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Review Article

Cerebral metabolic adaptation and ketone metabolism after brain injury

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The developing central nervous system has the capacity to metabolize ketone bodies. It was once accepted that on weaning, the 'post-weaned/adult' brain was limited solely to glucose metabolism. However, increasing evidence from conditions of inadequate glucose availability or increased energy demands has shown that the adult brain is not static in its fuel options. The objective of this review is to summarize the body of literature specifically regarding cerebral ketone metabolism at different ages, under conditions of starvation and after various pathologic conditions. The evidence presented supports the following findings: (1) there is an inverse relationship between age and the brain's capacity for ketone metabolism that continues well after weaning; (2) neuroprotective potentials of ketone administration have been shown for neurodegenerative conditions, epilepsy, hypoxia/ischemia, and traumatic brain injury; and (3) there is an age-related therapeutic potential for ketone as an alternative substrate. The concept of cerebral metabolic adaptation under various physiologic and pathologic conditions is not new, but it has taken the contribution of numerous studies over many years to break the previously accepted dogma of cerebral metabolism. Our emerging understanding of cerebral metabolism is far more complex than could have been imagined. It is clear that in addition to glucose, other substrates must be considered along with fuel interactions, metabolic challenges, and cerebral maturation.
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Introduction

Although glucose remains the primary cerebral metabolic substrate for adults under normal conditions, there are many physiologic and pathologic conditions that increase the availability of ketones and cerebral ketone metabolism (Owen *et al*, 1967; Hawkins *et al*, 1971; Dahlquist and Persson, 1976; Kreis and Ross, 1992; Vannucci and Vannucci, 2000). Ketone bodies (β-hydroxybutyrate, β-OHB and acetoacetate, AcAc, Figure 1) are the only endogenously circulating substrates that have been shown to contribute significantly to cerebral metabolism (Owen *et al*, 1967; Hawkins *et al*, 1971; Dahlquist and Persson, 1976). The brain's ability to increase its reliance on ketone bodies appears to be a form of *cerebral metabolic adaptation* (McIlwain, 1970).

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Ketogenic Cerebral Metabolic Adaptation and Cerebral Development

Ketone Metabolism

During development the brain 'switches' metabolic fuels from reliance on glucose *in utero*, lactate shortly after birth (Hellmann *et al*, 1982; Medina, 1985), to a combination of glucose and ketones during suckling, to primary reliance on glucose after weaning (Nehlig and de Vasconcelos, 1993). These changes in cerebral fuel use are accompanied by changes in circulating levels of substrates, cerebral transport of substrates, and metabolic enzyme activity for each substrate (Figure 2) and have been well characterized in the developing rodent brain.

The fetal brain relies on the maternal nutritional state, where under normal conditions glucose serves as the primary cerebral fuel (Guerra *et al*, 1967). Plasma glucose levels and metabolism in the fetus are relatively low compared with adult (Shambaugh *et al*, 1977), but glucose transporter 1 (GLUT1) transporter expression is comparable (Vannucci and Simpson, 2003; Figure 2). Although glycolytic activity is 38% greater in adults than fetal brain, the processing of glucose through the pentose

phosphate pathway was 164% higher within the fetal brain compared with adults (Guerra *et al*, 1967). Toward the end of gestation, the brain expression of ketone-metabolizing enzymes increases (Shambaugh *et al*, 1977) and plasma glucose decreases 49% with increase in β-OHB to 5.37 mmol/L, due to late gestation maternal fasting. *In vitro* experiments with graded concentrations of ^{14}C-β-OHB showed linear increase in $^{14}CO_2$, suggesting substrate availability is the limiting step (Shambaugh *et al*, 1977).

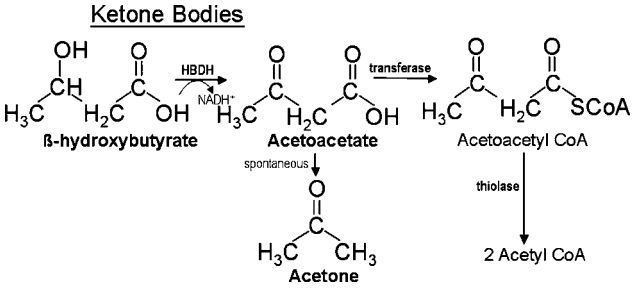

Figure 1 The chemical structures of the three ketone bodies: acetone, acetoacetate, and β-OHB. There are three enzymatic steps involved in processing β-OHB to TCA cycle entry as acetyl-CoA. D-3-Hydroxybutyrate dehydrogenase (HBDH) converts β-OHB into acetoacetate, which can spontaneously be converted into acetone. 3-Ketoacyl-CoA transferase converts acetoacetate into acetoacetyl-CoA. Acetoacetyl-CoA thiolase is the enzyme that converts acetoacetyl-CoA into acetyl-CoA, which can then enter the TCA cycle.

Immediately after birth and before suckling, the newborn depends on its own glycogen stores to provide glucose. Lactate is also metabolized during this time (Hellmann *et al*, 1982; Medina, 1985). Once suckling begins, circulating concentrations of ketones quickly increase to 0.3 to 1.5 mmol/L and metabolism of β-OHB becomes significant (Nehlig *et al*, 1991; Figure 2B). During this metabolic stage, there are a large number of monocarboxylate transporters (MCTs), which transport ketones, pyruvate, and lactate (Vannucci and Simpson, 2003). At the peak of ketone utilization, the brain's capacity to take up β-OHB is six times higher than the adult brain, as is the rate of ketone metabolism within the frontoparietal cortex (Cremer *et al*, 1976; Hawkins *et al*, 1971; Nehlig *et al*, 1991). The activities of the three enzymes involved in processing β-OHB into the Krebs cycle are two- to threefold higher during the suckling period (Booth *et al*, 1980; Leino *et al*, 1999; Page *et al*, 1971).

In addition to maturational differences in total cerebral ketone metabolism there are age-related *regional* differences in ketone metabolism. Unlike glucose metabolism, which can be quantitatively determined using ^{14}C-2-deoxy-D-glucose, no equivalent non-metabolizable analog exists for ketones.

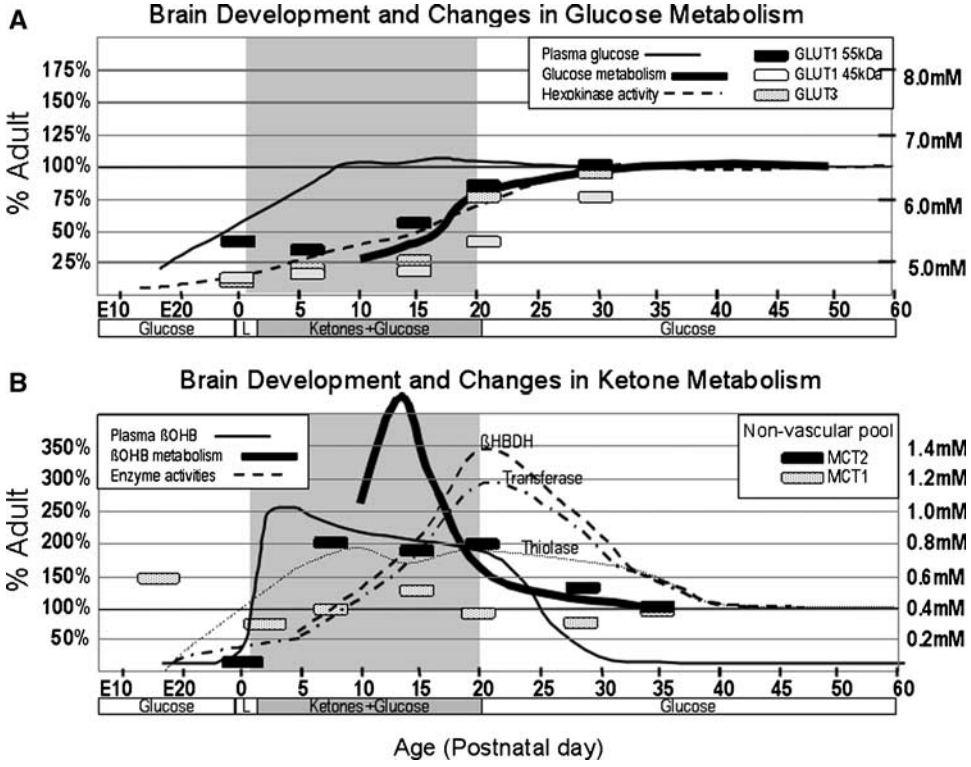

Figure 2 Changes in arterial concentrations, cerebral enzyme activities, and cerebral transporters for (**A**) glucose and (**B**) ketone metabolism with postnatal age. The shaded area of each graph represents the suckling period. Changes in arterial concentrations (solid lines) are indicated by the mmol/L values on the *y* axis (right). Enzymatic activities (broken lines) and transporter density changes (bars) are expressed as percentage of adult (Leong and Clark, 1984; Lockwood and Bailey, 1971; Nehlig *et al*, 1987, 1991; Page *et al*, 1971; Shambaugh *et al*, 1977; Vannucci, 1994; Vannucci and Simpson, 2003).

However, by using short ^{14}C-β-OHB infusions, autoradiographic images of cerebral uptake and metabolism of ketones can be acquired and analyzed qualitatively (Nehlig *et al*, 1991). In the suckling rat (postnatal day, PND18), the calculated rate of ketone metabolism is 0.3 μmol/g/min with ketones accounting for at least 30% of the total energy metabolism (Cremer and Heath, 1974). During cerebral maturation, autoradiographic images revealed homogeneous ketone metabolism, which reflect the global role of ketones for amino acid and lipid biosynthesis (Nehlig *et al*, 1991). However, by PND35 Nehlig reported the emergence of a regional pattern of ketone metabolism that was consistent with the pattern observed previously in adults (Hawkins and Biebuyck, 1979). Images of *glucose* metabolism show pronounced uptake in the cortex, with the highest metabolism in the superficial layers. In contrast, Hawkins and Biebuyck (1979) observed the greatest *ketone* uptake in the inner cortical layers, gradually decreasing toward the superficial layers. The authors concluded that ketone metabolism was limited to those specific regions by permeability and could therefore not completely substitute, but could supplement glucose metabolism.

Along with changes in regional uptake of ketones, there are maturational differences in the metabolic fates of ketones and cell-type preference. Oxidation of β-OHB to CO_2 decreases with cerebral maturation from 0.45 μmol/g per 2 h at PND18 to 0.25 μmol/g per2 h in adulthood, whereas glucose oxidation increases from 0.35 to 0.40 μmol/g per 2 h (Yeh *et al*, 1977). Synthesis of lipids from β-OHB and glucose decreases with development from 1.1 to 0.85 μmol/g per 2 h and 0.1 to 0.08 μmol/g per 2 h (Yeh *et al*, 1977; Patel and Owen 1977). Synthesis of glutamate, glutamine, and γ-aminobutyric acid from β-OHB was generally higher from birth to PND15, after which labeled glucose appeared more in these amino acids (De Vivo *et al*, 1975).

What A greater understanding of cell-type preference for ketone metabolism in relation to biosynthesis and energy production remains unclear. Data from ^{13}C-NMR studies using labeled ketones and glucose have demonstrated a glial and neuronal compartment of metabolism (Cruz and Cerdan, 1999). Upon ^{13}C-glucose infusion, 96% of neuronal and 52% of glial acetyl-CoA are labeled. Infusion with [l^{13}C]β-OHB shows 72% of acetyl-CoA in glia and only 48% in neurons labeled. Infusion with [1,2-$^{13}C_2$] acetate shows isotopomer labeling of glutamate, glutamine, and γ-aminobutyric acid that reflect metabolism through a pool characterized by glutamine synthase activity (i.e., the glial pool) (Cerdan *et al*, 1990). Acetate was found to enter almost exclusively the glial compartment (Waniewski and Martin, 1998; Kunnecke *et al*, 1993). The existence of compartmental differences in ketone metabolism with age remains unexplored at this time.

Glucose Metabolism

In contrast to the sharp developmental changes in ketone metabolism, changes in glucose metabolism with development are analogous to a slow crescendo (Figure 2A). The increase in plasma glucose (Vannucci and Simpson, 2003) precedes the increase in GLUTs. Glucose transporters 1 and 3 are the primary isoforms in the brain. The more heavily glycolsylated GLUT1 isoform (55 kDa) is found predominantly in the microvascular endothelium, whereas the less glycosylated isoform GLUT1 (45 kDa) is found in astrocytes (Maher, 1995). Glucose transporter 3 is the primary neuronal glucose transporter (Maher *et al*, 1992). Both hexokinase activity (Leong and Clark, 1984) and rates of glucose metabolism (Nehlig *et al*, 1987) also increase after glucose level increases in the plasma.

The time course of changes associated with transporter expression, metabolizing enzymes, and metabolic rates of a given substrate suggest that the developing brain is adapting to the changes in its metabolic environment, energy requirements, and hormonal signals (Nehlig and de Vasconcelos, 1993). Although not everyone would agree that developmental cerebral metabolism is a model of cerebral metabolic adaptation, consider the following experimental manipulations. If metabolic changes observed during development were 'adaptive' in response to alterations in substrate supply, then prolonging postnatal ketosis should result in prolonged elevations of ketone transporter expression, metabolizing enzymes, or metabolism. In fact, when dams are maintained on a high-fat diet during suckling, the postnatal ketosis is maintained above 1.5 mmol/L past PND35. At this same age, there is no difference in the hexokinase activity, but β-OHB dehydrogenase levels are 1.9 times greater than normal-fed age-matched controls (Sherman and Wilson, 1978). Similarly, maintenance of ketosis after weaning resulted in 40% increase in ^{3}H-β-OHB incorporation into brain lipids compared with age-matched normally weaned rats (Crane and Morgan, 1983). However, it has also been shown that high plasma ketone levels are not required for normal development of 3-oxoacid CoA transferase activity in the brain (Haney and Patel, 1985). This suggests a more hard-wired model to the time course of cerebral metabolic changes during development, one that is not entirely dependent on circulating substrate levels.

Ketogenic Cerebral Metabolic Adaptation and the Mature Brain

Changes in glucose availability through starvation or administration of the ketogenic diet have provided the earliest evidence of cerebral metabolic adaptation in the mature brain. Cerebral shifting to ketone metabolism requires (1) increasing the availability of

ketones, (2) increasing cerebral uptake of ketones, and (3) potentially increasing the activity of the necessary enzymes for ketone metabolism (Owen *et al*, 1967; Hasselbalch *et al*, 1995; Hawkins *et al*, 1971; Dahlquist and Persson, 1976). Each of these steps is addressed in greater detail below.

Plasma Levels

In normally fed adult mammals, β-OHB metabolism comprises $<3\%$ of total cerebral metabolism and is present in low circulating concentrations (0.1 mmol/L) with negligible uptake into the brain (Hawkins *et al*, 1971). However, plasma ketone levels can be increased four- to fivefold within 2 days via ketogenesis associated with starvation or administration of a ketogenic diet. Upon increasing β-OHB arterial concentrations, cerebral uptake is significantly enhanced by 4.9-fold in PND20 rats and 1.5-fold in adult rats (Dahlquist and Persson 1976; Hawkins *et al*, 1971). In contrast to the magnitude of ketone metabolism in the rodent brain, the human brain shows a significantly greater capacity to metabolize ketones. A 13-fold increase in cerebral uptake of β-OHB (not AcAc) was reported in adult humans after 3.5 days starvation, which accounts for approximately 35% of cerebral energy production (Hasselbalch *et al*, 1995).

Transporters

Non-vascular MCT1 and neuronal MCT2 expression remains constant throughout development (Vannucci and Simpson, 2003) and probably reflects their role in intercellular transport of lactate between neurons and astrocytes (Pellerin *et al*, 1998). In contrast, microvascular MCT1 decreases with cerebral maturation and is present at low levels in adulthood (Vannucci and Simpson, 2003). However, the rapid cerebral uptake of ketones upon availability reflects the concentration-dependent nature of the MCT transporter (Hawkins *et al*, 1971). This property allows for rapid 'switching' between fuels or combinations of fuels depending on their availability. In addition to the concentration-dependent uptake properties of the transporters, there is evidence of substrate-induced adaptive changes in MCT transporters. For example, adult rats on a ketogenic diet for 1 week can achieve 2 mmol/L plasma β-OHB levels within 24 h, which is sustained for 7 days. Despite the same plasma concentration of β-OHB at these time points, the permeability of ^{14}C-D-β-OHB was two times greater at 7 days than 24 h, suggesting that adult cerebral uptake of β-OHB changes with time in the presence of ketones (Moore *et al*, 1976).

Changes in MCT density may be another form of adaptation to increased plasma ketones. Immunogold electron microscopy revealed a eightfold increase in MCT1 labeling in adult endothelial cells after 4 weeks on a ketogenic diet (Leino *et al*, 2001). Although a time course of MCT upregulation has not been determined, this evidence demonstrates the capacity of the adult brain to upregulate brain MCTs. It has been reported in cultured colonic epithelial cells that MCT1 mRNA and protein upregulation can occur rapidly (12 to 24 h) in response to substrate application (Cuff *et al*, 2002).

Enzymatic Activity

After a decrease in glucose supply, the availability of total plasma ketones increases, as does its transport into the brain. However, demonstrating adaptation in ketone-metabolizing enzyme activities has been less conclusive. Early findings from Smith *et al* (1969) showed a 6.9-fold increase in mitochondrial β-OHB dehydrogenase (BDH) activity after 3 days of fasting in the adult rat brain. Subsequent studies have demonstrated a 30% decrease (Kante *et al*, 1990), a 38% decrease (Dahlquist *et al*, 1972), or no change (Pull and McIlwain, 1971) in BDH activity after a minimum of 72 h of starvation in adult rats. After more prolonged exposure to high-fat diet (10 weeks), Kante *et al* (1990) reported a 47% increase in BDH activity. Additionally, PND45 rats of mothers raised on high-fat diets maintained a 1.9-fold greater BDH activity than those of standard-fed mothers (Sherman and Wilson, 1978). Finally, the presence of substrate sodium butyrate increased BDH activity by 72% and increased 3-ketoacid-CoA transferase activity by 479% in cultured astrocytes (Poduslo, 1989). Collectively, these findings suggest that mitochondrial enzymes involved in ketone metabolism can be upregulated by levels of blood β-OHB in the adult brain; however the increased BDH activity is not necessary for increased ketone metabolism. This supports the interpretation that ketone metabolism is dependent on arterial ketone concentrations and the rate-limiting step remains its cerebral uptake.

Ketone Metabolism

It is known from some of the earliest studies in the field that prolonged starvation in humans results in increased plasma β-OHB levels and a subsequent decrease in the respiratory quotient from 1 to 0.63 (Owen *et al*, 1967). The decrease in cerebral uptake of glucose was accompanied by an increase in β-OHB uptake, demonstrating that ketone metabolism could provide 60% of the human brain's metabolic needs. The contribution of ketone metabolism toward total cerebral metabolism in the adult rat brain after 48 h of starvation is much less than that reported in humans, ranging from 15% (Dahlquist and Persson, 1976) to 25% (Ruderman *et al*, 1974). It is possible that this reflects a species difference in capacity for ketone metabolism, but

may equally be explained by the duration of the starvation or magnitude of ketosis achieved.

The increased oxidative metabolism of ketones has been observed primarily in the astrocytic pool (Melo *et al*, 2006). After implementation of the ketogenic diet in adult rats, 1-^{13}C-labeled glucose and [1,2-^{13}C]acetate were injected. Nuclear magnetic resonance spectroscopy revealed decreased neuronal oxidative metabolism of glucose and increased astrocytic metabolism of acetate. The increase in astrocytic metabolism was reflected by the increase in doublets for glutamate, glutamine, and γ-aminobutyric acid, as well as the increase in pyruvate carboxylation.

Collectively, these results from both cerebral development and starvation show the brain's capacity to adjust to physiologic changes in substrate availability. This fundamental understanding provides important insights into new potential avenues for treatment of neuropathologic conditions with alternative substrates.

Species Differences

Currently, the most complete profiles of developmental changes and starvation-induced changes in ketone transporters, enzymes, uptake, and metabolism have been reported in rats and humans. Although both these species show high neonatal levels of plasma ketones and significant cerebral uptake, other species do not show significant ketone metabolism during development or after starvation. Establishing these differences between species will be important for determining the best animal models tobe used when studying cerebral metabolism.

In the fetal lambs (PND3–18), newborn dog, and pig, ketones do not contribute significantly to cerebral metabolism. Arterial concentrations of ketones are low (Jones *et al*, 1975; Spitzer and Weng, 1972; Gentz *et al*, 1970). Although the arterial concentration of ketones in newborn dogs exceeds that of adult dogs, their metabolism contributes $<6\%$ of total cerebral oxygen consumption (Gregoire *et al*, 1978). Plasma ketone levels increase by 15% in the young lamb (Kammula, 1976), showed no change in puppies (Weng *et al*, 1973), and increased reasonably in piglets (Gentz *et al*, 1970) after starvation. Although previous reports suggested that the pig brain is deficient in acetoacetyl-CoA transferase (Tildon and Sevdalian, 1972), more recent studies by the authors using 50 mmol/L succinate concentrations revealed measurably low activity (Kahng *et al*, 1974). The low fetal and neonatal levels of ketones suggest that this substrate does not contribute significantly to normal brain development.

The human brain also possesses significant potential for ketone metabolism. The infant diet provides high levels of plasma ketones, which contribute 12.8% to cerebral development. After 9 h fasting, the infant plasma ketone levels increase to 0.84 mmol/L and contribute almost 30% of brain metabolism (Kraus *et al*, 1974). Prolonged starvation in human adults also shows significant cerebral ketone uptake (0.34 mmol/L), contributing almost 60% of the oxygen consumption (Owen *et al*, 1967). It has been proposed by Cahill (1982) that ketone metabolism may not be important in animals with lower brain/body ratios. Extending this hypothesis further, Hawkins *et al* (1986) posed an interesting notion that 'whether a requirement for the expansion of the cerebral cortex during the course of human evolution was the improved ability to use ketone bodies during time of need'.

Using Ketones in Central Nervous System Injuries: The Mature Brain

The adaptive cerebral metabolic changes that occur during development and starvation are examples of alterations that occur over prolonged periods of time. However, there is growing evidence that various conditions can induce rapid changes in both vascular and cellular transporters that ultimately would favor ketone metabolism (Table 1). Increased microvessel MCT2 expression was observed at 1 and 3 h (unpublished results) and at 6 and 24 h after traumatic brain injury (TBI) (Prins and Giza, 2006). Neuronal expression of MCT2 also increased within 6 h after noradrenaline application (Pierre *et al*, 2003). MCT1 expression was elevated within <24 h after ischemia (Tseng *et al*, 2003; Zhang *et al*, 2005), hemorrhagic shock with β-OHB resuscitation fluid (Lin *et al*, 2005), and hypoxia in cultured astrocytes (Vega *et al*, 2006).

In addition to transporter changes, ketone-metabolizing enzymes have also been reported to change rapidly in response to neuropathologic conditions. As early as 90 mins after application of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine to induce Parkinson- like degeneration, there is a 1.6-fold increase in BDH enzyme and 2.5-fold increase in whole brain β-OHB levels (Tieu *et al*, 2003). β-Hydroxybutyrate dehydrogenase activity was also induced after intermittent hypoxia in an age-dependent manner, with adults showing a 6% increase and PND18 rats showing a 37% increase in BDH activity (Trojan *et al*, 2000).

Collectively, these studies show the capacity of the MCT and ketone-metabolizing enzymes for dynamic changes in expression and suggest that the brain may be more receptive to ketone metabolism under these neuropathologic conditions. However, without accompanied increases in circulating ketone availability, these acute transporter and enzyme changes cannot be metabolically realized. Because ketone availability is low after weaning, it must be endogenously produced or exogenously provided.

Endogenous production of ketones by the liver (ketogenesis) is regulated by insulin, glucagon,

Table 1 Monocarboxylate changes after CNS injuries

Alterations	*Parameter*	*Time course*	*Age*	*Key findings*	*Reference*
Traumatic brain injury	MCT2	6, 24 h	PND35, -90 rat	Increased MCT2 expression in microvessels	Prins and Giza (2006)
Ischemia	MCT1	3, 21 days	Adult rat	Increased MCT1 in astrocytes and endothelia	Tseng *et al* (2003)
	MCT1, -2, -4	6 to 120 h 3 to 24 h	Adult rat	Increased MCT1, -2, -4 mRNA in infarct Increased MCT1 protein	Zhang *et al* (2005)
Hemorrhagic shock	MCT1	2 h	340 to 388 g rat	Increased MCT1 expression in animals resuscitated with β-OHB	Lin *et al* (2005)
Hypoxia	MCT1	1 day	Cultured astrocytes	Increased MCT1 expression 1 day after hypoxia	Vega *et al* (2006)
Diet-induced	MCT1 protein	4 to 6 weeks	150 to 190 g rat	Eight times increased MCT1 in brain after 4 weeks on ketogenic diet	Leino *et al* (2001)
Noradrenaline	MCT2 and MCT1	6 h	Cultured neurons	Within 2 h after noradrenaline application MCT2 expression increased	Pierre *et al* (2003)

CNS, central nervous system; MCT, monocarboxylate transporter.

cortisol, and catecholamines (Alberti *et al*, 1978). The relationship of these hormones to plasma ketones levels has not yet been profiled after various central nervous system (CNS) injuries. In the clinical setting, the body's ability to generate ketones may be further complicated by administration of drugs during patient management. For example, glucose is withheld from patients with CNS injuries for the first 24 to 48 h. Plasma β-OHB levels were reported to be around 0.2 mmol/L at 24 h after injury and then decrease to <0.1 mmol/L on days 3 to 14 (Ritter *et al*, 1996). This early period of starvation may be associated with ketogenesis and be reflected by the higher plasma ketone levels early after injury. The focus for CNS-injured patients has been management of arterial glucose levels with insulin (Vespa *et al*, 2006) and this process itself may suppress systemic ketogenesis (Fitch, 1988). The potential for hyperglycemia to exacerbate damage after ischemia (Fitch, 1988), human TBI (Laird *et al*, 2004; Jeremitsky *et al*, 2005; Cochran *et al*, 2003; Rovlias and Kotsou, 2000), or experimental TBI (Cherian *et al*, 1998) has long been appreciated. On the basis of these findings, management of plasma glucose within a specific range has been implemented among critically ill patients (van den Berghe *et al*, 2001), although the debate about the exact optimal range for glucose is ongoing (Vespa *et al*, 2006).

Using Ketones for Neuroprotection

Under neuropathologic conditions the energy demand and supply of the brain often become mismatched by changes in cerebral blood flow (CBF) and mitochondrial dysfunction. Glucose may no longer be sufficient during high-energy requirements, it may become less available, undeliverable, unable to be metabolized or in some cases may produce less favorable by-products. Under these circumstances, the metabolic role for glucose is altered and the brain may be unable to adapt effectively to changes in the primary fuel. However, by exogenously supplying ketones during this acute period when cerebral expression favors transport and cellular metabolism of ketones, the brain can be forced to 'shift' its reliance toward ketones. This approach has been successfuly applied to both rapidly developing pathologies (glutamate excitotoxicity, hypoxia/ischemia) and neurodegenerative conditions (Parkinson's disease, Alzheimer's disease) and more recently TBI (Table 2).

Glutamate excitotoxicity has been implicated in the cell death cascade of numerous neurodegenerative diseases and trauma. Glutamate is an important excitatory neurotransmitter, but when excessively present it over activates the glutamate receptors leading to intracellular calcium overload, production of free radicals, lipid peroxidation, and cell death (Choi, 1992). The administration of AcAc infusion for 14 days to both *in vitro* and *in vivo* rodent models of glutamate-induced neurotoxicity resulted in decreased neuronal damage (32%), reduced lesion volume (50%), and improved cellular ATP (Massieu *et al*, 2003). β-Hydroxybutyrate dehydrogenase and AcAc were both shown to protect neurons against kainic acid-induced cell death in mice (Noh *et al*, 2003) and in cultured mouse hippocampal cells (Noh *et al*, 2006). β-Hydroxybutyrate dehydrogenase infused intravenously before glutamate toxicity in the rat decreased lesion volume by 51% and reduced lipid peroxidation by 32% at 24 h (Mejia-Toiber *et al*, 2006). More recently, addition of 1 mmol/L β-OHB to an *in vitro* model of glutamate excitotoxicity increased cell survival through reduction of neuronal depolarization (Maalouf *et al*, 2007) The mechanism for ketones protection when administered before glutamate-induced excitotoxicity is likely the reduction of the free radical production (Figure 3, diamond 3).

Table 2 Ketone neuroprotection in various CNS injury models

Injury model	*Therapy*	*Species*	*Age*	*Key findings*	*Reference*
Glutamate toxicity *in vitro* and *in vivo*	Intravenous injection acetoacetate	Rats Cell culture	250 to 320 g	Decreased lesion volume, increased cell survival, improved ATP	Massieu *et al* (2003)
Glutamate toxicity	4 mmol/L β-OHB, 5 mmol/L acetoacetate	Cell culture		Increased cell survival by 27%	Noh *et al* (2006)
Kainic acid	Ketogenic diet	Mice	Not indicated	Increased hippocampal cell survival	Noh *et al* (2003)
Glutamate toxicity	Intravenous infusion β-OHB	Rats	250 to 320 g	51% decrease in lesion volume, 32% decrease in lipid peroxidation	Mejia-Toiber *et al* (2006)
Glutamate toxicity	1 mmol/L β-OHB/ 1 mmol/L acetoacetate	Dissociated rat neurons	1 to 3 weeks	Decreased neuronal death, decreased ROS production, increased NADH oxidation	Maalouf *et al* (2007)
Hypoxia tolerance	β-OHB	Mice	25 to 30 g	Pretreatment with ketones and hypoxia did not increase survival, but ketones with pre-hypoxia exposure increased survival 1.9 times compared with pre-hypoxia exposure alone	Rising and D'Alecy (1989)
Hypoxia	4 mmol/L β-OHB	Cell culture		Increased cell survival, decreased nuclei condensation, maintained mitochondrial membrane potential, decreased caspase 3 activation and PARP cleavage	Masuda *et al* (2005)
Hypoxia, anoxia,	Intravenous infusion β-OHB preinjury	Mice	5 weeks	Increased survival time	Suzuki *et al* (2001)
Global ischemia	Intravenous infusion β-OHB postinjury	Wistar rats	160 to 180 g	Decrease edema, improved ATP	
Focal cerebral Ischemia	Intravenous infusion β-OHB preinjury	Wistar rats	280 to 300 g	Permanent occlusion showed no protection, but transient ischemia showed decreased infarct area, edema, lipid peroxidation	Suzuki *et al* (2002)
Transient MCA	Ketogenic diet	Long Evans rats	300 g	Decreased infarct volume	Ritter *et al* (1996)
Traumatic brain injury	Intravenous Infusion β-OHB	Rats	350 to 400 g	Increased $^{14}CO_2$ production from ^{14}C-β-OHB, alleviated ATP decrease at 3 h	Prins *et al* (2004
Traumatic brain injury	Ketogenic diet	Rats	PND17, -35, -45, -65	Decreased contusion volume among PND35 and -45, decreased number fluorojade-positive cells	Prins *et al* (2005)
Alzheimer's *in vivo*	Ketogenic diet	Mice	3 months	Reduced total Aβ- levels by 25%, no difference in object recognition task	Van der Auwera *et al* (2005)
Alzheimer's	Ketogenic diet	Human		Improved cognitive testing	Reger *et al* (2004)
Alzheimer's *in vitro*	4 mmol/L β-OHB	Cell culture		Increased cell survival	Kashiwaya *et al* (2000)
Parkinson's *in vitro*	4 mmol/L β-OHB	Cell culture		Increased cell survival, cell size, neurites	
Parkinson's *in vivo*	Subcutaneous minipump infusion β-OHB	Mice	8 to 10 weeks	Increased dopaminergic neuronal survival, improved motor deficits, improved mitochondrial respiration	Tieu *et al* (2003)
Parkinson's *in vitro*	8 mmol/L β-OHB	Human cell culture		Increased survival, improved mitochondrial membrane potential, decreased cytochrome *c* release	Imamaura *et al* (2006)
Parkinson's *in vitro*	4 and 8 mmol/L β-OHB	Cell culture		Increased survival by 60%	Kweon *et al* (2004)
Amyotrophic lateral sclerosis	Ketogenic diet	Mice	P50	Increased preservation of motor neurons, better motor function	Zhao *et al* (2006)
Brain tumor-astrocytoma	Ketogenic diet/CR	Mice	8 to 10 weeks	Inhibited tumor growth, decreased angiogenesis, increased survival	Mukherjee *et al* (2002); Seyfried *et al* (2003); Zhou *et al* (2007)

β-HB, β-hydroxybutyrate; CR, calorie restricted; MCA, middle cerebral artery; PARP, poly-ADP-ribose polymerase; PND, postnatal day.

Hypoxic injury reduces oxygen availability and thus decreases oxidative glucose metabolism resulting in increased lactate production. High tolerance to hypoxia has been associated with increased plasma ketone levels (Eiger *et al*, 1980; D'Alecy *et al*, 1990). The use of the ketogenic diet for 3 weeks has shown to increase tolerance to *in vivo* hypoxia (Puchowicz *et al*, 2005). Administration of 4 mmol/L β-OHB to hypoxic hippocampal neurons decreased acute cell death, decreased the number of apoptotic

cells, maintained mitochondrial membrane potential, and decreased caspase-3 activation and poly-ADP-ribose polymerase cleavage (Masuda *et al*, 2005). The mechanism for neuroprotection after hypoxia is believed to be the reduction of glycolytically derived lactate associated with increased ketone metabolism, although decreased free radical damage and apoptotic cascade activation may also be involved (Figure 3, diamonds 3,1,7).

Ischemic injury restricts all substrate supply and oxidative metabolism and administration of glucose during reperfusion has been shown to exacerbate brain damage by increasing lactate accumulation resulting in lactate acidosis. Under these conditions, ketone metabolism has been shown to have neuroprotective effects (Table 2). The use of 1,3-butanediol in rats, which is readily converted into β-OHB in cells, has also been shown to improve neurologic recovery from ischemia (Marie *et al*, 1997). Pretreatment with 1,3-butanediol increased phosphocreatine and reduced lactate accumulation at 72 h after ischemia. In contrast to pre-injury manipulations, post-injury treatments have also demonstrated neuroprotection. Rats infused with β-OHB for 3 to 6 h starting immediately after bilateral carotid artery ligation showed decreased cerebral edema and tissue Na^+ content at 6 h and attenuated ATP reduction at 3 h (Suzuki *et al*, 2001). In this model, authors report a 40% decrease in CBF upon occlusion in the Wistar rat, suggesting that sufficient blood flow may remain to circulate the β-OHB. Infusion of β-OHB started immediately after transient occlusion of the middle cerebral artery showed approximately 50% reduction of cerebral infarct volume (Suzuki *et al*, 2002). Elevation of plasma β-OHB by 24 h of fasting resulted in 75% reduction of ischemia-induced infarct volume (Ritter *et al*, 1996). Adult rats fasted for 48 h before four-vessel occlusion showed decreased mortality, decrease post-traumatic seizures and decreased brain lactate (Marie *et al*, 1990). Although fasting related neuroprotection was also observed after hypoxia–ischemia in the adult rat, ketone administration alone failed to provide protection (Go *et al*, 1988). One of the proposed mechanisms of cell death after ischemia is glutamate excitotoxicity and similar modes of ketone neuroprotection may contribute to ischemic protection (Figure 3). However, it is important to keep in mind that during ischemia, blood flow ceases regionally and delivery of any substrate is limited. It is important for studies to show the time course of ketone delivery especially when ketone administration starts during the ischemic injury.

Traumatic brain injury is associated with an indiscriminate release of potassium and glutamate, transient elevation in glucose metabolism followed by prolonged glucose metabolic depression and reduction of ATP (Katayama *et al*, 1990; Kawamata *et al*, 1992; Yoshino *et al*, 1991; Lee *et al*, 1999). During this period of depressed glucose metabolism,

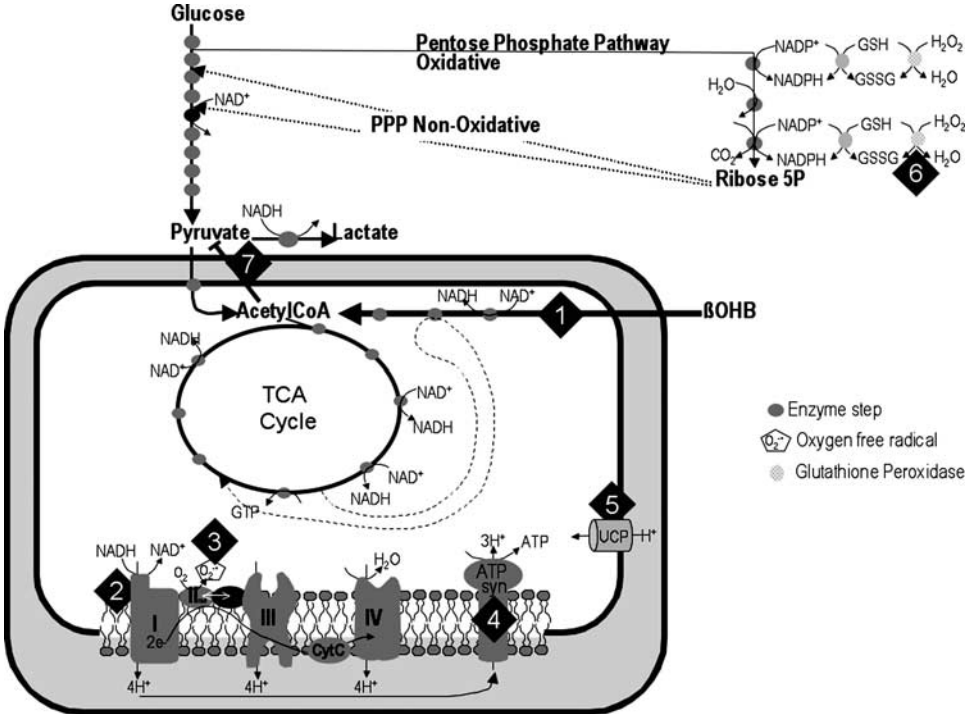

Figure 3 Summary of the properties of ketone metabolism that potentially contribute to neuroprotection. The numbers in black diamonds denote each mechanism. Ketones (1) require only three enzymatic steps to enter the TCA cycle; (2) reduce the NAD couple; (3) decrease free radical formation; (4) increase production of ATP; (5) increase mitochondrial uncoupling; (6) increase glutathione peroxidase activity; and (7) inhibit pyruvate entry into the TCA cycle. Abbreviation: UCP, uncoupling protein.

there is an increased flux of glucose through the pentose phosphate pathway, free radical production, and activation of poly-ADP-ribose polymerase-via DNA damage (Hall *et al*, 1993). The use of NAD^+ in poly ADP-ribose polymerase-mediated DNA repair process depletes cytosolic NAD^+ pool and can inhibit glyceraldehyde-3-phosphate dehydrogenase (a key enzyme in the glycolytic pathway). Under these conditions of impaired glycolytic metabolism, glucose becomes a less favorable *energy* substrate and shifting the brain toward ketone metabolism has been shown to provide neuroprotection. Exogenous administration of β-OHB (intravenously) immediately after adult TBI resulted in an 8.5-fold increase in β-OHB uptake, a greater than 10-fold increase in ^{14}C-β-OHB oxidation and alleviated the ipsilateral cortical decrease in ATP at 3 h after injury (Prins *et al*, 2004). The effectiveness of the ketogenic diet in reducing cortical contusion volume by 50% after focal TBI among PND30 and PND45 rats (Prins *et al*, 2005) has also been demonstrated. The immediate changes (1 h) in glucose metabolism and free radical production initiate ongoing changes in cerebral metabolism and damage after TBI. Although increased vascular expression of MCT2 has been shown during this time point, it is not the primary MCT in the endothelium. Changes in MCT1 expression after TBI may also contribute to use of ketone metabolism within hours after TBI. Although the neuroprotective potential of fasting after human TBI has not been documented, the presence of ketones has been shown to replace adequately systemic calories without generating hyperglycemia (Ritter *et al*, 1996). Finally, pretreatment with BD has been shown to reduce TBI and ischemia-induced brain edema as indicated by specific gravity measurements (Biros and Nordness, 1996). The preferential utilization of β-OHB is thought to reduce lactate production and thereby decrease cerebral edema (Figure 3, diamonds 1, 2, 3, 4, 7).

At this point it is important to note that many investigators routinely use overnight fasting in their experimental design before inducing various types of injuries. Although some studies utilize this as a variable in the study, others do not. In Ketogenic Cerebral Metabolic Adaptation and Cerebral Development section, many changes were initiated in the mature brain in response to fasting, starvation, or hypoglycemia. Starvation or fasting for 6 to 12 h will increase plasma levels of ketones significantly and can interfere with outcome measures, such as glucose metabolism. In addition, the increased presence of ketones before CNS injury induction can provide unintentional pre-injury 'neuroprotection,' which can interfere with studies examining neuroprotective effectiveness of drugs of interest. These changes should be considered when interpreting results involving pre-injury withholding of food.

The neuropathologies of glutamate toxicity, hypoxia/ischemia, and TBI all have a rapid cellular progression, which necessitates that ketones be delivered early to maximize its therapeutic effectiveness. This approach to early delivery also coincides with the early changes in ketone transporter expression observed after ischemia and TBI. Establishing the therapeutic window for ketone therapy will be critical in establishing its potential uses in clinical cases.

Unlike the rapid metabolic changes that are associated with brain insults, slower developing neurodegenerative conditions may also benefit from the metabolism of alternative substrates (Table 2). In the *in vitro* model of Parkinson's disease, 1-methyl-4-phenylpyridinium inhibits the activity of the mitochondrial NADH dehydrogenase complex, thereby decreasing electron transport activity and increasing production of free radicals. Administration of 4 mmol/L β-OHB increased the survival of cultured neurons from 1-methyl-4-phenylpyridinium toxicity (Kashiwaya *et al*, 2000). Another *in vitro* model of Parkinson's disease utilizes rotenone, which inhibits mitochondrial complex I. Application of 8 mmol/L β-OHB to this model of Parkinson's increased cell survival, improved mitochondrial membrane potential and reduced cytochrome *c* release in mouse neuronal cultures (Imamaura *et al*, 2006), and increased cell survival by 60% in human neuroblastoma cell culture (Kweon *et al*, 2004). More recently, 24 h infusion of β-OHB in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mice showed 45% decrease in motor deficits and a decrease in dopaminergic neurodegeneration (Tieu *et al*, 2003). Similarly, *in vitro* models of Alzheimer's disease have shown that $A\beta_{1\,to\,42}$ stimulates phosphorylation of pyruvate dehydrogenase, thereby blocking the entry of pyruvate into the tricarboxylic acid (TCA) cycle. Under these conditions, ketone metabolism can bypass the defective metabolic pathway and provide acetyl-CoA for energy production (Figure 3). Administration of 4 mmol/L β-OHB protected cultured mouse hippocampal neurons from $A\beta_{1\ to\ 42}$ toxicity (Kashiwaya *et al*, 2000). Ketones have also shown neuroprotection in *in vivo* models of Alzheimer's disease. Transgenic mice on the ketogenic diet for 43 days showed 25% decrease in amyloid-β deposition, but failed to show differences in object recognition task (Van der Auwera *et al*, 2005). Moreover, human patients with Alzheimer's or mild cognitive impairments placed on ketogenic diets showed improved performances in paragraph recall and on the Alzheimer's disease assessment scale test (Reger *et al*, 2004). It is important to note that while the *in vitro* Alzheimer studies allow one to study some of the mechanistic issues, *in vivo* Alzheimer's disease models with prolonged administration of ketones are critical to understanding the ultimate potential for recovery for this therapeutic approach.

In addition to Alzheimer's and Parkinson's diseases, amyotrophic lateral sclerosis and brain tumors have also shown positive response to ketone metabolism. Transgenic amyotrophic lateral sclerosis mice (mutant superoxide dismutase1, SOD1) maintained on a ketogenic diet starting PND50 showed improved motor performance and more motor neurons in the spinal cord than standard-fed animals (Zhao *et al*, 2006). In SOD1 mutant mice, mitochondrial dysfunction results in decreased ATP production, which is improved by 38% in the presence of β-OHB. Interestingly, the high glycolytic demands of brain tumors make them particularly susceptible to substrate deprivation under conditions of decreased glucose and increased ketone availability. Using a calorie-restrictive ketogenic diet, astrocytoma growth and angiogenesis were decreased and survival increased in mice (Mukherjee *et al*, 2002; Seyfried *et al*, 2003; Zhou *et al*, 2007).

Perhaps the most well-documented evidence of ketone neuroprotection has been observed among cases of childhood seizures or intractable epilepsy as reviewed recently by Gasior *et al* (2006) and Stafstrom (1999). This topic will be addressed in greater detail in the section on using ketones in CNS injuries in the developing brain.

Ketogenic Neuroprotective Mechanisms

There are unique properties of ketone metabolism that may make it a more suitable cerebral fuel under various neuropathologic conditions (Figure 3). First, there are only three enzymes involved in metabolizing β-OHB to acetyl-CoA, compared with the 11 biochemical steps to process glucose for TCA cycle entry (Figure 3, #1). Second, the presence of ketone bodies fundamentally alters mitochondrial metabolism by improving metabolic efficiency (Figure 3, #2). Ketones have been reported to decrease oxygen consumption, whereas increasing hydraulic work of the heart (Kashiwaya *et al*, 1994; Sato *et al*, 1995) and sperm motility (Lardy and Phillips, 1945). Ketone metabolism was shown to reduce the nicotinimide adenine dinucleotide (NAD) couple and oxidize the coenzyme Q couple, which increases the energy released by an electron traveling across the electron transport chain, ultimately increasing the G' of ATP hydrolysis (Figure 3, #4; Veech *et al*, 2001). Third, ketone metabolism can also decrease the production of free radicals by decreasing the reduced form of coenzyme Q, which decreases its reaction with O_2 to form superoxide $O_2^{-\bullet}$ (Figure 3, #3). Additionally, the reduction of NAD couple favors reduction of glutathione, which ultimately favors destruction of H_2O_2 by glutathione peroxidase reaction (Figure 3, #6, Krebs and Veech, 1969). The ketogenic diet has also been shown to increase mitochondrial uncoupling protein 2 expression by 55% with a comparable decrease in ROS production (Figure 3, #5) (Sullivan *et al*, 2004). Although the presence of ketones did not alter the degree of lipid peroxidation in normal hippocampi, there was a 50% increase in total antioxidant capacity. Specifically, animals maintained on the ketogenic diet showed a fourfold increase in glutathione peroxidase activity in rat hippocampi (Ziegler *et al*, 2003). Another potentially beneficial effect of cerebral ketone metabolism is its reported effect on CBF (Hasselbalch *et al*, 1996). Adult rats infused with sodium DL-β-OHB for 45 mins showed a 213% increase in global CBF.

The Weanling is Not an Adult: Age Differences in Ketone Metabolism After Weaning

The changes in cerebral metabolism that occur during brain development describe the 'natural cerebral capacity' to metabolize ketones, which decreas sharply after weaning. However, studies examining various post-weaned age groups have shown that age differences in the capacity of the brain to metabolize ketones continue well past weaning. For example, the ability to generate ketones endogenously is inversely proportional to age even after weaning. Ketogenesis after 48 h of starvation among PND57 rats showed a 95% increase in arterial β-OHB levels versus only an 81% increase among PND85 rats (Dahlquist and Persson, 1976). Similarly, there is a significantly greater production of ketones among 5-year-old children (28.6%) versus 10-year olds after 24 h of fasting (Saudubray *et al*, 1981).

In addition to age-related differences in ketogenesis, age-related differences in cerebral utilization of ketones has been observed after starvation or administration of ketones. On the basis of the developmental profiles in Figure 2, it is expected that a suckling animal will show significantly greater cerebral uptake of β-OHB than PND55 rats. However, age-related differences in uptake exist much later. PND57 rats show 61% greater cerebral uptake of β-OHB after 48 h of starvation compared with PND85 animals (Hawkins *et al*, 1971; Dahlquist and Persson, 1976). It has also been reported that at the same plasma level of β-OHB, PND35 rats show a 1.7-fold greater brain uptake index than PND50. Some of this age difference in uptake may be attributed to transporter expression or even transporter function. A decrease in the maximum transport velocity was observed with age when carotid injections of bolus D-β-hydroxy[3-^{14}C]butyrate were used to study ketone transport between 120 g (PND35) and 200 g (PND50) rats (Regen *et al*, 1983).

Using Ketones in Central Nervous System Injuries: The Developing Brain

Given these ongoing age-related differences in ketogenesis, transporter expression, and uptake,

the ability to increase metabolic reliance on ketones should be age-dependent, even after weaning. If so, this would have direct implications for the therapeutic potential of ketone administration to the injured, post-weaned developing brain. Research studies addressing neuroprotection with ketone metabolism after seizures, glutamate toxicity, ischemia, and TBI in the younger brain have begun to emerge (Table 3).

Perhaps the most well-established evidence for age-related differences in cerebral protection from ketones has arisen from its use in treating pediatric seizures and epilepsy (Table 3). The experimental research studies established significant decreases in seizure activity among younger animals compared with adults when a ketogenic diet was used. For example, after 20 days on the ketogenic diet/calorie-restricted diet, younger rats achieved the greatest plasma concentrations of ketones and showed the longest time to seizure onset after pentaylenetetrazole infusion (Bough *et al*, 1999). Similar protection was also observed after various types of seizure induction among PND16 mice after 10 days on the ketogenic diet compared with PND40 (Uhlemann and Neims, 1972). However, no protection was observed after 24 h on the ketogenic diet at PND25. After exposure to a volatile convulsant, flurothyl trifluroethyl ether, PND24 mice showed longer latencies to clonic seizures and showed lower mortality rates than PND51 mice (Rho *et al*, 1999). Although the exact mechanisms for this anti-seizure effect remains unknown, it has been proposed by Yudkoff *et al* (1997, 2001, 2004) that the effects of ketosis on glutamate metabolism may underlie this neuroprotection. The authors hypothesize that the metabolism of ketone bodies pulls oxaloacetate toward citrate production and away from transamination to aspartate. Under these conditions, the flux of glutamate to aspartate decreases and more glutamate is made available for γ-aminobutyric acid production, which may exert anti-epileptic properties. Concomitantly, ketosis may also result in production of glutamine, which is then released across the blood–brain barrier for leucine to provide the necessary cofactors for astrocytic glutamine synthetase reaction.

In addition to neuroprotection from seizures, administration of ketones has been shown to provide protection after hypoxia/ischemia (Table 3). As mentioned above in the section on Use of Ketones in Adult CNS Injuries, the study by Suzuki *et al* (2001) demonstrated decreased water, sodium, and lactate content with an alleviation of ATP and CBF depression after β-OHB treatment of ischemia. It is important to note that these studies were conducted in 160 to 180 g rats (approximately PND42), which re-emphasizes how even after 20 days post-weaning, the potential for ketogenic protection exists. In a glutamate toxicity model mentioned in the adult section, PND7-21 rat dissociated neurons showed decreased ROS-induced cell death with β-OHB or AcAc application (Maalouf *et al*, 2007). In a model of repeat hypoglycemic episodes, ketone-fed PND25 rats showed fewer fluorojade-positive cortical neurons after hypoglycemia than those maintained on standard diet (Yamada *et al*, 2005).

Table 3 Ketone neuroprotection in developmental CNS injury models

Injury model	*Therapy*	*Species*	*Age*	*Key findings*	*Reference*
Glutamate toxicity	1 mmol/L β-OHB/ 1 mmol/L acetoacetate	Dissociated rat neurons	1 to 3 weeks	Decreased neuronal death, decreased ROS production, increased NADH oxidation	Maalouf *et al* (2007)
Hypoglycemia	Ketogenic diet	Rats	PND25	Ketone-fed animals showed less neuronal loss than standard-fed	Yamada *et al* (2005)
Hypoxia, anoxia	Intravenous infusion β-OHB preinjury	Mice	5 weeks	Increased survival time	Suzuki *et al* (2001)
Global ischemia	Intravenos infusion β-OHB postinjury	Rats	160 to 180g	Decreased edema, improved ATP	
Seizures-flurothyl	Ketogenic diet	Mice	PND24	Ketone-fed juveniles showed longer	Rho *et al* (1999)
			PND52	Latencies to clonic seizures than adults and lower mortality rates	
Seizures	Ketogenic diet	Mice	PND16	Increased resistance to electroshock and bicuculline-induced seizures, no protection after PTZ-induced seizures	Uhlemann and Neims (1972)
Seizures-PTZ	Ketogenic diet/ calorie restriction	Rats	PND22, -28, -37, -63, -75, -126	Ketone/CR-fed showed greatest delay in onset of seizure among youngest age groups. CR alone also showed some protection	Bough *et al* (1999)
Traumatic brain injury	Ketogenic diet	Rats	PND17, -35, -45, -65	Decreased contusion volume among PND35 and -45, decreased number fluorojade-positive cells	Prins *et al* (2005)

β-OHB, β-hydroxybutyrate; CR, calorie restricted; CNS, central nervous system; NADH, nicotinimide adenine dinucleotide reduced; PND, postnatal day; PTZ, pentaylenetetrazole; ROS, reactive oxygen species.

Experimental models of TBI have also recently demonstrated age-dependent ketogenic neuroprotection (Prins *et al*, 2005; Table 3). Postnatal days 35 and 45 rats placed on a ketogenic diet immediately after controlled cortical impact injury for 7 days showed a 58 and 39% reduction in cortical contusion volume, respectively (Prins *et al*, 2005). Cross-sectional area of the remaining cortex was determined by tracing the contralateral and ipsilateral remaining cortex. Subtraction of the ipsi and contra corticies determined the area per section and total lesion volume was determined by integrating the area from each section with the distance between each section. The ketogenic diet had no significant effect on contusion volume in both PND17 and PND65 injured rats at 7 days post-injury. Both PND35 and -45 ketogenic-fed groups revealed fewer Fluorojade-positive cells in the cortex and hippocampus at 6 h and showed earlier decreases in plasma lactate compared with standard-fed animals. It remains unclear at this time why the youngest age group failed to show ketogenic neuroprotection after TBI. It is important to note, however, that this age group was the only group that had high circulating ketones at the time of the injury because they remained with their dams. This immature age group exhibited the highest level of ketone transporters and ketone metabolic activity. It is possible that at this age both glucose and ketones are used to meet the high-energy demands for cerebral development that ketones cannot sufficiently serve as a 'reserve' substrate after TBI (Nehlig, 2004). Collectively, these studies in seizures, ischemia, and TBI emphasize the importance of the age-dependent nature of the neuroprotective potentials of ketones. These post-weaned age groups may have the greatest potential for ketone metabolism manipulations and ultimately offers treatment options for pediatric neuropathologic conditions.

Adverse Effects of Ketones

The ketogenic diet was developed in the late 1920s (Bailey *et al*, 2005) based on a hypothesis that increased fat and low carbohydrates would decrease seizure activity. Although the majority of this review focuses on the therapeutic potentials of ketone bodies, there are some complications that accompany the administration of high-fat diets.

In terms of long-term outcomes, the majority of findings have emerged from the pediatric population due to its high use in childhood epilepsy. In general, patients tolerate the ketogenic diet well with mild side effects (Freeman *et al*, 2006). However, there are common early reactions that are usually associated with fat intolerance and dehydration and are managed with laxatives, decreased fiber intake and increased fluid intake. Early problems include dehydration, vomiting/nausea, diarrhea, constipation, and hypoglycemia. Complications that typically occur later include growth retardation due to insufficient protein levels, hepatic failure, vitamin/mineral deficiencies, immune dysfunction, renal stones, hypercholesterolemia, and cardiomyopathy.

The focus on ketogenic side effects has predominantly remained physiologic and very few studies have examined potential cognitive complications. One study examining obese women on the ketogenic diet for 28 days observed significant deficits on a high-order mental processing neuropsychologic test, but did not show problems on attention tasks (Wing *et al*, 1995). In addition to this clinical finding, an experimental study has also reported cognitive and behavioral adverse consequences. Zhao *et al* (2004) tested weanling rats maintained on a ketogenic diet for 30 days in the Morris water maze, open field test, brain weights, time to seizure onset, and hippocampal cell loss. No ketogenic effects were found in the activity, emotionality, or pathology, but there was significant reduction in seizure activity. The authors report that control rats on the ketogenic diet took four times longer to reach the platform than standard-fed control animals. Seizure-induced rats on the ketogenic diet also showed significantly longer escape latencies than standard-fed seizure animals. In a correspondence to the journal, Cunnane and Likhodii (2004) bring up the point that the diet used had a fat/protein/carbohydrate ratio that was two times greater than those used clinically. This comment emphasizes an important point that not all ketogenic diets are created equal, and examination of these differences will be important before therapeutic consideration.

In another study, PND30 rats were maintained on the ketogenic diet for 10 weeks and then tested in the open field, plus maze and nociception (Ziegler *et al*, 2005). Although no differences in anxiety were observed, the ketone-fed animals showed greater locomotor activity and hypernociception.

In contrast to the long-term adverse reactions, there are potentially biochemical consequences of ketone metabolism that may cause adverse effects acutely. Ketone metabolism increases the production of acetyl-CoA, inhibiting pyruvate dehydrogenase activity (Booth and Clark, 1981) and consequently glycolysis. Induction of hyperketonemia has been shown to decrease glucose metabolism in P20 rats by 20 to 35% (Miller, 1986), but did not alter glucose metabolism in adult rats despite the length of starvation (Corddrey *et al*, 1982; Crane *et al*, 1985). Three days fasting, 3-week starvation, or infusion of β-OHB resulted in 26, 54, and 66% decrease in cerebral glucose metabolism in human adults (Hasselbalch *et al*, 1994, 1996; Redies *et al*, 1989). These changes in glucose metabolism during increased ketone metabolism may be beneficial during acute stages of neuropathology. However, it is important to know how long decreases in glucose metabolism can be tolerated without long-term consequences, especially in the developing brain.

These adverse effects of the ketogenic diet must be taken into consideration when using ketones to treat CNS injuries, especially in those processes requiring long-term ketone therapy. Future studies will be necessary to address the long-term benefits and detrimental effects among the different types of ketone diets and among different age groups.

The historical trail of evidence for cerebral ketone metabolism covered in this review should encourage researchers to grapple with the evolving face of brain metabolism. The simplistic biochemical demonstration of glucose metabolism through glycolysis and TCA cycle is no longer-sufficient explanation for cellular metabolism. The significance of cerebral ketone metabolism and other alternative substrates must continue to be examined. The details of their metabolic fates and their interactive effects on glucose metabolism must be clarified before the full potential of alternative substrate therapy can be realized.

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