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Case report

Long-term ketone body therapy of severe multiple acyl-CoA dehydrogenase deficiency: A case report

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ABSTRACT

Objectives: Multiple acyl-CoA dehydrogenase deficiency (MADD) is the most severe disorder of mitochondrial fatty acid β-oxidation. Treatment of this disorder is difficult because the functional loss of the electron transfer flavoprotein makes energy supply from fatty acids impossible. Acetyl-CoA, provided by exogenous ketone bodies such as NaßHB, is the only treatment option in severe cases. Short-term therapy attempts have shown positive results. To our knowledge, no reports exist concerning long-term application of ketone body salts in patients with severe MADD.

Methods: This case report is a detailed retrospective metabolic analysis of a boy with severe MADD. Treatment with sodium β-hydroxybutyrate (NaβHB) started 8 d after birth using gradually increasing doses. In the initial phase, metabolic and acid-base parameters were checked multiple times a day. After 8 y of standardized therapy with 16 g NaβHB, substitution with calcium β-hydroxybutyrate (CaβHB) was attempted. In addition to the β-hydroxybutyrate (βHB) supplementation, continuous adjustments were made to the child's nutrition to provide necessary nutrients.

Results: Treatment with βHB salts leads to adverse effects like gastrointestinal discomfort and alkalosis. Measured concentrations of βHB were predominantly at 0.1 mmol/L or below detectable concentration. Nutritional therapy based on amino acid and acylcarnitine profiles is a necessary part of the therapy in MADD.

Conclusions: Therapy with NaβHB is lifesaving in cases of severe MADD but can have significant adverse effects. Supplementation with CaβHB led to gastrointestinal discomfort and had no additional positive clinical effect. The determined tolerable dose of βHB salt for long-term therapy was not high enough for a notable increase of βHB concentrations in blood.

Introduction

Multiple acyl-coenzyme A dehydrogenase deficiency (MADD), also known as glutaric aciduria type II, is an autosomal recessively inherited disorder of mitochondrial fatty acid oxidation [1,2]. The first description of this metabolic disease, which causes an increased urinary excretion of glutaric acid, was in 1976 [2]. The disorder is caused by a functional impairment of either the electron transfer flavoprotein with its two subunits (ETFA and ETFB) or of the ETF dehydrogenase (ETFDH; synonym ETF ubiquinone oxidoreductase, short ETF-QO) [3]. Clinically, MADD is characterized by a severe (cardio)myopathy, resulting from the dependency of muscle cells on fatty acid β-oxidation and by leukodystrophy [4–6]. A critical metabolic side effect of MADD is hyperammonemia, which is probably caused by the lack of acetyl-CoA from the fatty acid oxidation resulting in a decrease of *N*-acetyl-glutamate, which is an activator of the first step of the urea cycle. Hyperammonemia, toxic short-chain fatty acids, and a low level of ketone bodies play a role in the emergence of encephalopathy during episodes of acute decompensation [7]. The diagnosis of MADD is suggested by abnormal profiles of urinary organic acids and blood acylcarnitines [3,8]. Depending on the severity of the enzymatic defect, several phenotypes are described. Type I appears in the neonatal period and is characterized by additional congenital anomalies. Type II has the same time of onset but shows no anomalies. Type III has a mild (or later) onset of symptoms [9]. Apart from riboflavin-responsive forms of MADD, treatment is very difficult [6]. There is no causally efficacious therapy. The application of riboflavin, L-carnitine, glycine, and a diet with restricted intake of fat and protein can be helpful [3].

Several reports about the treatment of MADD with β-hydroxybutyrate (βHB) sodium salt (NaβHB) have been published. The first report was about a 12-mo-old girl treated using a maximum daily

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dose of 10 g βHB in combination with a low-fat and carbohydrate-rich diet [10]. In a summary report of three children with MADD 2 mo to 2 y of age, an intervention with 450 to 900 mg/kg body weight (bw) βHB resulted in considerable improvement during an observation period of 11 to 19 mo. In all cases, the intake of sodium βHB salt was not reported to be associated with adverse effects [4]. A 2.5-y-old boy with MADD and diffuse leukodystophy exhibited a rapid clinical improvement within 6 to 8 mo after using a daily βHB dosage of 600 mg/kg bw. Follow-up after 2.5 and 3 y revealed a further reduction of symptoms and improvement of the leukodystrophy. The treatment with sodium βHB was described as effective and safe [11]. The latest publication found no increase in blood ketone bodies on a daily oral dose of 900 mg/kg bw βHB in an 8-mo -old girl with severe MADD. A detection of ketone bodies and an improvement of clinical parameters was first observed after treatment with 2600 mg/kg bw βHB salt [12].

In the present study, the course of treatment with βHB salts in a patient with severe MADD over a time period of 10 y is described, presenting the first long-term report of ketone body therapy in MADD.

Methods

The case presentation is based on a retrospective data analysis of a patient with severe MADD during a 10-y observation period.

Case presentation

Patient history and medical observations

The 10-y-old boy was the first child born to parents of Turkish origin. Birth weight (3360 g), body length (52 cm), and head circumference (38 cm) were within the normal ranges. Seizures, respiratory insufficiency, and coma developed 5 d postnatally. Diagnostics revealed lactic acidosis (5.2 mmol/L), massive hyperammonemia (3136 μg/dL), and a pathologic profile of acylcarnitines characterized by an increase of short-, medium- and long-chain acylcarnitines (C4–C16). Hemodialysis followed by continuous venovenous hemodialysis and carbamoyl glutamic acid given to stimulate the urea cycle decreased the ammonia level to 127 μg/dL within 18 h and the child became more alert and active. Beyond the first critical event, a hyperammonemic coma 5 d after birth, the patient experienced problems during the first year of life, with the main problems being recurring vomiting, dehydration, and problems with the intake of nutrients.

Diagnosis of MADD was confirmed by metabolic, enzymatic, and molecular parameters. The genetic analyses showed a homozygous mutation for c.1141 G>C (G381 R) typical for a deficiency of ETF dehydrogenase.

The boy learned to walk within the second year of life. In the third year, he lost the ability to walk and there was increased spasticity of the lower extremities. The MADD-typical leukodystrophy was detected in a magnetic resonance tomography when the boy was 23 mo of age. Echocardiograms showed no signs of cardiomyopathy during the 10 y of treatment.

At 3 y of age, electroencephalography showed a highly pathologic pattern and an active generalized disposition to seizures. Treatment with a combination of the antiepileptics levetiracetam and ethosuximide led to a mostly clinical seizure-free period. For 2 y, the optimized pharmacologic and nutritional treatments have enabled the patient to be completely seizure free.

The child has developed kidney stones (100 % calcite; calcium carbonate) facilitated by severe alkalosis during NaβHB therapy.

In the course of treatment, the patient experienced episodes of metabolic decompensation nearly once a year. These deteriorations were caused by bacterial and fungal infections, especially port infections. A short interruption of enteral βHB therapy, for example, preceding and during general anaesthesia, was another reason for metabolic crisis.

Ketone body therapy

An increasing daily dose of NaβHB (Sigma-Aldrich Corporation, St. Louis, MO, USA; purity >99; racemat, D/L-form) from 0.2 to 2.7 g/kg bw (1.59–21.41 mmol D/L-βHB/kg bw) was administered during the first days of life (starting at 8 d postnatal). No critical clinical events occurred in the initial phase of the treatment; however, the patient developed metabolic alkalosis and hypernatremia. Even with high NaβHB doses, the concentration of βHB in the blood increased only minimally to 0.220 mmol/L, which is low, as depicted as a flat rise in the curve in Figure 1. During treatment with high doses of NaβHB, the patient developed massive alkalosis (pH 7.60; partial pressure of carbon dioxide 54 mm Hg, bicarbonate 52 mmol/L, base excess 27 mmol/L) and severe hypernatremia (165 mmol/L). A decrease of the NaβHB dosage was necessary to reduce the sodium load and to improve alkalosis.

In the following years, the dose of NaβHB in g/kg bw was slowly increased and reached a stable standard daily dose of 16 g NaβHB (1.4 g/kg bw; 126.89 mmol D/L-βHB) after 2 y and 5 mo. Higher doses were not tolerated because severe alkalosis (pH > 7.50) and severe hypernatremia (sodium > 150 mmol/L) developed. Current dosage corresponds to 0.8 g/kg bw daily. A renewed increase, like the initial phase, was not possible because of side effects like alkalosis and vomiting or further undesirable symptoms. Based on change in body weight, a steady reduction in dosage has occurred over time (Fig. 2).

An increased βHB concentration in serum with a maximum of 0.350 mmol/L was observed only in the first months of treatment. Later, the concentrations remained below the detection limit of 0.100 mmol/L in serum. Interruptions of NaβHB therapy led to metabolic problems like hyperammonemia and metabolic crisis.

Despite the execution of all possible therapeutic procedures, it was impossible to stabilize the positive development of the child. In the first 2 y, with high doses of NaβHB, the patient had an almost normal development and learned to walk without support. When NaβHB doses had to be decreased because of side effects, the patient lost the ability to walk. He is mentally disabled, probably because of the episode of severe hyperammonemia directly after birth and the later occurring leukodystrophy.

After 8 y of therapy with 16 g/d NaβHB, a new possibility of βHB intake, using a food-quality calcium salt available from a US manufacturer (KetoTech Inc., Seymour, IL, USA), was explored. The child was hospitalized for the test phase and

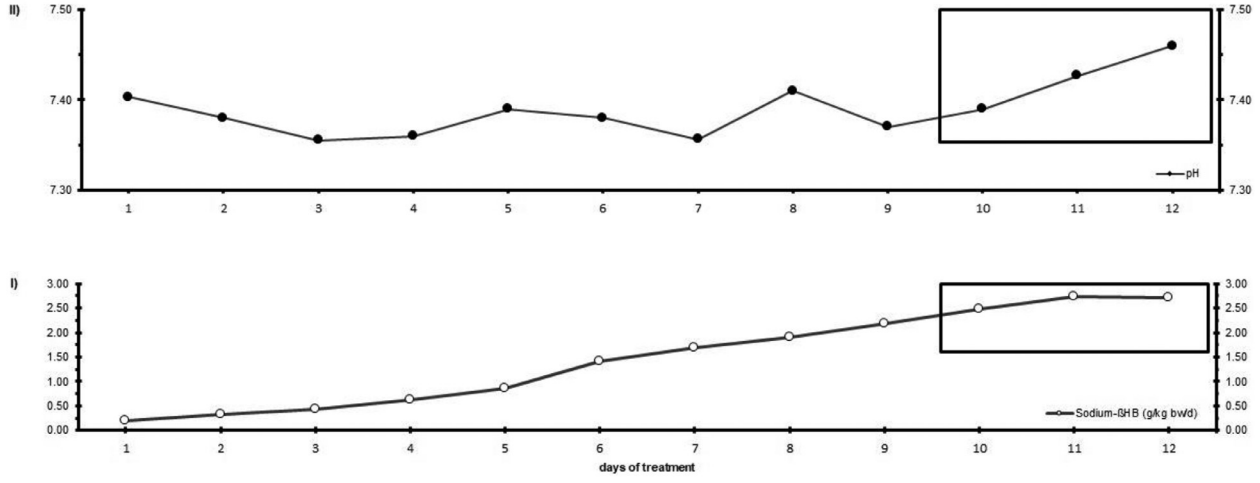

Fig. 1. First 12 d of treatment with NaβHB in a patient with severe MADD with a mark-up of critical phases. (A) Dose of NaβHB per day in g/kg bw and (B) pH during the initial phase of ketone body therapy. βHB, β-hydroxybutyrate; bw, body weight; MADD, multiple acyl-coenzyme A dehydrogenase deficiency; NaβHB, sodium β-hydroxybutyrate.

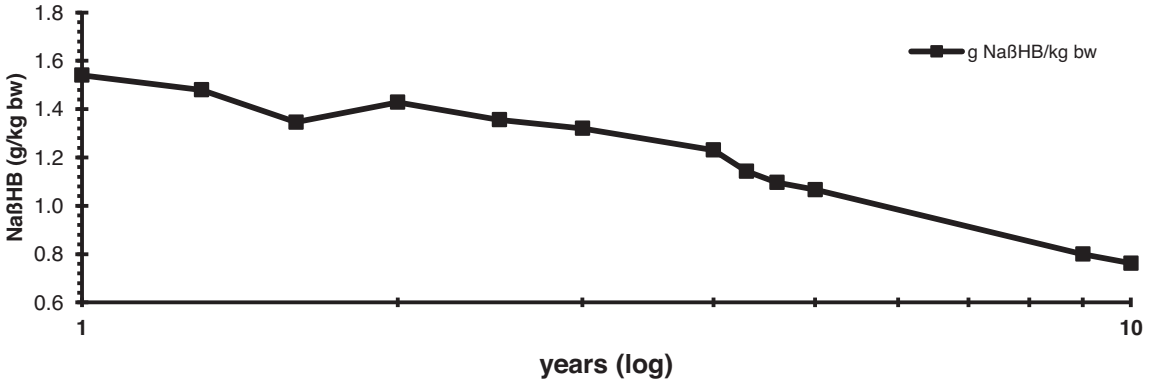

Fig. 2. A 10-y course of NaβHB supplementation (g/kg bw) in a patient with severe MADD. Bw, body weight; MADD, multiple acyl-coenzyme A dehydrogenase deficiency; NaβHB, sodium β-hydroxybutyrate.

Table 1
Composition of safflower and walnut oil including SFAs, MUFAs, and PUFAs in dependence of the administered oil dosage in mL/d

Oil type	Oil (mL/d)	Oil (g/d)	SFA (g)	MUFA (g)	C18:1 (g)	PUFA (g)	C18:2 (g)	ALA C18:3 (g)
Safflower oil	6	5.580	0.530	0.614	0.575	4.218	4.191	0.028
	8	7.440	0.707	0.818	0.766	5.625	5.587	0.037
	10	9.300	0.884	1.023	0.958	7.031	6.984	0.047
	12	11.160	1.060	1.228	1.149	8.437	8.381	0.056
	15	13.950	1.325	1.535	1.437	10.546	10.476	0.070
Walnut oil	6	5.532	0.581	1.062	1.012	3.623	2.899	0.675

ALA, α-linoleic acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

Table 2
Overview of the 10-y nutritional therapy including native protein, total fat, fat from normal food, critical amino acids (L-isoleucine, L-citrulline), and supplementation of essential fatty acids (safflower oil, walnut oil, ω-3 product)

Year	Native protein (g)	L-isoleucine (g)	L-citrulline (g)	Fat total (g)	Maximum fat from nutrition (g)	Safflower oil (mL)	Walnut oil (mL)	Ω-3 product (g)
2	11	0.2*	–	14.5	2.5	6	6	–
	11.5–14		2		1.5			
3	11.5	0.2*	2	12.8	1.5	6	6	–
	14	0.1†		13.5–14				
	16							
		0.1†						
4	17–18	0.1‡	2	12	5–6	6	–	4
	18–20							
	20	0.1*		14		8		2
5	23–25	0.1*	2	17	5–7	10	–	1
	25		6			10–12		0.5
6	25	0.1*	6	17	5–7	10–12	–	0.5
	27				7–8	10		
7	27	0.1*	6	17	7–8	10	–	0.5
8	27	0.1†	6	15	Only tube feeding	Use of a dietetic product containing fat		0.5
	28							
9	28	0.1†	6	17	4			–
	30			29				
10	30	0.1†	6	29	4			–

*Every 2 d.
†Daily.
‡As required.

given a starting dose of 10 g/d (40.60 mmol D/L-VHB/d) in addition to the ongoing therapy with NaβHB. While keeping an eye on metabolic parameters, a constant dose adjustment, 5 g of the βHB-salt (20.30 mmol D/L-βHB) at a time, was made in periods of 1 to 2 d. During the first 10 d of treatment, the additional therapy did not cause any specific clinical events. After 10 d with the maximum dose of 35 g CaβHB (142.12 mmol D/L-βHB/d) in addition to the 16 g/d NaβHB (total daily intake 269.01 mmol D/L-βHB), calcium levels (3.08 mmol/L) went off the scale and led to a metabolic alkalosis (pH 7.53). At the same time, serum showed a very small increase of βHB (maximum 0.18 mmol/L). The effect of the high-calcium content in serum was similar to the effect of the high-sodium content in the first alkalosis that occurred during the initiation phase with the NaβHB salt. Reducing CaβHB to 5 g/d showed a fast response and stabilization of blood parameters (calcium level 1.23 mmol/L; pH 7.36).

The patient developed increased diuresis and loose stools during the 2 mo of treatment with CaβHB, which led to discontinuation of the treatment, soon after which the gastrointestinal side effects were gone. The additional intake of CaβHB did not present any positive clinical effect such as an improvement of muscle performance or increased general health condition.

Nutrition and dietary supplements

The child is fed using a percutaneous endoscopic gastrostomy (PEG) tube and orally. In earlier years, food ingestion took place via a percutaneous endoscopic jejunal tube (PEJ) or *jejunal tube through*-percutaneous endoscopic gastrostomy (JET-PEG). Tube feeding is based on a special formula diet that excludes fat and limits critical amino acids (leucine, isoleucine, valine, and lysine). In the first 3 y, safflower and walnut oils provided essential fatty acids. Later, safflower oil and a

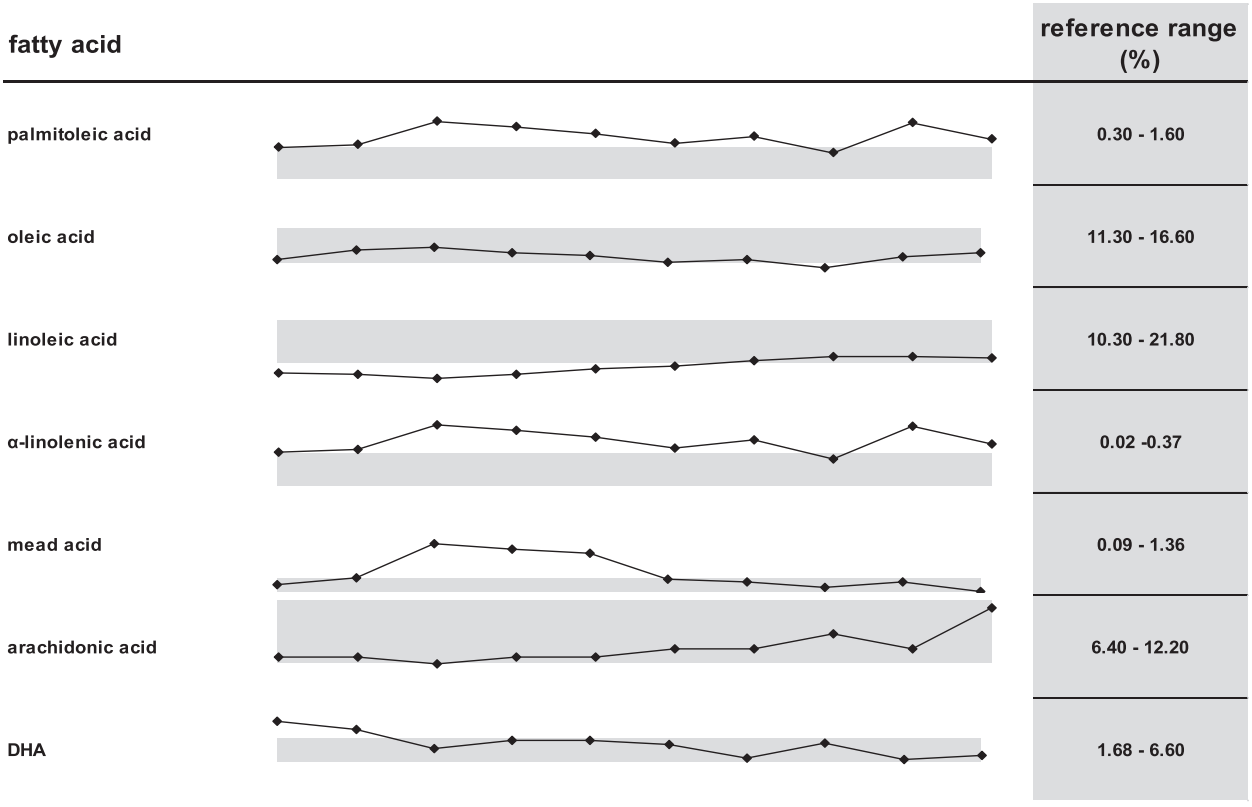

Fig. 3. Analysis of fatty acids in plasma phosphoglycerides with the respective reference range in a patient with severe MADD. The reference areas are shaded in gray. DHA, docosahexaenoic acid; MADD, multiple acyl-coenzyme A dehydrogenase deficiency.

carbohydrate-based docosahexaenoic acid (DHA) powder were used (Tables 1 and 2). There is a clear difference between the essential fatty acids (EFAs) in native safflower and walnut oil. Safflower oil contains more linoleic and oleic acids, whereas walnut oil provides a higher amount of α-linoleic acid (ALA).

The amount of safflower oil and walnut oil administered was based on EFA concentrations determined in plasma phospholipids. At the start of the nutritional therapy, especially linoleic acid was strongly decreased (<5 % of total fatty acid content; range: 10.30–21.80%) and mead acid increased (≤4.89 %; range: 0.09–1.36 %). A high amount of ALA with a low plasma linoleic acid content at the same time, led to the withdrawal of the walnut oil in the dietetic therapy (Tables 1 and 2). At first, the additional treatment with safflower oil showed no effect on the severe linoleic acid deficiency and the accompanying increased mead acid. The initially unclear cause for the stably low linoleic acid content in plasma was caused by the use of a high-oleic safflower oil, which is poor in linoleic acid (high oleic). Directly after changing to a native safflower oil with a high content in linoleic acid, there was an increase in plasma linoleic acid (>10.30 %; maximum 13.1%) and a simultaneous decrease in mead acid (<1.36 %; minimum 0.22%) detectable. The essential ω-3 fatty acids (ALA and docosahexaenoic acid) and the arachidonic acid were predominantly in the reference area (Fig. 3).

Nutritional fat and protein intake were strictly controlled in the first 7 y of life. Native protein from food was started at 11 g and increased to 30 g/d in small steps that were determined by body weight and blood parameters. A special amino acid supplement devoid of the critical amino acids was also used. Fat from natural sources directly from oral food intake was limited to 1.5 g in the first 3 y of life and increased to a maximum allowed intake of 8 g after 6 y. In year 8, the patient disliked oral food intake and had a phase of tube feeding only. In the following years, he was slowly trained back to normal ingestion of food in addition to the tube feed. Caused by the higher fat intake, a partial use of normal tube feeding for children with a balanced composition of fatty acids was possible, and the supplementation with safflower oil and an ω-3 product was no longer necessary. The higher intake of fat in the tube feeding is the cause for a limitation of fat in normal food (4 g/d) in the later phase of therapy. Overall, the daily nutrition specification, especially fat intake, was liberated in later years. A moderate fat-reduced diet with 15% fat in the daily energy (E%) supply was sufficient. Main energy source are still carbohydrates (75% daily energy; Table 2).

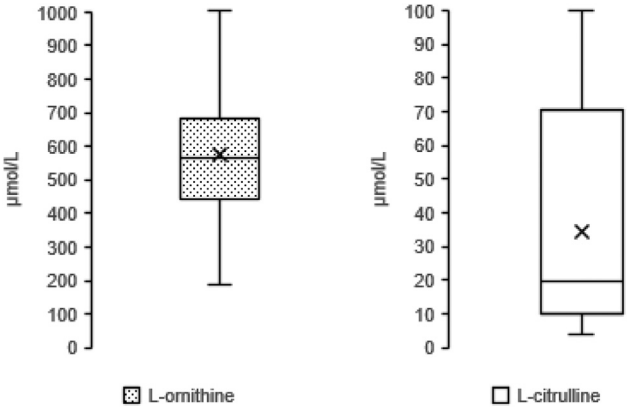

Fig. 4. Boxplot of L-ornithine and L-citrulline measured in a patient with severe MADD within a time period of 10 y (µmol/L; median, mean [×], SD; $n = 22$). MADD, multiple acyl-coenzyme A dehydrogenase deficiency.

Contingent on blood parameters, amino acids were added to the diet when required. The major deficit in essential amino acids was L-isoleucine (35.59 ± 18.16 µmol/L; median 28.50 µmol/L; reference range 50–100 µmol/L). To reach the requirement of the amino acid isoleucine, 0.1 g was added to the tube feed every day or every other day, depending on blood levels of isoleucine. Further, the not proteinogenic amino acid L-citrulline was supplemented according to blood parameters from year 2 on. In the first 5 y, a dosage of 2 g/d was necessary for stabilization of the blood parameter. In later years, with increased body weight, 6 g L-citrulline was added to the tube feeding. The measured value often showed a deficit of L-citrulline (34.64 ± 31.21 µmol/L; median 19.50 µmol/L; reference range 2– 30 µmol/L; Table 2 and Fig. 4). At the same time, a severe hyperornithinaemia

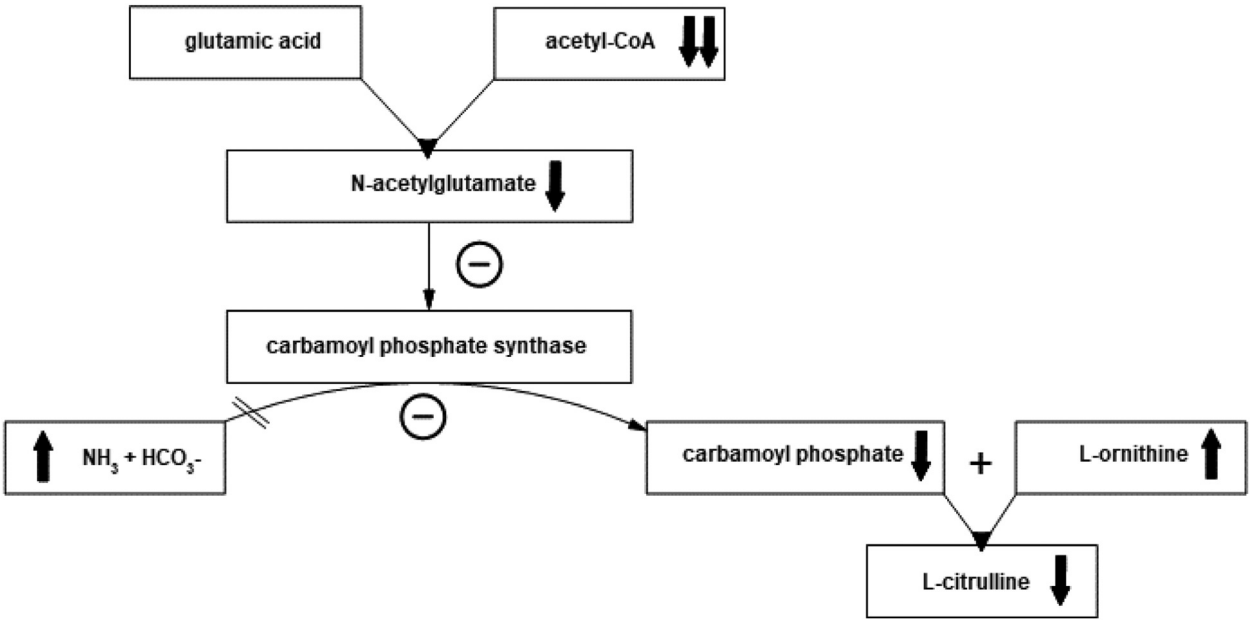

Fig. 5. Hypothesis of the influence of MADD on urea cycle caused by a decreased acetyl-CoA availability. The lack of acetyl-CoA reduces NAG, which is an activator for the carbamoyl phosphate synthase. This functional impairment leads to an increase of ammonia and L-ornithine with a corresponding decrease of L-citrulline. MADD, multiple acyl-coenzyme A dehydrogenase deficiency; NAG, *N*-acetyl-glutamate.

was present (55.69 ± 213.51 μmol/L; median 563.50 μmol/L; reference range 60–160 μmol/L; Fig. 4).

The concentrations of all other potentially critical amino acids in the blood were within the normal range or moderately increased. In most cases, a sufficient amino acid profile was reached using nutritional therapy. Glucose or maltodextrin were added to the tube feeding when energy intake needed to be increased.

The acylcarnitine profiles nearly normalized with therapy, with only mild increases in some acylcarnitines. Free carnitine was monitored to exclude a secondary deficiency. Supplementation with low-dose L-carnitin was needed several times. Furthermore, supplementation with coenzyme Q10 was started in the second year of life. The daily dose was 200 mg (2 × 100 mg).

Acylcarnitine and amino acid profiles established the foundation for the metabolic adjustments in nutritional therapy. In 10 y of nutritional advice, periodical adjustments of nutritional parameters were necessary. By default, changes in the nutrition plan were made two or three times a year and the modifications instructed on the telephone or in a personal counselling session (changes are shown in Table 2).

The body weight development of the child was at the third percentile during the first 5 y. With the start of the sixth year, the weight was continuously below this level. No weight loss was recorded throughout the therapy. Height, like in the body weight, was below peer average. In general, the weight-to-height development of the child is uncritical.

Discussion

Therapy for severe MADD is a multidisciplinary challenge. Several specialized medical fields and nutritionists must be involved in the treatment. In other reports of severe MADD treated with NaβHB as an external energy source, an increase in serum ßHB up to 0.343 mmol·L·0.360 mmol·L^{-1} after an intake of 200 mg·kg bw·150 mg·kg^{-1} bw is described. This comparatively high values resulted from a single-dose feeding and not from continuous tube feeding [4,11].

In this study, a maximum serum concentration of 0.350 mmol/L ßHB at a daily dose of ~2.5 g/kg in serum was observed only once, in the first months of therapy. Throughout the rest of the treatment, serum ßHB was at 0.100 mmol/L or not detectable. A case report from van Rijt et al. showed similar results in a female infant with MADD. The serum βHB reached an average of 0.150 mmol/L with a daily dose of 2.6 g/kg. In the initial phase from 0.45 to 1.4 g/kg daily, the βHB was largely <0.1 mmol/L [12]. The bolus intake of ketone body salts is less physiological and does not allow a well-balanced nutrition. Formation of feeding peaks in serum βHB should be avoided. A relatively high intake of exogenous ketone body salt is generally not sufficient to cover the energy deficit in severe MADD. The main reason could be the importance of fatty acids as an energy source for the heart, skeletal muscle, and other organs [13,14]. The heart generates up to 95% of the necessary energy from oxidative phosphorylation. During postnatal development, fatty acid oxidization becomes the primary energy source for generating adenosine triphosphate in the human heart [15]. Breakdown of the FAO leads to an inadequate supply of energy, which in turn makes a higher intake of alternative fuel, such as ketone bodies, necessary. The low βHB values in serum after ingestion of high doses of βHB salt indicates that a high amount of external βHB is needed if β-oxidation of fatty acids is impossible. For example, a 10-y-old boy with a preferred sedentary lifestyle requires 1600 kcal/d. To cover 30% of this daily energy requirement, the boy would need to ingest ~102 g D-βHB (which is ~204 g D/L-βHB; 1 g βHB = 4.71 kcal) [16,17]. The associated extremely high intake of the racemic sodium (~250 g; ~45.6 g sodium = 1618 mmol) or calcium salt (~244 g; ~ 39.7 g calcium = 991 mmol) is not acceptable and health risks are possible.

None of the available case reports described adverse events in direct relation to the NaβHB treatment [4,10–12]. The longest period reported was 3 y with consistently good tolerability of supplementation using βHB salt. Also, the treated child's renal function and electrolyte balance were inconspicuous [11].

Shifts in acid–base balance often occurred in the present case. A possible reason is the high intake of sodium or calcium as in the additional testing. One g of NaβHB provides 182 mg of sodium, which corresponds to 7.9 mval. A calcium βHB salt provides 163 mg/g calcium (8.1 mval). Daily treatment with 16 g of NaβHB leads to an intake of 2.9 g of sodium (126.9 mval). All acid–base imbalances were marked by an alkalosis. The tendency to acidosis was not detected during long-term treatment with lower doses. The effect of exogenous ketone bodies on the acid–base balance was described in animal models and humans in the 1980s [18–20].

In miniature pigs, an infusion of NaβHB (D/L) led to an increase in pH from 7.40 to 7.56 detecting an alkalizing effect of βHB sodium salt [18]. A test of a sodium acetoacetate in healthy individuals resulted in a rise in pH from 7.37 to 7.48 accompanied by an increase in bicarbonate and sodium in plasma [19]. In obese individuals, a sodium acetoacetate infusion raised pH from 7.36 to 7.40 [20]. The testing of a commercially available ketone body salt mixture of sodium and potassium βHB showed an increase in pH after a single intake in humans. In a parallel run test, a βHB ester compound demonstrated a contrary effect and lowered the pH of the blood. The mild alkalizing effect of the salt was attributed to βHB^- as a conjugated base, assuming a full dissociation of the ketone salt [21]. In this case, there were no appreciable concentrations of βHB in serum, thus excluding βHB as a possible trigger for alkalosis during treatment. High concentrations of cations and the resulting shift in the electrolyte household are suspected to lead to a modification of the acid–base balance. This was also suggested by the opposing effect the ketone body ester had on blood pH as shown by the comparative test in healthy adults [21]. Based on Stewart's acid–base approach, an increase in strong ion difference (SID) can induce a metabolic alkalosis. Specifically, a high cation load causes a high SID and metabolic alkalosis [22,23]. In view of published data and the present findings, the detected alkalosis is a ketone body salt–specific side effect that is potentially independent of the used counter ion. Our testing of a sodium and calcium βHB salt showed no difference in effect and high doses of both salts resulted in a metabolic alkalosis of the patient. To manage the problem in acute phases, a decrease of the SID is necessary. In the present case, a carbonic anhydrase inhibitor was used for higher sodium elimination for compensation of the secondary alkalosis. A higher intake of NaβHB to achieve higher βHB concentrations in serum was not possible because of the salt limitation and possible alkalosis.

The late onset gastrointestinal symptoms triggered by the CaβHB hint at a negative adaptive effect or tolerability of CaβHB. In standard calcium supplements, acute gastrointestinal side effects like constipation, abdominal pain, and severe diarrhea have been described. Generally, the reported symptoms are mild in nature. It is important to note that data acquisition for the adverse effects in publications was often self-reporting [24,25]. There was no indication of a time-lagged negative effect when a high dose of calcium supplement was taken. Although the formation of renal calculi is a potential side effect of CaβHB, the renal calculi in the present patient were not caused by the therapy because they occurred before the treatment with CaβHB started. Renal stone formation was triggered by the high sodium load during sodium βHB therapy and the reduction of solubility in the alkalotic urine. In addition, high intake of sodium is associated with a higher calcium loss and a potential risk for renal stones [26,27]. The additional alkalization of urine by the sodium salt fostered the formation of calcium carbonate stones (calcite) [28,29]. The combination of the higher calcium loss and the urine alkalization are a probable reason for the formation of calcite stones during therapy with NaβHB. Additionally, there are open discussions about an increased risk for cardiovascular events and a decrease in magnesium absorption caused by a high calcium intake [25,30,31]. Recommended dietary allowance of calcium in children 1 to 3 y of age is 700 mg; 1000 mg in ages 4 to 8 y; and 1300 mg in ages 9 to 13 y [32]. Supplementation using 10 g of CaβHB (~1630 mg Ca^{2+}), without considering any other food intake, clearly exceeds this recommendation. Adverse effects are therefore probable and should be monitored during therapy.

The severe linoleic acid deficiency and high value of mead acid at the same time was an important factor in nutritional therapy. The child showed no abnormalities typical for linoleic acid deficiency on his skin. A secondary lack of arachidonic acid, caused by the low linoleic acid, was not detected [33]. A correlation between decreased linoleic acid simultaneous with increased mead acid is a well-known fact. Mead acid, the only de novo synthesized polyunsaturated fatty acid of the human organism, is a marker for deficiency in EFAs like linoleic acid [34,35]. Supplementation with safflower oil to reach a higher concentration of linoleic acid in plasma failed initially. A closer look at the oil used ascertained that the amount of linoleic acid in the oil was very low. Apart from native safflower oils, there are high-oleic safflower oils available on the market. The high-oleic oils contain ≤75 % of oleic acid and only a small amount of linoleic acid (<15 %). An advantage of this composition is higher stability against temperature and oxidation [36,37]. These oils are not useful for supplementation of linoleic acid and should be avoided. The later use of a native safflower oil quickly eliminated the deficiency in linoleic acid.

A supplementation of coenzyme Q10 is still being discussed as therapy for MADD. There is a possibility of a secondary Q10 deficiency in MADD caused by ETFDH mutations, which lead to a myopathy [38]. The secondary Q10 deficiency is generally associated with the riboflavin-responsive type of MADD but is not detectable in all cases [39–41]. In the present case, supplementation with Q10 showed neither a notable effect on muscle performance nor an improvement of myopathy. However, a secondary deficiency in Q10 does not seem to be the main reason for muscle weakness in patients. The Q10 supplementation period was free from adverse effects. Plasma levels of the non-proteinogenic amino acid L-citrulline were determined in short intervals and supplemented accordingly. The preproduct of arginine synthesis is L-citrulline a product of ornithine and carbamoyl-phosphate, an intermediate product in the urea cycle that is important for nitrogen homeostasis [42,43]. The synthesis of carbamoyl-phosphate, a product of ammonia and bicarbonate, requires *N*-acetyl-glutamate (NAG) to activate the enzyme carbamoyl-synthase. A limitation of NAG, made possible in MADD by reduced formation of acetyl-CoA, leads to a reduction of L-citrulline and an increase in L-ornithine and ammonia [43]. The possible mechanisms are displayed in a flow chart in Figure 5. To avoid the cumulative effect of a deficit in acetyl-CoA and the effect on the urea cycle, monitoring L-citrulline and supplementation, in case of deficit, is necessary.

Nutritional therapy carried out by specially educated dietitians is essential to the treatment. Acylcarnitines and amino acid profiles are the foundation for nutritional adjustments in patients with MADD. The routine adaptation of fat and amino acids or protein intake to metabolic parameters is necessary during the child's development. The supplementation of carnitine is especially useful to avoid false acylcarnitine profiles with apparently normal findings. The continuous adaptation avoids very stringent food recommendations, making nutrition more compatible with everyday life. Despite the patient's relatively good physiological development, it was not possible to abstain from tube feeding.

It is important to note that this case study describes the effect of two ketone body salts in a single patient with severe MADD. Additional reports on the long-term effect of exogenous ketone bodies in healthy individuals and patients with metabolic diseases are necessary to adequately assess the effect of supplementation and therapy with βHB.

Conclusion

Therapy with NaβHB is a lifesaving treatment in cases of severe MADD, but it is not free from adverse effects. Side effects limit the supplementation dose and prevent ketone body supplementation that is high enough to compensate for the loss of β-oxidation. During therapy, close care and monitoring is necessary to react quickly to

unwanted metabolic changes. The routine check of electrolytes, acid-base parameters, and urine composition is essential when using a βHB-salt supplement. The intake of a calcium βHB salt had no additional positive clinical effect but generated adverse gastrointestinal symptoms. Nutritional therapy plays an important role in achieving satisfactory metabolic adjustments in MADD patients. New developments in ketone body therapy such as ketone body esters will be necessary for a sufficient supply in MADD.

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