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Fasting and Ketogenic Diet Effects on Audiogenic Seizure Susceptibility of Magnesium Deficient Rats[1]

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MAHONEY, A. W., D. G. HENDRICKS, N. BERNHARD AND D. V. SISSON. *Fasting and ketogenic diet effects on audiogenic seizure susceptibility of magnesium deficient rats.* PHARMACOL BIOCHEM BEHAV **18**(5) 683–687, 1983.—Because fasting and ketogenic diets decrease seizure susceptibility in epileptics, their anticonvulsant effects were studied using sound-induced seizures in the magnesium-deficient rat. Fasting markedly depressed seizure incidence and severity but did not affect latency (sec to seizure onset). High-fat diet increased incidence of audiogenic seizures and seizure severity, and decreased latency. Gavage of medium chain triglyceride, beta-hydroxybutyrate or glucose did not affect seizure incidence, seizure severity or latency. Nonspecific excitability level was not associated with treatment nor with seizure incidence, severity or latency time.

Ketogenic diet Epileptics Audiogenic seizure susceptibility Magnesium deficient diet Rats Fasting

ACCORDING to Peterman [14], it has been known since antiquity that fasting can prevent epileptic seizuring. Ketosis accompanies fasting and it can be caused by diet. Ketogenic diets have been used to control childhood seizures [9, 11, 13] and electroshock seizures in animals [1, 13, 15]. The mechanism of the anticonvulsant action of ketogenic diet has been reviewed [17]. The anticonvulsant effects of a ketogenic diet do not appear for a few days, even though blood ketone concentrations increase rapidly.

Withrow [17] concludes that the anticonvulsant effect of a ketogenic diet is associated with how blood ketone bodies relate to brain energy metabolism and is not the result of water, electrolyte, acid-base or lipid fluctuations in the brain. Ingestion of excess glucose, which blocks ketone production can rapidly negate the anticonvulsant effects of the diet.

The magnesium-deficient rat has been suggested as an animal model of seizure disorders [5]. Since common anticonvulsant drugs inhibit seizures in magnesium-deficient rats [5], we tested the effects of fasting and ketogenic diet on audiogenic-seizure susceptibility of this animal model, which has the advantage of being reversible by magnesium supplementation.

METHOD

Animal Care

Weanling female Sprague-Dawley rats (Simonsen Albinos, obtained from Simonsen Laboratories, Gilroy, CA) were used in experiments 1–5. Experiment 6 was conducted one year later as part of another series of experiments in which male animals were arbitrarily being used. All animals were fed magnesium-deficient diets (Table 1) for 17 days, at which time they were tested with horns for audiogenic seizure susceptibility, seizure severity, and nonspecific excitability level (NEL) [6]. (Adaptahorn (No. 374) manufactured by Edwards Company, Norwalk, Connecticut is designed for sounding an alarm in public places. There was a horn placed at either end of the animal cage.) The horns, located with the animal cage in a metal case having an observation window, sounded until the rat convulsed or for 90 sec, whichever occurred first. Testing was done selecting one animal each across groups until all had been tested. The rats were housed in stainless steel cages with wire bottoms and fronts in an animal room maintained at approximately 27°C. Demineralized water was offered ad lib in polyethylene bottles having rubber stoppers and stainless steel lick spouts. Lights were on from 0800 to 2000 hr.

Experiment 1

Fifty rats were fed diet 1 (Table 2) and after 17 days were divided into 5 groups of ten. The groups in the experiment were: (1) Untreated. (2) Saline—1 g of Tween 60 was emulsified with 100 g of 0.9% NaCl solution which was gavaged at 0.5 ml/100 g body weight. (3) (Medium chain triglyceride)

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TABLE 1

COMPOSITIONS OF DIETS USED IN EXPERIMENTS 1 TO 6 (g/kg)

Diet No.	1	2	3	4	5	6	7	8	9	10
Experiment No.	1–4, 6	2	2	2	3	3	3	5	5	5
Casein	200	250	250	250	245	245	245	245	245	245
Dextrose	628	428	428	428	118	118	118	550	7	4
Corn Oil	50	200	25	25	242	25	25	50	290	25
MCT[a]	—	—	175	—	—	217	—	—	—	268
OCT[b]	—	—	—	175	—	—	217	—	—	—
Vitamin Mixture[c]	22	22	22	22	22	22	22	22	22	22
Mineral Mixture[d]	50	50	50	50	50	50	50	50	50	50
a-cellulose[e]	50	50	50	50	323	323	323	83	386	386
kcal/g	3.76	4.65	4.62	4.62	3.77	3.73	3.73	3.77	3.74	3.73
%kcal as Fat	11	38	38	38	59	57	57	12	70	70

[a]Medium chain triglyceride; the fatty acids are primarily C_8 and C_{10}. Supplied by Mead Johnson Co., Evansville, IN 47721.

[b]Odd chain triglyceride; Triundecanoin. Supplied by PVO International, Inc., 416 Division Street, Boonton, NY 07005.

[c]Total Vitamin Supplement (U.S. Biochemicals, Im., Cleveland, OH). It contains (in g/kg) vitamin A concentrate (200,000 IU/g) 4.5, vitamin D concentrate (400,000 IU/g) 0.25, alpha tocophorol 5.0, ascorbic acid 45.0, myo-inositol 5.0, choline-HCl 75.0, menadione 2.25, paraminobenzoic acid 5.0, nisvin 4.5, riboflavin 1.0, pyridoxine-HCl 1.0, thiamin-HC1 1.0, vitamin B_{12} 0.00135, copantothenate 3.0, biotin 0.2 and glucose to make 1 kg.

[d]It contains (in g/kg) $CaCO_3$ 280, NaH_2PO_4 341, KCl 76.3, Zn SO_4-$7H_2O$ 1.176, $MnSO_4$-H_2O 3.42, Cu SO_4-$5H_2O$ 0.442, KI 0.004, Na_2MoO_4-$2H_2O$ 0.012, $CoCl_2$-$6H_2O$ 0.004 and dextrose to make 1 kg.

[e]Solka-Floc, unbleached, sold by Brown Company, Berlin, NH 03570.

30 g MCT, 70 g of 0.9% NaCl solution and 1 g Tween 60 were emulsified and gavaged as above. This protocol has been shown to increase blood ketone bodies approximately 400% 35 min after gavaging [2]. (4) Glucose—50% glucose solution was gavaged as above. (5) Fasted 20 hr—food cups were removed from the cages at 0500 hr the day before testing. The animals were gavaged 60 min before seizure testing.

Experiment 2

Sixty rats were divided into six groups of ten and fed diets 1 to 4 (Table 1) for 17 days. The groups were: (1) 5% LCT (long chain triglyceride, corn oil), (2) 5% LCT fasted 10 hr, (3) 5% LCT fasted 20 hr, (4) 20% LCT, (5) 20% MCT and (6) 20% OCT (odd chain triglyceride).

Experiment 3

Thirty rats were fed diet 1, and 30 were fed diets 5, 6 or 7 (Table 1) for 17 days. After this time, the rats fed diet 1 were divided into 3 groups of ten. One group was gavaged 1.5 ml of 1 M sodium beta-hydroxybutyrate (BHB) solution 60 min before seizure testing. One group was gavaged with 0.75 ml MCT emulsion (described in Experiment 1) 60 min before seizure testing. The remaining groups were seizure tested without further treatment.

Experiment 4

Forty rats were fed diet 1 (Table 1) for 18 days. Sixty min before seizure testing, they were gavaged with 1.5 ml saline, 0.75 ml MCT emulsion (described in Experiment 1), 0.75 ml 50 percent glucose solution or 1.5 ml 1 M BHB solution. The rats were retested for seizures 5 hr after gavaging.

Experiment 5

Forty rats were fed diet 8 (Table 1) and 2 groups of ten rats each were fed diet 9 or diet 10 for 17 days. Sixty min before seizure testing, 10 rats fed diet 8 were gavaged with 1.5 ml normal saline, another 10 rats fed diet 8 were gavaged with 1.5 ml MCT emulsion and another 10 rats fed diet 8 were gavaged with 1.5 ml 1 M BHB solution.

Experiment 6

Twenty-nine weanling male rats from an unrelated experiment were fed diet 1 (Table 1) for 17 days before being divided into 2 groups of 14 and 15 animals each. The first group was fasted 20 hr before seizure testing.

Statistical Analysis

The statistical analysis was conducted at two levels. First, each experiment was analyzed as a completely randomized design, and, second, a combined analysis was performed on sets of similar experiments.

A chi-square test was used to test for differences in the incidence of seizures from treatment to treatment. The analysis of variance was used to test for differences in seizure severity, seizure onset, and NEL. When the F test was significant, the least significant difference (LSD) test was applied to determine where the significant differences occurred.

RESULTS AND DISCUSSION

Fasting clearly reduced the susceptibility of magnesium-deficient rats to audiogenic seizures (Table 2). This fasting

TABLE 2

FASTING EFFECTS ON AUDIOGENIC SEIZURE SUSCEPTIBILITY AND NONSPECIFIC EXCITABILITY LEVEL (NEL) OF MAGNESIUM DEFICIENT RATS[a]

Experiment No.	No. Seized: No. Tested 1	2	6	Seizures (%)	Seizure Severity	Seizure[e] Onset (sec)	NEL
0 hr Fast	9:10[f]	9:12	8:15	70	1.9	16	34
10 hr Fast[b]	—	2:9	—	22	0.33	5	37
20 hr Fast[c]	2:10	2:9	2:14	18	0.39	23	37
Statistic[d]	9.9**	8.1*	4.9*	21.1**	F(2.72)=14**	F(2,31)=2.07	F(2,43)=1

[a]All rats were fed diet 1 (Table 1).
[b]The fasting time ranged from 9 to 13 hr.
[c]The fasting time ranged from 18.5 to 23.5 hr.
[d]For evaluating fasting effects in the experiments, chi square was used for testing statistical significance for seizure incidence, and analysis of variance was used for seizure severity, seizure onset, and NEL. One asterisk indicates $p0.05$ and two indicate $p0.01$.
[e]Reported for only those rats that convulsed.
[f]Number seized: number tested.

effect is further evidence that the magnesium-deficient rat may be useful as an animal model of seizure disorders in man. Severe food restriction (15.5 g vs. 5–6 g per day) but not a total fast gradually resulted in an increased sensitivity to electroconvulsive shock, and sensitivity to shock returned to normal immediately after refeeding [7]. It is tempting to suggest that the mechanism of seizuring is different between audio-induced in magnesium deficiency and electroconvulsive shock. The physiological differences between fasting and severe food restriction (fed about one third normal) may be important, however, in interpreting the results from these two models.

The high-fat diets did not decrease seizure susceptibility in magnesium deficient rats; instead, seizure susceptibility was increased by all of the high-fat diets studied (Table 3). This is in contrast with data on effects of high-fat diet on

TABLE 3

EFFECT OF AMOUNT AND TYPE OF DIETARY FAT ON SEIZURE SUSCEPTIBILITY AND NONSPECIFIC EXCITABILITY LEVEL (NEL) OF MAGNESIUM DEFICIENT RATS

	Long Chain Triglyceride[a]				Medium Chain Triglyceride[b]			Odd Chain Tryglyceride[c]		Statistic[d]	LSD 05/01
Fat, % of kcal	11	38	59	70	38	57	70	38	57	—	—
kcal/g diet	3.76	4.65	3.77	3.74	4.62	3.73	3.73	4.62	3.73	—	—
Experiment 2	9:12(1)[e]	8:9(2)	—	—	9:10(3)	—	—	7:10(4)	—	1.9	—
Experiment 3	6:12(1)	—	10:10(5)	—	—	6:6(6)	—	—	7:7(7)	12.3**	—
Experiment 5	4:12(8)	—	—	10:10(9)	—	—	10:10(10)	—	—	17.8**	—
Seizures, %	50	88	100	100	90	100	100	70	100	25.8**[g]	—
Seizure severity	3.0	2.6	3.8	3.9	2.7	4.0	3.9	3.0	3.6	F(3,94)= 15.2**[h]	0.7/1.0
Seizure onset, sec[f]	31	19	4	16	13	3	12	7	5	F(3,94)= 9.1*[i]	10/13
NEL	33	36	24	40	32	36	37	38	34	F(10,100)= 1.25	NS

[a]Corn oil.
[b]The fatty acids are mostly C_8 and C_{10}.
[c]The fatty acids are mostly C_9 and C_{11}.
[d]Chi square was used for testing statistical significance for seizure incidence, and analysis of variance was used for seizure severity, seizure onset and NEL. Two asterisks indicate $p0.01$. Least significant differences (LSD) were calculated when the F value was $p0.05$.
[e]Number seizured: number tested. The number in ( ) indicates the diet the rats consumed.
[f]Reported for only those rats that convulsed.
[g]Chi square value for fat level effect. The chi square value for the fat source effect was 6.6 ($p0.05$).
[h]F value for fat level effect. For fat source, using only the 38 and 57–59% of kcal levels, F(2,44)=0.34.
[i]F value for fat level effect. For fat source, using only the 38 and 57–59% of kcal levels, to F(2,44)=0.52.

TABLE 4

RESULTS OF GAVAGING MEDIUM CHAIN TRIGLYCERIDE (MCT) OR B-HYDROXYBUTYRATE (BHB) ON AUDIOGENIC SEIZURE INCIDENCES IN MAGNESIUM DEFICIENT RATS[a]

Treatment	Experiment Number 1	3	4	5	Seizures (%)	Seizure Severity	Seizure[b] Onset (sec)	NEL
Untreated	9:10	9:12	—	4:12	61	3.4	6	35
Saline	8:9	—	9:10(8:10)	1:10	62 (80)	3.7 (3.9)	21(53)	33
BHB	—	3:7	7:9(3:8)	4:10	53 (38)	3.2 (4.3)	38(74)	26
MCT	5:10	5:10	9:10(5:8)	3:10	55 (62)	3.8 (4.0)	22(47)	31
Glucose	7:10	—	10:10(6:8)	—	85 (75)	3.2 (4.0)	28(64)	35
Statistic[c]	5.5	0.1	2.5(4.0)	2.5	8.3	$F(4,162)=2.02$	$F(4,86)=1.21$	$F(4,160)=4.69^{**}$
LSD 05/01	—	—	—	—	—	NS	NS	5/7

[a]Rats in Experiments 1, 3, and 4 were fed diet 1 and those in Experiment 5 were fed diet 8 (Table 1). They were tested 1 hr after gavaging. In Experiment 4 the rats were retested 5 hr after gavaging and the results are in ( ). Some animals died after the first testing.

[b]Reported for only those rats that convulsed.

[c]Chi square was used for testing statistical significance for seizure incidence, and analysis of variance was used for seizure severity, seizure onset and NEL. Least significance differences (LSD) were calculated when the F value was $p0.01$ (two asterisks). NS means not statistically significant.

susceptibility to electroconvulsive shock in animals [1,15] and of Huttenlocher [12], who reported that a diet containing 60 percent of energy as MCT decreased epileptic seizures in six of 12 children studied. OCT was used in the present study because of its glucogenic effect [16]. In this study, no differences in seizure susceptibility were noted between animals fed the strongly ketogenic MCT, ketogenic LCT, or glucogenic OCT diets (Table 3). Although blood ketone concentrations were not assayed in this study, the diets containing 38 percent of their energy as fat from LCT or MCT should have produced mild ketonemia [3]. Assuming that the metabolic response of magnesium-deficient rats is similar to that of healthy ones, the animals fed diets containing 70 percent of the energy as MCT should have had pronounced ketonemia while those fed OCT would be anticipated to have had slight ketonemia [10]. Huttenlocher [11] concluded that 2 mM BHB must be present in the serum for the anticonvulsant effect of ketogenic diet in children. This is higher than would be anticipated using the dietary treatments reported here [3]. Since the anticonvulsant effect of ketogenic diet occurs within one week [17], the animals fed the high-fat diet should have had ketonemia of sufficient duration to prevent seizures. Again, it appears that the mechanism of seizuring differs between magnesium-deficiency and electroconvulsive shock.

Gavaging BHB, MCT or glucose did not reduce seizure incidence when the rats were tested 1 hr later (Table 4). In Experiment 4, however, there was evidence that seizure incidence was reduced by BHB when testing occurred 5 hr after gavaging. The amount of MCT gavaged has been shown to affect production of ketonemia in rats [2] and mice [8]. Since blood ketone concentrations were not measured, we assumed that the magnesium-deficient rat had a ketonemic response similar to that of healthy animals gavaged with MCT or BHB. Gavaging glucose resulted in a tendency for increased seizure incidence relative to other energy sources (Table 4). Gavaging BHB or MCT may have produced ketonemia of such short duration that brain energy metabolism remained unaltered and no anticonvulsant effect resulted [17]. Thus, it appears that any ketosis that may have been caused by these treatments is insufficient to explain the effects of fasting on seizure incidence observed in this study.

The latency (sec of sound before seizure) of seizures in the animals that convulsed was not affected by fasting but the severity of seizure was decreased (Table 2). The severity and latency of seizures were not affected by gavaging MCT, BHB or glucose (Table 4). High-fat diets, however, were associated with greater seizure severity and shortened latencies (Table 3). The latency effect was most pronounced in rats fed LCT. No statistically significant fat level effects were noted for these diets (Table 3). The correlation between latency time and seizure severity was -0.61 for rats fed the LCT diets. It can be concluded that seizure severity and latency time for rats that convulse were not strongly affected by the treatments studied here.

NEL was not correlated with any of the parameters of seizure assessment studied. Buck *et al.* [4] reported that NEL increased with the time that rats consumed a magnesium-deficient diet. It was greater at 14 days (when the animals were susceptible to audiogenic seizure) compared with magnesium-supplemented animals that were not seizure susceptible. It has been suggested that NEL is a good indicator of central nervous system excitability; it is inversely related to CSF magnesium concentration, and is not related to serum magnesium concentration [4]. Because fasting resulted in a significant change in seizure susceptibility but no change in NEL (Table 2), it may be concluded from this study that either NEL is not related to seizure activity or that the fasting effects on seizure susceptibility are mediated through a peripheral mechanism.

The potential of magnesium-deficiency induced seizure susceptibility as a model for seizure disorder in man, especially epilepsy, requires further evaluation. Although common anticonvulsant drugs [5] and fasting reduce seizuring in

the magnesium-deficient rat and in epileptic people, the lack of effect of ketogenic diet on the seizure susceptibility of the magnesium-deficient rat is evidence that the mechanism differs from that of epilepsy. Nevertheless, magnesium-deficiency induced susceptibility to seizures is interesting because no damage to brain tissue is apparent and the effect is reversible by magnesium supplementation.

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