High Plasma Branched-Chain Amino Acids: Aromatic Amino Acids Ratio in Children on the Ketogenic Diet: A Mechanism in Controlling Epilepsy

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Background : *The authors proposed that ketogenic diets will produce an increase in the ratio of branched chain amino acids (BCAAs) and aromatic amino acids (BCAAs) in plasma of children who are on the diets.*
Subjects and Method : *A sample of plasma amino acids sample before initiation of fasting and on day 10th of the dietary treatment was obtained in patients with refractory epilepsy who were newly admitted for initiation of ketogenic diet. Plasma amino acids were determined by high performance liquid chromatography equipment.*
Results : *There are 20 patients with refractory epilepsy participating in this study. Outcomes of ketogenic diet therapy were satisfactory. Nineteen cases out of 20 cases had a significantly higher ratio of plasma BCAAs: ARAAs during ketogenic diets than before the diet ($P < 0.001$).*
Conclusion : *The ketogenic diets produced an increased ratio of plasma BCAAs: ARAAs. Whether the increased ratio of plasma BCAAs: ARAAs plays an important role in controlling epilepsy is yet to be elucidated.*

Keywords : *Ketogenic diet, Refractory epilepsy, Plasma amino acid, Branched chain amino acid, Aromatic amino acid.*

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Ketogenic diets are now popularly prescribed as an additional therapy, besides antiepileptic drugs, in children who have refractory epilepsy[1]. Many centers have reported success of ketogenic diets in controlling epilepsy in this group of children[2-4]. The diet should be considered as an alternative therapy for children with difficult-to-control seizures. It is even more effective than many of the new anticonvulsant medications and is well tolerated by children and families[5]. When epilepsy is controlled after addition of the ketogenic diet, antiepileptic drugs can be tapered off. Some patients were reported to be antiepileptic drug-free within a couple of years[6]. Besides, developmental milestones in these children have caught up faster than before implementing the diets[7].

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How the ketogenic diet works in controlling epilepsy remains unknown. High blood glucose has been claimed as a triggering factor for seizures. Dehydration, ketone body levels and acidosis have all been considered as the determinants of the ketogenic diet efficacy, but why the diet works is still unknown.

To the authors' knowledge, there has not been a single study on plasma amino acid changes in patients on ketogenic diets. Limitation of carbohydrate and protein intakes as well as an increase in proportion of fat in the diets might result in changes in plasma amino acids. The amino acids that commonly interfere with brain functions are large aromatic amino acids (ARAAs) including tryptophan, phenylalanine and tyrosine. Whether they will increase the excitability potential of the brain is worth to studying.

The authors thus hypothesize that ketogenic diets will produce an increase in the ratio of

branched chain amino acids (BCAAs) and aromatic amino acids (ARAAs) in plasma of the children who are on the diets. Higher ratio of plasma BCAAs/ARAAs will probably result in decreasing ARAAs entering the central nervous system[8-10] and then decreasing seizures in these patients.

Subjects and Method

Patients who have been followed at the Neurology Clinic in the Department of Pediatrics, Faculty of Medicine Siriraj Hospital, Mahidol University and had refractory epilepsy were recruited into the study. Inclusion criteria were patients who continued to have seizures despite treatment with adequate doses of 2 or more antiepileptic drugs for a period of more than 4 months. These patients had been offered the ketogenic diet as additional therapy to antiepileptic drugs. Informed consent was obtained from the parents before the patients were enrolled in the study. All patients had to be hospitalized for at least 10 days for the initiation of the ketogenic diet.

After admission, a plasma amino acid sample before initiation of fasting and the diet treatment (or the pre-treatment sample) was collected from each patient. During this fasting period, the patient was closely monitored for signs and symptoms of hypoglycemia and dehydration. The fasting period usually lasted for 24-72 hours until urine ketone reached 4+. After either urine became ketotic or serum ketone body was equal to or above 1.6 mmol/L, then the ketogenic diet was started. There were two different ketogenic diet regimens in this study. A detailed constituent of each regimen is shown in Table 1.

Table 1. A detailed constituent of each regimen of the ketogenic diet

Regimen 1		Regimen 2	
Whipped cream	220 ml	Bacon	25 g
Eggs	1 egg	Eggs	1 egg
Tab multivitamin + mineral	1 tab	Soy bean oil	10 tsp
NaCl	tsp	Broccoli or collard green	50 g
Elixir KCl	1 tsp		
Water added up to	1,000 ml		
Total volume	1,000 ml		
Total energy	825 kcal		570 kcal
Total fat	85 g		58 g
Total protein	11.5 g		11 g
Total carbohydrate	7.2 g		2.2 g
Fat : nonfat	4.5:1		4.4:1

In brief, Regimen 1 contained mainly whipped cream and was used initially in small children who were still being bottle-fed or in older children who required nasogastric tube feeding. Regimen 2 was a regular fatty diet comprised of common foods available on the market, such as bacon, fatty meat, eggs, cooking oil etc. The fat: non-fat ratio of Regimen 1 and 2 were 4.5:1 and 4.4:1 respectively.

The ketogenic diet was started at one-fourth of the total caloric requirement. If the patient tolerated the diet well, then it was slowly increased to the total caloric requirement in a few days. Every child received a sugar-free multivitamin supplement and additional calcium. After the 10th day of the diet treatment, the second blood plasma amino acid was obtained in the morning before the meal for plasma amino acids. (the treatment sample).

The patients remained in the hospital until the parents or the caretakers were confident about preparing the patient's own ketogenic diet. After discharge, the parents were asked to continue keeping a seizure calendar and to measure the patient's urine ketones every morning. Parents were advised to consult the dietitian periodically over the phone when making an appropriate adjustment to the diet. All adverse events related to the diets were recorded at each follow-up visit. Antiepileptic drugs would be decreased or weaned off if the seizures decreased.

Plasma amino acids were determined by the high performance liquid chromatography (HPLC) equipment which was driven by a mini-metering pump, model 396/2796 (Duplex) of LDC Analytical, Inc. Picking cation-exchange of 5403 was used for separation of each amino acid. Proline, alanine and citrulline could not be clearly separated by this method. Plasma levels of leucine, isoleucine, valine, tryptophan, phenylalanine and tyrosine of the pre-treatment sample and treatment sample of each patients were analyzed.

Statistical Analysis

The ratio of BCAAs:ARAAs of the pre- treatment and the treatment samples from each patient were compared by pair-student T test.

Results

There were 20 patients with refractory epilepsy participating in this study. All of them were on a ketogenic diet Regimen 1 or Regimen 2 or a combination of both regimens. Characteristics of the 20 patients are listed in Table 2. There were 10 males and

Table 2. Characteristics of 20 patients on the ketogenic diet

No	Name	Sex	Age (yr)	Type of Epilepsy (yr)	Drugs of Treatment
1	SL	F	1	Partial Seizure	Phenobarbital + Dilantin
2	PK	F	6	Generalized Seizure	Lamictal + Tegretol
3	KS	F	1	Infantile Spasms	Vigabatrin + Phenobarbital
4	JT	F	1	Generalized Seizure	Dilantin + Lamictal + Depakine
5	BM	M	7	Myoclonic Seizure	Lamictal + Dilantin + Depakine
6	NV	M	8	Partial Seizure	Dilantin + Depakine + Topamax
7	KS	F	3	Generalized Seizure	Depakine + Topamax + Lamictal
8	PK	F	14	Generalized Seizure	Neurontin + Lamictal + Tegretol + Depakine + Rivotril
9	DS	M	2	Generalized Seizure	Topamax + Depakine + Rivotril + Dilantin
10	NT	F	6	Generalized Seizure	Topamax + Lamictal + Depakine
11	PK	F	2	Generalized Seizure	Topamax + Depakine
12	KK	M	10	Generalized Seizure	Dilantin + Depakine +Lamictal
13	TT	M	19	Generalized Seizure	Depakine + Topamax
14	VS	F	6	Generalized Seizure	Clonazepam + Dilantin + Depakine + Lamictal
15	PH	F	12	Generalized Seizure	Depakine + Rivotril + Tegretol + Topamax
16	BY	M	10	Atypical absence Seizure	Dilantin + Tegretol
17	TM	M	4	Generalized Seizure	Phenobarbital + Topamax
18	VK	M	4	Generalized Seizure	Depakine + Lamictal
19	AD	M	2	Partial Seizure	Depakine + Dilantin + Rivotril
20	AB	M	13	Partial Seizure	Tegretol + Topamax

10 females, with an age range from 1 year to 19 years. There were 13 patients with generalized seizures, 4 patientspatients with partial seizures, one with infantile spasms, and one each with myoclonic and atypical absence seizures. All the patients were on at least two antiepileptic drugs with adequate doses (documented by serum levels) for more than 4 months. Nine patients were on 2 antiepileptic drugs, eight patients were on 3 antiepileptic drugs, two patients were on 4 antiepileptic drugs and one patient was on 5 antiepileptic drugs.

Outcomes of ketogenic diet therapy were satisfactory. In brief, after 6 months of the ketogenic diet, 25% of the patients were seizure free, 50% had 90% seizure reduction, 19% had 50-90% seizure reduction and 6% had less than 50% seizure reduction.

Table 3 shows the levels of BCAAs and ARAAs of the pre-treatment and treatment samples.

Table 4 lists the ratio of sums of plasma leucine, isoleucine and valine (BCAAsS) to sums of plasma tryptophan, phenylalanine and tyrosine (ARAAS + s) in 20 patients before and during the ketogenic diet. Almost all cases, except case No. 3 and case No.4 had higher a ratio of plasma BCAAs: ARAAs during the ketogenic diet. The ratio was increased significantly more in the treatment samples than the pre-treatment samples. ($P < 0.001$)

Fig. 1 depicts the proposed mechanisms of the ketogenic diet in controlling seizures.

Discussion

A combination of the ketogenic diet with antiepileptic drugs has resulted in a high success of controlling refractory seizures. Muller Schwarze, et al recently showed that the ketogenic diet retarded epileptogenesis in an experimentalexperimental model[11]. The mechanism of action of the ketogenic diet seems to rely on changes in the brain's metabolism from that of a glucose-based energy substrate to a ketone-based substrate. The ketogenic diet may give rise to alterations in brain extracellular milieu, which serve to depress excitability and synchrony. There is increased evidence that ketone bodies may not be the primary biochemical factor responsible for controlling seizures in patients who were treated with the ketogenic diet. Cerebral acetone may contribute to seizure control in the ketogenic diet, but is unlikely to be the sole mechanism[12]. Likewise, Thio et al demonstrated that the anticonvulsant properties of the ketogenic diet did not result from a direct effect of ketone bodies on the primary voltage and ligand gated ion channels media-ting excitatory or inhibitory neurotransmission in the hippocampus[13]. Moreover, Likhodii et al have shown that seizure protection with the ketogenic diet did not improve with a higher level of ketosis[14]. Furthermore, Bough et al showed that beta-hydroxybutyrate is not directly involved in the anticonvulsant mechanism of the diet[15]. Until now the mechanism of action of the diet remains unknown and it is

Table 3. Plasma levels of branched-chain amino acids (BCAAs) and aromatic amino acids (ARAAs) before and after the ketogenic diet*

	Leucine		Isoleucine		Valine		Tryptophan		Phenylalanine		Tyrosine	
	a	b	a	b	a	b	a	b	a	b	a	b
1	129.95	125.42	50.71	56.05	45.61	24.80	10.53	11.07	68.63	37.20	55.36	26.82
2	147.64	189.25	56.63	80.81	45.53	66.88	15.12	8.68	58.56	52.82	67.68	43.18
3	208.28	149.50	96.44	69.98	22.36	43.53	14.68	13.46	67.36	55.47	38.79	31.97
4	151.46	98.30	71.53	39.93	30.82	28.07	19.54	82.87	69.39	34.42	38.91	18.99
5	68.08	127.51	38.12	57.50	8.53	61.26	8.02	3.99	39.03	44.12	32.14	25.08
6	146.22	113.78	71.06	54.65	65.43	71.79	10.49	7.81	53.06	40.96	53.85	44.99
7	146.72	150.34	68.92	84.68	64.32	116.17	27.73	24.54	82.62	68.92	68.86	68.11
8	58.01	72.78	22.82	23.36	16.69	27.76	6.25	9.00	28.73	29.86	25.78	18.47
9	130.81	111.44	57.42	52.94	26.51	38.16	8.86	3.77	41.25	27.83	8.25	20.91
10	257.22	280.09	190.89	127.18	80.3	165.63	52.24	88.10	153.35	127.30	22.15	100.87
11	83.84	71.18	38.00	34.52	23.03	31.44	8.33	10.41	37.67	31.14	47.48	26.79
12	66.40	76.22	39.07	40.99	21.93	36.69	16.04	5.81	37.95	18.72	25.25	14.01
13	121.84	205.09	46.79	116.53	33.87	82.56	11.31	107.57	50.57	62.82	52.16	53.00
14	0	10.22	79.92	173.67	0	71.74	0	0	34.74	29.92	30.90	70.72
15	265.24	458.93	126.94	233.71	103.30	245.88	56.24	52.11	153.57	259.39	116.09	106.57
16	118.86	264.17	51.41	123.56	17.83	99.36	6.39	13.57	51.21	62.39	36.00	43.97
17	447.50	733.20	200.72	330.41	115.48	304.06	111.26	43.15	225.52	116.40	198.72	204.27
18	223.44	172.06	106.49	113.44	110.03	133.24	0.75	1.06	104.19	96.53	107.12	82.19
19	82.84	228.67	36.94	117.35	23.45	61.47	13.24	18.88	46.50	64.76	37.65	60.07
20	511.90	340.92	226.39	162.06	102.83	107.59	807.17	237.68	145.66	96.65	106.28	62.38

* Values expressed as nmole/ml; a = before diet, b= during diet

Table 4. Ratio of branched chain amino acids (BCAAs) and aromatic amino acids (ARAAsS) before the ketogenic diet (Pre-treatment) and during ketogenic diet (Treatment)

	Pre-treatment	Treatment
Case 1	1.68	2.75
Case 2	1.77	3.22
Case 3	2.71	2.61
Case 4	1.99	1.22
Case 5	1.45	3.37
Case 6	2.41	2.56
Case 7	1.56	2.17
Case 8	1.61	2.16
Case 9	2.74	3.86
Case 10	1.61	1.81
Case 11	1.55	2.01
Case 12	1.61	3.99
Case 13	1.78	1.81
Case 14	1.22	2.54
Case 15	1.52	2.25
Case 16	2.01	4.06
Case 17	1.43	3.76
Case 18	2.08	2.33
Case 19	1.47	2.84
Case 20	0.79	1.54

difficult to assess which biochemical parameters should be monitored as adjustments are made to the diet[16].

It is frequently found that intakes of even a small amount of carbohydrates in the patients during the diet treatment will result in an increased frequency of seizures, while inhibition of carbohydrate intakes will result in a decreased frequency of seizures. Frequently, the seizure control appears even before the increase of serum ketones. Freeman et al found that the seizures in patients with Lennox Gastaut syndrome decreased rapidly after fasting[17]. Thus, other mechanisms that relate to the intake of carbohydrates may play a role in seizure control.

Certain dietary constituents of food are the precursors of central neurotransmitters. Serotonin, one of the neurotransmitters is synthesized from the amino acid L-tryptophan. It has been well established that increasing the brain tryptophan level will increase the level of serotonin as well as its major metabolite, 5-hydroxy indoleacetic acid[18]. Also it was demonstrated that consumption of a meal rich in carbohydrates and poor in protein will also raise central serotonin levels in rats[19]. Hasuo et al found that 5-hydroxytryptamine increases the efficacy of excitatory synaptic transmission in the hippocampal-septal circuit[20]. There are 2 groups of large neutral amino acids in plasma which are BCAAs (leucine, isoleucine and valine) and ARAAs (tryptophan, phenylalanine and tyrosine). These two groups compete for passage across the

blood brain barrier into the brain. Hence, the ratio of BCAAs:ARAAs is essential in predicting the amount of transportation of amino acids into the brain[21]. Several studies have demonstrated a relationship between changes in the plasma amino acid levels and stages of the consciousness[10,22,23]. The disturbance of plasma amino acid homeostasis attributed portal systemic encephalopathy was described as the quotient of the molar relation of the plasmatic concentration of BCAAs versus phenylalanine plus tyrosine[24]. Carbohydrate consumption leads to an increase in central serotonin by triggering the secretion of insulin from the pancreas, which causes most of the large neutral amino acids to uptake into the muscle, except tryptophan[25]. From the present study, the ratio of BCAAs:ARAAs during the diet increased significantly from the ratios of the pre-treatment samples. All the patients had decreased seizure frequency while on the ketogenic diet. It is possible that the higher ratio of BCAAs:ARAAs from the ketogenic diet may play an important role of seizure control than the effect of ketosis on seizure control. The authors proposed that the ketogenic diet exerts part of their antiepileptic effects through an increase of the ratio of BCAAs:ARAAs. The increase in plasmatic BCAAs to ARAAs ratio will result in an increase in BCAAs in the central nervous system (Fig. 1). However, why these changes result in decreasing seizures in these patients remains to be elucidated. Further studies on the exact mechanism of these plasma amino acids in the ketogenic diet in controlling refractory epilepsy are warranted.

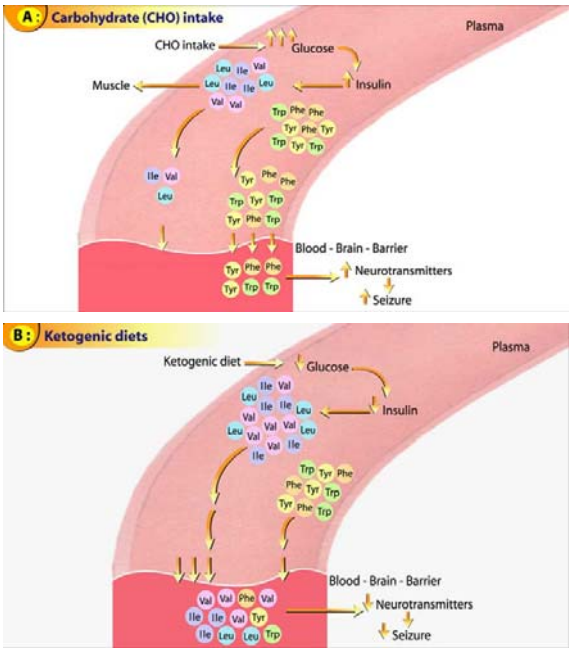

Fig. 1 Proposed mechanisms of the ketogenic diets in controlling seizures.

Conclusion

Children with refractory epilepsy who were on ketogenic diets had a high plasma branched-chain amino acids/aromatic amino acids ratio. This change might play a part in controlling epilepsy in these children.

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อัตราส่วนของกรดอะมิโน ชนิด branched ต่อกรดอะมิโนชนิด aromatic ในพลาสม่าเพิ่มขึ้นในเด็กโรคลมชัก ขณะได้รับอาหาร ketogenic

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คณะผู้ศึกษาได้ศึกษาระดับของกรดอะมิโนในพลาสม่าของผู้ป่วยโรคลมชักจำนวน 20 ราย ที่ดื้อต่อยากันชักที่ได้รับอาหารพิเศษคือ ketogenic โดยศึกษาระดับกรดอะมิโนก่อนที่จะเริ่มได้รับอาหาร และในวันที่ 10 ขณะที่ได้รับอาหารชนิดนี้ พบว่าผู้ป่วยส่วนใหญ่ตอบสนองดีต่ออาหาร และมีผู้ป่วย 19 รายใน 20 รายที่มีอัตราส่วนของกรดอะมิโนชนิด branched ต่อกรดอะมิโนชนิด aromatic เพิ่มขึ้นอย่างมีนัยสำคัญหลังได้รับอาหารชนิดนี้ อัตราส่วนของกรดอะมิโนชนิดที่เพิ่มขึ้นนี้อาจมีส่วนสำคัญในการควบคุมโรคลมชักได้