The Effects of a Ketogenic Diet on Behavioral Outcome after Controlled Cortical Impact Injury in the Juvenile and Adult Rat

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Abstract

The ketogenic diet has been shown to have unique properties that make it a more suitable cerebral fuel under various neuropathological conditions (e.g., starvation, ischemia, and traumatic brain injury (TBI)). Recently, age-dependent ketogenic neuroprotection was shown among postnatal day 35 (PND35) and PND45 rats after TBI, but not in PND17 and PND65 animals (Prins et al., 2005). The present study addresses the therapeutic potential of a ketogenic diet on motor and cognitive deficits after TBI. PND35 and PND75 rats received sham or controlled cortical impact (CCI) surgery and were placed on either standard (Std) or ketogenic (KG) diet for 7 days. Beam walking and the Morris water maze (MWM) were used to assess sensory motor function and cognition, respectively. PND35 CCI Std animals showed significantly longer traverse times than sham and CCI KG animals at the beginning of motor training. Footslip analysis revealed better performance among the sham and the CCI KG animals compared to the CCI Std group. In the MWM PND35 CCI KG animals showed significantly shorter escape latencies compared to CCI Std-fed animals. During the same time period there was no significant difference between sham animals and CCI KG animals. The therapeutic effect of the ketogenic diet on beam walking and cognitive performance was not observed in PND75 animals. This finding supports our theory about age-dependent utilization and effectiveness of ketones as an alternative fuel after TBI.

Key words: behavior; ketone metabolism; Morris water maze; traumatic brain injury

Introduction

THE ADULT BRAIN RELIES PRIMARILY on glucose for energy metabolism, but retains a limited capacity to utilize ketone bodies under conditions of starvation (Owen et al., 1967; Hasselbalch et al., 1996; Hawkins et al., 1971; Dahlquist and Persson, 1976), or when ketones are administered after ischemia (Marie et al., 1990; Suzuki et al., 2001) or traumatic brain injury (TBI) (Robertson et al., 1992; Prins et al., 2004, 2005). However, the capacity to utilize ketones is dependent on its availability, transport, and metabolism, all of which are significantly lower in the adult brain compared to the young brain (Lockwood and Bailey, 1971; Nehlig et al., 1987, 1991; Page et al., 1971; Vannucci and Simpson, 2003). Even after weaning the younger animal can produce more ketones endogenously (Dahlquist and Persson, 1976; Saudubary et al., 1981), has greater number of cerebral ketone transporters (Vannucci and Simpson, 2003), and greater enzymatic activity for ketone metabolism (Booth et al., 1980; Leino et al., 1999; Page et al., 1971).

Administration of a ketogenic diet and of ketone bodies has been shown to be neuroprotective in younger animals after seizures (Rho et al., 1999), TBI (Prins et al., 2005), and after glutamate toxicity (Maalouf et al., 2007). Recently, our laboratory revealed age-related differences in ketogenic neuroprotection and monocarboxylate transporter (MCT) expression after TBI. Postnatal day (PND) 35 and PND45 rats placed on a ketogenic (KG) diet for 7 days immediately after controlled cortical impact injury (CCI) showed a 58% and 39% reduction in cortical contusion volume, respectively, compared to age-matched injured control animals on a standard (Std) diet (Prins et al., 2005).

The age-dependent nature of the KG neuroprotection may be related to the greater expression of MCTs and activity of enzymes required for ketone metabolism in the younger brain (Cremer et al., 1976; Gerhart et al., 1997; Page et al., 1971). Our laboratory has also shown that the expression of MCT2 increases after CCI injury, both in PND35 and adult rats. However, the young animals showed an 80–88% greater expression compared to adult animals (Prins and Giza, 2006).

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Leino and associates (2001) also revealed that the KG diet itself increases the expression of MCT1. In addition, the activity of the enzymes required for ketone metabolism are two- to threefold greater during development compared to recently weaned or adult animals (Page et al., 1971; Lust et al., 2003).

While the KG diet was effective in increasing cellular survival within the injured core after TBI, the functional consequences of this remain unknown. Therefore, the current study expands upon these initial findings by hypothesizing that early treatment of TBI with a ketogenic diet will improve motor and cognitive recovery in an age-dependent manner.

Methods

Subjects

PND35 (137 ± 5.5 g) and PND75 (331 ± 3.1 g) ($n = 64$) male Sprague-Dawley rats were given sham surgery or CCI injury. Five of the PND75 animals were excluded from the study due to too mild or too severe injury. After surgery the animals were immediately placed on either a Std (Teklad #7013) or KG (Bioserv #F3666) diet for a week. All animals were returned to standard chow on post-injury day 7. The animals were randomly assigned to a group: sham Std diet, sham KG diet, CCI Std diet, or CCI KG diet ($n = 8$ per group per age, except for the sham KG group, which was $n = 5$). All groups were behaviorally assessed in the experimental design shown in Figure 1.

CCI injury

As characterized previously (Prins et al., 2004), the CCI injury model was used to generate a focal TBI to the left cortical hemisphere. The craniotomy was 6 mm in diameter, centered at -4 mm anteroposterior and 5 mm mid-lateral relative to the bregma. The injury compressed the brain 2 mm below the pial surface at 1.9 m/sec.

Beam walking

Three days prior to surgery, the animals were pre-trained to traverse a narrow beam (width 1.91 cm for PND75 and 1.27 cm for PND35) without footslips. The beam was 144 cm in length and elevated 60 cm above the surface. The animals were trained to escape loud white noise and an aversive bright light at the starting end of the beam and enter a darkened goal box at the end of the beam. The animals were allowed to stay in the goal box for 30 sec before the next trial. Each animal was given three trials per day. The animals were tested on the beam-walking apparatus from post-injury days

3–7. The animals' beam-walking ability was quantified by recording the traverse time and quantifying the number of right footslips per total steps (Feeney et al., 1982; Sutton et al., 1989). Two researchers assessed the ratings, and one was blind to the animals' treatment condition.

Morris water maze acquisition

The Morris water maze (MWM) has been previously described (Morris, 1984), and is used to assess cognitive performance following TBI (Hamm et al., 1993; Dixon et al., 1994; Prins and Hovda, 1998). See Prins and Hovda (1998) for descriptions of apparatus and settings.

The MWM testing was performed on post-injury days 10–16. The starting time was chosen in order to be sure that the animals treated with the KG diet would have a normal concentration of ketone bodies in their blood. The duration of the training period was chosen to make sure that the animals learned the task and were able to reach the "floor" in latency time (Prins and Hovda, 1998). Each animal received two blocks of training per day with an inter-block period of 21 min. Each block consisted of four consecutive trials in which the animal was released from each of the four quadrants in random order. The animals were given 45 sec to locate the platform, after which they were guided to it and remained on it for 30 sec. During the inter-block period, the animals were dried off and returned to a heated plastic cage. A computerized tracking system (San Diego Instruments, San Diego, CA) recorded the swim speed, swim path, distance, and time to locate the hidden platform. Analysis of swim speed in addition to cueing was used to determine the role of motor deficits in cognitive performance.

Morris water maze cueing

After training on post-injury day 11, the animals were given two blocks consisting of four cueing trials each to determine whether motor or visual deficits were confounding any perceived cognitive impairments. During the cueing trials, the platform was made visible by raising it 3.8 cm above the water. Each animal was released from each of the four release points in random order and allowed 10 sec to locate, swim to, and climb the visible platform.

Morris water maze probe trial

On post-injury day 17, the animals were given a probe trial, in which the hidden platform was removed from the tank. Each animal was given 45 sec to swim in the tank and the trial was recorded for zone analysis.

FIG. 1. Schematic diagram of the testing period for both age groups.

Circulating substrate concentration

Blood samples were not drawn from animals in the present study to avoid interference with behavioral performance. Previous results from our laboratory showed that there was no significant difference in plasma β -hydroxybutyrate (β -OHB) at 0, 1, 6, 24 hrs, and 7 days after injury in PND35, PND45, and PND65 animals maintained on a standard diet. However, age-matched animals on a ketogenic diet showed an immediate increase in β -OHB levels and a significant decrease in both glucose and lactate levels after injury (Prins et al., 2005).

Statistics

All data are expressed as mean $\pm$ SEM and were analyzed using SPSS software (SPSS, Inc., Chicago, IL). Comparison of traverse time (sec), footslip (%), weight (g), swim speed (sec), and latencies (sec), between the different age, diet, and injury groups was accomplished using two-way repeated measure analysis of variance (ANOVA).

Results

Weights

The average initial body weights for the PND35 and PND75 animals were 137 ± 5.5 g and 331 ± 3.1 g, respectively (Table 1). The overall ANOVA analysis showed a significant main effect for age (PND75 or PND35) [$F(1, 17) = 172.219$; $p < 0.05$]. Comparison within the two age groups showed no significant difference in initial weight pre-injury between any of the different groups (sham Std, sham KG, CCI Std, or CCI KG). On the last day of the KG treatment (post-injury day 7) the sham KG animals in both age groups and the PND75 CCI KG animals showed a 5% weight loss compared to their initial weight, while the PND35 CCI KG group showed a weight loss of 17%. At the same time point the PND75 sham and CCI animals on a Std diet had gained 7% and 5%, respectively, while the PND35 sham and CCI animals on a Std diet had gained 27% and 26%, respectively. After post-injury day 7, the KG animals (sham and CCI) from both age groups gained weight throughout the rest of the training. Repeated ANOVA analysis across all days showed no significant differences in the weight between the animals in the PND75 group. However, statistical comparison in the PND35 group showed a main effect of diet [$F(1, 15) = 58.170$, $p < 0.05$] and also that the weight of the animals was significantly effected by the interaction between injury group and diet [$F(1, 15) = 6.537$, $p < 0.05$].

Beam walking

All groups of both ages were able to traverse the beam and the traverse times decreased across the testing period (Fig. 2A).

There was no significant difference in traverse time during the pre-training between any groups or between the two ages. The overall analysis of differences in traverse times across all days post-injury showed a main effect of injury group (sham or injured) [$F(1, 45) = 5.233$, $p < 0.05$], but no effect of age or diet itself. The statistical comparison between the two sham groups (Std or KG) in both age groups showed no significant difference in traverse time across the training period (Fig. 2A and B). Looking at the diagram for the PND75 age group, the traverse time decreases slightly over time for all groups, and no distinct difference is visible at any time point between any of the groups. The statistical analysis proved this to be true. In the PND35 age group, no significant difference was observed across all days post-injury between any of the groups. However, the graph (Fig. 2B) shows an obvious difference between the CCI Std group and all the other groups on post-injury days 3 and 4. Therefore, a least significant difference (LSD) post-hoc analysis was conducted for this time period. The analysis showed that PND35 CCI Std animals took significantly longer [$F(3, 27) = 2.343$, $p < 0.05$] to traverse the beam compared to the other three groups on post-injury day 3, but not on post-injury day 4.

While all animals were able to traverse the beam within 30 sec or less, the quality of the traverse was determined by the number of footslips (Fig. 3A and B). Overall analysis for the pre-training showed no main effect of injury group, diet, age, or any interactions between the different categories. However, the overall analysis for the entire post-injury period showed a main effect of the interaction between injury group, diet, and age [$F(1, 43) = 4.586$, $p < 0.05$] and for the interaction between diet and age [$F(1, 43) = 11.262$, $p < 0.05$], but not for diet itself.

On post-injury day 3 (the first day of testing) the PND75 sham Std animals had an average of 2.63% footslips of the total number of right steps (Fig. 3A). This level of performance was seen throughout the testing period. PND75 sham KG, CCI Std, and CCI KG fed animals had a higher percentage of footslips of their total right steps on post-injury day 3 (10.5%, 44.6%, and 68.1%, respectively) compared to sham Std animals (Fig. 3A).

The statistical analysis within the PND75 group showed that there was a significant main effect for injury group (sham or injured) across all days [$F(1, 21) = 17.193$, $p < 0.05$] (Fig. 3A).

On post-injury day 3, the PND35 sham Std animals had an average of 31.2% footslips of the total number right steps (Fig. 3B). The performance improved to 8.54% by post-injury day 7. The PND35 sham KG group showed a slightly better performance with an initial average of 13.87% footslips of the total number of right steps, and at the end of the training period the footslips had decreased to 5.37% (Fig. 3B). PND35 CCI Std

TABLE 1. CHANGES IN BODY WEIGHT (MEAN $\pm$ SEM) FOR THE DIFFERENT INJURY/DIET GROUPS IN THE PND75 AND THE PND35 AGE GROUPS

	PND75 Sham Std	PND75 Sham KG	PND75 CCI Std	PND75 CCI KG	PND35 Sham Std	PND35 Sham KG	PND35 CCI Std	PND35 CCI KG
Initial body weight (g)	325 $\pm$ 2	352 $\pm$ 3	327 $\pm$ 9	334 $\pm$ 5	132 $\pm$ 9	133 $\pm$ 3	138 $\pm$ 13	142 $\pm$ 12
Body weight (g) after motor testing	351 $\pm$ 5	335 $\pm$ 5	343 $\pm$ 12	316 $\pm$ 3	180 $\pm$ 8	126 $\pm$ 7 ^a	183 $\pm$ 11	119 $\pm$ 12 ^a
Body weight (g) after MWM testing	384 $\pm$ 8	378 $\pm$ 5	374 $\pm$ 24	369 $\pm$ 10	245 $\pm$ 7	206 $\pm$ 3 ^a	249 $\pm$ 7	201 $\pm$ 7 ^a

^a $p < 0.05$ relative to age-matched standard-fed animals.

FIG. 2. Mean $\pm$ SEM traverse time for the (A) PND75 and (B) PND35 group between post-injury day -1 through 7. * $p < 0.05$ relative to sham Std and KG animals. † $p < 0.05$ relative to CCI KG animals.

animals showed serious motor impairments, with 78.90% of the right foot steps having slips on post-injury day 3. The ANOVA analysis showed a main effect for injury group and diet across all days [$F(1, 22) = 13.083$, and $F(1, 22) = 7.690$, $p < 0.05$ respectively]. This effect is probably the consequence of the significant difference (shown by LSD post-hoc analysis)

between the CCI Std group and the two sham groups during the entire training period ($p < 0.05$).

Interestingly, PND35 CCI KG animals showed no significant deficit relative to the performances of the sham animals (Std and KG) across the entire training period, while comparison between the injured KG and Std animals showed that

FIG. 3. Mean $\pm$ SEM footslips for the (A) PND75 and (B) PND35 group between post-injury day -1 through 7. * $p < 0.05$ relative to sham Std and KG animals. $^{\dagger}p < 0.05$ relative to CCI KG animals.

the beam-walking performance of CCI KG animals was enhanced. LSD post-hoc analysis showed that it was significantly better during post-injury days 3–6 ($p < 0.05$).

Morris water maze

All animals in the two age groups showed decreases in latency time during the first 2 days of training (Fig. 4A and B). Overall analysis showed a main effect for injury group and for the interaction between diet and age [$F(1, 42) = 24.141$ and

$F(1, 42) = 3.914$, $p < 0.05$, respectively]. Statistical analysis across all blocks between the two sham (Std or KG diet) groups in each age group did not show a significant difference (Fig. 4A and B).

The statistical analysis for the PND75 group showed a significant main effect for injury group across all blocks [$F(1, 22) = 5.028$, $p < 0.05$] (Fig. 4A). This difference is most likely due to the significantly better performance seen among the sham animals (Std and KG) after block 6 compared to the two injured groups (Std and KG). Statistical analysis across all

FIG. 4. Morris water maze mean $\pm$ SEM latencies for the (A) PND75 and (B) PND35 group between post-injury day -1 through 7. * $p < 0.05$ relative to sham Std and KG animals. † $p < 0.05$ relative to CCI KG animals.

blocks revealed that there was no significant difference in MWM performance between the two injured groups, regardless of diet.

Statistical analysis across all blocks for the PND35 animals revealed a significant main effect for injury group and diet [$F(1, 20) = 22.494$ and $F(1, 20) = 3.906$, $p < 0.05$ respectively] (Fig. 4B). These effects most likely come from the difference between the groups seen at and after block 6 (post-injury day 12) in the PND35 group. At this time point, PND35 CCI Std animals had latency times leveling at 22.63 ± 4.13 sec, while the CCI KG group showed escape latencies similar to sham Std animals (12.26 ± 2.20 sec). LSD post-hoc analy-

sis revealed that the CCI Std group had a significantly [$F(3, 24) = 4.330$, $p < 0.05$] greater latency time than shams (Std and KG) and CCI KG animals at this time point (Fig. 4B). During the same time point the PND35 CCI KG animals showed no significant difference compared to the two sham groups.

Morris water maze cueing

All animals regardless of age and injury were able to locate and climb onto the visual platform within 10 sec, indicating that none of the animals had visual or motor deficits. Analysis

TABLE 2. AVERAGE TIME IN SECONDS (MEAN $\pm$ SEM) SPENT IN EACH ZONE DURING THE PROBE TRIAL (POST-INJURY DAY 17) FOR EACH INJURY/DIET GROUP IN THE PND75 AND THE PND35 AGE GROUPS

Group	Zone 1	Zone 2	Zone 3	Zone 4
PND75 sham Std	0.45 $\pm$ 0.26	3.25 $\pm$ 0.67	6.0 $\pm$ 0.93	0.45 $\pm$ 0.45
PND75 sham KG	1.70 $\pm$ 1.01	1.45 $\pm$ 0.50	4.45 $\pm$ 1.44	2.40 $\pm$ 0.22
PND75 CCI Std	1.25 $\pm$ 0.43	3.0 $\pm$ 0.75	3.20 $\pm$ 0.24	2.65 $\pm$ 1.0
PND75 CCI KG	1.85 $\pm$ 1.32	0.90 $\pm$ 0.77	4.65 $\pm$ 2.70	2.65 $\pm$ 1.75
PND35 sham Std	1.48 $\pm$ 0.68	2.43 $\pm$ 0.68	4.45 $\pm$ 1.04	1.68 $\pm$ 0.59
PND35 sham KG	1.04 $\pm$ 0.59	1.60 $\pm$ 1.0	5.84 $\pm$ 1.32	1.52 $\pm$ 0.56
PND35 CCI Std	1.63 $\pm$ 0.37	1.85 $\pm$ 0.76	3.03 $\pm$ 1.06	3.45 $\pm$ 1.04
PND35 CCI KG	2.03 $\pm$ 1.08	2.06 $\pm$ 1.27	3.63 $\pm$ 1.21	2.31 $\pm$ 0.74

of the swim speeds revealed no significant difference due to injury group, age, diet or any interactions between the independent variables.

Morris water maze probe trial

The probe trial was used to determine the ability of the animal to remember the location of the platform 24 hrs after MWM training, and to determine search strategies when the platform was absent. In the PND75 group all of the sham Std and KG animals, 42% of the CCI Std animals, and 63% of the CCI KG animals went directly to the platform.

Among the PND35 animals 75% of the sham Std, 80% of the sham KG, 13% of the CCI Std, and 43% of the CCI KG animals went directly to the platform. See Table 2 for the time spent in each quadrant.

Discussion

The results of the current study show that administration of the ketogenic diet for 7 days beginning immediately after TBI significantly improves both motor and cognitive recovery in PND35 rats. This improvement was not observed among PND75 animals. These findings also support previous studies (Prins et al., 2004, 2005; Sullivan et al., 2004) showing that the ketogenic diet has an age-dependent neuroprotective effect.

The effects of the ketogenic diet on motor recovery

The results of the current motor experiments revealed only an acute deficit in traverse times among PND35 CCI Std rats,

but no deficits in the PND75 CCI (Std or KG) group. Earlier studies have shown an approximately two- to fourfold increase in traverse time, depending on the severity of the injury, for adult injured animals compared to sham animals at post-injury days 1 and 2 (Statler et al., 2006; Piot-Grosjean et al., 2001). The lack of detectable deficits in beam traverse times among PND75 CCI animals in the present study may be explained by the comparatively milder injury and/or the difference in beam width used. Previous studies have used a beam width of 2.5–2.7 cm compared to the 1.9 cm-wide-beam used in the present study for the PND75 group. A narrower beam may make it more difficult for even sham animals to traverse, and therefore may diminish any injury effects. Additionally, beam-walking testing starting on post-injury day 3 may have been too late to detect acute deficits.

While traverse times did not show significant deficits, the manner in which the animals traversed (footslips) did show acute deficits in the CCI Std and CCI KG groups for PND75 animals, and in the CCI Std group for PND35 animals.

PND35 animals administered the KG diet did not show traverse time deficits and demonstrated the fewest footslips compared to age-matched control animals. This result was not seen among PND75 animals maintained on the KG diet, and both traverse times (post-injury days 3–4) and footslips (post-injury days 3–7) were greater compared to the PND75 control group on the Std and the KG diet. This exacerbation of performance may be attributable to the increase in hyperactivity observed among PND100 rats on the KG diet (Ziegler et al., 2005). The same treatment has been shown to have the opposite effect in PND40 animals. Murphy and Burnham (2006) showed a decrease in activity level after 24 h in young rats kept on a KG diet. These and the present results show that treatment with a ketogenic diet can produce different outcomes, depending on cerebral maturation.

The effects of the ketogenic diet on cognitive recovery

Both PND35 and PND75 sham animals showed MWM acquisition patterns consistent with previous findings (Prins and Hovda, 1998). For example, all three groups in the PND35 age group showed longer latency times compared to their counterparts in the PND75 age group. However, the overall analysis of swim speeds revealed no significant difference. The fact that the younger animals showed longer latency times is most likely due to the juveniles' greater will to explore their surroundings.

CCI Std animals from both age groups also showed expected deficits in latency times. However, among CCI KG-treated groups, only the PND35 animals showed improvement. This cognitive improvement was not observed among PND75 CCI KG animals. While it is clear from the literature that ketone metabolism can contribute significantly to adult brain metabolism, it requires time. Following 48 h of starvation, arterial ketones reach 2.0 mM in adults and 2.58 mM in PND20 rats. Despite achieving similarly high circulating levels, the arteriovenous difference for ketones is -0.075 mM in adults and -0.174 mM for PND20 rats (Hawkins et al., 1971). It is only after 96 h of fasting that the adult brain shows -0.10 mM uptake of ketones. In the case of starvation or more slowly-evolving neuropathological conditions, ketone metabolism may have the same effect in the mature brain as in the immature.

FIG. 5. Histology. Comparison of contusion volume between study groups.

However, for the ketogenic diet to be neuroprotective under rapidly-evolving pathological conditions like TBI, it must get into the brain very shortly after administration and in sufficient quantity. Under the conditions of the present study, age-related neuroprotective differences may be explained by the enhanced ability of PND35 rat brains to utilize ketones as cerebral fuel. The normal developing brain has been shown to have greater expression of monocarboxylate transporters (MCTs) (Leino et al., 1999; Vannucci and Simpson, 2003), as well as greater activity of enzymes associated with ketone metabolism (Page et al., 1971). Following TBI, the microvessel expression of MCT2 and MCT1 has also been shown to be greater in the PND35 rat compared to adults (Prins and Giza, 2006; Prins et al., 2007), suggesting even greater capacity for ketone uptake after injury. These age-related differences in ketone uptake and metabolism likely contribute to the age-related neuroprotective effects following a rapidly evolving cerebral injury.

However, in contrast to these neuroprotective effects, Zhao and colleagues (2004) showed that prolonged administration (30 days) of the ketogenic diet in PND20 rats impaired cognitive behavior in both sham and seizure-induced animals. The inconsistency of this finding with our current results may be due to age, the shorter administration period (7 days), or differences in the MWM training design.

Ketogenic neuroprotection

There is an increasing body of literature demonstrating the neuroprotective effects of cerebral ketone metabolism under various neuropathological conditions (hypoxia, ischemia, glutamate excitotoxicity, TBI, Parkinson's disease, and Alzheimer's disease). A more detailed description of these findings has been recently reviewed (Prins, 2008).

TBI initiates secondary injury caused by a cascade of different events leading to changes in the intra- and extracellular environment of the brain (Hovda et al., 1992). Earlier studies have reported a depression in glucose metabolism (Hovda et al., 1991, 1992; Yoshino et al., 1991; Sutton et al., 1994), an increase in the production of reactive oxygen species (ROS) (Marklund et al., 2001), increased activation of the pentose phosphate pathway (PPP) (Bartnik et al., 2005), and an increase in poly ADP-ribose polymerase (PARP) activity (LaPlaca et al., 1999; Besson et al., 2003). The decrease in glucose metabolism may reflect altered glucose processing during a time of energy crisis. The increase in PARP activation is likely

to be due to repair of DNA damage caused by ROS. Since PARP requires NAD^+ to function, increased PARP activation has been shown to deplete cytosolic NAD^+ . Reduced NAD^+ can decrease glyceraldehyde-3-phosphate dehydrogenase activity (a key enzyme in the glycolytic pathway), which leads to glycolytic dysfunction. The increase in PPP activity suggests that more glucose-6-phosphate is utilized in the production of NADPH, which is used in the detoxification of ROS. These findings suggest that there is a change in the metabolic fate of glucose after TBI.

In our laboratory, we have shown a reduction in the contusion volume in PND35 CCI KG animals compared to both age-matched Std-fed animals and adult animals on KG or Std diet (Prins et al., 2005), which is consistent with the result in the current study (Fig. 5). This cellular preservation at the primary injury site may not alone contribute to the improved behavioral outcome, but it may lead to connections between the different areas of the brain that are intact and functional, which in turn would be important to the overall function of the brain. While the hippocampus was not directly impacted by the injury in the present study, the cells are still affected and will experience dynamic changes in metabolism, making them more vulnerable to functional impairments. This is consistent with the MWM deficits seen among CCI-injured animals in the present study.

The improved MWM performance among PND35 KG CCI animals may be related to improved hippocampal metabolism. Ketone metabolism has been shown to have unique properties that make it a favorable cerebral fuel under various conditions. Ketones have been shown to improve mitochondrial metabolic efficiency and decrease oxygen consumption, while increasing energy from the electron transport chain (Kashiwaya et al., 1994; Sato et al., 1995; Veech et al., 2001), and decreasing the production of free radicals (Sullivan et al., 2004; Ziegler et al., 2003). In addition, an increase in the cerebral uptake and utilization of ketones (Owen et al., 1967; Massieu et al., 2003), enhancement in the activity of ketone enzymes (Sokoloff, 1973), and the increase in expression of MCTs (Cremer et al., 1976) has been detected in animals maintained on a KG diet.

The lack of improved MWM performance among the adult animals treated with the ketogenic diet may be related to their decreased capacity to utilize ketones rapidly. Studies have shown a decrease in plasma ketone concentrations (Vannucci and Vannucci, 2000; Sokoloff, 1973; Hawkins et al., 1971; Lust et al., 2003), ketone enzymatic activity (Sokoloff, 1973; Lust

et al., 2003), and vascular MCT expression (Gerhart et al., 1997; Cremer et al., 1976; Leino et al., 1999) with cerebral maturation.

The findings of this study support the previously reported age-dependent nature of ketogenic neuroprotection after TBI, and expands this protection to include both improved motor and cognitive outcome after TBI among PND35 rats.

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Author Disclosure Statement

No competing financial interests exist.

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