The Ketogenic Diet Is Well Tolerated and Can Be Effective in Patients with Argininosuccinate Lyase Deficiency and Refractory Epilepsy

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Abstract Argininosuccinate lyase (ASL) deficiency (MIM 608310, McKusick 207900) is a rare disorder of the urea cycle, which leads to a deficiency of arginine and hyperammonemia. Epilepsy is a frequent complication of this disorder. A ketogenic diet (KD) can be a very effective therapy for refractory epilepsy, and it has been widely used in children. Until now, no experiences with the KD in patients with urea cycle defects have been reported.

We present two cases of patients with ASL deficiency and refractory epilepsy who were treated with a KD. In both patients, the KD was initiated during a hospital admission and the fat percentage of the diet was increased to above 90% in five equal steps. In patient 1, during the KD the protein intake was continued as before, and in patient 2 the natural protein was increased with 0,2 g/kg/day while the protein from the amino acid supplement (UCD-2[®], Milupa) was decreased with 0,3 g/kg/day. During and after

the introduction of the KD, all biochemical parameters reflecting urea cycle function and ammonia levels were stable in both patients and no signs of derangement were detected. On the KD, patient 1 demonstrated a reduction in seizure frequency of >50%, and an increase in well-being. In patient 2, no effects of the KD on the seizure frequency were noted and after 6 months the KD was discontinued.

Concluding, the KD does not cause metabolic derangement, is well tolerated, and can be effective in patients with ASL deficiency who are treated with a protein restriction.

Abbreviations

ASA	Argininosuccinic acid
ASL deficiency	Argininosuccinate – lyase deficiency
EEG	Electroencephalogram
KD	Ketogenic diet
UCD	Urea cycle disorder

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Introduction

We present two patients with a urea cycle defect and refractory epilepsy. The usual treatment consists of supplementation of L-arginine, the missing enzyme product; restriction of natural protein intake to reduce the nitrogen load, often with a part replaced by special essential amino acid mixtures in order to avoid their limitation of protein synthesis; if needed drugs to stimulate alternate pathways of nitrogen excretion. Treatment of epilepsy of these patients is hampered by the relative contraindication of valproate as this may increase the ammonia level and may give rise to liver damage. Due to the fact that the epilepsy was difficult to treat, a ketogenic diet (KD) was initiated in both patients.

Patient 1

A now 5-year-old boy presented with hyperammonemia in the first 48 h of life. The maximum ammonia level was 1,085 $\mu\text{mol/l}$. Treatment consisting of peritoneal dialysis and administration of sodium benzoate and arginine was started immediately, and ammonia levels normalized within 24 h. Urine and plasma argininosuccinic acid (ASA) and plasma citrulline and glutamine were strongly elevated, and mutation analysis demonstrated heterozygosity for a c.857 A.G (Q286R) and a c.447-1 G > A mutation in the argininosuccinate lyase gene (ASL, EC 4.3.2.1), confirming the diagnosis argininosuccinate lyase deficiency (ASL deficiency, MIM 608310, McKusick 207900).

After the serious clinical presentation in the first week of life and during treatment with a protein restricted diet, sodium phenylbutyrate and arginine, no further episodes of metabolic decompensation occurred and the patient demonstrated normal psychomotor development until he developed severe and refractory epilepsy at the age of 18 months. In the following years, his epilepsy did not respond to levetiracetam, carbamazepine, clobazam, or ethosuximide. Severe seizures necessitated the use of diazepam on average twice weekly. His development was fully arrested after the start of the epilepsy. The electroencephalogram (EEG) showed almost continuous presence of high-voltage, specific epileptiform discharges. Cerebral MRI at age 2 years and 8 months demonstrated bilateral loss of tissue at the nucleus caudatus and atrophy of the frontal cortex.

Patient 2

This male patient presented on the third day of life with hyperammonemia resulting in seizures and coma. The highest ammonia level was 847 $\mu\text{mol/l}$, which rapidly normalized after treatment with sodium benzoate, sodium phenylacetate, arginine, and carnitine. The diagnosis of argininosuccinate lyase deficiency was made based on the amino acid profile, with strongly elevated glutamine and very low arginine levels and a clearly increased argininosuccinic acid in the urine. In this patient, milestones were normal at 12 months of age notwithstanding some hypertonia. At the age of 2.5 years, he developed severe refractory epilepsy, and during the following years he demonstrated little development. He developed a status epilepticus requiring high-dose midazolam and artificial ventilation at the age of 7 years. Cerebral MRI at ages 6 months and 2 years demonstrated no abnormalities, and an MRI at age 7 years and 8 month, just before the start of the ketogenic diet, demonstrated loss of tissue at the nucleus caudatus and minor myelinisation abnormalities.

Introduction of the Ketogenic Diet

Patient 1 was four years old, his weight was 17.5 kg, and he was fed by gastrostomy at the start of the KD. He had a daily intake of 1.0 g per kg of natural protein and 0.4 g per kg of protein from an essential amino acid supplement (UCD-2[®] Milupa) to meet his total requirements of essential amino acids. Energy intake was adequate for his age (1,350 calories per day, divided into five portions of tube-feeding) and the dietary fat intake was normal with 40% of the total energy amount. He was treated with sodium benzoate 1,200 mg four times per day (274 mg/kg/day) and L-Arginine 1,500 mg four times per day (324 mg/kg/day). The patient was admitted to the hospital for the introduction of the KD. In the KD of this patient, the protein intake was continued as before. With the use of Ketocal[®] (Nutricia) and a fat emulsion (Calogen[®] Nutricia), the amount of fat in the diet was raised to a total of 90% in five equal steps.

During the introduction of the KD, the total amount of calories per day was raised with 200 calories per day because of weight loss during the introduction of the KD (and to avoid catabolism c.q. protein breakdown as a consequence). With a total of 90% fat in the diet, the patient reached the state of ketosis. This enteral feeding was well tolerated except for diarrhea in the first days for which a fiber supplement was added to the feeding.

Patient 2 was 7 years old with a weight of 25.6 kg at the start of the KD. He had a daily intake of 0.8 g per kg of natural protein and 0.5 g per kg of protein from an essential amino acid supplement (UCD-2[®], Milupa) (UCD2[®]) to meet his total requirements of essential amino acids. Energy intake was adequate for his age (1,875 kcal per day distributed over six meals in a combination of tube feeding per gastrostomy and normal meals), and the dietary fat intake was normal with 34% of the total energy amount. He was treated with Arginine HCl 3,900 mg four times per day (600 mg/kg/day).

In the KD of this patient, the protein intake was 1 g of natural protein and 0.2 g per kg from an essential amino acid supplement (UCD-2[®], Milupa). With the use of Ketocal[®], the amount of fat in the diet was raised to a total of 92% in five equal steps. The diet was started as total tube feeding in a period of epileptic decompensation, then after clinical improvement, partly changed to ketogenic normal oral meals equivalent in fat, protein, and energy.

With a total of 92% fat in the diet, the patient reached only moderate ketosis (1-2+ in urine). LCT fat was partly replaced by MCT fat (3 ml Liquigen[®] equal to 1.5 MCT fat per kg b.w.), which resulted in a somewhat higher ketosis rate (1-3+ in urine). This feeding was well tolerated and after 3 months reduced in energy with 10% because of weight gain above the usual growth rate. Medication was thoroughly checked for carbohydrate content.

Safety and Effect

During and after the introduction of the KD in both patients all biochemical parameters reflecting urea cycle function and ammonia levels were stable, and no signs of derangement were detected. Levels of essential amino acids were within the normal range, with a slight decrease of alanine and an increase of branched chain amino acids, which may result from the increased gluconeogenesis during the KD (Table 1.) All measurements were in fed state due to the frequency of the feedings. Blood glucose concentrations remained within aimed range (> 4 mmol/L). Measurements of bicarbonate in patient 1 and CO_2 in patient 2 demonstrated no metabolic acidosis during ketosis.

Before the initiation of the KD, patient 1 demonstrated an average of 0.5–3 seizures per day and diazepam was administered on average twice weekly. A decrease of the frequency of seizures was noted after the first week of introduction of the KD. In the 3 months period after introduction of the KD the patient demonstrated a 50% reduction in seizure frequency. The administration of diazepam for severe seizures is still necessary twice weekly. However, with the decrease in seizure frequency there is a remarkable improvement in his well-being. He is more alert and now communicates more actively with his parents.

In patient 2, no effects on seizure frequency were noted during the application of the KD and after 6 months the KD was discontinued and the enteral feeding was gradually converted into his original feeding in five equal steps.

Discussion

ASL deficiency is a rare autosomal recessive disorder of the urea cycle caused by the deficiency of the enzyme argininosuccinate lyase, which leads to a deficiency of

arginine (and fumarate/malate in the cytosol) and to hyperammonemia. Patients with ASL deficiency may present with either a severe neonatal form or a late onset form (Erez et al. 2011). Epilepsy is a frequent complication of ASL deficiency, both in patients with a clinical presentation and in those detected with newborn screening (Ficicioglu et al. 2009; Grioni et al. 2011).

A KD can be a very effective therapy for refractory epilepsy (Bough 2008; Kossoff et al. 2009), and it has been widely used in children. It was first formulated by Wilder (1921), however only frequently used since the 1990s. It has been known since the time of Hippocrates that fasting is an effective treatment for seizures, and the KD was designed to mimic the fasting state. Despite intensive research in recent years, the mechanism by which the diet protects against seizures remains unknown. Recent hypotheses state that while epileptic foci are hypometabolic areas, metabolic derangement leads to synaptic instability and the development of seizures. Initiation of KD leads to upregulation of several pathways, including energy metabolism genes, mitochondrial biogenesis and an increase in energy reserves which results in more resistant brain tissue to metabolic stress and an increase in seizure threshold (Bough 2008).

To the best of our knowledge, no reports of the use of a KD in urea cycle defects could be found. Furthermore, a question posted on the Metab-L, a mailing list on inborn errors of metabolism (<http://www.daneel.franken.de/metab-l>) did result in negative advice but no experiences were reported. Until now, the effects of the KD have only been described in other metabolic disorders leading to refractory epilepsy (Klepper 2008). Because of the very poor quality of life due to the refractory epilepsy, a trial with a KD was started in both patients. Patients with urea cycle defects are prone for decompensation when they are fasting, due to protein catabolism exceeding its synthesis. While KD induces a

Table 1 Plasma amino acids before start KD and after reaching ketosis

		Glutamine ref 373–709 $\mu\text{mol/l}$	Arginine ref 38–98 $\mu\text{mol/l}$	Alanine ref 158–314 $\mu\text{mol/l}$	Valine ref 133–73 $\mu\text{mol/l}$	Leucine ref 64–164 $\mu\text{mol/l}$	Isoleucine ref 31–83 $\mu\text{mol/l}$
Pat 1	<i>n</i>	<i>N</i> = 7	<i>N</i> = 7	<i>N</i> = 7	<i>N</i> = 7	<i>N</i> = 6	<i>N</i> = 7
6 months before start KD	mean (range)	541 (466–635)	79 (30–119)	344 (229–485)	141 (112–181)	64 (48–88)	33 (15–52)
Pat 1	<i>n</i>	<i>N</i> = 8	<i>N</i> = 8	<i>N</i> = 8	<i>N</i> = 8	<i>N</i> = 8	<i>N</i> = 8
6 months after reaching ketosis	mean (range)	565 (432–718)	58 (22–201)	152 (75–225)	227 (126–288)	113 (41–173)	74 (27–113)
Pat 2	<i>n</i>	<i>N</i> = 3	<i>N</i> = 2	<i>N</i> = 2	<i>N</i> = 2	na	<i>N</i> = 2
6 months before start KD	mean (range)	516 (502–538)	80 (72–87)	341 (306–376)	227 (203–250)		65 (48–82)
Pat 2	<i>n</i>	<i>N</i> = 3	<i>N</i> = 3	<i>N</i> = 3	<i>N</i> = 3	<i>N</i> = 1	<i>N</i> = 3
6 months after reaching ketosis	mean (range)	460 (326–517)	60 (29–106)	132 (103–156)	224 (186–264)	109	70 (61–85)

state of ketosis, it is due to administration of a high fat diet and not to catabolism. No signs of metabolic decompensation were detected in our patients.

In general, numerous adverse effects are seen in KD including weight loss, gastroesophageal reflux, constipation, and diarrhea (Coppola et al. 2009). Diarrhea is seen in approximately 13% of patients receiving KD (Neal et al. 2008). Side effects in patient 1 were indeed diarrhea, and gastroesophageal reflux for which he was treated with omeprazole. Also, he suffered from calciuria necessitating oral potassium citrate. However, the positive effects of the KD on his well-being outweigh the adverse affects by far.

Conclusion

We conclude that the KD does not cause metabolic decompensation, is well tolerated and can be effective in patients who suffer from ASL deficiency and who are treated with protein restriction. The KD may well be an acceptable option for patients with refractory epilepsy due to other inborn errors of metabolism.

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