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Detrimental Effects of the Ketogenic Diet on Cognitive Function in Rats

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ABSTRACT

The ketogenic diet (KD) is a high-fat, low-carbohydrate, and low-protein diet that is widely used to treat epilepsy in children. Although the KD has been shown to be efficacious in the treatment of childhood epilepsy, the long-term effects of the KD on brain development are not clear. The objective of this study was to examine the long-term effects of the KD on visual-spatial memory, activity level, and emotionality in immature rats after status epilepticus (SE). Weanling rats were subjected to lithium/pilocarpine-induced SE or saline injections and were then randomized to either the KD or regular rat diet, both fed *ad libitum*. One month later, rats were evaluated for visual-spatial memory in the water maze, activity level in the open field test, and emotionality with the handling test. Spontaneous recurrent seizures were measured using videotaping, and seizure susceptibility was tested with flurothyl inhalation. Brains were weighed and examined for mossy fiber sprouting and cell loss. Although rats treated with the KD were active and seemed healthy, their weight gain was substantially lower than that in rats that received regular rat diet. The KD reduced the number of spontaneous seizures but had no discernible effect on flurothyl seizure susceptibility. KD-fed rats, with or without SE, had significantly impaired visual-spatial learning and memory compared with rats that were fed regular diet. The KD had minimal effects on activity level and emotionality. Rats that were treated with the KD had significantly impaired brain growth. No differences in pathology scores between the KD and regular diet groups were seen after SE. Despite reducing the number of spontaneous seizures after SE, the KD resulted in severe impairment in visual-spatial memory and decreased brain growth, with no effect on mossy fiber sprouting. This study raises concerns about the long-term effects of the KD on brain development. **(*Pediatr Res* 55: 498–506, 2004)**

Abbreviations

BHB, β-hydroxybutyrate
KD, ketogenic diet
NoSE-Cont, no status epilepticus, control diet group
NoSE-KD, no status epilepticus, ketogenic diet group
P, postnatal day
SE, status epilepticus
SE-Cont, status epilepticus, control diet group
SE-KD, status epilepticus, ketogenic diet group

The ketogenic diet (KD) is a high-fat, low-carbohydrate, and low-protein diet that is now widely used to treat epilepsy (1–4). Although the diet has not undergone the rigorous evaluation process required for marketing of antiepileptic drugs, there is now a considerable body of evidence indicating that the KD is efficacious in the treatment of epilepsy (1, 2, 5, 6).

Despite the use of the KD over several decades, the long-term effects of the KD on cognition are not yet known. Anecdotal reports suggest improvements in alertness, cognitive function, and behavior (2), although impairments in patients have also been reported (7). In a prospective study on the effects of the KD, Pulsifer *et al.* (8) found that developmental quotients in children on the diet improved after 1 y. One of the difficulties in evaluating cognitive effects of the KD in children is that the diet is rarely continued in children who do not respond with a reduction in seizure frequency (5, 9). The improvement in cognitive function therefore may represent the effects of reduced seizures, a positive cognitive effect of the diet, or a combination of these factors. Because the KD is not administered to healthy children, it is unlikely that clinical studies will provide definitive answers about the effects of the KD on cognitive function.

In this regard, experimental animal models may provide insight into the effects of the KD on cognitive function. The

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KD is well tolerated by rats and has been shown by most authors to suppress seizures in some rodent models of seizures (10–17). In this study, we examined the cognitive and behavioral effects of the KD when administered to immature rats with and without a history of status epilepticus (SE). We report that KD treatment is associated with serious adverse effects on spatial learning and memory.

METHODS

Animals. A total of 60 male Sprague-Dawley rats were used in the experiments. Rats were housed with their litter until weaning at postnatal day (P) 20. After SE, they were group-housed (two per cage) in plastic cages under diurnal lighting conditions with lights on from 0700 to 1900 h. All efforts were made to minimize animal suffering, and all animal procedures were in compliance with National Institutes of Health and Children's Hospital Boston guidelines. The protocol was reviewed and approved by the Animal Care and Use Committee of Children's Hospital Boston.

Lithium-pilocarpine–induced SE. SE was induced by using the lithium-pilocarpine model, which reproduces the main clinical and neuropathologic features of human temporal lobe epilepsy. Neuronal damage occurs mainly in the hippocampus, dentate gyrus, piriform and entorhinal cortices, septum, thalamus, amygdala, and neocortex. Pretreatment with lithium chloride potentiates the pilocarpine effect. Previous studies have demonstrated that lithium-pilocarpine does not produce substantial hippocampal damage during the first 2 wk of life (18, 19). We therefore elected to induce SE in weanling, P20 animals that roughly correspond in age to a human toddler (20).

At P19, 40 rats received an i.p. injection of lithium chloride, 127 mg/kg; 18–19 h later, rats received an s.c. injection of pilocarpine hydrochloride, 60 mg/kg, for induction of SE; the controls ($n = 20$) received an injection of the same amount of normal saline. After pilocarpine injection, all rats progressed to SE. The onset of SE was characterized by initial immobility and chewing followed by repetitive clonic activity of the trunk and limbs. The rats then developed repeated rearing with forelimb clonus and falling interspersed with immobility, chewing, and myoclonic jerks occurring singularly or in series. Fifteen (37.5%) of the SE rats died during or within 72 h of the SE. This mortality rate is not unusual for lithium-pilocarpine–treated rats in this age range (18). None of the controls died. The 25 surviving rats were randomly divided into two groups: SE KD (SE-KD) group ($n = 13$) and SE control diet (SE-Cont) group ($n = 12$). All had SE for at least 5 h after pilocarpine injection, and the two treatment groups did not differ in regard to duration or severity of the seizures. The 20 rats that received an injection of normal saline were also divided into two groups: no SE KD (NoSE-KD) group ($n = 10$) and No SE control diet (NoSE-Cont) group ($n = 10$).

Diets. All control animals were fed rodent chow (Purina #5001), whereas experimental rats were fed a modified high-fat (78.8% of calories derived from fat) meal. A description of the constituents of both the KD (Bio-Serve, Frenchtown, NJ, U.S.A.; Formula Rat Diet AIN-76 Modified, High-Fat, product #F3666) and normal diets is provided in Table 1. In the KD diet, the proximate profile was protein 8.36%, fat 78.80%, and carbohydrate 0.76% for a fat:(protein + carbohydrate) ratio of 8.6:1. This diet has been used by other investigators and has been demonstrated to result in elevated '24-hydroxybutyrate (BHB) levels (10, 14). Water was provided for all animals *ad libitum* throughout the studies.

Water maze. All rats were tested in the water maze 30 d after initiation of the KD between P53 and P57. Rats remained on the diet throughout the testing. A plastic circular swimming pool (200 cm diameter; 50 cm high) was filled to a depth of 25 cm with water. A total of 200 mL of evaporated milk was added to make the water opaque and prevent visualization of the platform. Four points on the rim of the pool were designated north (N), south (S), east (E), and west (W), thus dividing the pool into four quadrants (NW, NE, SE, SW). An 8 × 8-cm Plexiglas platform, onto which the rat could escape, was positioned in the center of one of the quadrants, 1 cm below the water surface. Because of the marked differences in weight, it was not possible to blind the groups to the examiner.

One day before the test, each rat was placed in the pool for 60 s without the platform present; this free swim enabled the rat to become habituated to the training environment. On days 1–4, rats were trained for 24 trials (six trials a day) to locate and escape onto the submerged platform. For each rat, the quadrant in which the platform was located remained constant, but the point of immersion into the pool varied between N, E, S, and W in a quasi-random order for the 24 trials so that the rat was not able to predict the platform location from the point at which it was placed into the pool. The latency from immersion into the pool to escape onto the platform was recorded for each trial, and the observer also manually recorded the route taken by the rat to reach the platform. All trials also were videotaped for subsequent analysis of swimming path. On mounting the platform, the rats were given a 30-s rest period, after which the next trial was started. If the rat did not find the platform in 120 s, then it was manually placed on the platform

Table 1. *Composition of control and KD*

Constituent	KD (%)	Normal diet (%)	Normal diet (%)
Lard	47.5	Saturated fat	1.5
Butter	19.95		
Corn oil	11.4	Unsaturated fat	8.5
Protein	9.5	Protein	23.4
Fiber	5.0	Fiber	5.3
Ash*	3.8	Ash	6.9
Vitamin mix†	2.09		
Dextrose	0.76	Carbohydrates	49.0

* The formula contains the following mineral mix (g/kg): calcium phosphate, dibasic 500.0, potassium citrate 220.0, sucrose 117.53, sodium chloride 74.0, potassium sulfate 52.0, magnesium oxide 24.0, ferric citrate 6.0, magnesium carbonate 3.5, zinc carbonate 1.6, chromium potassium sulfate 0.55, calcium phosphate, tribasic 0.5, cupric carbonate 0.3, potassium iodate 0.01, and sodium selenite 0.01.

† The vitamin mix contain the following (g/kg): sucrose 971.73, vitamin E acetate 20.0, niacin 3.0, calcium pantothenate 1.6, retinyl palmitate 1.0, pyridoxine 0.7, riboflavin 0.6, thiamine 0.6, vitamin D (400,000 IU/g) 0.25, folic acid 0.2, menadion sodium bisulfite (33%) 0.2, vitamin B_{12} (1%) 0.1, and biotin 0.02.

for a 30-s rest. At the start of each trial, the rat was held facing the perimeter and dropped into the pool to ensure immersion.

One day after completion of the last latency trial, the platform was removed and animals were placed in the water maze in the quadrant opposite to where the platform had previously been located. The path and the time spent in the quadrant where the platform had been previously placed were recorded. In this part of the water maze, the probe test, normal animals typically spend more time in the quadrant where the platform had been previously located than in the other quadrants.

The Morris water maze is a test of hippocampal-dependent spatial memory (21, 22), the closest parallel to episodic memory in humans (23–25). The testing procedure used during the 4 d of locating the hidden platform provides a measure of spatial reference memory, whereas the probe trial is a measure of the strength of spatial learning (25, 26). The water maze offers advantages over other tests that measure memory in that it is not necessary to use food deprivation or food rewards to motivate the behavior of the animals during the spatial learning procedure. This test has been used extensively in our laboratory (27, 28).

Open field test. After completion of the water maze, the rats were evaluated for activity level in the open field test (29–31). The open field test is a measure of the animals' reaction to a novel environment. Rats were placed in a closed area with the floor divided into 64 equal squares (10 cm^2). The testing area was cleaned after each trial, and ambient lighting and noise remained constant throughout both testing days; testing was done between 1000 and 1200 h. The number of squares crossed in 2 min was measured during 2 consecutive days of testing. A duration of 2 min provides a short enough interval to keep the environment novel; longer periods of time leads to habituation to the environment.

Handling test. On P80–81, emotional responses were systematically studied by observation of the reaction of the rat to nonstressful handling (slow rubbing of the hand along the rat's back) and to stressful handling (brisk rubbing against the grain of the rat's fur on the back sides with a gloved hand) (32). In addition, mildly painful stimulations were applied in a quantified manner by pinching the tail with rubber tweezers at the tip and the base. A score of 1 indicated that the rat initially struggled but become calm within 15 s. A score of 2 indicated that the rat struggled >15 s. A score of 3 indicated that the rat struggled >15 s and exhibited defensive reactions consisting of one or more of the following: piloerection, flattening of the ears against the head, attempting to bite, or backing away from the examiner. A score of 4 indicated that the rat struggled for >15 s and exhibited flight behaviors of loud vocalization or wild running. The animals were always tested between 1200 and 1600 h by the same examiner. The total score for nonstressful, stressful, and painful handling over 2 consecutive days of testing was calculated.

Flurothyl-induced seizures. Seizures were induced by flurothyl inhalation as described previously (27, 30). Flurothyl (bis-2,2,2-triflurothyl ether; Aldrich Chemical Co., Milwaukee, WI, U.S.A.) is a rapidly acting CNS stimulant that produces seizures within minutes of exposure. At P80, rats were placed in a small, plastic, cylindrical chamber (radius = 7.5 cm, height = 18 cm). Liquid flurothyl was delivered through a plastic syringe and dripped onto filter paper in the center of the chamber. The flurothyl was administered *via* a pump at a constant rate of 3 mL/h. The chamber was flushed with room air and cleaned between trials. Rats were exposed to flurothyl until tonic extension of both the forelimbs and hindlimbs was observed. Rats were then quickly removed from the chamber and allowed to recover in room air until they returned to their baseline activity level. After recovery, rats were returned to their cages. The latencies to the first bout of forelimb clonus and hindlimb clonus were recorded.

Ketosis. Ketosis was assayed by spectrophotometrically measuring the levels of BHB present in blood plasma using a StatSite meter (GDS Technologies, Elkhart, IN, U.S.A.). Blood samples (0.2–0.3 mL) were collected from the tail vein of the rat at the same time of day (between 1100 and 1600 h) after >60 d of maintenance on respective diets. Samples were immediately transferred to 3-mL Li^+-heparin Vacutainers and centrifuged at 2000 × *g* for 5 min. BHB levels were determined using 28 μL of plasma.

Spontaneous seizures. Animals were videotaped for 6 h for the presence of spontaneous seizure for 4 consecutive days at P55. Blinded observers who were not familiar with the study design (Y.H. and Q.Z.) reviewed the tapes and recorded the number of seizures.

Histology. After completion of the behavioral testing, the rats were killed with a lethal dose of pentobarbital, and the brains were removed, weighed, sectioned, and stained using thionin and Timm stain for the axons and terminals of the dentate granule cell axons, the so-called mossy fibers. After an overdose with sodium pentobarbital (100 mg/kg), rats were perfused transcardially with 200 mL of sodium sulfide perfusion medium (2.925 g of Na_2S, 2.975 g of NaH_2PO_4, H_2O in 500 mL of distilled H_2O) followed by 200 mL of 4% paraformaldehyde. Brains were weighed, and the distances from the frontal lobes to the occipital region and across the parietal region were measured. The brains were then postfixed in paraformaldehyde for 24 h and then placed in a 30% sucrose solution until the brains sank to the bottom of the chamber. Coronal sections through the entire extent of the hippocampus were cut at 40 μm on a freezing microtome, and sections were stored in PBS (pH 7.3). Every fourth section was stained for mossy fibers by using the Timm stain, and alternate sections were stained with thionin for cell counting.

The sections were developed in the dark for 40–50 min in a solution of 50% Arabic gum (120 mL), 10 mL of citric acid (51 g/100 mL H_2O), 10 mL of sodium citrate (47 g/100 mL H_2O), 3.47 g of hydroquinone in 60 mL, and 212.25 mg of $AgNO_3$. Slides from control animals were always stained at the same time as slides from animals with serial seizures or SE. After washing, the slides were dehydrated in alcohol, cleared in xylene, and mounted on slides with Permount.

Timm staining was analyzed in a semiquantitative scale ranging from 0 to 5 for terminal sprouting in CA3 and the supragranular region as previously described (33–35). Timm staining in the pyramidal and infrapyramidal CA3 regions and the supragranular region was assessed on each section from the septal area, where the two blades of the dentate were equal and

formed a V shape (2.8 mm posterior from the bregma) to a point approximately 3.8 mm posterior to the bregma (36). Assessment of the Timm score in the supragranular region was done in the inferior blade of the dentate, avoiding the edge and crest of the gyrus. Both hippocampi of the specimens were analyzed, with the score given to the CA3 and supragranular regions reflecting the mean for the two sides. Although seizure-related brain damage is widespread, for the purposes of consistency and reproducibility, we limited quantification of sprouting to an area of the hippocampus with well-documented cell loss or synaptic reorganization (27, 30).

Thionin slides were analyzed for cell loss in the CA3, the CA1, and the hilus using a semiquantitative scoring system ranging from 0 to 5: a score of 0 indicated no lesion; 1 indicated scattered cell loss, 2 indicated moderate cell loss occurring in clusters, 3 indicated substantial cell loss disrupting normal architecture of the region, 4 indicated extensive loss of cells with only remnants of the cell layer remaining, and 5 indicated total loss of neurons (35). Five brain sections at the level of the dorsal hippocampus on both sides were examined, and the scores were added to obtain a total score for each region (5 sections $\times$ 2 hippocampi = 10 hippocampi per rat). This score was divided by 10 to determine a mean score per hippocampus per rat. All histologic scoring was done by an investigator (G.L.H.) who was blinded to treatment group.

Statistical analysis. Latencies to the escape platform in the four groups were evaluated for variance (Bartlett's test for equal variance) and normality (Gaussian-shaped distribution) using the Kolmogorov-Smirnov goodness-of-fit test. Differences between groups in escape latencies to the water maze platform for each day of testing were assessed using the two-way ANOVA. Mean times to the escape platform for each day of testing, time spent in the target quadrant during the probe test, and swimming speed were compared in the four groups using the ANOVA. *Post hoc* comparisons were made using the *t* test. The histology scores were also analyzed for variance and distribution. When the data passed the normality test ($p > 0.10$), group means were compared with the ANOVA. The nonparametric Kruskal-Wallis was used for the handling test.

RESULTS

Rats seemed to return to their baseline behavior by 24 h after the SE. At the time the animals were randomized to the four treatment groups, there were no differences in body weight (SE-KD 46.29 $\pm$ 1.07 g; SE-Cont 42.93 $\pm$ 1.37 g, NoSE-KD 42.68 $\pm$ 1.52 g, NoSE-Cont 42.5 $\pm$ 1.67 g; $p = 0.159$). The animals tolerated the diet well, with none of the animals losing weight after starting the KD or regular rat chow. As previously described (10, 37, 38), animals that consumed the KD gained substantially less weight than the controls in both the SE and NoSE groups (weights at P57: SE-KD 123.5 $\pm$ 9.06 g; SE-Cont 205.6 $\pm$ 8.60 g, NoSE-KD 117.1 $\pm$ 8.10 g, NoSE-Cont 251.1 $\pm$ 9.52 g; $p < 0.001$). Both the SE and NoSE groups were significantly lighter than the corresponding groups that received regular rat chow ($p < 0.001$). Although small and thin with oily fur, the animals were active and showed no evidence of infectious or respiratory complications. None of the animals died after the initial bout of SE.

Water maze. Both the controls and SE rats swam well without any impairment of mobility. As demonstrated in Figure 1, all four groups demonstrated improvements in water maze performance during the 4 d of testing with decreases in latency to the platform ($F = 27.82$, $p < 0.001$). There were marked differences in time to platform among the four groups ($F = 18.26$, $p < 0.001$). Rats that were subjected to SE performed substantially more poorly than non-SE rats ($F = 25.98$, $p < 0.001$).

Animals that were administered the KD had substantially worse performances in the water maze than animals that received regular rat chow. In the SE group, the KD group was impaired compared with the rats that received regular chow ($F = 5.25$, $p = 0.003$). Likewise, in the NoSE groups, animals that received the KD did worse in the water maze than the NoSE rats that received regular rat chow ($F = 25.31$, $p < 0.001$). The detrimental effects of the KD were dramatic. Animals that were not subjected to SE and received the KD did as poorly in the water maze as SE rats that received the control diet.

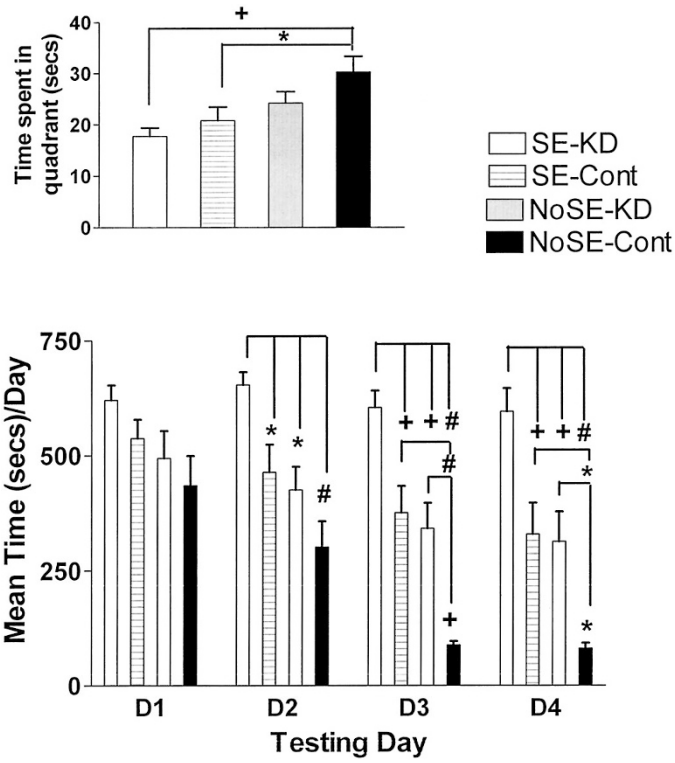

Figure 1. Comparison of escape latencies to platform in the water maze (mean $\pm$ SE) in SE and animals that were administered either the KD or the regular diet. The abscissa lists the day of training; the ordinate is mean time for each day of testing. Both the SE and No-SE animals had shorter latencies to the escape platform over the 4 d of testing. Both the SE and non-SE rats that received the KD performed substantially poorer than corresponding groups that received regular chow. The insert is the result of the probe test with mean time ($\pm$SE) spent in the target platform. SE rats spent less time in the target quadrant than non-SE rats. $^{*}p < 0.05$; $^{+}p < 0.01$; $^{\#}p < 0.001$. SE-KD, status epilepticus, ketogenic diet group; SE-Cont, status epilepticus, control diet group; NoSE-KD, no status epilepticus, ketogenic diet group; NoSE-Cont, no status epilepticus, control diet group.

The probe test also showed differences between groups ($F = 4.92$, $p = 0.005$). As can be seen by the insert of Figure 1, SE animals spent less time in the target quadrant than NoSE animals, with the KD groups in both instances spending shorter periods in the target quadrant than the corresponding groups that received the regular diet. Statistical significance was reached only for the comparisons between the two SE groups *versus* the NoSE group that received regular rat chow.

As shown in Figure 2, we addressed the question of whether the differences in water maze spatial learning could be related to swimming speed. There were differences between groups ($F = 3.281$, $p = 0.031$), with KD rats swimming faster than rats that received the regular diet. However, statistical significance was reached only between the SE-KD and SE-Cont groups, with the SE-KD rats swimming faster than SE animals that received regular chow. Thus, although the SE-KD rats swam faster than the SE-Cont animals, they took longer to find the platform than the SE-Cont. In addition, there was no difference in motivation to find the platform, as indicated by time to find the platform on the first trial with the escape platform present (SE-KD 102.30 ± 10.15 s, SE-Cont 108.81 ± 7.55 s, NoSE-KD 99.90 ± 13.40 s, NoSE-Cont 105.51 ± 10.23 s; $p = 0.933$).

Taken together, these results show that SE is associated with a deficit in hippocampal-dependent spatial learning and memory. KD treatment significantly exacerbated this cognitive impairment. In addition, even the KD alone (NoSE group) was associated with impaired spatial memory.

Open field test. As shown in Figure 3, animals on the KD were more active on the first day of the open field test compared with the non-KD groups ($F = 3.523$, $p = 0.023$). However, no statistical differences were noted on the second day of testing or when the two daily scores were combined.

Handling test. Total mean score for the handling tests differed, with the NoSE-KD showing more aggressiveness and irritability when handled than the SE group that received the regular rat chow (SE-KD 4.54, SE-Cont 4.33, NoSE-KD 5.70, NoSE-Cont 4.60; $p = 0.005$).

Spontaneous recurrent seizures. During the course of daily handling or observation, spontaneous seizures were observed in 2 of 11 of the SE-KD group and 6 of 10 of the SE-Cont group, a statistically nonsignificant difference. Seizure frequency was low and did not differ statistically between the SE-KD (0.2/h) and the SE-Cont (0.3). The seizures were typically brief, lasting <15 s in all cases except one. The seizures consisted of forelimb clonus. One rat from the SE-KD group had a seizure that lasted approximately 60 s with forelimb clonus, rearing, falling, and tonic posturing.

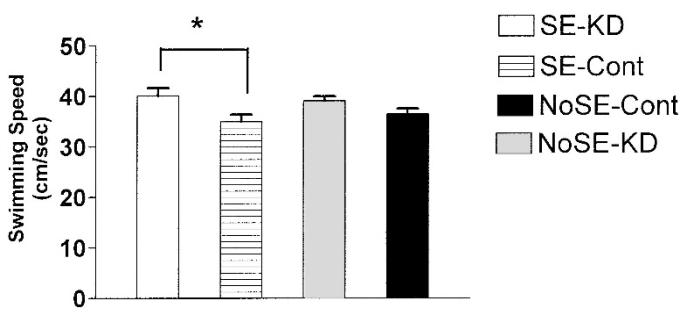

Figure 2. Swimming speed in the four treatment groups. The abscissa lists the groups; the ordinate is mean swimming time. Swimming speed was comparable in the four groups, with the KD-treated animals swimming slightly faster than animals that were given regular rat chow. The groups did differ ($p = 0.038$) with *post hoc* testing demonstrating a difference between the SE-KD group and SE-Cont group (*$p < 0.05$).

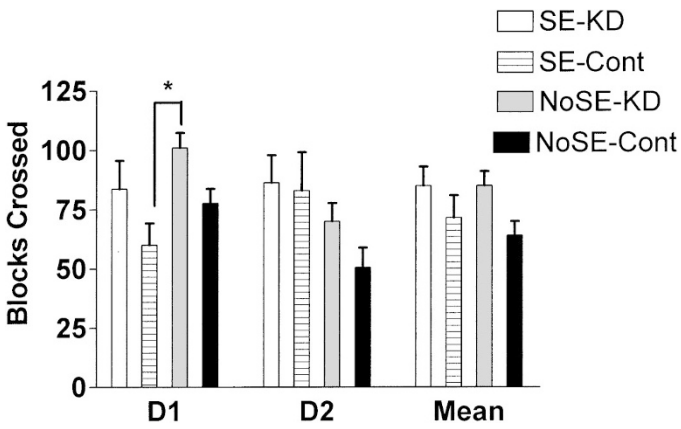

Figure 3. Comparison of open field test in the four treatment groups. On day 1 of testing, rats that received the KD were more active than corresponding groups that received regular rat chow ($p = 0.023$; *$p < 0.05$).

BHB levels. As expected from other studies (37, 39), BHB levels were significantly higher in the KD groups than in the rats that received regular rat chow (KD group 3.398 ± 0.817 mmol/L, regular chow 0.348 ± 0.070 mmol/L; $t = 3.930$, $p < 0.001$).

Seizure threshold. Flurothyl inhalation induced myoclonic and tonic seizures in all of the animals. There were no differences between onset of myoclonic seizures in the four groups. Onset to tonic seizures was longer in the NoSE-KD than in the SE-KD group (Fig. 4). Seizure severity seemed similar in all four groups. The KD therefore did not alter seizure threshold in a model of generalized seizures.

Brain weights and histology. There were substantial differences between brain weight in the four groups, with the SE-KD brains being lighter than the other groups (Fig. 5). Brain lengths and widths also differed, with the SE-KD rats having smaller brains than the other groups (length $p = 0.024$; width $p = 0.022$).

Animals with SE had higher cell loss scores in all three brain regions than the NoSE groups (Fig. 6). The presence or absence of the KD had no effect on cell loss scores. Animals with SE also had higher Timm scores in both CA3 and the supragranular region than the NoSE groups. Examples of the histologic patterns are shown in Figure 7. There were no differences between the KD and regular chow groups.

DISCUSSION

The main finding of this study is that young rats that were treated with the KD diet for 1 mo had deficient spatial learning and memory in the Morris water maze, regardless of whether they experienced lithium-pilocarpine–induced SE. The spatial learning deficit was worse in KD-treated animals that had SE, even though those rats had a modest reduction in the number of

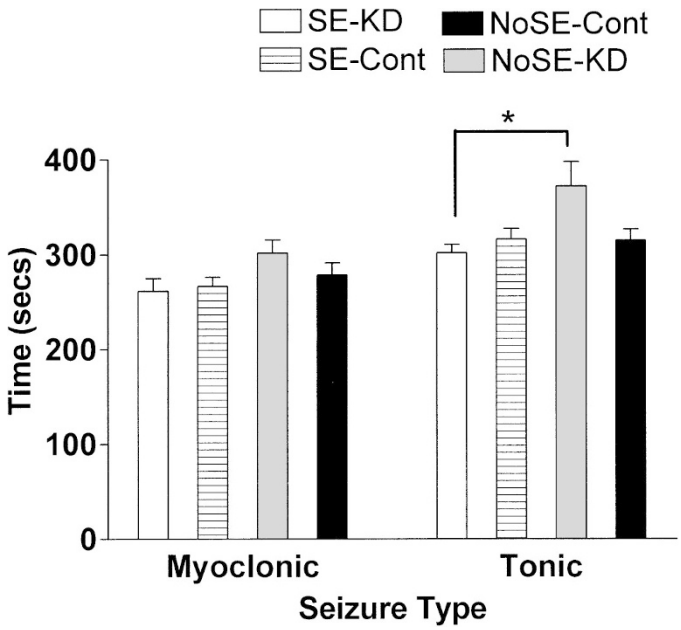

Figure 4. Comparison of time to onset of myoclonic and tonic seizures in the four treatment groups. Time to onset of tonic seizures was higher in the NoSE-KD than in the SE-KD group (*$p < 0.05$).

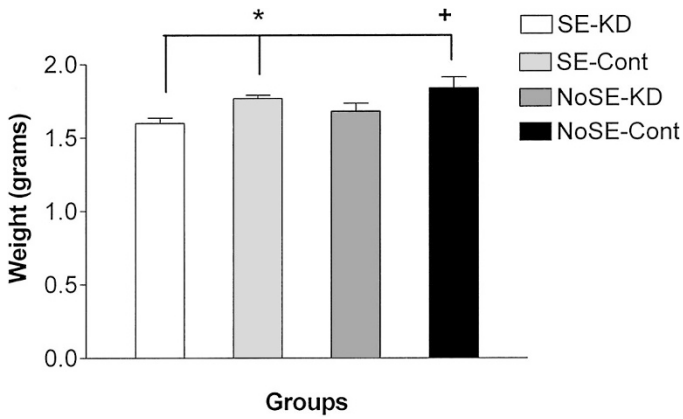

Figure 5. Comparison of brain weights in the four treatment groups. The groups differed ($p = 0.003$), with the SE-KD having lower brain weights than the SE-Cont and NoSE-Cont groups (*$p < 0.05$; **$p < 0.01$).

seizures. These findings raise concerns about the safety of the KD in young animals—and possibly humans—from the cognitive viewpoint. As previously described (34, 35), rats that underwent SE at P20 and were tested during adolescence had a substantial impairment of visual-spatial memory in the water maze, a task that is hippocampus-dependent. The SE animals had pathologic lesions of the hippocampus with cell loss and sprouting of the mossy fibers into the supragranular region of the dentate gyrus. In the present study, the KD exacerbated the impairment of spatial memory in rats that were subjected to SE. In addition, control rats that were not subjected to SE but received the KD were substantially impaired in the water maze–learning task. The impaired water maze performance was not secondary to poor swimming ability. The KD rats actually swam faster than the control animals but were significantly slower in getting to the platform than the controls. Likewise, motivation to find the escape platform was not impaired in the SE-KD group. Rather, the deficits in performance seemed to be related to the lack of ability to learn and remember the platform location. The degree of impairment in spatial memory was remarkable, especially because spontaneous seizure frequency was lower in the SE-KD group than in rats that received regular rat chow. In our study, SE was associated with a similar degree of mossy fiber sprouting, regardless of dietary treatment. Similarly, there was no difference in SE-related cell loss in KD- or control-fed rats. The KD by itself did not cause either cell loss or mossy fiber sprouting.

Our findings differ somewhat from other studies that used the KD after SE. Muller-Schwartze *et al.* (40) found that administration of the KD after kainic acid–induced SE reduced both spontaneous seizure frequency and mossy fiber sprouting. Likewise, Noh *et al.* (41) found that the KD inhibited apoptosis after kainic acid–induced SE.

Other investigators have also reported cognitive deficits associated with either a high-fat diet (42, 43) or the KD (10). Su *et al.* (10) found that rats that were treated with the KD after kainic acid–induced SE showed greater deficits in spatial memory than rats that were given regular rat chow. The authors found that impaired water maze performance occurred despite a reduction in seizures in animals on the KD. It is interesting that Su *et al.* (10) found no discernible histologic damage after the SE. Our study confirms and expands these findings by demonstrating that KD-treated rats that were not subjected to SE also show impairment in spatial learning. Rats that were subjected to SE in our study had substantially more histologic damage than the rats in the Su *et al.* (10) study, demonstrating that the KD can be detrimental regardless of the degree of histologic damage. However, it should be noted that the agent used to induce SE differed in the two studies, as did the age of SE induction. Cognitive deficits associated with the KD might also be model and age dependent. In the kindling model, there was no difference in water maze learning or open field test performance between KD-fed and control-fed adult rats (15).

One of the striking findings in the rats that were treated with the KD was poor growth, a phenomenon reported by other investigators who studied the KD (10, 37, 38). Although none of the rats lost weight during the study, there were large differences in body and brain weight and size between the KD-treated and rats that received regular chow. The adverse consequences of the KD in this study therefore may be related to protein or protein-calorie malnutrition. In the diet used here, protein composed only 8% of the diet, which is less than half of the protein content of the regular diet.

It is known that protein-calorie malnutrition can produce morphologic, neurochemical, neurophysiologic, or functional alterations in the developing brain (44–50). However, despite these changes in brain development, cognitive effects of malnutrition have been variable (51) with impairment of learning and spatial memory after early malnutrition described by some authors (52–58), whereas other have not found any differences (59–62). Timing of the cognitive task seems to be an important factor: spatial learning ability of undernourished young rats was substantially impaired compared with performance after nutritional rehabilitation (59).

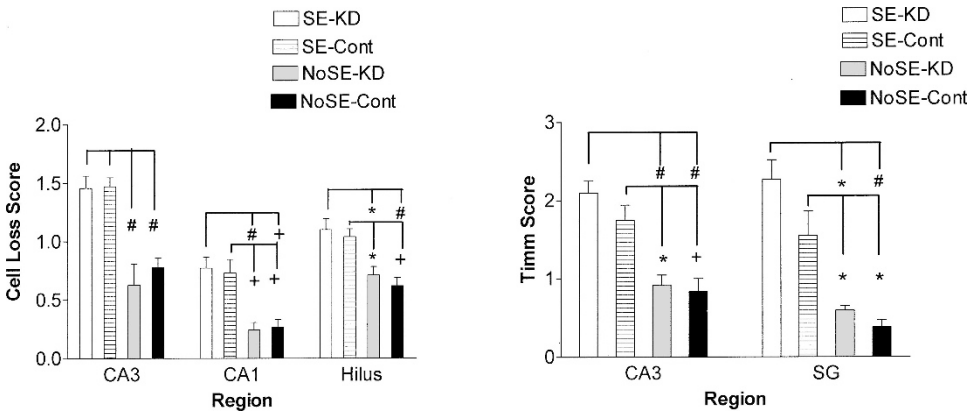

Figure 6. Comparison of cell loss scores and Timm scores in the four groups of animals. (*A*) Animals with SE had higher cell loss scores in all three brain regions than the NoSE groups. The presence or absence of the KD had no effect on cell loss scores. (*B*) Animals with SE had higher Timm scores in both CA3 and the supragranular region (SG) than the NoSE groups. There were no differences between the KD and regular chow groups.

Because we did not vary the protein content in the KD or gavage feed animals to maintain appropriate caloric intake, we cannot determine how much, if any, of the spatial memory deficits were secondary to undernutrition as opposed to a detrimental effect of the high fat content. It is also possible that insufficient vitamins in the formula or the type of fat used to induce the ketosis contributed to the cognitive impairment (63). Weight loss has not always been observed in rats that were treated with the KD (15, 64, 65). It is also possible to design the study so that control and KD rats maintain similar weights (14). Future studies on the cognitive effects of the KD should try to correct for the poor weight gain seen in this study.

Although it is difficult to extrapolate rodent studies to the human condition, a number of similarities between our study and the clinical situation may cause some concern. In children, weight loss is a stated goal of KD treatment and protein intake is limited (66). At the Johns Hopkins Hospital, Vining *et al.* (67) limit caloric intake to 75% of the recommended daily allowance. Stunting of growth therefore is common, particularly in children who started at a younger age (67, 68). Indeed, growth curves in children on the KD often closely resemble those seen in our rats. Because clinical studies have demonstrated detrimental effects of high-fat diets on cognitive function (7, 69), it is possible that the cognitive deficits are multifactorial, involving both undernutrition and the high fat content of the KD. It is also possible that the response to the KD is species specific and the KD is more deleterious in rats than in humans. However, considering our findings, further studies that critically evaluate cognitive function in children on the KD are needed.

Anecdotally, children who are started on the KD have been reported to have improvements in behavior and mood (2), although in adults, high-fat diets have been associated with significant deterioration of mood (69). Here we found that animals that received the KD were more active and demonstrated higher emotionality scores than rats that received regular rat chow, although the differences from the controls were slight. Although we saw no beneficial effect on emotionality or activity level, as with cognitive impairment, it is possible that the response to the KD is species specific.

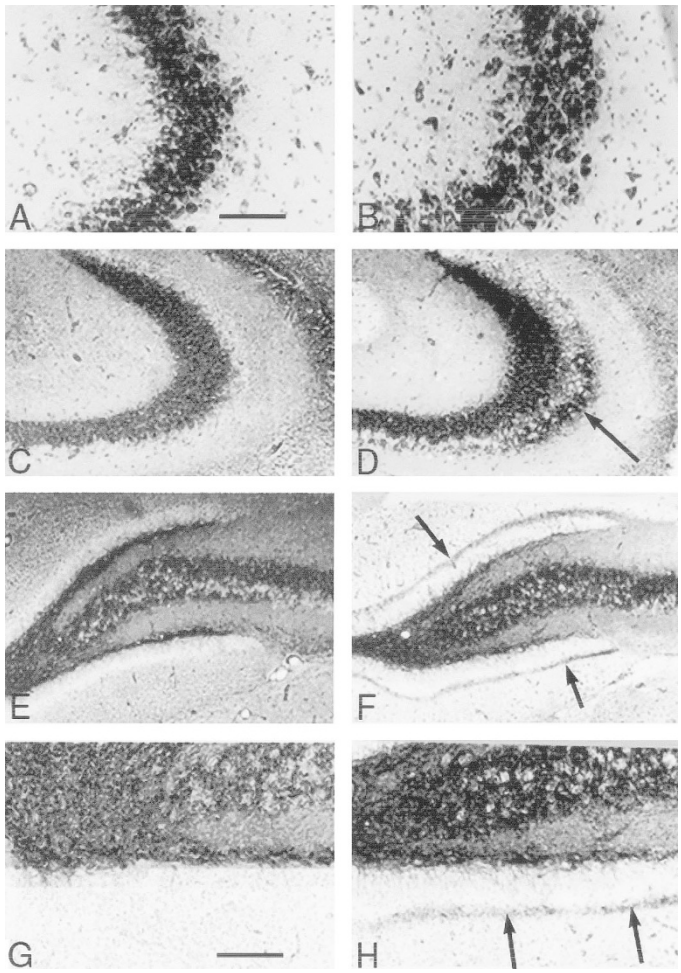

Figure 7. Examples of hippocampal histology in Cont (*A*, *C*, *E*, *G*) and SE (*B*, *D*, *F*, *H*) rats that were treated with the KD. *A* and *B* demonstrate the CA3 region of the hippocampus stained with thionin. Note the patchy cell loss in the SE rat (*B*). *C* through *H* specimens are stained with Timm solution, which causes zinc-filled mossy fibers and terminals to stain black. *C* and D are from the CA3 region of the hippocampus. Aberrant staining in the pyramidal cell layer from the SE rat is denoted in *D* by the arrow. *E* and *F* show Timm staining in the dentate granule cell layer. *F* shows sprouting of the mossy fibers in the supragranular region of the dentate gyrus arrows. *G* and *H* are higher power photomicrographs of the dentate granule cell layer. Sprouting of mossy fibers in the supragranular region of the dentate gyrus is shown by arrows in the rat with SE (*H*). Calibration for *A–F* = 70 μm; *G–H* = 40 μm.

There is little question that in both children and rodents, the KD can reduce seizure frequency. What is less clear is the long-term consequences of a high-fat, low-carbohydrate, and low-protein diet on brain development.

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