the intracellular pH in the presence of 20 mmol/L lactate (Munsch and Pape, 1999; Ruusuvuori et al., 2010).

GLYCOGEN STORES AND INHIBITION OF GLYCOGEN PHOSPHORYLASE

Glycogen phosphorylase catalyzes the rate-limiting step in glycogenolysis in animals by releasing glucose-1-phosphate from

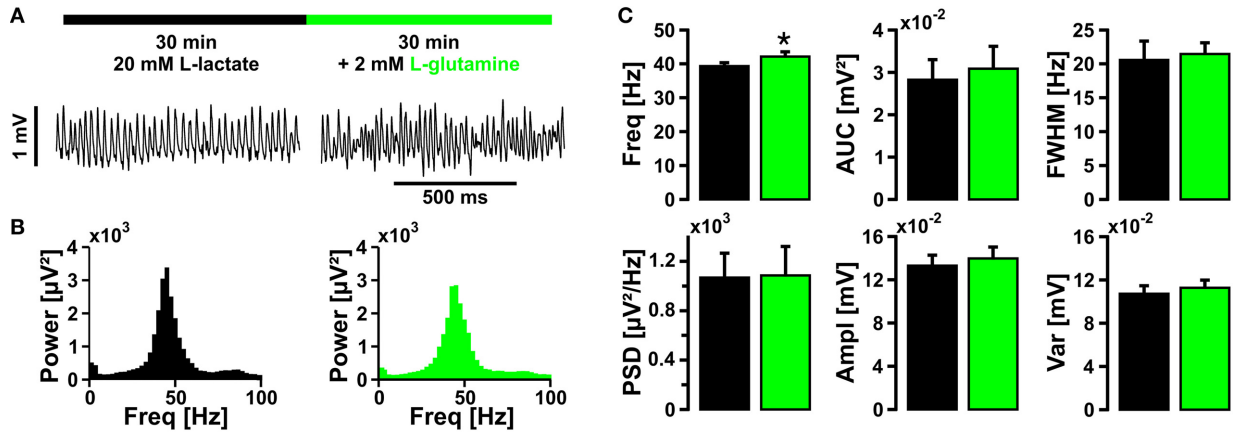


FIGURE 5 | Gamma oscillations in the presence of lactate and glutamine. (A) Gamma oscillations were induced by bath application of acetylcholine (2 μmol/L) and physostigmine (400 nmol/L) in the presence of 20 mmol/L lactate (black bar); after 30 min, 2 mmol/L glutamine was added (green bar). Local field potentials (LFP) were recorded in stratum pyramidale of the CA3 subfield (sample traces). **(B)** Corresponding power spectra of sample traces shown in **(A)** were calculated from 100 s taken at the end of each data segment. **(C)** Gamma oscillations were analyzed for various parameters, i.e., peak frequency (Freq), area under curve (AUC), full width at half maximum (FWHM), peak power spectral density (PSD), amplitude (Ampl), and variance of the amplitude (Var) ($n = 19$, $N = 4$). Note that glutamine has only a minor effect on the frequency of gamma oscillations. Paired *t*-test. Statistical significance is marked by asterisks ($P < 0.05$).

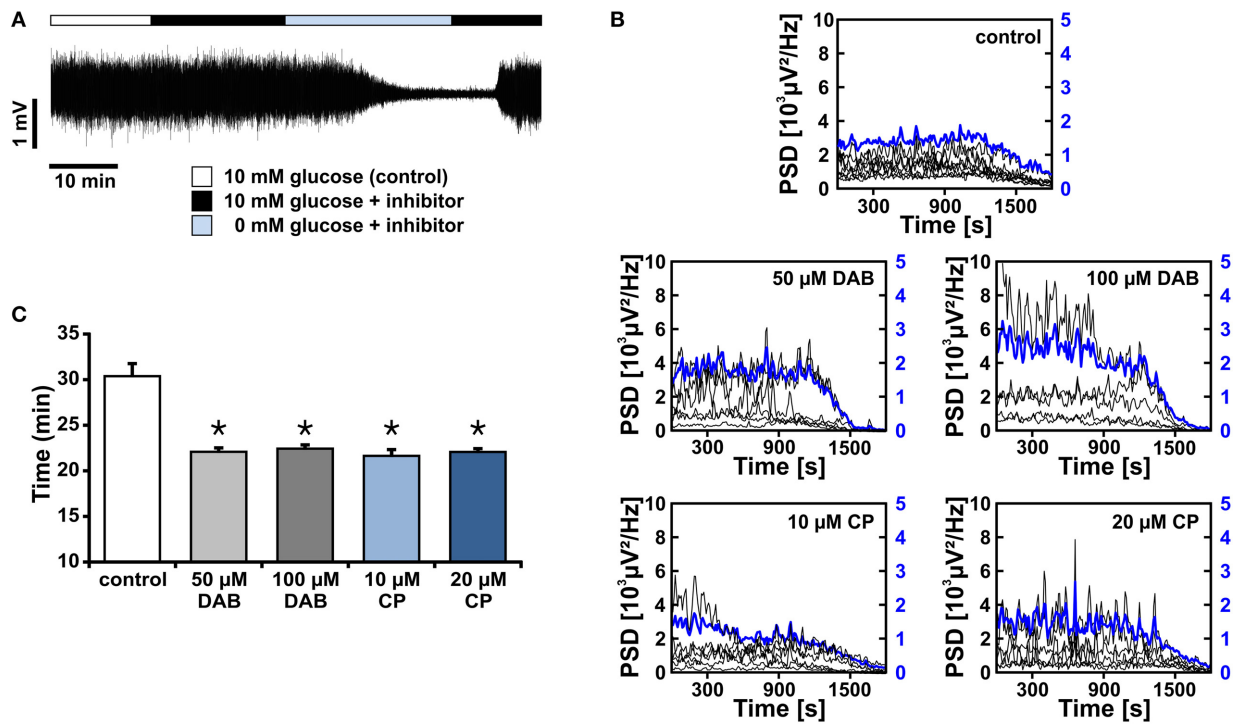


FIGURE 6 | Gamma oscillations and glycogen stores during glucose deprivation. (A) Gamma oscillations were induced by bath application of acetylcholine (2 μmol/L) and physostigmine (400 nmol/L) in the presence of 10 mmol/L glucose (white bar). Subsequently, inhibitors of glycogen phosphorylase, DAB (50 μmol/l or 100 μmol/L) or CP-316819 (10 μmol/L or 20 μmol/L) were applied, in the presence (black bar) or absence (light blue bar) of glucose. Note that the standard gas mixture (95% O2 and 5% CO2) was continuously present. Local field potentials (LFP) were recorded in stratum pyramidale of the CA3 subfield subfield (sample trace). **(B)** The peak power spectral density (μV^2/Hz) for each recording trace is shown in black (scaling on left y-axis), the average of all recordings is shown in blue (scaling on right y-axis). Power spectra were calculated every 10 s and plotted over time. **(C)** The points in time are given for complete suppression of gamma oscillations, i.e., power reaching a threshold defined as the mean of the last 100 s plus 1 standard deviation, according to the protocol given in **(A)** (control, $n = 10$, $N = 3$; DAB, $n = 6$, $N = 3$, and $n = 5$, $N = 3$; CP-316819, $n = 6$, $N = 3$, and $n = 5$, $N = 2$). Note that inhibition of glycogen phosphorylase accelerates the decay of gamma oscillations during glucose deprivation. Kruskal Wallis ANOVA on ranks. Statistical significance vs. control is marked by asterisks ($P < 0.05$).

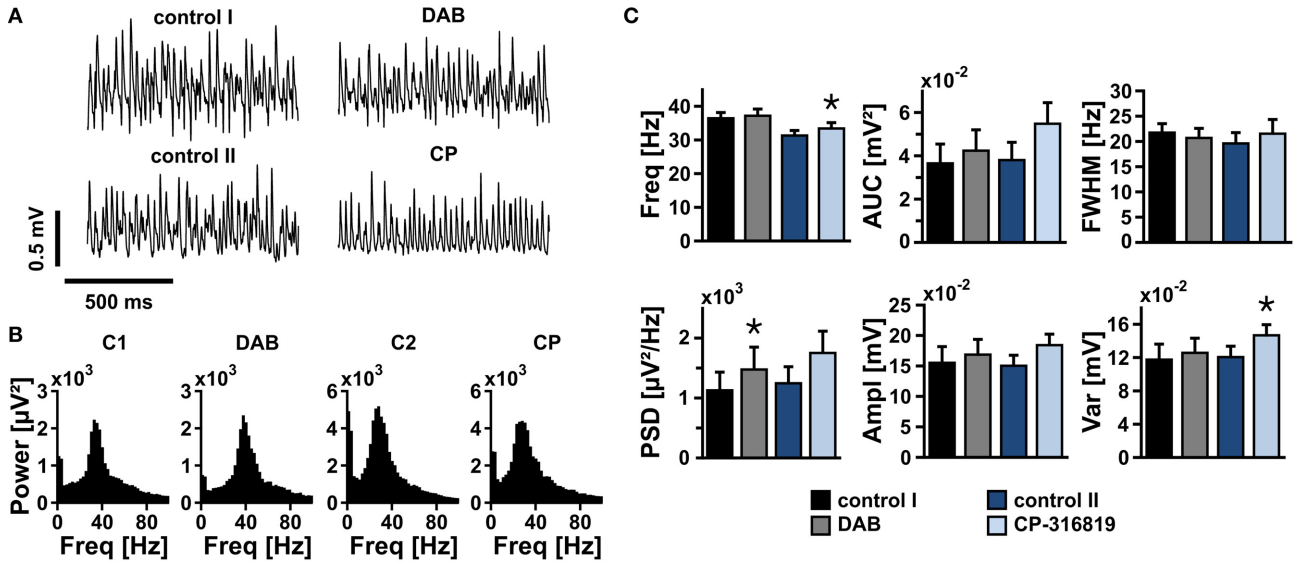


FIGURE 7 | Gamma oscillations and glycogen turnover. (A) Gamma oscillations were induced by bath application of acetylcholine (2 µmol/L) and physostigmine (400 nmol/L) in the presence of 10 mmol/L glucose. After 15 min, inhibitors of glycogen phosphorylase, DAB (50 µmol/l or 100 µmol/L) or CP-316819 (10 µmol/L or 20 µmol/L) were added to the recording solution. Local field potentials (LFP) were recorded in stratum pyramidale of the CA3 subfield (sample traces). **(B)** Corresponding power spectra of sample traces shown in **(A)** were calculated from 100 s taken at the end of each data segment. **(C)** Gamma oscillations were analyzed for various parameters, i.e., peak frequency (Freq), area under curve (AUC), full width at half maximum (FWHM), peak power spectral density (PSD), amplitude (Ampl), and variance of the amplitude (Var) (control 1 and DAB, $n = 13$, $N = 4$; control 2 and CP-316819, $n = 10$, $N = 3$). Note that inhibition of glycogen phosphorylase in the presence of 10 mmol/L glucose has only minor effects on gamma oscillations. Paired *t*-test or Wilcoxon signed rank test. Statistical significance is marked by asterisks ($P < 0.05$).

the terminal alpha-1,4-glycosidic bond, finally supporting various intracellular metabolic pathways (Dienel and Cruz, 2014). It is widely accepted that glycogen is stored in astrocytes (Roberts, 2007; Dienel and Cruz, 2014). However, glycogen has been also found in localized compartments of neurons such as synaptic boutons and dendritic spines (Fiala et al., 2003), and there is recent evidence that neurons have an active glycogen metabolism (Saez et al., 2014). Several studies support two roles of glycogen as an energy substrate. The first role is to supply energy for regular neuronal activity (Swanson, 1992; Dienel et al., 2002; Kong et al., 2002; Gibbs et al., 2006; Choi et al., 2012; Duran et al., 2013). The second role is to provide glucose equivalents when supply with glucose or oxygen is limited, such as during hypoglycemia or ischemia/hypoxia (Wender et al., 2000; Choi et al., 2003; Saez et al., 2014). The glycogen metabolism might primarily occur in astrocytes that finally provide lactate to neurons (Dringen et al., 1993; Dienel and Cruz, 2014).

In our study, glycogen breakdown significantly delayed the decay of gamma oscillations in the absence of glucose for about 8 min. This indicates (i) a considerable glycogen reserve in slice cultures that might be larger than *in vivo* (Cater et al., 2001; Kong et al., 2002), and (ii) quite effective mechanisms to mobilize and metabolize astrocytic and neuronal glycogen during gamma oscillations. However, inhibition of glycogen phosphorylase had only minor effects on gamma oscillations in the presence of glucose. Thus, our data suggest that glycogen serves as an important fuel reserve to sustain gamma oscillations and thus higher brain functions for a transient period under pathological conditions.

REFERENCES

Abdelmalik, P. A., Shannon, P., Yiu, A., Liang, P., Adamchik, Y., Weisspapir, M., et al. (2007). Hypoglycemic seizures during transient hypoglycemia exacerbate hippocampal dysfunction. *Neurobiol. Dis.* 26, 646–660. doi: 10.1016/j.nbd.2007.03.002

Attwell, D., Buchan, A. M., Charpak, S., Lauritzen, M., MacVicar, B. A., and Newman, E. A. (2010). Glial and neuronal control of brain blood flow. *Nature* 468, 232–243. doi: 10.1038/nature09613

Bahr, B. A., Kessler, M., Rivera, S., Vanderklish, P. W., Hall, R. A., Mutneja, M. S., et al. (1995). Stable maintenance of glutamate receptors and other synaptic components in long-term hippocampal slices. *Hippocampus* 5, 425–439. doi: 10.1002/hipo.450050505

Bak, L. K., Schousboe, A., and Waagepetersen, H. S. (2006). The glutamate/GABA-glutamine cycle: aspects of transport, neurotransmitter homeostasis and ammonia transfer. *J. Neurochem.* 98, 641–653. doi: 10.1111/j.1471-4159.2006.03913.x

Barros, L. F. (2013). Metabolic signaling by lactate in the brain. *Trends Neurosci.* 36, 396–404. doi: 10.1016/j.tins.2013.04.002

Bartos, M., Vida, I., and Jonas, P. (2007). Synaptic mechanisms of synchronized gamma oscillations in inhibitory interneuron networks. *Nat. Rev. Neurosci.* 8, 45–56. doi: 10.1038/nrn2044

Bouzier-Sore, A.-K., Voisin, P., Canioni, P., Magistretti, P. J., and Pellerin, L. (2003). Lactate is a preferential oxidative energy substrate over glucose for neurons in culture. *J. Cereb. Blood Flow Metab.* 23, 1298–1306. doi: 10.1097/01.WCB.0000091761.61714

Carter, B. C., and Bean, B. P. (2009). Sodium entry during action potentials of mammalian neurons: incomplete inactivation and reduced metabolic efficiency in fast-spiking neurons. *Neuron* 64, 898–909. doi: 10.1016/j.neuron.2009.12.011

Cater, H. L., Benham, C. D., and Sundstrom, L. E. (2001). Neuroprotective role of monocarboxylate transport during glucose deprivation in slice cultures of rat hippocampus. *J. Physiol.* 531, 459–466. doi: 10.1111/j.1469-7793.2001.0459i.x

Cater, H. L., Chandratheva, A., Benham, C. D., Morrison, B. III., and Sundstrom, L. E. (2003). Lactate and glucose as energy substrates during, and after, oxygen deprivation in rat hippocampal acute and cultured slices. *J. Neurochem.* 87, 1381–1390. doi: 10.1046/j.1471-4159.2003.02100.x

Chih, C.-P., and Roberts, Jr. E. L. (2003). Energy substrates for neurons during neural activity: a critical review of the astrocyte-neuron lactate shuttle hypothesis. *J. Cereb. Blood Flow Metab.* 23, 1263–1281. doi: 10.1097/01.WCB.0000081369.51727.6F

Choi, H. B., Gordon, G. R. J., Zhou, N., Tai, C., Rungta, R. L., Martinez, J., et al. (2012). Metabolic communication between astrocytes and neurons via bicarbonate-responsive soluble adenylyl cyclase. *Neuron* 75, 1094–1104. doi: 10.1016/j.neuron.2012.08.032

Choi, I.-Y., Seaquist, E. R., and Gruetter, R. (2003). Effect of hypoglycemia on brain glycogen metabolism *in vivo*. *J. Neurosci. Res.* 72, 25–32. doi: 10.1002/jnr.10574

Csicsvari, J., Hirase, H., Czurkó, A., Mamiya, A., and Buzsáki, G. (1999). Oscillatory coupling of hippocampal pyramidal cells and interneurons in the behaving rat. *J. Neurosci.* 19, 274–287.

De Simoni, A., Griesinger, C. B., and Edwards, F. A. (2003). Development of rat CA1 neurones in acute *versus* organotypic slices: role of experience in synaptic morphology and activity. *J. Physiol.* 550, 135–147. doi: 10.1113/jphysiol.2003.039099

Dienel, G. A. (2012). Brain lactate metabolism: the discoveries and the controversies. *J. Cereb. Blood Flow Metab.* 32, 1107–1138. doi: 10.1038/jcbfm.2011.175

Dienel, G. A., Ball, K. K., and Cruz, N. F. (2007). A glycogen phosphorylase inhibitor selectively enhances local rates of glucose utilization in brain during sensory stimulation of conscious rats: implications for glycogen turnover. *J. Neurochem.* 102, 466–478. doi: 10.1111/j.1471-4159.2007.04595.x

Dienel, G. A., and Cruz, N. F. (2014). Contributions of glycogen to astrocytic energetics during brain activation. *Metab. Brain Dis.* doi: 10.1007/s11011-014-9493-8. [Epub ahead of print].

Dienel, G. A., Wang, R. Y., and Cruz, N. F. (2002). Generalized sensory stimulation of conscious rats increases labeling of oxidative pathways of glucose metabolism when the brain glucose-oxygen uptake ratio rises. *J. Cereb. Blood Flow Metab.* 22, 1490–1502. doi: 10.1097/00004647-200212000-00009

Dringen, R., Gebhardt, R., and Hamprecht, B. (1993). Glycogen in astrocytes: possible function as lactate supply for neighboring cells. *Brain Res.* 623, 208–214. doi: 10.1016/0006-8993(93)91429-V

Duran, J., Saez, I., Gruart, A., Guinovart, J. J., and Delgado-García, J. M. (2013). Impairment in long-term memory formation and learning-dependent synaptic plasticity in mice lacking glycogen synthase in the brain. *J. Cereb. Blood Flow Metab.* 33, 550–556. doi: 10.1038/jcbfm.2012.200

Erecińska, M., and Silver, I. A. (2001). Tissue oxygen tension and brain sensitivity to hypoxia. *Respir. Physiol.* 128, 263–276. doi: 10.1016/S0034-5687(01)00306-1

Fiala, J. C., Kirov, S. A., Feinberg, M. D., Petrak, L. J., George, P., Goddard, C. A., et al. (2003). Timing of neuronal and glial ultrastructure disruption during brain slice preparation and recovery *in vitro*. *J. Comp. Neurol.* 465, 90–103. doi: 10.1002/cne.10825

Fisahn, A., Pike, F. G., Buhl, E. H., and Paulsen, O. (1998). Cholinergic induction of network oscillations at 40 Hz in the hippocampus *in vitro*. *Nature* 394, 186–189. doi: 10.1038/28179

Fisahn, A., Yamada, M., Duttaroy, A., Gan, J.-W., Deng, C.-X., McBain, C. J., et al. (2002). Muscarinic induction of hippocampal gamma oscillations requires coupling of the M1 receptor to two mixed cation currents. *Neuron* 33, 615–624. doi: 10.1016/S0896-6273(02)00587-1

Galeffi, F., Foster, K. A., Sadgrove, M. P., Beaver, C. J., and Turner, D. A. (2007). Lactate uptake contributes to the NAD(P)H biphasic response and tissue oxygen response during synaptic stimulation in area CA1 of rat hippocampal slices. *J. Neurochem.* 103, 2449–2461. doi: 10.1111/j.1471-4159.2007.04939.x

Gandhi, G. K., Cruz, N. F., Ball, K. K., and Dienel, G. A. (2009). Astrocytes are poised for lactate trafficking and release from activated brain and for supply of glucose to neurons. *J. Neurochem.* 111, 522–536. doi: 10.1111/j.1471-4159.2009.06333.x

Gibbs, M. E., Anderson, D. G., and Hertz, L. (2006). Inhibition of glycogenolysis in astrocytes interrupts memory consolidation in young chickens. *Glia* 54, 214–222. doi: 10.1002/glia.20377

Gloveli, T., Dugladze, T., Saha, S., Monyer, H., Heinemann, U., Traub, R. D., et al. (2005). Differential involvement of oriens/pyramidale interneurones in hippocampal network oscillations *in vitro*. *J. Physiol.* 562, 131–147. doi: 10.1113/jphysiol.2004.073007

Gulyás, A. I., Buzsáki, G., Freund, T. F., and Hirase, H. (2006). Populations of hippocampal inhibitory neurons express different levels of cytochrome c. *Eur. J. Neurosci.* 23, 2581–2594. doi: 10.1111/j.1460-9568.2006.04814.x

Gulyás, A. I., Szabó, G. G., Ulbert, I., Holderith, N., Monyer, H., Erdélyi, F., et al. (2010). Parvalbumin-containing fast-spiking basket cells generate the field potential oscillations induced by cholinergic receptor activation in the hippocampus. *J. Neurosci.* 30, 15134–15145. doi: 10.1523/JNEUROSCI.4104-10.2010

Hájos, N., Pálhalmi, J., Mann, E. O., Németh, B., Paulsen, O., and Freund, T. F. (2004). Spike timing of distinct types of GABAergic interneuron during hippocampal gamma oscillations *in vitro*. *J. Neurosci.* 24, 9127–9137. doi: 10.1523/JNEUROSCI.2113-04.2004

Hájos, N., Papp, E. C., Acsády, L., Levey, A. I., and Freund, T. F. (1998). Distinct interneuron types express m2 muscarinic receptor immunoreactivity on their dendrites or axon terminals in the hippocampus. *Neuroscience* 82, 355–376. doi: 10.1016/S0306-4522(97)00300-X

Hájos, N., and Paulsen, O. (2009). Network mechanisms of gamma oscillations in the CA3 region of the hippocampus. *Neural Netw.* 22, 1113–1119. doi: 10.1016/j.neunet.2009.07.024

Halestrap, A. P. (2013). The SLC16 gene family - structure, role and regulation in health and disease. *Mol. Aspects Med.* 34, 337–349. doi: 10.1016/j.mam.2012.05.003

Hall, C. N., Klein-Flügge, M. C., Howarth, C., and Attwell, D. (2012). Oxidative phosphorylation, not glycolysis, powers presynaptic and postsynaptic mechanisms underlying brain information processing. *J. Neurosci.* 32, 8940–8951. doi: 10.1523/JNEUROSCI.0026-12.2012

Hertz, L., Gibbs, M. E., and Dienel, G. A. (2014). Fluxes of lactate into, from, and among gap junction-coupled astrocytes and their interaction with noradrenaline. *Front. Neurosci.* 8:261. doi: 10.3389/fnins.2014.00261

Hu, H., and Jonas, P. (2014). A supercritical density of Na^+ channels ensures fast signaling in GABAergic interneuron axons. *Nat. Neurosci.* 17, 686–693. doi: 10.1038/nn.3678

Huchzermeyer, C., Albus, K., Gabriel, H.-J., Otáhal, J., Taubenberger, N., Heinemann, U., et al. (2008). Gamma oscillations and spontaneous network activity in the hippocampus are highly sensitive to decreases in pO_2 and concomitant changes in mitochondrial redox state. *J. Neurosci.* 28, 1153–1162. doi: 10.1523/JNEUROSCI.4105-07.2008

Huchzermeyer, C., Berndt, N., Holzhütter, H.-G., and Kann, O. (2013). Oxygen consumption rates during three different neuronal activity states in the hippocampal CA3 network. *J. Cereb. Blood Flow Metab.* 33, 263–271. doi: 10.1038/jcbfm.2012.165

Ide, K., Schmalbruch, I. K., Quistorff, B., Horn, A., and Secher, N. H. (2000). Lactate, glucose and O_2 uptake in human brain during recovery from maximal exercise. *J. Physiol.* 522, 159–164. doi: 10.1111/j.1469-7793.2000.t01-2-00159.xm

Ivanov, A. I., Malkov, A. E., Waseem, T., Mukhtarov, M., Buldakova, S., Gubkina, O., et al. (2014). Glycolysis and oxidative phosphorylation in neurons and astrocytes during network activity in hippocampal slices. *J. Cereb. Blood Flow Metab.* 34, 397–407. doi: 10.1038/jcbfm.2013.222

Ivanov, A., Mukhtarov, M., Bregestovski, P., and Zilberter, Y. (2011). Lactate effectively covers energy demands during neuronal network activity in neonatal hippocampal slices. *Front. Neuroenergetics* 3:2. doi: 10.3389/fnene.2011.00002

Kageyama, G. H., and Wong-Riley, M. T. T. (1982). Histochemical localization of cytochrome oxidase in the hippocampus: correlation with specific neuronal types and afferent pathways. *Neuroscience* 7, 2337–2361. doi: 10.1016/0306-4522(82)90199-3

Kann, O. (2012). The energy demand of fast neuronal network oscillations: insights from brain slice preparations. *Front. Pharmacol.* 2:90. doi: 10.3389/fphar.2011.00090

Kann, O., Huchzermeyer, C., Kovács, R., Wirtz, S., and Schuelke, M. (2011). Gamma oscillations in the hippocampus require high complex I gene expression and strong functional performance of mitochondria. *Brain* 134, 345–358. doi: 10.1093/brain/awq333

Kann, O., and Kovács, R. (2007). Mitochondria and neuronal activity. *Am. J. Physiol. Cell Physiol.* 292, C641–C657. doi: 10.1152/ajpcell.00222.2006

Kann, O., Kovács, R., and Heinemann, U. (2003a). Metabotropic receptor-mediated Ca^{2+} signaling elevates mitochondrial Ca^{2+} and stimulates oxidative metabolism in hippocampal slice cultures. *J. Neurophysiol.* 90, 613–621. doi: 10.1152/jn.00042.2003

Kann, O., Papageorgiou, I. E., and Draguhn, A. (2014). Highly energized inhibitory interneurons are a central element for information processing in cortical networks. *J. Cereb. Blood Flow Metab.* 34, 1270–1282. doi: 10.1038/jcbfm.2014.104

Kann, O., Schuchmann, S., Buchheim, K., and Heinemann, U. (2003b). Coupling of neuronal activity and mitochondrial metabolism as revealed by NAD(P)H fluorescence signals in organotypic hippocampal slice cultures of the rat. *Neuroscience* 119, 87–100. doi: 10.1016/S0306-4522(03)00026-5

Kong, J., Shepel, P. N., Holden, C. P., Mackiewicz, M., Pack, A. I., and Geiger, J. D. (2002). Brain glycogen decreases with increased periods of wakefulness: implications for homeostatic drive to sleep. *J. Neurosci.* 22, 5581–5587.

Lehmann, D., Faber, P. L., Achermann, P., Jeanmonod, D., Gianotti, L. R. R., and Pizzagalli, D. (2001). Brain sources of EEG gamma frequency during volitionally meditation-induced, altered states of consciousness, and experience of the self. *Psychiatry Res.* 108, 111–121. doi: 10.1016/S0925-4927(01)00116-0

Li, C.-L., and McIlwain, H. (1957). Maintenance of resting membrane potentials in slices of mammalian cerebral cortex and other tissues *in vitro*. *J. Physiol.* 139, 178–190.

Lutz, A., Greischar, L. L., Rawlings, N. B., Ricard, M., and Davidson, R. J. (2004). Long-term meditators self-induce high-amplitude gamma synchrony during mental practice. *Proc. Natl. Acad. Sci. U.S.A.* 101, 16369–16373. doi: 10.1073/pnas.0407401101

Montgomery, S. M., and Buzsáki, G. (2007). Gamma oscillations dynamically couple hippocampal CA3 and CA1 regions during memory task performance. *Proc. Natl. Acad. Sci. U.S.A.* 104, 14495–14500. doi: 10.1073/pnas.0701826104

Munsch, T., and Pape, H.-C. (1999). Modulation of the hyperpolarization-activated cation current of rat thalamic relay neurones by intracellular pH. *J. Physiol.* 519, 493–504. doi: 10.1111/j.1469-7793.1999.0493m.x

Niessing, J., Ebisch, B., Schmidt, K. E., Niessing, M., Singer, W., and Galuske, R. A. W. (2005). Hemodynamic signals correlate tightly with synchronized gamma oscillations. *Science* 309, 948–951. doi: 10.1126/science.1110948

Nishida, M., Juhász, C., Sood, S., Chugani, H. T., and Asano, E. (2008). Cortical glucose metabolism positively correlates with gamma-oscillations in nonlesional focal epilepsy. *Neuroimage* 42, 1275–1284. doi: 10.1016/j.neuroimage.2008.06.027

Oren, I., Hájos, N., and Paulsen, O. (2010). Identification of the current generator underlying cholinergically induced gamma frequency field potential oscillations in the hippocampal CA3 region. *J. Physiol.* 588, 785–797. doi: 10.1113/jphysiol.2009.180851

Overgaard, M., Rasmussen, P., Bohm, A. M., Seifert, T., Brassard, P., Zaar, M., et al. (2012). Hypoxia and exercise provoke both lactate release and lactate oxidation by the human brain. *FASEB J.* 26, 3012–3020. doi: 10.1096/fj.11-191999

Paulsen, O., and Moser, E. I. (1998). A model of hippocampal memory encoding and retrieval: GABAergic control of synaptic plasticity. *Trends Neurosci.* 21, 273–278. doi: 10.1016/S0166-2236(97)01205-8

Pellerin, L., and Magistretti, P. J. (2012). Sweet sixteen for ANLS. *J. Cereb. Blood Flow Metab.* 32, 1152–1166. doi: 10.1038/jcbfm.2011.149

Penttonen, M., Kamondi, A., Acsády, L., and Buzsáki, G. (1998). Gamma frequency oscillation in the hippocampus of the rat: intracellular analysis *in vivo*. *Eur. J. Neurosci.* 10, 718–728. doi: 10.1046/j.1460-9568.1998.00096.x

Ribak, C. E., Seress, L., and Leranth, C. (1993). Electron microscopic immunocytochemical study of the distribution of parvalbumin-containing neurons and axon terminals in the primate dentate gyrus and Ammon's horn. *J. Comp. Neurol.* 327, 298–321. doi: 10.1002/cne.903270211

Roberts, E. L. Jr. (2007). "The support of energy metabolism in the central nervous system with substrates other than glucose," in *Handbook of Neurochemistry and Molecular Neurobiology*, eds A. Lajtha, G. Gido, and G. A. Dienel (Berlin; Heidelberg: Springer-Verlag), 137–179. doi: 10.1007/978-0-387-30411-3_7

Rolfe, D. F. S., and Brown, G. C. (1997). Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiol. Rev.* 77, 731–758.

Ruusuvuori, E., Kirilkin, I., Pandya, N., and Kaila, K. (2010). Spontaneous network events driven by depolarizing GABA action in neonatal hippocampal slices are not attributable to deficient mitochondrial energy metabolism. *J. Neurosci.* 30, 15638–15642. doi: 10.1523/JNEUROSCI.3355-10.2010

Saez, I., Duran, J., Sinadinos, C., Beltran, A., Yanes, O., Tevy, M. F., et al. (2014). Neurons have an active glycogen metabolism that contributes to tolerance to hypoxia. *J. Cereb. Blood Flow Metab.* 34, 945–955. doi: 10.1038/jcbfm.2014.33

Schurr, A., and Gozal, E. (2012). Aerobic production and utilization of lactate satisfy increased energy demands upon neuronal activation in hippocampal slices and provide neuroprotection against oxidative stress. *Front. Pharmacol.* 2:96. doi: 10.3389/fphar.2011.00096

Schurr, A., Miller, J. J., Payne, R. S., and Rigor, B. M. (1999). An increase in lactate output by brain tissue serves to meet the energy needs of glutamate-activated neurons. *J. Neurosci.* 19, 34–39.

Schurr, A., and Payne, R. S. (2007). Lactate, not pyruvate, is neuronal aerobic glycolysis end product: an *in vitro* electrophysiological study. *Neuroscience* 147, 613–619. doi: 10.1016/j.neuroscience.2007.05.002

Schurr, A., West, C. A., and Rigor, B. M. (1988). Lactate-supported synaptic function in the rat hippocampal slice preparation. *Science* 240, 1326–1328. doi: 10.1126/science.3375817

Shulman, R. G., Hyder, F., and Rothman, D. L. (2001). Lactate efflux and the neuroenergetic basis of brain function. *NMR Biomed.* 14, 389–396. doi: 10.1002/nbm.741

Sickmann, H. M., Walls, A. B., Schousboe, A., Bouman, S. D., and Waagepetersen, H. S. (2009). Functional significance of brain glycogen in sustaining glutamatergic neurotransmission. *J. Neurochem.* 109 Suppl. 1, 80–86. doi: 10.1111/j.1471-4159.2009.05915.x

Simpson, I. A., Carruthers, A., and Vannucci, S. J. (2007). Supply and demand in cerebral energy metabolism: the role of nutrient transporters. *J. Cereb. Blood Flow Metab.* 27, 1766–1791. doi: 10.1038/sj.jcbfm.9600521

Stittsworth, J. D. Jr., and Lanthorn, T. H. (1993). Lactate mimics only some effects of D-glucose on epileptic depolarization and long-term synaptic failure. *Brain Res.* 630, 21–27. doi: 10.1016/0006-8993(93)90637-3

Suh, S. W., Bergher, J. P., Anderson, C. M., Treadway, J. L., Fosgerau, K., and Swanson, R. A. (2007). Astrocyte glycogen sustains neuronal activity during hypoglycemia: studies with the glycogen phosphorylase inhibitor CP-316,819 (*[R-R*,S*]*-5-chloro-*N*-[2-hydroxy-3-(methoxymethylamino)-3-oxo-1-(phenylmethyl)pro pyl]-1*H*-indole-2-carboxamide). *J. Pharmacol. Exp. Ther.* 321, 45–50. doi: 10.1124/jpet.106.115550

Suzuki, A., Stern, S. A., Bozdagi, O., Huntley, G. W., Walker, R. H., Magistretti, P. J., et al. (2011). Astrocyte-neuron lactate transport is required for long-term memory formation. *Cell* 144, 810–823. doi: 10.1016/j.cell.2011.02.018

Swanson, R. A. (1992). Physiologic coupling of glial glycogen metabolism to neuronal activity in brain. *Can. J. Physiol. Pharmacol.* 70 Suppl., S138–S144. doi: 10.1139/y92-255

Takács, V. T., Szönyi, A., Freund, T. F., Nyiri, G., and Gulyás, A. I. (2014). Quantitative ultrastructural analysis of basket and axo-axonic cell terminals in the mouse hippocampus. *Brain Struct. Funct.* doi: 10.1007/s00429-013-0692-6. [Epub ahead of print].

Traub, R. D., Bibbig, A., Fisahn, A., LeBeau, F. E. N., Whittington, M. A., and Buhl, E. H. (2000). A model of gamma-frequency network oscillations induced in the rat CA3 region by carbachol *in vitro*. *Eur. J. Neurosci.* 12, 4093–4106. doi: 10.1046/j.1460-9568.2000.00300.x

Traub, R. D., Kopell, N., Bibbig, A., Buhl, E. H., LeBeau, F. E. N., and Whittington, M. A. (2001). Gap junctions between interneuron dendrites can enhance synchrony of gamma oscillations in distributed networks. *J. Neurosci.* 21, 9478–9486.

Uhlhaas, P. J., and Singer, W. (2010). Abnormal neural oscillations and synchrony in schizophrenia. *Nat. Rev. Neurosci.* 11, 100–113. doi: 10.1038/nrn2774

van Vugt, M. K., Schulze-Bonhage, A., Litt, B., Brandt, A., and Kahana, M. J. (2010). Hippocampal gamma oscillations increase with memory load. *J. Neurosci.* 30, 2694–2699. doi: 10.1523/JNEUROSCI.0567-09.2010

Waagepetersen, H. S., Bakken, I. J., Larsson, O. M., Sonnewald, U., and Schousboe, A. (1998). Metabolism of lactate in cultured GABAergic neurons studied by ^{13}C nuclear magnetic resonance spectroscopy. *J. Cereb. Blood Flow Metab.* 18, 109–117. doi: 10.1097/00004647-199801000-00011

Walls, A. B., Sickmann, H. M., Brown, A., Bouman, S. D., Ransom, B., Schousboe, A., et al. (2008). Characterization of 1,4-dideoxy-1,4-imino-d-arabinitol (DAB) as an inhibitor of brain glycogen shunt activity. *J. Neurochem.* 105, 1462–1470. doi: 10.1111/j.1471-4159.2008.05250.x

Wender, R., Brown, A. M., Fern, R., Swanson, R. A., Farrell, K., and Ransom, B. R. (2000). Astrocytic glycogen influences axon function and survival during glucose deprivation in central white matter. *J. Neurosci.* 20, 6804–6810.

Whittaker, R. G., Turnbull, D. M., Whittington, M. A., and Cunningham, M. O. (2011). Impaired mitochondrial function abolishes gamma oscillations in the

hippocampus through an effect on fast-spiking interneurons. *Brain* 134, e180. doi: 10.1093/brain/awr018

Whittington, M. A., and Traub, R. D. (2003). *Interneuron diversity series*: inhibitory interneurons and network oscillations *in vitro*. *Trends Neurosci.* 26, 676–682. doi: 10.1016/j.tins.2003.09.016

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 14 October 2014; paper pending published: 01 November 2014; accepted: 18 November 2014; published online: 05 December 2014.

*Citation: Galow LV, Schneider J, Lewen A, Ta T-T, Papageorgiou IE and Kann O (2014) Energy substrates that fuel fast neuronal network oscillations. Front. Neurosci. **8**:398. doi: 10.3389/fnins.2014.00398*

This article was submitted to Neuroenergetics, Nutrition and Brain Health, a section of the journal Frontiers in Neuroscience.

frontiers in NEUROSCIENCE

OPINION ARTICLE
published: 09 December 2014
doi: 10.3389/fnins.2014.00407

The mitochondrial L-lactate dehydrogenase affair

Salvatore Passarella*, Gianluca Paventi and Roberto Pizzuto

Department of Medicine and Health Sciences, University of Molise, Campobasso, Italy
**Correspondence: passarel@unimol.it*

Edited by:
Avital Schurr, University of Louisville, USA

Reviewed by:
Oliver Kann, University of Heidelberg, Germany
George A. Brooks, University of California, USA

Keywords: mitochondria, L-lactate dehydrogenase, L-lactate metabolism, gastrocnemious muscle mitochondria, energy metabolism

The existence of a mitochondrial L-lactate dehydrogenase (m-L-LDH) suggested by Dianzani (1951), was shown by Baba and Sharma (1971) with the enzyme located in the mitochondrial matrix; later Brooks et al. (1999) proposed the intracellular lactate shuttle and in the third millennium the existence of m-L-LDH was definitively been confirmed in mammalian, plant and yeast mitochondria as reviewed by Schurr (2006), Passarella et al. (2008), and Brooks (2009), being its existence finally recognized by inclusion of m-L-LDH in the Mitocarta (http://www.broadinstitute.org/pubs/MitoCarta/index.html). The experimental strategy to be used to show whether and how L-lactate can enter mitochondria to be metabolized is well-established and has been applied to a variety of mitochondria including heart (Brooks et al., 1999; Valenti et al., 2002), liver (Brooks et al., 1999; de Bari et al., 2004), skeletal muscle (Dubouchaud et al., 2000; de Bari et al., 2008; Passarella et al., 2008) plant (Paventi et al., 2007), brain (Schurr, 2006; Atlante et al., 2007; Schurr and Payne, 2007; Hashimoto et al., 2008), and cancer cells (de Bari et al., 2010a; Pizzuto et al., 2012). Thus, it is a matter for considerable surprise that the overwhelming evidence for an m-L-LDH located inside mitochondria is not by now universally accepted (Rasmussen et al., 2002; Sahlin et al., 2002; Ponsot et al., 2005; Gladden, 2007; Yoshida et al., 2007; Elustondo et al., 2013).

Using correctly applied procedures, metabolism of L-lactate via the m-L-LDH can be investigated in mitochondria (but also in permeabilized cells, cell homogenates, and mitoplasts) in a few hours. Obviously, caution must be used to control that mitochondrial coupling was not impaired. This simple strategy includes both L-lactate uptake measurements and measurements of mitochondrial processes occurring after L-lactate uptake due to the occurrence of an enzyme i.e., m-L-LDH, which can metabolize the imported L-lactate with in some cases export of the newly synthesized metabolites (for some details see Passarella et al., 2003). The measurements include:

(1) Swelling measurements which provide initial evidence that L-lactate can enter mitochondria; importantly, the stereospecificity of the process and the inhibition of swelling found due to non-penetrant compounds strongly suggest that L-lactate uptake occurs in a carrier-mediated manner. Obviously a carrier-mediated transport itself suggests that L-lactate is metabolized inside mitochondria.
(2) Measurements of the increase in the redox state of the intramitochondrial pyridine nucleotides found as a result of the addition of L-lactate to the mitochondrial samples; such an increase itself shows that mitochondrial metabolism occurs inside the organelles via the NAD^+ dependent m-L-LDH. Having established, by applying the control strength criterion (see Passarella et al., 2003), that the rate of NAD^+ reduction mirrors that of L-lactate transport across the mitochondrial membrane, the transport kinetics can be investigated including their pH and temperature dependence. Importantly the presence of carrier/s devoted to transport L-lactate across the mitochondrial membrane postulates that m-L-LDH is located inside mitochondria. Changes in NAD^+/NADH redox state should be modulated by certain ionophores under conditions designed to selectively affect ΔpH and $\Delta\Psi$ as well as inhibited by a variety of non-penetrant compounds. In the former case the nature of the energy dependence of the transport can be established; in the latter the inhibition profiles could be used to ascertain further whether the L-lactate carriers, the pyruvate carrier and other carriers differ from one another. In some cases this has been shown: in distinction with others we have shown that two separate carriers transport pyruvate and L-lactate into rat liver mitochondria (de Bari et al., 2004). The point is that α-cyano-hydroxycinnamate (α-CCN^-) can inhibit the uptake of both pyruvate and L-lactate, but the pyruvate carrier is inhibited at a concentration (25 μM) at which no inhibition of L-lactate transport occurs.
(3) Measurements of oxygen consumption by coupled purified mitochondria due to L-lactate addition. To conclude that oxygen consumption depends on the NAD^+ dependent m-L-LDH inhibition by the complex I inhibitor rotenone as well as by oxalate/oxamate, inhibitors of L-LDH must be found.
(4) Proton efflux and increase of membrane potential could be also found as a result of L-lactate uptake and metabolism. Conversely proton uptake occurs as a result of L-lactate addition to mitochondria previously treated with an inhibitor cocktail used to prevent any energy metabolism. These effects show the existence of

L-lactate energy metabolism via m-L-LDH and of a proton compensated L-lactate symport.

(5) Measurements can be made to show the efflux of a variety of metabolites newly synthesized inside mitochondria due to externally added L-lactate. This could occur via antiporters, separate from the L-lactate, D-lactate, and pyruvate carriers. The *in vitro* reconstruction of the L-lactate/pyruvate shuttle and of gluconeogenesis (Valenti et al., 2002; de Bari et al., 2004) has been shown and suggests that the mitochondrial L-lactate metabolism is associated with gluconeogenesis occurring perhaps together with L-lactate oxidation to pyruvate in the cytosol. However, even efflux of certain metabolites via unidentified carrier/s show the occurrence of intramitochondrial L-lactate metabolism (Pizzuto et al., 2012).

Use could be made of arsenite, inhibitor of the pyruvate dehydrogenase, to rule out that oxygen consumption, proton efflux, $\Delta\Psi$ increase and metabolite traffic derives solely from the metabolism of pyruvate generated from L-lactate in external mitochondrial compartments and taken up in the matrix.

Last, but not least, (a) enzymatic assay (b) immunological analysis, and (c) confocal fluorescence microscopy can be used: (a) allows for initial information about enzyme kinetics features; (b) and (c) can reveal the existence of m-L-LDH even if inactive, but have the limitation that they are of no value in dissecting L-lactate metabolism.

A part of this strategy has unsuccessfully been applied in other laboratories with the conclusion that m-L-LDH either does not exist or is localized in the outer mitochondrial compartments. This has led to the mistaken conclusion that L-lactate is not a mitochondrial metabolite despite all the evidence to the contrary. In this case skeletal muscle mitochondria were investigated. Our opinion is that perhaps the investigators concerned were not able to isolate coupled mitochondria, a task that is far from easy in particular with skeletal muscle samples, and that they were not careful enough in selecting reaction media and in using inhibitors at the right concentrations. For instance the failure to measure oxygen consumption as a result of L-lactate addition to skeletal muscle mitochondria (Elustondo et al., 2013) could be due to the presence of 5 mM $MgSO_4$in the medium used to prepare isolated skeletal muscle mitochondria: sulfate is known to enter mitochondria causing efflux of intramitochondrial phosphate, malate, and succinate (Crompton et al., 1974, 1975). Moreover, 60 mM lactobionate included in the medium used to measure oxygen uptake by mitochondria is expected to prevent L-lactate uptake due to its chemical structure and high concentration. Finally, no control has been reported that c and m-L-LDH dehydrogenase are insensitive to 60 mM lactobionate. Of course, it is impossible to mimic cytosol totally with the medium used for *in vitro* experiments, however if findings observed in medium free of compounds expected to affect the mitochondria, "disappear" in another medium a detailed examination of the experimental conditions is needed.

Our opinion is that, having established that mitochondria are purified and coupled, a simple protocol, used in the experiment show here (**Figure 1**), if properly followed will definitively ascertain the existence of m-L-LDH as well as its localization.

Rabbit gastrocnemius muscle is rapidly isolated (5–10 min) after killing the animals and put immediately in ice-cold KCl medium (0.1 KCl, 50 mM Tris-HCl, 5 mM $MgCl_2$, 1 mM EDTA, 1 mM ATP, pH 7.5). Mitochondria (RGM) are isolated as in Lee et al. (1993) with the exclusion of protease treatment and immediately checked for their intactness by showing that no reduction of absorbance at 334 nm occurs as a result of NADH addition. m-L-LDH activity is assayed in RGM solubilized with 0.1% Triton X-100 (TX-100) as the decrease in absorbance of NADH after pyruvate addition (**Figure 1A**). No absorbance change occurs when pyruvate is added to intact mitochondria, this showing that m-L-LDH is located in the inner mitochondrial compartments. Surprisingly enough, such a simple assay was not reported in a paper in which the m-L-LDH existence was denied since "the distribution of L-LDH activity among the fractions paralleled that of pyruvate kinase" (Rasmussen et al., 2002); notice that the occurrence of a mitochondrial pyruvate kinase was later shown (Pizzuto et al., 2010). In another case the L-LDH activity was considered negligible (Yoshida et al., 2007). Thus, in our opinion it is easy to dismiss the possibility that the m-L-LDH is located in the outer mitochondrial membrane/intermembrane space: no NADH oxidation occurs when pyruvate is added to purified mitochondria, whereas treatment of mitochondria with TX-100 results in NADH oxidation via Complex 1 with rate increased by addition of pyruvate which reacts with NADH via m-L-LDH.

According to (2), the existence of a m-L-LDH localized in the inner mitochondrial compartments can be simply established by checking the ability of externally added L-lactate to reduce the intramitochondrial NAD^+ (**Figure 1B**); in this case addition of L-lactate to mitochondria (previously treated with the uncoupler FCCP, which favors the oxidation of the intramitochondrial NADH, and with the complex I inhibitor rotenone to prevent any oxidation of the newly formed NADH) results in an increase of the NAD(P)H fluorescence. The involvement of L-LDH in this process is confirmed by the inhibition due to oxamate, an inhibitor of LDH.

Consistently, immunological analysis shows that in mitochondria free of cytosolic contamination (no tubulin, a marker of the cytosolic fraction) contains a protein recognized by the L-LDH antibody (inset **Figure 1**).

We wonder why other investigators who deny the existence of mitochondrial L-LDH did not carry out these simple experiments. Perhaps their views are colored by the mistaken belief, based on incorrect thermodynamic arguments, that mitochondria cannot import L-lactate. Indeed both Sahlin et al. (2002) and Rasmussen et al. (2002) argue at the idea of L-lactate conversion to pyruvate inside mitochondria is not feasible on the basis of thermodynamic principles. They point to a much higher reduction of the NAD^+/NADH redox couple inside mitochondria; so much higher that in fact it would theoretically eliminate the possibility of L-lactate to pyruvate conversion. Sahlin et al. (2002) go on

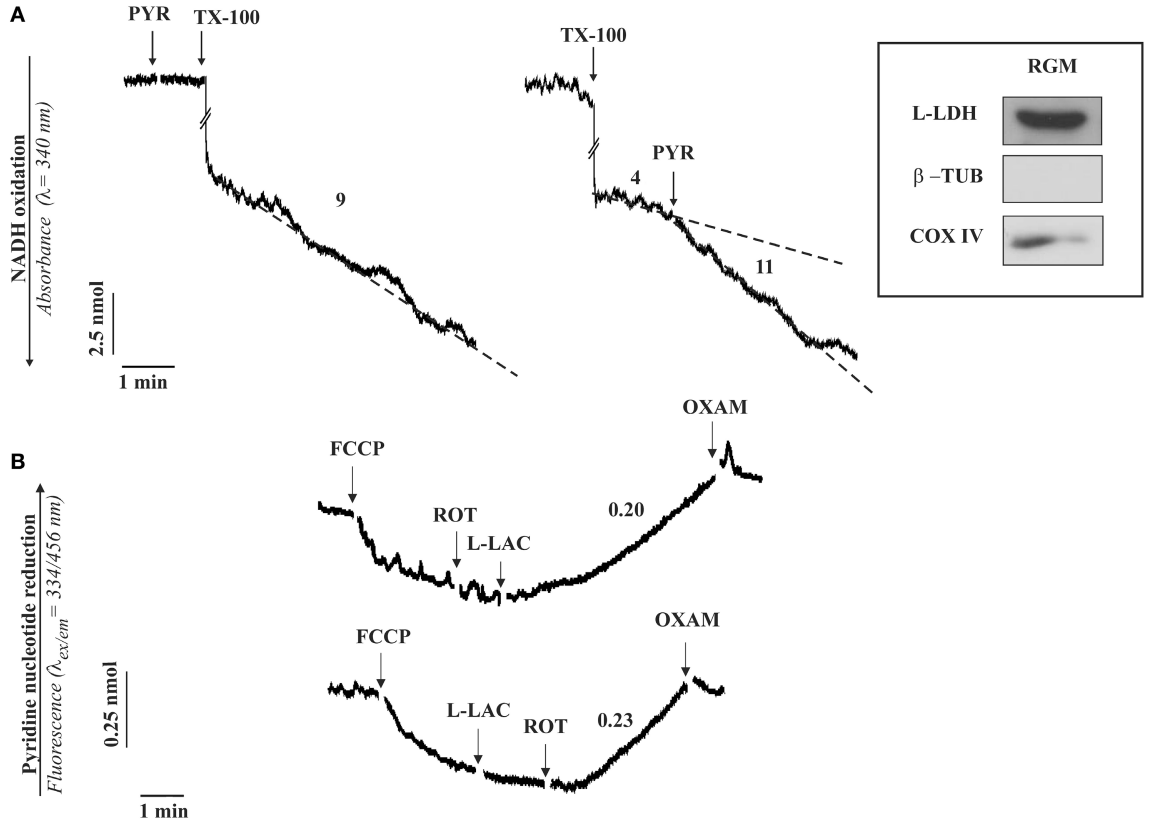


FIGURE 1 | The occurrence of a L-LDH (A) and L-lactate metabolism (B) in isolated rabbit gastrocnemius mitochondria (RGM). (A) Enzymatic assay of L-LDH in RGM. RGM (0.15 mg protein) were incubated at 25°C in 2 ml standard medium consisting of 0.2 M sucrose, 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM Na-EDTA, 20 mM HEPES-TRIS (pH 7.2) in the presence of NADH (0.2 mM) and the absorbance (λ = 340 nm) was continuously monitored. At the time indicated by the arrows the following additions were made: pyruvate (PYR, 1 mM), Triton X-100 (TX-100, 0.1%). Numbers along curves are rates expressed as nmol NAD(P)H oxidized/min x mg mitochondrial protein. **(B)** Fluorimetric investigation of the redox state of the mitochondrial pyridine nucleotides caused by L-lactate (L-LAC) addition to RGM. RGM (0.5 mg protein), were incubated at 25°C in 2 ml standard medium and reduction of pyridine nucleotides was followed fluorimetrically (λ_{ex} = 334 nm; λ_{em} = 456 nm) as a function of time. At the arrows the following additions were made: FCCP (1 μM), rotenone (2 μg), L-LAC (5 mM), oxamate (OXAM, 5 mM). Numbers along curves are rates expressed as nmol of $NAD(P)^+$ reduced/min × mg mitochondrial protein. Inset: immunodetection of L-LDH in RGM. Solubilized protein (35 μg) from mitochondrial fraction was analyzed by Western blotting as described in Pizzuto et al. (2012). Membrane blots were incubated with polyclonal anti-L-LDH, anti-COX-IV and anti-β-tubulin (β-TUB). COX-IV and β-TUB were used as mitochondrial and cytosolic markers, respectively. The experiment shown in this figure has been described in Passarella et al. (2008) and reported in de Bari et al. (2008).

to suggest that if m-L-LDH were present in the mitochondrial matrix, it would lead to a futile cycle in which pyruvate would be reduced to L-lactate in mitochondria and vice versa in the cytosol, oxidizing mitochondrial NADH and finally removing the driving force for the electron transport chain. However, given that in brain mitochondria the NAD^+ concentration is 8–20 fold higher than that of NADH and that pyruvate is actively oxidized via pyruvate dehydrogenase, we suggested (Atlante et al., 2007) that m-L-LDH *in vivo* catalyzes essentially L-lactate oxidation. Ultimately, the removal of the oxidation product by carrier-mediated transport and mitochondrial metabolism overcomes any thermodynamic difficulty. In this case, our results were consistent with the postulate/proposal of Schurr (2006) that L-lactate is the only major product of cerebral glycolysis and that it can be metabolized inside mitochondria. On the other hand glucose oxidation to L-lactate is expected to occur when oxidative phosphorylation is reduced since citrate and/or other citric cycle intermediates are required outside mitochondria for anabolism to occur (e.g., see Pizzuto et al., 2012). In this case anaerobic glycolysis is expected to provide ATP and L-lactate to play in mitochondria an anaplerotic role and/or to be transferred to other cells in the intercellular shuttle.

Notice that a putative L-lactate oxidase, located in the intermembrane space of rat liver mitochondria has been shown (de Bari et al., 2010b). This enzyme gives H_2O_2 and pyruvate and could be candidate to the new role proposed for L-lactate as "lactormone," i.e., in Brooks' term (Hashimoto and Brooks, 2008) as a cell-signaling molecule that is involved in the adaptive response to exercise.

To gain further insight into the physiological role of mitochondrial L-LDH in intact cells such as neurons and myocytes, experiments in which pyruvate but not L-lactate metabolism is prevented should be carried out, otherwise mitochondria should be used. Likely low

α-cyanocinnamate concentrations and arsenite could be used to prevent any pyruvate metabolism. Notice that in Hep G2 cells as in cancer cells, the mitochondrial pyruvate carrier does not work properly (Pizzuto et al., 2012). On the other hand, if pyruvate metabolism cannot be prevented one should be forced to use isolated mitochondria. Indeed, it was claimed that L-lactate uptake by mitochondria and intramitochondrial metabolism was not feasible, due to thermodynamics reasons (see above), but mitochondria did not know this ... and took up L-lactate! We are waiting for further progress!

REFERENCES

Atlante, A., de Bari, L., Bobba, A., Marra, E., and Passarella, S. (2007). Transport and metabolism of L-lactate occur in mitochondria from cerebellar granule cells and are modified in cells undergoing low potassium dependent apoptosis. *Biochim. Biophys. Acta* 1767, 1285–1299. doi: 10.1016/j.bbabio.2007.08.003

Baba, N., and Sharma, H. M. (1971). Histochemistry of lactic dehydrogenase in heart and pectoralis muscles of rat. *J. Cell Biol.* 51, 621–635. doi: 10.1083/jcb.51.3.621

Brooks, G. A. (2009). Cell-cell and intracellular lactate shuttles. *J. Physiol.* 587, 5591–5600. doi: 10.1113/jphysiol.2009.178350

Brooks, G. A., Dubouchaud, H., Brown, M., Sicurello, J. P., and Butz, C. E. (1999). Role of mitochondrial lactate dehydrogenase and lactate oxidation in the intracellular lactate shuttle. *Proc. Natl. Acad. Sci. U.S.A.* 96, 1129–1134. doi: 10.1073/pnas.96.3.1129

Crompton, M., Palmieri, F., Capano, M., and Quagliariello, E. (1974). The transport of sulphate and sulphite in rat liver mitochondria. *Biochem. J.* 142, 127–137.

Crompton, M., Palmieri, F., Capano, M., and Quagliariello, E. (1975). A kinetic study of sulphate transport in rat liver mitochondria. *Biochem. J.* 146, 667–673.

de Bari, L., Atlante, A., Valenti, D., and Passarella, S. (2004). Partial reconstruction of *in vitro* gluconeogenesis arising from mitochondrial L-lactate uptake/metabolism and oxaloacetate export via novel L-lactate translocators. *Biochem. J.* 380, 231–242. doi: 10.1042/BJ20031981

de Bari, L., Chieppa, G., Marra, E., and Passarella, S. (2010a). L-lactate metabolism can occur in normal and cancer prostate cells via the novel mitochondrial L-lactate dehydrogenase. *Int. J. Oncol.* 37, 1607–1620. doi: 10.3892/ijo-00000815

de Bari, L., Chieppa, G., Paventi, G., Pizzuto, R., Valenti, D., Atlante, A., et al. (2008). "The lactate-mitochondria affair," in *Proceedings of Bari International Symposium on "Mitochondrial Physiology and Pathology" IUBMB S1/2008* (Bari).

de Bari, L., Valenti, D., Atlante, A., and Passarella, S. (2010b). Lactate generates hydrogen peroxide in purified rat liver mitochondria due to the putative L-lactate oxidase localized in the intermembrane space. *FEBS Lett.* 584, 2285–2290. doi: 10.1016/j.febslet.2010.03.038

Dianzani, M. U. (1951). La ripartizione sistema ossidante dell'acido lattico nelle cellule del fegato e del rene di ratti normali e di ratti con degenerazione grassa del fegato. *Arch. Fisiol.* 50, 181–186.

Dubouchaud, H., Butterfield, G. E., Wolfel, E. E., Bergman, B. C., and Brooks, G. A. (2000). Endurance training, expression, and physiology of LDH, MCT1, and MCT4 in human skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* 278, E571–E579.

Elustondo, P. A., White, A. E., Hughes, M. E., Brebner, K., Pavlov, E., and Kane, D. A. (2013). Physical and functional association of lactate dehydrogenase (LDH) with skeletal muscle mitochondria. *J. Biol. Chem.* 288, 25309–25317. doi: 10.1074/jbc.M113.476648

Gladden, L. B. (2007). Is there an intracellular lactate shuttle in skeletal muscle? *J. Physiol.* 582: 899. doi: 10.1113/jphysiol.2007.138487

Hashimoto, T., and Brooks, G. A. (2008). Mitochondrial lactate oxidation complex and an adaptive role for lactate production. *Med. Sci. Sport Exer.* 40, 486–494. doi: 10.1249/MSS.0b013e31815fcb04

Hashimoto, T., Hussien, R., Cho., H.-S., Kaufer D. and Brooks, G. A. (2008). Evidence for a mitochondrial lactate oxidation complex in rat neurons: a crucial component for a brain lactate shuttle. *PloS-One* 3:e2915. doi: 10.1371/journal.pone.0002915

Lee, C. P., Martens, M. E., and Tsang, S. H. (1993). "Small scale preparation of skeletal muscle mitochondria and its application in the study of human disease," in *Methods in Toxicology*, Vol. 2, eds D. Jones and L. Lash (New York, NY: Academic Press), 70–83.

Passarella, S., Atlante, A., Valenti, D., and de Bari, L. (2003). The role of mitochondrial transport in energy metabolism. *Mitochondrion* 2, 319–343. doi: 10.1016/S1567-7249(03)00008-4

Passarella, S., de Bari, L., Valenti, D., Pizzuto, R., Paventi, G., and Atlante, A. (2008). Mitochondria and L-lactate metabolism. *FEBS Lett.* 582, 3569–3576. doi: 10.1016/j.febslet.2008.09.042

Paventi, G., Pizzuto, R., Chieppa, G., and Passarella, S. (2007). L-Lactate metabolism in potato tuber mitochondria. *FEBS J.* 274, 1459–1469. doi: 10.1111/j.1742-4658.2007.05687.x

Pizzuto, R., Paventi, G., Atlante, A., and Passarella, S. (2010). Pyruvate kinase in pig liver mitochondria. *Archiv. Biochem. Biophys.* 495, 42–48. doi: 10.1016/j.abb.2009.12.016

Pizzuto, R., Paventi, G., Porcile, C., Sarnataro, D., Daniele, A., and Passarella, S. (2012). L-Lactate metabolism in HEP G2 cell mitochondria due to the L-lactate dehydrogenase determines the occurrence of the lactate/pyruvate shuttle and the appearance of oxaloacetate, malate and citrate outside mitochondria. *Biochim. Biophys. Acta* 1817, 1679–1690. doi: 10.1016/j.bbabio.2012.05.010

Ponsot, E., Zoll, J., N'Guessan, B., Ribera, F., Lampert, E., Richard, R., et al. (2005). Mitochondrial tissue specificity of substrates utilization in rat cardiac and skeletal muscles. *J. Cell. Physiol.* 203, 479–486. doi: 10.1002/jcp.20245

Rasmussen, H. N., van Hall, G., and Rasmussen, U. F. (2002). Lactate dehydrogenase is not a mitochondrial enzyme in human and mouse vastus lateralis muscle. *J. Physiol.* 541, 575–580. doi: 10.1113/jphysiol.2002.019216

Sahlin, K., Fernström, M., Svensson, M., and Tonkonogi, M. (2002). No evidence of an intracellular lactate shuttle in rat skeletal muscle. *J. Physiol.* 541, 569–574 doi: 10.1113/jphysiol.2002.016683

Schurr, A. (2006). Lactate: the ultimate cerebral oxidative energy substrate? *J. Cereb. Blood Flow Metab.* 26, 142–152 doi: 10.1038/sj.jcbfm.9600174

Schurr, A., and Payne, R. S. (2007). Lactate, not pyruvate, is neuronal aerobic glycolysis end product: an *in vitro* electrophysiological study. *Neuroscience* 147, 613–619 doi: 10.1016/j.neuroscience.2007.05.002

Valenti, D., de Bari, L., Atlante, A., and Passarella, S. (2002). L-lactate transport into rat heart mitochondria and reconstruction of the L-lactate/pyruvate shuttle. *Biochem. J.* 364, 101–104.

Yoshida, Y., Holloway, G. P., Ljubicic, V., Hatta, H., Spriet, L. L., Hood, D. A., et al. (2007). Negligible direct lactate oxidation in subsarcolemmal and intermyofibrillar mitochondria obtained from red and white rat skeletal muscle. *J. Physiol.* 582, 1317–1335. doi: 10.1113/jphysiol.2007.135095

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 27 September 2014; accepted: 22 November 2014; published online: 09 December 2014.
*Citation: Passarella S, Paventi G and Pizzuto R (2014) The mitochondrial L-lactate dehydrogenase affair. Front. Neurosci. **8**:407. doi: 10.3389/fnins.2014.00407*
This article was submitted to Neuroenergetics, Nutrition and Brain Health, a section of the journal Frontiers in Neuroscience.

frontiers in
NEUROSCIENCE

OPINION ARTICLE
published: 05 January 2015
doi: 10.3389/fnins.2014.00430

The role of membrane acid/base transporters and carbonic anhydrases for cellular pH and metabolic processes

Joachim W. Deitmer[1]*, Shefeeq M. Theparambil[1], Iván Ruminot[1] and Holger M. Becker[2]

[1] *General Zoology, FB Biology, University of Kaiserslautern, Kaiserslautern, Germany*
[2] *Zoology/Membrane Transport, FB Biology, University of Kaiserslautern, Kaiserslautern, Germany*
**Correspondence: deitmer@biologie.uni-kl.de*

Edited by:
Avital Schurr, University of Louisville, USA

Reviewed by:
Bernardo Victor Alvarez, Universidad Nacional De La Plata, Argentina
Mitchell Chesler, New York University School of Medicine, USA
Eva Maria Ruusuvuori, University of Helsinki, Finland

Keywords: pH, bicarbonate, lactate, transport metabolon, metabolism

ACIDOSIS AND PROTON HOMEOSTASIS IN CELLS AND TISSUES

Acidosis in the brain may severely impair a variety of functions, including synaptic transmission, metabolic energy supply, membrane transport and other processes (Ruusuvuori and Kaila, 2014).

Transport of acid–base equivalents across the cell membrane of neurons and glial cells also results in pH changes in the extracellular spaces. Cytosolic and extracellular buffer capacity and the activity of carbonic anhydrases contribute to shape pH changes, which can be elicited by neuronal activity, neurotransmitters and neuromodulators, metabolic processes, active cellular pH regulation, and secondary transporters carrying acid–base equivalents, and in turn these pH changes can affect neuronal functions (Deitmer and Rose, 1996; Chesler, 2003). The free H^+ concentration in cells is in the nanomolar range, and the high buffer capacity of cells provides a reservoir of acid equivalents in the millimolar range. In other words, there is a pool of protons in rapid exchange between buffer sites and free solution, with 10^5 or more protons being buffered for each proton in solution. At a blood pH of 7.4, and 7.2–7.3 in the extracellular spaces of brain tissue (Cragg et al., 1977; Ruusuvuori and Kaila, 2014), and with a negative membrane potential of between −50 and −90 mV in mammalian brain cells, H^+ has to be continuously extruded to maintain a physiological cytosolic pH of 7.0–7.3. Nevertheless, pH changes may peak well outside this range, at least for short time periods, and may be considered as H^+ signals, sometimes even with neurotransmitter function (Deitmer and Rose, 1996; Du et al., 2014). The net extrusion of acid from neurons and glial cells is accomplished by secondary active transport, wherein the efflux of H^+ or the influx of HCO_3^- is coupled to Na^+ influx, utilizing energy stored in the transmembrane Na^+ gradient. pH regulation in these cells involves a variety of membrane acid–base carriers, including sodium–hydrogen exchange, sodium–bicarbonate cotransport, and sodium-dependent and sodium-independent chloride–bicarbonate exchange. In addition, there are a number of acid/base-coupled carriers, which are linked to the transport of metabolites, such as lactate and amino acids. The lactate transport via monocarboxylate transporters (MCTs) has been suggested to play a major role for the supply of energy to neurons, and led to the "Astrocyte-to-Neuron Lactate Shuttle Hypothesis" (ANLSH; Pellerin and Magistretti, 1994).

LACTATE SHUTTLE AND ACID/BASE TRANSPORT METABOLON

Lactate, pyruvate, and ketone bodies are transported into and out of cells via MCTs (*SLC*16), of which 14 isoforms have been described. The first four of these 14 isoforms (MCT1-4) have been shown to transport monocarboxylates together with H^+ in a 1:1 stoichiometry. MCT1 is the ubiquitous isoform that is found in nearly all tissues, where it could either operate as a lactate importer or exporter, and has an intermediate K_m value of 3–5 mM for L-lactate (Bröer et al., 1998). MCT2, the high-affinity carrier, is mainly found in neurons, and MCT4, the low-affinity, high-capacity carrier, has been reported for glial cells in the brain.

The lactate shuttle hypothesis suggests that lactate is produced and exported by glial cells, in particular astrocytes, under normoxic conditions, and taken up by neurons for further metabolization (Pellerin and Magistretti, 1994). The ANLSH infers that astrocytes help to supply energetic substrates for neurons to meet their energy requirements, especially during enhanced neuronal activity. There is substantial evidence, both *in vitro* and *in vivo*, that lactate indeed can substitute for glucose to maintain neuronal functions, such as e.g., synaptic transmission and memory formation (Schurr et al., 1988; Suzuki et al., 2011). During energy deprivation, the addition of monocarboxylates has been shown to restore synaptic function and to be neuroprotective *in vivo*, in acute rodent brain slices, isolated optic nerve and neuronal cultures (Izumi et al., 1997; Schurr et al., 1997; Cater et al., 2001; Wyss et al., 2011). The finding that glucose is preferentially taken up by astrocytes and at higher rates than by neighboring neurons (Barros et al., 2009; Jakoby et al., 2014), implying that some energetic substrate has to be passed on to neurons, as they are the main energy consumers, also supports the ANLSH. More recently, lactate production and supply to neuronal axons have been suggested also for oligodendrocytes in the mammalian central nervous system

(Fünfschilling et al., 2012; Lee et al., 2012), indicating that astrocytes and oligodendrocytes form a metabolic network with neurons to maintain neuronal function.

A transport metabolon has been defined as a supramolecular complex of sequential metabolic enzymes and cellular structural elements in which metabolites are passed from one active site to another without complete equilibration with the bulk cellular fluids (Srere, 1985). First evidence for a transport metabolon, formed between carbonic anhydrase (CA) and an acid/base transporter was found for CAII and the Cl^-/HCO_3^- exchanger AE1 (Kifor et al., 1993; Vince and Reithmeier, 1998). Since then, various acid/base transporters have been reported to interact with different isoforms of carbonic anhydrase: For the electrogenic Na^+/HCO_3^- cotransporter, NBCe1, both functional (Becker and Deitmer, 2007; Schüler et al., 2011) and physical (Gross et al., 2002; Alvarez et al., 2003; Pushkin et al., 2004) interaction with different CA isoforms has been suggested. All of these interactions have in common that CA-mediated augmentation of transport activity requires the catalytic activity of the different CA isoforms.

An entirely different form of transport metabolon has first been detected, when expressing MCT1 and CAII in *Xenopus* oocytes (Becker et al., 2005). The presence of CAII indeed more than doubled the rate of lactate transport, and the CAII-induced augmentation of MCT activity persisted in the absence of CO_2/HCO_3^-, and was insensitive to inhibition of CAII catalytic activity with EZA, and was still present with the catalytically inactive mutant CAII-V143Y (Becker et al., 2005, 2011; Becker and Deitmer, 2008), suggesting that the augmentation of MCT activity does not depend on the reversible conversion of CO_2 and HCO_3^-/H^+ by CAII. No interaction between CAII and rat MCT2 could be detected, when the enzyme was injected into oocytes co-expressing MCT2 together with its trafficking protein embigin (Klier et al., 2011). Cytosolic CAII was shown to bind to the C-terminal tail of MCT1, which presumably positions the enzyme close enough to the pore of the transporter for efficient H^+ shuttling (Stridh et al., 2012). The binding of CAII to a glutamic acid cluster within the MCT C-terminal may also explain the isoform specificity of the interaction between MCTs and CAII, since rat MCT4, but not MCT2, possesses a similar cluster of three glutamate residues.

Augmentation of MCT activity by extracellular CAs has also been found in the brain: By inhibition of extracellular CA activity with benzolamide and an antiserum against CAIV, respectively, Svichar and Chesler (2003) could show a significant reduction in lactate-induced intracellular acidification in rat hippocampal pyramidal neurons and in cultured astrocytes.

CA ACTIVITY MEDIATES BETWEEN DIFFERENT FORMS OF METABOLIC ACIDOSIS

Carbonic anhydrases play a vital role in acid/base kinetics and mediate between acid production by oxidative phosphorylation in form of CO_2 and acid production by anaerobic glycolysis. When CO_2 increases in the cell, e.g., due to oxidative phosphorylation in mitochondria, it can leave the cell by freely diffusing through the cell membrane, or it can be converted to H^+ and HCO_3^-, with the rate of conversion depending on catalytic activity of cytosolic CA. Most cells express CAII, which is the fastest isoform, and either CAIV and/or CAXIV, which are fast extracellular isoforms in the brain. CAIV has recently been shown to display intracellular activity in addition, which would further contribute to high intracellular CA activity (Schneider et al., 2013). With this enzymatic equipment, neurons and glial cells can produce considerable amounts of H^+, which can be extruded by either NHE or MCT. Extracellular CA activity can convert part of extracellular CO_2 to H^+ and HCO_3^-, the latter being substrate for NBC to be transported into and out of the cell. Thus, additional HCO_3^- can be delivered to, or removed from, the cytosol, in particular in astrocytes, which can have a robust expression of NBC, which mediates a high bicarbonate sensitivity of the cells, to further compensate metabolically produced H^+ (Theparambil et al., 2014).

Furthermore, both extra- and intracellular CA isoforms, as e.g., CAIV, can form transport metabolons with the bicarbonate- and proton-coupled carriers (see above). In mouse retina, CAXIV co-localized with anion exchanger isoform 3 (AE3) in Müller and horizontal cells, and physical and functional interaction between the CAXIV and AE3 was shown (Casey et al., 2009). Disruption of transport metabolon function, as suggested to occur after CAIV mutation, can interfere with photoreceptor maintenance and pH regulation in the retina (Yang et al., 2005; Alvarez et al., 2007). Whether other extracellular CA isoforms, which have been detected in brain tissue, also form functional metabolons with MCT and/or NBC, is still unknown. Interestingly, cytosolic CAI and CAIII, which are expressed by some cells, can enhance NBC activity in *Xenopus* oocytes (Schüler et al., 2011), but not MCT transport activity (Becker and Deitmer, 2008). In addition, by stabilizing the H^+ gradient, NBC can support lactate transport via MCT, when expressed together in oocytes (Becker et al., 2004).

From these and other results, it can be concluded that brain cells, and quite possibly other cell types in other tissues, use a whole network of acid/base-coupled membrane carriers and different CA isoforms to regulate intracellular pH, which links acid/base status, H^+ buffering, energy metabolism, and H^+/HCO_3^--coupled membrane transport. Thus, acid/base-coupled metabolite transport is coupled to pH regulation, and both are linked to CA activity and to non-catalytic functions of CA.

CONCLUSIONS AND PERSPECTIVES

Regulation of metabolism in organisms is not only complex, but also involves a large number of enzymes and membrane transporters, which may form networks to enhance their efficacy. Lactate, as a metabolic intermediate from glucose or glycogen breakdown, appears to play a major role as energetic substrate shuttled between cells and tissues, both under hypoxic and normoxic conditions. The membrane transport of lactate via monocarboxylate transporter occurs in cotransport with H^+, which is a substrate, a signal and a modulator of other metabolic processes. Lactate transporter form a "transport metabolon" with carbonic anhydrases, which not only provide a rapid equilibrium between CO_2, HCO_3^-, and H^+, but in addition enhance lactate transport by a non-enzymatic interaction, which requires physical binding as found

in frog oocytes as expression system for the proteins involved. Carbonic anhydrases mediate between different states of metabolic acidosis, induced by glycolysis and oxidative phosphorylation, and play a relay function in coupling pH regulation and metabolism.

ACKNOWLEDGMENT

The work this article is base on has been supported by grants from the Deutsche Forschungsgemeinschaft.

REFERENCES

Alvarez, B. V., Loiselle, F. B., Supuran, C. T., Schwartz, G. J., and Casey, J. R. (2003). Direct extracellular interaction between carbonic anhydrase IV and the human NBC1 sodium/bicarbonate cotransporter. *Biochemistry* 42, 12321–12329. doi: 10.1021/bi0353124

Alvarez, B. V., Vithana, E. N., Yang, Z., Koh, A. H., Yeung, K., Yong, V., et al. (2007). Identification and characterization of a novel mutation in the carbonic anhydrase IV gene that causes retinitis. *Invest. Ophthalmol. Vis. Sci.* 48, 3459–3468. doi: 10.1167/iovs.06-1515

Barros, L. F., Courjaret, R., Jakoby, P., Loaiza, A., Lohr, C., and Deitmer, J. W. (2009). Preferential transport and metabolism of glucose in Bergmann glia over Purkinje cells: a multiphoton study of cerebellar slices. *Glia* 57, 962–970. doi: 10.1002/glia.20820

Becker, H., Bröer, S., and Deitmer, J. W. (2004). Facilitated lactate transport by MCT1 when coexpressed with the sodium bicarbonate cotransporter (NBC) in *Xenopus* oocytes. *Biophys. J.* 86, 235–247. doi: 10.1016/S0006-3495(04)74099-0

Becker, H. M., and Deitmer, J. W. (2007). Carbonic anhydrase II increases the activity of the human electrogenic Na^+/HCO_3^- cotransporter. *J. Biol. Chem.* 282, 13508–13521. doi: 10.1074/jbc.M700066200

Becker, H. M., and Deitmer, J. W. (2008). Nonenzymatic proton handling by carbonic anhydrase II during H^+-lactate cotransport via monocarboxylate transporter 1. *J. Biol. Chem.* 283, 21655–21667. doi: 10.1074/jbc.M802134200

Becker, H. M., Hirnet, D., Fecher-Trost, C., Sültemeyer, D., and Deitmer, J. W. (2005). Transport activity of MCT1 expressed in *Xenopus* oocytes is increased by interaction with carbonic anhydrase. *J. Biol. Chem.* 280, 39882–39889. doi: 10.1074/jbc.M503081200

Becker, H. M., Klier, M., Schüler, C., McKenna, R., and Deitmer, J. W. (2011). Intramolecular proton shuttle supports not only catalytic but also noncatalytic function of carbonic anhydrase II. *Proc. Natl. Acad. Sci. U.S.A.* 108, 3071–3076. doi: 10.1073/pnas.1014293108

Bröer, S., Schneider, H., Bröer, A., and Deitmer, J. W. (1998). Characterization of the monocarboxylate transporter 1 expressed in *Xenopus laevis* oocytes by changes in cytosolic pH. *Biochem. J.* 333, 167–174.

Casey, J. R., Sly, W. S., Shah, G. N., and Alvarez, B. V. (2009). Bicarbonate homeostasis in excitable tissues: role of AE3 Cl^-/HCO_3^- exchanger and carbonic anhydrase XIV interaction. *Am. J. Physiol. Cell Physiol.* 297, 1091–1102. doi: 10.1152/ajpcell.00177.2009

Cater, H. L., Benham, C. D., and Sundstrom, L. E. (2001). Neuroprotective role of monocarboxylate transport during glucose deprivation in slice cultures of rat hippocampus. *J. Physiol.* 531, 459–466. doi: 10.1111/j.1469-7793.2001.0459i.x

Chesler, M. (2003). Regulation and modulation of pH in the brain. *Physiol. Rev.* 83, 1183–1221. doi: 10.1152/physrev.00010.2003

Cragg, P., Patterson, L., and Purves, M. J. (1977). The pH of brain extracellular fluid in the cat. *J. Physiol.* 272, 137–166.

Deitmer, J. W., and Rose, C. R. (1996). pH regulation and proton signalling by glial cells. *Prog. Neurobiol.* 48, 73–103. doi: 10.1016/0301-0082(95)00039-9

Du, J., Reznikov, L. R., Price, M. P., Zha, X. M., Lu, Y., Moninger, T. O., et al. (2014). Protons are a neurotransmitter that regulates synaptic plasticity in the lateral amygdala. *Proc. Natl. Acad. Sci. U.S.A.* 111, 8961–8966. doi: 10.1073/pnas.1407018111

Fünfschilling, U., Supplie, L. M., Mahad, D., Boretius, S., Saab, A. S., Edgar, J., et al. (2012). Glycolytic oligodendrocytes maintain myelin and long-term axonal integrity. *Nature* 485, 517–521. doi: 10.1038/nature11007

Gross, E., Pushkin, A., Abuladze, N., Fedotoff, O., and Kurtz, I. (2002). Regulation of the sodium bicarbonate cotransporter kNBC1 function: role of Asp986, Asp988 and kNBC1-carbonic anhydrase II binding. *J. Physiol.* 544, 679–685. doi: 10.1113/jphysiol.2002.029777

Izumi, Y., Benz, A. M., Katsuki, H., and Zorumski, C. F. (1997). Endogenous monocarboxylates sustain hippocampal synaptic function and morphological integrity during energy deprivation. *J. Neurosci.* 17, 9448–9457.

Jakoby, P., Schmidt, E., Ruminot, I., Gutiérrez, R., Barros, L. F., and Deitmer, J. W. (2014). Higher transport and metabolism of glucose in astrocytes compared with neurons: a multiphoton study of hippocampal and cerebellar tissue slices. *Cereb. Cortex* 24, 222–31. doi: 10.1093/cercor/bhs309

Kifor, G., Toon, M. R., Janoshazi, A., and Solomon, A. K. (1993). Interaction between red cell membrane band 3 and cytosolic carbonic anhydrase. *J. Membr. Biol.* 134, 169–79. doi: 10.1093/cercor/bhs309

Klier, M., Schüler, C., Halestrap, A. P., Sly, W. S., Deitmer, J. W., and Becker, H. M. (2011). Transport activity of the high-affinity monocarboxylate transporter MCT2 is enhanced by extracellular carbonic anhydrase IV but not by intracellular carbonic anhydrase II. *J. Biol. Chem.* 286, 27781–27791. doi: 10.1074/jbc.M111.255331

Lee, Y., Morrison, B. M., Li, Y., Lengacher, S., Farah, M. H., Hoffman, P. N., et al. (2012). Oligodendroglia metabolically support axons and contribute to neurodegeneration. *Nature* 487, 443–448. doi: 10.1038/nature11314

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. doi: 10.1073/pnas.91.22.10625

Pushkin, A., Abuladze, N., Gross, E., Newman, D., Tatishchev, S., Lee, I., et al. (2004). Molecular mechanism of kNBC1-carbonic anhydrase II interaction in proximal tubule cells. *J. Physiol.* 559, 55–65. doi: 10.1113/jphysiol.2004.065110

Ruusuvuori, E., and Kaila, K. (2014). Carbonic anhydrases and brain pH in the control of neuronal excitability. *Subcell. Biochem.* 75, 271–290. doi: 10.1007/978-94-007-7359-2_14

Schneider, H.-P., Alt, M. D., Klier, M., Spiess, A., Andes, F. T., Waheed, A., et al. (2013). GPI-anchored carbonic anhydrase IV displays both intra- and extracellular activity in cRNA-injected oocytes and in mouse neurons. *Proc. Natl. Acad. Sci. U.S.A.* 110, 1494–1499. doi: 10.1073/pnas.1221213110

Schüler, C., Becker, H. M., McKenna, R., and Deitmer, J. W. (2011). Transport activity of the sodium bicarbonate cotransporter NBCe1 is enhanced by different isoforms of carbonic anhydrase. *PLoS ONE* 6:e27167. doi: 10.1371/journal.pone.0027167

Schurr, A., Payne, R. S., Miller, J. J., and Rigor, B. M. (1997). Brain lactate is an obligatory aerobic energy substrate for functional recovery after hypoxia: further *in vitro* validation. *J. Neurochem.* 69, 423–426. doi: 10.1046/j.1471-4159.1997.69010423.x

Schurr, A., West, C. A., and Rigor, B. M. (1988). Lactate-supported synaptic function in the rat hippocampal slice preparation. *Science* 240, 1326–1328. doi: 10.1126/science.3375817

Srere, P. (1985). The metabolon. *Trends Biochem. Sci.* 13, 313–314.

Stridh, M. H., Alt, M. D., Wittmann, S., Heidtmann, H., Aggarwal, M., Riederer, B., et al. (2012). Lactate flux in astrocytes is enhanced by a non-catalytic action of carbonic anhydrase II. *J. Physiol.* 590, 2333–2351. doi: 10.1113/jphysiol.2011.220152

Suzuki, A., Stern, S. A., Bozdagi, O., Huntley, G. W., Walker, R. H., Magistretti, P. J., et al. (2011). Astrocyte-neuron lactate transport is required for long-term memory formation. *Cell* 144, 810–823. doi: 10.1016/j.cell.2011.02.018

Svichar, N., and Chesler, M. (2003). Surface carbonic anhydrase activity on astrocytes and neurons facilitates lactate transport. *Glia* 41, 415–419. doi: 10.1002/glia.10187

Theparambil, S. M., Ruminot, I., Schneider, H.-P., Shull, G. E., and Deitmer, J. W. (2014). The electrogenic sodium bicarbonate cotransporter NBCe1 is a high-affinity bicarbonate carrier in cortical astrocytes. *J. Neurosci.* 34, 1148–1157. doi: 10.1523/JNEUROSCI.2377-13.2014

Vince, J. W., and Reithmeier, R. A. F. (1998). Carbonic anhydrase II binds to the carboxyl terminus of human band 3, the erythrocyte $C1^-/HCO_3^-$ exchanger. *J. Biol. Chem.* 273, 28430–28437. doi: 10.1074/jbc.273.43.28430

Wyss, M. T., Jolivet, R., Buck, A., Magistretti, P. J., and Weber, B. (2011). *In vivo* evidence for lactate as a neuronal energy source. *J. Neurosci.* 31, 7477–7485. doi: 10.1523/JNEUROSCI.0415-11.2011

Yang, Z., Alvarez, B. V., Chakarova, C., Jiang, L., Karan, G., Frederick, J. M., et al. (2005). Mutant

carbonic anhydrase 4 impairs pH regulation and causes retinal photoreceptor degeneration. *Hum. Mol. Genet.* 14, 255–265. doi: 10.1093/hmg/ddi023

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 28 October 2014; accepted: 09 December 2014; published online: 05 January 2015.
*Citation: Deitmer JW, Theparambil SM, Ruminot I and Becker HM (2015) The role of membrane acid/base transporters and carbonic anhydrases for cellular pH and metabolic processes. Front. Neurosci. **8**:430. doi: 10.3389/fnins.2014.00430*
This article was submitted to Neuroenergetics, Nutrition and Brain Health, a section of the journal Frontiers in Neuroscience.

frontiers in
NUTRITION

OPINION ARTICLE
published: 05 January 2015
doi: 10.3389/fnut.2014.00027

Lactate and cancer: revisiting the Warburg effect in an era of lactate shuttling

Matthew L. Goodwin[1,2]*, L. Bruce Gladden[3], Maarten W. N. Nijsten[4] and Kevin B. Jones[1,2]

[1] *Department of Orthopaedics, University of Utah, Salt Lake City, UT, USA*
[2] *Huntsman Cancer Institute, Salt Lake City, UT, USA*
[3] *School of Kinesiology, Auburn University, Auburn, AL, USA*
[4] *Department of Critical Care, University Medical Center Groningen, Groningen, Netherlands*
**Correspondence: matthewlgoodwin@gmail.com*

Edited by:
Avital Schurr, University of Louisville, USA

Reviewed by:
Avital Schurr, University of Louisville, USA
Joachim Deitmer, University of Kaisersautern, Germany

Keywords: glucose, LDH, metabolism, lactate shuttle, oxygen, VEGF, HIF-1α

INTRODUCTION

Despite causing over half a million deaths per year, cancer continues to elude our understanding and evade our therapeutic approaches. Our comprehension of cancer metabolism lags woefully behind other areas of cancer research. Starting with the seminal experiments done in the 1920s by the Cori's and Warburg (1, 2), tumors are often described as being glucose avid tissues that produce lactate despite adequate oxygen (O_2) tension (i.e., the "Warburg Effect"). Only recently, we have begun to understand that tumor cell metabolism is significantly more complicated. Specifically, insight from the study of lactate metabolism has shed light on the peculiar metabolic nature of tumor cells. Here, we present a brief overview of some of the recent developments in the ever expanding literature on lactate metabolism and cancer.

LACTATE METABOLISM

First discovered in 1780 by Carl Wilhelm Scheele, lactate was found to be elevated in the muscles of hunted stags in 1808 (3). Later, experiments by Pasteur (4), Meyerhof (5), and A.V. Hill (6) led to widespread understanding of the glycolytic pathway and the notion that lack of O_2 led to fermentation and lactate accumulation. Out of this early work grew the idea that lactate was a waste that must be cleared from the muscles and blood, preferably by being converted to glucose in the liver via the Cori cycle. However, over the last 50 years, it has been demonstrated in numerous experiments that lactate is both a potent fuel and signaling molecule, and it is constantly being produced and circulated throughout the body, often when there is adequate O_2 (7). Despite this evidence, lactate as a "hypoxic waste product" is still erroneously taught in many medical schools to this day.

While lack of adequate O_2 forbids the continuation of oxidative phosphorylation, the notion that lactate production was solely the result of inadequate O_2 began to change in the 1960s. In a series of elegant experiments in dog muscle, Wendell Stainsby's group provided evidence that the lactate-releasing canine muscle was not dysoxic (8). Despite criticism over some of his techniques the concept proved correct: lactate formation due to lack of O_2 is often the exception rather than the rule, even in critically ill patients. The lactate dehydrogenase (LDH) reaction is a rapid, near-equilibrium reaction that lies heavily in the direction of lactate; any time glycolysis is active, lactate is formed and equilibrates with local lactate gradients. Lactate equilibrates mainly by diffusing across membranes via monocarboxylate transporters (MCTs). In lactate-producing tissues or situations, this often means exporting lactate into circulation, where both local and distant tissues can take it up and use it as a fuel.

This observation that lactate is constantly being produced and consumed formed the basis of the cell-to-cell lactate shuttle, a hypothesis originally introduced by George Brooks in 1984 (9). His widely accepted hypothesis posits that lactate is the key intermediate metabolite in whole body metabolism. It is well described in the literature that lactate can readily replace glucose as a fuel for almost all cells of the body (any cell with mitochondria), including heart, liver, muscle, and even brain (10). So well-supported in so many different experimental settings is his hypothesis that it can now firmly be called "Lactate Shuttle Theory" (11). Finally, more recent work has shown that lactate is also a potent signaling molecule, triggering the stabilization of hypoxia inducible factor-1α (HIF-1α), and subsequently increasing expression of vascular endothelial growth factor (VEGF), resulting in angiogenesis (**Figure 1**). This new understanding is only now beginning to be explored in tumor models (see Lactate and Cancer: Convergence and Recent Studies below).

TUMOR METABOLISM

Unlike lactate metabolism, the study of tumors dates back to as long ago as 1600 BC with descriptions of breast masses (12). However, the modern era of tumor metabolism began in the 1920s with experiments by the Cori's and Otto Warburg (1, 2). Briefly, the Cori's showed that the axillary vein draining a hen wing with a sarcoma had a higher lactate and lower glucose when compared to the non-tumor limb. Taking a similar approach, Warburg et al. (2) measured arteriovenous (a–v) differences across tumor beds in rat tumor models. He showed that the vein always had more lactate and less glucose than the artery feeding the tumor, suggesting a net lactate output in presumably normoxic tumor beds. These early investigators clearly recognized the "Warburg

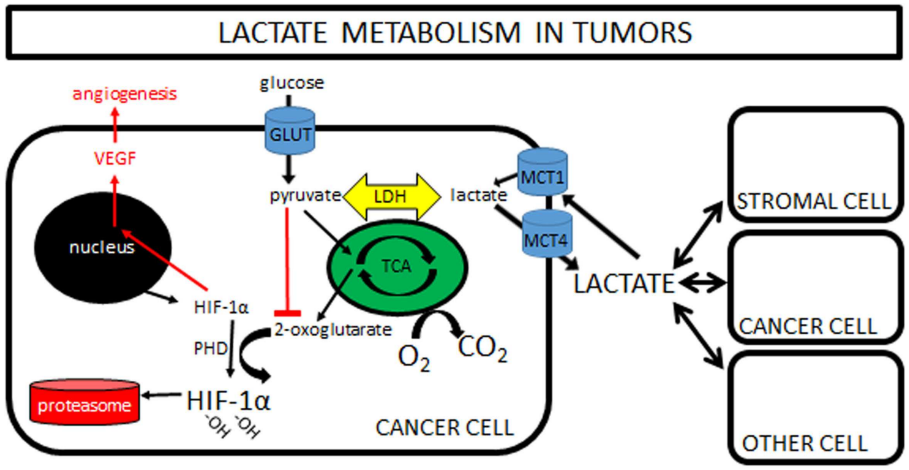


FIGURE 1 | Lactate metabolism in tumors: a simplified cartoon showing lactate being shuttled to and from cancer cells and its potential role as a signaling molecule in driving angiogenesis. Increasing pyruvate inhibits formation of 2-oxoglutarate, with the net effect of less degradation of HIF-1α in the proteasome and increased VEGF and angiogenesis. Note that pyruvate can be increased by hypoxia "backing up" the TCA cycle, or by importation of lactate via MCT1s. Some lactate shuttling likely is present between cancer cells and (1) other cancer cells within the tumor, (2) tumor stromal cells, and/or (3) non-tumor cells both local and distant from the tumor. Note that the traditional "Warburg Effect" describes tumors relying heavily on glucose uptake (via GLUTs) with subsequent lactate exportation (via MCT4s) in normoxia. A "Reverse Warburg Effect" describes lactate production from stromal cells, which is then taken up and used by local cancer cells. Some unique combination of these pathways is likely present within each tumor, highlighting the need for further *in vivo* experimentation. (Note that MCTs can transport lactate either direction; MCT1s are typically expressed in cells importing lactate, while MCT4s are expressed in cells exporting lactate.)

Effect," the "unusual" behavior whereby tumors produce lactate in a normoxic environment.

Do all tumors exhibit the Warburg Effect? In the current era of genomics and the understanding that cancer represents hundreds or thousands of different genotypes, it seems unlikely that all tumor cells would behave in an identical manner. Even in cells of the same clonal expansion, the metabolism of any one cell will vary depending on its local microenvironment. For example, cancer cells at a hypoxic core might use glucose and produce lactate, while cells on the periphery, close to a robust vascular and O_2 supply, might take up this lactate and oxidize it as a fuel. This concept of shuttling lactate between cancer cells was first introduced by Sonveaux et al. (13). A similar case has been made for a "Reverse Warburg" effect, whereby stromal cells are proposed to produce lactate that tumor cells then take up and oxidize (14).

LACTATE AND CANCER: CONVERGENCE AND RECENT STUDIES

In an insightful commentary on the work done by Sonveaux et al. (13), Semenza poignantly questioned, "Was there any precedent that should have alerted us to this symbiotic relationship between aerobic and hypoxic cancer cells? Of course: the well known recycling of lactate in exercising muscle" (15). Cancer cell metabolism has long been investigated in cultured cells *in vitro*, typically with non-physiological conditions (e.g., media with 25 mM glucose instead of 5 mM for cells that have been passaged many times) that hinder translation to therapeutic models. While great insight can be gained from experiments *in vitro*, it is critical that studies also examine tumors in the context of their local microenvironment. We propose that more studies of animal tumor models *in vivo* are needed to bridge the current gap from bench to bedside.

While one can debate which cells may or may not exhibit a Warburg Effect (16), it is clear that many tumors are glucose avid [the basis of positron emission tomography (PET) scans] and subsequently produce lactate. In a provocative piece in 2009, Nijsten and van Dam (17) presented a hypothetical treatment whereby glucose might be systemically lowered and lactate provided exogenously. If tumors are glucose consumers/lactate producers *and* almost all other tissues in the body can actively take up and use lactate as a fuel, why not systemically induce hypoglycemia to starve tumor cells while providing lactate as a salvage fuel for other tissues? While the idea of inducing hypoglycemia *in vivo* in this manner seems daunting, proof of concept for this idea came from a recent case report out of their hospital (18). This case reports on a patient who walked into the emergency department with extreme hypoglycemia [(glucose) = 13 mg/dL). Not only was he neither comatose nor dead but also was he alert and oriented. Upon first sampling, his blood lactate was 25 mM, likely serving as a salvage fuel for his vital organs, particularly his brain. Work to investigate this concept of a "lactate-protected hypoglycemia" should be pursued.

Finally, lactate also has a role as a potent signaling molecule. This is of particular interest in tumor metabolism, as high-lactate levels are often associated with a worse prognosis [e.g., Ref. (19)]. One proposed mechanism for this poor prognosis is increased angiogenesis. Any change in lactate immediately equilibrates with pyruvate through LDH and vice versa. Accumulating pyruvate inhibits the formation of 2-oxoglutarate, the molecule responsible for targeting HIF-1α for degradation in

the proteasome. When lactate (pyruvate) levels increase, HIF-1α drives angiogenesis via VEGF expression. It should be emphasized that HIF-1α stabilization can be driven by lactate or hypoxia independently (**Figure 1**).

This lactate-to-VEGF pathway has been shown to be independent of O_2 tension and appears to be an appealing target for potential anti-tumor therapies. De Saedeleer et al. (20) showed that oxidative tumor cells *in vitro* activate HIF-1α via importation of lactate. In another study in glycolytic glioma tumor cells *in vitro*, lactate exposure increased HIF-1α levels independent of hypoxia (21); in a similar study *in vivo* (mice), intraperitoneal lactate administration enhanced xenografted tumor growth, metastasis, and vascularity (22). Finally, another group inhibited MCTs in Lewis Lung carcinoma mice, driving a twofold reduction in vascularity in <2 weeks (13).

In an effort to understand the role of lactate in the tumor microenvironment, the KB Jones Lab at Utah introduced a new transgenic mouse model [alveolar soft parts sarcoma (ASPS)]. In this model, the ASPS oncogene was bred into mice that subsequently developed tumors that were indistinguishable histologically from human ASPS tumors. These vascular tumors had high levels of HIF-1α despite being normoxic throughout. Remarkably, these tumors demonstrated a dramatic increase in vascularity when the mice were given daily intraperitoneal injections of lactate for 2 weeks. Perhaps most interesting was the finding that these tumors formed only within the cranial vault in mice, which was also the area of highest lactate concentration in the mouse (23).

CONCLUSION AND FUTURE DIRECTIONS

Current understandings of lactate and tumor metabolism are now converging, seemingly providing as many questions as answers. Lactate is both a potent fuel (oxidative) and signaling molecule (angiogenesis) in most tissues throughout the body. Early work in tumor models suggests that lactate may be either generated and exported or imported and used as a fuel and potent signaling molecule. Lactate-protected hypoglycemia may be a viable strategy in tumors that exhibit a "Warburg Effect," while MCT inhibitors may be useful in tumors whose angiogenesis is driven by lactate (e.g., ASPS). Most tumors likely lie somewhere between these two extremes, and either or both may soon serve as important adjuvant therapies. It is critical that more studies investigate the metabolic behavior of specific tumors with models *in vivo*. Only when we understand the metabolic behavior of tumors *in vivo* can we then begin to understand how to effectively target them therapeutically.

REFERENCES

1. Cori CF, Cori GT. The carbohydrate metabolism of tumors II. Changes in the sugar, lactic acid, and CO2-combioning power of blood passing through a tumor. *J Biol Chem* (1925) **65**:397–405.
2. Warburg O, Wind F, Negelein E. The metabolism of tumors in the body. *J Gen Physiol* (1927) **8**:519–30. doi:10.1085/jgp.8.6.519
3. Gladden LB. 200th anniversary of lactate research in muscle. *Exerc Sport Sci Rev* (2008) **36**:109–15. doi:10.1097/JES.0b013e31817c0038
4. Pasteur L. Memoire sur la fermentation appeleelactique. *Ann Chim Phys* (1858) **52**:404–18.
5. Meyerhof O. Recent investigations on the aerobic and an-aerobic metabolism of carbohydrates. *J Gen Physiol* (1927) **8**(6):531–42. doi:10.1085/jgp.8.6.531
6. Bassett DR Jr. Scientific contributions of A. V. Hill: exercise physiology pioneer. *J Appl Physiol (1985)* (2002) **93**(5):1567–82.
7. Gladden LB. Lactate metabolism: a new paradigm for the third millennium. *J Physiol* (2004) **558**(Pt 1):5–30. doi:10.1113/jphysiol.2003.058701
8. Jöbsis F, Stainsby W. Oxidation of NADH during contractions of circulated mammalian skeletal muscle. *Respir Physiol* (1968) **4**:292–300. doi:10.1016/0034-5687(68)90035-2
9. Brooks GA. Lactate production under fully aerobic conditions: the lactate shuttle during rest and exercise. *Fed Proc* (1986) **45**(13):2924–9.
10. Schurr A, West CA, Rigor BM. Lactate-supported synaptic function in the rat hippocampal slice preparation. *Science* (1988) **240**(4857):1326–8. doi:10.1126/science.3375817
11. Gladden LB. Current trends in lactate metabolism: introduction. *Med Sci Sports Exerc* (2008) **40**(3):475–6. doi:10.1249/MSS.0b013e31816154c9
12. American Cancer Society. *The History of Cancer*. ACS (2002). Available from: http://www.cancer.org/cancer/cancerbasics/thehistoryofcancer/index?
13. Sonveaux P, Vegran F, Schroeder T, Wergin MC, Verrax J, Rabbani ZN, et al. Targeting lactate-fueled respiration selectively kills hypoxic tumor cells in mice. *J Clin Invest* (2008) **118**:3930–42. doi:10.1172/JCI36843
14. Pavlides S, Whitaker-Menezes D, Castello-Cros R, Flomenberg N, Witkiewicz AK, Frank PG, et al. The reverse Warburg effect: aerobic glycolysis in cancer associated fibroblasts and the tumor stroma. *Cell Cycle* (2009) **8**(23):3984–4001. doi:10.4161/cc.8.23.10238
15. Semenza GL. Tumor metabolism: cancer cells give and take lactate. *J Clin Invest* (2008) **118**:3835–7. doi:10.1172/JCI37373
16. Zu XL, Guppy M. Cancer metabolism: facts, fantasy, and fiction. *Biochem Biophys Res Commun* (2004) **313**(3):459–65. doi:10.1016/j.bbrc.2003.11.136
17. Nijsten MW, van Dam GM. Hypothesis: using the Warburg effect against cancer by reducing glucose and providing lactate. *Med Hypotheses* (2009) **73**(1):48–51. doi:10.1016/j.mehy.2009.01.041
18. Oldenbeuving G, McDonald JR, Goodwin ML, Sayilir R, Reijngoud DJ, Gladden LB, et al. A patient with acute liver failure and extreme hypoglycaemia with lactic acidosis who was not in a coma: causes and consequences of lactate-protected hypoglycaemia. *Anesth Intensive Care* (2014) **42**(4):507–11.
19. Brizel DM, Schroeder T, Scher RL, Walenta S, Clough RW, Dewhirst MW, et al. Elevated tumor lactate concentrations predict for an increased risk of metastases in head-and-neck cancer. *Int J Radiat Oncol Biol Phys* (2001) **51**:349–53. doi:10.1016/S0360-3016(01)01630-3
20. De Saedeleer CJ, Copetti T, Porporato PE, Verrax J, Feron O, Sonveaux P. Lactate activates HIF-1 in oxidative but not in Warburg-phenotype human tumor cells. *PLoS One* (2012) **7**:e46571. doi:10.1371/journal.pone.0046571
21. Lu H, Forbes RA, Verma A. Hypoxia-inducible factor 1 activation by aerobic glycolysis implicates the Warburg effect in carcinogenesis. *J Biol Chem* (2002) **277**:23111–5. doi:10.1074/jbc.M202487200
22. Bonuccelli G, Tsirigos A, Whitaker-Menezes D, Pavlides S, Pestell RG, Chiavarina B, et al. Ketones and lactate "fuel" tumor growth and metastasis: evidence that epithelial cancer cells use oxidative mitochondrial metabolism. *Cell Cycle* (2010) **9**:3506–14. doi:10.4161/cc.9.17.12731
23. Goodwin ML, Jin H, Straessler K, Smith-Fry K, Zhu JF, Monument MJ, et al. Modeling alveolar soft part sarcomagenesis in the mouse: a role for lactate in the tumor microenvironment. *Cancer Cell* (2014) **26**(6):851–62. doi:10.1016/j.ccell.2014.10.003

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 14 November 2014; accepted: 06 December 2014; published online: 05 January 2015.
*Citation: Goodwin ML, Gladden LB, Nijsten MWN and Jones KB (2015) Lactate and cancer: revisiting the Warburg effect in an era of lactate shuttling. Front. Nutr. **1**:27. doi: 10.3389/fnut.2014.00027*
This article was submitted to Neuroenergetics, Nutrition and Brain Health, a section of the journal Frontiers in Nutrition.

frontiers in NEUROSCIENCE

REVIEW ARTICLE
published: 09 February 2015
doi: 10.3389/fnins.2015.00023

Methylglyoxal, the dark side of glycolysis

Igor Allaman[1]*, Mireille Bélanger[1] and Pierre J. Magistretti[2,1]

[1] *Laboratory of Neuroenergetics and Cellular Dynamics, Brain Mind Institute, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland*
[2] *Division of Biological and Environmental Sciences and Engineering, King Abdullah University of Science and Technology, Thuwal, Saudi Arabia*

Edited by:
Avital Schurr, University of Louisville, USA

Reviewed by:
Neil Sims, Flinders University, Australia
Juan P. Bolanos, University of Salamanca-Consejo Superior de Investigaciones Científicas, Spain

****Correspondence:***
Igor Allaman, EPFL, SV/ Brain Mind Institute/LNDC, AAB 117, Station 19, CH-1015 Lausanne, Switzerland
e-mail: igor.allaman@epfl.ch

Glucose is the main energy substrate for the brain. There is now extensive evidence indicating that the metabolic profile of neural cells with regard to glucose utilization and glycolysis rate is not homogenous, with a marked propensity for glycolytic glucose processing in astrocytes compared to neurons. Methylglyoxal, a highly reactive dicarbonyl compound, is inevitably formed as a by-product of glycolysis. Methylglyoxal is a major cell-permeant precursor of advanced glycation end-products (AGEs), which are associated with several pathologies including diabetes, aging and neurodegenerative diseases. In normal situations, cells are protected against methylglyoxal toxicity by different mechanisms and in particular the glyoxalase system, which represents the most important pathway for the detoxification of methylglyoxal. While the neurotoxic effects of methylglyoxal and AGEs are well characterized, our understanding the glyoxalase system in the brain is more scattered. Considering the high energy requirements (i.e., glucose) of the brain, one should expect that the cerebral glyoxalase system is adequately fitted to handle methylglyoxal toxicity. This review focuses on our actual knowledge on the cellular aspects of the glyoxalase system in brain cells, in particular with regard to its activity in astrocytes and neurons. A main emerging concept is that these two neural cell types have different and energetically adapted glyoxalase defense mechanisms which may serve as protective mechanism against methylglyoxal-induced cellular damage.

Keywords: methylglyoxal, neuron, astrocyte, triosephosphate, advanced-glycation end-products (AGEs), glutathione

INTRODUCTION

The brain has high energy requirements and glucose is the main energy substrate for the brain. About 20% of the oxygen consumed by the human body are dedicated to cerebral functions (Mink et al., 1981), yet the brain represents around only 2% of the total body mass (Molina and DiMaio, 2012). Maintenance and restoration of ion gradients dissipated by signaling processes such as postsynaptic and action potentials, as well as uptake and recycling of neurotransmitters, are the main processes contributing to the high energy needs of the awake brain (Jolivet et al., 2009; Harris et al., 2012; Hyder et al., 2013). For instance, it has been estimated that glutamate-mediated neurotransmission is responsible for most of the energy expended in the gray matter (Sibson et al., 1998; Shulman et al., 2004; Hyder et al., 2006), highlighting the close relationship between brain activity, glutamatergic neurotransmission, energy requirements and glucose utilization.

While neurons have high energy requirements, astrocytes are generally considered to account for around 20% of the awake brain's energy expenditure (Harris et al., 2012; Hyder et al., 2013). Intriguingly, experimental evidence demonstrates that the amount of glucose that astrocytes actually take up is disproportionally high in comparison to their energy requirements. For example, in acute cerebellar slices, the uptake of fluorescent glucose analogs is several-fold higher in Bergmann glia than in Purkinje cells (Barros et al., 2009; Jakoby et al., 2014). Nevertheless, one should mention that the stereospecificity of such fluorescent glucose analogs has been questioned (Yamamoto et al., 2011). In the resting rat brain, other studies have shown that astrocytes are responsible for approximately half of glucose uptake (Nehlig et al., 2004; Chuquet et al., 2010), and that this proportion increases even further upon functional activation (Chuquet et al., 2010). Such observations may be seen as paradoxical since neurons, not astrocytes, have the highest energy needs. A simple explanation for such an apparent paradox is the transfer of glycolysis-derived energy substrates from astrocytes to neurons. For instance, according to the astrocyte-neuron lactate shuttle (ANLS), glutamate uptake into astrocytes during synaptic activation (i.e., glutamatergic neurotransmission) stimulates glucose uptake and aerobic glycolysis in astrocytes. Lactate produced through astrocytic aerobic glycolysis is then shuttled to neurons where it is used to produce energy in the mitochondria, i.e., lactate is converted to pyruvate which is then processed in the tricarboxylic acid (TCA) cycle, hence bypassing neuronal glycolysis (Magistretti, 2009; Bélanger et al., 2011a; Pellerin and Magistretti, 2012) (**Figure 1**, see Figure legend for more details).

In line with this, here is now strong evidence demonstrating that cerebral glucose consumption is not homogenous for all neural cell types and that metabolic specialization takes place in terms of energy metabolism between neuron and glia (see e.g., Bélanger et al., 2011a). Consistent with their higher energy requirements, neurons sustain a high rate of oxidative metabolism compared to glial cells (Lebon et al., 2002; Itoh et al., 2003; Bouzier-Sore

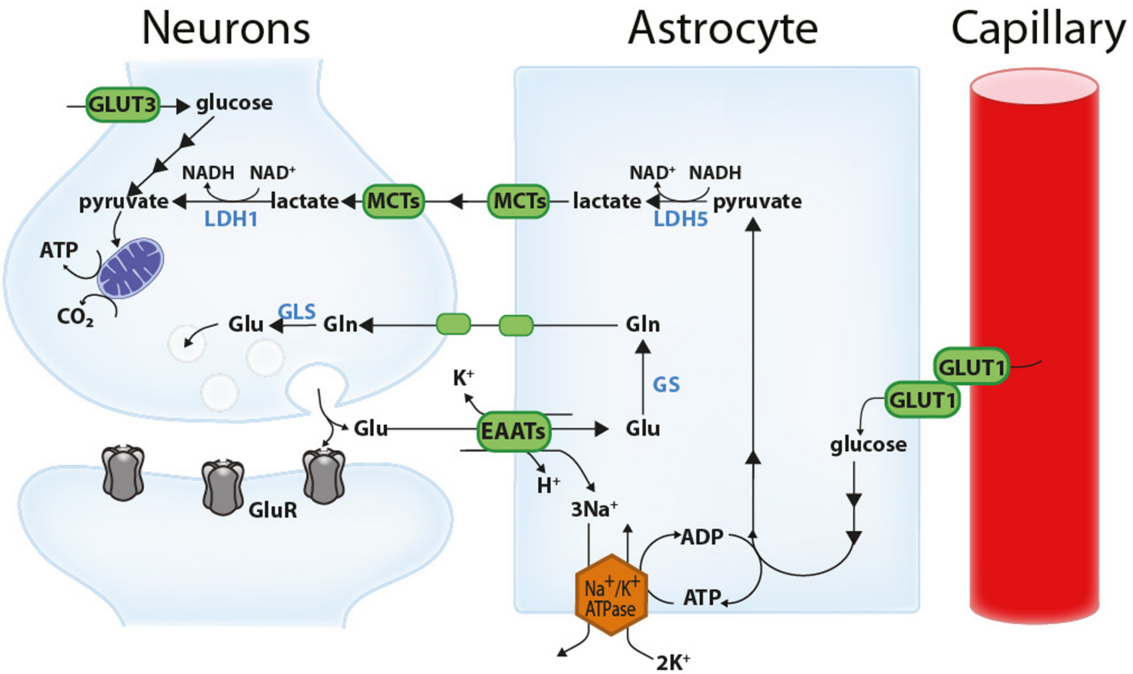


FIGURE 1 | Schematic representation of the astrocyte-neuron lactate shuttle (ANLS). Glutamate (Glu) released at the synapse activates glutamatergic receptors (GluR) and is associated with important energy expenditures in neuronal compartments. A large proportion of the glutamate released at the synapse is taken up by astrocytes via excitatory amino acid transporters (EAATs, more specifically GLT-1 and GLAST) together with 3 Na^+ ions. This Na^+ is extruded by the action of the Na^+/K^+ ATPase, consuming ATP. This triggers non oxidative glucose utilization in astrocytes and glucose uptake from the circulation through the glucose transporter GLUT1 expressed by both capillary endothelial cells and astrocytes. Glycolytically-derived pyruvate is converted to lactate by lactate dehydrogenase 5 (LDH5; mainly expressed in astrocytes) and shuttled to neurons through monocarboxylate transporters (mainly MCT1 and MCT4 in astrocytes and MCT2 in neurons). In neurons, this lactate can be used as an energy substrate following its conversion to pyruvate by LDH1 (mainly expressed in neurons). Neurons can also take up glucose via the neuronal glucose transporter 3 (GLUT3). Concomitantly, astrocytes participate in the recycling of synaptic glutamate via the glutamate-glutamine cycle. Following its uptake by astrocytes, glutamate is converted to glutamine (Gln) by the action of glutamine synthetase (GS) and shuttled to neurons where it is converted back to glutamate by glutaminases (GLS). Reproduced with permission from Bélanger et al. (2011a).

et al., 2006; Boumezbeur et al., 2010a). Interestingly, a large body of evidence shows that neurons can efficiently use lactate as an energy substrate (Schurr et al., 1997; Bouzier et al., 2000; Qu et al., 2000; Serres et al., 2005; Boumezbeur et al., 2010b) and even show a preference for lactate over glucose when both substrates are present (Itoh et al., 2003; Bouzier-Sore et al., 2006). By contrast, although astrocytes display lower rates of oxidative metabolism compared to neurons, they avidly take up glucose and characteristically present a high glycolytic rate (Itoh et al., 2003; Herrero-Mendez et al., 2009; Bittner et al., 2010). A large portion of the glucose entering the glycolytic pathway in astrocytes is released as lactate in the extracellular space (Pellerin and Magistretti, 1994; Itoh et al., 2003; Serres et al., 2005; Bouzier-Sore et al., 2006; Lovatt et al., 2007). Another striking difference between astrocytes and neurons is the presence of glycogen reserves almost exclusively in the glial compartment, the mobilization of which also leads to lactate release in the extracellular space (see e.g., Dringen et al., 1993; Allaman, 2009; Bélanger et al., 2011a). Even though the ANLS hypothesis is compatible with some glucose utilization by neurons, it (the ANLS) has been challenged by some studies claiming that glucose is the main, if not the exclusive, energy substrate for oxidative metabolism in neurons (see e.g., Chih and Roberts, 2003; Hertz et al., 2007; Dienel, 2012; Patel et al., 2014).

In addition to this, astrocytes are known to display high metabolic plasticity in terms of glucose utilization, when compared to neurons (Pellerin and Magistretti, 1994; Almeida et al., 2001; Prapong et al., 2002; Porras et al., 2004; Gavillet et al., 2008; Allaman et al., 2010; Belanger et al., 2010). One striking example is the differential energetic response of astrocytes and neurons following the inhibition of mitochondrial respiration by nitric oxide (NO). In neurons, NO induces a massive intracellular ATP depletion and leads to apoptosis. By contrast, astrocytes respond to NO by increasing their glycolysis rate, which restrains the fall in ATP levels and prevents apoptosis (Almeida et al., 2001). A critical element accounting for the glycolytic response in astrocytes is the synthesis of fructose-2,6-bisphosphate—a potent activator of the glycolytic enzyme phosphofructokinase-1—by the enzyme Pfkfb (Almeida et al., 2004) (**Figure 2**). While Pfkfb is highly expressed in astrocytes, it is virtually absent in neurons due to its constant proteasomal degradation, therefore impeding glycolysis stimulation in neurons (Herrero-Mendez et al., 2009). Nevertheless, the ability of neurons to increase their glucose utilization rate in different conditions has also been described (see e.g., Dienel, 2012;

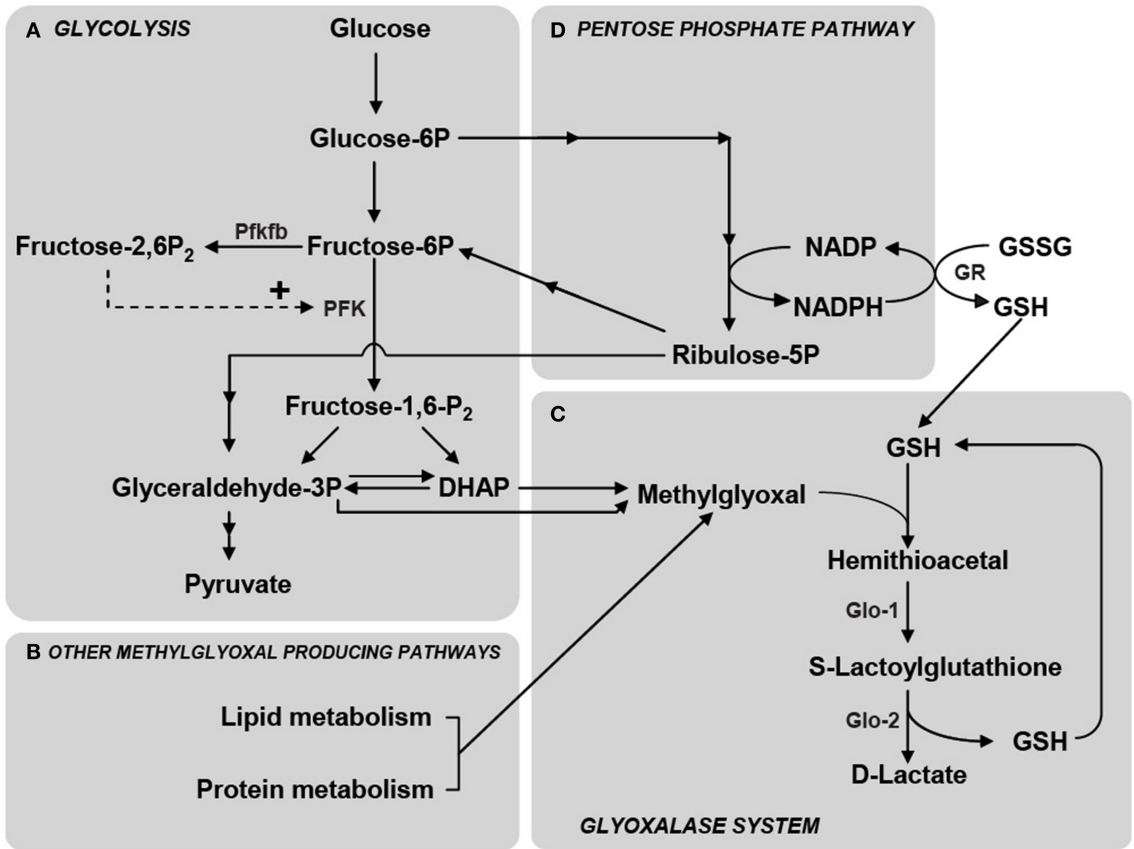


FIGURE 2 | Schematic representation of the main metabolic pathways involved in MG production and elimination. (A) MG is formed mainly by the fragmentation of the glycolytic intermediates glyceraldehyde-3-phosphate (Glyceraldehyde-3P) and dihydroxyacetone phosphate (DHAP), but also from the metabolism of lipids and proteins **(B)**. Phosphofructokinase-1 (PFK) is the rate-limiting step of glycolysis and thus constitutes an important regulatory site, one of its most potent allosteric activators being Fructose-2, 6-bisphosphate (Fructose-2,6-P_2). Fructose-2,6-P_2 levels are controlled by the enzyme 6-phosphofructose-2-kinase/fructose-2,6-bisphosphatase (Pfkfb) which is most abundantly expressed in astrocytes compared to neurons (Herrero-Mendez et al., 2009). **(C)** MG is detoxified principally via the glyoxalase system which consists of the enzymes Glyoxalase-1 (Glo-1) and Glyoxalase-2 (Glo-2). The first step in MG detoxification requires its spontaneous reaction with reduced glutathione (GSH) to form a hemithioacetal which is used as a substrate by Glo-1 to form S-Lactoylglutathione. Glo-2 then catalyzes the transformation of S-Lactoylglutathione into D-Lactate, recycling GSH in the process. **(D)** The pentose phosphate pathway is linked to MG detoxification via the formation of NADPH which is required for the recycling of GSH from its oxidized form (GSSG) via the action of glutathione reductase (GR). Reproduced with permission from Bélanger et al. (2011b).

Patel et al., 2014). The abovementioned observations, in conjunction with transcriptome analysis of brain cells isolated by fluorescence-activated cell sorting (FACS) revealing weaker and differential expression of glycolytic enzymes in neurons than in astrocytes (Lovatt et al., 2007; Cahoy et al., 2008; Zhang et al., 2014), sustain the view of astrocytes as a prevalent site for the glycolytic processing of glucose in the brain.

By opposition to one's common belief, glycolysis is not an innocuous metabolic pathway for cells, since it inevitably produces methylglyoxal (MG) as a by-product. The accumulation of the cell-permeant MG is highly deleterious, since this compound is one of the most potent glycating agents produced in cells. It readily reacts with proteins, lipids and nucleic acids to form advanced glycation end products (AGEs). AGEs are implicated in various pathophysiological mechanisms, including those associated with diabetic complications (cataracts, retinopathy, nephropathy, angiopathy), aging, and neurodegenerative disorders (Wautier and Guillausseau, 2001; Munch et al., 2012). In normal conditions, MG detoxification is mainly achieved by the ubiquitous enzymatic glyoxalase system that keeps MG concentration at low non-toxic cellular levels.

Since the brain has very high energy requirements in order to maintain neural cells function, brain cells are more likely to be exposed to MG compared with other cell types. Therefore, MG handling by neural cells has to be tightly regulated in order to prevent irreversible MG toxicity.

MG AND THE GLYOXALASE SYSTEM

MG, a dicarbonyl compound, is a ubiquitous product of cellular metabolism being therefore present in all cells, either under normal or pathological conditions. Enzymatic and non-enzymatic routes are known to produce MG. The rate of MG formation depends on the organism, tissue, cell metabolism and physiological conditions.

While MG can be produced as a by-product of protein and fatty acid metabolism (Thornalley, 1996; Kalapos, 1999; Vander Jagt and Hunsaker, 2003), the glycolytic pathway represents the most important endogenous source of MG via the fragmentation of the triosephosphates glyceraldehyde-3-phosphate (GAP) and dihydroxyacetone phosphate (DHAP) (Richard, 1993) (**Figure 2**). It is estimated that 0.1–0.4% of the glycolytic flux results in MG production (Kalapos, 2008a) and that nonenzymatic MG formation rate, from GAP and DHAP, is 0.1 mM per day in rat tissues (Richard, 1991). Cerebrospinal fluid levels of MG have been estimated to be between 10 and 20 μM (Kuhla et al., 2005), and cellular levels of free MG are typically in the low μM range (Rabbani and Thornalley, 2010). Importantly, as MG is highly reactive its half life is short in a biological environment and therefore, at the time and site of production local concentrations may be significantly higher (Kalapos, 2008b). As an example of the potency at which MG reacts with biological samples, addition of 1 μM [^{14}C]MG to human plasma *ex vivo* produced complete and irreversible binding of MG to plasma protein within 24 h at 37°C (Thornalley, 2005). Consistently, up to 90–99% of cellular MG is bound to macromolecules, and assessment of total (free + bound) MG, suggested that cellular concentrations up to 300 μM can be reached (Thornalley, 1996; Chaplen et al., 1998).

High levels of MG occur when the concentrations of their precursors are elevated, such as in hyperglycemia, impaired glucose utilization and triosephosphate isomerase deficiency (Ahmed et al., 2003a).

As previously mentioned, MG is one of the most potent glycating agents present in cells making its accumulation highly deleterious. For instance, MG readily reacts with lipids, nucleic acids and with lysine and arginine residues of proteins to form AGEs such as argpyrimidine, hydroimidazolone MG-H1, MG-derived lysine dimer and N^{ϵ}-(1-carboxyethyl)lysine (Thornalley, 2005, 2007; Rabbani and Thornalley, 2010). Besides the direct changes in protein function by MG modifications, AGE-modified proteins also exert cellular effects via their interaction with specific AGE receptors [RAGE (receptor for AGE)] (Grillo and Colombatto, 2008; Daroux et al., 2010), which triggers an inflammatory response at the cellular level, also accounting for AGE toxicity. AGEs play an important role in various pathophysiological mechanisms, including those associated with diabetic complications, aging, and neurodegenerative disorders (Wautier and Guillausseau, 2001; Ramasamy et al., 2005; Goldin et al., 2006; Munch et al., 2012).

In order to avoid the toxic effects of MG, cells possess different detoxifying mechanisms such as the glyoxalase, aldose reductase, aldehyde dehydrogenase and carbonyl reductase pathways (Thornalley, 1993; Kalapos, 1999; Vander Jagt and Hunsaker, 2003). Undoubtedly, the glyoxalase system, an ubiquitous enzymatic pathway, is the main detoxifying system for MG and other reactive dicarbonyl compounds in eukaryotic cells, thereby playing a major role the cellular defense against glycation and oxidative stress (Thornalley, 1993; Kalapos, 2008b). It detoxifies MG through two sequential enzymatic reactions catalyzed by glyoxalase-1 (Glo-1) and glyoxalase-2 (Glo-2), using glutathione as a co-factor. Glo-1 converts the hemithioacetal formed by the non-enzymatic reaction of reduced glutathione (GSH) with MG, to S-D-lactoylglutathione. This compound is then metabolized to D-Lactate (the poorly metabolizable enantiomer of L-lactate) by Glo-2, which recycles glutathione in the process (**Figure 2**) (Thornalley, 1993). Since S-D-lactoylglutathione is a non-toxic compound, metabolism of the dicarbonyl compound by Glo-1 represents a crucial step for MG detoxification, implying that Glo-1 activity indirectly determines MG toxicity and the rate of AGEs formation. One should also consider that GSH recycling occurs as S-D-lactoylglutathione is metabolized to D-Lactate. This implies that large increases of MG levels or low Glo-2 activity may result in S-D-lactoylglutathione accumulation, keeping GSH trapped, hence potentially leading to decreased GSH availability for other cellular processes such as defense against oxidative stress (Dringen, 2000).

GLYOXALASE SYSTEM IN NEURONS AND ASTROCYTES

Direct assessment of the intrinsic glyoxalase system capacities in both neurons and astrocytes has been done using mouse primary cortical cultures (Bélanger et al., 2011b). In this model, both Glo-1 and Glo-2 enzymes activities are significantly higher in astrocytes compared to neurons, i.e., Glo-1 and Glo-2 displayed respectively 9.8 times higher and 2.5 higher activities in astrocytes as compared to neurons.

In both cell types, Glo-1 activity rate was markedly higher compared to Glo-2 supporting the view that the rapid conversion of MG and GSH to S-D-Lactoylglutathione represents a critical step in the cellular defense against MG toxicity (Thornalley, 2008). A similar difference in the levels of Glo-1 and Glo-2 expression was also observed in C6 glioma cell lines (Hansen et al., 2012). Consistent with the differential Glo-1 activity between astrocytes and neurons, concentration-response curve analysis demonstrated that astrocytes detoxify exogenously added MG to D-lactate more efficiently than neurons (Bélanger et al., 2011b). Glo-1 enrichment in astrocytes compared to neurons was confirmed *in vivo* in the cerebral cortex of mice by immunofluorescence labeling (Bélanger et al., 2011b). Consistent with *in vitro* results, while Glo-1 immunoreactivity could be observed in both astrocytes (GFAP-positive cells) and neurons (NeuN-positive cells), the strongest levels of Glo-1 immunostaining was found in GFAP-positive cells. At the structural level, Glo-1 immunoreactivity in astrocytes was observed to be present the cell body as well as in the main astrocytic processes, i.e., along the GFAP-positive filaments.

Consistent with this observation, cell specific FACS demonstrated a 2-fold enrichment of Glo-1 activity in acutely isolated brain cortical astrocytes compared to other brain cortical cells (Bélanger et al., 2011b). Using a similar technique, Cahoy et al. (2008) demonstrated a 2.5-fold enrichment of Glo-1 mRNA expression in acutely isolated mouse astrocytes (Cahoy et al., 2008). In the human brain samples, both Glo-1 positive astrocytes and neurons were observed (Chen et al., 2004; Kuhla et al., 2006a).

Together, these results demonstrate a consistently highly efficient glyoxalase system in (cortical) astrocytes compared to neurons in both *in vitro* and *in vivo* situations. Moreover, this suggests that such a cell-specific distribution has physiological significance in the intact brain.

EXOGENOUS MG IS HIGHLY TOXIC FOR NEURONS, RED-OX DEPENDENCY

Consistent with a less active glyoxalase system, neurons are more vulnerable than astrocytes to MG toxicity. Indeed, exogenously added MG at concentrations between 250 and 750 μM is detrimental to neuronal viability in hippocampal, cortical and primary sensory cortex cultures or in SH-SY5Y neuroblastoma cells (Di et al., 2004, 2008; de Arriba et al., 2007; Bélanger et al., 2011b; Radu et al., 2012), whereas concentrations up to 1 mM do not impact cellular integrity in astrocytic cultures or in C6 glioma cells (Bélanger et al., 2011b; Hansen et al., 2012). Direct comparison of MG toxicity in primary cultures of mouse cortical neurons and astrocytes evidenced a 6-fold higher susceptibility toward MG toxicity in neurons compared to astrocytes (Bélanger et al., 2011b). Even more strikingly, when co-cultured with astrocytes neurons are significantly protected from MG toxicity (added at concentration s of up to 2 mM) further highlighting the high capacity of astrocytes to detoxify extracellular sources of MG, protecting neurons in the process (Bélanger et al., 2011b).

Neuronal MG toxicity is associated with AGEs production and accumulation (Bélanger et al., 2011b) but is also involved in oxidative stress-mediated cell death. Indeed, intracellular glutathione and NADPH levels, as well as glutathione-dependent antioxidant processes, are strongly decreased in cultured neuronal cell preparation following MG application, leading to cellular oxidative stress and intracellular accumulation of reactive oxygen species (Di et al., 2004, 2008; de Arriba et al., 2007). As an example, co-treatment of MG with the antioxidant, and glutathione precursor, N-acetylcysteine (NAC) prevents MG-mediated neuronal death in hippocampal cultures (Di et al., 2004). This can be explained by the requirement of GSH for MG detoxification. Indeed, although GSH is recycled back during the processing of MG though the glyoxalase system at the Glo-2 step, exposure to MG is likely to cause a transient GSH depletion since part of the intracellular GSH is kept trapped in the form of S-D-lactoylglutathione during the process, especially since Glo-2 displays much slower activity rates than Glo-1 in both astrocytes and neurons (see above) (**Figure 2**). This transient GSH depletion resulting from glyoxalase system activity may therefore have important consequences for the cell's oxidative stress status [e.g., increasing oxidized glutathione (GSSG)/GSH and NADP/NADPH ratios], since glutathione represents one of the most important antioxidants in mammalian cells (Dringen, 2000). In line with this, GSSG was recently found to directly inactivate Glo-1 through covalent modification (Birkenmeier et al., 2010).

It has previously been reported that glutathione concentrations are higher in astrocytes than in neurons (Dringen, 2000). Such differences in glutathione metabolism, together with higher Glo-1 and -2 activities, may explain the greater resistance of astrocytes against MG toxicity compared to neurons. In agreement with this, astrocytes red-ox indexes (glutathione and NADPH levels) were not severely compromised even when cultured astrocytes were exposed to milimolar concentrations of MG (Bélanger et al., 2011b).

IMPACT OF MANIPULATING Glo-1 ACTIVITY IN ASTROCYTES AND NEURONS

The observation that exogenous MG is highly damaging to neurons compared to astrocytes raises the question of the capacity of these cells to control intracellular MG production and detoxification. Experiments conducted *in vitro* on neural cells in which Glo-1 activity was decreased gave interesting clues about this question. Using RNA interference strategies Bélanger and collaborators observed that a similar decrease in Glo-1 activity (80%) does not impact astrocytic and neuronal cell viability to the same extent using primary mouse cortical cultures as a model (Bélanger et al., 2011b). For instance, Glo-1 inhibition resulted in a significant loss of neuronal viability (35%) that was associated with increased AGEs accumulation, while no deleterious effects were measured in astrocytes. Of note, due to the high Glo-1 expression levels in astrocytes, a 80% decrease in Glo-1 expression in these cells leaves Glo-1 expression at the same levels of expression than those measured in control neuronal cultures. This observation is consistent with a study conducted on SH-SY5Y cells in which chronically elevated MG concentrations induced by Glo-1 pharmacological inhibition with p-bromobenzylglutathione cyclopentyl diester (pBrBzGSCp2) also resulted in a decreased cellular viability (by 25%) (Kuhla et al., 2006b).

Considering the high susceptibility of neurons to Glo-1 silencing as described above, Bélanger et al. sought to determine whether Glo-1 upregulation could conversely protect neurons against various cellular stresses and in particular MG (Bélanger et al., 2011b). Intriguingly, they did not observed significant protection of cultured cortical neurons against exogenous MG toxicity following lentiviral-mediated Glo-1 overexpression, despite a large increase in enzymatic activity (6.8-fold increase). This result suggests that low expression level of Glo-1 is not the only factor accounting for the high neuronal vulnerability to MG toxicity. As discussed above, cultured neurons display particularly low levels of glutathione and a poor capacity to replenish the glutathione pool following exposure to MG, which may also explain neuronal susceptibility toward MG insults. Low glutathione levels may therefore represent a limiting factor for MG detoxification in neurons, in which case Glo-1 overexpression *per se* would not be sufficient to provide neuroprotective properties.

INCREASED GLYCOLYSIS OVERWHELMS NEURONAL GLYOXALASE SYSTEM CAPACITY

As previously stated, the poor capacity of neurons to upregulate glycolysis (Almeida et al., 2001) is partially explained by the constitutive downregulation of Pfkfb, more specifically Pfkfb3 also known as PFK-2 isoform 3, which is responsible for the generation of fructose-2,6-bisphosphate, a key regulator of glycolysis (Herrero-Mendez et al., 2009) (**Figure 2**). Considering that, as described above, neurons have low MG detoxification capacity, the low protein expression levels of Pfkfb3 may be viewed as a neuronal regulatory mechanism preventing MG damages, since MG production is tightly dependent upon the glycolytic rate. In order to get insights about such hypothesis, Bélanger and collaborators manipulated glucose utilization in neuronal cultures taking advantage of the observation made by Herrero-Mendez and collaborators that overexpression of Pfkfb3 results in an increased

glycolytic rate in cultured rat neurons (Herrero-Mendez et al., 2009). In primary cultures of cortical neurons, it was observed that increased lentiviral-mediated Pfkfb3 expression resulted in a limited but significant stimulation of glucose utilization (20%) (Bélanger et al., 2011b). Remarkably, this stimulation of the neuronal glycolytic rate coincided with a significant increase in intracellular MG levels and enhanced levels of argpyrimidine-modified proteins, which are MG-specific AGE product. In this context, it is worth mentioning that Herrero-Mendez and collaborators demonstrated in their initial work that an increase in neuronal glycolytic rate (i.e., induced by Pfkfb3 overexpression) was accompanied by a marked decrease in the oxidation of glucose through the pentose phosphate pathway (a metabolic route involved in the regeneration of reduced glutathione through the formation of reducing equivalent in the form of NADPH, **Figure 2**). Hence, less NADPH is available to regenerate the small pool of neuronal GSH, which may further compromise the efficiency of the glyoxalase system (Herrero-Mendez et al., 2009). Altogether, these results support the notion that even a modest increase in neuronal glycolytic rate can readily overwhelm the neuronal glyoxalase system, which results in the accumulation of MG and thereupon in protein damage.

GLYOXALASE SYSTEM IN BRAIN DISORDERS

Historically, most research on the glyoxalase system in diseases has focused on the importance of MG detoxification to prevent cellular damage due to the glycation of proteins and nucleic acids. These studies have implicated high concentrations of MG and/or low Glo-1 activity in the etiology of metabolic disorders, such as diabetes (Wautier and Guillausseau, 2001; Ramasamy et al., 2005; Goldin et al., 2006).

When looking more specifically at pathologies affecting the brain, modulations of the glyoxalase system have been observed in aging as well as in various neuropathological conditions, including ischemia, epilepsy, Alzheimer's disease and Parkinson's disease (Ramasamy et al., 2005; Kuhla et al., 2007; Kurz et al., 2011; Distler and Palmer, 2012). For instance, starting from the fifth decade Glo-1 expression was reported to decrease progressively in the human brain (Kuhla et al., 2006a), in correlation with increased AGEs levels in neurons and astrocytes (Luth et al., 2005). In line with this, over-expression of the Glo-1 homolog in *C. elegans* (ortholog CeGly) decreases MG-dependent toxic effects associated with the loss Glo-1 activity that occurs with age, and prolonged *C. elegans* life span (Morcos et al., 2008). Incidentally, decrease in glutathione levels during aging may also exacerbate the deleterious effects of declining Glo-1 expression (Currais and Maher, 2013). In line with this, the concentration of GSH in human lenses decreases with age (Kamei, 1993) which correlates with an increase of the MG-related AGE content (Ahmed et al., 2003b). Hence, reduced levels of glutathione in aging may not only have consequences for its function as an antioxidant, i.e., red-ox defense mechanisms, but also for its role as co-factor of the glyoxalase system (Currais and Maher, 2013).

As mentioned, impaired Glo-1 activity also contributes to the pathogenesis of neurodegenerative disorders. MG accumulation is tightly linked oxidative stress and AGE formation, which are major factors involved in many of these age-dependent pathologies. In Alzheimer's disease (AD), a marked increase in AGE accumulation is observed at later stages, which correlates with a decrease below normal levels of Glo-1 (Luth et al., 2005; Kuhla et al., 2007). In line with this, cerebrospinal fluid of patients affected by AD presents high levels of MG (Kuhla et al., 2005). A striking observation is the co-localization of AGEs with amyloid-β plaques and neurons containing hyperphosphorylated tau in AD brains, while astrocytes are also affected as they show increased AGE levels (Wong et al., 2001; Luth et al., 2005). Such observations suggest an impairment of the glyoxalase system in AD which may be involved in the vicious circle of amyloid-β deposition, AGE formation and oxidative stress associated with this disease. Nevertheless, further studies are required to delineate the specific effects of the glyoxalase system alteration on different neural cell types. Glo-1 expression was also shown to be modulated in ischemia. Indeed, following permanent middle cerebral artery occlusion, temporal and spatial changes in Glo-1 immunoreactivity in endothelial cells, neurons and astrocytes were observed (Pieroh et al., 2014).

Interestingly, higher levels of baseline MG derivatives in the serum were associated with a faster rate of cognitive decline in ederly individuals, indicating that peripheral MG metabolism imbalances may also affect brain pathopysiology (Beeri et al., 2011).

The functional defense role of the glyoxalase pathway has been exploited as a potential therapeutic target, through strategies aiming at reducing MG concentrations and/or enhancing the glyoxalase system in brain diseases. For example, More and collaborators developed a synthetic co-factor for Glo-1 (ψ-GSH) that crosses the blood brain barrier (BBB) (More et al., 2013). This molecule was shown to mitigate Alzheimer's disease indicators in a transgenic mouse model (APP/PS1). For instance, it was observed that intraperitoneal administration of ψ-GSH completely averts the development of spatial mnemonic and long-term cognitive/cued-recall impairment in these mice. Moreover, amyloid-β deposition and oxidative stress indicators were also drastically reduced in the ψ-GSH-treated APP/PS1 mouse. While a detailed characterization of the selectivity of ψ-GSH mode of action *in vivo* remains to be fully established, ψ-GSH represents a promising drug for the treatment of diseases associated with a glyoxalase dysfunction, including neurodegenerative pathologies. Increasing Glo activities in ischemia has also been shown to be an effective neuroprotective strategy. In a recent study, Glo proteins were coupled to the Tat (transactivator of transcription) peptide as a vehicle to provide Glo-1 and Glo-2 exogenously to cells of the central nervous system (Shin et al., 2014). These Tat–Glo proteins were intraperitoneally injected 30 min before performing transient forebrain ischaemia in gerbils. Increased intracerebral Glo levels were detected, indicating the ability of the agent to cross the BBB. Importantly, cell viability tests performed 7 and 14 days after ischaemia, demonstrated a neuroprotective effect of Tat-Glo proteins delivery. Interestingly, it was noted that enhancing the glyoxalase system as a whole via the simultaneous injection of Tat–Glo-1 and Tat–Glo-2 displayed the highest protection level. Such examples demonstrate the importance of the glyoxalase dysfunction in AD and ischemia, but also represent a proof of concept for the development of strategies aiming to improve the

efficiency or restore the glyoxalase defense system in the context of brain disorders.

Finally, recent studies from several laboratories also suggested that modulations of MG concentration and Glo-1 activity are involved in schizophrenia, autism, anxiety, depression, sleep and pain phenotypes (Hambsch et al., 2010; Bierhaus et al., 2012; Distler and Palmer, 2012; Distler et al., 2012, 2013; Jakubcakova et al., 2013), further pointing to the role of the glyoxalase system as an important element in brain physiology and pathology.

PHYSIOLOGICAL ROLES OF MG AND THE GLYOXALASE SYSTEM

The glutathione dependence as a co-factor for Glo-1, the non-enzymatic origin of MG from triosephosphates and the fact that the end-product is D- rather than L-lactate, isolate MG and Glo-(1 and 2) from glycolysis, leaving this system orphan in function. So is there any physiological cerebral function for this pathway, apart from its importance as a detoxifying system? There is now emerging evidence that the answer is yes. For instance, modulation of Glo-1 activity was shown to regulate anxiety-like behavior and seizure-susceptibility in mice. These effects are likely to be mediated through the regulation of MG levels, as MG was demonstrated to act as a competitive partial agonist for GABAA (γ-aminobutyric acid A) receptors (Distler et al., 2012; McMurray et al., 2014). MG was also shown to modulate the activities other proteins such as the voltage-gated sodium channel Nav1.8 or the TRPA1 ion channel (Bierhaus et al., 2012; Andersson et al., 2013). In a recent study, it was shown that D-lactate supports the viability of dopaminergic neurons *in vitro* by promoting the maintenance of mitochondrial potential (Toyoda et al., 2014). Interestingly, the latter work was linked to the discovery of DJ-1 (also known as PARK7) as a human homolog of a novel type of glyoxalase (Glo-3), that converts MG to lactic acid in the absence of glutathione (Lee et al., 2012; Toyoda et al., 2014). DJ-1 has been linked with the onset of Parkinson's disease, which is associated with mitochondrial decline in dopaminergic neurons of the substantia nigra, suggesting a possible mitochondrial regulatory role of MG metabolism in dopaminergic neurons.

CONCLUDING REMARKS

Altogether, the observations reviewed in this article support the notion that there are major differences in the glyoxalase system of astrocytes and neurons, which impact their respective capacity for cellular defense against glycation. As they are mainly based on *in vitro* observations, these findings remain to be fully established in the *in vivo* situation. Nevertheless, there is converging and convincing evidence demonstrating that astrocytes display a much more efficient glyoxalase system than neurons in cultures as well as higher Glo-1 immunoreactivity and activity in the mouse brain (Bélanger et al., 2011b). Interestingly, when compared to the high MG-producing CHO cells or various other cell types (Ranganathan et al., 1995; Shinohara et al., 1998; Wu et al., 2001; Ahmed et al., 2003a; Kumagai et al., 2009), Glo-1 activity rates in cultured astrocytic (Bélanger et al., 2011b) were shown to be several fold higher, supporting the notion of a particularly efficient glyoxalase system in astrocytes.

As previously mentioned, glutathione levels are significantly higher in astrocytes than in neurons (Dringen, 2000; Bélanger et al., 2011b). Since MG detoxification via the glyoxalase pathway requires glutathione as a co-factor, these differences in glutathione metabolism, together with a higher glyoxalase activity, endow astrocytes with a optimized glyoxalase machinery and thus a greater resistance to exogenous MG compared to neurons. Consistently, numerous studies demonstrated a high neuronal susceptibility to MG toxicity, which was associated with AGEs production and oxidative stress leading to apoptosis (Kikuchi et al., 1999; Di et al., 2004; Di Loreto et al., 2008; Chen et al., 2010; Bélanger et al., 2011b; Radu et al., 2012).

The differential Glo-1 activity observed in astrocytes and neurons may be linked to their dissimilar metabolic energetic profile. As previously mentioned, astrocytes display high metabolic plasticity in terms of glucose utilization, when compared to neurons (Pellerin and Magistretti, 1994; Almeida et al., 2001; Prapong et al., 2002; Porras et al., 2004; Gavillet et al., 2008; Allaman et al., 2010; Belanger et al., 2010). In particular, astrocytes, are able to up-regulate their glycolytic rate upon the synthesis of fructose-2,6-bisphosphate by the enzyme Pfkfb3 (Almeida et al., 2004), an enzyme that is virtually absent in neurons due to its constant proteasomal degradation (Herrero-Mendez et al., 2009). Strikingly, enhancing neuronal glycolysis via Pfkfb3 overexpression leads to oxidative stress and apoptosis (Herrero-Mendez et al., 2009). The observation that even a moderate increase in neuronal glycolysis, subsequent to Pfkfb3 overexpression, was sufficient to significantly increase MG and AGEs levels (Bélanger et al., 2011b) indicates a role of MG production in the neuronal toxicity associated with an increase in the glycolytic rate. Hence, one can conceive that neurons constitutively suppress pathways potentially leading to MG formation because of their limited capacity to face deleterious dicarbonyl stress. Similarly to Pfkfb, expression of the enzymatic machinery for glycogen synthesis is tightly suppressed in neuron, explaining the virtual absence of glycogen in this cell type. Importantly, failure to down regulate glycogen synthesis machinery results in neuronal apoptosis (Vilchez et al., 2007). Therefore, glycolysis and glycogen metabolism pathways both of which are directly linked to triosephosphates metabolism are tightly repressed in neurons and lead to apoptosis if activated.

These observations may explain why astrocyte can sustain a higher basal glycolytic rate than neurons. They may also provide insights into the reason why neurons depend on astrocytes-derived lactate to satisfy their energy needs, as proposed by the ANLS model and substantiated by several *in vitro*, *ex vivo*, and *in vivo* data (Kasischke et al., 2004; Porras et al., 2004; Rouach et al., 2008; Magistretti, 2009; Chuquet et al., 2010). Considering that glucose metabolism inevitably leads to MG formation, lactate utilization may provide a mean for neurons to limit MG toxicity by reducing their glucose utilization.

According to this hypothesis, neurons meet their high energetic requirements by utilizing astrocyte-derived lactate (produced from extracellular glucose or glycogen pools), which can be oxidized in the TCA cycle following its conversion to pyruvate. Such a scenario allows neurons to generate energy on demand without compromising their antioxidant status (through the mobilization of GSH by Glo-1), and leaves astrocytes with

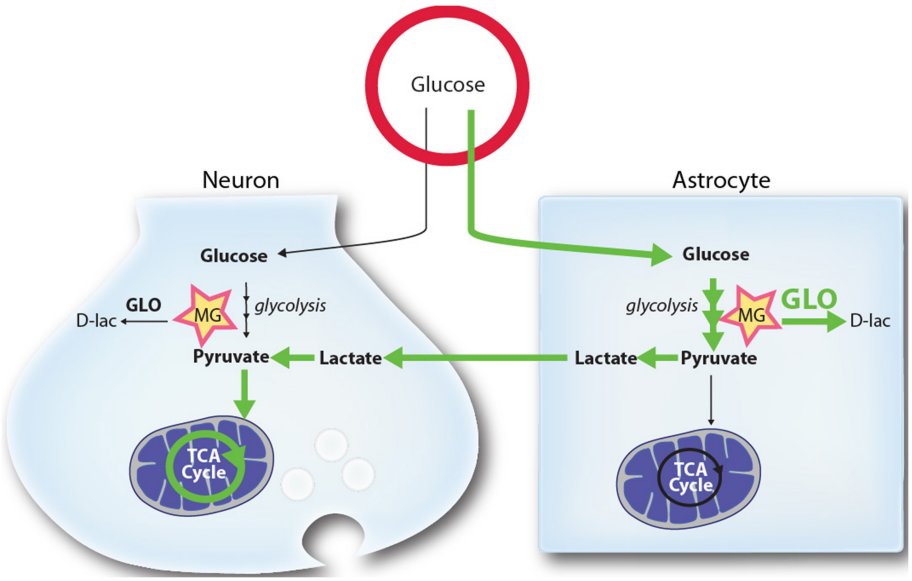


FIGURE 3 | Schematic representation of the proposed mechanism by which higher glycolytic rates in astrocytes may provide a mechanism limiting MG toxicity in neurons. Glycolysis in both astrocytes and neurons leads to the production of the toxic MG by-product. MG is detoxified by both cell types through the glyoxalase system (GLO), producing D-lactate (D-lac). In normal and stimulated conditions (ANLS) glucose utilization and its processing through the glycolysis is tilted toward astrocytes, which release L-lactate (lactate) that can be used by neurons as a mitochondrial energy substrate. Due to the high activity of the MG-detoxifying glyoxalase system in astrocytes, these cells are well equipped to handle MG accumulation and toxicity. Because they display lower glyoxalase system activity, neurons benefit from the glycolytic processing of glucose in astrocytes since they are spared from: (1) MG accumulation and toxicity (2) alterations in their antioxidant status (through the mobilization of GSH by Glo-1), and (3) the burden of mounting an enzymatic system to process MG. See text for more details. Green arrows highlight the prevalent routes of glucose utilization in brain cells.

most of the MG burden (**Figure 3**). In line with this, the observation that astrocytes protect neurons against MG toxicity in a co-culture model (Bélanger et al., 2011b), suggests another level of astrocyte-neuron cooperativity. For instance, a highly efficient glyoxalase system in astrocytes may reinforce neuronal protection against MG potentially leaking from the periphery, the cerebrospinal fluid or surrounding cells. As a whole, such view support the concept of an "outsourced glycolysis" to astrocytes in the brain as suggested by Barros (2013).

Interestingly, evidence shows that in astrocytes, genes involved in glycogen, glycolysis and glutamate uptake are expressed through a common metabolic program (Brunet et al., 2010; Mamczur et al., 2014). Similarly, the expression of genes involved in glutathione metabolism together with Glo-1 appears to be under the control of Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) that is highly expressed in astrocytes (Thimmulappa et al., 2002; Vargas and Johnson, 2009; Xue et al., 2012). This further supports the notion of a differential, complementary and coherent metabolic phenotypic differentiation between astrocytes and neurons with regards to neuroenergetics.

Consistent with its important role in controlling MG detoxification, glyoxalase system dysfunctions have been associated with various brain pathologies and in particular neurodegenerative diseases (Ramasamy et al., 2005; Kuhla et al., 2007; Kurz et al., 2011; Distler and Palmer, 2012). Astrocytes are key players in the maintenance of brain homeostasis, and several cooperative metabolic processes between astrocytes and neurons have been identified (Belanger and Magistretti, 2009; Allaman et al., 2011), which include among others energy substrate delivery (e.g., ANLS), defense against oxidative stress and excitotoxicity (Rothstein et al., 1996; Johnson et al., 2008; Vargas and Johnson, 2009; Bélanger et al., 2011a; Pellerin and Magistretti, 2012). Therefore, glyoxalase system dysfunctions may not only impact neuronal viability directly (if occurring in neurons) but also indirectly (if occurring in astrocytes) through the alterations of cooperative processes between astrocytes and neurons. As exemplified with ψ-GSH and Tat-Glo delivery in the context of AD and ischemia, strategies aiming to re-establish or boost the glyoxalase system efficiency will therefore represent potentially powerful therapeutically approaches for MG-associated brain pathologies.

It can be concluded that there is now strong evidence showing that the glyoxalase pathway differs significantly between astrocytes and neurons in a way that renders neurons more vulnerable to MG and AGE accumulation. These observations pinpoint the notion that a metabolic specialization is taking place in brain cells, i.e., astrocytes being more glycolytic than neurons, which may help protecting neurons from MG toxicity. Moreover, more than being a dead-end pathway the glyoxalase system may be important in the regulation of cerebral functions. Undoubtedly, future studies will help to shed light of the importance of this system in brain physiology and pathophysiology.

ACKNOWLEDGMENTS

This work was supported by grants from Swiss National Science Foundation (FNRS) (no. 310030B-148169/1), from the NCCR Synapsy and from the Biaggi and Panacée Foundations to Pierre J. Magistretti.

REFERENCES

Ahmed, N., Battah, S., Karachalias, N., Babaei-Jadidi, R., Horanyi, M., Baroti, K., et al. (2003a). Increased formation of methylglyoxal and protein glycation, oxidation and nitrosation in triosephosphate isomerase deficiency. *Biochim. Biophys. Acta* 1639, 121–132. doi: 10.1016/j.bbadis.2003.08.002

Ahmed, N., Thornalley, P. J., Dawczynski, J., Franke, S., Strobel, J., Stein, G., et al. (2003b). Methylglyoxal-derived hydroimidazolone advanced glycation end-products of human lens proteins. *Invest. Ophthalmol. Vis. Sci.* 44, 5287–5292. doi: 10.1167/iovs.03-0573

Allaman, I. (2009). "Glial glycogen metabolism," in *Encyclopedia of Neuroscience*, ed L. R. Squire (Oxford: Academic Press), 811–818.

Allaman, I., Belanger, M., and Magistretti, P. J. (2011). Astrocyte-neuron metabolic relationships: for better and for worse. *Trends Neurosci.* 34, 76–87. doi: 10.1016/j.tins.2010.12.001

Allaman, I., Gavillet, M., Belanger, M., Laroche, T., Viertl, D., Lashuel, H. A., et al. (2010). Amyloid-beta aggregates cause alterations of astrocytic metabolic phenotype: impact on neuronal viability. *J. Neurosci.* 30, 3326–3338. doi: 10.1523/JNEUROSCI.5098-09.2010

Almeida, A., Almeida, J., Bolanos, J. P., and Moncada, S. (2001). Different responses of astrocytes and neurons to nitric oxide: the role of glycolytically generated ATP in astrocyte protection. *Proc. Natl. Acad. Sci. U.S.A.* 98, 15294–15299. doi: 10.1073/pnas.261560998

Almeida, A., Moncada, S., and Bolanos, J. P. (2004). Nitric oxide switches on glycolysis through the AMP protein kinase and 6-phosphofructo-2-kinase pathway. *Nat. Cell Biol.* 6, 45–51. doi: 10.1038/ncb1080

Andersson, D. A., Gentry, C., Light, E., Vastani, N., Vallortigara, J., Bierhaus, A., et al. (2013). Methylglyoxal evokes pain by stimulating TRPA1. *PLoS ONE* 8:e77986. doi: 10.1371/journal.pone.0077986

Barros, L. F. (2013). Metabolic signaling by lactate in the brain. *Trends Neurosci.* 36, 396–404. doi: 10.1016/j.tins.2013.04.002

Barros, L. F., Courjaret, R., Jakoby, P., Loaiza, A., Lohr, C., and Deitmer, J. W. (2009). Preferential transport and metabolism of glucose in Bergmann glia over Purkinje cells: a multiphoton study of cerebellar slices. *Glia* 57, 962–970. doi: 10.1002/glia.20820

Beeri, M. S., Moshier, E., Schmeidler, J., Godbold, J., Uribarri, J., Reddy, S., et al. (2011). Serum concentration of an inflammatory glycotoxin, methylglyoxal, is associated with increased cognitive decline in elderly individuals. *Mech. Ageing Dev.* 132, 583–587. doi: 10.1016/j.mad.2011.10.007

Belanger, M., Allaman, I., and Magistretti, P. J. (2010). Differential effects of pro- and anti-inflammatory cytokines alone or in combinations on the metabolic profile of astrocytes. *J. Neurochem.* 116, 564–576. doi: 10.1111/j.1471-4159.2010.07135.x

Bélanger, M., Allaman, I., and Magistretti, P. J. (2011a). Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation. *Cell Metab.* 14, 724–738. doi: 10.1016/j.cmet.2011.08.016

Belanger, M., and Magistretti, P. J. (2009). The role of astroglia in neuroprotection. *Dialogues Clin. Neurosci.* 11, 281–295.

Bélanger, M., Yang, J., Petit, J. M., Laroche, T., Magistretti, P. J., and Allaman, I. (2011b). Role of the glyoxalase system in astrocyte-mediated neuroprotection. *J. Neurosci.* 31, 18338–18352. doi: 10.1523/JNEUROSCI.1249-11.2011

Bierhaus, A., Fleming, T., Stoyanov, S., Leffler, A., Babes, A., Neacsu, C., et al. (2012). Methylglyoxal modification of Nav1.8 facilitates nociceptive neuron firing and causes hyperalgesia in diabetic neuropathy. *Nat. Med.* 18, 926–933. doi: 10.1038/nm.2750

Birkenmeier, G., Stegemann, C., Hoffmann, R., Gunther, R., Huse, K., and Birkemeyer, C. (2010). Posttranslational modification of human glyoxalase 1 indicates redox-dependent regulation. *PLoS ONE* 5:e10399. doi: 10.1371/journal.pone.0010399

Bittner, C. X., Loaiza, A., Ruminot, I., Larenas, V., Sotelo-Hitschfeld, T., Gutierrez, R., et al. (2010). High resolution measurement of the glycolytic rate. *Front. Neuroenergetics* 2:26. doi: 10.3389/fnene.2010.00026

Boumezbeur, F., Mason, G. F., de Graaf, R. A., Behar, K. L., Cline, G. W., Shulman, G. I., et al. (2010a). Altered brain mitochondrial metabolism in healthy aging as assessed by *in vivo* magnetic resonance spectroscopy. *J. Cereb. Blood Flow Metab.* 30, 211–221. doi: 10.1038/jcbfm.2009.197

Boumezbeur, F., Petersen, K. F., Cline, G. W., Mason, G. F., Behar, K. L., Shulman, G. I., et al. (2010b). The contribution of blood lactate to brain energy metabolism in humans measured by dynamic 13C nuclear magnetic resonance spectroscopy. *J. Neurosci.* 30, 13983–13991. doi: 10.1523/JNEUROSCI.2040-10.2010

Bouzier, A. K., Thiaudiere, E., Biran, M., Rouland, R., Canioni, P., and Merle, M. (2000). The metabolism of [3-(13)C]lactate in the rat brain is specific of a pyruvate carboxylase-deprived compartment. *J. Neurochem.* 75, 480–486. doi: 10.1046/j.1471-4159.2000.0750480.x

Bouzier-Sore, A. K., Voisin, P., Bouchaud, V., Bezancon, E., Franconi, J. M., and Pellerin, L. (2006). Competition between glucose and lactate as oxidative energy substrates in both neurons and astrocytes: a comparative NMR study. *Eur. J. Neurosci.* 24, 1687–1694. doi: 10.1111/j.1460-9568.2006.05056.x

Brunet, J. F., Allaman, I., Magistretti, P. J., and Pellerin, L. (2010). Glycogen metabolism as a marker of astrocyte differentiation. *J. Cereb. Blood Flow Metab.* 30, 51–55. doi: 10.1038/jcbfm.2009.207

Cahoy, J. D., Emery, B., Kaushal, A., Foo, L. C., Zamanian, J. L., Christopherson, K. S., et al. (2008). A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. *J. Neurosci.* 28, 264–278. doi: 10.1523/JNEUROSCI.4178-07.2008

Chaplen, F. W., Fahl, W. E., and Cameron, D. C. (1998). Evidence of high levels of methylglyoxal in cultured Chinese hamster ovary cells. *Proc. Natl. Acad. Sci. U.S.A.* 95, 5533–5538. doi: 10.1073/pnas.95.10.5533

Chen, F., Wollmer, M. A., Hoerndli, F., Munch, G., Kuhla, B., Rogaev, E. I., et al. (2004). Role for glyoxalase I in Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A.* 101, 7687–7692. doi: 10.1073/pnas.0402338101

Chen, Y. J., Huang, X. B., Li, Z. X., Yin, L. L., Chen, W. Q., and Li, L. (2010). Tenuigenin protects cultured hippocampal neurons against methylglyoxal-induced neurotoxicity. *Eur. J. Pharmacol.* 645, 1–8. doi: 10.1016/j.ejphar.2010.06.034

Chih, C. P., and Roberts, E. L., Jr. (2003). Energy substrates for neurons during neural activity: a critical review of the astrocyte-neuron lactate shuttle hypothesis. *J. Cereb. Blood Flow Metab.* 23, 1263–1281. doi: 10.1097/01.WCB.0000081369.51727.6F

Chuquet, J., Quilichini, P., Nimchinsky, E. A., and Buzsaki, G. (2010). Predominant enhancement of glucose uptake in astrocytes versus neurons during activation of the somatosensory cortex. *J. Neurosci.* 30, 15298–15303. doi: 10.1523/JNEUROSCI.0762-10.2010

Currais, A., and Maher, P. (2013). Functional consequences of age-dependent changes in glutathione status in the brain. *Antioxid. Redox Signal.* 19, 813–822. doi: 10.1089/ars.2012.4996

Daroux, M., Prevost, G., Maillard-Lefebvre, H., Gaxatte, C., D'Agati, V. D., Schmidt, A. M., et al. (2010). Advanced glycation end-products: implications for diabetic and non-diabetic nephropathies. *Diabetes Metab.* 36, 1–10. doi: 10.1016/j.diabet.2009.06.005

de Arriba, S. G., Stuchbury, G., Yarin, J., Burnell, J., Loske, C., and Munch, G. (2007). Methylglyoxal impairs glucose metabolism and leads to energy depletion in neuronal cells–protection by carbonyl scavengers. *Neurobiol. Aging* 28, 1044–1050. doi: 10.1016/j.neurobiolaging.2006.05.007

Di, L. S., Caracciolo, V., Colafarina, S., Sebastiani, P., Gasbarri, A., and Amicarelli, F. (2004). Methylglyoxal induces oxidative stress-dependent cell injury and up-regulation of interleukin-1beta and nerve growth factor in cultured hippocampal neuronal cells. *Brain Res.* 1006, 157–167. doi: 10.1016/j.brainres.2004.01.066

Di, L. S., Zimmitti, V., Sebastiani, P., Cervelli, C., Falone, S., and Amicarelli, F. (2008). Methylglyoxal causes strong weakening of detoxifying capacity and apoptotic cell death in rat hippocampal neurons. *Int. J. Biochem. Cell Biol.* 40, 245–257. doi: 10.1016/j.biocel.2007.07.019

Dienel, G. A. (2012). Brain lactate metabolism: the discoveries and the controversies. *J. Cereb. Blood Flow Metab.* 32, 1107–1138. doi: 10.1038/jcbfm.2011.175

Di Loreto, S., Zimmitti, V., Sebastiani, P., Cervelli, C., Falone, S., and Amicarelli, F. (2008). Methylglyoxal causes strong weakening of detoxifying capacity and apoptotic cell death in rat hippocampal neurons. *Int. J. Biochem. Cell Biol.* 40, 245–257. doi: 10.1016/j.biocel.2007.07.019

Distler, M. G., Gorfinkle, N., Papale, L. A., Wuenschell, G. E., Termini, J., Escayg, A., et al. (2013). Glyoxalase 1 and its substrate methylglyoxal are novel

regulators of seizure susceptibility. *Epilepsia* 54, 649–657. doi: 10.1111/epi.12121

Distler, M. G., and Palmer, A. A. (2012). Role of Glyoxalase 1 (Glo-1) and methylglyoxal (MG) in behavior: recent advances and mechanistic insights. *Front. Genet.* 3:250. doi: 10.3389/fgene.2012.00250

Distler, M. G., Plant, L. D., Sokoloff, G., Hawk, A. J., Aneas, I., Wuenschell, G. E., et al. (2012). Glyoxalase 1 increases anxiety by reducing GABAA receptor agonist methylglyoxal. *J. Clin. Invest.* 122, 2306–2315. doi: 10.1172/JCI61319

Dringen, R. (2000). Metabolism and functions of glutathione in brain. *Prog. Neurobiol.* 62, 649–671. doi: 10.1016/S0301-0082(99)00060-X

Dringen, R., Gebhardt, R., and Hamprecht, B. (1993). Glycogen in astrocytes: possible function as lactate supply for neighboring cells. *Brain Res.* 623, 208–214. doi: 10.1016/0006-8993(93)91429-V

Gavillet, M., Allaman, I., and Magistretti, P. J. (2008). Modulation of astrocytic metabolic phenotype by proinflammatory cytokines. *Glia* 56, 975–989. doi: 10.1002/glia.20671

Goldin, A., Beckman, J. A., Schmidt, A. M., and Creager, M. A. (2006). Advanced glycation end products: sparking the development of diabetic vascular injury. *Circulation* 114, 597–605. doi: 10.1161/CIRCULATIONAHA.106.621854

Grillo, M. A., and Colombatto, S. (2008). Advanced glycation end-products (AGEs): involvement in aging and in neurodegenerative diseases. *Amino Acids* 35, 29–36. doi: 10.1007/s00726-007-0606-0

Hambsch, B., Chen, B. G., Brenndorfer, J., Meyer, M., Avrabos, C., Maccarrone, G., et al. (2010). Methylglyoxal-mediated anxiolysis involves increased protein modification and elevated expression of glyoxalase 1 in the brain. *J. Neurochem.* 113, 1240–1251. doi: 10.1111/j.1471-4159.2010.06693.x

Hansen, F., de Souza, D. F., Silveira, S. L., Hoefel, A. L., Fontoura, J. B., Tramontina, A. C., et al. (2012). Methylglyoxal alters glucose metabolism and increases AGEs content in C6 glioma cells. *Metab. Brain Dis.* 27, 531–539. doi: 10.1007/s11011-012-9329-3

Harris, J. J., Jolivet, R., and Attwell, D. (2012). Synaptic energy use and supply. *Neuron* 75, 762–777. doi: 10.1016/j.neuron.2012.08.019

Herrero-Mendez, A., Almeida, A., Fernandez, E., Maestre, C., Moncada, S., and Bolanos, J. P. (2009). The bioenergetic and antioxidant status of neurons is controlled by continuous degradation of a key glycolytic enzyme by APC/C-Cdh1. *Nat. Cell Biol.* 11, 747–752. doi: 10.1038/ncb1881

Hertz, L., Peng, L., and Dienel, G. A. (2007). Energy metabolism in astrocytes: high rate of oxidative metabolism and spatiotemporal dependence on glycolysis/glycogenolysis. *J. Cereb. Blood Flow Metab.* 27, 219–249. doi: 10.1038/sj.jcbfm.9600343

Hyder, F., Patel, A. B., Gjedde, A., Rothman, D. L., Behar, K. L., and Shulman, R. G. (2006). Neuronal-glial glucose oxidation and glutamatergic-GABAergic function. *J. Cereb. Blood Flow Metab.* 26, 865–877. doi: 10.1038/sj.jcbfm.9600263

Hyder, F., Rothman, D. L., and Bennett, M. R. (2013). Cortical energy demands of signaling and nonsignaling components in brain are conserved across mammalian species and activity levels. *Proc. Natl. Acad. Sci. U.S.A.* 110, 3549–3554. doi: 10.1073/pnas.1214912110

Itoh, Y., Esaki, T., Shimoji, K., Cook, M., Law, M. J., Kaufman, E., et al. (2003). Dichloroacetate effects on glucose and lactate oxidation by neurons and astroglia *in vitro* and on glucose utilization by brain *in vivo*. *Proc. Natl. Acad. Sci. U.S.A.* 100, 4879–4884. doi: 10.1073/pnas.0831078100

Jakoby, P., Schmidt, E., Ruminot, I., Gutierrez, R., Barros, L. F., and Deitmer, J. W. (2014). Higher transport and metabolism of glucose in astrocytes compared with neurons: a multiphoton study of hippocampal and cerebellar tissue slices. *Cereb. Cortex* 24, 222–231. doi: 10.1093/cercor/bhs309

Jakubcakova, V., Curzi, M. L., Flachskamm, C., Hambsch, B., Landgraf, R., and Kimura, M. (2013). The glycolytic metabolite methylglyoxal induces changes in vigilance by generating low-amplitude non-REM sleep. *J. Psychopharmacol.* 27, 1070–1075. doi: 10.1177/0269881113495596

Johnson, J. A., Johnson, D. A., Kraft, A. D., Calkins, M. J., Jakel, R. J., Vargas, M. R., et al. (2008). The Nrf2-ARE pathway: an indicator and modulator of oxidative stress in neurodegeneration. *Ann. N.Y. Acad. Sci.* 1147:61–69. doi: 10.1196/annals.1427.036

Jolivet, R., Magistretti, P. J., and Weber, B. (2009). Deciphering neuron-glia compartmentalization in cortical energy metabolism. *Front. Neuroenergetics* 1:4. doi: 10.3389/neuro.14.004.2009

Kalapos, M. P. (1999). Methylglyoxal in living organisms: chemistry, biochemistry, toxicology and biological implications. *Toxicol. Lett.* 110, 145–175. doi: 10.1016/S0378-4274(99)00160-5

Kalapos, M. P. (2008a). Methylglyoxal and glucose metabolism: a historical perspective and future avenues for research. *Drug Metabol. Drug Interact.* 23, 69–91. doi: 10.1515/DMDI.2008.23.1-2.69

Kalapos, M. P. (2008b). The tandem of free radicals and methylglyoxal. *Chem. Biol. Interact.* 171, 251–271. doi: 10.1016/j.cbi.2007.11.009

Kamei, A. (1993). Glutathione levels of the human crystalline lens in aging and its antioxidant effect against the oxidation of lens proteins. *Biol. Pharm. Bull.* 16, 870–875. doi: 10.1248/bpb.16.870

Kasischke, K. A., Vishwasrao, H. D., Fisher, P. J., Zipfel, W. R., and Webb, W. W. (2004). Neural activity triggers neuronal oxidative metabolism followed by astrocytic glycolysis. *Science* 305, 99–103. doi: 10.1126/science.1096485

Kikuchi, S., Shinpo, K., Moriwaka, F., Makita, Z., Miyata, T., and Tashiro, K. (1999). Neurotoxicity of methylglyoxal and 3-deoxyglucosone on cultured cortical neurons: synergism between glycation and oxidative stress, possibly involved in neurodegenerative diseases. *J. Neurosci. Res.* 57, 280–289. doi: 10.1002/(SICI)1097-4547(19990715)57:2<280::AID-JNR14>3.0.CO;2-U

Kuhla, B., Boeck, K., Luth, H. J., Schmidt, A., Weigle, B., Schmitz, M., et al. (2006a). Age-dependent changes of glyoxalase I expression in human brain. *Neurobiol. Aging* 27, 815–822. doi: 10.1016/j.neurobiolaging.2005.04.006

Kuhla, B., Boeck, K., Schmidt, A., Ogunlade, V., Arendt, T., Munch, G., et al. (2007). Age- and stage-dependent glyoxalase I expression and its activity in normal and Alzheimer's disease brains. *Neurobiol. Aging* 28, 29–41. doi: 10.1016/j.neurobiolaging.2005.11.007

Kuhla, B., Luth, H. J., Haferburg, D., Boeck, K., Arendt, T., and Munch, G. (2005). Methylglyoxal, glyoxal, and their detoxification in Alzheimer's disease. *Ann. N.Y. Acad. Sci.* 1043:211–216. doi: 10.1196/annals.1333.026

Kuhla, B., Luth, H. J., Haferburg, D., Weick, M., Reichenbach, A., Arendt, T., et al. (2006b). Pathological effects of glyoxalase I inhibition in SH-SY5Y neuroblastoma cells. *J. Neurosci. Res.* 83, 1591–1600. doi: 10.1002/jnr.20838

Kumagai, T., Nangaku, M., Kojima, I., Nagai, R., Ingelfinger, J. R., Miyata, T., et al. (2009). Glyoxalase I overexpression ameliorates renal ischemia-reperfusion injury in rats. *Am. J. Physiol. Renal Physiol.* 296, F912–F921. doi: 10.1152/ajprenal.90575.2008

Kurz, A., Rabbani, N., Walter, M., Bonin, M., Thornalley, P., Auburger, G., et al. (2011). Alpha-synuclein deficiency leads to increased glyoxalase I expression and glycation stress. *Cell. Mol. Life Sci.* 68, 721–733. doi: 10.1007/s00018-010-0483-7

Lebon, V., Petersen, K. F., Cline, G. W., Shen, J., Mason, G. F., Dufour, S., et al. (2002). Astroglial contribution to brain energy metabolism in humans revealed by 13C nuclear magnetic resonance spectroscopy: elucidation of the dominant pathway for neurotransmitter glutamate repletion and measurement of astrocytic oxidative metabolism. *J. Neurosci.* 22, 1523–1531.

Lee, J. Y., Song, J., Kwon, K., Jang, S., Kim, C., Baek, K., et al. (2012). Human DJ-1 and its homologs are novel glyoxalases. *Hum. Mol. Genet.* 21, 3215–3225. doi: 10.1093/hmg/dds155

Lovatt, D., Sonnewald, U., Waagepetersen, H. S., Schousboe, A., He, W., Lin, J. H., et al. (2007). The transcriptome and metabolic gene signature of protoplasmic astrocytes in the adult murine cortex. *J. Neurosci.* 27, 12255–12266. doi: 10.1523/JNEUROSCI.3404-07.2007

Luth, H. J., Ogunlade, V., Kuhla, B., Kientsch-Engel, R., Stahl, P., Webster, J., et al. (2005). Age- and stage-dependent accumulation of advanced glycation end products in intracellular deposits in normal and Alzheimer's disease brains. *Cereb. Cortex* 15, 211–220. doi: 10.1093/cercor/bhh123

Magistretti, P. J. (2009). Role of glutamate in neuron-glia metabolic coupling. *Am. J. Clin. Nutr.* 90, 875S–880S. doi: 10.3945/ajcn.2009.27462CC

Mamczur, P., Borsuk, B., Paszko, J., Sas, Z., Mozrzymas, J., Wisniewski, J. R., et al. (2014). Astrocyte-neuron crosstalk regulates the expression and subcellular localization of carbohydrate metabolism enzymes. *Glia* 63, 328–340. doi: 10.1002/glia.22753

McMurray, K. M., Distler, M. G., Sidhu, P. S., Cook, J. M., Arnold, L. A., Palmer, A. A., et al. (2014). Glo-1 inhibitors for neuropsychiatric and anti-epileptic drug development. *Biochem. Soc. Trans.* 42, 461–467. doi: 10.1042/BST20140027

Mink, J. W., Blumenschine, R. J., and Adams, D. B. (1981). Ratio of central nervous system to body metabolism in vertebrates: its constancy and functional basis. *Am. J. Physiol.* 241, R203–R212.

Molina, D. K., and DiMaio, V. J. (2012). Normal organ weights in men: part II-the brain, lungs, liver, spleen, and kidneys. *Am. J. Forensic Med. Pathol.* 33, 368–372. doi: 10.1097/PAF.0b013e31823d29ad

Morcos, M., Du, X., Pfisterer, F., Hutter, H., Sayed, A. A., Thornalley, P., et al. (2008). Glyoxalase-1 prevents mitochondrial protein modification and enhances lifespan in *Caenorhabditis elegans*. *Aging Cell* 7, 260–269. doi: 10.1111/j.1474-9726.2008.00371.x

More, S. S., Vartak, A. P., and Vince, R. (2013). Restoration of glyoxalase enzyme activity precludes cognitive dysfunction in a mouse model of Alzheimer's disease. *ACS Chem. Neurosci.* 4, 330–338. doi: 10.1021/cn3001679

Munch, G., Westcott, B., Menini, T., and Gugliucci, A. (2012). Advanced glycation endproducts and their pathogenic roles in neurological disorders. *Amino Acids* 42, 1221–1236. doi: 10.1007/s00726-010-0777-y

Nehlig, A., Wittendorp-Rechenmann, E., and Lam, C. D. (2004). Selective uptake of [14C]2-deoxyglucose by neurons and astrocytes: high-resolution microautoradiographic imaging by cellular 14C-trajectography combined with immunohistochemistry. *J. Cereb. Blood Flow Metab.* 24, 1004–1014. doi: 10.1097/01.WCB.0000128533.84196.D8

Patel, A. B., Lai, J. C., Chowdhury, G. M., Hyder, F., Rothman, D. L., Shulman, R. G., et al. (2014). Direct evidence for activity-dependent glucose phosphorylation in neurons with implications for the astrocyte-to-neuron lactate shuttle. *Proc. Natl. Acad. Sci. U.S.A.* 111, 5385–5390. doi: 10.1073/pnas.1403576111

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. doi: 10.1073/pnas.91.22.10625

Pellerin, L., and Magistretti, P. J. (2012). Sweet sixteen for ANLS. *J. Cereb. Blood Flow Metab.* 32, 1152–1166. doi: 10.1038/jcbfm.2011.149

Pieroh, P., Birkenmeier, G., and Dehghani, F. (2014). The temporal and spatial dynamics of glyoxalase I following excitoxicity and brain ischaemia. *Biochem. Soc. Trans.* 42, 534–537. doi: 10.1042/BST20140022

Porras, O. H., Loaiza, A., and Barros, L. F. (2004). Glutamate mediates acute glucose transport inhibition in hippocampal neurons. *J. Neurosci.* 24, 9669–9673. doi: 10.1523/JNEUROSCI.1882-04.2004

Prapong, T., Buss, J., Hsu, W. H., Heine, P., West, G. H., and Uemura, E. (2002). Amyloid beta-peptide decreases neuronal glucose uptake despite causing increase in GLUT3 mRNA transcription and GLUT3 translocation to the plasma membrane. *Exp. Neurol.* 174, 253–258. doi: 10.1006/exnr.2001.7861

Qu, H., Haberg, A., Haraldseth, O., Unsgard, G., and Sonnewald, U. (2000). (13)C MR spectroscopy study of lactate as substrate for rat brain. *Dev. Neurosci.* 22, 429–436. doi: 10.1159/000017472

Rabbani, N., and Thornalley, P. J. (2010). Methylglyoxal, glyoxalase 1 and the dicarbonyl proteome. *Amino Acids* 42, 1133–1142. doi: 10.1007/s00726-010-0783-0

Radu, B. M., Dumitrescu, D. I., Mustaciosu, C. C., and Radu, M. (2012). Dual effect of methylglyoxal on the intracellular Ca(2+) signaling and neurite outgrowth in mouse sensory neurons. *Cell. Mol. Neurobiol.* 32, 1047–1057. doi: 10.1007/s10571-012-9823-5

Ramasamy, R., Vannucci, S. J., Yan, S. S., Herold, K., Yan, S. F., and Schmidt, A. M. (2005). Advanced glycation end products and RAGE: a common thread in aging, diabetes, neurodegeneration, and inflammation. *Glycobiology* 15, 16R–28R. doi: 10.1093/glycob/cwi053

Ranganathan, S., Walsh, E. S., and Tew, K. D. (1995). Glyoxalase I in detoxification: studies using a glyoxalase I transfectant cell line. *Biochem. J.* 309, 127–131.

Richard, J. P. (1991). Kinetic parameters for the elimination reaction catalyzed by triosephosphate isomerase and an estimation of the reaction's physiological significance. *Biochemistry* 30, 4581–4585. doi: 10.1021/bi00232a031

Richard, J. P. (1993). Mechanism for the formation of methylglyoxal from triosephosphates. *Biochem. Soc. Trans.* 21, 549–553. doi: 10.1042/bst0210549

Rothstein, J. D., Dykes-Hoberg, M., Pardo, C. A., Bristol, L. A., Jin, L., Kuncl, R. W., et al. (1996). Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. *Neuron* 16, 675–686. doi: 10.1016/S0896-6273(00)80086-0

Rouach, N., Koulakoff, A., Abudara, V., Willecke, K., and Giaume, C. (2008). Astroglial metabolic networks sustain hippocampal synaptic transmission. *Science* 322, 1551–1555. doi: 10.1126/science.1164022

Schurr, A., Payne, R. S., Miller, J. J., and Rigor, B. M. (1997). Brain lactate is an obligatory aerobic energy substrate for functional recovery after hypoxia: further *in vitro* validation. *J. Neurochem.* 69, 423–426. doi: 10.1046/j.1471-4159.1997.69010423.x

Serres, S., Bezancon, E., Franconi, J. M., and Merle, M. (2005). *Ex vivo* NMR study of lactate metabolism in rat brain under various depressed states. *J. Neurosci. Res.* 79, 19–25. doi: 10.1002/jnr.20277

Shin, M. J., Kim, D. W., Lee, Y. P., Ahn, E. H., Jo, H. S., Kim, D. S., et al. (2014). Tat-glyoxalase protein inhibits against ischemic neuronal cell damage and ameliorates ischemic injury. *Free Radic. Biol. Med.* 67, 195–210. doi: 10.1016/j.freeradbiomed.2013.10.815

Shinohara, M., Thornalley, P. J., Giardino, I., Beisswenger, P., Thorpe, S. R., Onorato, J., et al. (1998). Overexpression of glyoxalase-I in bovine endothelial cells inhibits intracellular advanced glycation endproduct formation and prevents hyperglycemia-induced increases in macromolecular endocytosis. *J. Clin. Invest.* 101, 1142–1147. doi: 10.1172/JCI119885

Shulman, R. G., Rothman, D. L., Behar, K. L., and Hyder, F. (2004). Energetic basis of brain activity: implications for neuroimaging. *Trends Neurosci.* 27, 489–495. doi: 10.1016/j.tins.2004.06.005

Sibson, N. R., Shen, J., Mason, G. F., Rothman, D. L., Behar, K. L., and Shulman, R. G. (1998). Functional energy metabolism: *in vivo* 13C-NMR spectroscopy evidence for coupling of cerebral glucose consumption and glutamatergic neuronalactivity. *Dev. Neurosci.* 20, 321–330. doi: 10.1159/000017327

Thimmulappa, R. K., Mai, K. H., Srisuma, S., Kensler, T. W., Yamamoto, M., and Biswal, S. (2002). Identification of Nrf2-regulated genes induced by the chemopreventive agent sulforaphane by oligonucleotide microarray. *Cancer Res.* 62, 5196–5203.

Thornalley, P. J. (1993). The glyoxalase system in health and disease. *Mol. Aspects Med.* 14, 287–371. doi: 10.1016/0098-2997(93)90002-U

Thornalley, P. J. (1996). Pharmacology of methylglyoxal: formation, modification of proteins and nucleic acids, and enzymatic detoxification–a role in pathogenesis and antiproliferative chemotherapy. *Gen. Pharmacol.* 27, 565–573. doi: 10.1016/0306-3623(95)02054-3

Thornalley, P. J. (2005). Dicarbonyl intermediates in the maillard reaction. *Ann. N.Y. Acad. Sci.* 1043:111–117. doi: 10.1196/annals.1333.014

Thornalley, P. J. (2007). Endogenous alpha-oxoaldehydes and formation of protein and nucleotide advanced glycation endproducts in tissue damage. *Novartis Found. Symp.* 285, 229–243. doi: 10.1002/9780470511848.ch17

Thornalley, P. J. (2008). Protein and nucleotide damage by glyoxal and methylglyoxal in physiological systems–role in ageing and disease. *Drug Metabol. Drug Interact.* 23, 125–150. doi: 10.1515/DMDI.2008.23.1-2.125

Toyoda, Y., Erkut, C., Pan-Montojo, F., Boland, S., Stewart, M. P., Muller, D. J., et al. (2014). Products of the Parkinson's disease-related glyoxalase DJ-1, D-lactate and glycolate, support mitochondrial membrane potential and neuronal survival. *Biol. Open* 3, 777–784. doi: 10.1242/bio.20149399

Vander Jagt, D. L., and Hunsaker, L. A. (2003). Methylglyoxal metabolism and diabetic complications: roles of aldose reductase, glyoxalase-I, betaine aldehyde dehydrogenase and 2-oxoaldehyde dehydrogenase. *Chem. Biol. Interact.* 143–144, 341–351. doi: 10.1016/S0009-2797(02)00212-0

Vargas, M. R., and Johnson, J. A. (2009). The Nrf2-ARE cytoprotective pathway in astrocytes. *Expert Rev. Mol. Med.* 11, e17. doi: 10.1017/S1462399409001094

Vilchez, D., Ros, S., Cifuentes, D., Pujadas, L., Valles, J., Garcia-Fojeda, B., et al. (2007). Mechanism suppressing glycogen synthesis in neurons and its demise in progressive myoclonus epilepsy. *Nat. Neurosci.* 10, 1407–1413. doi: 10.1038/nn1998

Wautier, J. L., and Guillausseau, P. J. (2001). Advanced glycation end products, their receptors and diabetic angiopathy. *Diabetes Metab.* 27, 535–542. doi: DM-11-2001-27-5-1262-3636-101019-ART1

Wong, A., Luth, H. J., Deuther-Conrad, W., Dukic-Stefanovic, S., Gasic-Milenkovic, J., Arendt, T., et al. (2001). Advanced glycation endproducts co-localize with inducible nitric oxide synthase in Alzheimer's disease. *Brain Res.* 920, 32–40. doi: 10.1016/S0006-8993(01)02872-4

Wu, L., Eftekharpour, E., Davies, G. F., Roesler, W. J., and Juurlink, B. H. (2001). Troglitazone selectively inhibits glyoxalase I gene expression. *Diabetologia* 44, 2004–2012. doi: 10.1007/s001250100004

Xue, M., Rabbani, N., Momiji, H., Imbasi, P., Anwar, M. M., Kitteringham, N., et al. (2012). Transcriptional control of glyoxalase 1 by Nrf2 provides a stress-responsive defence against dicarbonyl glycation. *Biochem. J.* 443, 213–222. doi: 10.1042/BJ20111648

Yamamoto, T., Tanaka, S., Suga, S., Watanabe, S., Nagatomo, K., Sasaki, A., et al. (2011). Syntheses of 2-NBDG analogues for monitoring stereoselective uptake of D-glucose. *Bioorg. Med. Chem. Lett.* 21, 4088–4096. doi: 10.1016/j.bmcl.2011.04.148

Zhang, Y., Chen, K., Sloan, S. A., Bennett, M. L., Scholze, A. R., O'Keeffe, S., et al. (2014). An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *J. Neurosci.* 34, 11929–11947. doi: 10.1523/JNEUROSCI.1860-14.2014

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 23 October 2014; accepted: 13 January 2015; published online: 09 February 2015.
*Citation: Allaman I, Bélanger M and Magistretti PJ (2015) Methylglyoxal, the dark side of glycolysis. Front. Neurosci. **9**:23. doi: 10.3389/fnins.2015.00023*

This article was submitted to Neuroenergetics, Nutrition and Brain Health, a section of the journal Frontiers in Neuroscience.

frontiers in
NEUROSCIENCE

REVIEW ARTICLE
published: 09 February 2015
doi: 10.3389/fnins.2014.00408

Cerebral metabolism following traumatic brain injury: new discoveries with implications for treatment

George A. Brooks[1]* and Neil A. Martin[2]

[1] *Exercise Physiology Laboratory, Department of Integrative Biology, University of California, Berkeley, Berkeley, CA, USA*
[2] *Department of Neurosurgery, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA, USA*

Edited by:
Suzanne L. Dickson, The Sahlgrenska Academy at the University of Gothenburg, Sweden

Reviewed by:
Avital Schurr, University of Louisville, USA
L. Bruce Gladden, Auburn University, USA

****Correspondence:***
George A. Brooks, Department of Integrative Biology, University of California, Berkeley, 5101 VLSB, Berkeley, CA 94720-3140, USA
e-mail: gbrooks@berkeley.edu

Because it is the product of glycolysis and main substrate for mitochondrial respiration, lactate is the central metabolic intermediate in cerebral energy substrate delivery. Our recent studies on healthy controls and patients following traumatic brain injury (TBI) using [6,6-$^{2}H_2$]glucose and [3-^{13}C]lactate, along with cerebral blood flow (CBF) and arterial-venous (jugular bulb) difference measurements for oxygen, metabolite levels, isotopic enrichments and $^{13}CO_2$ show a massive and previously unrecognized mobilization of lactate from corporeal (muscle, skin, and other) glycogen reserves in TBI patients who were studied 5.7 ± 2.2 days after injury at which time brain oxygen consumption and glucose uptake ($CMRO_2$ and CMRgluc, respectively) were depressed. By tracking the incorporation of the ^{13}C from lactate tracer we found that gluconeogenesis (GNG) from lactate accounted for 67.1 ± 6.9%, of whole-body glucose appearance rate (Ra) in TBI, which was compared to 15.2 ± 2.8% (mean ± SD, respectively) in healthy, well-nourished controls. Standard of care treatment of TBI patients in state-of-the-art facilities by talented and dedicated heath care professionals reveals presence of a catabolic Body Energy State (BES). Results are interpreted to mean that additional nutritive support is required to fuel the body and brain following TBI. Use of a diagnostic to monitor BES to provide health care professionals with actionable data in providing nutritive formulations to fuel the body and brain and achieve exquisite glycemic control are discussed. In particular, the advantages of using inorganic and organic lactate salts, esters and other compounds are examined. To date, several investigations on brain-injured patients with intact hepatic and renal functions show that compared to dextrose + insulin treatment, exogenous lactate infusion results in normal glycemia.

Keywords: lactate shuttle, gluconeogenesis, trauma, brain fuel, brain metabolism

GLYCOLYSIS MAKES LACTATE CONTINUOUSLY

As described by editor Schurr in the introductory chapter, the conclusion that glycolysis makes lactate is a central issue; by definition, glycolysis makes lactate (Schurr, 2014). Whether glycolysis produces acid, or not, is a different, and perhaps unresolved issue. That glycolysis produces lactate normally, under fully aerobic conditions is perhaps best exemplified by results of studies on resting muscle (Stanley et al., 1986; Richardson et al., 1998; Bergman et al., 1999b), the healthy beating heart (Gertz et al., 1981, 1988; Bergman et al., 2009), the normally functioning brain (van Hall et al., 2009; Wyss et al., 2011; Glenn et al., 2015), and in other diverse tissues following enteral carbohydrate nutrition (Foster, 1984; Meyer et al., 2002). That lactate production is greatly increased during muscular exercise can be readily observed (Stanley et al., 1985; Mazzeo et al., 1986; Bergman et al., 1999b; Messonnier et al., 2013), but again lactate production in working muscle during graded exercise up to maximum is not due to oxygen insufficiency (Richardson et al., 1998), and for that matter, the elevation in circulating lactate concentration during prolonged exercise cannot be attributed solely to net lactate release from working muscle (Ahlborg and Felig, 1982; Brooks et al., 1998). Rather, some other large tissue bed, perhaps the integument (Johnson and Fusaro, 1972) is responsible for elevated blood lactate concentration and turnover rates when there is no net lactate release from working muscle (Ahlborg and Felig, 1982; Brooks et al., 1998).

Importance of the fact that lactate can be produced in particular cellular compartments and from those sites can enter the interstitium and vasculature from where it can reach adjacent or anatomically distributed cells, organs, and tissues to affect important functions was recognized in 1984 (Brooks, 1984). At that time data from several sources were evaluated in light of original data on glucose and lactate fluxes in resting and exercising, untrained and endurance trained laboratory rats (Brooks and Donovan, 1983; Donovan and Brooks, 1983). The exchange of lactate among cellular sites of production and removal was termed the "Lactate Shuttle" (Brooks, 1984).

In humans and other mammals the extent of lactate production is typically overlooked because removal (disposal) rate

balances production (appearance) rate. In other words, with typical concentration measurement technology low and stable blood lactate levels belie high turnover (production and removal) rates. And, even when arterial-venous [a-v] concentration differences and tissue blood flow measurements are available, in the absence of isotope tracer technology turnover rate is unknown because of rapid turnover within the tissue bed of interest. Restated, basing conclusions about the dynamics of lactate or any other metabolite solely on concentration data is intellectually equivalent to taking the US census in the morning and evening of the same day, and then upon seeing no significant change in population numbers concluding that nothing happened in the population during that day. Happily, many entered the population, and regrettably also, some exited. Hence, concentration data provide an incomplete view of lactate turnover (flux) except in conditions of sudden and large changes when homeostatic imbalances in the relationship between production and removal are indicated.

In resting or working muscle (Stanley et al., 1986; Bergman et al., 1999b), the heart in normal sinus rhythm at rest or under load (Gertz et al., 1981, 1988; Bergman et al., 2009), and in other tissues such as skin (Johnson and Fusaro, 1972), lactate production and net release are the norm. Restated, idling or working skeletal muscles and heart simultaneously produce and consume lactate (Stanley et al., 1986; Bergman et al., 1999b) as shown by means of simultaneous tissue blood flow arterial-venous difference ([a-v], [a-v]Δ, or AVD) measures for lactate concentration and isotopic enrichment (Stanley et al., 1986). Depending on conditions such as nutritive state, power output, duration of activity, and arterial lactate concentration, working muscle may switch from net release to consumption (Brooks et al., 1998; Bergman et al., 1999b). As well, tissues other than working muscle can produce lactate, and if arterial lactate concentration rises sufficiently, working muscle can become a lactate consumer as seen in men exercising at sea level, on acute exposure to 4300 m altitude, and after 3 weeks of acclimatization to 4300 m altitude on Pikes Peak (Brooks et al., 1998). For the heart, net uptake is typical (Gertz et al., 1988), with few reports of lactate net release from the working heart (Gertz et al., 1981). In the example shown (**Figure 1**), net lactate release from the legs of a trained bicyclist causes arterial lactate concentration to rise at exercise onset. However, as exercise continues, lactate clearance mechanisms, including lactate uptake and oxidation by the heart reduce the circulating lactate load. Data in **Figure 1** also show that myocardial lactate uptake and oxidation are concentration dependent. In the figure "chemical lactate" extraction is equal to "net uptake" based on [a-v] for chemical lactate concentration and myocardial blood flow, which is equivalent to the cerebral metabolic rate of lactate (CMRlac) terms used in studies of cerebral lactate metabolism employing measurements of [a-v] and cerebral blood flow (CBF). The dependence of cellular lactate exchange on concentration difference is due to the fact that cell membrane lactate transport proteins (Garcia et al., 1994, 1995) are symports that co-transport lactate anions and hydrogen ions (Roth and Brooks, 1990a,b). Typically then, cells release lactate because of intracellular glycolysis and lactate accumulation, whereas lactate consumer cells such as heart, liver, kidneys, and red skeletal muscle contain a high mitochondrial density which lowers intracellular [lactate] relative to arterial and interstitial [lactate]. In the brain, as in other tissues lactate exchange among neurons and other cell types depends not only on the metabolic rates within cells, but also on the extracellular environment which is influenced by cellular metabolism as well as vascular delivery and removal.

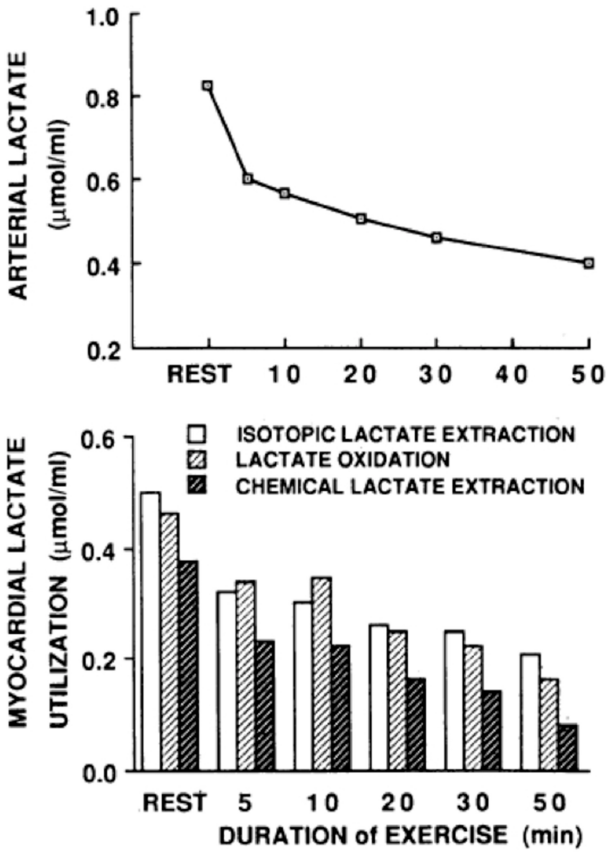


FIGURE 1 | Arterial lactate level (upper graph) and a comparison of myocardial tracer-measured (isotopic) lactate extraction, lactate oxidation, and chemical extraction based on (a-v) and myocardial blood flow (lower graph) are shown for a trained bicyclist whose arterial [lactate] fell during moderate-intensity exercise. The results show that net lactate exchange measurements underestimate total lactate production, that most lactate produced is oxidized, and that lactate uptake is concentration dependent. From Gertz et al. (1988).

Given the existing literature on lactate metabolism in human muscle and heart it should not be surprising that the healthy human brain displays similar features (Glenn et al., 2003, 2015; van Hall et al., 2009; Wyss et al., 2011), brain lactate turnover is the norm and occurs during net release as well as net uptake. In brain, as well as other tissues, glucose is taken up, glycolysis occurs, and lactate is produced. However, because lactate is also taken up, the CMR for lactate depends on the balance of cerebral production plus uptake vs. disposal, which is mainly oxidation (Glenn et al., 2015).

Science is difficult enough, and in the case of lactate metabolism understanding is complicated by the use of different

terms in different fields. Hence, we need to define terms as they appear in this review. For brain, cerebral metabolic rate for glucose (CMRgluc) equals the arterial-venous difference for glucose (i.e., [a-v]gluc) times CBF: CMRgluc = (AVDgluc) (CBF). Similarly, for lactate (CMRlac) equals the arterial-venous difference ([a-v]lac) times CBF: CMRlac = (AVDlac) (CBF). As already noted, in skeletal (Stanley et al., 1986; Bergman et al., 1999b) and cardiac muscle (Gertz et al., 1988; Bergman et al., 2009), and lung (Johnson et al., 2012) physiology, the term net lactate exchange is equivalent to CMRlac and in these instances the negative sign (−) indicates net lactate release, whereas a positive sign (+) indicates net lactate uptake. For **Figure 2** on cerebral net lactate exchange (>0 sign, and dark area) indicates net cerebral lactate release, whereas the (<0) sign and light area indicates lactate uptake as shown for control subjects and patients after suffering traumatic brain injury (TBI). In the first days following injury most patients display net cerebral lactate uptake transitioning to net release as in controls after several days of intensive care. This said, whether positive or negative, the CMR for lactate underestimates production because of simultaneous production and removal within brain tissue (van Hall et al., 2009; Glenn et al., 2015) as it does in other tissues such as skeletal muscle (Stanley et al., 1986; Bergman et al., 1999b) and heart (Gertz et al., 1988).

As illustrated by the use of equivalent terms "net glucose uptake" and "CMRgluc" in muscle and brain metabolism fields, a larger hurdle must be negotiated to achieve understanding of the significance of tissue turnover. For tissues such as muscle and brain that are net glucose consumers, tracer- and chemical measures of glucose yield equivalent results because glucose is not produced in muscle (Bergman et al., 1999a) or brain (Glenn et al., 2015). However, muscle (Stanley et al., 1986; Bergman et al., 1999b), and brain (van Hall et al., 2009; Glenn et al., 2015) simultaneously consume and produce lactate. Therefore, to know muscle or brain lactate turnover rate both uptake and release need to be measured and summed to calculate total lactate production.

Realization that tissue "net lactate" and "CMRlac" provide data on only part of the story leads to insight into meaning of the term "lactate production," and its use, or misuse as too often investigators will mistake the terms lactate "accumulation" and "net release" to mean "lactate production." Such misuse of terminology is more than careless; the misuse reflects lack of understanding and can lead to misinterpretation of data and missed opportunities for intervention in the intensive care unit (ICU).

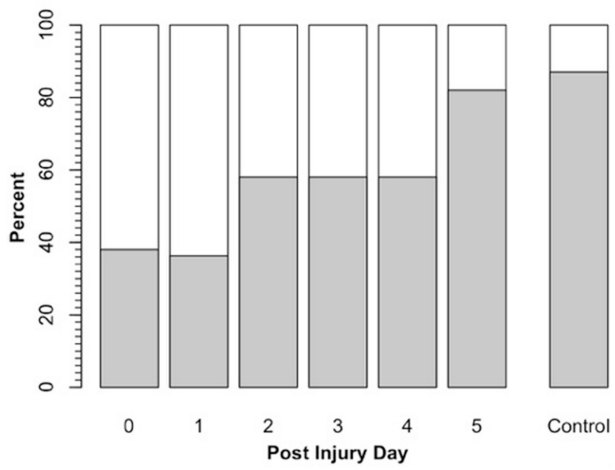


FIGURE 2 | Cerebral metabolic rate (CMR) for chemical lactate [CMRlac = (CBF) (a-v)lac] over time in patients with severe TBI. To illustrate the change in CMRlac over time, data presented as percentage of patients demonstrating net cerebral lactate uptake (i.e., CMRlac < 0, white area) compared percentage of patients demonstrating cerebral net lactate release (i.e., CMRlac > 0, dark area). Patients display wide variability and significant changes over time with regression to control values of net cerebral lactate release over time. As illustrated in **Figure 1**, CMRlac underestimates total lactate production. Redrawn from Glenn et al. (2003) and ongoing studies with control values courtesy of T. C. Glenn.

GLYCOLYSIS AND "HYPERGLYCOLYSIS" AFTER CEREBRAL INJURY

Glucose uptake and glycolysis are features of normal cerebral metabolism, but trauma affects CMRgluc and glycolysis in specific ways. In particular, there is a relative increase in glycolysis, a "hyper-glycolysis" that is a recognized feature of cerebral metabolism immediately after injury (Bergsneider et al., 1997). As part of the sympathetically mediated fight and flight mechanism (Selye, 1950; Selye and Fortier, 1950), both glycogen and glucose are precursors to glycolytic flux. However, compared to muscle in which glycogen content is typically 1.25 g/100 g (1 mMol/100 g tissue) (Hultman, 1967; Krssak et al., 2000), and liver in which glycogen storage can be several times greater than in muscles of well-nourished individuals (20–40 mMol/100 g tissue) (Nilsson and Hultman, 1974; Roden et al., 2001), cerebral glycogen content is very small (0.3–0.4 μMol/100 g tissue) (Oz et al., 2007). Accordingly, it is understandable that cerebral glycogen is rapidly recruited and depleted following acute injury. Limited cerebral glycogen reserves point to the importance of the continuous delivery of carbohydrate energy sources to the brain, always (Glenn et al., 2003; van Hall et al., 2009), and especially following injury (Glenn et al., 2003; Vespa et al., 2012). Not to confuse cerebral substrate delivery and use with the US Federal Budget, the human brain is definitely "pay as you go," with deficits in oxygen, substrate, both or either, having significant effects on tissue function and viability.

In the field of cerebral metabolism, the term "hyperglycolysis" is a relative term used to connote the extent of metabolic crisis following injury. Following injury, depression of brain functions are indicated by the decrements in cerebral oxygen consumption rate ($CMRO_2$) as well as glucose uptake and use (CMRgluc). Hence, the presence of hyperglycolysis is indicated by a fall in the normal molar ratio (MR), or "Metabolic Ratio" of 6/1 for $CMRO_2$/CMRgluc, the stoichiometry for complete glucose oxidation being given as: $6\ O_2 + C_6H_{12}O_2 \rightarrow 6\ CO_2 + 6\ H_2O$. The fall in cerebral MR following injury is indicative of a relative increase in the role of glycolysis leading to cerebral lactate production (Glenn et al., 2015), as well as an increase in pentose phosphate pathway activity (Dusick et al., 2007). Reiterated, the state of "hyperglycolysis" indicates an increase in glycolysis relative to cerebral oxygen consumption.

Because brain cells depend on glycolysis from carbohydrate energy sources, while at the same time possessing limited capacity for glycogen reserves, the importance of cerebral glucose delivery in health and disease cannot be overstated. The homeostatic setpoint for blood glucose concentration is 5 mM (≈100 mg%), whereas typical circulating blood [lactate] is <1 mM (<9 mg%), and [pyruvate] and [β-OH butyrate] are in the μM range (<1 mg%). Restated, vascular glucose mass is 10-fold or more greater than the circulating alternative CHO energy sources combined. Hence, it is to be expected that maintaining euglycemia from exogenous nutritional support is of major importance in the neural ICU. This can perhaps be illustrated best by the better outcomes of TBI patients as a result of careful assessment and early enteral and parenteral nutritional support (Wang et al., 2013).

However, without knowledge of the metabolic and nutritional status of the individual patient, the practice of medicine using means-based administration of nutritional support and insulin therapy (van den Berghe et al., 2001) can result in over- and underfeeding, hyper- and hypoglycemia (Van den Berghe et al., 2006; Henderson and Finfer, 2009; Marik, 2009; Myburgh and Chittock, 2009) because assessments of the patient are based solely on blood metabolite concentration measurements. The use of [glucose] and the treatment of hyper- and hypoglycemia with insulin without consideration of individual flux can potentially result in medical care that creates the condition that is then subsequently treated. Infusion of exogenous glucose at a rate greater than patients' individual glucose turnover will cause hyperglycemia. This is then treated using insulin to maintain glycemia. A better approach would be to utilize the autoregulatory functionality that is intact (Glenn et al., 2014) and monitor glucose production rate (i.e., Ra) to control the individual patient's [glucose]. Given current and anticipated technological advances, such as stable isotope technology and rapid analysis, it should be possible for attending clinicians to continually monitor an individual patient's body energy state (BES) and to deliver precision nutritive care by adjusting enteral and parenteral nutrient delivery over the course of treatment. Restated, while the field of nutrition and pathophysiological metabolic pathways is pursued heavily by scientists and physicians worldwide, and late vs. early enteral and parenteral guidelines are desired for the treatment of the critically ill and injured (Casaer et al., 2011b), means based titrating blood [glucose] with insulin (Meijering et al., 2006) or standardized equations describing caloric needs for healthy individuals (Harris and Benedict, 1918) cannot describe the intra-patient metabolic and nutritional variability (Glenn et al., 2014).

HINTS AT ELEVATED CEREBRAL LACTATE METABOLISM POST-INJURY

Brain injuries are complicated and produce highly variable effects, not only between patients, but temporally within patients with seemingly confounding and contradictory effects. For example, hyperglycolysis is typical post-TBI (Bergsneider et al., 1997), and was observed by Glenn et al., who saw TBI to significantly decrease cerebral MR (Glenn et al., 2003). However, even though injury depressed cerebral MR, many TBI patients demonstrated cerebral net lactate uptake in the hours post injury (**Figure 2**). Hence, puzzlingly hyperglycolysis coincided with a positive CMRlac, or increased net lactate uptake. UCLA investigators took the apparently paradoxical results to indicate that something extraordinary was happening to brain CHO metabolism post injury (Glenn et al., 2003).

That the human brain could take up and use lactate as a fuel, not simply take up and accumulate lactate was entirely consistent with extensive literature reports on cerebral metabolism in laboratory rodents and rodent brain slices and tissue preparations. Not only do astrocytes and neurons contain lactate transporters (MCTs) necessary for cellular uptake (Pellerin et al., 2005; Pierre and Pellerin, 2005), but importantly also mitochondria of neurons contain MCTs and other components of the mitochondrial lactate oxidation complex (mLOC) (Hashimoto et al., 2008) necessary for neuronal lactate oxidation. Again, as described in the introductory chapter (Schurr, 2014), in comparison to glucose, rodent brain slices and neuronal preparations preferentially take up and oxidize lactate in comparison to glucose (Schurr, 2006, 2008; Schurr and Payne, 2007). Although controversial a decade ago (Glenn et al., 2003), the hint of cerebral lactate oxidation following injury has been elaborated upon with results of basic science studies on laboratory animals (Schurr and Payne, 2007) and clinical research studies on humans (Smith et al., 2003; Gallagher et al., 2009; Ichai et al., 2009, 2013; Wyss et al., 2011; Jalloh et al., 2013; Bouzat et al., 2014; Glenn et al., 2014).

BODY SUPPORTS THE BRAIN

Along the way to studying cerebral glucose-lactate interactions in patients following TBI an unexpected result emerged on our TBI patients given standard of care treatment in state of the art facilities by the dedicated and highly trained health care professionals. This was the herculean effort of the body to mobilize energy resources for the injured brain (Glenn et al., 2014). With reference **Figure 2**, patients were enrolled in our isotope tracer studies involving use of primed-continuous infusions of [6,6-$^{2}H_{2}$]glucose (i.e., D2-glucose) and [3-^{13}C]lactate to track whole-body and cerebral glucose-lactate interactions in TBI. Patients were studied as soon as permission of legal representatives could be obtained, usually 96–140 h after injury at which time numerous parameters were approaching control levels. The time lag had to do with mechanics of securing permission of patients' legal representatives to conduct studies on patients receiving standard of care treatment. Consequently, at the time of study arterial glucose (**Figure 3A**) and lactate (**Figure 3B**) concentrations in patients were not significantly different from those in fasting control subjects. However, compared to values in control subjects, in TBI patients CMRgluc was significantly depressed (**Figure 4A**) ($p < 0.01$), whereas consistent with **Figure 2** values, CMRlac was similar to control values (**Figure 4B**).

As with glucose and lactate concentration levels, whole body glucose turnover (Ra shown) in treated TBI patients was similar to values in controls (**Figure 5A**). Due to variability in TBI patients, the slightly higher glucose Ra in patients was not significantly different ($P = 0.06$), but trends toward indicating a hypermetabolic state, or, in comparison to exercising controls, a moderate intensity of exercise similar to a brisk walk. Again the intra-patient variability demonstrated that some patients were more hypermetabolic than others, and therefore would

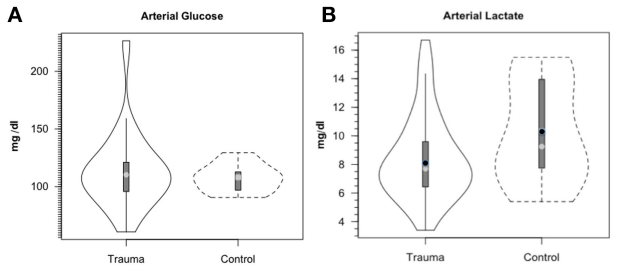


FIGURE 3 | Arterial glucose (A) and lactate concentrations (B) in control and TBI patients. Values were constant over time, so mean values for min 60, 90, and 120 min are shown. Solid lines represent TBI patients ($n = 12$) while dashed lines are normal control subjects ($n = 6$). This and subsequent figures depict the following components: median (light circle), mean (dark circle), standard deviation (heavy vertical bar), box-plot whisker (thin vertical bar) and a kernel density estimation of the data distribution (replacing the box-plot's rectangular depiction) following Hintze and Nelson (1998) as visualized by R package "Caroline" (Schruth, 2012). When coincident, the median circle symbol obscures the mean symbol. TBI patients demonstrated arterial glucose and lactate levels similar to those in healthy control subjects. From Glenn et al. (2014).

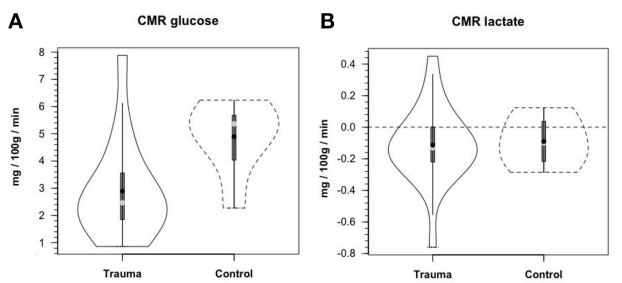


FIGURE 4 | Cerebral metabolic rates (CMR) for glucose (A) and lactate (B), in control and TBI patients; CMR = (Metabolite AVD) (Cerebral Blood Flow), alternatively termed net exchange. Values were constant over time, so mean values for min 60, 90, and 120 min are shown. Solid lines represent TBI patients ($n = 12$) while dashed lines are normal control subjects (n–6); other aspects of the figure described in the legend to **Figure 3**, above. Figures show net glucose uptake and net lactate release. Compared to values in controls, CMRgluc, but not CMRlac, was depressed in TBI patients. From Glenn et al. (2015).

require increased nutritional support as their body energy stores are depleted more rapidly. However, even though arterial [lactate] was similar in control subjects and patients, blood lactate turnover (Ra shown) was elevated by ≈90% in TBI patients compared to controls (**Figure 5B**). Because in this investigation we lacked AVD and blood flow measurements across tissues other than brain, we can only speculate that the sites of lactate release were muscle (Bergman et al., 1999b) and the integument (Johnson and Fusaro, 1972) as is found in normal physiology. Since blood glucose and lactate concentrations were similar in controls and patients the natural tendency was to conclude that not much was happening other than blood glucose homeostasis had been achieved. However, such a conclusion would not have been correct.

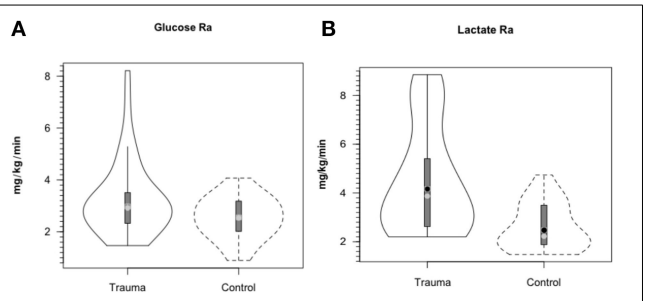


FIGURE 5 | Hepatic (+ renal) glucose production, Ra (A) and body lactate appearance rate (B) in control and TBI patients. Values were constant over time, so mean values for min 60, 90, and 120 min are shown. Solid lines represent TBI patients while dashed lines are normal control subjects. Due to variability in TBI patients, the tendency for higher glucose production was NSD ($P = 0.06$). However, whole-body production was ≈ 90% higher in TBI patients than healthy controls ($P < 0.01$). From (Glenn et al., 2014).

Because we could measure the incorporation of ^{13}C into blood glucose from infused [3-^{13}C]lactate, the rate of gluconeogenesis (GNG) from lactate and the percentage of circulating glucose formed from lactate could be measured. Compared to values of 15.2% of glucose Ra from lactate in control subjects, most (67.1%) circulating glucose in TBI patients came from lactate (**Figure 6**). These dramatic, and fundamentally paradigm changing results made possible because of the use of dual isotope tracer studies would not have been envisioned from contemporary measures of blood metabolite concentrations (**Figures 3A,B**). With the linked-goals of reducing time in the intensive care unit and improving patient outcomes, and recognition that TBI patients given best standard of care treatment are in a catabolic state, presents challenges, but offers opportunities to improve TBI patient care and improve patient outcomes by providing nutritive support to the injured, but recovering brain.

BRAIN RUNS ON LACTATE BOTH DIRECTLY AND INDIRECTLY

The central role of glucose in sustaining brain metabolism has long been recognized (Scheinberg and Stead, 1949; Scheinberg et al., 1965; Cahill et al., 1968; Sokoloff, 1973; Dienel and Hertz, 2001), with classic and contemporary results (Glenn et al., 2003, 2015) in accord. These rare, but extremely important data contribute in major ways to the understanding of cerebral metabolism in normal and pathological conditions, including the formation of dietary reference intakes for carbohydrate nutrition (Medicine, 2005), the values for Estimated Daily Requirement (EAR) and Recommended dietary Allowance (RDA = EAR ± 2SD) for carbohydrate to provide cerebral needs 100 and 130 g/day, respectively.

Contemporary measurements show that despite hyperglycolysis on injury, CMRgluc is decreased in the days following TBI (**Figure 4A**) (Glenn et al., 2015). Again, in the patients studied, chemical concentration based measurement of CMRlac was not significantly different from control values (**Figure 4B**) ($P > 0.05$). Like decreased $CMRO_2$, decreased CMRgluc following TBI may reflect the presence of intrinsic cerebral mechanisms to allow the injured brain to "rest" in recovery. Alternatively, decreased

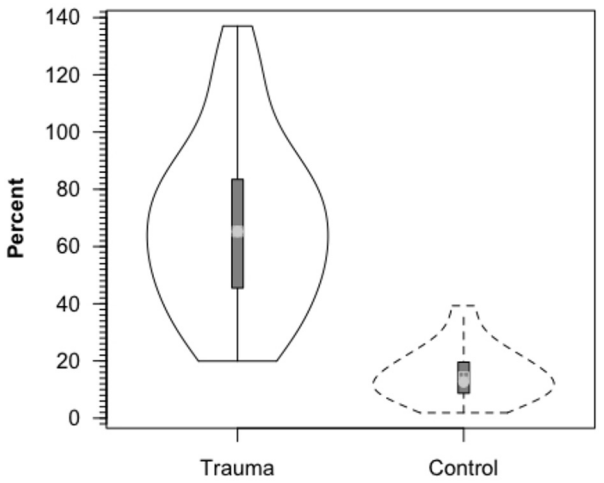


FIGURE 6 | Percent contribution of lactate to glucose production (gluconeogenesis, GNG) in healthy controls (dashed lines, $n = 6$) and TBI patients (solid lines, $n = 12$). Values significantly greater following TBI, $p < 0.05$. Trauma caused a major change in gluconeogenesis from lactate. From Glenn et al. (2014).

cerebral metabolism following injury may be representative of an injury-induced metabolic crisis, the latter being associated with poor patient outcomes (Stein et al., 2012). Hence, the emphasis in this review is to optimize substrate availability to promote healing of the injured brain.

By comparison with results from studies on healthy control subjects the importance of GNG from lactate in supplying glucose for the brain is shown in **Figure 7**. In TBI patients the pattern of glucose production from hepatic gluconeogenesis (GNG) vs. glycogenolysis (GLY) is very different from that in healthy, postabsorptive control subjects in whom GNG contributes only 16% of hepatic glucose Ra whereas GNG contributes 64% to glucose Ra in TBI. Clearly, following TBI endogenously produced lactate definitively supports brain metabolism via GNG; we term this "indirect" brain fueling following TBI (Glenn et al., 2015). However, lactate is also directly taken up and oxidized by injured and healthy brains; we term this "direct" brain fueling by lactate.

Until advent of advanced technologies, such as the use of isotope tracers (van Hall et al., 2009; Glenn et al., 2015), cerebral lactate metabolism in healthy and brain-injured individuals was anticipated from results of AVDlac and CMRlac measurements (**Figures 2**, **4B**) and experimentation on animal brain preparations (Schurr, 2008), but the extent of cerebral lactate turnover could not be assessed because of simultaneous lactate production and oxidative disposal within the tissue.

Figure 8A shows cerebral lactate fractional exaction (FExlac) to approximate 10% in both healthy control subjects and those suffering TBI (Glenn et al., 2015). Incidentally, the FEx for glucose also approximates 10% in healthy subjects and TBI patients (Glenn et al., 2015), so the value of 10% FEx for lactate is in the range of biological plausibility. Nonetheless, knowing CBF, arterial [lactate] and FExlac, cerebral tracer-measured lactate uptake (TMUlac) can be determined and compared to $^{13}CO_2$ excretion. With our moderate and severe TBI patients studied 5.7 ± 2.2 days after injury tracer-measured cerebral lactate uptake was not different from values measured in healthy control subjects (**Figure 8B**). Then, knowing and summing net lactate release (CMRlac) and cerebral tracer-measured lactate uptake (TMUlac), total cerebral lactate production can be determined (**Figure 9**). As shown in **Figure 9**, the conceptual model of cerebral lactate metabolism developed from seeing concentration-based depictions, such as arterial [glucose] and [lactate] (**Figure 3**), and CMRgluc and CMRlac (**Figure 4**), is very different from that developed from knowledge of total cerebral lactate production (**Figure 9**). To reiterate, new information of whole-body and cerebral glucose-lactate interactions show that glucose metabolism is suppressed following TBI, but lactate metabolism is intact. This knowledge provides impetus to explore the possibility of supporting cerebral carbohydrate metabolism and improving patient outcomes following injury by providing formulations containing lactate and other monocarboxylates.

Based on measurements of cerebral tracer-measured lactate uptake (TMU) (**Figure 8B**) and simultaneously measured $^{13}CO_2$ excretion into the jugular bulb, cerebral lactate oxidation is essentially 100% (**Figure 10**). Among various things the data indicate that lactate entering the brains of healthy controls and TBI patients is not simply stored, but utilized as a fuel energy source. Importantly, the results (**Figure 11**) show that the brain oxidizes lactate directly, which is supported by evidence of the mLOC being present in the only species (rat) examined to date (Hashimoto et al., 2008).

With the benefit of simultaneous CBF as well as arterial and jugular bulb concentration and isotope enrichment measurements for glucose and lactate it has been possible to partition total cerebral carbohydrate uptake in healthy controls and TBI patients (**Figure 11**). These results are consistent with what has been known about cerebral metabolism, but importantly they also provide novel insights into cerebral carbohydrate metabolism in healthy control subjects and TBI patients as the sum of glucose and lactate uptake equals total cerebral carbohydrate metabolism.

In healthy controls cerebral glucose uptake accounts for 15–25% of blood glucose disposal (Scheinberg and Stead, 1949; Scheinberg et al., 1965; Cahill et al., 1968; Sokoloff, 1973; Dienel and Hertz, 2001), and for the first time this has been validated using tracer technology (Glenn et al., 2015). The percentage varies depending on conditions, with time since eating a major factor because GNG is suppressed by the arrival of dietary energy, CHO and macronutrients (Trimmer et al., 2002). Now, because of our simultaneous measurements of body and brain glucose and lactate fluxes we gain additional insights into total cerebral carbohydrate metabolism, and therefore introduce the term CMRCHO. In well-nourished postabsorptive controls glucose production from hepatic glycogen provides 76% of cerebral carbohydrate needs, with 15% of the total of glucose produced from lactate via gluconeogenesis, the latter representing the indirect contribution of lactate. Direct cerebral lactate uptake represented 9% of cerebral CHO needs in control subjects (**Figure 11A**). In contrast, while CMRgluc is suppressed following brain injury, and glucose

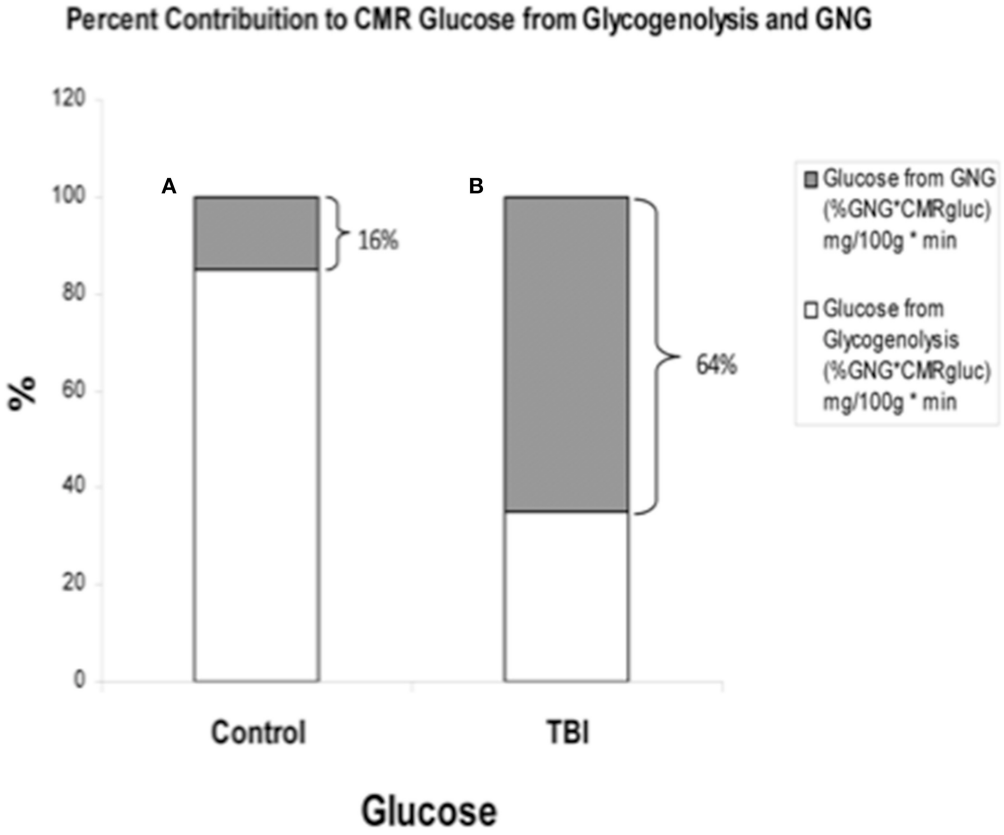


FIGURE 7 | By supporting gluconeogenesis, lactate indirectly supports CMR glucose. The relative contributions to CMR glucose from hepatic gluconeogenesis (GNG), and glucose from hepatic glycogenolysis in healthy control subjects (Left, **A**), and TBI patients (Right, **B**). A comparison of **(A)** (control) and **(B)** (TBI) shows the large increase in percentage cerebral glucose uptake contributed by GNG from lactate following TBI. From (Glenn et al., 2015).

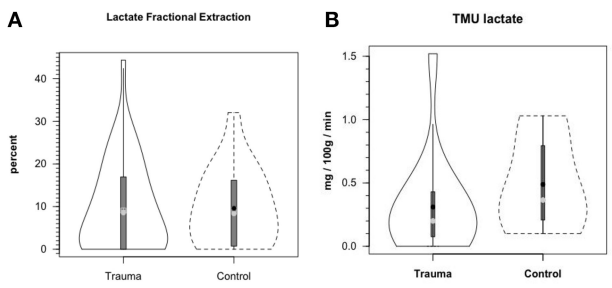


FIGURE 8 | Cerebral lactate fractional extraction (A) and tracer-measured cerebral lactate uptake (B) in healthy control subjects and TBI patients. Solid lines represent TBI patients while dashed lines are normal control subjects. Both fractional extraction and lactate uptake are preserved in TBI patients indicating plausibility of increasing cerebral lactate uptake by raising arterial [lactate] by means of exogenous lactate infusion. From (Glenn et al., 2015).

still represents 90% of CMRCHO, gluconeogenesis from lactate provides 58% CHO energy supply, and the relative contribution of lactate uptake remains at 10% (**Figure 11B**). Hence, most energy supplied to the injured brain comes directly, or indirectly from lactate.

Like **Figures 4A**, **6** shows that CMRgluc is suppressed in TBI patients. However, besides the effect of TBI on cerebral CHO needs and CMRgluc, the side-by-side comparison in **Figure 7** shows a very different pattern of cerebral carbohydrate energy source in patients following TBI. While glucose still provided most CHO-energy, most (59%) glucose came indirectly from lactate via GNG, and 9% came from direct lactate uptake in TBI patients making the total lactate or lactate-derived contribution 68% of total CHO energy as opposed to 25% in controls.

CLINICAL APPLICATIONS: EXOGENOUS LACTATE INFUSION STUDIES

BRAIN NEEDS

As described here and in related papers (Glenn et al., 2014, 2015), several recent realizations lead to the question: "is vascularly infused lactate an alternative brain fuel?" On one hand, if lactate is the major oxidative energy source, how can it be considered as an alternative when it is, in fact "the fuel?" However, on the other hand, if cerebral glycolysis is blocked after injury, then lactate formulations are substances that health care givers can administer to supplement, or augment vascular nutrient delivery to an injured brain. In the latter case, formulations containing lactate salts, esters and other compounds would effectively

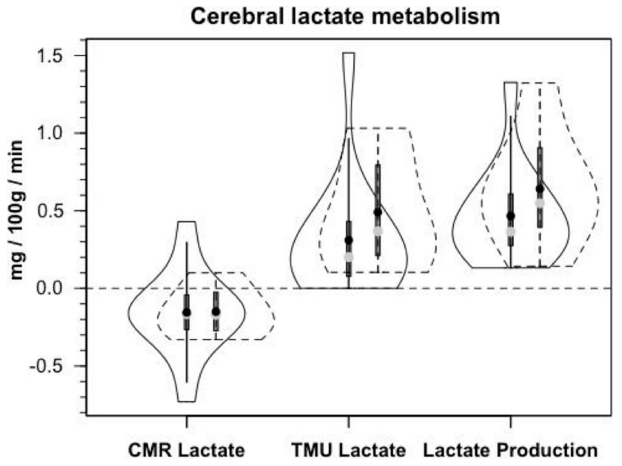


FIGURE 9 | Components of cerebral lactate metabolism: Release (CMRlac), Tracer-Measured Uptake and Total Cerebral Lactate Production = TMU-CMRlac. Solid lines represent TBI patients while dashed lines are normal control subjects. The use of [^{13}-13C]lactate tracer allow a very different view of cerebral lact production. Should CMRlac be taken as a measure of lactate production, total lactate production would be grossly underestimated. Regardless, whether estimated from CMR or total lactate production, control subjects and TBI patients show similar capacities for lactate uptake, release and production. From Glenn et al. (2015).

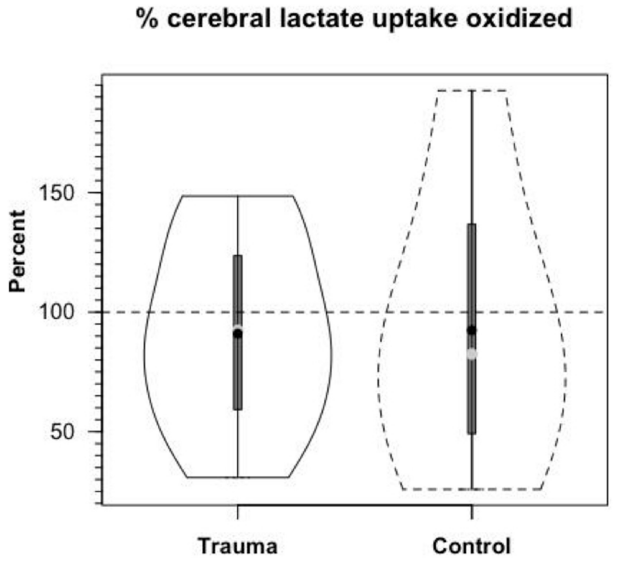


FIGURE 10 | Cerebral lactate Oxidation in control and TBI patients. Values given in relative terms (% lactate uptake that is oxidized). Lactate taken up by healthy controls and TBI patients is oxidized directly within the tissue. Values corrected for the contribution to cerebral release of $^{13}CO_2$ from the oxidation of ^{13}C-glucose produced from circulating [3-^{13}C]lactate. From (Glenn et al., 2015).

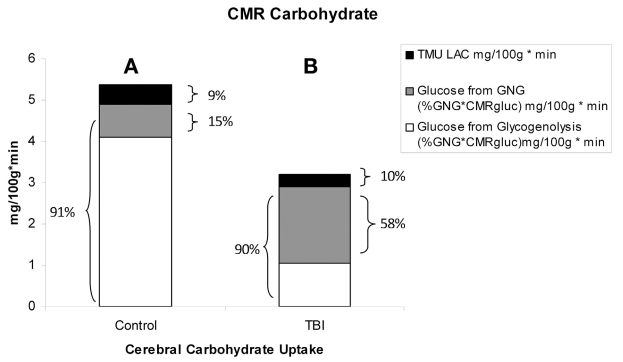


FIGURE 11 | Absolute and relative contributions to total cerebral carbohydrate (CHO) from lactate, glucose from gluconeogenesis (GNG), and glucose from hepatic glycogenolysis (GLY = Glucose Ra – GNGgluc) in healthy control subjects (Left, A), and TBI patients (Right, B). Compared to **(A)**, panel **(B)** shows the decrease in total CHO use and CMRgluc following TBI, but increased contributions of lactate and glucose from GNG to total cerebral CHO uptake after TBI. A comparison of **(A)** (control) and **(B)** (TBI) shows the large increase in percentage cerebral CHO uptake contributed by lactate, directly, or indirectly, in comparison to healthy controls who have hepatic GLY available for to support CMRgluc. From Glenn et al. (2015).

augment the typical dextrose solutions currently used to support TBI patients. At a minimum, the exogenous glucose should not be administered at a rate greater than the glucose turnover rate of the individual patient. Such a practice would inevitably result in hyperglycemia and require insulin treatment. Seemingly, a better alternative would be to support glycemia by providing lactate and other monocarboxylate formulations that would allow the liver and kidneys to maintain euglycemia as is typically seen with exogenous lactate infusions (Miller et al., 2002a,b; Bouzat et al., 2014).

Although not widely recognized, exogenous vascular infusion of sodium lactate-lactic acid mixtures has been utilized to study substrate kinetics in dogs (Issekutz and Miller, 1962), rats (Guo et al., 2009; Johnson et al., 2011, 2012), and humans (Miller et al., 2002a,b, 2005; Ichai et al., 2009, 2013). The latter studies established short-term safety of the method of infusing concentrated lactate solutions for several hours into healthy men with good renal function to manage the sodium load and respiratory alkalosis (Miller et al., 2005). Importantly, studies have commenced with the intent of determining the effects of sodium lactate infusion in cerebral metabolism following TBI (Ichai et al., 2009; Bouzat et al., 2014; Glenn et al., 2015). In addition to the infusion of sodium lactate, investigators have experimented with infusion of hypertonic sodium pyruvate into swine to evaluate viability of that approach for cardiac resuscitation and neuroprotection following cardiac arrest (Sharma et al., 2005, 2008; Ryou et al., 2009, 2010, 2012). In control beagles, nominal arterial lactate and pyruvate concentrations are similar to those in humans (1.0 and 0.1 mM, respectively), but, as used, sodium pyruvate infusion increases arterial pyruvate and lactate concentrations to 3.5 and 8 mM, respectively (Sharma et al., 2005), and so pyruvate infusion may be an effective tool in managing blood and tissue [L]/[P] while raising arterial [L] and [P].

LACTATE AND ITS ALTERNATIVES FOR BRAIN FUELING

Above we discussed some of the issues related to fueling the injured brain, either by providing glucose or an alternative

such as lactate or keto acids such as pyruvate, acetoacetate or β-hydroxybutyrate. Aside from what glycolysis produces and neurons respire, numerous factors need consideration, some of which are described here.

Lactate: We have advanced the idea that lactate enters the mitochondrial reticulum via one or several MCT isoforms and interacts with the mitochondrial Krebs Cycle and respiratory apparatus via a mLOC in muscle (Brooks et al., 1999a,b; Dubouchaud et al., 2000; Hashimoto et al., 2006) and brain (Hashimoto et al., 2008), and others have advanced the idea that pyruvate enters the mitochondrial matrix via pyruvate carrier isoforms (Bricker et al., 2012; Herzig et al., 2012). However, all seem to agree that the monocarboxylates lactate and pyruvate traverse cell membranes via MCT1 or MCT4 (Brooks, 2009; Divakaruni et al., 2013). Plasma membrane MCT isoforms have different affinities for monocarboxylates (Roth and Brooks, 1990a,b), but it is clear also that in blood the monocarboxylates appear in concentrations that differ by an order of magnitude or more. For instance, the nominal blood lactate/pyruvate ratio ([L]/[P] or L/P) in arterial blood of < 10 can rise to > 300 in venous effluent of working human muscle (Henderson et al., 2007). Related to the L/P in blood are the effects of LDH in erythrocytes and the lung parenchyma that raise the L/P during each circulatory passage (Johnson et al., 2011, 2012).

Operating under the assumption that because it is the product of glycolysis and biological brain fuel (Schurr, 2014), we (Miller et al., 2002a,b; Glenn et al., 2014) and others (Oddo et al., 2012; Ichai et al., 2013; Bouzat et al., 2014) experimented on supplementing nutritive supply to the injured brain with formulations containing lactate or lactate anion. As described above, lactate supports glycemia without causing hyperglycemia because of hepatic autoregulation of blood [glucose]. Further, lactate is taken up directly and oxidized by the healthy as well as injured brain, and, importantly, in the ranges studied, uptake is not saturation limited (**Figure 8A**). Importantly, and in contrast to the case for working skeletal muscle in which lactate supplementation substitutes for glucose, following TBI in which glycolysis is partially blocked, lactate supplementation via a 2 mM lactate clamp did not decrease CMRgluc, but instead raised total cerebral CHO uptake (unpublished data). This so called "sparing" of blood glucose can then potentially be used for other cerebral metabolic priorities of glucose such as the neuroprotective pentose phosphate pathway (Bartnik et al., 2005; Dusick et al., 2007).

Short, several hour infusions of sodium lactate appear to result in manageable sodium and alkalotic stresses in healthy controls (Miller et al., 2005) and TBI patients with good renal and respiratory function (Bouzat et al., 2014). Likely, however, for longer durations infusions of other lactate formulations including organic salts (arginyl lactate), esters (glycerol tri-lactate or N-acetyl lactate) or other compounds will need to be developed and tested for safety and effectiveness in human subjects.

Pyruvate: To reiterate from above, Mallet and colleagues have developed sodium pyruvate infusion procedures and have done extensive testing in animal models (Sharma et al., 2008; Ryou et al., 2012). Typically the investigators utilize 2 M sodium pyruvate solutions for infusion via central venous lines (Sharma et al., 2008). However, sodium pyruvate solutions are unstable and give rise to noxious degradation products. To our knowledge, neither sodium pyruvate nor pyruvate derivatives such as ethyl pyruvate and N-acetyl pyruvate have been tested for safety and effectiveness in human subjects. Moreover, the biological rationale for infusing pyruvate is questionable because it is rapidly converted to lactate in blood (Sharma et al., 2005; Johnson et al., 2011, 2012) and so the question arises what is the effective moiety taken up by healthy and injured tissues.

Another advantage of exogenous lactate over pyruvate supplementation relates to another chronic problem in the management of TBI patients, that is the complication of hyperglycemia with dextrose infusion and poor control of glycemia (Vespa et al., 2012). A potential advantage of infusing lactate, a gluconeogenic precursor, as opposed to dextrose for maintaining glycemic control is to take advantage of hepatic autoregulation of glucose Ra. At present, extant data indicates that in both healthy individuals (Miller et al., 2002a,b) and TBI patients exogenous lactate infusion does not result in hyperglycemia (Bouzat et al., 2014). In contrast, sodium pyruvate infusion results in hyperglycemia in beagles (Sharma et al., 2005).

Given that both lactate and pyruvate are likely to traverse plasma membrane, and hence blood-brain barrier thresholds by MCTs that are capable of transporting either monocarboxylate, a logical suggestion is to consider infusing mixtures of both lactate and pyruvate, particularly if it is found efficacious to affect the circulating L/P. For the present, however, given the liabilities of infusing pyruvate compounds, there seems to be no practical advantage of infusing pyruvate over lactate. In this context some would argue that pyruvate is an effective ROS scavenger and is neuro- and cardio protective (Mallet and Sun, 2003; Sharma et al., 2008), but similar claims can be made for lactate (Rice et al., 2002; Holloway et al., 2007; Schurr and Gozal, 2011; Alessandri et al., 2012; Herzog et al., 2013; Bouzat et al., 2014).

Ketones: The "ketones" acetoacetate and β-hydroxybutyrate cross plasma membrane and blood-brain barriers via facilitated, MCT-mediated transport (Roth and Brooks, 1990a,b), but the specialized MCT isoform appears to be MCT2 (Prins and Giza, 2006). Because acetoacetate and β-hydroxybutyrate gain access to neurons via a unique isoform, thus minimizing crosstalk and competition for transport among MCT isoforms, the approach of using ketogenic diets to support cerebral healing in pediatric populations is an area of active research (Prins and Hovda, 2009; Prins, 2012). Therefore, by extension of ongoing efforts it is entirely reasonable to consider augmenting neuroplegic solutions for iv infusion in the treatment of acute TBI with acetoacetate and β-hydroxybutyrate.

As with other candidates for inclusion in neuroplegic solutions acetoacetate and β-hydroxybutyrate would have advantages and disadvantages. The ketones would contribute to whole body and cerebral substrate availability, and thus would represent direct fuel energy sources for the body and brain. However, ketones would be unlikely to contribute to the maintenance of glycemia via GNG. As well, acetoacetate and β-hydroxybutyrate would by necessity be delivered as sodium salts with efficacy dependent on good renal function.

BODY AND BRAIN IN COMPETITION FOR NUTRIENTS IN THE ICU [BODY ENERGY RESOURCE BUT NUTRIENT SINK]

Despite being treated according to current standard of care protocols, in state of the art facilities, by talented and dedicated health care professionals, as indicated by the very high rate of gluconeogenesis (**Figure 5**), our patients were undernourished. In our companion reports (Glenn et al., 2014, 2015) we describe herculean efforts of the body, liver and kidneys to maintain glycemia via GNG. Above we described possible uses of neuroplegic formulations to sustain the injured brain and promote healing, but efficacy of supporting the injured brain by providing intravenous support needs to be considered within the context of overall, whole-body nutrition. Whether delivered via a central or peripheral line, parenteral nutrition will go to where the circulation takes it. Relative to other tissues, the brain is hypermetabolic and is relatively well perfused in healthy and severely brain injured individuals. However, most enteral and parenteral nutrition will go elsewhere. For example, infused lactate is removed mainly by oxidation in skeletal muscle (Bergman et al., 1999b; Miller et al., 2002a,b; Emhoff et al., 2013). Hence, despite valiant efforts to support the injured brain, both directly and indirectly from endogenous sources (Glenn et al., 2014, 2015), so far as parenteral nutrition is concerned the body is in competition with the brain for resources. Hence, it is to be anticipated that the intravenous infusion of neuroplegic solutions following TBI need to be considered on the background of overall enteral nutrition so that overall nutrient and energy availability is sufficient to meet needs of the brain and other tissues whether they be healthy or injured.

Assessing nutritional adequacy of patients in the ICU is a technical challenge. At best, results of indirect calorimetry are difficult to interpret and the technology is difficult to employ, especially in head injury cases, or when a patient is artificially ventilated. Standardized equations (Harris and Benedict, 1918) force regression to population means, but cannot be expected to precisely meet individual patient needs. The use of nitrogen balance, albumin and prealbumin measurements are also difficult to employ and there are significant time lags between measurements and ongoing changes in nitrogen balance. No wonder the literature is rich with controversy over the benefits, or liabilities of providing enteral or parenteral nutrition to patients (McClave et al., 1998; Petros and Engelmann, 2006; Griffiths, 2007; Loh and Griffiths, 2009; Casaer et al., 2011a). However Wang et al. (2013) show in a meta-analysis review of TBI treatments that providing enteral and parenteral nutritive support early result in better patient outcomes (Wang et al., 2013). In our view, such uncertainty involves an inability to deliver personalized, precision care to patients. Hence, new methods of assessing BES in the ICU need to be found to aid clinicians in delivering energy and nutrients sufficient to cover needs for the body and brain. In this regard, %GNG is an excellent candidate biomarker for assessing BES in the ICU, but technology needs to be developed and validated for clinical use.

THE CONFUSED STATE OF UNDERSTANDING ON LACTATE CLEARANCE

Results of our investigations indicate that caution needs to be applied when using the term "lactate clearance" as a biomarker of the severity of traumatic injury (Zhang and Xu, 2014). Without the use of isotope tracers, clearance is calculated as net metabolite change over time with lactatemia and lactic acidosis harbingers of poor outcome. In our investigation we infused [3–13C]lactate tracer to determine metabolic clearance rate (MCR = Rd/[lactate]a), units being (ml/kg/min). In our investigation lactate production (Ra) and disposal (Rd) rates were significantly elevated following TBI (**Figure 4B**) (Glenn et al., 2014), while arterial lactate concentration ([lactate]a) was the same in control subjects and TBI patients (**Figure 3B**). This means that lactate MCR was very high and significantly greater, not lower, in TBI patients compared to controls. Additional tracer studies will be required to establish the relationship between lactate MCR and outcome following TBI.

SUMMARY

Trauma to the brain results in a metabolic crisis (Stein et al., 2012; Vespa et al., 2012), or crises as the mechanism of injury may involve trauma to other body parts. The injured person is typically hypermetabolic even if cerebral metabolism is suppressed following injury (Glenn et al., 2003). Together, our results (Glenn et al., 2014, 2015) and those of others (Gallagher et al., 2009; van Hall et al., 2009; Jalloh et al., 2013; Bouzat et al., 2014) indicate a significant role for lactate in cerebral normo- and pathophysiology. Given that CMRgluc is suppressed following cerebral injury, it's possible to take advantage of the Lactate Shuttle mechanism of supplying energy to bypass the restriction in glycolytic flux and spare limited glucose reserves for other functions such as pentose phosphate pathway activity.

As reviewed above, sodium lactate infusion is a logical first step in translating new knowledge of cerebral lactate metabolism into procedures and practices to provide nutritive support to the injured brain. However, other lactate compounds or formulations containing additional or other amendments may offer still greater potential to nourish the injured brain in the context of an injured or malnourished body. In the space available we have shown new and dramatic results on body-brain interactions in moderate and severe TBI patients. Those results not only show direct and indirect nutritive support of the body for the brain, but the results should alert scientists and clinicians alike to the possibility that attempts to provide parenteral, vascular, support for the brain will be met by an eagerly needy, and far larger body corpus that can easily outcompete the injured brain for glucose and monocarboxylate alternatives. Therefore, by using glucose production rate and %GNG as biomarkers for BES in individual patients health care providers would have actionable data upon which to personalize individual patient needs for delivery of energy and nutrients required to support both brain and body needs. In practical terms, with knowledge of BES health care professionals would have knowledge to provide energy and macronutrients to meet corporal needs, as indicated by %GNG in the range of 25% (Horning and Brooks, 2012a,b). Then, via parenteral routes monocarboxylate formulations designed specifically to supplement cerebral energy needs could be delivered in an effort to support cerebral repair and recovery following injury (Horning and Brooks, 2012a,b).

Today the field of precision medicine focuses predominately on genomics, but all variations of the omics, (i.e., application of panomic analysis to individual disease) will participate (NIH,

2011) in efforts to utilize the concept of precision medicine to improve health care delivery, particularly in the area of managing trauma. In fact, other omics constituting precision medicine (e.g., proteomics, metabolomics, and now fluxomics), as illustrated through the use of %GNG as a biomarker for BES and treatment using formulations targeting injured tissue, already might be areas of interest. Indeed, segments of the biotech, medtech, digital health, and life sciences industries are spearheading medical applications and exploring opportunities. With basis of knowing an individual patient's BES, health care providers will be able to practice precision medicine through use of personalized prescriptions to deliver exogenous enteral and parenteral nutritive formulations to maintain a body energy state, that neither over- or underfeeds patients, but, importantly, provides directed nutrient support for the injured brain. A personalized approach to precision body energy state will therefore enable the clinicians to support both the body and the brain during recovery from trauma.

ACKNOWLEDGMENTS

Research supported by: the UCLA Brain Injury Research Center and award PO1NS058489 from the National Institute of Neurological Disorders and Stroke (NINDS), and a gift from CytoSport, Inc. of Benicia, CA. The authors thank the following for reading and commenting on the manuscript: Michael A. Horning, and Daniel Kane. We thank Thomas Glenn and David McArthur for contributing **Figure 2**.

REFERENCES

Ahlborg, G., and Felig, P. (1982). Lactate and glucose exchange across the forearm, legs, and splanchnic bed during and after prolonged leg exercise. *J. Clin. Invest.* 69, 45–54. doi: 10.1172/JCI110440

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. doi: 10.1089/neu.2011.2067

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. doi: 10.1089/neu.2005.22.1052

Bergman, B. C., Butterfield, G. E., Wolfel, E. E., Lopaschuk, G. D., Casazza, G. A., Horning, M. A., et al. (1999a). Muscle net glucose uptake and glucose kinetics after endurance training in men. *Am. J. Physiol.* 277(1 Pt 1): E81–E92.

Bergman, B. C., Tsvetkova, T., Lowes, B., and Wolfel, E. E. (2009). Myocardial glucose and lactate metabolism during rest and atrial pacing in humans. *J. Physiol.* 587(Pt 9): 2087–2099. doi: 10.1113/jphysiol.2008.168286

Bergman, B. C., Wolfel, E. E., Butterfield, G. E., Lopaschuk, G. D., Casazza, G. A., Horning, M. A., et al. (1999b). Active muscle and whole body lactate kinetics after endurance training in men. *J. Appl. Physiol.* 87, 1684–1696.

Bergsneider, M., Hovda, D. A., Shalmon, E., Kelly, D. F., Vespa, P. M., Martin, N. A., et al. (1997). Cerebral hyperglycolysis following severe traumatic brain injury in humans: a positron emission tomography study. *J. Neurosurg.* 86, 241–251. doi: 10.3171/jns.1997.86.2.0241

Bouzat, P., Sala, N., Suys, T., Zerlauth, J. B., Marques-Vidal, P., Feihl, F., et al. (2014). Cerebral metabolic effects of exogenous lactate supplementation on the injured human brain. *Intensive Care Med.* 40, 412–421. doi: 10.1007/s00134-013-3203-6

Bricker, D. K., Taylor, E. B., Schell, J. C., Orsak, T., Boutron, A., Chen, Y. C., et al. (2012). A mitochondrial pyruvate carrier required for pyruvate uptake in yeast, Drosophila, and humans. *Science* 337, 96–100. doi: 10.1126/science.1218099

Brooks, G. A. (1984). "Lactate: glycolytic end product and oxidative substrate during sustained exercise in mammals—the lactate shuttle," in *Comparative Physiology and Biochemistry - Current Topics and Trends*, Vol. A, *Respiration - Metabolism - Circulation*, ed R. Gilles (Berlin: Springer Verlag), 208–218.

Brooks, G. A. (2009). Cell-cell and intracellular lactate shuttles. *J. Physiol.* 587(Pt 23): 5591–5600. doi: 10.1113/jphysiol.2009.178350

Brooks, G. A., Brown, M. A., Butz, C. E., Sicurello, J. P., and Dubouchaud, H. (1999a). Cardiac and skeletal muscle mitochondria have a monocarboxylate transporter MCT1. *J. Appl. Physiol. (1985)* 87, 1713–1718.

Brooks, G. A., and Donovan, C. M. (1983). Effect of endurance training on glucose kinetics during exercise. *Am. J. Physiol.* 244, E505–E512.

Brooks, G. A., Dubouchaud, H., Brown, M., Sicurello, J. P., and Butz, C. E. (1999b). Role of mitochondrial lactate dehydrogenase and lactate oxidation in the intracellular lactate shuttle. *Proc. Natl. Acad. Sci. U.S.A.* 96, 1129–1134. doi: 10.1073/pnas.96.3.1129

Brooks, G. A., Wolfel, E. E., Butterfield, G. E., Cymerman, A., Roberts, A. C., Mazzeo, R. S., et al. (1998). Poor relationship between arterial [lactate] and leg net release during exercise at 4,300 m altitude. *Am. J. Physiol.* 275(4 Pt 2): R1192–R1201.

Cahill, G. J. Jr., Owen, O. E., and Morgan, A. P. (1968). The consumption of fuels during prolonged starvation. *Adv. Enzyme Regul.* 6, 143–150. doi: 10.1016/0065-2571(68)90011-3

Casaer, M. P., Hermans, G., Wilmer, A., and Van den Berghe, G. (2011a). Impact of early parenteral nutrition completing enteral nutrition in adult critically ill patients (EPaNIC trial): a study protocol and statistical analysis plan for a randomized controlled trial. *Trials* 12, 21. doi: 10.1186/1745-6215-12-21

Casaer, M. P., Mesotten, D., Hermans, G., Wouters, P. J., Schetz, M., Meyfroidt, G., et al. (2011b). Early versus late parenteral nutrition in critically ill adults. *N. Engl. J. Med.* 365, 506–517. doi: 10.1056/NEJMoa1102662

Dienel, G. A., and Hertz, L. (2001). Glucose and lactate metabolism during brain activation. *J. Neurosci. Res.* 66, 824–838. doi: 10.1002/jnr.10079

Divakaruni, A. S., Wiley, S. E., Rogers, G. W., Andreyev, A. Y., Petrosyan, S., Loviscach, M., et al. (2013). Thiazolidinediones are acute, specific inhibitors of the mitochondrial pyruvate carrier. *Proc. Natl. Acad. Sci. U.S.A.* 110, 5422–5427. doi: 10.1073/pnas.1303360110

Donovan, C. M., and Brooks, G. A. (1983). Endurance training affects lactate clearance, not lactate production. *Am. J. Physiol.* 244, E83–E92.

Dubouchaud, H., Butterfield, G. E., Wolfel, E. E., Bergman, B. C., and Brooks, G. A. (2000). Endurance training, expression, and physiology of LDH, MCT1, and MCT4 in human skeletal muscle. *Am. J. Physiol.* 278, E571–E579.

Dusick, J. R., Glenn, T. C., Lee, W. N., Vespa, P. M., Kelly, D. F., Lee, S. M., et al. (2007). Increased pentose phosphate pathway flux after clinical traumatic brain injury: a [1,2-13C2]glucose labeling study in humans. *J. Cereb. Blood Flow Metab.* 27, 1593–1602. doi: 10.1038/sj.jcbfm.9600458

Emhoff, C. A., Messonnier, L. A., Horning, M. A., Fattor, J. A., Carlson, T. J., and Brooks, G. A. (2013). Direct and indirect lactate oxidation in trained and untrained men. *J. Appl. Physiol.* 115, 829–838. doi: 10.1152/japplphysiol.00538.2013

Foster, D. W. (1984). Banting lecture 1984. From glycogen to ketones–and back. *Diabetes* 33, 1188–1199. doi: 10.2337/diab.33.12.1188

Gallagher, C. N., Carpenter, K. L., Grice, P., Howe, D. J., Mason, A., Timofeev, I., et al. (2009). The human brain utilizes lactate via the tricarboxylic acid cycle: a 13C-labelled microdialysis and high-resolution nuclear magnetic resonance study. *Brain* 132(Pt 10): 2839–2849. doi: 10.1093/brain/awp202

Garcia, C. K., Brown, M. S., Pathak, R. K., and Goldstein, J. L. (1995). cDNA cloning of MCT2, a second monocarboxylate transporter expressed in different cells than MCT1. *J. Biol. Chem.* 270, 1843–1849. doi: 10.1074/jbc.270.4.1843

Garcia, C. K., Goldstein, J. L., Pathak, R. K., Anderson, R. G., and Brown, M. S. (1994). Molecular characterization of a membrane transporter for lactate, pyruvate, and other monocarboxylates: implications for the Cori cycle. *Cell* 76, 865–873. doi: 10.1016/0092-8674(94)90361-1

Gertz, E. W., Wisneski, J. A., Neese, R., Bristow, J. D., Searle, G. L., and Hanlon, J. T. (1981). Myocardial lactate metabolism: evidence of lactate release during net chemical extraction in man. *Circulation* 63, 1273–1279. doi: 10.1161/01.CIR.63.6.1273

Gertz, E. W., Wisneski, J. A., Stanley, W. C., and Neese, R. A. (1988). Myocardial substrate utilization during exercise in humans. Dual carbon-labeled carbohydrate isotope experiments. *J. Clin. Invest.* 82, 2017–2025. doi: 10.1172/JCI113822

Glenn, T. C., Kelly, D. F., Boscardin, W. J., McArthur, D. L., Vespa, P., Oertel, M., et al. (2003). Energy dysfunction as a predictor of outcome after moderate or severe head injury: indices of oxygen, glucose, and lactate metabolism. *J. Cereb. Blood Flow Metab.* 23, 1239–1250. doi: 10.1097/01.WCB.0000089833.23606.7F

Glenn, T. C., Martin, N. A., Hovda, D. A., Vespa, P., Johnson, M. L., Horning, M. A., et al. (2015). Lactate; brain fuel following traumatic brain injury. *J. Neurotrauma.* [Epub ahead of print].

Glenn, T. C., Martin, N. A., Mcarthur, D. L., Hovda, D., Vespa, P. M. M., Horning, M. A., et al. (2014). Endogenous nutritive support following traumatic brain injury: peripheral lactate production for glucose supply via gluconeogenesis. *J. Neurotrauma.* doi: 10.1089/neu.2014.3482. [Epub ahead of print].

Griffiths, R. D. (2007). Too much of a good thing: the curse of overfeeding. *Crit. Care* 11, 176. doi: 10.1186/cc6165

Guo, J., Parise, R. A., Joseph, E., Egorin, M. J., Lazo, J. S., Prochownik, E. V., et al. (2009). Efficacy, pharmacokinetics, tisssue distribution, and metabolism of the Myc-Max disruptor, 10058-F4 [Z,E]-5-[4-ethylbenzylidine]-2-thioxothiazolidin-4-one, in mice. *Cancer Chemother. Pharmacol.* 63, 615–625. doi: 10.1007/s00280-008-0774-y

Harris, J. A., and Benedict, F. G. (1918). A biometric study of human basal metabolism. *Proc. Natl. Acad. Sci. U.S.A.* 4, 370–373. doi: 10.1073/pnas.4.12.370

Hashimoto, T., Hussien, R., and Brooks, G. A. (2006). Colocalization of MCT1, CD147, and LDH in mitochondrial inner membrane of L6 muscle cells: evidence of a mitochondrial lactate oxidation complex. *Am. J. Physiol.* 290, E1237–E1244. doi: 10.1152/ajpendo.00594.2005

Hashimoto, T., Hussien, R., Cho, H. S., Kaufer, D., and Brooks, G. A. (2008). Evidence for the mitochondrial lactate oxidation complex in rat neurons: demonstration of an essential component of brain lactate shuttles. *PLoS ONE* 3:e2915. doi: 10.1371/journal.pone.0002915

Henderson, G. C., Horning, M. A., Wallis, G. A., and Brooks, G. A. (2007). Pyruvate metabolism in working human skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* 292, 317–325. doi: 10.1152/ajpendo.00363.2006

Henderson, W. R., and Finfer, S. (2009). Differences in outcome between the NICE-SUGAR and Leuven trials: possible methodological explanations. *Crit. Care Resusc.* 11, 175–177.

Herzig, S., Raemy, E., Montessuit, S., Veuthey, J. L., Zamboni, N., Westermann, B., et al. (2012). Identification and functional expression of the mitochondrial pyruvate carrier. *Science* 337, 93–96. doi: 10.1126/science.1218530

Herzog, R. I., Jiang, L., Herman, P., Zhao, C., Sanganahalli, B. G., Mason, G. F., et al. (2013). Lactate preserves neuronal metabolism and function following antecedent recurrent hypoglycemia. *J. Clin. Invest.* 123, 1988–1998. doi: 10.1172/JCI65105

Hintze, J. L., and Nelson, R. D. (1998). Violin plots: a box plot-density trace synergism. *Am. Stat.* 52, 181–184.

Holloway, R., Zhou, Z., Harvey, H. B., Levasseur, J. E., Rice, A. C., Sun, D., et al. (2007). Effect of lactate therapy upon cognitive deficits after traumatic brain injury in the rat. *Acta Neurochirur.* 149, 919–927. discussion: 927. doi: 10.1007/s00701-007-1241-y

Horning, M. A., and Brooks, G. A. (2012a). *Formulations and Methods to Provide Nutrition to Human and other Patients.* Alexandria, VA: US Patent and Trade Office.

Horning, M. A., and Brooks, G. A. (2012b). *Systems and Methods to Estimate Nutritional Needs of Human and other Patients.* US 8′827,490 B2. Alexandria, VA: US Patent and Trade Office.

Hultman, E. A. (1967). Physiological role of muscle glycogen in man. *Physiol. Muscular Exercise* (Suppl. 1), I99–I112.

Ichai, C., Armando, G., Orban, J. C., Berthier, F., Rami, L., Samat-Long, C., et al. (2009). Sodium lactate versus mannitol in the treatment of intracranial hypertensive episodes in severe traumatic brain-injured patients. *Intensive Care Med.* 35, 471–479. doi: 10.1007/s00134-008-1283-5

Ichai, C., Payen, J. F., Orban, J. C., Quintard, H., Roth, H., Legrand, R., et al. (2013). Half-molar sodium lactate infusion to prevent intracranial hypertensive episodes in severe traumatic brain injured patients: a randomized controlled trial. *Intensive Care Med.* 39, 1413–1422. doi: 10.1007/s00134-013-2978-9

Issekutz, B. J., and Miller, H. (1962). Plasma free fatty acids during exercise and the effect of lactic acid. *Proc. Soc. Exp. Biol. Med.* 110, 237–239.

Jalloh, I., Helmy, A., Shannon, R. J., Gallagher, C. N., Menon, D. K., Carpenter, K. L., et al. (2013). Lactate uptake by the injured human brain: evidence from an arteriovenous gradient and cerebral microdialysis study. *J. Neurotrauma* 30, 2031–2037. doi: 10.1089/neu.2013.2947

Johnson, J. A., and Fusaro, R. M. (1972). The role of the skin in carbohydrate metabolism. *Adv. Metab. Disord.* 60, 1–55. doi: 10.1016/B978-0-12-027306-5.50006-1

Johnson, M. L., Emhoff, C. A., Horning, M. A., and Brooks, G. A. (2012). Transpulmonary lactate shuttle. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 302, R143–R149. doi: 10.1152/ajpregu.00402.2011

Johnson, M. L., Hussien, R., Horning, M. A., and Brooks, G. A. (2011). Transpulmonary pyruvate kinetics. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 301, R769–R774. doi: 10.1152/ajpregu.00206.2011

Krssak, M., Petersen, K. F., Bergeron, R., Price, T., Laurent, D., Rothman, D. L., et al. (2000). Intramuscular glycogen and intramyocellular lipid utilization during prolonged exercise and recovery in man: a 13C and 1H nuclear magnetic resonance spectroscopy study. *J. Clin. Endocrinol. Metab.* 85, 748–754. doi: 10.1210/jc.85.2.748

Loh, N. H. W., and Griffiths, R. D. (2009). *The Curse of Overfeeding and the Blight of Underfeeding*. New York, NY: Springer.

Mallet, R. T., and Sun, J. (2003). Antioxidant properties of myocardial fuels. *Mol. Cell. Biochem.* 253, 103–111. doi: 10.1023/A:1026009519783

Marik, P. E. (2009). Glycemic control in critically ill patients: What to do post NICE-SUGAR? *World J. Gastrointest. Surg.* 1, 3–5. doi: 10.4240/wjgs.v1.i1.3

Mazzeo, R. S., Brooks, G. A., Schoeller, D. A., and Budinger, T. F. (1986). Disposal of blood [1-13C]lactate in humans during rest and exercise. *J. Appl. Physiol.* 60, 232–241.

McClave, S. A., Lowen, C. C., Kleber, M. J., Nicholson, J. F., Jimmerson, S. C., McConnell, J. W., et al. (1998). Are patients fed appropriately according to their caloric requirements? *J. Parenter. Enteral Nutr.* 22, 375–381. doi: 10.1177/0148607198022006375

Medicine, I. O. (2005). *Dietary Reference Intakes: Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids.* Washington, DC: The National Academies Press.

Meijering, S., Corstjens, A. M., Tulleken, J. E., Meertens, J. H., Zijlstra, J. G., and Ligtenberg, J. J. (2006). Towards a feasible algorithm for tight glycaemic control in critically ill patients: a systematic review of the literature. *Crit. Care* 10, R19. doi: 10.1186/cc3981

Messonnier, L. A., Emhoff, C. A., Fattor, J. A., Horning, M. A., Carlson, T. J., and Brooks, G. A. (2013). Lactate kinetics at the lactate threshold in trained and untrained men. *J. Appl. Physiol. (1985)* 114, 1593–1602. doi: 10.1152/japplphysiol.00043.2013

Meyer, C., Dostou, J. M., Welle, S. L., and Gerich, J. E. (2002). Role of human liver, kidney, and skeletal muscle in postprandial glucose homeostasis. *Am. J. Physiol. Endocrinol. Metab.* 282, E419–E427. doi: 10.1152/ajpendo.00032.2001

Miller, B. F., Fattor, J. A., Jacobs, K. A., Horning, M. A., Navazio, F., Lindinger, M. I., et al. (2002a). Lactate and glucose interactions during rest and exercise in men: effect of exogenous lactate infusion. *J. Physiol.* 544(Pt 3): 963–975. doi: 10.1113/jphysiol.2002.027128

Miller, B. F., Fattor, J. A., Jacobs, K. A., Horning, M. A., Suh, S. H., Navazio, F., et al. (2002b). Metabolic and cardiorespiratory responses to the lactate clamp. *Am. J. Physiol. Endocrinol. Metab.* 283, E889–E898. doi: 10.1152/ajpendo.00266.2002

Miller, B. F., Lindinger, M. I., Fattor, J. A., Jacobs, K. A., Leblanc, P. J., Duong, M., et al. (2005). Hematological and acid-base changes in men during prolonged exercise with and without sodium-lactate infusion. *J. Appl. Physiol.* 98, 856–865. doi: 10.1152/japplphysiol.00753.2004

Myburgh, J. A., and Chittock, D. R. (2009). Differences in outcome between the NICE-SUGAR and Leuven trials: biological mechanisms of intensive glucose control in critically ill patients. *Crit. Care Resusc* 11, 178–179.

NIH. (2011). *Toward Precision Medicine: Building a Knowledge Network for Biomedical Research and a New Taxonomy of Disease.* Washington, DC: DHHS.

Nilsson, L. H., and Hultman, E. (1974). Liver and muscle glycogen in man after glucose and fructose infusion. *Scand. J. Clin. Lab. Invest.* 33, 5–10. doi: 10.3109/00365517409114190

Oddo, M., Levine, J. M., Frangos, S., Maloney-Wilensky, E., Carrera, E., Daniel, R. T., et al. (2012). Brain lactate metabolism in humans with subarachnoid hemorrhage. *Stroke* 43, 1418–1421. doi: 10.1161/STROKEAHA.111.648568

Oz, G., Seaquist, E. R., Kumar, A., Criego, A. B., Benedict, L. E., Rao, J. P., et al. (2007). Human brain glycogen content and metabolism: implications on its role in brain energy metabolism. *Am. J. Physiol. Endocrinol. Metab.* 292, E946–E951. doi: 10.1152/ajpendo.00424.2006

Pellerin, L., Bergersen, L. H., Halestrap, A. P., and Pierre, K. (2005). Cellular and subcellular distribution of monocarboxylate transporters in cultured brain cells and in the adult brain. *J. Neurosci. Res.* 79, 55–64. doi: 10.1002/jnr.20307

Petros, S., and Engelmann, L. (2006). Enteral nutrition delivery and energy expenditure in medical intensive care patients. *Clin. Nutr.* 25, 51–59. doi: 10.1016/j.clnu.2005.08.013

Pierre, K., and Pellerin, L. (2005). Monocarboxylate transporters in the central nervous system: distribution, regulation and function. *J. Neurochem.* 94, 1–14. doi: 10.1111/j.1471-4159.2005.03168.x

Prins, M. L. (2012). Cerebral ketone metabolism during development and injury. *Epilepsy Res.* 100, 218–223. doi: 10.1016/j.eplepsyres.2011.09.027

Prins, M. L., and Giza, C. C. (2006). Induction of monocarboxylate transporter 2 expression and ketone transport following traumatic brain injury in juvenile and adult rats. *Dev. Neurosci.* 28, 447–456. doi: 10.1159/000094170

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. doi: 10.1089/neu.2008.0769

Rice, A. C., Zsoldos, R., Chen, T., Wilson, M. S., Alessandri, B., Hamm, R. J., et al. (2002). Lactate administration attenuates cognitive deficits following traumatic brain injury. *Brain Res.* 928, 156–159. doi: 10.1016/S0006-8993(01)03299-1

Richardson, R. S., Noyszewski, E. A., Leigh, J. S., and Wagner, P. D. (1998). Lactate efflux from exercising human skeletal muscle: role of intracellular PO2. *J. Appl. Physiol.* 85, 627–634.

Roden, M., Petersen, K. F., and Shulman, G. I. (2001). Nuclear magnetic resonance studies of hepatic glucose metabolism in humans. *Recent Prog. Horm. Res.* 56, 219–237. doi: 10.1210/rp.56.1.219

Roth, D. A., and Brooks, G. A. (1990a). Lactate and pyruvate transport is dominated by a pH gradient-sensitive carrier in rat skeletal muscle sarcolemmal vesicles. *Arch. Biochem. Biophys.* 279, 386–394. doi: 10.1016/0003-9861(90)90506-T

Roth, D. A., and Brooks, G. A. (1990b). Lactate transport is mediated by a membrane-bound carrier in rat skeletal muscle sarcolemmal vesicles. *Arch. Biochem. Biophys.* 279, 377–385. doi: 10.1016/0003-9861(90)90505-S

Ryou, M. G., Flaherty, D. C., Hoxha, B., Gurji, H., Sun, J., Hodge, L. M., et al. (2010). Pyruvate-enriched cardioplegia suppresses cardiopulmonary bypass-induced myocardial inflammation. *Ann. Thorac. Surg.* 90, 1529–1535. doi: 10.1016/j.athoracsur.2010.06.010

Ryou, M. G., Flaherty, D. C., Hoxha, B., Sun, J., Gurji, H., Rodriguez, S., et al. (2009). Pyruvate-fortified cardioplegia evokes myocardial erythropoietin signaling in swine undergoing cardiopulmonary bypass. *Am. J. Physiol. Heart Circ. Physiol.* 297, H1914–H1922. doi: 10.1152/ajpheart.01213.2008

Ryou, M. G., Liu, R., Ren, M., Sun, J., Mallet, R. T., and Yang, S. H. (2012). Pyruvate protects the brain against ischemia-reperfusion injury by activating the erythropoietin signaling pathway. *Stroke* 43, 1101–1107. doi: 10.1161/STROKEAHA.111.620088

Scheinberg, P., Bourne, B., and Reinmuth, O. M. (1965). Human cerebral lactate and pyruvate extraction. I. Control subjects. *Arch. Neurol.* 12, 246–250. doi: 10.1001/archneur.1965.00460270022003

Scheinberg, P., and Stead, E. A. (1949). The cerebral blood flow in male subjects as measured by the nitrous oxide technique. normal values for blood flow, oxygen utilization, glucose utilization, and peripheral resistance, with observations on the effect of tilting and anxiety. *J. Clin. Invest.* 28(5 Pt 2): 1163–1171. doi: 10.1172/JCI102150

Schruth, D. M. (2012). *Caroline: A Collection of Database, Data Structure, Visualization, and Utility Functions for R, R package version 0.7.4.* Available online at: http://CRAN.R-project.org/package=caroline.

Schurr, A. (2006). Lactate: the ultimate cerebral oxidative energy substrate? *J. Cereb. Blood Flow Metab.* 26, 142–152. doi: 10.1038/sj.jcbfm.9600174

Schurr, A. (2008). Lactate: a major and crucial player in normal function of both muscle and brain. *J. Physiol.* 586(Pt 11): 2665–2666. doi: 10.1113/jphysiol.2008.155416

Schurr, A. (2014). Cerebral glycolysis: a century of persistent misunderstanding and misconception. *Front. Neurosci.* 8:360. doi: 10.3389/fnins.2014.00360

Schurr, A., and Gozal, E. (2011). Aerobic production and utilization of lactate satisfy increased energy demands upon neuronal activation in hippocampal slices and provide neuroprotection against oxidative stress. *Front. Pharmacol.* 2:96. doi: 10.3389/fphar.2011.00096

Schurr, A., and Payne, R. S. (2007). Lactate, not pyruvate, is neuronal aerobic glycolysis end product: an *in vitro* electrophysiological study. *Neuroscience* 147, 613–619. doi: 10.1016/j.neuroscience.2007.05.002

Selye, H. (1950). Stress and the general adaptation syndrome. *Br. Med. J.* 1, 1383–1392. doi: 10.1136/bmj.1.4667.1383

Selye, H., and Fortier, C. (1950). Adaptive reaction to stress. *Psychosom. Med.* 12, 149–157. doi: 10.1097/00006842-195005000-00003

Sharma, A. B., Barlow, M. A., Yang, S. H., Simpkins, J. W., and Mallet, R. T. (2008). Pyruvate enhances neurological recovery following cardiopulmonary arrest and resuscitation. *Resuscitation* 76, 108–119. doi: 10.1016/j.resuscitation.2007.04.028

Sharma, A. B., Knott, E. M., Bi, J., Martinez, R. R., Sun, J., and Mallet, R. T. (2005). Pyruvate improves cardiac electromechanical and metabolic recovery from cardiopulmonary arrest and resuscitation. *Resuscitation* 66, 71–81. doi: 10.1016/j.resuscitation.2004.12.016

Smith, D., Pernet, A., Hallett, W. A., Bingham, E., Marsden, P. K., and Amiel, S. A. (2003). Lactate: a preferred fuel for human brain metabolism *in vivo*. *J. Cereb. Blood Flow Metab.* 23, 658–664. doi: 10.1097/01.WCB.0000063991.19746.11

Sokoloff, L. (1973). Metabolism of ketone bodies by the brain. *Annu. Rev. Med.* 24, 271–280. doi: 10.1146/annurev.me.24.020173.001415

Stanley, W. C., Gertz, E. W., Wisneski, J. A., Morris, D. L., Neese, R. A., and Brooks, G. A. (1985). Systemic lactate kinetics during graded exercise in man. *Am. J. Physiol.* 249(6 Pt 1): E595–E602.

Stanley, W. C., Gertz, E. W., Wisneski, J. A., Neese, R. A., Morris, D. L., and Brooks, G. A. (1986). Lactate extraction during net lactate release in legs of humans during exercise. *J. Appl. Physiol.* 60, 1116–1120.

Stein, N. R., McArthur, D. L., Etchepare, M., and Vespa, P. M. (2012). Early cerebral metabolic crisis after TBI influences outcome despite adequate hemodynamic resuscitation. *Neurocrit. Care* 17, 49–57. doi: 10.1007/s12028-012-9708-y

Trimmer, J. K., Schwarz, J. M., Casazza, G. A., Horning, M. A., Rodriguez, N., and Brooks, G. A. (2002). Measurement of gluconeogenesis in exercising men by mass isotopomer distribution analysis. *J. Appl. Physiol.* 93, 233–241. doi: 10.1152/japplphysiol.01050.2001

Van den Berghe, G., Wilmer, A., Milants, I., Wouters, P. J., Bouckaert, B., Bruyninckx, F., et al. (2006). Intensive insulin therapy in mixed medical/surgical intensive care units: benefit versus harm. *Diabetes* 55, 3151–3159. doi: 10.2337/db06-0855

van den Berghe, G., Wouters, P., Weekers, F., Verwaest, C., Bruyninckx, F., Schetz, M., et al. (2001). Intensive insulin therapy in critically ill patients. *N. Engl. J. Med.* 345, 1359–1367. doi: 10.1056/NEJMoa011300

van Hall, G., Stromstad, M., Rasmussen, P., Jans, O., Zaar, M., Gam, C., et al. (2009). Blood lactate is an important energy source for the human brain. *J. Cereb. Blood Flow Metab.* 29, 1121–1129. doi: 10.1038/jcbfm.2009.35

Vespa, P., McArthur, D. L., Stein, N., Huang, S. C., Shao, W., Filippou, M., et al. (2012). Tight glycemic control increases metabolic distress in traumatic brain injury: a randomized controlled within-subjects trial. *Crit. Care Med.* 40, 1923–1929. doi: 10.1097/CCM.0b013e31824e0fcc

Wang, X., Dong, Y., Han, X., Qi, X. Q., Huang, C. G., and Hou, L. J. (2013). Nutritional support for patients sustaining traumatic brain injury: a systematic review and meta-analysis of prospective studies. *PLoS ONE* 8:e58838. doi: 10.1371/journal.pone.0058838

Wyss, M. T., Jolivet, R., Buck, A., Magistretti, P. J., and Weber, B. (2011). *In vivo* evidence for lactate as a neuronal energy source. *J. Neurosci.* 31, 7477–7485. doi: 10.1523/JNEUROSCI.0415-11.2011

Zhang, Z., and Xu, X. (2014). Lactate clearance is a useful biomarker for the prediction of all-cause mortality in critically ill patients: a systematic review and meta-analysis*. *Crit. Care Med.* 42, 2118–2125. doi: 10.1097/CCM.0000000000000405

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 07 October 2014; accepted: 23 November 2014; published online: 09 February 2015.

Citation: Brooks GA and Martin NA (2015) Cerebral metabolism following traumatic brain injury: new discoveries with implications for treatment. Front. Neurosci. ***8****:408. doi: 10.3389/fnins.2014.00408*

This article was submitted to Neuroenergetics, Nutrition and Brain Health, a section of the journal Frontiers in Neuroscience.

frontiers in
NEUROSCIENCE

OPINION ARTICLE
published: 17 February 2015
doi: 10.3389/fnins.2015.00017

A unique array of neuroprotective effects of pyruvate in neuropathology

Yuri Zilberter*, Olena Gubkina and Anton I. Ivanov

Institut de Neurosciences des Systèmes, Aix Marseille Université, Inserm UMR_S 1106, Marseille, France
**Correspondence: yuri.zilberter@univ-amu.fr*

Edited by:
Pierre J. Magistretti, École Polytechnique Fédérale de Lausanne, Switzerland

Reviewed by:
Avital Schurr, University of Louisville, USA
Evelyne Gozal, University of Louisville, USA

Keywords: energy metabolism, pyruvate, neurodegeneration, oxidative stress, PARP-1, NAD, neuroinflammation

The three common signature characteristics of many neurological diseases are brain hypometabolism, oxidative stress, and neuroinflammation (Melo et al., 2011; Cai et al., 2012; Heneka et al., 2014). In order to be efficient, successful treatment should target all three pathologies simultaneously. Pyruvate seems to be an ideal candidate for such a treatment because of its unique combination of neuroprotective effects (**Figure 1**). In this opinion paper, we attempt to review and summarize recent information concerning these effects and their significance for neuroprotection.

PYRUVATE ENHANCES THE BRAIN-TO-BLOOD GLUTAMATE EFFLUX

Perisynaptic astrocytes normally provide fast take-up of glutamate released during synaptic activity. In pathological conditions however, extracellular glutamate levels can be abnormally high and neurotoxic (Wang and Qin, 2010). Part of this glutamate can be cleared via glutamate transporters located in the capillary endothelial cells that form the blood-brain-barrier. The efficacy of such efflux depends on the glutamate concentration gradient between blood and interstitial fluid (Teichberg et al., 2009). Meanwhile, blood glutamate content can be lowered by activation of a blood-resident enzyme glutamate–pyruvate transaminase that in the presence of pyruvate transforms glutamate into 2-ketoglutarate (Gottlieb et al., 2003), thus reducing the glutamate blood concentration. This should favor the glutamate flux from the interstitial fluid to the blood. Therefore, extracellular glutamate levels can be controlled in part by blood pyruvate, which can enhance the brain-to-blood glutamate efflux.

Indeed, Zlotnik and co-authors demonstrated (Zlotnik et al., 2008, 2012) that intravenous injection of pyruvate after traumatic brain injury in rats led to a transient decrease in blood glutamate levels and significantly improved neurological outcome during the first days following injury as well as hippocampal neuron survival at 30 days after injury.

One of the most severe acute neurological conditions, associated with excessive glutamate release, is the status epilepticus (SE). Glutamate-induced excitotoxicity is largely based on massive influx of Ca^{2+} via glutamate receptors, which seems to be a necessary step in the overall process of neuronal degeneration and the acute neuronal cell death that occurs after SE. Morphological analysis of the rat brain after pilocarpine-induced SE demonstrates that the hippocampal subfield CA1 and the hilus of dentate gyrus are particularly susceptible to neuronal cell loss. SE-induced neuronal loss in CA1 was largely prevented in rats treated with pyruvate plus oxaloacetate (i.p. injection 30 min after development of SE) (Carvalho et al., 2011). Moreover, neuronal damage in the dentate gyrus was prevented in rats that received pyruvate alone while oxaloacetate alone did not reveal any neuroprotective effects. The authors related the observed beneficial effects to the blood glutamate scavenging, although other capabilities of pyruvate could also influence the positive outcome.

PYRUVATE NON-ENZYMATICALLY SCAVENGES H_2O_2

Pyruvate in relatively small concentrations (<1 mM) protects neurons against H_2O_2–induced toxicity (Desagher et al., 1997). This effect is not related to the pyruvate's function as an energy substrate but rather to its ability to non-enzymatically interact with H_2O_2 producing acetate, water, and carbon dioxide (Holleman, 1904). The antioxidant effects of pyruvate and other alpha-ketoacids has been confirmed both *in vitro* in several cell types including neurons and *in vivo* in whole organs such as heart or kidney (Desagher et al., 1997; Das, 2006). Accumulation of reactive oxygen species (ROS) is a prominent feature of oxidative stress and by scavenging ROS pyruvate may substantially reduce the toxic consequences of this pathological event.

ANTI-INFLAMMATORY ACTION OF PYRUVATE

Many studies on different organs provided evidence that pyruvate (ethyl pyruvate) is an effective anti-inflammatory agent (reviewed in Kao and Fink, 2010). They demonstrated that pyruvate treatment down-regulates activation of the pro-inflammatory transcription factor, NF-kB, as well as the expression of several pro-inflammatory proteins, such as tumor necrosis factor (TNF), interleukin 6 (IL-6) and others (Das, 2006; Kao and Fink, 2010). The mechanism of this pyruvate effect is yet unclear although it may be explained, at least partly, by the pyruvate antioxidant properties as well as by the pyruvate-induced inhibition of poly-ADP ribose polymerase-1

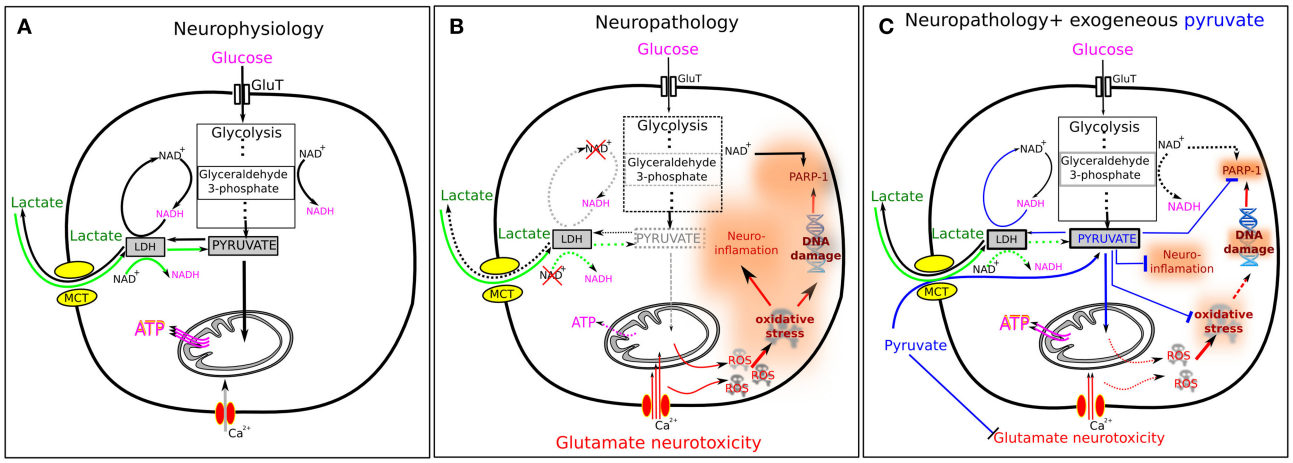


FIGURE 1 | Pyruvate and lactate in normal and pathological conditions. **(A)** In normal conditions, glucose enters the cell via glucose transporters (GluT) and is metabolized in a 10-step glycolysis. Endogenous pyruvate is a final product of glycolysis and the main energy substrate for ATP generation in mitochondria. Excessive for mitochondria pyruvate can be transformed to lactate by lactate dehydrogenase (LDH) in the presence of NADH. Extracellular lactate can enter the cell via monocarboxylate transporters (MCT). Lactate then is converted to pyruvate by LDH in the presence of NAD^+ (green arrows). This explains why extracellular lactate may serve efficiently as the energy fuel for brain cells. **(B)** The situation is changed radically under pathological conditions. A number of neurological disorders are characterized by the oxidative stress and increased level of interstitial glutamate—both factors inducing strong excitotoxicity. Oxidative stress results from excessive presence of ROS, while glutamate induces cellular overload with Ca^{2+} ions. ROS induce DNA damage leading to the overactivation of poly-ADP ribose polymerase-1 (PARP-1) that results in depletion of cytosolic NAD^+. ROS also activate the pro-inflammatory transcription factor NF-kB inducing neuroinflammation. Depletion of NAD^+ induces inhibition of glycolysis since the glycolysis step 6, conversion of glyceraldehyde 3-phosphate to 3-phosphoglycerate, requires two molecules of NAD^+. This results in the insufficient outcome of pyruvate and decline in the mitochondrial ATP production. Moreover, NAD^+ depletion makes ineffectual the conversion of lactate to pyruvate (dashed green arrows) and lactate cannot serve as the energy substrate anymore. **(C)** Exogenous pyruvate is able to ameliorate many impaired cellular functions described in **(B)**. In blood, pyruvate activates a blood resident enzyme glutamate–pyruvate transaminase which transforms glutamate into 2-ketoglutarate and thus lowers the blood glutamate level. This results in an enhanced efflux of glutamate from brain parenchyma that reduces neuronal overload with Ca^{2+} ions. Pyruvate reacts directly with H_2O_2producing acetate, H_2O and CO_2 and thus reducing oxidative stress. Pyruvate inhibits PARP-1 overactivation that prevents depletion of NAD^+ and thus promotes glycolysis (glyceraldehyde-3-phosphate depending step). It also inhibits expression of several pro-inflammatory proteins, such as tumor necrosis factor (TNF), interleukin 6 (IL-6) and others. All these effects explain the neuroprotective properties of pyruvate.

(PARP-1) overactivation (see below and **Figure 1C**).

PYRUVATE ENHANCES GLYCOGEN CONTENT IN ASTROCYTES

Pyruvate supplementation prior to glucose deprivation significantly protected synaptic function against the deleterious effects of hypoglycemia in brain slices (Shetty et al., 2012). The authors associated beneficial effect of pyruvate with both increased glycogen content during pyruvate pretreatment and subsequent glycogen utilization during glucose deprivation leading to the increased ATP levels. Interestingly, both extra glucose and lactate pretreatment also increased the glycogen content, although significantly less efficiently than pyruvate. However, neither lactate nor extra glucose pretreatment was sufficient to provide the protective effect on synaptic transmission during glucose deprivation.

Pyruvate chronic supplementation also strongly increased the glycogen content of cortical tissue *in vivo* in the Alzheimer's disease mouse model (APPswe/PS1dE9) (Zilberter et al., 2013).

PYRUVATE PROVIDES NEUROPROTECTION AGAINST DAMAGE INDUCED BY POLY-ADP RIBOSE POLYMERASE-1 OVERACTIVATION

Poly-ADP ribose polymerase 1 (PARP-1) synthesizes polymers of ADP-ribose that are implicated in regulation of a number of cellular processes including modulation of transcription, DNA repair, neuronal survival and death (Smith et al., 2013). Importantly, to generate polymers of ADP-ribose PARP-1 consumes cytoplasmic NAD^+. In various neurological disorders, excessive activation of PARP-1 by oxidative stress has been documented (Ma et al., 2012). This process compromised cell survival via activation of pro-death pathways by ADP-ribose polymers and by creating energy deficit via depletion of cytoplasmic NAD^+ that was followed by inhibition of glycolysis and ATP production (see **Figure 1B**).

It has been also reported recently that PARP-1 directly inhibits hexokinase (Andrabi et al., 2014), increasing its potential for blocking glycolysis. Importantly, Ying and colleagues reported (Ying et al., 2002) that exogenous TCA cycle substrates (including pyruvate) administration following PARP-1 activation reduced cell death in the astrocyte–neuron cultures from approximately 70% to 30%.

Similar neuroprotective effects of pyruvate was reported *in vivo* in transient cerebral ischemia and severe hypoglycemia models, in which PARP-1 had been shown to be a key mediator of neurotoxicity (Suh et al., 2003; Moroni and Chiarugi,

2009). In these models, pyruvate treatment either completely prevented the neuronal loss or reduced it by 70–90% (Lee et al., 2001; Suh et al., 2005). Brain damage reduction due to pyruvate treatment was also reported in the rodent model of traumatic brain injury with documented prominent oxidative stress, PARP-1 overactivation and loss of NAD^+ (Satchell et al., 2003; Clark et al., 2007; Fukushima et al., 2009; Sharma et al., 2009). Venous infusion of pyruvate after controlled arterial hemorrhage in swine reduced oxidative stress and PARP fragmentation in the brain (Mongan et al., 2003). Although elucidating the exact mechanisms of pyruvate neuroprotection was beyond the scope of these studies, the authors suggested that the pyruvate action includes the ROS scavenging, NAD^+ replenishment, recovering the pyruvate-dehydrogenase activity and direct mitochondrial fueling.

Interestingly, PARP-1 overactivation was also demonstrated in the brain of transgenic Alzheimer's disease mouse model (Abeti et al., 2011). In mixed cultures of neurons and glial cells, β-amyloid peptide, the major neurotoxic agent in the pathophysiology of Alzheimer's disease, evokes oxidative stress followed by hyperactivation of PARP-1, depolarization of mitochondrial membrane and finally cell death. (Abeti and Duchen, 2012). Addition of pyruvate to culture medium of β-amyloid treated cells prevented the mitochondrial membrane potential loss (Abramov and Duchen, 2005) and improved cell survival (Alvarez et al., 2003).

One reasonable explanation for the efficient pyruvate action may be in its antioxidant properties. Since PARP-1 is activated in response to oxidative damage to DNA, reducing oxidative stress would decrease PARP-1 activity resulting in NAD^+ depletion. In addition, exogenous pyruvate can provide energy in conditions when glycolysis intensity is reduced due to a low cytoplasmic NAD^+. Indeed, pyruvate is a "direct" energy substrate for mitochondria, while lactate needs to be converted first to pyruvate in the reaction dependent on the availability of cytoplasmic NAD^+. Importantly, mitochondrial pool of NAD^+, indispensible for pyruvate metabolism in mitochondria, is maintained for at least 24 h when cytoplasmic NAD^+ is depleted (Stein and Imai, 2012), thus ensuring energy production.

ANTIEPILEPTIC EFFECTS OF PYRUVATE

Recently, a robust antiepileptic effect of pyruvate (combined with antioxidants ascorbic acid and alpha-tocopherol) treatment has been revealed in the genetic model of temporal lobe epilepsy (Simeone et al., 2014). In addition, the authors showed that a single pretreatment of wild-type mice with these drugs reduced the severity of kainate-induced events resulting in 100% protection from severe tonic–clonic seizures. Unfortunately, the authors did not determine the contribution of each applied drug to the antiepileptic effect. To the best of our knowledge neither ascorbic acid nor alpha-tocopherol expresses significant antiepileptic action (Waldbaum and Patel, 2010). Therefore, we believe that pyruvate is the major player in the Simeone's work and the pyruvate antiepeileptic effect is presumably reinforced by complementary antioxidants.

Neuronal hyperactivity leading to abnormal oscillations and epilepsy, characteristic for Alzheimer's disease (Amatniek et al., 2006; Noebels, 2011), has been observed as well in different mouse models of the disease (Palop and Mucke, 2009). We found (Minkeviciene et al., 2009; Zilberter et al., 2013) that the general reason for hyperactivity may be the Aβ-induced modification of basic neuronal properties, such as the resting membrane potential and reversal potential of GABA-induced currents, presumably evoked by energy metabolism imbalance. Critically, in the presence of pyruvate, Aβ failed to induce its deleterious effects on the cellular parameters. Moreover, pyruvate chronic dietary supplementation considerably reduced epileptic phenotype in APP/PS1 mice (Zilberter et al., 2013). In another Alzheimer's disease model (3xTg-AD mice), chronic pyruvate treatment reduced both oxidative stress and hyperexcitability, and inhibited short and long-term memory deficits (Isopi et al., 2014).

CONCLUSIONS

Oxidative stress and metabolic dysfunction are significant pathogenic factors contributing to neurological disorders. Pyruvate may be a unique therapeutic tool for correcting neuronal network abnormalities developing due to these factors. Combination of the following properties validates this conclusion: (i) Oxidative stress is the general feature of neurological disorders and is associated with accumulation of ROS. Pyruvate is a potent scavenger of ROS and its contribution to the antioxidant defense system becomes significant during neuropathologies; (ii) The oxidative stress-induced overactivation of PARP-1 results in the depletion of cytosolic NAD^+ and inhibition of glycolysis that evokes energy deficiency and frequently results in a cell death. Pyruvate significantly abates overactivation of PARP-1. In addition, as pyruvate is a direct substrate for mitochondrial metabolism and its oxidation does not depend on the cytoplasmic redox state, pyruvate bypasses restrictions imposed by PARP-1 and can restore energy deficiency in such conditions; (iii) Pyruvate reduces the blood glutamate level, facilitating the glutamate efflux from brain tissue through the blood-brain barrier thus reducing the glutamate-induced neurotoxicity; (iv) Pyruvate augements glycogen stores, thus increasing neuronal tolerance to ischemia and hypoglycemia; (v) Neuroinflammation is a common attribute of a number of neuropathologies. Pyruvate reveals a potent anti-inflammatory action; (vi) Pyruvate prevents neural network hyperexcitability.

We conclude that pyruvate, in addition to its well-recognized function in energy metabolism, is a powerful neuroprotector, the potential therapeutic significance of which is yet widely underrated.

ACKNOWLEDGMENTS

We thank Drs. Tanya Zilberter and Misha Zilberter for their help in manuscript preparation. Our work was supported by the Alzheimer's Association research grant NESAD-12-242486.

REFERENCES

Abeti, R., Abramov, A. Y., and Duchen, M. R. (2011). Beta-amyloid activates PARP causing astrocytic metabolic failure and neuronal death. *Brain* 134, 1658–1672. doi: 10.1093/brain/awr104

Abeti, R., and Duchen, M. R. (2012). Activation of PARP by oxidative stress induced by beta-amyloid: implications for Alzheimer's disease. *Neurochem.*

Res. 37, 2589–2596. doi: 10.1007/s11064-012-0895-x

Abramov, A. Y., and Duchen, M. R. (2005). The role of an astrocytic NADPH oxidase in the neurotoxicity of amyloid beta peptides. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 360, 2309–2314. doi: 10.1098/rstb.2005.1766

Alvarez, G., Ramos, M., Ruiz, F., Satrustegui, J., and Bogonez, E. (2003). Pyruvate protection against beta-amyloid-induced neuronal death: role of mitochondrial redox state. *J. Neurosci. Res.* 73, 260–269. doi: 10.1002/jnr.10648

Amatniek, J. C., Hauser, W. A., Delcastillo-Castaneda, C., Jacobs, D. M., Marder, K., Bell, K., et al. (2006). Incidence and predictors of seizures in patients with Alzheimer's disease. *Epilepsia* 47, 867–872. doi: 10.1111/j.1528-1167.2006.00554.x

Andrabi, S. A., Umanah, G. K., Chang, C., Stevens, D. A., Karuppagounder, S. S., Gagne, J. P., et al. (2014). Poly(ADP-ribose) polymerase-dependent energy depletion occurs through inhibition of glycolysis. *Proc. Natl. Acad. Sci. U.S.A.* 111, 10209–10214. doi: 10.1073/pnas.1405158111

Cai, H., Cong, W. N., Ji, S., Rothman, S., Maudsley, S., and Martin, B. (2012). Metabolic dysfunction in Alzheimer's disease and related neurodegenerative disorders. *Curr. Alzheimer Res.* 9, 5–17. doi: 10.2174/156720512799015064

Carvalho, A. S., Torres, L. B., Persike, D. S., Fernandes, M. J., Amado, D., Naffah-Mazzacoratti Mda, G., et al. (2011). Neuroprotective effect of pyruvate and oxaloacetate during pilocarpine induced status epilepticus in rats. *Neurochem. Int.* 58, 385–390. doi: 10.1016/j.neuint.2010.12.014

Clark, R. S., Vagni, V. A., Nathaniel, P. D., Jenkins, L. W., Dixon, C. E., and Szabo, C. (2007). Local administration of the poly(ADP-ribose) polymerase inhibitor INO-1001 prevents NAD+ depletion and improves water maze performance after traumatic brain injury in mice. *J. Neurotrauma* 24, 1399–1405. doi: 10.1089/neu.2007.0305

Das, U. N. (2006). Is pyruvate an endogenous anti-inflammatory molecule? *Nutrition* 22, 965–972. doi: 10.1016/j.nut.2006.05.009

Desagher, S., Glowinski, J., and Premont, J. (1997). Pyruvate protects neurons against hydrogen peroxide-induced toxicity. *J. Neurosci.* 17, 9060–9067.

Fukushima, M., Lee, S. M., Moro, N., Hovda, D. A., and Sutton, R. L. (2009). Metabolic and histologic effects of sodium pyruvate treatment in the rat after cortical contusion injury. *J. Neurotrauma* 26, 1095–1110. doi: 10.1089/neu.2008.0771

Gottlieb, M., Wang, Y., and Teichberg, V. I. (2003). Blood-mediated scavenging of cerebrospinal fluid glutamate. *J. Neurochem.* 87, 119–126. doi: 10.1046/j.1471-4159.2003.01972.x

Heneka, M. T., Kummer, M. P., and Latz, E. (2014). Innate immune activation in neurodegenerative disease. *Nat. Rev. Immunol.* 14, 463–477. doi: 10.1038/nri3705

Holleman, A. F. (1904). Notice sur l'action de l'eau oxygénée sur les acides α−cétoniques et sur les dicétones 1. 2. *Recueil des Travaux Chimiques des Pays-Bas et de la Belgique* 23, 169–172. doi: 10.1002/recl.19040230504

Isopi, E., Granzotto, A., Corona, C., Bomba, M., Ciavardelli, D., Curcio, M., et al. (2014). Pyruvate prevents the development of age-dependent cognitive deficits in a mouse model of Alzheimer's disease without reducing amyloid and tau pathology. *Neurobiol. Dis.* doi: 10.1016/j.nbd.2014.11.013. [Epub ahead of print].

Kao, K. K., and Fink, M. P. (2010). The biochemical basis for the anti-inflammatory and cytoprotective actions of ethyl pyruvate and related compounds. *Biochem. Pharmacol.* 80, 151–159. doi: 10.1016/j.bcp.2010.03.007

Lee, J. Y., Kim, Y. H., and Koh, J. Y. (2001). Protection by pyruvate against transient forebrain ischemia in rats. *J. Neurosci.* 21:RC171.

Ma, Y., Chen, H., He, X., Nie, H., Hong, Y., Sheng, C., et al. (2012). NAD+ metabolism and NAD(+)-dependent enzymes: promising therapeutic targets for neurological diseases. *Curr. Drug Targets* 13, 222–229. doi: 10.2174/138945012799201711

Melo, A., Monteiro, L., Lima, R. M., Oliveira, D. M., Cerqueira, M. D., and El-Bacha, R. S. (2011). Oxidative stress in neurodegenerative diseases: mechanisms and therapeutic perspectives. *Oxid. Med. Cell. Longev.* 2011:467180. doi: 10.1155/2011/467180

Minkeviciene, R., Rheims, S., Dobszay, M. B., Zilberter, M., Hartikainen, J., Fülöp, L., et al. (2009). Fibrillar β-amyloid-induced hyperexcitability of cortical and hippocampal neurons triggers progressive epilepsy. *J. Neurosci.* 29, 3453–3462. doi: 10.1523/JNEUROSCI.5215-08.2009

Mongan, P. D., Karaian, J., Van Der Schuur, B. M., Via, D. K., and Sharma, P. (2003). Pyruvate prevents poly-ADP ribose polymerase (PARP) activation, oxidative damage, and pyruvate dehydrogenase deactivation during hemorrhagic shock in swine. *J. Surg. Res.* 112, 180–188. doi: 10.1016/S0022-4804(03)00148-3

Moroni, F., and Chiarugi, A. (2009). Post-ischemic brain damage: targeting PARP-1 within the ischemic neurovascular units as a realistic avenue to stroke treatment. *FEBS J.* 276, 36–45. doi: 10.1111/j.1742-4658.2008.06768.x

Noebels, J. (2011). A perfect storm: converging paths of epilepsy and Alzheimer's dementia intersect in the hippocampal formation. *Epilepsia* (Suppl. 1), 39–46. doi: 10.1111/j.1528-1167.2010.02909.x

Palop, J. J., and Mucke, L. (2009). Epilepsy and cognitive impairments in Alzheimer disease. *Arch. Neurol.* 66, 435–440. doi: 10.1001/archneurol.2009.15

Satchell, M. A., Zhang, X., Kochanek, P. M., Dixon, C. E., Jenkins, L. W., Melick, J., et al. (2003). A dual role for poly-ADP-ribosylation in spatial memory acquisition after traumatic brain injury in mice involving NAD+ depletion and ribosylation of 14-3-3gamma. *J. Neurochem.* 85, 697–708. doi: 10.1046/j.1471-4159.2003.01707.x

Sharma, P., Benford, B., Li, Z. Z., and Ling, G. S. (2009). Role of pyruvate dehydrogenase complex in traumatic brain injury and Measurement of pyruvate dehydrogenase enzyme by dipstick test. *J. Emerg. Trauma Shock* 2, 67–72. doi: 10.4103/0974-2700.50739

Shetty, P. K., Sadgrove, M. P., Galeffi, F., and Turner, D. A. (2012). Pyruvate incubation enhances glycogen stores and sustains neuronal function during subsequent glucose deprivation. *Neurobiol. Dis.* 45, 177–187. doi: 10.1016/j.nbd.2011.08.002

Simeone, K. A., Matthews, S. A., Samson, K. K., and Simeone, T. A. (2014). Targeting deficiencies in mitochondrial respiratory complex I and functional uncoupling exerts anti-seizure effects in a genetic model of temporal lobe epilepsy and in a model of acute temporal lobe seizures. *Exp. Neurol.* 251, 84–90. doi: 10.1016/j.expneurol.2013.11.005

Smith, J. A., Park, S., Krause, J. S., and Banik, N. L. (2013). Oxidative stress, DNA damage, and the telomeric complex as therapeutic targets in acute neurodegeneration. *Neurochem. Int.* 62, 764–775. doi: 10.1016/j.neuint.2013.02.013

Stein, L. R., and Imai, S. (2012). The dynamic regulation of NAD metabolism in mitochondria. *Trends Endocrinol. Metab.* 23, 420–428. doi: 10.1016/j.tem.2012.06.005

Suh, S. W., Aoyama, K., Chen, Y., Garnier, P., Matsumori, Y., Gum, E., et al. (2003). Hypoglycemic neuronal death and cognitive impairment are prevented by poly(ADP-ribose) polymerase inhibitors administered after hypoglycemia. *J. Neurosci.* 23, 10681–10690.

Suh, S. W., Aoyama, K., Matsumori, Y., Liu, J., and Swanson, R. A. (2005). Pyruvate administered after severe hypoglycemia reduces neuronal death and cognitive impairment. *Diabetes* 54, 1452–1458. doi: 10.2337/diabetes.54.5.1452

Teichberg, V. I., Cohen-Kashi-Malina, K., Cooper, I., and Zlotnik, A. (2009). Homeostasis of glutamate in brain fluids: an accelerated brain-to-blood efflux of excess glutamate is produced by blood glutamate scavenging and offers protection from neuropathologies. *Neuroscience* 158, 301–308. doi: 10.1016/j.neuroscience.2008.02.075

Waldbaum, S., and Patel, M. (2010). Mitochondria, oxidative stress, and temporal lobe epilepsy. *Epilepsy Res.* 88, 23–45. doi: 10.1016/j.eplepsyres.2009.09.020

Wang, Y., and Qin, Z. H. (2010). Molecular and cellular mechanisms of excitotoxic neuronal death. *Apoptosis* 15, 1382–1402. doi: 10.1007/s10495-010-0481-0

Ying, W., Chen, Y., Alano, C. C., and Swanson, R. A. (2002). Tricarboxylic acid cycle substrates prevent PARP-mediated death of neurons and astrocytes. *J. Cereb. Blood Flow Metab.* 22, 774–779. doi: 10.1097/00004647-200207000-00002

Zilberter, M., Ivanov, A., Ziyatdinova, S., Mukhtarov, M., Malkov, A., Alpar, A., et al. (2013). Dietary energy substrates reverse early neuronal hyperactivity in a mouse model of Alzheimer's disease. *J. Neurochem.* 125, 157–171. doi: 10.1111/jnc.12127

Zlotnik, A., Gurevich, B., Cherniavsky, E., Tkachov, S., Matuzani-Ruban, A., Leon, A., et al. (2008). The contribution of the blood glutamate scavenging activity of pyruvate to its neuroprotective properties in a rat model of closed head injury. *Neurochem. Res.* 33, 1044–1050. doi: 10.1007/s11064-007-9548-x

Zlotnik, A., Sinelnikov, I., Gruenbaum, B. F., Gruenbaum, S. E., Dubilet, M., Dubilet, E., et al. (2012). Effect of glutamate and blood glutamate scavengers oxaloacetate and pyruvate

on neurological outcome and pathohistology of the hippocampus after traumatic brain injury in rats. *Anesthesiology* 116, 73–83. doi: 10.1097/ALN.0b013e31823d7731

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 27 October 2014; accepted: 12 January 2015; published online: 17 February 2015.
*Citation: Zilberter Y, Gubkina O and Ivanov AI (2015) A unique array of neuroprotective effects of pyruvate in neuropathology. Front. Neurosci. **9**:17. doi: 10.3389/fnins.2015.00017*
This article was submitted to Neuroenergetics, Nutrition and Brain Health, a section of the journal Frontiers in Neuroscience.

frontiers in
NEUROSCIENCE

PERSPECTIVE ARTICLE
published: 27 February 2015
doi: 10.3389/fnins.2015.00022

Lactate is always the end product of glycolysis

Matthew J. Rogatzki[1], Brian S. Ferguson[2], Matthew L. Goodwin[3] and L. Bruce Gladden[4]*

[1] Department of Health and Human Performance, University of Wisconsin-Platteville, Platteville, WI, USA
[2] Department of Biomedical Sciences, University of Missouri, Columbia, MO, USA
[3] Department of Orthopaedics, and Huntsman Cancer Institute, University of Utah, Salt Lake City, UT, USA
[4] School of Kinesiology, Auburn University, Auburn, AL, USA

Edited by:
Pierre Magistretti, École polytechnique fédérale de Lausanne, Switzerland

Reviewed by:
Avital Schurr, University of Louisville, USA
George A. Brooks, University of California, USA

***Correspondence:**
L. Bruce Gladden, School of Kinesiology, Auburn University, 301 Wire Road, Auburn, AL 36849, USA
e-mail: gladdlb@auburn.edu

Through much of the history of metabolism, lactate (La^-) has been considered merely a dead-end waste product during periods of dysoxia. Congruently, the end product of glycolysis has been viewed dichotomously: pyruvate in the presence of adequate oxygenation, La^- in the absence of adequate oxygenation. In contrast, given the near-equilibrium nature of the lactate dehydrogenase (LDH) reaction and that LDH has a much higher activity than the putative regulatory enzymes of the glycolytic and oxidative pathways, we contend that La^- is always the end product of glycolysis. Cellular La^- accumulation, as opposed to flux, is dependent on (1) the rate of glycolysis, (2) oxidative enzyme activity, (3) cellular O_2 level, and (4) the net rate of La^- transport into (influx) or out of (efflux) the cell. For intracellular metabolism, we reintroduce the Cytosol-to-Mitochondria Lactate Shuttle. Our proposition, analogous to the phosphocreatine shuttle, purports that pyruvate, NAD^+, NADH, and La^- are held uniformly near equilibrium throughout the cell cytosol due to the high activity of LDH. La^- is always the end product of glycolysis and represents the primary diffusing species capable of spatially linking glycolysis to oxidative phosphorylation.

Keywords: aerobic, anaerobic, lactate dehydrogenase, mitochondria, NADH, pyruvate, cytosolic lactate shuttle

INTRODUCTION

In the nineteenth century, ≈80 years after the discovery of lactate (La^-) by Scheele (Kompanje et al., 2007), Louis Pasteur noticed that facultative yeast cells grew more under aerobic than anaerobic conditions, yet the consumption of sugar was decreased and fermentation to alcohol was less under aerobic conditions (Pasteur, 1861). Previously, Pasteur (1858) had recognized that some types of yeast fermented sugar to La^- under anaerobic, but not aerobic conditions. This phenomenon (for both alcohol and La^- fermentation) has been called the Pasteur Effect (Barnett and Entian, 2005). A parallel phenomenon was discovered in skeletal muscle and whole animals. For skeletal muscle Fletcher and Hopkins (1907) reported that La^- accrued in anaerobic frog muscles at rest. During stimulation, La^- concentration ($[La^-]$) increased rapidly in anaerobic amphibian muscle, but disappeared when these fatigued muscles were allowed to recover in an oxygen (O_2) rich environment. Subsequently, Meyerhof demonstrated conclusively that glycogen was the precursor of La^- in isolated muscles, and the full glycolytic pathway was elucidated by the early 1940s (Meyerhof, 1942; Brooks and Gladden, 2003). The traditional dogma was built upon this framework and other research on hypoxia: Pyruvate is the end product of glycolysis under aerobic conditions and La^- is the end product when O_2 is insufficient. Schurr (2006) discussed this dogma from the viewpoint of brain metabolism.

It is widely accepted that intracellular PO_2 values of ≈0.5 Torr or less result in O_2-limited oxidative phosphorylation, a condition termed dysoxia (Connett et al., 1990), with ensuing La^- production and accumulation. However, Stainsby and Welch (1966) reported La^- efflux from an ostensibly well-oxygenated contracting muscle. Subsequently, Jöbsis and Stainsby (1968) observed La^- production and release from a contracting canine skeletal muscle while the NAD^+/NADH redox couple was becoming more oxidized, an indication of adequate O_2 supply. Using a different approach, myoglobin cryomicrospectroscopy, to determine PO_2 in dog gracilis muscle contracting at progressively faster rates, Connett et al. (1986) found increasing La^- efflux without evidence of dysoxia; the lowest PO_2 values were generally on the order of 2 Torr. Richardson et al. (1998) used proton magnetic resonance spectroscopy (MRS) to determine myoglobin saturation (and thereby intracellular PO_2) in humans during graded exercise. In parallel experiments with the same type of exercise, La^- efflux was determined via arteriovenous concentration differences and blood flow. They found La^- efflux in the presence of intracellular PO_2 levels (∼3 Torr) that should not limit oxidative phosphorylation. Véga et al. (1998) also reported that isolated, stimulated nerve tissue releases lactate during aerobic conditions.

These findings, along with other abundant circumstantial evidence indicate that net La^- production and efflux from cells can occur under aerobic conditions (Gladden, 2004a,b). In fact, Brooks (2000) proposed that "lactate was produced all the time in fully oxygenated cells and tissues." Schurr (2006) discussed this proposition in detail, proposing that "glycolysis always proceeds to its final step, the LDH reaction and the formation of lactate" in brain tissue but most likely in many other tissues as well. Subsequently, Schurr and Payne (2007) and

Schurr and Gozal (2012) provided supportive experimental evidence for this postulate in hippocampal brain slices. Here, we embrace this concept, proposing that even in the absence of net La^- accumulation, and in the presence of plentiful O_2, La^- is the natural end product of glycolysis. Importantly, we use basic biochemical principles to undergird this concept and re-introduce the Cytosol-to-Mitochondria Lactate Shuttle.

THE LDH REACTION IS A NEAR EQUILIBRIUM REACTION

La^- is formed in the following reaction that is catalyzed by the enzyme lactate dehydrogenase (LDH):

$$\text{Pyruvate}^- + \text{NADH} + \text{H}^+ \leftrightarrow \text{Lactate}^- + \text{NAD}^+$$

The equilibrium constant is strongly in favor of La^- ($1.62 \times 10^{11}\ M^{-1}$) (Lambeth and Kushmerick, 2002), and LDH activity is high relative to the putative regulatory enzymes in the glycolytic pathway in skeletal muscle (Connett and Sahlin, 2011), liver, kidney, cardiac muscle, spleen, and fat (Shonk and Boxer, 1964), brain (Iwangoff et al., 1980; Morland et al., 2007), and both malignant and benign mammary tumors (Larner and Rutherford, 1978; Balinsky et al., 1984). Importantly, LDH activity is also high in comparison to the putative regulatory enzymes of pyruvate oxidation; see Spriet et al. (2000) for skeletal muscle, Morland et al. (2007) for brain, and Marie and Shinjo (2011) for brain cancer. While measures of tissue La^- to pyruvate ratios are scarce, some example values are ≈7:1 for liver (Liaw et al., 1985), ≈10–13:1 for resting skeletal muscle (Sahlin et al., 1976; Liaw et al., 1985), and values as high as 159:1 in skeletal muscle immediately following exhaustive dynamic exercise (Sahlin et al., 1976). Reference values for the La^- to pyruvate ratio in the brain, using microdialysis probes, average 23:1 (Reinstrup et al., 2000; Sahuquillo et al., 2014). Typically, the ratio rises following traumatic brain injury, even in the absence of ischemia or low tissue PO_2 {≥ 25(Sahuquillo et al., 2014); ≥40 (Vespa et al., 2005)}. Despite standardization of techniques, microdialysis values do not necessarily reflect real tissue concentrations (Sahuquillo et al., 2014). Nevertheless, these La^- to pyruvate microdialysis values for human brain are not far afield from values (≈13:1) obtained on rat brain homogenates (Ponten et al., 1973). Overall, the high [La^-] relative to [pyruvate] even with adequate O_2 supply, reinforces the role of LDH activity in determining La^- appearance. The high LDH activity and La^--leaning equilibrium constant of the LDH reaction are key elements in the proposition that La^- is the major end product of glycolysis under essentially all metabolic conditions. Simply put, any time glycolysis is operative, regardless of local oxygen tension, La^- is being formed in most types of tissues. However, the amount of La^- produced and actually accumulated (i.e., an increased [La^-]) can be altered by factors such as O_2 tension, metabolic rate, available mitochondrial activity, and other factors.

FATES OF PYRUVATE

Potential fates of pyruvate are listed below. We propose that none of these processes occurs at a rate that matches the initial conversion of pyruvate to La^-, thus ensuring that La^- is always the end product of glycolysis.

1. Efflux from the cell primarily via monocarboxylate transporters (MCTs). However, La^- is always present in a higher concentration than pyruvate and will depart cells at a faster rate than will pyruvate.
2. Conversion to alanine via the near equilibrium alanine aminotransferase reaction which has an equilibrium constant of about 1 (Tiidus et al., 2012), so alanine concentration should approximate pyruvate concentration and the conversion of pyruvate to alanine should not detract from the conversion of pyruvate to La^-.
3. Gluconeogenic/Glyconeogenic reactions. In gluconeogenic tissues, pyruvate can be converted to oxaloacetate in a reaction catalyzed by pyruvate carboxylase (Pascoe and Gladden, 1996). In skeletal muscle glyconeogenesis, pyruvate can be converted to malate with catalysis by malic enzyme (Pascoe and Gladden, 1996) or more likely to phosphoenolpyruvate via reversal of the pyruvate kinase reaction (Donovan and Pagliassotti, 2000). These reactions represent "reversal" of glycolysis and they begin with La^-, the natural end product of glycolysis. In the brain, glycogen is most abundant in astrocytes and sparse to negligible in neurons (Cataldo and Broadwell, 1986). Although pyruvate carboxylase is expressed in cultured astroglial cells, oligodendrocytes, microglial cells, and ependymocytes (Murin et al., 2009), we are unaware of any information on the ability of any of these cells to synthesize glycogen from La^-.
4. Transport across the mitochondrial inner membrane with subsequent conversion to Acetyl-CoA via the pyruvate dehydrogenase (PDH) reaction followed by entry into the tricarboxylic acid cycle and oxidation. Pyruvate crosses the inner mitochondrial membrane via simple diffusion and facilitated diffusion; the transporters are an MCT (Hashimoto et al., 2006) and the mitochondrial pyruvate carrier (Divakaruni and Murphy, 2012). For ongoing oxidation of pyruvate, NADH shuttling into the mitochondrial matrix by the malate-aspartate and glycerol phosphate shuttles is equally important as pyruvate transport.

The constant presence of La^- and its accumulation during periods of glycolytic stimulation is evidence that the LDH reaction predominates over these alternative fates of pyruvate.

Figure 1 illustrates a model of intracellular metabolism which we call the "Cytosol-to-Mitochondria Lactate Shuttle"; its origin can be traced to a review of La^- metabolism by Stainsby and Brooks (1990). Because of the high LDH activity and an equilibrium constant far in the direction of La^-, La^- is always the predominant result of glycolysis. However, formation of La^- is not synonymous with La^- accumulation and increased [La^-]. Mitochondria constitute a sink for pyruvate and under conditions of slow glycolytic activity with ample O_2, oxidation in most cells is sufficient to closely match production by glycolysis; transmembrane La^- flux will vary between slow release and slow uptake with release being the more typical condition. In a manner analogous to creatine kinase and the Phosphocreatine Shuttle, LDH holds pyruvate and La^- in equilibrium throughout the cell cytosol. In this scenario, La^- is the primary species that travels to the neighborhood of the mitochondrial reticulum,

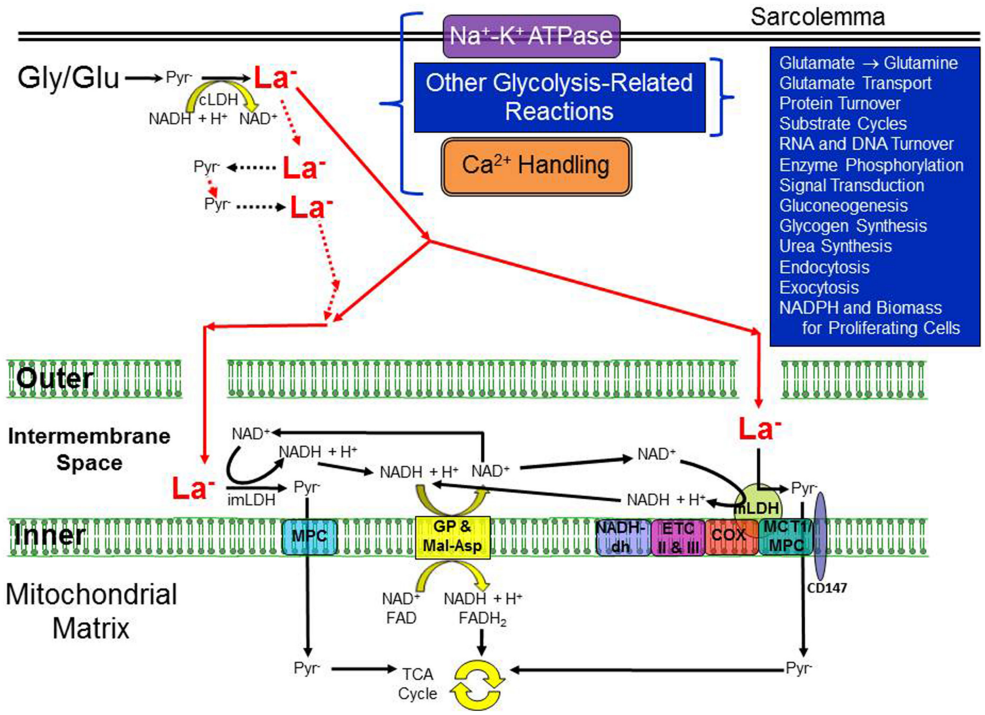


FIGURE 1 | Illustration of the essential elements of the re-introduced Cytosol-to-Mitochondria Lactate Shuttle. A high activity of cytosolic LDH is considered to guarantee La^- formation in the cytosol under virtually all conditions but especially during periods of increased glycolytic activity. Not all cells would necessarily exhibit all of the processes shown in the upper right quadrant. La^- can be formed throughout the cytosol; two particular locations are noted for which there is evidence of compartmentation with glycolysis, one in association with the Na^+-K^+-ATPase pump in the sarcolemma and the other for skeletal and cardiac muscle, the Ca^{2+}-ATPase in the sarcoplasmic reticulum membrane. The sarcolemma is illustrated by the thick double lines at the top of the cartoon whereas the inner and outer mitochondrial membranes are dramatically enlarged to demonstrate possible La^- pathways. The gaps in the outer mitochondrial membrane illustrate that it is freely permeable to most small molecules (but probably not permeable to LDH). La^- is shown in bold and red, and larger than pyruvate (Pyr^-) to indicate that La^- is typically present in much higher concentration than Pyr^- (i.e., a high La^-/Pyr^- ratio). Whether La^- is converted back to Pyr^- outside the intermembrane space, inside the space, or via a mitochondrial LDH, the resulting NADH + H^+ would be shuttled across the inner mitochondrial membrane via the malate-aspartate and glycerol phosphate shuttles. Pyr^- could be transported across the inner mitochondrial membrane by either a mitochondrial pyruvate carrier (MPC) or a monocarboxylate transporter (MCT), both of which have been identified in the inner membrane. COX indicates cytochrome oxidase; cLDH, cytosolic lactate dehydrogenase; CD147, single-span transmembrane glycoprotein; ETC II and III, electron transport chain complexes II and III; Gly, glycogen; Glu, glucose; imLDH, LDH in the intermembrane space; Inner, inner mitochondrial membrane; La^-, lactate; MCT1, monocarboxylate transporter 1; mLDH, mitochondrial LDH; MPC, mitochondrial pyruvate carrier; NADH-dh, NADH dehydrogenase complex I; Outer, outer mitochondrial membrane; Pyr^-, pyruvate. Conceived from (1) Stainsby and Brooks (1990), (2) Hashimoto et al. (2006), and (3) Gladden (2008).

most likely to the intermembrane space where LDH is attached to the outer side of the inner mitochondrial membrane (Hashimoto et al., 2006; Gladden, 2008). Here, La^- is converted to pyruvate for entry into the mitochondria, given the relative "sink" for pyruvate. Simultaneously, NADH is regenerated from the reversal of the LDH reaction and its pair of electrons is shuttled across the inner mitochondrial membrane by the malate-aspartate and glycerol phosphate shuttles. An important difference from the Phosphocreatine Shuttle is that two key components, La^- and pyruvate, unlike phosphocreatine, can cross the plasma membrane and leave the cell.

The Cytosol-to-Mitochondria paradigm posits that La^- is always formed during glycolysis, even if La^- is not accumulating and $[La^-]$ is stable. Of course, if O_2 is so low that oxidative phosphorylation is inhibited, then La^- production will exceed the rate at which oxidative metabolism can use pyruvate and NADH, causing $[La^-]$ and La^- efflux to rise. Also, if glycolytic activity increases even with ample O_2 levels, as in skeletal muscle contracting at a moderate intensity or perhaps in activated astrocytes (Pellerin and Magistretti, 2011), La^- production will not be matched by pyruvate oxidation and $[La^-]$ will rise as will transport of La^- out of the cell. Similarly, if glycolytic enzyme activity is enhanced and/or mitochondrial function (oxidative enzyme activity) is downregulated such that glycolysis is favored over oxidation, there will be an ongoing mismatch between La^- production and subsequent pyruvate and NADH oxidation resulting in elevated $[La^-]$ and La^- efflux. This latter situation is observed in "Warburg" cancer cells (Semenza, 2008) and in COPD patients during whole body exercise *in vivo* (Maltais et al., 1996).

With endurance exercise training, skeletal muscle mitochondrial content is increased (Holloszy and Coyle, 1984), and there is now a larger sink for pyruvate. Increased mitochondrial oxidative activity requires lower levels of stimulators (e.g., ADP) for a particular oxidative phosphorylation rate; these same stimuli

are allosteric stimulators of key glycolytic enzymes so glycolysis is reduced. Additionally, if La^- membrane transport is inhibited, particularly in cells that already have a mismatch in which glycolysis is favored over oxidative metabolism, it is likely that cellular [La^-] will rise with potentially deleterious effects on the cell (Le Floch et al., 2011). Further, strong inhibition of total LDH activity in glycolytic cells should prevent equilibrium and thereby reduce La^- production, accumulation, and efflux (Fantin et al., 2006). However, the effect of changing the LDH isozyme pattern independent of inhibition or reduction of total LDH activity is still yet to be fully resolved (Downer et al., 2006).

FUTURE DIRECTIONS: INFLUENCE OF LDH ISOFORM AND APPLICATIONS TO TUMOR METABOLISM

What impact does LDH isoform have and how might this knowledge be applied to the treatment of diseases with altered metabolism, like cancers?

First, LDH is a tetrameric enzyme composed of two protein subunits which total approximately 135 kDa (Cahn et al., 1962). The tetramer can assemble as five separate isozymes by forming all combinations of the M (muscle) form (product of the Ldh-A gene) or the H (heart) form (product of the Ldh-B gene) producing: M_4 (= A_4 = LDH5), M_3H_1 (= A_3B_1 = LDH_4), M_2H_2 (= A_2B_2 = LDH3), M_1H_3 (= A_1B_3 = LDH2), and H_4 (= B_4 = LDH1). Results from investigations *in vitro* indicate differing kinetic properties with respect to substrate affinity and inhibition among these isozymes. The M-dominated isozymes have 3.5–7 times higher K_m-values for pyruvate and La^- than the H-dominated forms. Further, the H_4 types are inhibited by pyruvate at concentrations above ∼0.2 mM while the M_4 types are little affected by pyruvate concentrations as high as 5 mM (Plagemann et al., 1960; Stambaugh and Post, 1966; Quistorff and Grunnet, 2011b). The H_4 isozyme is inhibited by [La^-] above 20–40 mM while the M_4 isozyme is less inhibited by high [La^-] (Stambaugh and Post, 1966). These points have been offered as evidence for functional differences in cellular metabolism of various tissues with the heart forms promoting oxidation while the muscle forms facilitate formation of La^- (Cahn et al., 1962). The LDH isozyme distribution found in nature fits with these characteristics determined *in vitro*. For example, fast-twitch, glycolytic, type II skeletal muscle fibers have a greater proportion of M-type LDH isozyme whereas slow-twitch, oxidative, type I skeletal muscles as well as cardiac muscle have a greater proportion of the H-type LDH isozyme (Van Hall, 2000). Congruently, endurance exercise training decreases the proportion of the M-type LDH isozyme in the trained muscles (Van Hall, 2000). In the brain, astrocytes (which are postulated to have a higher glycolytic metabolism), have a greater proportion of the M-type LDH isozyme, whereas neurons (which are asserted to have a higher oxidative metabolism), have a greater proportion of the H-type LDH isozyme (Schurr, 2006; Pellerin and Magistretti, 2011). In tumors, glycolytic "Warburg-type" cells have a greater proportion of M-type LDH isozyme while more oxidative cancer cells have a greater proportion of H-type LDH isozyme (Semenza, 2008). So, the circumstantial evidence of LDH isozyme distribution patterns coincides with the perceived function of the LDH isozymes as determined *in vitro*.

The evidence cited above has led to the conclusion that LDH isozyme pattern is a causative factor in La^- metabolism. To further elucidate the role of LDH isozyme apportionment as a coordinator of La^- metabolism, Summermatter et al. (2013) undertook an investigation to test the role of peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) as a regulator of LDH isozyme subtype expression. PGC-1α is known to be important in the coordination of cellular energy metabolism (Wu et al., 1999). In response to a variety of stimuli, PGC-1α stimulates mitochondrial biogenesis, promotes transition of skeletal muscle to a more oxidative phenotype, and contributes to altered carbohydrate and lipid metabolism (Liang and Ward, 2006).

Summermatter et al. (2013) studied muscle-specific PGC-1α transgenic mice as well as muscle-specific PGC-1α knockout mice and found (1) lower blood [La^-] in the transgenic animals, and higher blood [La^-] in the knockout animals in response to endurance exercise, and (2) reduced expression of M-type LDH in the transgenic animals and reduced H-type LDH in the knockout animals. These authors concluded, as their title asserts, that "skeletal muscle PGC-1 α controls whole-body La^- homeostasis through estrogen-related receptor α-dependent activation of LDH B and repression of LDH A." In their view, the LDH isozyme pattern is a major player in whole body metabolism of La^-.

However, there are under-appreciated admonitions regarding LDH isozyme functions and their potential roles in metabolism. First, the aforementioned kinetic properties for LDH isoforms were determined *in vitro* at 20 or 25°C, and the K_m-values for pyruvate increase with temperature, approximately doubling at 37°C compared to 25°C (Latner et al., 1966; Quistorff and Grunnet, 2011b). Previously, Newsholme and Leech (1983), Van Hall (2000), Newsholme (2004), Gladden (2008), and Quistorff and Grunnet (2011a), have raised significant questions about the role of LDH isozyme profiles in La^- production vs. utilization, noting that: (1) enzymes do not change the equilibrium constant of a reaction; (2) the LDH reaction is near equilibrium, minimizing allosteric effects; (3) differences in LDH isozyme function *in vivo* are possibly quite small because of the higher physiological temperatures and binding to structures or other proteins; (4) the concentrations of La^- and pyruvate needed for LDH inhibition *in vitro* are much higher than the highest concentrations observed *in vivo*; and (5) LDH inhibition *in vitro* may be due to traces of the enol form of pyruvate that are less likely to be present *in vivo*.

Although Summermatter et al. (2013) state with conviction that LDH isoform pattern is a major factor in whole body La^- metabolism, there is a fatal flaw in their design. They ignored the fact that PGC-1 α transgenic mice have increased mitochondrial proliferation and oxidative phosphorylation enzymes, whereas PGC-1α knockout mice have significant reductions in cytochrome oxidase and citrate synthase activities (Arany et al., 2005). In our opinion, these changes in mitochondrial function, the previously noted high total LDH activity irrespective of isozyme pattern, and the near equilibrium nature of this reaction render the conclusions of Summermatter et al. (2013) untenable. Therefore, we conclude that the exact physiological and biochemical roles of LDH isozymes *in vivo* remain to be definitively elucidated.

Finally, with regard to tumor metabolism, understanding that La^- is the end product of glycolysis is paramount to designing interventions for targeting cancers. Briefly, experiments by Cori and Cori (1925) and by Warburg et al. (1927) showed that tumors appeared to be avidly consuming glucose and producing La^-. Subsequent dogma in tumor metabolism has held that tumors exhibit a "Warburg Effect," producing and exporting La^-. However, we now know that not only do different tumor types handle La^- differently (some are net producers; some are net consumers), but even within a single tumor there may be shuttling between different cell types; a cell to cell La^- shuttle (Semenza, 2008). Many cancer cells are poor consumers of lactate (Sonveaux et al., 2008) sparking speculation that a La^--protected hypoglycemia may be therapeutic (Nijsten and van Dam, 2009). In contrast, some tumors avidly use La^- as a fuel, and respond to supplemental La^- with increased proliferation and vascularity, likely a direct result of upregulation of vascular endothelial growth factor (VEGF) and hypoxia-inducible factor 1α (HIF-1α). In a recent study on an animal model of a sarcoma, Goodwin et al. (2014) reported that La^- drove sarcomagenesis in the absence of hypoxia. Amazingly, our understanding of La^- metabolism in cancer remains unsettled almost 90 years after Warburg's first studies.

CONCLUSIONS

Our understanding of La^- formation has changed drastically since its discovery. Traditionally, pyruvate has been thought to be the end product of glycolysis when O_2 is present and La^- the end product during periods of dysoxia. In the late twentieth century and early twenty-first century it was discovered that O_2 is not limiting to oxidative phosphorylation under most cellular conditions, and La^- is indeed produced even when there is no limitation on the rate of O_2 delivery to mitochondria. Further reflection on the activity of the LDH enzyme and the equilibrium constant of its reaction advance the proposition that La^- is the primary end product of glycolysis under most, if not all metabolic conditions in most cells. The role of the different LDH isozymes in metabolism is not as clearly evident as most researchers suggest, and we conclude that their exact function remains undiscovered. Whether or not we are correct about the Cytosol-to-Mitochondria Lactate Shuttle as described here and the uncertain role of the LDH isoforms will be difficult to evaluate under conditions *in vivo*. One approach is modeling *in silico*. Understanding the exact mechanisms of glycolysis and La^- metabolism will not only deepen our understanding of metabolism in healthy tissues, but will also lend insight into diseased or injured tissues, with the most obvious applications being the deranged carbohydrate metabolism present in cancer cells (Vander Heiden et al., 2009) and cerebral metabolism following traumatic brain injury (Brooks and Martin, 2014).

REFERENCES

Arany, Z., He, H., Lin, J., Hoyer, K., Handschin, C., Toka, O., et al. (2005). Transcriptional coactivator PGC-1α controls the energy state and contractile function of cardiac muscle. *Cell Metab.* 1, 259–271. doi: 10.1016/j.cmet.2005.03.002

Balinsky, D., Platz, C. E., and Lewis, J. W. (1984). Enzyme activities in normal, dysplastic, and cancerous human breast tissues. *J. Natl. Cancer Inst.* 72, 217–224.

Barnett, J. A., and Entian, K. D. (2005). A history of research on yeasts 9: regulation of sugar metabolism. *Yeast* 22, 835–894. doi: 10.1002/yea.1249

Brooks, G. A. (2000). Intra-and extra-cellular lactate shuttles. *Med. Sci. Sports. Exerc.* 32, 790–799. doi: 10.1097/00005768-200004000-00011

Brooks, G. A., and Gladden, L. B. (2003). "The metabolic systems: anaerobic metabolism (glycolytic and phosphagen)," in *Exercise Physiology. People and Ideas,* ed C. M. Tipton (New York, NY: Oxford University Press), 322–360.

Brooks, G. A., and Martin, N. A. (2014). Cerebral metabolism following traumatic brain injury: new discoveries with implications for treatment. *Front. Neurosci.* 8:408. doi: 10.3389/fnins.2014.00408

Cahn, R., Zwilling, E., Kaplan, N., and Levine, L. (1962). Nature and development of lactic dehydrogenases the two major types of this enzyme form molecular hybrids which change in makeup during development. *Science* 136, 962–969. doi: 10.1126/science.136.3520.962

Cataldo, A. M., and Broadwell, R. D. (1986). Cytochemical identification of cerebral glycogen and glucose−6−phosphatase activity under normal and experimental conditions: I. Neurons and glia. *J. Electron Microsc. Tech.* 3, 413–437. doi: 10.1002/jemt.1060030406

Connett, R., Gayeski, T., and Honig, C. (1986). Lactate efflux is unrelated to intracellular PO_2 in a working red muscle *in situ*. *J. Appl. Physiol.* 61, 402–408.

Connett, R., Honig, C., Gayeski, T., and Brooks, G. (1990). Defining hypoxia: a systems view of VO_2, glycolysis, energetics, and intracellular PO_2. *J. Appl. Physiol.* 68, 833–842.

Connett, R. J., and Sahlin, K. (2011). "Control of glycolysis and glycogen metabolism," in *Handbook of Physiology, Section 12, Exercise: Regulation and Integration of Multiple Systems,* eds L. B. Rowell and J. T. Shepherd (New York, NY: Oxford University Press), 870–911.

Cori, C. F., and Cori, G. T. (1925). The carbohydrate metabolism of tumors II. Changes in the sugar, lactic acid, and CO_2-Combining Power of Blood Passing through a tumor. *J. Biol. Chem.* 65, 397–405.

Divakaruni, A. S., and Murphy, A. N. (2012). A mitochondrial mystery, solved. *Science* 337, 41–43. doi: 10.1126/science.1225601

Donovan, C. M., and Pagliassotti, M. J. (2000). Quantitative assessment of pathways for lactate disposal in skeletal muscle fiber types. *Med. Sci. Sports Exerc.* 32, 772–777. doi: 10.1097/00005768-200004000-00009

Downer, J., Sevinsky, J. R., Ahn, N. G., Resing, K. A., and Betterton, M. (2006). Incorporating expression data in metabolic modeling: a case study of lactate dehydrogenase. *J. Theor. Biol.* 240, 464–474. doi: 10.1016/j.jtbi.2005.10.007

Fantin, V. R., St-Pierre, J., and Leder, P. (2006). Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance. *Cancer Cell* 9, 425–434. doi: 10.1016/j.ccr.2006.04.023

Fletcher, W. M., and Hopkins, F. G. (1907). Lactic acid in amphibian muscle. *J. Physiol.* 35, 247–309. doi: 10.1113/jphysiol.1907.sp001194

Gladden, L. B. (2004a). Lactate metabolism: a new paradigm for the third millennium. *J. Physiol.* 558, 5–30. doi: 10.1113/jphysiol.2003.058701

Gladden, L. B. (2004b). "Lactate metabolism during exercise," in *Principles of Exercise Biochemistry*, *3rd Edn.*, ed J. R. Poortmans (Basel: Karger), 152–196.

Gladden, L. B. (2008). 200th anniversary of lactate research in muscle. *Exerc. Sport Sci. Rev.* 36, 109–115. doi: 10.1097/JES.0b013e31817c0038

Goodwin, M. L., Jin, H., Straessler, K., Smith-Fry, K., Zhu, J., Monument, M. J., et al. (2014). Modeling alveolar soft part sarcomagenesis in the mouse: a role for lactate in the tumor microenvironment. *Cancer Cell* 26, 851–862. doi: 10.1016/j.ccell.2014.10.003

Hashimoto, T., Hussien, R., and Brooks, G. A. (2006). Colocalization of MCT1, CD147, and LDH in mitochondrial inner membrane of L6 muscle cells: evidence of a mitochondrial lactate oxidation complex. *Am. J. Physiol. Endocrinol. Metab.* 290, E1237–E1244. doi: 10.1152/ajpendo.00594.2005

Holloszy, J. O., and Coyle, E. F. (1984). Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *J. Appl. Physiol.* 56, 831–838.

Iwangoff, P., Armbruster, R., Enz, A., and Meier-Ruge, W. (1980). Glycolytic enzymes from human autoptic brain cortex: normal aged and demented cases. *Mech. Ageing. Dev.* 14, 203–209. doi: 10.1016/0047-6374(80)90120-7

Jöbsis, F., and Stainsby, W. (1968). Oxidation of NADH during contractions of circulated mammalian skeletal muscle. *Respir. Physiol.* 4, 292–300. doi: 10.1016/0034-5687(68)90035-2

Kompanje, E., Jansen, T., van der Hoven, B., and Bakker, J. (2007). The first demonstration of lactic acid in human blood in shock by Johann Joseph Scherer (1814–1869) in January 1843. *Inten. Care Med.* 33, 1967–1971. doi: 10.1007/s00134-007-0788-7

Lambeth, M. J., and Kushmerick, M. J. (2002). A computational model for glycogenolysis in skeletal muscle. *Ann. Biomed. Eng.* 30, 808–827. doi: 10.1114/1.1492813

Larner, E. H., and Rutherford, C. L. (1978). Application of a microchemical technique to the elucidation of enzyme activity profiles within single human mammary tumors. *Cancer* 41, 1863–1870.

Latner, A., Siddiqui, S., and Skillen, A. (1966). Pyruvate inhibition of lactate dehydrogenase activity in human tissue extracts. *Science* 154, 527–529.

Le Floch, R., Chiche, J., Marchiq, I., Naiken, T., Ilc, K., Murray, C. M., et al. (2011). CD147 subunit of lactate/H+ symporters MCT1 and hypoxia-inducible MCT4 is critical for energetics and growth of glycolytic tumors. *Proc. Natl. Acad. Sci. U.S.A.* 108, 16663–16668. doi: 10.1073/pnas.1106123108

Liang, H., and Ward, W. F. (2006). PGC-1α: a key regulator of energy metabolism. *Adv. Physiol. Educ.* 30, 145–151. doi: 10.1152/advan.00052.2006

Liaw, K.-Y., Wei, T.-C., Hsu, S.-C., and Lin, J.-K. (1985). Effect of severe injury and critical illness on high-energy phosphates in human liver and muscle. *J. Trauma Injury Infect. Crit. Care* 25, 628–633. doi: 10.1097/00005373-198507000-00009

Maltais, F., Simard, A.-A., Simard, C., Jobin, J., Desgagnés, P., and Leblanc, P. (1996). Oxidative capacity of the skeletal muscle and lactic acid kinetics during exercise in normal subjects and in patients with COPD. *Am. J. Respir. Crit. Care Med.* 153, 288–293. doi: 10.1164/ajrccm.153.1.8542131

Marie, S. K. N., and Shinjo, S. M. O. (2011). Metabolism and brain cancer. *Clinics* 66, 33–43. doi: 10.1590/S1807-59322011001300005

Meyerhof, O. (1942). "Intermediary carbohydrate metabolism," in *A Symposium on Respiratory Enzymes* (Madison, WI: The University of Wisconsin Press), 3–15.

Morland, C., Henjum, S., Iversen, E. G., Skrede, K. K., and Hassel, B. (2007). Evidence for a higher glycolytic than oxidative metabolic activity in white matter of rat brain. *Neurochem. Int.* 50, 703–709. doi: 10.1016/j.neuint.2007.01.003

Murin, R., Cesar, M., Kowtharapu, B. S., Verleysdonk, S., and Hamprecht, B. (2009). Expression of pyruvate carboxylase in cultured oligodendroglial, microglial and ependymal cells. *Neurochem. Res.* 34, 480–489. doi: 10.1007/s11064-008-9806-6

Newsholme, E. A. (2004). "Enzymes, energy and endurance: some provocative thoughts," in *Principles of Exercise Biochemistrym, 3rd, Rev Edn.* Vol. 46, Medicine and Sport Science, ed J. R. Poortmans (Basel: Karger Medical and Scientific Publishers), 1–35.

Newsholme, E. A., and Leech, A. R. (1983). "Catabolism of carbohydrates," in *Biochemistry for the Medical Sciences* (New York, NY: John Wiley & Sons), 167–245.

Nijsten, M. W. N., and van Dam, G. M. (2009). Hypothesis: using the Warburg effect against cancer by reducing glucose and providing lactate. *Med. Hypotheses* 73, 48–51. doi: 10.1016/j.mehy.2009.01.041

Pascoe, D. D., and Gladden, L. B. (1996). Muscle glycogen resynthesis after short term, high intensity exercise and resistance exercise. *Sports Med.* 21, 98–118. doi: 10.2165/00007256-199621020-00003

Pasteur, L. (1858). Memoire sur la fermentation appelée lactique. *Ann. Chim. Phys.* 52, 404–418.

Pasteur, L. (1861). Expériences et vues nouvelles sur la nature des fermentations. *C. R. Hebd. Seances Acad. Sci. Paris* 53, 1260–1264.

Pellerin, L., and Magistretti, P. J. (2011). Sweet sixteen for ANLS. *J. Cereb. Blood Flow Metab.* 32, 1152–1166. doi: 10.1038/jcbfm.2011.149

Plagemann, P. G., Gregory, K. F., and Wróblewski, F. (1960). The electrophoretically distinct forms of mammalian lactic dehydrogenase II. Properties and interrelationships of rabbit and human lactic dehydrogenase isozymes. *J. Biol. Chem.* 235, 2288–2293.

Ponten, U., Ratcheson, R., Salford, L., and Siesj,ö, B. (1973). Optimal freezing conditions for cerebral metabolites in rats. *J. Neurochem.* 21, 1127–1138. doi: 10.1111/j.1471-4159.1973.tb07567.x

Quistorff, B., and Grunnet, N. (2011a). High brain lactate is not caused by a shift in the lactate dehydrogenase A/B ratio. *Proc. Natl. Acad. Sci. U.S.A.* 108, E21. doi: 10.1073/pnas.1017750108

Quistorff, B., and Grunnet, N. (2011b). The Isoenzyme Pattern of LDH Does Not Play a Physiological Role; Except Perhaps During Fast Transitions in Energy Metabolism. Albany, NY: Aging 3.

Reinstrup, P., Ståhl, N., Mellergård, P., Uski, T., Ungerstedt, U., and Nordström, C.-H. (2000). Intracerebral microdialysis in clinical practice: baseline values for chemical markers during wakefulness, anesthesia, and neurosurgery. *Neurosurgery* 47, 701–709. doi: 10.1097/00006123-200009000-00035

Richardson, R. S., Noyszewski, E. A., Leigh, J. S., and Wagner, P. D. (1998). Lactate efflux from exercising human skeletal muscle: role of intracellular. *J. Appl. Physiol.* 85, 627–634.

Sahlin, K., Harris, R., Nylind, B., and Hultman, E. (1976). Lactate content and pH in muscle samples obtained after dynamic exercise. *Pflügers Arch.* 367, 143–149. doi: 10.1007/BF00585150

Sahuquillo, J., Merino, M.-A., Sánchez-Guerrero, A., Arikan, F., Vidal-Jorge, M., Martínez-Valverde, T., et al. (2014). Lactate and the lactate-to-pyruvate molar ratio cannot be used as independent biomarkers for monitoring brain energetic metabolism: a microdialysis study in patients with traumatic brain injuries. *PloS ONE* 9:e102540. doi: 10.1371/journal.pone.0102540

Schurr, A. (2006). Lactate: the ultimate cerebral oxidative energy substrate? *J. Cereb. Blood Flow Metab.* 26, 142–152. doi: 10.1038/sj.jcbfm.9600174

Schurr, A., and Gozal, E. (2012). Aerobic production and utilization of lactate satisfy increased energy demands upon neuronal activation in hippocampal slices and provide neuroprotection against oxidative stress. *Front. Pharmacol.* 2:96. doi: 10.3389/fphar.2011.00096

Schurr, A., and Payne, R. (2007). Lactate, not pyruvate, is neuronal aerobic glycolysis end product: an *in vitro* electrophysiological study. *Neuroscience* 147, 613–619. doi: 10.1016/j.neuroscience.2007.05.002

Semenza, G. L. (2008). Tumor metabolism: cancer cells give and take lactate. *J. Clin. Investig.* 118, 3835–3837. doi: 10.1172/JCI37373

Shonk, C. E., and Boxer, G. E. (1964). Enzyme patterns in human tissues. I. Methods for the determination of glycolytic enzymes. *Cancer Res.* 24,709–721.

Sonveaux, P., Végran, F., Schroeder, T., Wergin, M. C., Verrax, J., Rabbani, Z. N., et al. (2008). Targeting lactate-fueled respiration selectively kills hypoxic tumor cells in mice. *J. Clin. Investig.* 118, 3930–3942. doi: 10.1172/JCI36843

Spriet, L. L., Howlett, R. A., and Heigenhauser, G. (2000). An enzymatic approach to lactate production in human skeletal muscle during exercise. *Med. Sci. Sports Exerc.* 32, 756–763. doi: 10.1097/00005768-200004000-00007

Stainsby, W. N., and Brooks, G. A. (1990). Control of lactic acid metabolism in contracting muscles and during exercise. *Exerc. Sport Sci. Rev.* 18, 29–64. doi: 10.1249/00003677-199001000-00005

Stainsby, W. N., and Welch, H. G. (1966). Lactate metabolism of contracting dog skeletal muscle *in situ*. *Am. J. Physiol.* 211, 177–183.

Stambaugh, R., and Post, D. (1966). Substrate and product inhibition of rabbit muscle lactic dehydrogenase heart (H4) and muscle (M4) isozymes. *J. Biol. Chem.* 241, 1462–1467.

Summermatter, S., Santos, G., Pérez-Schindler, J., and Handschin, C. (2013). Skeletal muscle PGC-1α controls whole-body lactate homeostasis through estrogen-related receptor α-dependent activation of LDH B and repression of LDH A. *Proc. Natl. Acad. Sci. U.S.A.* 110, 8738–8743. doi: 10.1073/pnas.1212976110

Tiidus, P., Tupling, A. R., and Houston, M. (2012). *Biochemistry Primer for Exercise Science, 4th Edn*. Champaign, IL: Human Kinetics, p. 250.

Vander Heiden, M. G., Cantley, L. C., and Thompson, C. B. (2009). Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* 324, 1029–1033. doi: 10.1126/science.1160809

Van Hall, G. (2000). Lactate as a fuel for mitochondrial respiration. *Acta Physiol. Scand.* 168, 643–656. doi: 10.1046/j.1365-201x.2000.00716.x

Véga, C., Poitry-Yamate, C. L., Jirounek, P., Tsacopoulos, M., and Coles, J. A. (1998). Lactate is released and taken up by isolated rabbit vagus nerve during aerobic metabolism. *J. Neurochem.* 71, 330–337. doi: 10.1046/j.1471-4159.1998.71010330.x

Vespa, P., Bergsneider, M., Hattori, N., Wu, H.-M., Huang, S.-C., Martin, N. A., et al. (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. doi: 10.1038/sj.jcbfm.9600073

Warburg, O., Wind, F., and Negelein, E. (1927). The metabolism of tumors in the body. *J. Gen. Physiol.* 8, 519–530. doi: 10.1085/jgp.8.6.519

Wu, Z., Puigserver, P., Andersson, U., Zhang, C., Adelmant, G., Mootha, V., et al. (1999). Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell* 98, 115–124. doi: 10.1016/S0092-8674(00)80611-X

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 31 October 2014; accepted: 13 January 2015; published online: 27 February 2015.
Citation: Rogatzki MJ, Ferguson BS, Goodwin ML and Gladden LB (2015) Lactate is always the end product of glycolysis. Front. Neurosci. ***9****:22. doi: 10.3389/fnins.2015.00022*

This article was submitted to Neuroenergetics, Nutrition and Brain Health, a section of the journal Frontiers in Neuroscience.

frontiers in Neuroscience

REVIEW
published: 08 April 2015
doi: 10.3389/fnins.2015.00112

Glycolysis and the significance of lactate in traumatic brain injury

Keri L. H. Carpenter[1,2]*, *Ibrahim Jalloh*[1] and *Peter J. Hutchinson*[1,2]

[1] Division of Neurosurgery, Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK, [2] Wolfson Brain Imaging Centre, Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK

In traumatic brain injury (TBI) patients, elevation of the brain extracellular lactate concentration and the lactate/pyruvate ratio are well-recognized, and are associated statistically with unfavorable clinical outcome. Brain extracellular lactate was conventionally regarded as a waste product of glucose, when glucose is metabolized via glycolysis (Embden-Meyerhof-Parnas pathway) to pyruvate, followed by conversion to lactate by the action of lactate dehydrogenase, and export of lactate into the extracellular fluid. In TBI, glycolytic lactate is ascribed to hypoxia or mitochondrial dysfunction, although the precise nature of the latter is incompletely understood. Seemingly in contrast to lactate's association with unfavorable outcome is a growing body of evidence that lactate can be beneficial. The idea that the brain can utilize lactate by feeding into the tricarboxylic acid (TCA) cycle of neurons, first published two decades ago, has become known as the astrocyte-neuron lactate shuttle hypothesis. Direct evidence of brain utilization of lactate was first obtained 5 years ago in a cerebral microdialysis study in TBI patients, where administration of ^{13}C-labeled lactate via the microdialysis catheter and simultaneous collection of the emerging microdialysates, with ^{13}C NMR analysis, revealed ^{13}C labeling in glutamine consistent with lactate utilization via the TCA cycle. This suggests that where neurons are too damaged to utilize the lactate produced from glucose by astrocytes, i.e., uncoupling of neuronal and glial metabolism, high extracellular levels of lactate would accumulate, explaining the association between high lactate and poor outcome. Recently, an intravenous exogenous lactate supplementation study in TBI patients revealed evidence for a beneficial effect judged by surrogate endpoints. Here we review the current state of knowledge about glycolysis and lactate in TBI, how it can be measured in patients, and whether it can be modulated to achieve better clinical outcome.

Keywords: traumatic brain injury (human), cerebral energy metabolism, glycolysis, lactate, pyruvate, glucose, microdialysis

OPEN ACCESS

Edited by:
Avital Schurr,
University of Louisville, USA

Reviewed by:
Avital Schurr,
University of Louisville, USA
Oliver Kann,
University of Heidelberg, Germany
Lorenz Hirt,
University of Lausanne, Switzerland

***Correspondence:**
Keri L. H. Carpenter,
Division of Neurosurgery,
Department of Clinical Neurosciences,
University of Cambridge, Box 167,
Cambridge Biomedical Campus,
Cambridge CB2 0QQ, UK
klc1000@wbic.cam.ac.uk

Specialty section:
This article was submitted to Neuroenergetics, Nutrition and Brain Health, a section of the journal Frontiers in Neuroscience

Received: *07 November 2014*
Accepted: *16 March 2015*
Published: *08 April 2015*

Citation:
Carpenter KLH, Jalloh I and Hutchinson PJ (2015) Glycolysis and the significance of lactate in traumatic brain injury. Front. Neurosci. 9:112. doi: 10.3389/fnins.2015.00112

Introduction

Cerebral metabolism following injury appears to differ from that in normal brain, although the full extent and nature of these changes are poorly understood, especially in man.

Traumatic brain injury (TBI) results from the action of external mechanical forces that cause macroscopic tissue damage at the time of injury and initiate cellular processes that evolve over the hours and days that follow. It is a heterogeneous disorder that includes a range of macroscopic tissue pathologies including hematomas, contusions, and edema. Often these

lesions are sufficiently large to require urgent surgical evacuation to prevent fatal compression of vital brain structures. Occasionally, even in the presence of severe TBI, defined clinically as patients presenting in coma, there is little in the way of macroscopic damage to the brain evident on CT scans. This illustrates the impact that pathophysiology at the microscopic level has on cellular and neurological function. These processes are multifactorial and include disturbed ion hemostasis (Unterberg et al., 2004), excitotoxicity (Katayama et al., 1990; Kawamata et al., 1992), cell wall and mitochondrial disruption (Xiong et al., 1997; Lewen et al., 2001), inflammation, and derangements in oxidative energy metabolism (Jalloh et al., 2014).

Patients with severe TBI frequently have injuries to other parts of the body such as the lungs or limb bones and are typically managed in an intensive care setting (Chesnut et al., 1993; Stocchetti et al., 1996). They are medically complex patients with a myriad of attendant issues that need to be considered, for example, nutrition, respiratory care, and deep-vein thrombosis prophylaxis, in addition to complications associated with TBI such as seizures, hydrocephalus, and endocrine dysfunction. Overall, TBI is one of the leading causes of mortality and morbidity in young adults. The most recent large observational studies report unfavorable outcomes from severe TBI ranging between 54 and 66% (Roozenbeek et al., 2013).

The best-known metabolic characteristics of injured brain are a high lactate concentration and a high lactate/pyruvate (L/P) ratio, in the brain extracellular fluid. An L/P ratio greater than 25 is interpreted as arising from high glycolytic activity indicative of hypoxia or mitochondrial dysfunction (Timofeev et al., 2011a), although the precise nature of the latter is incompletely understood. A recent study in 233 TBI patients demonstrated that L/P ratio >25 predicted unfavorable clinical outcome in a multivariate analysis in addition to previously known predictors of outcome (Timofeev et al., 2011a). Compared with normal brain, the course of the glycolytic pathway from glucose to lactate differs after injury, with in some cases an increase in the proportion of lactate produced via diversion of glucose-6-phosphate through the pentose phosphate pathway (PPP), also termed hexose monophosphate shunt, compared with direct glycolysis that even so remains the major route (Bartnik et al., 2005, 2007; Dusick et al., 2007; Jalloh et al., 2013).

Measurement of Glycolysis and Lactate

Glycolysis is a multi-stage pathway with many facets, and there are various biochemical approaches to measuring glycolysis. Microdialysis is a tool for monitoring cerebral metabolism. Monitoring of severe TBI patients in neurocritical care may include intracranial pressure, brain tissue oxygen and extracellular chemistry using microdialysis. The latter possesses a semi-permeable membrane that is continuously perfused with fluid, allowing molecules to diffuse across the membrane, to and from the brain's extracellular space. Clinically, the catheter is perfused with a physiological salt solution and the returning fluid (microdialysate) is analyzed at the bedside utilizing automated enzymatic colorimetric assays to measure endogenous glucose, lactate, pyruvate, glutamate, and glycerol. In this way microdialysis has been used to monitor glucose delivery to the brain and, using L/P ratio, as a marker of the balance between "aerobic" (referring to TCA cycle) and "anaerobic" metabolism (referring to glycolysis culminating in lactate). A schematic diagram of the microdialysis catheter is shown in **Figure 1**.

In the laboratory, a classic method of measuring "true glycolysis" (the Embden-Meyerhof-Parnas pathway, also termed the Embden-Meyerhof pathway), is to measure radioactive water produced from [5-^{3}H]-glucose, used originally in *ex vivo* organ

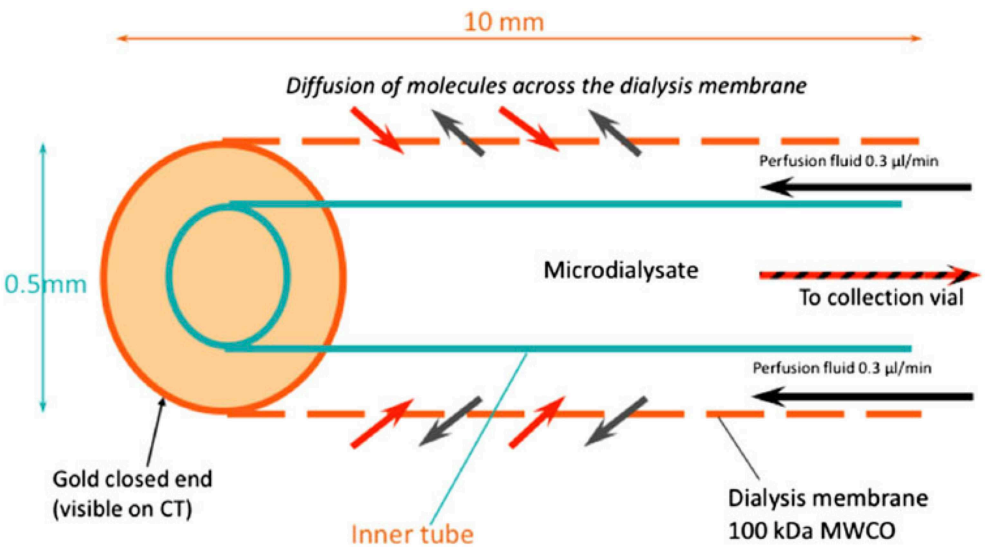


FIGURE 1 | Schematic of the microdialysis catheter tip. Substances in the extracellular fluid outside the catheter tip are able to diffuse across the microdialysis membrane to be collected for analysis. Abbreviation: MWCO, nominal molecular weight cut-off of the microdialysis membrane. Originally published by Shannon et al. (2013) in J Pharmacokinet Pharmacodyn 40: 343–458 under a Creative Commons Attribution Licence.

(heart) perfusion (Neely et al., 1972) and more recently *in vitro* in cell cultures (e.g., De Bock et al., 2013). A limitation of this assay is that it does not measure conversion of glucose all the way through to pyruvate, and since it uses long-lived radioactivity in the form of tritium it is unsuitable for use in humans. A possible alternative would be to adapt the method by using the stable isotope deuterium in place of radioactive tritium and quantify the deuterated water by mass spectrometry. However, this would require a specialized type of mass spectrometry that is not widely available.

Besides the above, there are various other techniques and instrumentation for measuring aspects of glycolysis in the laboratory, reviewed recently (TeSlaa and Teitell, 2014). These are performed under controlled conditions, often in cell cultures with specific inhibitors to tie down particular components of biochemistry, although general principles such as measurement *in vitro* of extracellular levels of lactate, glucose and concentration of oxygen are also shared with neurocritical care monitoring *in vivo*. We have already mentioned microdialysis (above) for measuring brain extracellular glucose, lactate etc., and brain tissue oxygen ($PbtO_2$) can be measured alongside using an intracranial oxygen sensor (Timofeev et al., 2011b; Shannon et al., 2013; Jalloh et al., 2015). Extracellular acidification measurement, employed *in vitro*, has also been performed in patients using the Neurotrend sensor to measure brain extracellular pH, although no further measurements are possible as the manufacturer (Diametrics, Buckinghamshire, UK) has discontinued this sensor and no alternative clinically approved intracranial pH sensor currently exists (Timofeev et al., 2013). Another glycolysis-related measurement is uptake of ^{18}F-deoxyglucose (FDG) by cells. FDG is a glucose analog that becomes phosphorylated inside the cells and retained without further metabolism. FDG is more often employed in patients (as opposed to cell cultures), with clinical imaging by positron emission tomography (PET) as exemplified below.

Various studies of FDG-PET have been carried out in TBI patients, for example (Vespa et al., 2005; Hutchinson et al., 2009). Established computation methods allow cerebral metabolic rate of glucose (CMRglc, expressed in units of μmol/100 g tissue/min) to be determined for the desired regions of interest (ROIs), e.g., a 2 cm ROI around the microdialysis catheter tip (Hutchinson et al., 2009). While FDG-PET cannot distinguish glycolysis from TCA cycle metabolism, the use of microdialysis provides information on extracellular lactate, pyruvate and glucose concentrations and allows inferences to be made. A combined FDG-PET and microdialysis study showed significant positive correlations of CMRglc with lactate and pyruvate concentrations, no relationship between CMRglc and L/P ratio, and a weak inverse trend for CMRglc with glucose concentrations in the microdialysates (**Figure 2**) (Hutchinson et al., 2009). The study concluded (with the caveat of being a small study in 17 patients) that in TBI brain, an increase in glucose metabolism leads to increases in both lactate and pyruvate, as opposed to a shift toward "anaerobic" metabolism (Hutchinson et al., 2009). Only 2 of the 17 patients in this study showed very high L/P ratio exceeding 40 (Hutchinson et al., 2009), a state known as metabolic crisis. In a different study of microdialysis combined with PET (FDG and triple oxygen), a higher proportion (7 out of 19) of patients showed metabolic crisis (L/P ratio >40) though only a single patient showed regional ischemia (Vespa et al., 2005). Unlike (Hutchinson et al., 2009), there was no correlation between microdialysis parameters and regional CMRglc (Vespa et al., 2005). The apparent disparity between the two studies' results was suggested to have stemmed at least partly from the different proportions of metabolic crisis patients (Hutchinson et al., 2009).

As diffusion across the microdialysis membrane is bidirectional, microdialysis can also be used to deliver molecules ("retrodialysis" e.g., ^{13}C-labeled substrates), thereby microdosing a region of interest around the catheter tip, whilst simultaneously collecting the products in the emerging microdialysate, for subsequent NMR analysis. In this way, we have infused 1,2-$^{13}C_2$ glucose into TBI patients' brains via the microdialysis catheter to compare production of glycolytic 2,3-$^{13}C_2$ lactate vs. PPP-derived 3-^{13}C lactate (Jalloh et al., 2015). This study was carried out with brain tissue oxygen ($PbtO_2$) measured simultaneously in the vicinity of the microdialysis catheter, shedding light on the relationship of local oxygen concentration to glycolytic- and PPP- generated lactate. Also, the ^{13}C-labeling enabled newly synthesized lactate to be distinguished from "old" lactate, a differentiation otherwise impossible without labeling. The findings of the study (Jalloh et al., 2015) are discussed in the section entitled "*Origins of lactate in brain,*" below. We have also shown that infusion of 3-^{13}C lactate or 2-^{13}C acetate into the brains of TBI patients via the microdialysis catheter produced ^{13}C signals for glutamine C4, C3, and C2 in the emerging microdialysates (analyzed by NMR), indicating TCA cycle operation followed by conversion of glutamate to glutamine (see **Figure 3** and section entitled "*Brain utilization of lactate,*" below) (Gallagher et al., 2009). Microdialysis can thus be used to manipulate the immediate microenvironment around the catheter by adding chosen metabolic substrates that enter the relevant biochemical pathways at different stages allowing specific stages of the process to be investigated.

Brain TCA cycle flux in humans has been measured employing *in vivo* magnetic resonance spectroscopy (MRS) using ^{13}C-labeled glucose (typically 1-^{13}C glucose or 1,6-$^{13}C_2$ glucose) (Rothman et al., 2011). The TCA cycle flux is calculated by kinetic modeling of ^{13}C labeling in glutamine and glutamate in brain measured by MRS, during ^{13}C labeled glucose intravenous infusion. The technique has been mostly applied to healthy volunteers. A few *in vivo* MRS studies of ^{13}C-labeling have been carried out in patients with various pathologies, but the technique has not yet been applied to the severe TBI field. Only a few centers worldwide have neurocritical care units adjacent to MRI scanners equipped for MRS and the expertise to support fully ventilated severe TBI patients through the procedure, and moreover, ^{13}C measurement of TCA cycle is itself a highly specialized area within the *in vivo* MRS field. *In vivo* brain MRS studies of ^{13}C labeling are extendable to other intravenously administered substrates; for example ^{13}C acetate has been used (Lebon et al., 2002), and *in vivo* MRS has been used to examine ^{13}C lactate utilization in the TCA cycle, by measuring ^{13}C labeling kinetics in glutamate and glutamine in healthy volunteers (Boumezbeur et al., 2010). Reviewing the latter study, Rothman et al. (2011) commented as

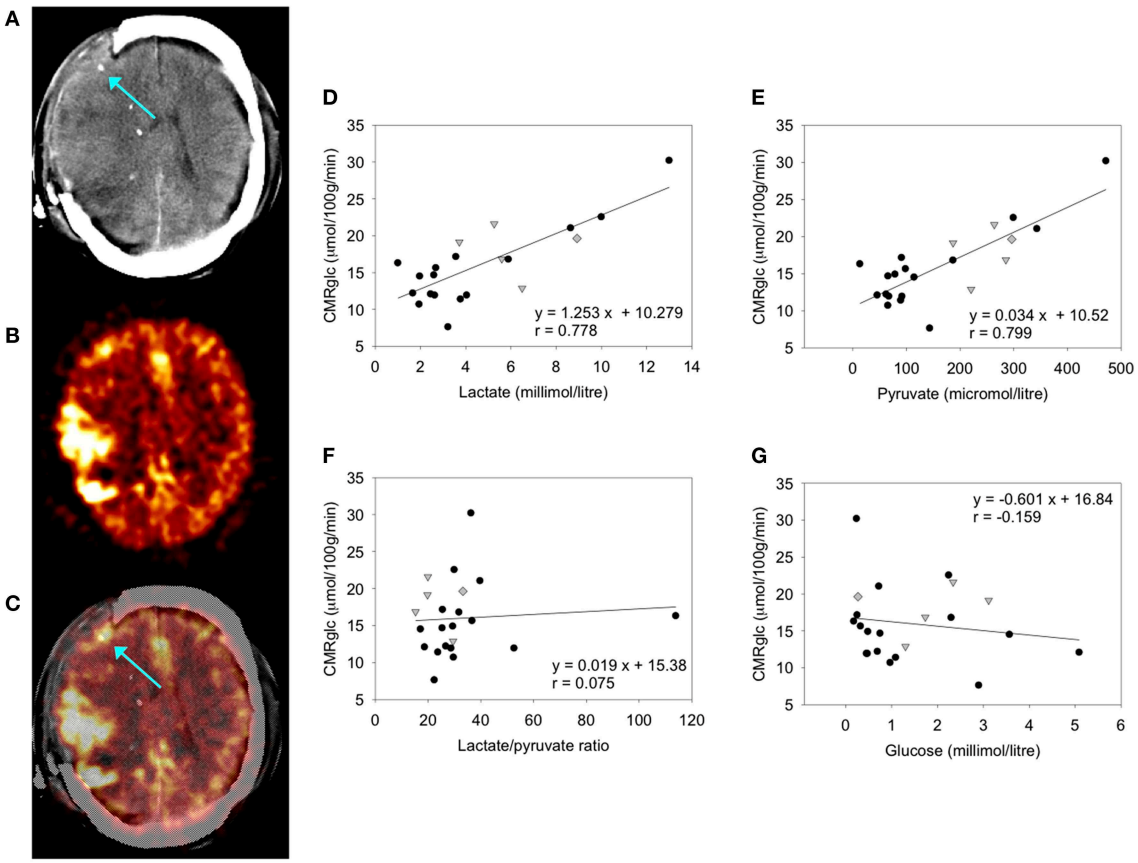


FIGURE 2 | FDG-PET measurement of CMRglc and its relationship to brain microdialysate composition: (A–C) FDG-PET CMRglc map demonstrating relatively high FDG uptake at sites of injury, in contrast to less injured areas of the brain. (A) Computed tomography (CT) scan showing gold tip of microdialysis catheter (indicated by arrow). **(B)** Co-registered FDG-PET CMRglc map showing high FDG uptake at sites of injury. **(C)** Overlay of CT and co-registered CMRglc map, showing microdialysis catheter tip location (arrow). **(D–F)** Graphs illustrating relationships by linear regression (for 22 ROIs in 17 TBI patients) between FDG-PET derived CMRglc and the microdialysis parameters measured during the scan **(D)** lactate, **(E)** pyruvate, **(F)** lactate/pyruvate (L/P) ratio, and **(G)** glucose. For the linear regressions in **(D–G)**, corresponding values of p (ANOVA) are <0.0001, <0.0001, 0.74, and 0.48, respectively. Data-points from catheters at craniotomy sites (four patients) are differentiated by gray triangles. Data-points from a second FDG-PET scan (one patient) are differentiated by gray diamonds. All other data-points are depicted as black circles (catheters inserted via cranial access device). Linear regressions presented on the graphs are for the entire (combined black plus gray symbols) dataset consisting of all 22 ROIs. Originally published by Hutchinson et al. (2009) in Acta Neurochir (Wien) 151: 51–61, and reproduced with kind permission of Springer Science+Business Media.

follows. "Relative consumption of plasma lactate between neurons and astrocytes is similar to that of glucose (Boumezbeur et al., 2010). The calculation of the lactate metabolic capacity is in good agreement with recent AV difference studies using isotopically labeled lactate as a tracer, further confirming the potential importance of plasma lactate as a substrate for brain metabolism (van Hall et al., 2009)."

More readily achievable than ^{13}C *in vivo* MRS measurements of TCA cycle flux are ^{1}H *in vivo* MRS measurements of endogenous molecules. ^{1}H *in vivo* MRS in TBI clinically in patients (**Figure 4**) (Marino et al., 2007) have shown that the most abundant signal is N-acetylaspartate (NAA), a mitochondrial marker. As absolute quantification is difficult, NAA is often expressed as a ratio to creatine, or sometimes to choline (Marino et al., 2007; Maddock and Buonocore, 2012). While lactate is abundant extracellularly (at millimol/L concentrations readily measurable on microdialysis), it is, in contrast, much less evident on ^{1}H *in vivo* MRS of normal or TBI brain. Moreover, depending on the choice of echo time for MRS, these small lactate signals can virtually disappear or appear inverted. Lactate elevation can be seen on ^{1}H *in vivo* MRS in pathological states, e.g., in tumors. In TBI brain, lactate elevation can be seen on ^{1}H MRS in some but not all instances (Marino et al., 2007).

Another *in vivo* modality is ^{31}P MRS, which can inform on ATP in tissues, including brain, by measuring the phosphocreatine/ATP ratio and inorganic phosphate/ATP ratio (Garnett et al., 2001) or by ^{31}P magnetization-transfer methods (Befroy et al., 2012). ^{31}P has a natural abundance of 100% of all phosphorus atoms. While ATP is produced at a much higher yield (per molecule of glucose) by the combination of glycolysis plus

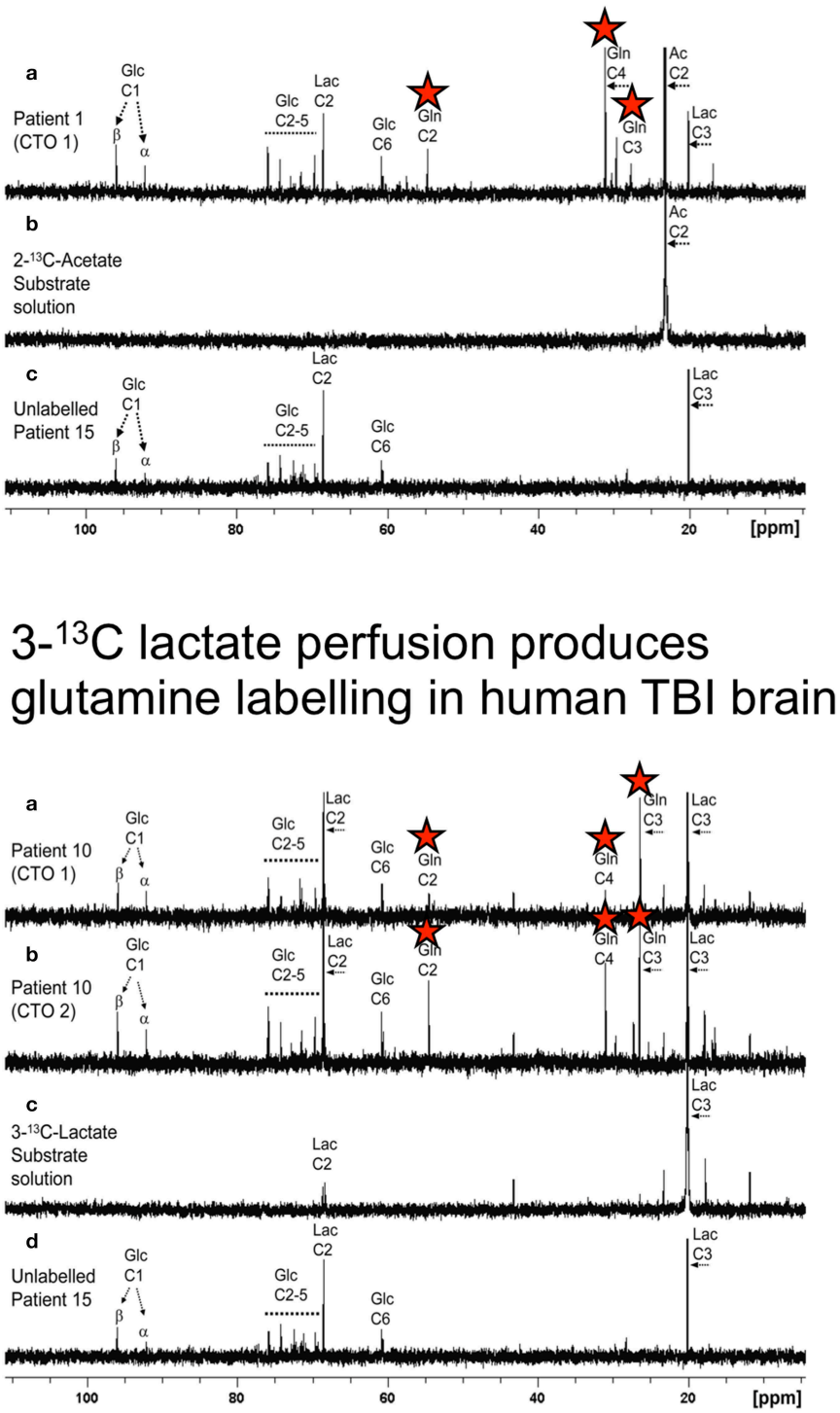


FIGURE 3 | Upper panel: (a) Example of ^{13}C NMR spectrum of brain microdialysate from a TBI patient, who received perfusion with 2-^{13}C acetate (4 mM) by a microdialysis catheter via a craniotomy (CTO); red stars indicate ^{13}C signals for glutamine C4, C3, and C2 indicating metabolism via TCA cycle. (b) ^{13}C NMR spectrum of the 2-^{13}C acetate substrate solution prior to perfusing. **(c)** ^{13}C NMR spectrum of brain microdialysate from an unlabeled patient whose microdialysis catheter was

(Continued)

FIGURE 3 | Continued
perfused with plain perfusion fluid without labeled substrate. Lower panel: **(a,b)** Examples of ^{13}C NMR spectra of brain microdialysates from a TBI patient, who received perfusion with 3-^{13}C lactate (4 mM) by microdialysis catheters via a craniotomy (CTO); red stars indicate ^{13}C signals for glutamine C4, C3, and C2 indicating metabolism via TCA cycle. **(c)** ^{13}C NMR spectrum of the 3-^{13}C lactate substrate solution prior to perfusing. **(d)** ^{13}C NMR spectrum of brain microdialysate from an unlabeled patient [as in Upper panel **(c)**]. Originally published by Gallagher et al. (2009) in Brain 132: 2839–2849, and reproduced with permission of Oxford Journals.

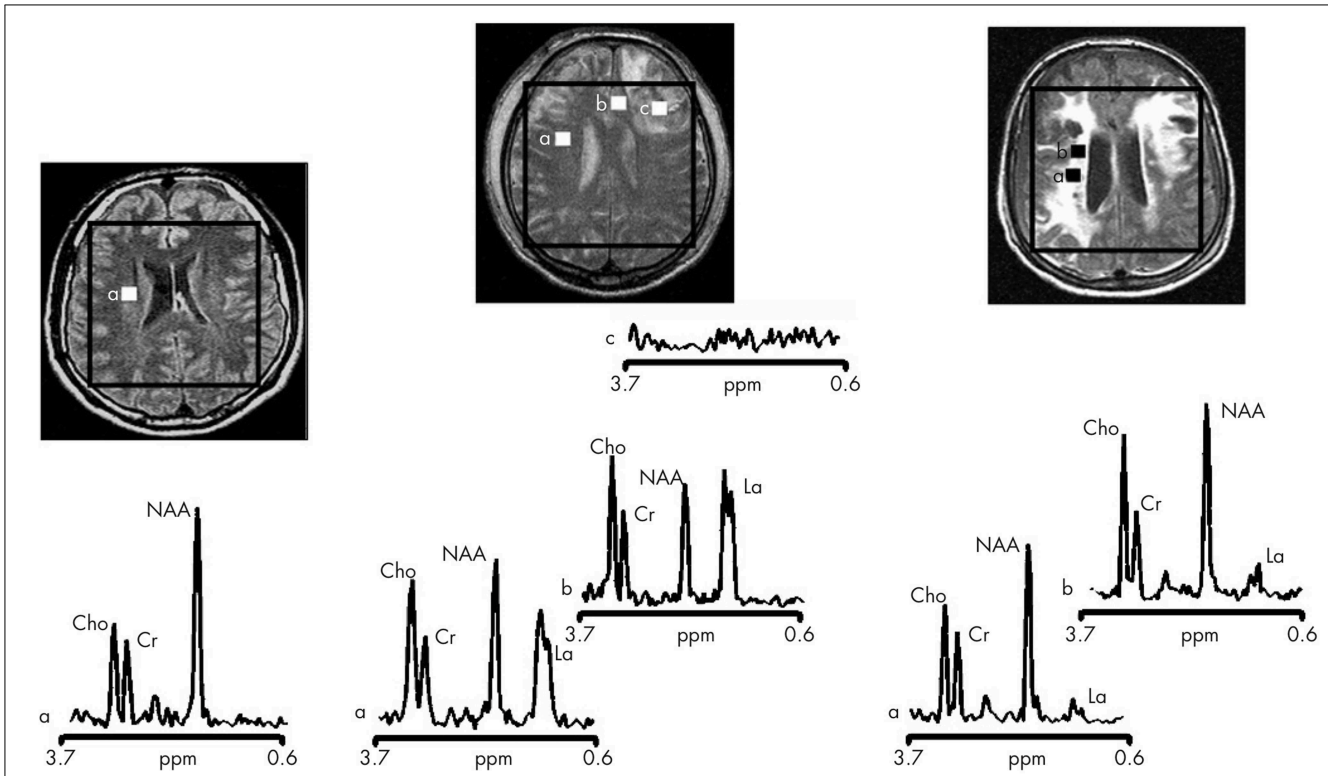


FIGURE 4 | Proton magnetic resonance spectra and conventional magnetic resonance images showing the volume of interest for spectroscopic imaging of a normal control (left panel), Patient 1 (central panel), and Patient 8 (right panel) with traumatic brain injury (TBI). On conventional MRI, Patient 1 shows a focal hematoma in the frontal left hemisphere and patient 8 shows diffuse MRI abnormalities. Spectra show decreases of N-acetylaspartate (NAA) and increases of choline (Cho) and lactate (La) in patients with TBI (a and b in central and right panels) with respect to the normal control (a in left panel). The spectra of Patient 1 (central panel) show more pronounced metabolic abnormalities than those of Patient 8 (right panel), despite the fact that Patient 8 showed markedly more abnormalities on conventional MRI. In the spectra of Patient 1 (central panel), metabolic abnormalities are clearly evident in the normal appearing brain. Finally, in Patient 1, voxels inside the focal hematoma (c in central panel) were excluded to avoid the artifacts that could be derived by the cerebral haemorrhagic contusion. Cr, creatine. Reproduced from J Neurol Neurosurg Psychiatry, Marino et al. 78: 501–507 (2007) with permission from BMJ Publishing Group Ltd.

mitochondrial metabolism than by glycolysis alone, the latter is thought to represent a quick source of ATP under stress-response conditions such as TBI. A combination of modalities is needed in order to differentiate between glycolysis and mitochondrial metabolism as sources of ATP in brain.

Origins of Lactate in Brain

The majority of lactate in the brain is regarded as "glycolytic," originating from glucose metabolism *in situ*, by the Embden-Meyerhof pathway, to pyruvate, followed by conversion of pyruvate to lactate by the action of lactate dehydrogenase (LDH). Other routes to brain lactate, including synthesis via the PPP and uptake of lactate from the circulation, are discussed below.

There is some disparity in nomenclature in the brain metabolism literature, which undoubtedly adds confusion for any non-biochemist readers, or even for biochemists unfamiliar with the brain injury field. In the brain injury literature, glycolysis culminating in lactate is often termed "anaerobic metabolism," though often without supporting evidence regarding the oxygen status in the tissue concerned. In old studies brain injury was often associated with hypoxia/ischemia (real or assumed), although more recently advances in neurocritical care mean that overt hypoxia/ischemia is usually avoided. Even so, microvascular ischemia appears to exist in some cases (Newcombe et al., 2013), as do episodes of hypoxia (Timofeev et al., 2011b). We regard hypoxia as $PbtO_2$ <20 mmHg, with severe hypoxia as $PbtO_2$ <10 mmHg.

High brain lactate concentrations may result from mitochondrial dysfunction forcing cells to rely on glycolysis (Embden-Meyerhof pathway) to generate ATP. Without functional mitochondrial electron transport chains (ETC) and shuttles, the conversion (mediated by LDH) of pyruvate to lactate is necessary to recycle NADH to NAD^+ to enable glycolysis to continue. Hypoxia prevents mitochondria from functioning properly. Since molecular oxygen (O_2) is the terminal electron acceptor of the ETC, adequate O_2 presence is vital for mitochondrial function. However, mitochondria may become dysfunctional even in the presence of O_2, for instance if any of the ETC components are damaged, or if the TCA cycle (which feeds the ETC) is compromised, or if the mitochondrial membranes become leaky. The concentration of lactate in the brain depends not only on lactate production but also lactate consumption. We have previously commented, "Low extracellular lactate levels, associated with better outcomes (Timofeev et al., 2011a), might be because astrocytic glycolysis-derived lactate is being efficiently taken up by neurons and utilized via the TCA cycle (Gallagher et al., 2009). Conversely, high extracellular lactate may result if neurons are too damaged to efficiently utilize the lactate being produced by astrocytes, i.e., uncoupling of neuronal and glial metabolism" (Carpenter et al., 2014).

In classic biochemical terms, "glycolysis" is the Embden-Meyerhof pathway from glucose to pyruvate, whereas the pyruvate to lactate step is a subsequent conversion that is not part of glycolysis proper. Pyruvate does not necessarily produce lactate, as pyruvate can be converted into acetyl CoA and thence enter the TCA cycle. Also, lactate can be converted to pyruvate, and thence to acetyl CoA and the TCA cycle (see Section entitled "*Utilization of lactate,*" below). The description "glycolytic" is often loosely applied to cells or tissues that produce abundant lactate, e.g., injured regions of brain or tumors, and these cells/tissues also may have apparently elevated PPP as well as high glycolysis. In tumors, and especially in cancer cell lines *in vitro*, it has long been recognized that the high glycolysis often occurs despite a ready supply of oxygen, a phenomenon known as the Warburg effect (Warburg, 1956), often termed "aerobic glycolysis" in the tumor literature (Bensinger and Christofk, 2012). In a study of 24 TBI patients, Sala et al. (2013) judged that elevations in brain extracellular lactate were predominantly "glycolytic," with "hypoxic" lactate elevation in a minority. These authors stated their criteria for "hypoxic lactate" as microdialysate lactate >4 mmol/L with $PbtO_2$ <20 mmHg, and "glycolytic lactate" as microdialysate lactate >4 mmol/L with microdialysate pyruvate >119 mmol/L. It is relevant to comment that the actual origins of the lactate were not determined in this study. Therefore, the brain extracellular lactate detected may have included a minor portion arising via the PPP rather than direct glycolysis, and moreover some of the lactate may have entered the brain from the circulation; see below for further information. Results from CT perfusion (with iohexol contrast agent) available for a subset of (16 out of 24) patients in the study by Sala et al. suggested that "glycolytic" lactate was associated with hyperaemic brain perfusion, and "hypoxic" lactate with "diffuse oligaemia" (Sala et al., 2013).

Relevant to the balance between lactate and pyruvate is the nature of the LDH enzyme. Two distinct subunits are combined to form the five tetrameric isoenzymes of LDH. Subunit LDH1 ("heart type," found in neurons) preferentially drives lactate to pyruvate, while subunit LDH5 ("muscle type") is present in "glycolytic tissues" and in both neurons and astrocytes, and preferentially drives pyruvate to lactate (Bittar et al., 1996).

The PPP is a complex detour starting from glucose-6-phosphate (hence its alternative name "hexose monophosphate shunt") bypassing some of the steps of glycolysis in the metabolism of glucose, and its key features have been summarized as follows by Jalloh et al. (2015). "The key enzyme for the PPP, glucose-6-phosphate dehydrogenase, which is responsible for the rate-limiting step, is present in most tissues and cell types, and is regarded as a "housekeeping" enzyme (Pandolfi et al., 1995; Riganti et al., 2012). The PPP does not consume or produce ATP and does not require molecular oxygen. In the early "oxidative phase" of the PPP, the first carbon of the glucose skeleton is lost as carbon dioxide, and nicotinamide adenine dinucleotide phosphate ($NADP^+$) is converted to NADPH. The latter participates in reductive biosynthetic reactions, such as lipid synthesis and in producing the reduced form of glutathione and thioredoxin which are cofactors for glutathione peroxidase and peroxiredoxins respectively, both of which scavenge hydroperoxides, thereby combatting oxidative stress. Among the many intermediates of the later "non-oxidative" phase of the PPP is ribose 5-phosphate, used for nucleic acid synthesis. PPP activity after TBI has been suggested to play a protective role, promoting synthesis of nucleic acids and fatty acids for tissue repair and combatting oxidative stress (Ben-Yoseph et al., 1996; Bartnik et al., 2005)."

Microdialysis perfusion with 1,2-$^{13}C_2$ glucose, in TBI patients' brains, and, for comparison, in "normal" brain in non-TBI patients, with high-resolution ^{13}C NMR analysis of the emerging microdialysates, has enabled comparison of lactate production by glycolysis (evidenced by 2,3-$^{13}C_2$ lactate), and the PPP (evidenced by 3-^{13}C lactate) (Jalloh et al., 2015). Doubly labeled lactate (with ^{13}C next to ^{13}C) produces characteristic doublet signals on ^{13}C NMR, distinct from singlets due to single ^{13}C labeling (^{13}C next to ^{12}C). A schematic of the biosynthetic pathway labeling patterns is shown in **Figure 5** (Carpenter et al., 2014; Jalloh et al., 2015), and illustrative examples of ^{13}C NMR spectra in **Figure 6** (Jalloh et al., 2015). The natural abundance of ^{13}C is 1.1% of all carbon atoms, so the probability of two endogenous ^{13}C atoms occurring next to each other naturally is $1.1 \times 1.1\% = 0.01\%$, thus the doublet signature, for doubly ^{13}C labeled lactate, is essentially free from background. The singly ^{13}C labeled lactate results were expressed after background-subtraction of the natural lactate's contribution to ^{13}C NMR singlet signals. Glycolysis was always the major source of lactate and the PPP the minor source. The conclusions were as follows (Jalloh et al., 2015), and the graphs (**Figures 7**, **8**) are from the same paper. "The minor pathway, PPP-derived lactate production, was statistically not significantly different in the TBI brain than in normal brain. However, several of the TBI individuals showed PPP-derived lactate elevation above the range observed in the normal brain (**Figure 7**). There was a shift in glucose metabolism from glycolysis to PPP with decreasing brain tissue oxygen ($PbtO_2$) concentrations (**Figure 8**). The findings raise interesting questions about the roles of the PPP and glycolysis after TBI, and whether they can

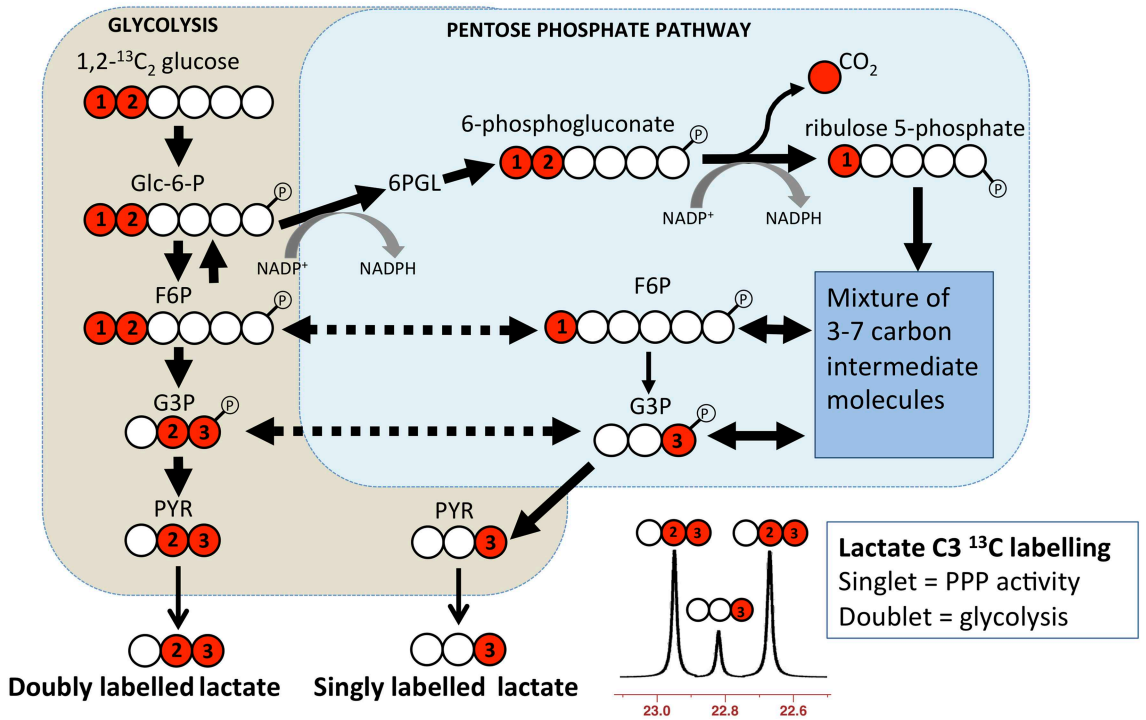


FIGURE 5 | Simplified schematic of steps in glycolysis and the pentose phosphate pathway (PPP), showing ^{13}C labeling patterns resulting from 1,2-$^{13}C_2$ glucose substrate. Red fills indicate ^{13}C atoms. Abbreviations: Glc-6-P, glucose-6-phosphate; 6PGL, 6-phosphogluconolactone; F6P, fructose-6-phosphate; G3P, glyceraldehyde-3-phosphate; PYR, pyruvate. Originally published by Carpenter et al. (2014) in Eur J Pharm Sci 57: 87–97 under a Creative Commons Attribution Licence.

be manipulated to enhance the potentially reparative and antioxidant role of the PPP and achieve a better outcome for the patient. The ^{13}C methodology developed here provides a means of distinguishing recently synthesized lactate and its biosynthetic origin, and at the same time measuring local oxygen tension alongside. ^{13}C-labeled microdialysis with 1,2-$^{13}C_2$ glucose as substrate may thus find a methodological role in studies of hyperoxia or strategies to optimize perfusion and mitochondrial function. This is the first time that a comparison between glycolysis and the PPP has been carried out directly in the human brain."

Studies in adult rats and brain slices suggested that the PPP increased after hypoxia (Domanska-Janik, 1988). Contrastingly, other researchers found that, in neonatal rats, the PPP decreased after hypoxia-ischemia, suggesting that inability to upregulate the PPP may render neonatal brain vulnerable to oxidative stress (Brekke et al., 2014).

Although the shared gateway into both the Embden-Meyerhof pathway and the PPP is via the action of hexokinase (HK) converting glucose to glucose-6-phosphate (G6P), there are several isoforms of that enzyme. HKI (dubbed "the brain HK") seems to predestine G6P for subsequent processing via the Embden-Meyerhof pathway, while HKII ("the muscle HK") has a more complex role and under some circumstances seems to predispose toward the PPP. There is evidence to suggest HK1 being located bound to mitochondria. The interaction of HKs with mitochondria is not static, but is regulated by factors such as glucose, G-6-P and kinases such as protein kinase B (PKB, also known as Akt) and glycogen synthase kinase 3 (GSK-3). Accordingly, HKII has been suggested to translocate between the cytosol, where it channels G6P into the PPP, and binding to mitochondria, where it predisposes G6P to "glycolysis and oxidative phosphorylation" (John et al., 2011). It has been suggested that shifts in expression (and/or activity) between HKI and HKII may be a contributing factor (e.g., in injury or disease) to changes in the balance between the Embden-Meyerhof pathway and the PPP (John et al., 2011).

Glucose-6-phosphate dehydrogenase (G6PD, also known as G6PDH), the enzyme responsible for the rate-limiting step of the PPP, is known to exhibit many gene mutations, some of which are manifest as various degrees of G6PD enzyme deficiency (Notaro et al., 2000; Cappellini and Fiorelli, 2008). The G6PD gene is on the X-chromosome, and has long been recognized in the context of the phenomenon of the mosaic of X-chromosome inactivation in females (Beutler et al., 1962), termed Lyon's Hypothesis or Law (Lyon, 1961; Gendrel and Heard, 2011;

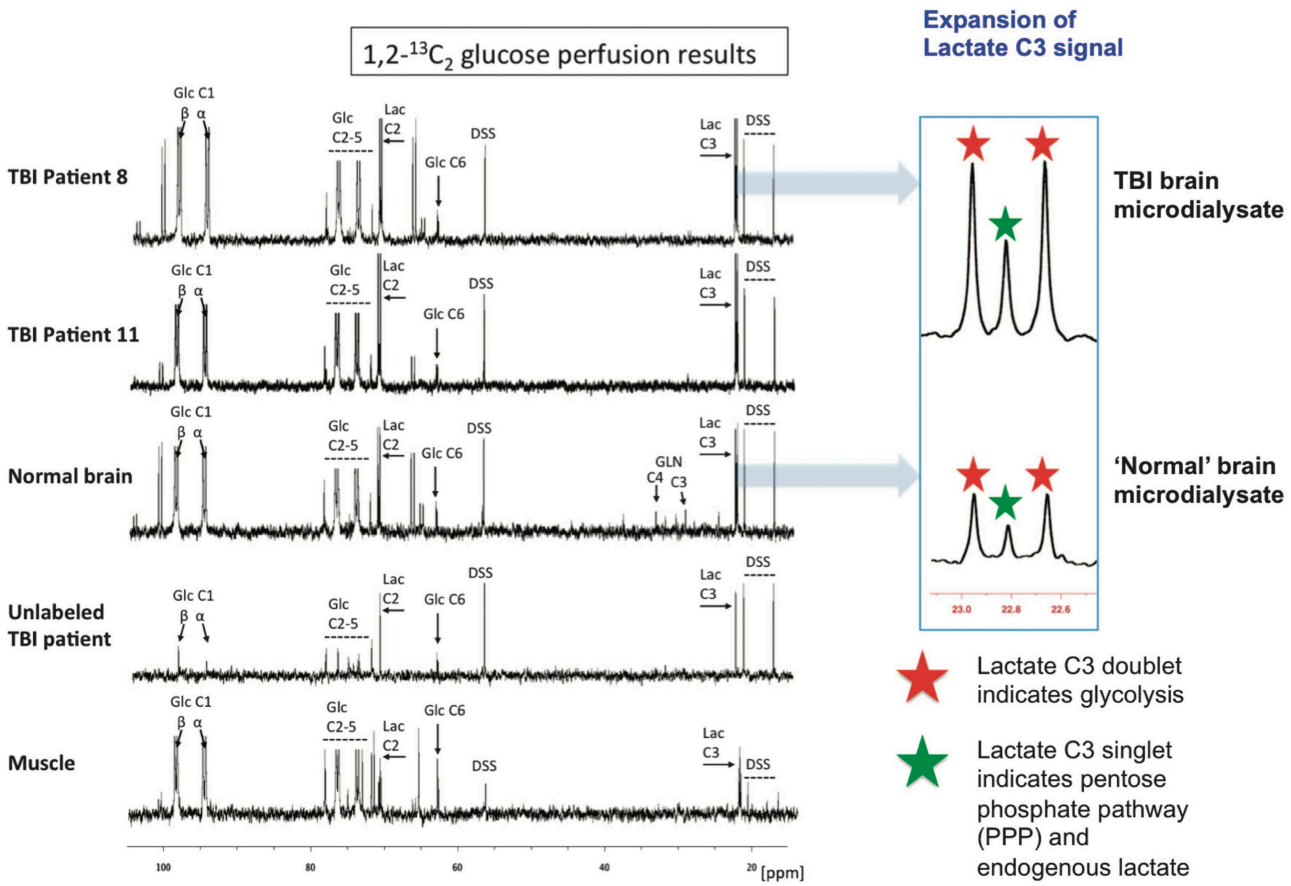


FIGURE 6 | Illustrative examples of ^{13}C NMR spectra for microdialysates from patients who received perfusion with 1,2-$^{13}C_2$ glucose (4 mmol/L): TBI brain (uppermost two spectra, 24-h perfusion), "normal" brain (third spectrum, 24-h perfusion), and muscle (bottom spectrum, 8-h perfusion). An example of brain microdialysate from an unlabeled TBI patient with plain (unsupplemented) perfusion fluid is shown for comparison (fourth spectrum). Examples of expansion of the lactate C3 signal are shown for TBI brain and "normal" brain. Red stars indicate lactate C3 doublet (due to glycolytic 2,3-13C2 lactate) and green stars indicate C3 singlet (due to pentose phosphate pathway-derived 3-^{13}C lactate plus endogenous lactate). Glucose (Glc), lactate (Lac), 4,4-dimethyl-4-silapentane-1-sulfonate sodium salt (DSS, the internal reference standard). Spectra were run from −20 to +250 p.p.m. The main reference DSS signal at 0 p.p.m. is not shown in the range illustrated. Originally published by Jalloh et al. (2015) in J Cereb Blood Flow Metab 35: 111–120, and reproduced with permission of Nature Publishing Group.

Harper, 2011). In female embryo early development, inactivation occurs randomly of one of the two X-chromosomes in each cell, then tissues and organs develop as mosaics consisting of clonal patches of cells that maintain their original inactivation state during subsequent cell divisions. G6PD can be inhibited by dehydroepiandrosterone (DHEA) (Rodriguez-Rodriguez et al., 2013). DHEA, a neurosteroid, is synthesized in brain, and also outside of brain (mainly by adrenal glands). DHEA is considered neuroprotective, and, in human brain tissue, there was a tendency for lower DHEA levels in subjects with Alzheimer's disease than in non-dementia similarly-aged controls (Schumacher et al., 2003). Endogenous DHEA levels are regarded as declining with age, and highest in males and lower in females, based on circulating levels which show individual variations and pathology-related variations (Samaras et al., 2013). DHEA in brain tissue is less well-understood. The above factors regarding G6PD and DHEA might thus contribute to variability in the PPP vs. glycolysis as sources of lactate.

Another source of lactate in the brain, besides production *in situ* from glucose via glycolysis (major pathway) and PPP (minor pathway) is uptake of lactate from the circulation (Ide et al., 2000; Overgaard et al., 2012). The brain shows periods of net uptake and net export of lactate. This has been studied by arteriovenous (AV) difference in TBI patients by Jalloh et al. (2013). Their conclusions were as follows (Jalloh et al., 2013). "Our findings suggest that the injured brain takes up lactate, which can be oxidatively metabolized (Gallagher et al., 2009). Lactate uptake occurs despite relatively high brain lactate levels after TBI suggesting up-regulation of MCT transporters. Glucose delivery to brain cells is maintained during periods of lactate uptake. Hence, lactate uptake may reflect an adaptive response to the increased energy demands and change in metabolic priorities of the injured brain. The injured brain's capacity to use endogenous lactate as an alternative fuel implies that exogenous lactate may be therapeutic in TBI patients. Accordingly, rats given intravenous lactate after fluid percussion injury performed better than those given

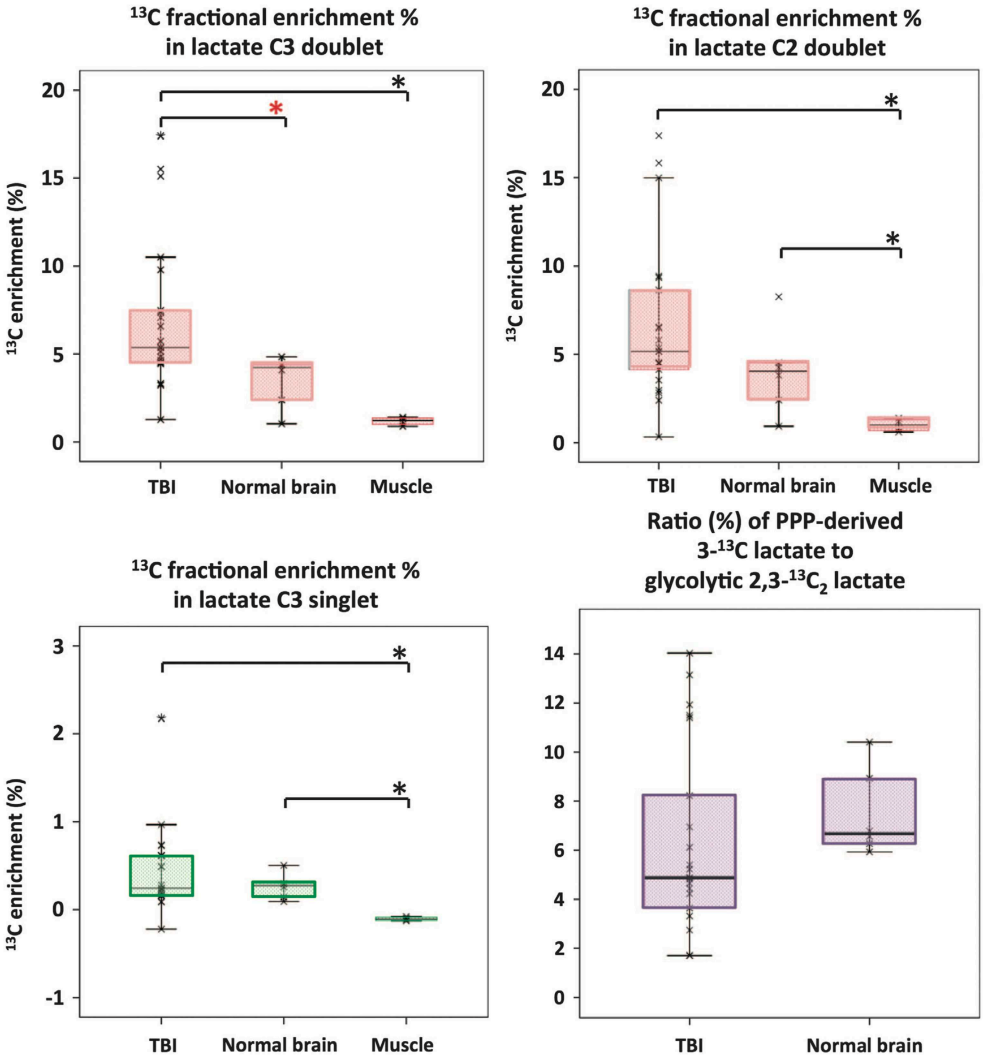


FIGURE 7 | Microdialysate NMR measurements of ^{13}C labeling: results from perfusion for 24-h- (brain: TBI or "normal") or 8-h perfusion (muscle) with 1,2-$^{13}C_2$glucose (4 mmol/L). Red asterisks denote $P < 0.01$ for TBI vs. "normal" brain (Mann–Whitney); other comparisons asterisked in black denote $P < 0.05$. Individual data points are shown by × symbols. Number of patients: 15 TBI, six "normal" brain, and four muscle. Originally published by Jalloh et al. (2015) in J Cereb Blood Flow Metab 35: 111–120, and reproduced with permission of Nature Publishing Group.

saline (Rice et al., 2002; Holloway et al., 2007). Glycemic control of neurocritical care patients is necessary to avoid both hypo- and hyperglycaemia, although tight glycaemic control may be too restrictive for optimal cerebral metabolism and less rigid control may be preferable (Kramer et al., 2012). Lactate administration may have a role in supporting energy metabolism in this context. Ongoing and future clinical studies will elucidate whether lactate administration improves outcomes."

A possible contribution to lactate in brain is via the TCA cycle (Cruz and Cerdan, 1999; Tyson et al., 2003; Sonnewald, 2014). The TCA cycle intermediates malate and oxaloacetate (OAA) can be converted to pyruvate by the action of malic enzyme; also OAA can be converted by the action of phosphoenolpyruvate carboxykinase (PEPCK) plus pyruvate kinase (PK) to pyruvate, then LDH can convert pyruvate to lactate. Anaplerosis (i.e., topping-up) of TCA cycle intermediates is coupled to cataplerosis (i.e., spin-out) from the TCA cycle, and Sonnewald has recently hypothesized, "cataplerosis in the brain is achieved by exporting the lactate generated from TCA cycle intermediates into the blood and perivascular area.... This shifts the generally accepted paradigm of lactate generation as simply derived from glycolysis to that of oxidation and might present an alternative explanation for aerobic glycolysis" (Sonnewald, 2014). Whether this is a significant route of lactate production in human TBI brain is not (to our knowledge) reported in the literature.

Utilization of Lactate in TBI

While glucose is recognized as the primary energy substrate in most organs, recent evidence suggests that the situation in the

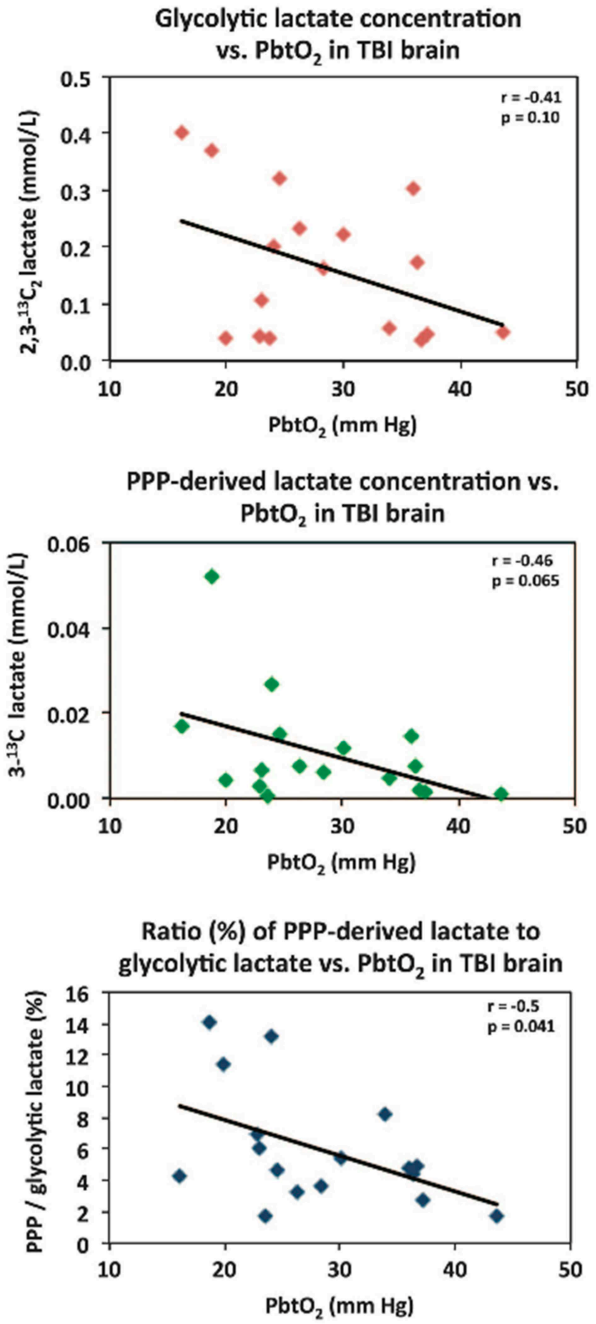


FIGURE 8 | Relationships in TBI brain for glycolytic lactate and pentose phosphate pathway (PPP)-derived lactate vs. $PbtO_2$. Concentrations of glycolytic 2,3-$^{13}C_2$ lactate (upper panel) and PPP-derived 3-^{13}C lactate (middle panel) plotted vs. $PbtO_2$. Lower panel: ratio (%) of PPP-derived 3-^{13}C lactate to glycolytic 2,3-$^{13}C_2$ lactate, plotted vs. $PbtO_2$. Each data point represents the results of NMR analysis of the combined contents of 24 × 1 h of microdialysate collection vials from one microdialysis catheter, plotted against the corresponding $PbtO_2$ concentration expressed in mmHg, measured using a Licox oxygen probe placed alongside the microdialysis catheter in the brain. Lines are fitted by linear regression (statistics shown are Pearson's correlation coefficient r and analysis of variance *P*–value). Data are from 13 TBI patients. Four of these thirteen had a second period of monitoring, making 17 data points in total for each correlation. Originally published by Jalloh et al. (2015) in J Cereb Blood Flow Metab 35: 111–120, and reproduced with permission of Nature Publishing Group.

brain is considerably more complex, particularly in relation to the main cell types. Traditionally it has been believed that both glia and neurons metabolize glucose as the preferred substrate via glycolysis to pyruvate, which is converted to acetyl CoA and enters the TCA cycle resulting in the generation of ATP by oxidative phosphorylation. Recent evidence suggests, however, that the neurons may utilize lactate (classically perceived as a waste product) as an energy substrate. **Figure 9** outlines a scheme of metabolic trafficking between astrocytes and neurons (Gallagher et al., 2009). Glucose (Glc) from the vasculature is metabolized to lactate (Lac) in astrocytes, exported into the extracellular fluid, taken up by neurons and processed (via pyruvate and acetate) by the TCA cycle. This spins off glutamate (Glt), which is released and then taken up by astrocytes, which convert it to glutamine (Gln), which is released into the extracellular fluid and taken up by neurons, which re-convert it to glutamate (Gallagher et al., 2009). This theory, which has become known as the astrocyte-neuron lactate shuttle (ANLS) hypothesis was proposed by Pellerin and Magistretti (1994) as a result of *in vitro* studies and has been later supported by studies in animals, (e.g., Tyson et al., 2003). Moreover, our recent microdialysis studies using ^{13}C-labeling have demonstrated that the injured human brain can metabolize lactate via the TCA cycle (Gallagher et al., 2009). We suggested that low extracellular lactate levels, with better outcomes, might be because astrocytic glycolysis-derived lactate is being efficiently taken up by neurons and utilized via the TCA cycle (Gallagher et al., 2009). Conversely, where neurons are too damaged to utilize the lactate produced from glucose by astrocytes, i.e., uncoupling of neuronal and glial metabolism, high extracellular levels of lactate would accumulate, with poor outcome (Carpenter et al., 2014).

Although the ANLS hypothesis is still somewhat controversial, in recent years the concept of lactate as a brain energy substrate has been extended even further by Schurr in the postulation that lactate (rather than pyruvate) may be the true substrate for mitochondrial respiration (Schurr, 2006). In Schurr's postulated scheme, the glycolytic route in the cytosol, from glucose to pyruvate then by LDH-mediated conversion to lactate, is followed by uptake of lactate by a monocarboxylate transporter (MCT) in the mitochondrial outer membrane, then by conversion of lactate to pyruvate (by LDH1?) in the mitochondrial inner membrane (Schurr, 2006). The rest of the biochemical pathway of mitochondrial respiration (pyruvate to acetate CoA, TCA cycle, ETC and ATP synthesis) then follows on.

More recent studies of the significance of lactate led Suzuki et al. to propose, "astrocyte-neuron lactate transport is essential for long term synaptic plasticity, long-term memory, and its underlying molecular and synaptic changes" (Suzuki et al., 2011). Lactate transport may thus have important implications for pathologies with cognitive deficits. Very recently, Galow et al. showed that gamma oscillations, required for complex neuronal processing, can be fuelled by energy-rich substrates, most effectively by high concentrations of glucose, but also, to a somewhat lower degree, by high concentrations of lactate or pyruvate (Galow et al., 2014).

A recent study by Bouzat et al. showed evidence for beneficial effects (judged by surrogate markers) resulting from intravenous lactate administration in 15 TBI patients (Bouzat et al., 2014). A

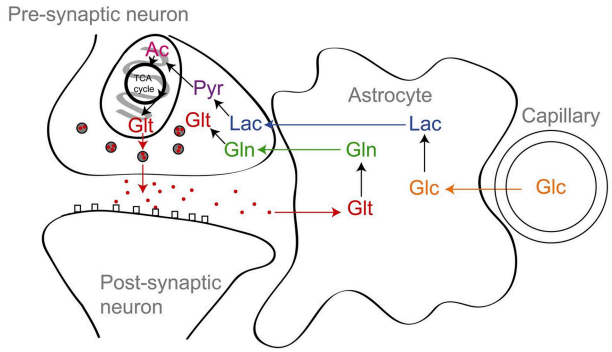
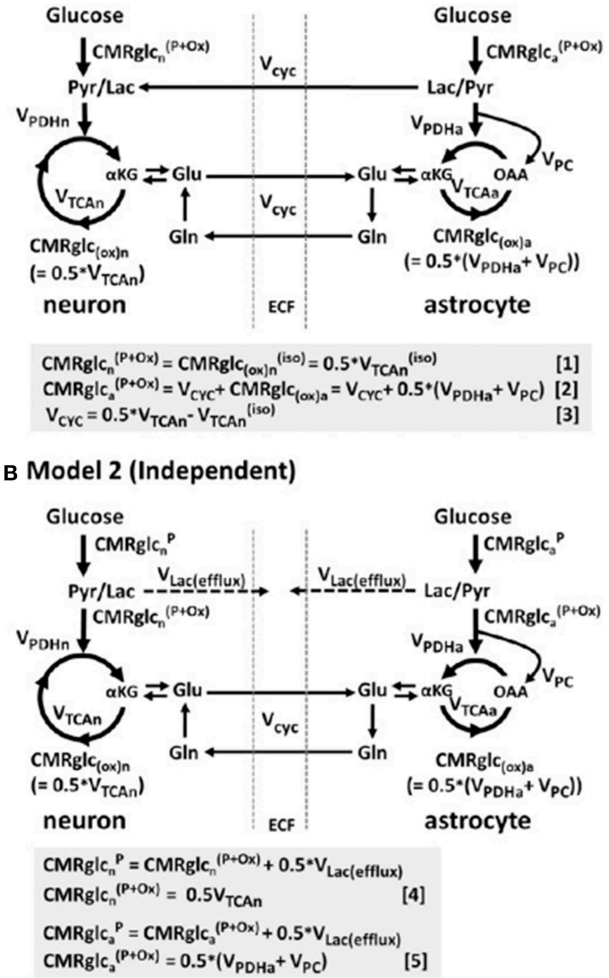


FIGURE 9 | Glucose (Glc) from the vasculature is metabolized to lactate (Lac) in astrocytes, exported into the extracellular fluid, taken up by neurons and processed [via pyruvate (Pyr) and acetate (Ac)] by the TCA cycle. This spins off glutamate (Glt), which is released and then taken up by astrocytes, which convert it to glutamine (Gln), which is released into the extracellular fluid and taken up by neurons, which re-convert it to glutamate. For details of the relevant membrane transporters (see Chih and Roberts, 2003; Gallagher et al., 2009; Pellerin and Magistretti, 2012). Besides Lac production *in situ* from Glc, Lac may also be taken up from the circulation, (see Ide et al., 2000; Overgaard et al., 2012; Jalloh et al., 2013). For typical concentrations of the above species and $PbtO_2$ (see Gallagher et al., 2009; Timofeev et al., 2011a,b). Originally published by Gallagher et al. (2009) in Brain 132: 2839–2849, and reproduced with permission of Oxford Journals.



$$CMRglc_n^{(P+Ox)} = CMRglc_{(ox)n}^{(iso)} = 0.5*V_{TCAn}^{(iso)} \quad [1]$$
$$CMRglc_a^{(P+Ox)} = V_{CYC} + CMRglc_{(ox)a} = V_{CYC} + 0.5*(V_{PDHa} + V_{PC}) \quad [2]$$
$$V_{CYC} = 0.5*V_{TCAn} - V_{TCAn}^{(iso)} \quad [3]$$
$$CMRglc_n^{P} = CMRglc_n^{(P+Ox)} + 0.5*V_{Lac(efflux)}$$
$$CMRglc_n^{(P+Ox)} = 0.5V_{TCAn} \quad [4]$$
$$CMRglc_a^{P} = CMRglc_a^{(P+Ox)} + 0.5*V_{Lac(efflux)}$$
$$CMRglc_a^{(P+Ox)} = 0.5*(V_{PDHa} + V_{PC}) \quad [5]$$

FIGURE 10 | Schematic depiction of two neuroenergetics models under consideration to account for the 1:1 flux relationship between increments in the rate of glutamate/glutamine cycling (V_{cyc}) and the TCA cycle flux in neurons (V_{TCAn}). (A) ANLS-type model (Model 1). **(B)** Independent-type model (Model 2) in which neurons and astrocytes take up and oxidize glucose according to their respective energy needs. Phosphorylated glucose not oxidized within the cell may be effluxed as lactate, $V_{Lac(efflux)}$, which is shown by dashed lines. The results of this study in rat brain suggested that neurons are capable of supporting a substantial fraction of their substrate requirements by direct uptake and phosphorylation of glucose. Originally published by Patel et al. (2014) in Proc Natl Acad Sci USA 111: 5385–5390, and reproduced with permission from Proc Natl Acad Sci USA.

mixed effects linear regression model was used to evaluate the results. The treatment caused increases in brain microdialysate lactate (coefficient 0.47 mmol/L, 95% confidence interval 0.31–0.63 mmol/L), pyruvate [13.1 (8.78–17.4) μmol/L], and glucose [0.1 (0.04–0.16) mmol/L; all $p < 0.01$]. Reductions in brain microdialysate glutamate [−0.95 (−1.94–0.06) mmol/L, $p = 0.06$] and ICP [−0.86 (−1.47–0.24) mmHg, $p < 0.01$] were also observed (Bouzat et al., 2014). The lactate solution administered was hypertonic, and it was not resolved how much of the apparent benefit on ICP was due to this property or due to the actual lactate itself.

An earlier study by Ichai et al. in TBI patients showed that hyperosmolar sodium lactate solution (504 mmol/L; 1100 mosm/L) given intravenously was more effective at lowering ICP than a mannitol solution with an equivalent osmotic load (1160 mosm/L) (Ichai et al., 2009). Also, compared with saline solution (0.9%), a 0.5 mol/L sodium lactate solution was more effective at reducing the occurrence of raised ICP episodes in TBI patients (Ichai et al., 2013). In a model of TBI–controlled cortical impact (CCI) in rats–infusion of lactate (100 mmol/L) showed significantly improved cerebral blood flow (CBF), significantly reduced lesion volume, and no significant difference in extracellular glutamate concentration, when compared to CCI with saline (0.9%) infusion control (Alessandri et al., 2012).

Furthermore, such studies of intravenous lactate and the concept of the ANLS model have elicited controversy, and other models have been proposed (Dienel, 2014). Much of the evidence for the ANLS model is summarized in a review (Pellerin and Magistretti, 2012). A recent kinetic modeling study in rats (using non-radioactive ^{19}F-FDG and 1,6-$^{13}C_2$ glucose) compared an ANLS model with an "independent" model in which neurons and astrocytes take up and oxidize glucose according to their respective energy needs (Patel et al., 2014) (**Figure 10**). The results did not support the ANLS astrocytic lactate production shuttling to neurons to provide a major neuronal fuel, but instead favored the "independent" model with neuronal glucose-derived pyruvate as the major oxidative fuel for activated neurons (Patel et al., 2014).

Compatible with the conclusion of Patel et al. (2014) is an *in vitro* study in mouse hippocampal slices with

electrophysiological stimulation, in the presence of glucose compared with pyruvate or, in some instances, lactate (Ivanov et al., 2014). The prime measures were changes in NAD(P)H and tissue oxygen, other measures being FAD, intracellular pH and Ca^{2+}, and ATP (Ivanov et al., 2014). Distinction of neuronal contributions was achieved using inhibitors. These authors concluded, "Our data do not support the hypothesis of a preferential utilization of astrocyte-released lactate by neurons during network activation in slices–instead we show that during such activity glucose is an effective energy substrate for both neurons and astrocytes" (Ivanov et al., 2014).

Utilization of glucose but not lactate has been reported to correlate with N-methyl-D-aspartate (NMDA)-induced neurotransmission in mouse cerebellar neurons *in vitro* (Bak et al., 2009). These authors have suggested, "the role of extracellular lactate may be to support basal metabolism in neurons rather than neurotransmission activity *per se* mandating a revision of how we perceive the role of lactate in cerebral energy metabolism" (Bak et al., 2009).

Whether the above findings *in vivo* in rats, and *in vitro* in mouse brain slices and mouse neurons are relevant to human TBI is as yet unknown.

While it is still debated (Dienel, 2014) whether or not lactate is a "preferred fuel" (sparing glucose) after TBI or whether brain energetics are improved as a result of intravenous lactate, it is evident from ^{13}C labeling studies that animal and human brain (injured and non-injured) can utilize lactate via the TCA cycle, judged by production of the appropriate ^{13}C labeling patterns in glutamate and/or glutamine (Tyson et al., 2003; Gallagher et al., 2009; Boumezbeur et al., 2010). At face value, the idea that intravenous administration of exogenous lactate may be beneficial for TBI patients (Ichai et al., 2009, 2013; Bouzat et al., 2014) seems to conflict with the association between high (endogenous) brain extracellular lactate and poor outcome (Timofeev et al., 2011a). We have previously suggested that where build-up of (endogenous) lactate occurs, this may be because the neurons are too damaged to utilize it, which might explain the association between high endogenous extracellular lactate and poor outcome (Carpenter et al., 2014). A possible rationalization of the apparently beneficial effect of lactate infusion (Ichai et al., 2009, 2013; Bouzat et al., 2014) vs. the unfavorable association of high endogenous lactate (Timofeev et al., 2011a) might be that if $PbtO_2$ is adequate, and if mitochondria are functioning, then brain cells can use lactate as a feedstock, via conversion to pyruvate and then acetate, for the TCA cycle and ultimately ATP production.

Whether other "alternative fuels" (apart from lactate) might be relevant in treating TBI is unknown. It has been suggested that sodium pyruvate might be preferable to sodium lactate, particularly as pyruvate would bypass the redox step from lactate to pyruvate (Dienel, 2014). However, pyruvate in solution is known to self-react, forming various products including a dimer (parapyruvate) that can, at least under some circumstances, inhibit alpha-ketoglutarate dehydrogenase, an enzyme in the TCA cycle (Margolis and Coxon, 1986). An alternative form of pyruvate that has been suggested is ethyl pyruvate which is reportedly more stable in solution and has anti-inflammatory effects distinct from sodium pyruvate (Fink, 2007).

Conclusions and Future Prospects

Understanding of the roles of glycolysis and lactate in the human brain are continuing to evolve. From the original concept of lactate as merely a waste product, with adverse associations in injured brain, it is becoming increasingly recognized as a potential source of energy for the brain. Having the right balance between glycolysis (and PPP) and mitochondrial metabolism is vital. Controlling this balance is becoming recognized as a therapeutic target in treating TBI patients during neurocritical care. For interpreting the levels of brain extracellular lactate during multimodality monitoring it is important to also take into account the other parameters, including brain extracellular pyruvate and glucose levels, as well as circulating levels of glucose and lactate in blood. Infusion and perfusion studies, and labeling with ^{13}C with detection by *ex vivo* NMR or *in vivo* MRS, have potential to shed further light on cerebral energy metabolism in human brain and may help suggest strategies for improving TBI treatment protocols for better clinical outcomes.

Acknowledgments

We gratefully acknowledge financial support as follows. Research support: the Medical Research Council (MRC, Grant Nos. G0600986 ID79068 and G1002277 ID98489) and the National Institute for Health Research Biomedical Research Centre (NIHR BRC) Cambridge (Neuroscience Theme; Brain Injury and Repair Theme). Authors' support: KLHC—NIHR BRC Cambridge (Neuroscience Theme; Brain Injury and Repair Theme); IJ—MRC (Grant no. G1002277 ID 98489) and NIHR BRC Cambridge; PJH—NIHR Research Professorship, Academy of Medical Sciences/Health Foundation Senior Surgical Scientist Fellowship, and the NIHR BRC Cambridge

References

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. doi: 10.1089/neu.2011.2067

Bak, L. K., Walls, A. B., Schousboe, A., Ring, A., Sonnewald, U., and Waagepetersen, H. S. (2009). Neuronal glucose but not lactate utilization is positively correlated with NMDA-induced neurotransmission and fluctuations in cytosolic Ca2+ levels. *J. Neurochem.* 109(Suppl. 1), 87–93. doi: 10.1111/j.1471-4159.2009.05943.x

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. doi: 10.1089/neu.2006.0210

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. doi: 10.1089/neu.2005.22.1052

Befroy, D. E., Rothman, D. L., Petersen, K. F., and Shulman, G. I. (2012). (31)P-magnetization transfer magnetic resonance spectroscopy measurements of *in vivo* metabolism. *Diabetes* 61, 2669–2678. doi: 10.2337/db12-0558

Bensinger, S. J., and Christofk, H. R. (2012). New aspects of the Warburg effect in cancer cell biology. *Semin. Cell Dev. Biol.* 23, 352–361. doi: 10.1016/j.semcdb.2012.02.003

Ben-Yoseph, O., Boxer, P. A., and Ross, B. D. (1996). Assessment of the role of the glutathione and pentose phosphate pathways in the protection of primary cerebrocortical cultures from oxidative stress. *J. Neurochem.* 66, 2329–2337. doi: 10.1046/j.1471-4159.1996.66062329.x

Beutler, E., Yeh, M., and Fairbanks, V. F. (1962). The normal human female as a mosaic of X-chromosome activity: studies using the gene for C-6-PD-deficiency as a marker. *Proc. Natl. Acad. Sci. U.S.A.* 48, 9–16. doi: 10.1073/pnas.48.1.9

Bittar, P. G., Charnay, Y., Pellerin, L., Bouras, C., and Magistretti, P. J. (1996). Selective distribution of lactate dehydrogenase isoenzymes in neurons and astrocytes of human brain. *J. Cereb. Blood Flow Metab.* 16, 1079–1089. doi: 10.1097/00004647-199611000-00001

Boumezbeur, F., Petersen, K. F., Cline, G. W., Mason, G. F., Behar, K. L., Shulman, G. I., et al. (2010). The contribution of blood lactate to brain energy metabolism in humans measured by dynamic 13C nuclear magnetic resonance spectroscopy. *J. Neurosci.* 30, 13983–13991. doi: 10.1523/JNEUROSCI.2040-10.2010

Bouzat, P., Sala, N., Suys, T., Zerlauth, J. B., Marques-Vidal, P., Feihl, F., et al. (2014). Cerebral metabolic effects of exogenous lactate supplementation on the injured human brain. *Intensive Care Med.* 40, 412–421. doi: 10.1007/s00134-013-3203-6

Brekke, E. M., Morken, T. S., Wideroe, M., Haberg, A. K., Brubakk, A. M., and Sonnewald, U. (2014). The pentose phosphate pathway and pyruvate carboxylation after neonatal hypoxic-ischemic brain injury. *J. Cereb. Blood Flow Metab.* 34, 724–734. doi: 10.1038/jcbfm.2014.8

Cappellini, M. D., and Fiorelli, G. (2008). Glucose-6-phosphate dehydrogenase deficiency. *Lancet* 371, 64–74. doi: 10.1016/S0140-6736(08)60073-2

Carpenter, K. L., Jalloh, I., Gallagher, C. N., Grice, P., Howe, D. J., Mason, A., et al. (2014). (13)C-labelled microdialysis studies of cerebral metabolism in TBI patients. *Eur. J. Pharm. Sci.* 57, 87–97. doi: 10.1016/j.ejps.2013.12.012

Chesnut, R. M., Marshall, L. F., Klauber, M. R., Blunt, B. A., Baldwin, N., Eisenberg, H. M., et al. (1993). The role of secondary brain injury in determining outcome from severe head injury. *J. Trauma* 34, 216–222. doi: 10.1097/00005373-199302000-00006

Chih, C. P., and Roberts, Jr. E. L. (2003). Energy substrates for neurons during neural activity: a critical review of the astrocyte-neuron lactate shuttle hypothesis. *J. Cereb. Blood Flow Metab.* 23, 1263–1281. doi: 10.1097/01.WCB.0000081369.51727.6F

Cruz, F., and Cerdan, S. (1999). Quantitative 13C NMR studies of metabolic compartmentation in the adult mammalian brain. *NMR Biomed.* 12, 451–462.

De Bock, K., Georgiadou, M., Schoors, S., Kuchnio, A., Wong, B. W., Cantelmo, A. R., et al. (2013). Role of PFKFB3-driven glycolysis in vessel sprouting. *Cell* 154, 651–663. doi: 10.1016/j.cell.2013.06.037

Dienel, G. A. (2014). Lactate shuttling and lactate use as fuel after traumatic brain injury: metabolic considerations. *J. Cereb. Blood Flow Metab.* 34, 1736–1748. doi: 10.1038/jcbfm.2014.153

Domanska-Janik, K. (1988). Hexose monophosphate pathway activity in normal and hypoxic rat brain. *Resuscitation* 16, 79–90. doi: 10.1016/0300-9572(88)90073-1

Dusick, J. R., Glenn, T. C., Lee, W. N., Vespa, P. M., Kelly, D. F., Lee, S. M., et al. (2007). Increased pentose phosphate pathway flux after clinical traumatic brain injury: a [1,2-13C2]glucose labeling study in humans. *J. Cereb. Blood Flow Metab.* 27, 1593–1602. doi: 10.1038/sj.jcbfm.9600458

Fink, M. P. (2007). Ethyl pyruvate: a novel anti-inflammatory agent. *J. Intern. Med.* 261, 349–362. doi: 10.1111/j.1365-2796.2007.01789.x

Gallagher, C. N., Carpenter, K. L., Grice, P., Howe, D. J., Mason, A., Timofeev, I., et al. (2009). The human brain utilizes lactate via the tricarboxylic acid cycle: a 13C-labelled microdialysis and high-resolution nuclear magnetic resonance study. *Brain* 132(Pt 10), 2839–2849. doi: 10.1093/brain/awp202

Galow, L. V., Schneider, J., Lewen, A., Ta, T. T., Papageorgiou, I. E., and Kann, O. (2014). Energy substrates that fuel fast neuronal network oscillations. *Front. Neurosci.* 8:398. doi: 10.3389/fnins.2014.00398

Garnett, M. R., Corkill, R. G., Blamire, A. M., Rajagopalan, B., Manners, D. N., Young, J. D., et al. (2001). Altered cellular metabolism following traumatic brain injury: a magnetic resonance spectroscopy study. *J. Neurotrauma* 18, 231–240. doi: 10.1089/08977150151070838

Gendrel, A. V., and Heard, E. (2011). Fifty years of X-inactivation research. *Development* 138, 5049–5055. doi: 10.1242/dev.068320

Harper, P. S. (2011). Mary Lyon and the hypothesis of random X chromosome inactivation. *Hum. Genet.* 130, 169–174. doi: 10.1007/s00439-011-1013-x

Holloway, R., Zhou, Z., Harvey, H. B., Levasseur, J. E., Rice, A. C., Sun, D., et al. (2007). Effect of lactate therapy upon cognitive deficits after traumatic brain injury in the rat. *Acta Neurochir. (Wien)* 149, 919–927; discussion 927. doi: 10.1007/s00701-007-1241-y

Hutchinson, P. J., O'Connell, M. T., Seal, A., Nortje, J., Timofeev, I., Al-Rawi, P. G., et al. (2009). A combined microdialysis and FDG-PET study of glucose metabolism in head injury. *Acta Neurochir. (Wien)* 151, 51–61; discussion 61. doi: 10.1007/s00701-008-0169-1

Ichai, C., Armando, G., Orban, J. C., Berthier, F., Rami, L., Samat-Long, C., et al. (2009). Sodium lactate versus mannitol in the treatment of intracranial hypertensive episodes in severe traumatic brain-injured patients. *Intensive Care Med.* 35, 471–479. doi: 10.1007/s00134-008-1283-5

Ichai, C., Payen, J. F., Orban, J. C., Quintard, H., Roth, H., Legrand, R., et al. (2013). Half-molar sodium lactate infusion to prevent intracranial hypertensive episodes in severe traumatic brain injured patients: a randomized controlled trial. *Intensive Care Med.* 39, 1413–1422. doi: 10.1007/s00134-013-2978-9

Ide, K., Schmalbruch, I. K., Quistorff, B., Horn, A., and Secher, N. H. (2000). Lactate, glucose and O2 uptake in human brain during recovery from maximal exercise. *J. Physiol.* 522(Pt 1), 159–164. doi: 10.1111/j.1469-7793.2000.t01-2-00159.xm

Ivanov, A. I., Malkov, A. E., Waseem, T., Mukhtarov, M., Buldakova, S., Gubkina, O., et al. (2014). Glycolysis and oxidative phosphorylation in neurons and astrocytes during network activity in hippocampal slices. *J. Cereb. Blood Flow Metab.* 34, 397–407. doi: 10.1038/jcbfm.2013.222

Jalloh, I., Carpenter, K. L., Grice, P., Howe, D. J., Mason, A., Gallagher, C. N., et al. (2015). Glycolysis and the pentose phosphate pathway after human traumatic brain injury: microdialysis studies using 1,2-(13)C2 glucose. *J. Cereb. Blood Flow Metab.* 35, 111–120. doi: 10.1038/jcbfm.2014.177

Jalloh, I., Carpenter, K. L., Helmy, A., Carpenter, T. A., Menon, D. K., and Hutchinson, P. J. (2014). Glucose metabolism following human traumatic brain injury: methods of assessment and pathophysiological findings. *Metab. Brain Dis.* doi: 10.1007/s11011-014-9628-y. [Epub ahead of print].

Jalloh, I., Helmy, A., Shannon, R. J., Gallagher, C. N., Menon, D. K., Carpenter, K. L., et al. (2013). Lactate uptake by the injured human brain: evidence from an arteriovenous gradient and cerebral microdialysis study. *J. Neurotrauma* 30, 2031–2037. doi: 10.1089/neu.2013.2947

John, S., Weiss, J. N., and Ribalet, B. (2011). Subcellular localization of hexokinases I and II directs the metabolic fate of glucose. *PLoS ONE* 6:e17674. doi: 10.1371/journal.pone.0017674

Katayama, Y., Becker, D. P., Tamura, T., and Hovda, D. A. (1990). Massive increases in extracellular potassium and the indiscriminate release of glutamate following concussive brain injury. *J. Neurosurg.* 73, 889–900. doi: 10.3171/jns.1990.73.6.0889

Kawamata, T., Katayama, Y., Hovda, D. A., Yoshino, A., and Becker, D. P. (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. doi: 10.1038/jcbfm.1992.3

Kramer, A. H., Roberts, D. J., and Zygun, D. A. (2012). Optimal glycemic control in neurocritical care patients: a systematic review and meta-analysis. *Crit. Care* 16, R203. doi: 10.1186/cc11812

Lebon, V., Petersen, K. F., Cline, G. W., Shen, J., Mason, G. F., Dufour, S., et al. (2002). Astroglial contribution to brain energy metabolism in humans revealed by 13C nuclear magnetic resonance spectroscopy: elucidation of the dominant pathway for neurotransmitter glutamate repletion and measurement of astrocytic oxidative metabolism. *J. Neurosci.* 22, 1523–1531.

Lewen, A., Fujimura, M., Sugawara, T., Matz, P., Copin, J. C., and Chan, P. H. (2001). Oxidative stress-dependent release of mitochondrial cytochrome c after traumatic brain injury. *J. Cereb. Blood Flow Metab.* 21, 914–920. doi: 10.1097/00004647-200108000-00003

Lyon, M. F. (1961). Gene action in the X-chromosome of the mouse (*Mus musculus* L.). *Nature* 190, 372–373. doi: 10.1038/190372a0

Maddock, R. J., and Buonocore, M. H. (2012). MR spectroscopic studies of the brain in psychiatric disorders. *Curr. Top. Behav. Neurosci.* 11, 199–251. doi: 10.1007/7854_2011_197

Margolis, S. A., and Coxon, B. (1986). Identification and quantitation of the impurities in sodium pyruvate. *Anal. Chem.* 58, 2504–2510. doi: 10.1021/ac00125a033

Marino, S., Zei, E., Battaglini, M., Vittori, C., Buscalferri, A., Bramanti, P., et al. (2007). Acute metabolic brain changes following traumatic brain injury and their relevance to clinical severity and outcome. *J. Neurol. Neurosurg. Psychiatry* 78, 501–507. doi: 10.1136/jnnp.2006.099796

Neely, J. R., Denton, R. M., England, P. J., and Randle, P. J. (1972). The effects of increased heart work on the tricarboxylate cycle and its interactions with glycolysis in the perfused rat heart. *Biochem. J.* 128, 147–159.

Newcombe, V. F., Williams, G. B., Outtrim, J. G., Chatfield, D., Gulia Abate, M., Geeraerts, T., et al. (2013). Microstructural basis of contusion expansion in traumatic brain injury: insights from diffusion tensor imaging. *J. Cereb. Blood Flow Metab.* 33, 855–862. doi: 10.1038/jcbfm.2013.11

Notaro, R., Afolayan, A., and Luzzatto, L. (2000). Human mutations in glucose 6-phosphate dehydrogenase reflect evolutionary history. *FASEB J.* 14, 485–494.

Overgaard, M., Rasmussen, P., Bohm, A. M., Seifert, T., Brassard, P., Zaar, M., et al. (2012). Hypoxia and exercise provoke both lactate release and lactate oxidation by the human brain. *FASEB J.* 26, 3012–3020. doi: 10.1096/fj.11-191999

Pandolfi, P. P., Sonati, F., Rivi, R., Mason, P., Grosveld, F., and Luzzatto, L. (1995). Targeted disruption of the housekeeping gene encoding glucose 6-phosphate dehydrogenase (G6PD): G6PD is dispensable for pentose synthesis but essential for defense against oxidative stress. *EMBO J.* 14, 5209–5215.

Patel, A. B., Lai, J. C., Chowdhury, G. M., Hyder, F., Rothman, D. L., Shulman, R. G., et al. (2014). Direct evidence for activity-dependent glucose phosphorylation in neurons with implications for the astrocyte-to-neuron lactate shuttle. *Proc. Natl. Acad. Sci. U.S.A.* 111, 5385–5390. doi: 10.1073/pnas.1403576111

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. doi: 10.1073/pnas.91.22.10625

Pellerin, L., and Magistretti, P. J. (2012). Sweet sixteen for ANLS. *J. Cereb. Blood Flow Metab.* 32, 1152–1166. doi: 10.1038/jcbfm.2011.149

Rice, A. C., Zsoldos, R., Chen, T., Wilson, M. S., Alessandri, B., Hamm, R. J., et al. (2002). Lactate administration attenuates cognitive deficits following traumatic brain injury. *Brain Res.* 928, 156–159. doi: 10.1016/S0006-8993(01)03299-1

Riganti, C., Gazzano, E., Polimeni, M., Aldieri, E., and Ghigo, D. (2012). The pentose phosphate pathway: an antioxidant defense and a crossroad in tumor cell fate. *Free Radic. Biol. Med.* 53, 421–436. doi: 10.1016/j.freeradbiomed.2012.05.006

Rodriguez-Rodriguez, P., Fernandez, E., and Bolanos, J. P. (2013). Underestimation of the pentose-phosphate pathway in intact primary neurons as revealed by metabolic flux analysis. *J. Cereb. Blood Flow Metab.* 33, 1843–1845. doi: 10.1038/jcbfm.2013.168

Roozenbeek, B., Maas, A. I., and Menon, D. K. (2013). Changing patterns in the epidemiology of traumatic brain injury. *Nat. Rev. Neurol.* 9, 231–236. doi: 10.1038/nrneurol.2013.22

Rothman, D. L., De Feyter, H. M., de Graaf, R. A., Mason, G. F., and Behar, K. L. (2011). 13C MRS studies of neuroenergetics and neurotransmitter cycling in humans. *NMR Biomed.* 24, 943–957. doi: 10.1002/nbm.1772

Sala, N., Suys, T., Zerlauth, J. B., Bouzat, P., Messerer, M., Bloch, J., et al. (2013). Cerebral extracellular lactate increase is predominantly nonischemic in patients with severe traumatic brain injury. *J. Cereb. Blood Flow Metab.* 33, 1815–1822. doi: 10.1038/jcbfm.2013.142

Samaras, N., Samaras, D., Frangos, E., Forster, A., and Philippe, J. (2013). A review of age-related dehydroepiandrosterone decline and its association with well-known geriatric syndromes: is treatment beneficial? *Rejuvenation Res.* 16, 285–294. doi: 10.1089/rej.2013.1425

Schumacher, M., Weill-Engerer, S., Liere, P., Robert, F., Franklin, R. J., Garcia-Segura, L. M., et al. (2003). Steroid hormones and neurosteroids in normal and pathological aging of the nervous system. *Prog. Neurobiol.* 71, 3–29. doi: 10.1016/j.pneurobio.2003.09.004

Schurr, A. (2006). Lactate: the ultimate cerebral oxidative energy substrate? *J. Cereb. Blood Flow Metab.* 26, 142–152. doi: 10.1038/sj.jcbfm.9600174

Shannon, R. J., Carpenter, K. L., Guilfoyle, M. R., Helmy, A., and Hutchinson, P. J. (2013). Cerebral microdialysis in clinical studies of drugs: pharmacokinetic applications. *J. Pharmacokinet. Pharmacodyn.* 40, 343–358. doi: 10.1007/s10928-013-9306-4

Sonnewald, U. (2014). Glutamate synthesis has to be matched by its degradation - where do all the carbons go? *J. Neurochem.* 131, 399–406. doi: 10.1111/jnc.12812

Stocchetti, N., Furlan, A., and Volta, F. (1996). Hypoxemia and arterial hypotension at the accident scene in head injury. *J. Trauma* 40, 764–767. doi: 10.1097/00005373-199605000-00014

Suzuki, A., Stern, S. A., Bozdagi, O., Huntley, G. W., Walker, R. H., Magistretti, P. J., et al. (2011). Astrocyte-neuron lactate transport is required for long-term memory formation. *Cell* 144, 810–823. doi: 10.1016/j.cell.2011.02.018

TeSlaa, T., and Teitell, M. A. (2014). Techniques to monitor glycolysis. *Methods Enzymol.* 542, 91–114. doi: 10.1016/B978-0-12-416618-9.00005-4

Timofeev, I., Carpenter, K. L., Nortje, J., Al-Rawi, P. G., O'Connell, M. T., Czosnyka, M., et al. (2011a). Cerebral extracellular chemistry and outcome following traumatic brain injury: a microdialysis study of 223 patients. *Brain* 134(Pt 2), 484–494. doi: 10.1093/brain/awq353

Timofeev, I., Czosnyka, M., Carpenter, K. L., Nortje, J., Kirkpatrick, P. J., Al-Rawi, P. G., et al. (2011b). Interaction between brain chemistry and physiology after traumatic brain injury: impact of autoregulation and microdialysis catheter location. *J. Neurotrauma* 28, 849–860. doi: 10.1089/neu.2010.1656

Timofeev, I., Nortje, J., Al-Rawi, P. G., Hutchinson, P. J., and Gupta, A. K. (2013). Extracellular brain pH with or without hypoxia is a marker of profound metabolic derangement and increased mortality after traumatic brain injury. *J. Cereb. Blood Flow Metab.* 33, 422–427. doi: 10.1038/jcbfm.2012.186

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. doi: 10.1016/j.brainres.2003.08.027

Unterberg, A. W., Stover, J., Kress, B., and Kiening, K. L. (2004). Edema and brain trauma. *Neuroscience* 129, 1021–1029. doi: 10.1016/j.neuroscience.2004.06.046

van Hall, G., Stromstad, M., Rasmussen, P., Jans, O., Zaar, M., Gam, C., et al. (2009). Blood lactate is an important energy source for the human brain. *J. Cereb. Blood Flow Metab.* 29, 1121–1129. doi: 10.1038/jcbfm.2009.35

Vespa, P., Bergsneider, M., Hattori, N., Wu, H. M., Huang, S. C., Martin, N. A., et al. (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. doi: 10.1038/sj.jcbfm.9600073

Warburg, O. (1956). On the origin of cancer cells. *Science* 123, 309–314. doi: 10.1126/science.123.3191.309

Xiong, Y., Gu, Q., Peterson, P. L., Muizelaar, J. P., and Lee, C. P. (1997). Mitochondrial dysfunction and calcium perturbation induced by traumatic brain injury. *J. Neurotrauma* 14, 23–34. doi: 10.1089/neu.1997.14.23

Conflict of Interest Statement: Dr Peter J. Hutchinson is a Director of Technicam. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.