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Metabolic alterations in developing brain after injury – knowns and unknowns

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Abstract

Brain development is a highly orchestrated complex process. The developing brain utilizes many substrates including glucose, ketone bodies, lactate, fatty acids and amino acids for energy, cell division and the biosynthesis of nucleotides, proteins and lipids. Metabolism is crucial to provide energy for all cellular processes required for brain development and function including ATP formation, synaptogenesis, synthesis, release and uptake of neurotransmitters, maintaining ionic gradients and redox status, and myelination. The rapidly growing population of infants and children with neurodevelopmental and cognitive impairments and life-long disability resulting from developmental brain injury is a significant public health concern. Brain injury in infants and children can have devastating effects because the injury is superimposed on the high metabolic demands of the developing brain. Acute injury in the pediatric brain can derail, halt or lead to dysregulation of the complex and highly regulated normal developmental processes. This paper provides a brief review of metabolism in developing brain and alterations found clinically and in animal models of developmental brain injury. The metabolic changes observed in three major categories of injury that can result in life-long cognitive and neurological disabilities, including neonatal hypoxia-ischemia, pediatric traumatic brain injury, and brain injury secondary to prematurity are reviewed.

Introduction

The mammalian brain is dependent on a constant supply of oxygen and nutrients, both from dietary intake and provided by other organs, which are delivered to the brain via circulation [1, 2]. The developing brain controls the function of body organs through neurotransmission, while simultaneously facing the metabolic demands of its' own growth and maturation. Brain development is an energy expensive process; hence, during infancy and childhood there is a continuous increase in cerebral blood flow and glucose utilization [3, 4].

Glucose and substrates present in mammalian milk including ketones, fatty acids and glycerol are used to meet the high metabolic demands of the developing brain, whereas adult

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brain uses glucose as the primary energy substrate [2–4]. Recognition that the immature brain uses a variety of substrates for energy and biosynthetic processes led to extensive research to characterize temporal and regional changes in brain development and metabolism [3–5].

Early studies demonstrated high circulating levels and uptake of ketones and lactate [2, 5, 6] as well as glucose, whereas positron emission tomography (PET) studies revealed regional differences in uptake and use of glucose that correlated with developmental maturation of brain function [3, 4, 7].

Uptake and utilization of substrates depends on the presence of specific glucose and monocarboxylic acid transporters (MCT) and the activity of enzymes required for metabolism (see Table 1). Glucose and the MCT transporters responsible for lactate and ketone body uptake show regional and temporal changes in both human [8] and rodent brain [9] which parallel the developmental transition from lower glucose use in suckling brain to the high glucose use by adult brain (Table 1). The enzymes for substrate use undergo a coordinated maturation; with those for ketone body utilization peaking during early development then decreasing after the suckling period in rodents, while enzymes for glucose metabolism and TCA cycle activity increase several-fold from birth and mature between 20–40 days of age in rodents [1, 10–12]. Rates of TCA cycle flux and neurotransmitter cycling increase at least 3-fold from postnatal day 10 to 30 [13]. (Table 1).

The developing brain utilizes many substrates including glucose, ketone bodies, lactate, fatty acids and amino acids for energy and biosynthesis of lipids and proteins [1, 5, 6, 10, 13]. Metabolism is crucial to provide energy for all cellular processes including ATP formation, cell division, synaptogenesis, synthesis, release and uptake of neurotransmitters, maintaining ionic gradients, and maintaining reduced glutathione and redox status [1]. Metabolism is also crucial for synthesis of proteins, nucleic acids, carbohydrates and membrane lipids needed for mitochondrial function and myelination [1]. Although oxidation of glucose via the TCA cycle for energy is lower in developing brain, metabolism of glucose via the pentose phosphate pathway is higher than in adults and provides necessary precursors for nucleotide synthesis and the NADPH required for lipid biosynthesis and maintaining reduced glutathione [1, 10, 14, 15]. The ability to utilize ketones, lactate, pyruvate, free fatty acids and glycerol and certain amino acids also enables the developing brain to survive episodes of hypoglycemia [5, 6].

Developmental Brain Injuries that Can Lead to Poor Neurodevelopmental Outcome

A significant public health concern is the rapidly growing population of infants and children with neurodevelopmental and cognitive impairments [16–18]. Due to advancements in both neonatal and intensive care, more premature infants and children with neonatal or childhood brain injury survive with life-long disability [16,17]. A common misconception is that children with brain injury fare better than adults [19]. However, the pathology differs from adults, because in infants and children injury is superimposed on the highly orchestrated processes and high metabolic demands of the developing brain [19–25]. Acute injury in the

pediatric brain can derail or even halt normal developmental processes due to energy failure, disrupted cell division and synaptogenesis, impaired ionic gradients and depleted antioxidant capacity which can further impair metabolism [26–30].

The overall goal of this paper is to provide a brief review of metabolism in developing brain and the alterations found clinically and in animal models of developmental brain injury. We focus on three major categories of injury that can result in life-long cognitive and neurological disabilities, including brain injury secondary to prematurity, neonatal hypoxia-ischemia, and pediatric traumatic brain injury.

Brain Injury Related to Prematurity

Worldwide approximately 15 million infants are born prematurely, with ~1.5 million annual deaths attributable to preterm birth, especially in those very premature infants (<28 weeks gestation) [31]. Survival rates for premature neonates have increased over the last decades, with improvements in neonatal intensive care. However, the long-term morbidity from premature birth, especially as related to brain injury and abnormalities in brain development, has not significantly decreased. The neurologic deficits in premature infants are diverse, and include cerebral palsy, intellectual disability, sensory deficits, learning disabilities, attention deficits and problems with executive function [32, 33]. Many of these deficits persist throughout childhood and adolescence, requiring assistance in school and support for daily living activities. A longitudinal study showed that 72% of adolescents born very prematurely (<750 g birth weight) had school performance difficulties even without sensory or IQ impairments [33]. It is estimated that in the United States, ~5,000 annual cases of cerebral palsy, and ~10–20,000 cases of serious learning disabilities result from premature birth [32]. One of the most common conditions leading to neurologic injury in premature infants is periventricular leukomalacia (PVL), with diffuse white matter damage resulting from ischemia or inflammation in the vulnerable, immature white matter [32]. This process is believed to account for the majority of motor, cognitive and behavioral sequelae of prematurity; however, the biological basis for brain injury secondary to prematurity is complex and multifactorial. Animal models demonstrate the negative effects of prematurity on growth and maturation of multiple cell types throughout the brain, including neurons, astrocytes, and oligodendrocytes [29].

Neonatal Hypoxia-Ischemia

Neonatal hypoxia-ischemia (HI), which occurs in 1.8–6 per 1,000 live births, is a significant public health problem [26, 34–36]. A large proportion (~25%) of infants with perinatal hypoxia-ischemia develop life-long disabilities [37], which include intellectual disability, impaired learning, memory and executive function, seizure disorders and varying degrees of motor impairment [38, 39]. HI results in significant mortality and accounted for ~ 9% of all infant deaths in the US in 2005 [40]. Hypoxic-ischemic encephalopathy (HIE) is due to decreased delivery of oxygen and nutrients to brain, lactic acidosis and decreased clearance of CO_2 [26]. MRI and MRS studies reveal significant injury to deep gray matter including thalamus, hippocampus, putamen and basal ganglia; cortical loss is also observed but there is a relative sparing of cortex compared to other regions [26, 41–43]. While the causes of the

initial hypoxia-ischemia are multifactorial, the insult triggers a delayed cascade of molecular events resulting in progressing brain injury over time [26]. This insult leads to rapid energy failure, followed by a transient period of normalization of function for several hours, and a later secondary energy failure that ultimately can result in cell death [23, 24]. Hypothermia, the current therapeutic modality to prevent or attenuate secondary energy failure must be initiated within the first 6 hours of life and continued for 72 hr [24, 43]. Many randomized clinical trials demonstrated that therapeutic hypothermia decreases mortality without increasing disability in surviving children [16, 44]. While many prospective and retrospective studies show that therapeutic hypothermia is safe, assessing the long term outcomes of this intervention (up to adulthood) is still ongoing. A recent report by Massaro et al., [45] shows that this intervention is now offered to children with mild form of HIE. .

Pediatric Traumatic Brain Injury

Trauma is the leading cause of death in children ages 1–18, and traumatic brain injury (TBI) is the major determinant of functional outcome in injured children (NIH 1998). In the United States each year, approximately ½ million children sustain TBI with ~35,000 hospitalizations, and ~3,000 pediatric deaths from brain trauma [46]. Over the last 10 years, the rates of TBI-related Emergency Department visits have increased across all age groups, with children 0–4 years of age having the highest rate of any age group, and adolescents 15–19 years of age having the second highest rate [46]. Overall, the vast majority of TBI occurs in children and young adults [46].

The pathobiology of the response to biomechanical injury in a developing brain is distinct from that in adult TBI, and may require age-specific treatments [47]. In addition, while post-injury recovery in the adult is defined by return to pre-morbid functional level, the developing brain is dynamic. Developing children are expected to be continuously learning new skills, making the burden of TBI even greater. As a result, survivors of pediatric TBI suffer from many long-term physical, cognitive, psychological and social impairments [17, 18], and many of these deficits can persist into adulthood [48–50].

Alterations in Brain Metabolism After Developmental Brain Injury

Acute injury including neonatal hypoxia-ischemia and traumatic brain injury leads to rapid energy failure which is followed by a transient normalization of function and then a later secondary energy failure that ultimately leads to brain injury and poor neurodevelopmental outcome [23, 24]. Acute alterations in energy metabolism and prolonged metabolic dysregulation after neonatal and pediatric brain injury leave the brain vulnerable and unable to support many processes essential for normal development [20–24, 51]. Any injury superimposed on the high metabolic requirements of the rapidly developing brain can compromise normal developmental processes and lead to neurodevelopmental outcomes ranging from mild to severe learning disabilities [20–24]. Very premature infants often have neurodevelopmental disabilities; however, metabolic alterations in brain of premature infants are not well characterized.

Importance of Proton Magnetic Resonance Spectroscopy in Assessing Brain Injury

Magnetic Resonance Imaging (MRI) and proton Magnetic Resonance Spectroscopy (^{1}H-MRS) have become essential tools for studying brain injury in infants, children and adults [17–19, 47, 48]. ^{1}H-MRS is a non-invasive method for measuring metabolites that provide information about the structural and metabolic integrity of brain tissue. Importantly, ^{1}H-MRS can detect localized metabolic abnormalities, even in tissue that appears anatomically normal on other forms of MR imaging [52, 53]. Some studies have determined the absolute concentration of metabolites. Numerous studies over the past two decades have demonstrated that ratios of metabolites provide diagnostically relevant information about the severity of injury and are predictive of neurological outcome [20–22, 51, 52, 54].

N-acetylaspartate (NAA) is an amino acid synthesized in neuronal mitochondria which increases throughout early brain development, reaching a plateau at ~ 2 to 3 years of age [55, 56]. Reductions in NAA reflect severe alterations in neuronal mitochondrial function [56] and may reflect loss of viable neurons [55, 56]. NAA is released by neurons and taken up by oligodendroglial cells which use the acetyl moiety as a substrate for the synthesis of myelin lipids [1]. NAA can be detected in areas of active myelination during development [57, 58]. The presence of lactate in brain reflects anaerobic metabolism including impaired TCA cycle activity [56–58]. Presence of lactate is one of the earliest markers of brain injury in infants [56] and persistently elevated lactate is predictive of poor outcome [23]. The creatine-phosphocreatine pathway is essential for buffering ATP levels in brain [1]. Although Cr levels are considered to be relatively constant, decreased total and/or phosphocreatine levels reflect impaired energy status. Choline levels (tCho or Cho) increase in developing brain and the peak observed include free choline and choline containing phospholipids which are essential components of cell membranes and myelin [59]. Decreased choline can reflect cell loss; whereas increased choline can be a maker of increased membrane turnover, cellular proliferation and/or gliosis after injury [56, 59]. Myo-inositol is a key glial osmolyte that can increase in brain in response to edema [59]. Inositol containing phospholipids have important roles in signaling pathways involved in differentiation and cell growth in brain [60, 61].

The interpretation of ^{1}H spectroscopy in pediatric brain injury is challenging, as metabolite levels can change throughout normal brain development [62], with rapid maturational changes in the first year of life and slower changes through adolescence [63–65]. Ultimately, post-traumatic changes in ^{1}H-MRS in infants and children must be interpreted by comparison to age-matched reference data [55]. Although alterations in metabolite ratios provide useful clinical information, they do not give an in depth understanding of the mechanisms underlying energy failure subsequent to HI.

^{13}C-Nuclear Magnetic Resonance Spectroscopy is Used to Determine Brain Metabolism

^{13}C-Nuclear Magnetic Resonance (^{13}C-NMR) spectroscopy is the most powerful technique for determining energy metabolism and neurotransmitter synthesis in brain [1, 66]. Specifically labeled forms of ^{13}C-glucose are used to determine metabolism via glycolysis, the pentose phosphate pathway, synthesis of neurotransmitters glutamate and GABA and metabolism via the pyruvate carboxylase pathway and synthesis of glutamine in astrocytes [66]. Alterations in neuron → astrocyte and astrocyte → neuron trafficking of metabolites can also be determined by ^{13}C-NMR since individual carbons of glutamate, glutamine and GABA are labeled from neuronal specific and glial specific pathways [66]. Since ^{13}C-acetate is selectively taken up into astrocytes this precursor is used to determine metabolism via the astrocyte TCA cycle and trafficking from astrocytes → neurons [66]. Studies using ex vivo ^{13}C-NMR have provided important information about alterations in specific pathways of metabolism in preclinical studies of brain injury [10, 66–69]. There are fewer in vivo studies and due to technical considerations including relatively low sensitivity, and due to the limitations using high field magnets there are a limited number of human studies. However, the greatly increased sensitivity of newer hyperpolarized ^{13}C-NMR techniques holds great promise for determining metabolism in human brain. ^{13}C-NMR can provide a functional readout of alterations in metabolism via neuronal and glial pathways in brain after injury and determine the efficacy of neuroprotective therapies.

Metabolic Alterations Associated with Prematurity

Clinical studies using magnetic resonance imaging (MRI) demonstrate significantly reduced gray matter volume (both cortex and subcortical structures), diffuse white matter injury, and abnormal development of temporal lobes and cerebellum in very premature infants [42, 70]. ^{1}H-MRS revealed decreased NAA levels in brain of very premature infants at term equivalent age and later time points [51, 71], consistent with changes observed in a recent animal study [72]. Preterm infants had lower GABA and glutamate in right frontal lobe than term controls and altered neonatal resting-state connectivity, (a measure of neural activity determined by assessing fluctuations in blood oxygen level dependent signal) [73, 74]. MRS showed increased lactate in areas of diffuse white matter injury in preterm infants and increased glutamine in punctate white matter lesions [75]. Bapat et al. [76] recently reported that decreased ratios of NAA/Cho in hippocampus, cortex and subventricular zone and decreased NAA/Myo-inositol ratio in subventricular zone in very low birth weight infants were associated with cognitive and language delay assessed at 18–22 months. Bluml et al. [30] reported altered metabolic maturation of white matter and disturbed synchronization of white matter and grey matter maturation that could contribute to neurological problems in preterm infants. These recent studies provide strong evidence of alterations in metabolism and connectivity, and asynchronous development in premature brain [30, 51, 71, 73, 76]. To date the metabolic alterations in brain of premature infants are not understood and remain poorly characterized. There is a paucity of preclinical data regarding specific alterations in transporter levels, substrate use, oxidative energy metabolism and antioxidant capacity (Table 2).

Metabolic Alterations in Neonatal Hypoxia-Ischemia

Magnetic Resonance Imaging (MRI) and proton Magnetic Resonance Spectroscopy (^{1}H-MRS) have become essential tools for studying brain injury in infants with hypoxia-ischemia [17–19, 72]. Such studies have provided important insights regarding brain changes after HI, and studies that also included neurological assessment at 12 months have demonstrated the power of ^{1}H-MRS data in predicting outcome [20–22]. A number of studies have reported that ratios of metabolites provide diagnostically relevant information and are predictive of neurological outcome [20–22]. A meta-analysis of prognostic accuracy of MR biomarkers and correlation with neurodevelopmental outcome at 1 year (32 studies; 860 infants) demonstrated that MR spectroscopy was a better diagnostic tool than conventional MRI [77, 78]. Ratios of Lac/choline in basal nuclei [20] and Lac/NAA accurately predicted adverse neurodevelopmental outcome at 1 year with high specificity and predictive value. [79]. Very early peaks in lactate can normalize; however, the persistence of high lactate in brain is associated with poor outcome [23, 56] (Table 2).

A recent high resolution ^{1}H-MRS provided new insights into injury and information about the differences in brain in animals treated with hypothermia and normothermia after HI [80]. Biomarkers related to the differential injury pattern were identified in CD1 mice with right carotid artery occlusion and HI at postnatal day (PND) 7, and identified metabolites that distinguished between brains less damaged and more damaged after HI [80]. Changes in malate and aspartate are particularly relevant to metabolism since they suggest impairment in the activity of the malate-aspartate shuttle, which is essential for transferring reducing equivalents from the cytosol to the mitochondria to be used for energy [80, 81].

Studies using ^{13}C-NMR spectroscopy are a powerful tool to determine overall energy metabolism, neurotransmitter synthesis, alterations in cell specific metabolic pathways and neuronal-glial interactions. Morken et al. [14] used ^{13}C-NMR to determine the differences in metabolism in normal neonatal and adult brain (Table 1). To date ^{13}C-NMR has not been used clinically in infants; however, it was recently used in animal studies to determine alterations in specific metabolic pathways following HI [82, 83]. Morken et al. [83] using the clinically relevant carotid artery ligation and hypoxia in PND 7 rat pup (Rice Vannucci model [84]) showed that following HI metabolism via the pentose phosphate pathway (PPP) was reduced bilaterally and metabolism via pyruvate carboxylase (PC) was reduced in the ipsilateral (hypoxic-ischemic) side of brain (Table 2). The PPP pathway is particularly high in developing brain to provide precursors for nucleotide formation, and the NADPH for lipid synthesis and for maintaining reduced glutathione [14]. Thus, a significant decrease in metabolism via this pathway can explain, in part, the decreased brain volume, white matter abnormalities and susceptibility to oxidative stress after HI.

Morken et al. [82] found that metabolism via the pyruvate carboxylase pathway in astrocytes was decreased after HI. This pathway has the essential role of adding net carbons to the TCA cycle (anaplerosis) and provides substrate in the form of glutamine to neurons to replenish carbons in the neuronal TCA cycle, since intermediates are continuously drained from the cycle for synthesis of glutamate and GABA [1, 85] Neonatal HI led to a prolonged depression in mitochondrial metabolism [82]. Mitochondrial metabolism was decreased in

the ipsilateral side at ≤6 hours post HI and was reduced in neurons but not in astrocytes in the contralateral side [82]. This study provided new insight into sex differences since male pups had lower astrocytic mitochondrial metabolism than females immediately after HI; whereas, mitochondrial metabolism was reduced longer in females and in both neurons and astrocytes [82]. (See Table 2 for details).

Metabolic Alterations After Pediatric Traumatic Brain Injury

A number of adult clinical studies have correlated the extent of metabolic change seen by ^{1}H-MRS with the severity of injury and neurologic outcome after TBI [52, 54]. After TBI, dramatic reductions in NAA are often observed and correlate with either neuronal cell loss and/or impaired neuronal energy metabolism [55]. Reductions in NAA or in NAA/Creatine and NAA/Choline ratios have been shown to correlate with greater injury severity and worse neurological outcomes after pediatric TBI [63, 86].

Lactate presence in remote (uninjured) areas of the occipital cortex after TBI in children is strongly associated with poor neurological outcome [86]. Multiple studies have reported the strong association between lactate peaks on ^{1}H-MRS and poor long-term neurological outcome (up to several years) after pediatric TBI [63, 86–88] (Table 2). More recently, this association has also been observed in pediatric victims of abusive head trauma, with infants that show elevated lactate on ^{1}H-MRS having worse early neurological outcome [89]. These investigators suggested that lactate presence is more common after non-accidental TBI in children than in accidental TBI [90, 91], and that the lactate peaks could represent either primary injury or secondary hypoxic-ischemic injury [89, 90]. Importantly, using a combination of ^{1}H-MRS metabolites may provide even greater predictive value in both accidental and non-accidental pediatric TBI. For example, in a series of 90 infants who had confirmed non-accidental TBI, a logistic regression model that used the combination of initial Glasgow Coma Score (GCS), presence of retinal hemorrhages, and lactate and NAA values on spectroscopy, was able to predict poor neurologic outcome in 100% of the cases [90]. In another study that included both accidental and non-accidental TBI, ^{1}H-MRS changes were more predictive of outcomes than clinical findings [88]. One of the few studies to compare ^{1}H-MRS metabolites with a detailed panel of intelligence and neuropsychologic testing found that the NAA/creatine and choline/creatine ratio, and presence of lactate were predictive of long-term cognitive outcome [88].

Preclinical studies using ^{1}H-MRS in TBI models offer several potential advantages, such as the ability to obtain data very early (<4h) after injury in order to define the time course of post-traumatic metabolic dysfunction. In clinical studies, the average time to obtain the first ^{1}H-MRS imaging is ~1 week, often due to the instability of children after moderate to severe TBI, limiting the ability to obtain lengthy MRI/MRS. Using ^{1}H-MRS in preclinical studies, with both early and serial imaging, will allow testing of neuroprotective strategies aimed at reducing cerebral energy failure after TBI. However, there are very few studies using this approach in pediatric TBI models. Casey et al. [92] used a model of focal TBI (controlled cortical impact, CCI) in immature rats to evaluate spectroscopic changes at 4h, 24h and 7d after injury. The ipsilateral (injured) hemisphere showed early (4h, 24h) increases in lactate/creatine ratios, and delayed (24h, 7d) decreases in NAA/creatine ratios,

with significant decreases in the NAA/lactate ratio at all times studied, in this model of developmental TBI (Table 2). The metabolic alterations occurred early (4 hrs) and prior to significant cell death demonstrating potentially reversible alterations of energy metabolism. Another study used both ^{1}H and phosphorus (^{31}P) NMR spectroscopy to evaluate neurochemical changes and test the effect of a ketogenic diet on brain metabolism after CCI in juvenile (PND 35) and adult rats [93]. The results showed that initial alterations in brain metabolism were seen earlier (6h) in adult rats than in juvenile rats (24h). Furthermore, the ketogenic diet significantly increased NAA and reduced lactate levels, and improved ATP, creatine and phosphocreatine levels at 24h after TBI in only in the juvenile rats. In a model of more diffuse injury (fluid percussion injury, FPI), investigators used ^{31}P MR spectroscopy at 4h after TBI in immature (PND 7, PND 14, PND 21) and adult rats [94]. In contrast to the finding in adult rats, in the immature rats, there were no significant changes in the ^{31}P MR spectra, which showed relatively normal pH, intracellular free magnesium (iMg) and phosphocreatine/inorganic phosphate (PCr/Pi) ratios at an early time point (4 hrs) after TBI. However, no studies to date have determined these metabolites at later time points after injury. In summary, although there are limited studies using ^{1}H-MRS in pediatric TBI animal models, the results show the translational potential for utilizing this technique to both define the timeline and degree of metabolic dysfunction after injury, and to evaluate the efficacy of novel neuroprotective strategies targeting metabolic rescue.

To date ^{13}C-NMR has been employed in limited number of preclinical studies of TBI and the majority of these studies were performed in adult animals [69]. Our group used a model of moderate-to-severe brain trauma (CCI) in PND 21–22 day old rats and assessed oxidative glucose metabolism at between 5 and 6 hrs after TBI. Oxidative glucose metabolism via pyruvate dehydrogenase was delayed in both injured and contralateral sides of the brain compared to sham animals, and there was delayed hypermetabolism of glutamate, glutamine and GABA in both sides of injured brain [68]; (Table 2). These findings significantly differ from ^{13}C-NMR in studies adult animals which did not find any differences in oxidative glucose metabolism in glutamatergic neurons in CCI-TBI [95]. Using a fluid percussion model of diffuse brain TBI, in adult rats Bartnik and colleagues [96] demonstrated decreased oxidative glucose metabolism in both astrocytes and neurons as early at 3.5 hrs post TBI. Both adult and immature brain TBI studies demonstrate that in general oxidative metabolism at 24hrs post TBI was comparable to sham operated animals [95–97]. However, the only differences at 24 hr post TBI found in the studies were age specific with decrease ^{13}C label incorporation into glutamine in adult brains, and decreased ^{13}C label incorporation into glutamate in immature brain. These differences may reflect differential cell-specific alterations in response to injury. Additional studies employing multiple ^{13}C-labled substrates will be particularly useful for further delineating alterations in neuronal and glial metabolic pathways after TBI in developing brain

Alternative Substrates and Metabolic Modifiers in Brain Injury Models

A number of studies have tested the potential of alternative energy substrates and modulators of metabolism for preventing injury and improving metabolic and functional outcomes. Compounds that improve brain function and/or decrease injury volume when administered after injury are the logical therapeutic approaches. Treatment after injury with creatine

monohydrate [98], acetyl-L-carnitine [99], or ketogenic diets [100] have led to improved locomotor and/or cognitive function in young rodents after brain injury (See Table 3). Compounds that can be metabolized for energy in brain, including ketones [93, 101], acetyl-L-carnitine [102], and triheptanoin [103] can lead to improved energy status. Therapy with branched chain amino acids (BCAA) after fluid percussion TBI improved synaptic efficiency and cognitive performance in adult mice presumably by normalizing brain levels of these amino acids which have a role in glutamate metabolism [104]. BCAA therapy as well as triheptanoin which provides anaplerotic substrates to brain and can decreased seizure activity [103, 105] also appear to be potentially useful treatments for pediatric brain injury. A recent study [148] using an electron scavenger specifically targeted to neuronal mitochondrial (XJB-5-131-- a hemigramicidin-nitroxide) demonstrated that this compound prevented mitochondrial cardiolipin oxidation and caspase activation. This therapy decreased neuronal death, behavioral deficits and cortical lesion volume in PND 17 rats after CCI-TBI.

Conclusion

Clinical studies using ^{1}H-MRS in infants and children have greatly increased the knowledge of temporal and regional changes in brain after injury and the relationship of energy failure to neurologic outcome. Advances in imaging techniques are leading to a better understanding of specific alterations that occur subsequent to prematurity, neonatal and childhood injury including alterations in resting state and brain connectivity. Animal studies have led to an increased understanding of the mechanisms leading to both acute and ongoing injury in developing brain. These include alterations in metabolism via neuronal and glial specific pathways in brain, changes in the expression, level and activity of key proteins involved in development, metabolism and signaling pathways, and the extent of oxidative stress in specific brain injury models. However, there are still many gaps in knowledge including a lack of sufficiently detailed information about temporal alterations in brain energetics and metabolism, the effects of age at time of injury (prenatal, neonatal, during synaptogenesis and rapid myelination or at the peak of myelination), and the effects of diet before, during and after injury.

Our understanding of energy metabolism in developing brain stems from studies of temporal changes in transporter levels and function, enzymatic activity, and changes in cell populations and mitochondrial function.. In vivo and ex vivo PET and ^{13}C-NMR spectroscopy have greatly increased our knowledge of regional changes during development and metabolism in developing brain. To date there are relatively few ^{13}C-NMR studies of metabolism via cell specific pathways, neurotransmitter synthesis, and neuron $\rightarrow$ astrocyte and astrocyte $\rightarrow$ neuron metabolic trafficking in developing brain. Many newer tools including genetically modified knock-out, knock-in and inducible Cre-mice, BOLD and resting state MRI, proteomic and lipidomic techniques and imaging including matrix-assisted laser desorption/ionization-mass spectrometry imaging (MALDI-MSI) to determine regional alterations in metabolites have great potential to increase the understanding of the normal developmental changes in brain metabolism, alterations resulting from injury and effects of therapies.

Future Directions

Additional clinical studies using ^{1}H-MRS and advanced neuroimaging techniques will lead to increased understanding of the regional, temporal, and functional changes resulting from prematurity, neonatal hypoxia-ischemia and traumatic brain injury in infants and children. Clinical studies that determine brain changes in conjunction with long-term neurological outcome are particularly important for identifying biomarkers associated with functional outcome. The recent identification of metabolites in plasma, including fumaric acid and propanoic acid, that correlate with, and are potential biomarkers for long term functional outcome in a nonhuman primate model of neonatal HIE is very promising [159]. Clinical studies using conventional ^{13}C-NMR spectroscopy or hyperpolarized ^{13}C-NMR would provide much needed information about alterations in metabolic pathways of specific importance to developing brain and the extent of alterations in astrocytes, neurons and the trafficking of metabolites between these cells. Additional preclinical studies using animal models are essential for increasing the understanding of mechanisms of injury and efficacy of neuroprotective therapies.

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Abbreviations used

BBB	blood-brain barrier
BHB	β-hydroxybutyrate
BHBdehase	β-hydroxybutyrate dehydrogenase
CCI	controlled cortical impact traumatic brain injury
Cho or tCho	total choline
Cr or tCr	total creatine
Gln	glutamine
Glu	glutamate
Glx	glutamate and glutamine
GPC	glycerophosphocholine
HI	hypoxia-ischemia
HIE	hypoxic-ischemic encephalopathy

^{1}H-MRS	proton magnetic resonance spectroscopy
Lac	lactate
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
NAA	N-acetylaspartate
PC	pyruvate carboxylase
PDH	pyruvate dehydrogenase
PND	postnatal day
PPP	pentose phosphate pathway
TBI	traumatic brain injury

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Table 1

Metabolism of substrates in developing versus adult brain

Substrate	Developing brain compared to adult brain	References
Glucose	Overall metabolism/substrate use:	
	• Glucose oxidation developing ≪ adult brain	[106–108]
	• Glucose uptake can account for 50% or less of cerebral oxygen consumption in the newborn period. (newborn Baboons -- in the first 50 hr of life)	[109]
	• 12 infants (age 11 days–12 months) uptake ~ 1.04 ml/min/mg brain rain tissue in nine anesthetized human infants.	[110]
	Transport:	
	• Glucose uptake developing < adult brain	[111]
	• GLUT 3 developing ≪ adult brain	[9]
	• GLUT1 developing < adult brain	[9]
	Enzymes:	
	• Pyruvate dehydrogenase (PDH), citrate synthase, and NAD^+ -linked isocitrate dehydrogenase (ICDH) increase rapidly ~ 4–5-fold in cortex from birth to PND 20 with adult values reached between 20–40 days; varies increase in fumarase was ~ 3-fold; increases varied by enzyme & brain region	[106]
	• KGDHC activity increases 3–4-fold in brain regions between PND 2–4 and PND 30	[11]
	• Pentose phosphate pathway (PPP) enzyme glucose 6-phosphate dehydrogenase (reports vary); Cortex: highest at PND5 declined gradually to adult levels at PND 60;	[15]
	• Glucose 6-phosphate dehydrogenase Cortex: PND 5 & PND 10 same and ~ 25% increase at PND 21	[112]
	• GPDH activity in female > male brain at PND 5, 20 and 60	[15]
	• $NADP^+$-isocitrate dehydrogenase high at birth and decline ~ 50% by PND 30	[113]
	• Citrate synthase increases ~ fourfold in the first four weeks after birth and remains high in adult brain	[10]
	• 6-phosphogluconate dehydrogenase; Cortex increase ~ 30% between PND5 to peak at PND 10 – 21; decreases ~30% by PND 90; increase in the first few weeks after birth and subsequently decline	[112]
	• Overall PPP activity developing brain > adult	[10, 14]
	Metabolism – specific pathways and cells:	
	• Glucose oxidation developing ≪ adult brain (PND 7 rat versus 250 g rats) ^{13}C-glucose metabolism	[14]
	• Glucose → Acetyl CoA developing brain < adult	[14]
	• Increase in TCA cycle flux from PND which closely parallels increase in KGDHC activity	[12]
	• Rates of TCA cycle flux and neurotransmitter cycling increase at least 3-fold from postnatal day 10 to 30	[13]
	• Metabolism via pyruvate carboxylase (PC) developing < adult brain	[14]
	• proportion of glucose metabolism to anaplerosis/PDH PND 7 > adult	[14]
	• Ratio of PPP/total glucose metabolism higher in developing brain than adult	[14]

Substrate	Developing brain compared to adult brain	References
	• More glucose prioritized to PPP & PC in developing versus adult brain	[14]
	• Glutamate transfer from Neurons → Astrocytes P 7 < adult brain	[14]
	• Glutamine transfer from Astrocytes → Glutamatergic Neurons p7 > adult	[14]
	• Glutamine transfer from Astrocytes → GABAergic Neurons p7 < adult	[14]
	• Glucose oxidation in synaptosomes PND 17–18 ~ 66% of adult rate	[114]
Ketones	Overall metabolism/substrate use:	
	• Developing > adult brain	[9, 115, 116]
	• Ketone body metabolism supported ~ 48 to 76% of the energy in PND 16–28 rat brain	[115]
	Transport:	
	• MCT1 developing > adult brain	[9]
	• High uptake of BHB into brain in suckling rats	[115, 117, 118]
	• Human infants – uptake of ketone bodies > than adult values reported	[2]
	Enzymes:	
	• Beta hydroxybutyrate dehydrogenase (BHBdehase) enzyme activity in cortex increases 5 fold from birth to PND 25; activity is 3–4-fold higher at PND 15 > mature brain	[108, 115, 119]
	• CoA transferase increase 4–5-fold from birth to PND 20; decreases 4-fold in adult	[108] [120]
	• BHBdehase enzyme activity developing brain > adult brain; similar pattern in most regions	[106]
	Metabolism:	[120–122]
	• 3-hydroxybutyrate is a preferred substrate for sterol synthesis in developing brain	[119]
	• Oxidation of BHB was higher in synaptosomes from PND 17–18 > adult rat brain	[114, 123]
	• Ketone bodies are incorporated into amino acids in developing brain	[124]
	• Loss of activity in the ketone body metabolizing enzyme CoA transferase lead to developmental disability in human infants	[125]
	• Metabolism of [1,2-^{13}C]acetate developing brain < adults	[14]
	• Infused BHB accounted for 62% of neuronal oxidation in awake young adult rats	[126]
Lactate	Transport:	
	• Cerebral uptake of lactate was observed at birth in newborn Baboons	[109]
	Overall metabolism/substrate use:	
	• 70% of energy was from lactate oxidation in p6 rat brain	[107]
	• synaptosomal lactate oxidation at p17–18 was 20% > adults	[114]
Fatty acids	Overall metabolism/substrate use:	

Substrate	Developing brain compared to adult brain	References
	• Developing brain ≫ adult brain	[127]
	Transport:	
	• Palmitate uptake into most brain regions increase ~ 2-fold between PND 15-PND 20 in gray and white matter regions; uptake corresponds to the time course of myelination	[128]
	• Palmitate uptake declined 4–5-fold in gray matter and 7–10-fold in white matter by PND 38 days and reached adult levels by 3 months.	[128]
	Metabolism:	
	• The white/gray ratio for palmitate uptake declined significantly between 20 > 38 days & 38> 90 days of age	[128]
	• Palmitate is incorporated into proteins, lipids including phospholipids, neutral lipids and fatty acids, and gangliosides, and amino acids and TCA cycle intermediates	[127]
	• High rate of synthesis of saturated and monounsaturated fatty acids, such as palmitate, in the PND 6-PND14 developing brain (not taken up intact)	[127]
	• Synthesis of longer chain PUFA is from dietary precursors 18:2ω6 and 18:3ω3	[129]
	• ^{13}C-Octanoate accounted for 20% of total brain oxidative energy production in normal adult rat brain; metabolized in astrocytes > neurons	[130]
	• Whole body fatty acid oxidation high in PND 21 rat pup; oleic acid had highest oxidation rate (the major FA in human milk)	[131, 132]
Amino acids	Transport:	
	• Significant cerebral uptakes for histidine and arginine in infants	[2]
	• Amino acids taken up across BBB into developing brain and use in PND 15rat ~ = adult	[133] [118]

Table 2

Metabolic alterations after injury to developing brain

Injury Type	Clinical or Models	Metabolic alterations	References
Premature brain injury	**Clinical**	**^{1}H spectra MRS and NMR data**	
		• preterm infants with birth weight 500–1500g compared to term neonates born at 37–41 weeks of gestation; decreased concentrations of GABA and glutamate in the frontal cortex in preterm neonates compared to term	[73]
		• preterm infants born at 25 weeks of gestation had decreased NAA/Cho in subventricular zone, frontal right cortex and right hippocampus;	[76]
		• cerebellar volume was decreased in preterm infants born at 25–31 weeks of gestation; decreased NAA/Cho in cerebellum;	[134]
	Models		
	Chronic hypoxia	• white matter NAA was decreased at PND 18 and PND 30 mice following chronic hypoxia from PND 3-PND 11;	[72]
	PND 3–11 mice	• PND10 had increased activity of LDH, no difference was observed in the activity of citrate synthase, alpha-ketoglutarate dehydrogenase; many enzymes altered at PND 30	[135]
	Carotid artery ligation PND 5 mice	• Ventriculomegaly, thinning of the corpus collosum, dereased myelin basic protein on the side of ligation,	[136]
Neonatal Hypoxia Ischemia	**Clinical**	**^{1}H spectra and MRS and NMR data**	
		• Newborn infants with HI: increased cerebral Lac is an early indication of clinical brain injury	[56, 137]
		• Persistence of high Lac is associated with poor outcome	[23, 138]
		• Perinatal HI with severe or fatal outcomes had increased Lac, decreased total Cho (tCho) and n-acetylaspartate (NAA)	[23]
		^{1}H-MRS results and & neurological outcome at 6 months or 1 year:	
		• Meta-analysis of prognostic accuracy of MR biomarkers and correlation with neurodevelopmental outcome at 1 year (32 studies; 860 infants); Lac/NAA in deep gray matter had an 82% overall pooled sensitivity and 95% specificity and was a better diagnostic tool than conventional MRI. Lac/NAA was most accurate quantitative MR biomarker for prediction of neurological outcome.	[77]
		• Poor outcome associated with presence of Lac, lower NAA/Cr & lower NAA/Cho	[63,139]
		• Increased Lac and decreased NAA most common findings in infants with neurological abnormalities at 12 months; highest significance was Lac/choline in basal nuclei. (Mental Development Index of the Bayley Scores of Infant Development at 12 months)	[22]
		• Maximal cerebral peak area of Lac/NAA accurately predicted adverse outcome at 1 year	[79]
		• Increased Lac and decreased Cr in HIE; mild lesions, Lac/Cr < 0.5, mild to moderate lesion, Lac/Cr 0.5–1.5; severe lesions, Lac/Cr >1.5	[140]
		• MRS metabolite ratio of Lac/NAA deep grey matter best predictor of adverse outcome	[21]
		• Changes in Lac, NAA & Cho in HIE; highest Cho and Lac levels in gray matter differentiated non-survivors from survivors	[141]

Injury Type	Clinical or Models	Metabolic alterations	References
		• Lac/Cr correlated with clinical grading in moderate & severe HIE	[142]
		• Myo-inositol/Cr and Lac/Cr were higher in infants with abnormal MRI and poor outcome	[143]
		• Glx in basal ganglia elevated in severe HIE; Glx/NAA and Glx/Cho ratios higher in severe outcome than in mild-moderate group	[144]
	Models		
	Piglet	• Piglet model of HI, increases in Lac/NAA, Lac/tCho and Lac/tCr were observed up to 7 days after injury	[145]
	PND 7 rat pup	• PND 7 rat pup; Lac concentration in ipsilateral hemispheres was high and remained high during the first 48 h after injury; NAA in ipsilateral side decreased to 55 ± 14% during hypoxia, recovered during early post hypoxic period, and decreased to 61 ± 25% and 41 ± 28% at 24 and 48 h post HI respectively	[146]
	PND 7 rat pup	• PND 7 rat pup (75 min HI) significant reductions in reduced glutathione (GSH), myo-inositol, taurine, and total creatine (tCr) in the ipsilateral hippocampus at 24 hr post HI; no significant changes in cortex of HI pups compared with the controls	[147]
	PND 7 mouse	• PND 7 mouse pups (30 min HI); alterations in formate, acetate, NAAG, fumarate, succinate, glutamine, isoleucine, histidine, malate, ascorbate and taurine, differed after hypothermic vs normothermic recovery	[80]
		^{13}C-Glucose and acetate metabolism – after H/I in PND 7 rat brain	
	PND 7 rat pup	• Metabolism via pentose phosphate pathway (PPP) was reduced bilaterally after HI; metabolism via Pyruvate carboxylase (PC) reduced in ipsilateral side	[83]
		• Mitochondrial metabolism decreased in ipsilateral side ≤6 hours post HI	[82]
		• mitochondrial metabolism reduced in neurons but not in astrocytes in contralateral side	[82]
		• Glutamate transfer from Neurons → Astrocytes increased in the contralateral, but not in ipsilateral side at 0 hour; reduced in both sides at 6 hours after HI.	[82]
		• Glutamine transfer from Astrocytes → Glutamatergic neurons was not altered in both hemispheres	[82]
		• Glutamine transfer from Astrocytes → GABAergic neurons was increased in ipsilateral side immediately after H/I	[82]
		• Metabolism via PC in astrocytes (anaplerosis) decreased after H/I	[82]
		• Partial pyruvate recycling pathway was increased directly after HI.	[82]
		• Male pups had lower astrocytic mitochondrial metabolism than females immediately after HI,	[10] [82]
		• Mitochondrial metabolism was reduced longer in females and in both neurons and astrocytes.	[82]
Traumatic brain injury (TBI)	**Clinical**	**^{1}H-MRS**	
		• Diffuse axonal injury is characterized by decreased NAA/Cr and increased Cho/Cr; NAA/Cr was decreased more in children with poor outcome.	[53]
		• Increased in glutamate following TBI regardless of outcome	[149]
		• Myo-inositol increased after TBI in children and elevated levels correlate to poor neurological outcomes;	[150]
	Models	• **Controlled cortical impact (CCI)**	

Injury Type	Clinical or Models	Metabolic alterations	References
		^{1}H spectroscopy in cortex and injured hippocampus –	
	PND 16–17 rats	• increased Lac/Cr (4 hrs), decreased NAA/Cr at 24 hrs and 7days;	[92]
		^{13}C-Glucose metabolism	
	PND 16–17 rats	• 24 hr after TBI, mitochondrial metabolism was comparable to shams	[97]
		• Decreased neuronal metabolism and/or glutaminase – decreased label in Glutamate	[97]
	PND 21–22 rats	• 6 hr post TBI, delayed mitochondrial metabolism via PDH and PC with later increase labeling in ipsilateral and contralateral hemispheres	[68]
		• Glutamate transfer from Neurons → Astrocytes is delayed and later increased in the both sides of injured brain compared to sham	[68]
		• Glutamine transfer from Astrocytes → Glutamatergic neurons is altered in both hemispheres	[68]
	PND 17 rats	• Proteomics analyses – 2 weeks post TBI decreased expression of mitochondrial proteins – voltage-dependent anion channel 1 and 2; pyruvate dehydrogenase subunits E1alpha and beta	[151]
		• Pyruvate dehydrogenase activity and mitochondrial respiratory control ratio were decreased at 4 hr post TBI	[152]
	PND 17 rats	• Lipid metabolism – increased oxidation of cardiolipin promoting apoptosis	[153]
	PND 35 rats	• Increased MCT2 expression in cerebral vasculature at 24hrs after TBI	[154]
		• Decreased ATP, Total Creatine and Creatine at 24 hr after TBI	[93]
	PND 21 mice	• Glutathione Peroxidase did not change at 24 hr after TBI	[155]

Table 3

Effects of alternative substrates and metabolic modifiers in brain injury models

Substrates	Experimental paradigm	Alterations	Refs.
Ketones	TBI – adult rats	3hr continuous infusion of BHB alleviated decrease in ATP	[101]
Ketogenic diet	TBI - PND 35 rat	• Improved ATP, creatine, phosphocreatine, total creatine;	[93]
		• Improved behavioral outcome (beam walking; Morris water maze performance)	[160]
		• Decreased contusion volume	[100]
Carnitine and acetyl-L-carnitine	Carnitine PND 7 HI rat	• Carnitine treatment during HI decreased injury volume and number of dead cells; post treatment had no effect	[156]
		• HI led to abnormal ratio of acyl-CoA:CoA; pretreatment with carnitine prevented changes in acyl-CoA:CoA, prevented increases in glutamate, glycine, superoxide, prevented loss of cardiolipin	[157]
	Acetyl-L-carnitine PND 21 rats	• Treatment with acetyl-L-carnitine after TBI decreased lesion size and improved beam walking performance and novel object recognition tests	[99]
	PND 21 normal rats	• ^{13}C-acetyl-L-carnitine was metabolized primarily in GABAgeric neurons and astrocytes in immature brain	[102]
Creatine	HI in PND 10 mice	• Supplementing diet at weaning with 1% or 3% creatine monohydrate decreased infarct size, improved locomotor and exploratory behavior and spatial memory	[98]
Branched chain amino acids (BCAA)	Fluid percussion trauma adult mice	• TBI reduced BCAA content in brain, decreased synaptic efficiency (EPSP) and impaired cognition; treatment with branched chain amino acids (BCAA) normalized synaptic efficiency and improved cognition	[104]
Triheptanoin/trioctanoin	Epilepsy adult mice	• Triheptanoin partially restored TCA cycle metabolites	[103]
		• Triheptanoin increased anaplerotic metabolites	[105]
N-3 fatty acid enriched triglyceride emulsions	PND 10 mice after HI	• N-3 fatty acid enriched triglyceride emulsions administered 0–2 hr after HI decreased lesion size	[158]
		• Tri-DHA (docosahexaenoic acid) was protective; tri-EPA (eicosapentanoeic acid) was not effective	
XJB-5-131 (electron scavenger targeted to neuronal mitochondria)	TBI – PND 17 rats	• Prevented cardiolipin oxidation and caspase activation;	[161]
		• Improved mitochondrial function, decreased neuronal cell death, behavioral deficits and cortical lesion volume	