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ORIGINAL ARTICLE

Management and outcome in 75 individuals with long-chain fatty acid oxidation defects: results from a workshop

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Summary At present, long-chain fatty acid oxidation (FAO) defects are diagnosed in a number of countries by newborn screening using tandem mass spectrometry. In the majority of cases, affected newborns are asymptomatic at time of diagnosis and acute clinical presentations can be avoided by early preventive measures. Because evidence-based studies on management of long-chain FAO defects are lacking, we carried out a retrospective analysis of 75 patients from 18 metabolic centres in Germany, Switzerland, Austria and the Netherlands with special regard to treatment and disease outcome. Dietary treatment is effective in many patients and can prevent acute metabolic derangements and prevent or reverse severe long-term complications such as cardiomyopathy. However, 38% of patients with very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency had intermittent muscle weakness and pain despite adhering to therapy.

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Seventy-six per cent of patients with disorders of the mitochondrial trifunctional protein (TFP)-complex including long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency, had long-term myopathic symptoms. Of these, 21% had irreversible peripheral neuropathy and 43% had retinopathy. The main principle of treatment was a fat-reduced and fat-modified diet. Fat restriction differed among patients with different enzyme defects and was strictest in disorders of the TFP-complex. Patients with a medium-chain fat-based diet received supplementation of essential long-chain fatty acids. l-Carnitine was supplemented in about half of the patients, but in none of the patients with VLCAD deficiency identified by newborn screening. In summary, in this cohort the treatment regimen was adapted to the severity of the underlying enzyme defect and thus differed among the group of long-chain FAO defects.

Abbreviations

CPT II(D)	carnitine palmitoyl transferase II (deficiency)
FAO	fatty acid oxidation
LCHAD(D)	long-chain 3-hydroxy-acyl-CoA dehydrogenase (deficiency)
LCT	long-chain triglycerides (long-chain fat)
LKAT(D)	long-chain 3-ketoacyl-CoA thiolase (deficiency)
MCT	medium-chain triglycerides
TFP(D)	mitochondrial trifunctional protein (deficiency)
VLCAD(D)	very long-chain acyl-CoA dehydrogenase (deficiency)

Introduction

Disorders in long-chain fatty acid β-oxidation can affect several enzymes of the mitochondrial β-oxidation or the transport of long-chain fatty acids into mitochondria. Important enzymes involved in the transport of fatty acids into the mitochondrial matrix are carnitine palmitoyl-CoA transferase I (CPT I), carnitine acylcarnitine translocase (CACT) and carnitine palmitoyl-CoA transferase II (CPT II). Within the mitochondria, each β-oxidation cycle of straight-chain fatty acids consists of four enzyme reactions that are chain-length-specific. For long-chain fatty acids (C_{18}–C_{14} acyl-CoA) from diet or from endogenous lipolysis, the initial degradation step in humans is catalysed by very long-chain acyl-CoA dehydrogenase (VLCAD). The following three enzymatic reactions are catalysed by the mitochondrial trifunctional protein (TFP). This multienzyme complex is composed of β-subunits harbouring long-chain enoyl-CoA hydratase and long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) activities and β-subunits harbouring long-chain 3-ketoacyl-CoA thiolase (LKAT) activity, assembled as a heterooctamer (Ushikubo et al. 1996). Mutations in either subunit can result in TFP deficiency with reduced activity of all three TFP enzymes. However, a mutation within the catalytic domain of one of these enzymes results in isolated enzyme deficiency. A common example of the latter is the c.1528G>C mutation in the TFP α-subunit gene, homozygosity of which is responsible for isolated LCHAD deficiency (LCHADD) (Den boer et al. 2002). Only recently, two patients with isolated LKAT deficiency have been identified (Das et al. 2006; Sander et al. 2005). Disorders of the TFP-complex therefore comprise general TFP deficiency (TFPD), isolated LCHADD and isolated LKAT deficiency (LKATD).

Energy production from long-chain fatty acids is impaired in all of the long-chain fatty acid oxidation defects. Clinical symptoms therefore mainly develop during episodes of illness or fasting and affect organs that use long-chain fat as the primary energy source

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such as the heart and skeletal muscles (Ørngreen et al. 2004). In addition, impaired glucose production during catabolism results in hypoglycaemia (Spiekerkoetter et al. 2006).

Fatty acid oxidation disorders are now widely recognized in newborn screening programmes in many countries, leading to a much larger number of diagnosed patients (Wilcken et al. 2003). The majority of patients are asymptomatic at the time of diagnosis. VLCAD deficiency (VLCADD) is the most common disorder of long-chain fatty acid oxidation and disease prevalence has been calculated at about 1:50 000 to 1:100 000 (Spiekerkoetter et al. 2003), while LCHADD/TFPD occur with a prevalence of about 1:110 000 (Das et al. 2006). Even before the screening era, different phenotypes of varying severity and ages of onset were distinguished for long-chain fatty acid oxidation defects (Andresen et al. 1999; Gregersen et al. 2001). The most severe phenotype presents with an acute metabolic encephalopathy and cardiomyopathy in the first days of life. Attenuated phenotypes comprise illness-induced hypoketotic hypoglycaemia and exercise-induced skeletal myopathy as well as episodic rhabdomyolysis. In addition neuropathic symptoms such as peripheral neuropathy and retinopathy are observed in disorders of the TFP-complex (Spiekerkoetter et al. 2004). The latter are not reversible with treatment (Spiekerkoetter et al. 2004), but retention of retinal function and visual acuity was observed in some patients.

Proposals for the management of long-chain fatty acid oxidation disorders have been controversial, especially since individuals identified through newborn screening are mostly asymptomatic. Because evidence-based studies on the treatment of long-chain FAOD and treatment recommendations are lacking, data on 75 patients were collected from 18 metabolic centres in Germany, Switzerland, Austria and the Netherlands with the focus on dietary treatment and outcome. The goal of this study was to outline current expert practice as basis for further prospective studies and the development of approved treatment guidelines.

Methods and results

Questionnaires were sent to 18 metabolic centres in Germany, Switzerland, Austria and the Netherlands in order to collect data on patients with long-chain fatty acid oxidation defects. Seventy-five questionnaires were completed and returned. No data are available about the correct number of patients followed at the respective institutions, some of whom might have died or have been lost during follow-up. A total of 75 subjects with long-chain fatty acid oxidation defects were classified by diagnosis as outlined in Table 1. Diagnosis in all subjects was confirmed by enzyme and/or molecular analysis. All data from the questionnaires were discussed in detail during a two-day workshop.

Patients

The patients designated ‘asymptomatic’ were identified either by newborn screening or by family screening after an index case had been diagnosed because of clinical symptoms or by newborn screening. All remained asymptomatic during follow-up. Within the group of symptomatic patients, 41 were diagnosed clinically before tandem mass spectrometry (MS/MS) screening was available and another 11 were recognized in newborn screening. These 11 patients presented with clinical symptoms in the first days of life, in some patients even before screening results were available. An additional two patients identified by screening developed symptoms during follow-up.

Among the complete group of 75 patients, 32 (42.7%) were identified by newborn screening, 2 further patients by family screening (32 [+2]) (Table 1). Approximately half of all patients were affected by a disorder of the TFP-complex (38/75; 50.7%). Another 32 (42.7%) of the 75 patients of patients had VLCADD. The total number of patients identified per year increased in parallel with the implementation of newborn screening programmes. More than 50% of all patients from our study were younger than 6 years of age (Fig. 1).

Consanguinity was documented in 7/53 (13.2%) families (data available in 53 families). Information about the ethnic background was available for 62 patients, of whom 47 (75.8%) were of caucasian origin (German, Austrian, Dutch or Swiss), 9 (14.5%) of Turkish origin and 6 (9.7%) from other populations. Pregnancy was complicated in 10/62 (16.1%) cases, but specific complications associated with fatty acid oxidation defects such as maternal HELLP (haemolysis, elevated liver enzymes, low platelets) syndrome or maternal AFLP (acute fatty liver of pregnancy) were reported for only five mothers. Among them, four offspring suffered from LCHADD, one from TFPD. Reduced birth weight or preterm birth was observed in 19/66 (28.8%) cases (12/16 patients with LCHADD, 5/7 with other deficiencies of the TFP-complex and 2/28 patients with VLCADD).

Diagnosis in patients with VLCADD was confirmed by either enzyme analysis and/or molecular analysis.

Table 1 Cohort of patients

Patients	VLCADD	LCHADD	LKATD	TFPD	CPTIID	Total
Total	32	27	1	10	5	75
Identified by newborn screening	18 (+2)[a]	7	1	3	3	32 (+2)
Asymptomatic at diagnosis	17/20	4/7	0	0	2/3	23/34
Became symptomatic during follow-up	0/17	1/4	0	0	1/2	2/23

[a] Including 2 patients identified by family screening.

As already reported in the literature, molecular genetic heterogeneity was also documented in our cases (data not shown). All patients with isolated LCHADD were homozygous for the c.1528G>C mutation in *HADHA*, the gene for the TFP-α subunit. Patients with general TFPD were characterized either by reduced activities of LCHAD and 3-ketoacyl-CoA thiolase or by mutations in *HADHA* or *HADHB*, the genes for the TFP-α or TFP-β subunit, respectively, and molecular genetic heterogeneity was also present among these cases (data not shown).

In symptomatic patients the clinical presentation at the time of diagnosis varied with respect to severity and age of onset. Patients with VLCADD and disorders of the TFP-complex presented with cardiomyopathy, arrhythmias, acute metabolic encephalopathy, hypoglycaemia or myopathy (Table 2). In addition, those with a disorder of the TFP-complex had neuropathic long-term complications such as peripheral neuropathy and retinopathy. In general, TFPD and isolated LCHADD could not be distinguished in their acute clinical presentation and long-term complications (Table 2). However, survival in the group of TFP-deficient patients was much lower because of a majority of severe phenotypes. Peripheral neuropathy occurred in 1 surviving patient with general TFPD (3/4) and in 2/13 patients with isolated LCHADD. Retinopathy was observed in 5/13 LCHADD patients and in 1 surviving TFPD patient.

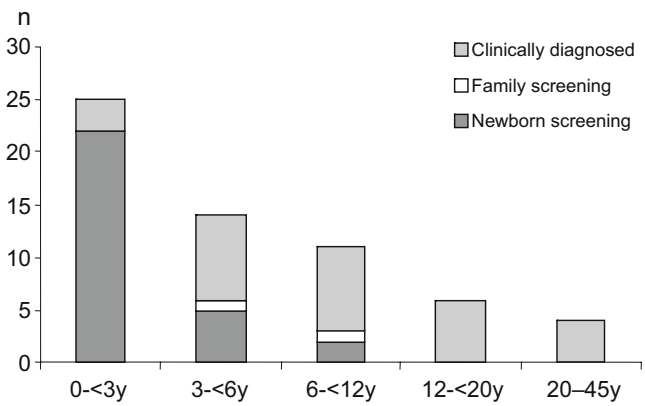

Fig. 1 Age distribution and mode of diagnosis in 75 individuals included in this study

The vast majority of patients with VLCADD (17/20) identified by newborn screening were asymptomatic at the time of diagnosis. These patients as well as their siblings detected by family screening remained asymptomatic in follow-up to 7 years of age. Four asymptomatic newborns were diagnosed with LCHADD by screening (4/7), and three remained asymptomatic during a 2-year follow-up (3/4) (Table 2). All three patients with general TFPD identified by newborn screening were already symptomatic at the time of diagnosis.

Two of 30 VLCADD patients died because of VLCADD-associated symptoms during metabolic derangement; both of them were identified before the newborn screening era. In isolated LCHADD, 3/20 died and they were both identified before the screening era. All those patients with general TFPD who presented in the neonatal period died (5/7). Two of them were identified by newborn screening. Isolated LKATD was diagnosed in only one patient, who also died.

Of the five patients with CPT II deficiency, three were identified by newborn screening. One of them was followed until 5 years of age and remained asymptomatic. The two other children developed severe hepatopathy, one of them also cardiomyopathy, and died within the first year of life. Two clinically diagnosed patients exhibited myopathic symptoms with severe rhabdomyolysis with onset at the ages of 10 and 15 years, respectively. Whereas the first patient was diagnosed at the age of 11 years, one year after onset of symptoms, the second was diagnosed correctly only at the age of 45 years. No signs of cardiomyopathy or hepatopathy were observed in these two patients. Because of the heterogeneous clinical presentation observed in our cohort, consistent with the reported literature, and our limited experience due to the small number of patients, the study group did not further evaluate disease management in the patients with CPT II deficiency.

Table 2 Clinical presentation

	VLCADD	LCHADD	LKATD	TFPD	CPTIID
Data available	30/32	20/27	1/1	7/10	5/5
Identified by screening	20/30	7/20	1/1	3/7	3/5
Symptomatic	3/20	4/7	1/1	3/3	2/3
Cardiomyopathy	0/3	1/4	NA	3/3	1/3
Arrhythmias	2[a]/3	NA	NA	NA	0/3
Reye syndrome	0/3	1/4	NA	2/3	0/3
Hypoglycaemia	1/3	3/4	1/1	1/3	0/3
Hepatopathy/-megaly	1/3	NA	NA	NA	2/3
Hypotonia/myopathy	1/3	2/4	1/1	2[b]/3	1/3
Deceased (at age)	0/20	0/7	1/1 (6 weeks)	2/3 (3 days, 8 days)	2/3 6 months, 7 months)
Clinically diagnosed	10/30	13/20		4/7	2/5
Age at diagnosis	1 day–36 years (median 2.5 months)	3 days–11 years (median 5 months)		1 day–4.5 years (median 1 year)	11 years, 45 y
Cardiomyopathy	6/10	7/13		1/4	0/2
Arrhythmias	3/10	1/13		0/4	0/2
Reye syndrome	1/10	6/13		0/4	0/2
Hypoglycaemia	6/10	13/13		2/4	0/2
Hypotonia/myopathy	4/10	12/13		2/4	2/2
Retinopathy	0/10	5/13		1/4	0/2
Neuropathy	0/10	2/13		1/4	0/2
Deceased (at age)	2/10 (2 months, 3 months)	3/13 3 days, 4 months, 4 years)		3/4 2 days, 7 days, 5 months)	0/2

The numbers after the slash signify in how many patients data were available for the information requested.
NA, not available
[a] 1/2 presented with QT syndrome.
[b] 1/2 presented with apnoea and hypoventilation.

Dietary treatment

Fat-reduced/fat-modified diet

Detailed information on dietary treatment was unfortunately not provided in all questionnaires. Accordingly, the number of patients analysed for their dietary treatment is indicated in the heading of each patient group.

VLCAD deficiency (n=27) In our cohort, 25/27 patients were on a long-chain triglyceride (LCT)-restricted diet. Most of them (21/27) received fat modification by supplementation with medium-chain triglycerides (MCT) (Table 3). In 9/22 patients additional carbohydrates were supplemented in order to ensure euglycaemia between meals, resulting in a hypercaloric diet; 3/22 were on continuous overnight nasogastric tube feeding.

All asymptomatic infants below 4 months of age received a fat-modified diet; however, only 3/10 were fed a special formula low in LCT and high in MCT (Basic-f, [Milupa, Friedrichsdorf, Germany] with MCT added, or Monogen, [SHS, Heilbronn, Germany]) (Table 4). The remaining infants were on a diet consisting of equal volumes of breast milk (or infant formula) and MCT-containing formula (Table 4).

LCHAD deficiency (n=14) LCT intake was restricted in 13/14 patients with LCHAD deficiency. All patients were supplemented with MCT. Eleven of 14 received additional carbohydrates; 2/14 were on continuous overnight nasogastric tube feeding.

Six out of 7 patients with LCHADD identified by newborn screening completely received a special formula low in LCT and high in MCT (Basic-f with MCT added, or Monogen). Only one asymptomatic individual with LCHADD received a diet consisting of a mixture of an MCT-containing formula and a standard infant formula.

General TFP deficiency (n=6) In 3/5 patients the long-chain fat intake was restricted; 5/6 were supplemented with MCT. All patients received additional carbohydrates resulting in a hypercaloric diet, and 2/4 were on continuous overnight nasogastric tube feeding.

Table 3 Fat-restricted and fat-modified diet in FAO disorders:daily fat content

	VLCADD	LCHADD	LKATD	TFPD	CPTIID
LCT					
Data available	27/32	14/27	1/1	5/7	5/5
Intake restriction	25/27	13/14	0/1	3/5	4/5
Maximum amount given (g/kg per day)	0.8–2.1	0.6–2.3	0.5	NA	0.5
MCT					
Data available	27/32	17/27	1/1	6/7	3/5
MCT supplement	21/27	17/17	1/1	5/6	3/3
MCT supplement in screened newborns	13/18	7/7	1/1	3/3	3/3
Amount MCT (g/kg per day)	0.7–3.3	0.5–2.6	2	1.8–4	4.5[a]

NA, data not available.
[a] Information available only in one patient.

General dietary and pharmacological measures

Micronutrient and essential fatty acid supplements

Supplementation of vitamins and minerals was not generally done. In most cases vitamin and mineral consumption from food was documented and blood concentrations of fat-soluble vitamins and minerals were monitored.

Because of the very strict reduction of long-chain fat in the diet, essential long-chain fatty acids were supplemented in most patients. Walnut, soy or wheat-germ oils were preferred as source of essential fatty acids, because they contain linoleic ($C_{18:2}$ ω-6) and linolenic ($C_{18:3}$ ω-3) acids at an ideal ratio. The amount of essential fatty acids was given according to the D-A-CH dietary recommendations (German/Austrian/Swiss Nutrition Societies 2000).

l-Carnitine

Data on l-carnitine supplementation were available in 52 patients. Twenty-eight subjects (54%) received l-carnitine. Twenty-three of these were diagnosed before the screening era; only 5/28 (17.9%) identified by newborn screening received carnitine supplementation and only 1 of these 5 suffered from VLCADD. This patient was supplemented with 30 mg/kg l-carnitine per day for just 1 week when blood free carnitine concentration was as low as 3 μmol/L. The other 4 on l-carnitine treatment presented with a disorder of the TFP complex and received l-carnitine at a dose of 50–120 mg/kg per day. Only 2 of these 4 patients presented with decreased free carnitine in blood when supplementation was started.

Out of the 9 symptomatic patients with VLCADD and carnitine supplementation (9/23; 39%), 7 (78%)

Table 4 Fat-modified diet in FAO disorders up to 4 months

	VLCADD	LCHADD	LKATD	TFPD	CPTIID
Asymptomatic infants					
Data available	10/15 (+2)[a]	4/4			1/2
MCT-containing formula + essential fatty acids	3/10	3/4			1/1
Breast milk/infant formula and MCT-containing formula[b]: 50–50%	6/10				
Fat-free and infant formula[c]: 50–50%	1/10	1/4			
Symptomatic infants					
Data available	5/10	3/4	1/1	1/2	2/2
MCT-containing formula + essential fatty acids	4/5	3/3	1/1	1/2	
Breast milk/infant formula and MCT-containing formula[b]: 50–50%	1/5	0			

[a] 15 identified by newborn screening, 2 identified by family screening.
[b] MCT-containing formula. Special formula low in LCT and high in MCT:
P Basic f, Milupa + 2.0 g MCT oil per 100 ml; amounts per 100 ml: LCT: 0.06 g, MCT 2.00 g.
P Monogen, SHS: amounts per 100 ml: LCT 0.21 g, MCT 1.89 g.
[c] Fat-free formula: Basic f, Milupa without MCT: LCT 0.06 g.

had decreased free carnitine in blood before start of treatment. Out of the 14 symptomatic patients with a disorder of the TFP-complex (14/23; 61%), only 5 (36%) had reduced free carnitine in blood before start of treatment. l-Carnitine was supplemented at 5–100 mg/kg per day in this group.

In summary, in 15/28 patients (54%) supplemented with l-carnitine, blood free carnitine concentration was decreased before treatment.

Other types of treatment

Docosahexanoic acid (DHA) (n=1)

Because of progressive retinopathy diagnosed at the age of 20 months, one patient with LCHADD was supplemented with DHA ($C_{22:6}$ ω-3). Supplementation was started at the age of 11 years with a follow-up of only 6 months with no signs of improvement. Otherwise, DHA was not routinely supplemented in our cohort of patients.

Triheptanoine (n=2)

One patient with TFPD and severe cardiomyopathy was supplemented with triheptanoine, an anaplerotic odd-chain fatty acid triglyceride (Roe et al. 2002), but died at 5 months due to cardiac failure. Another patient with LCHADD was started on triheptanoine at the age of 5 years. Follow-up had been only for a few months at the time of this study.

Avoidance of fasting and recommended maximum fasting periods

Avoidance of fasting in order to prevent endogenous lipolysis was recommended for all patients. Feeding intervals varied with age. In the neonatal period, feeding every 3–4 h, also during nights, was recommended. Overnight continuous nasogastric tube feeding with glucose polymer or infant formula was not generally recommended by attending physicians. In a few patients, but not before completion of the first year of life, uncooked cornstarch was added to the late-evening meal in order to extend the overnight fasting interval (Table 5).

Compliance and outcome

Adherence to treatment as estimated by the attending physician was considered optimal in 20/27 (74%) patients with VLCADD (data available in 27 patients) and in 20/23 (87%) patients with LCHADD/TFPD. However, in 5/27 (20%) patients with VLCADD, and in 2/23 (9%) patients with disorders of the TFP-complex compliance was reported to be suboptimal. Despite this highly rated compliance, 38% of subjects (5/13) with VLCADD presented with intermittent symptoms of skeletal myopathy such as muscle weakness, muscle pain and/or myoglobinuria. Persisting or intermittent skeletal myopathy was observed in 11/17 (65%) subjects with disorders of the TFP complex and good compliance. Three of 14 surviving TFP-deficient patients (including LCHADD) (21%) developed peripheral neuropathy and 6/14 (43%) had retinopathy. However a great number of patients died too early to be able to evaluate this symptom.

In the 5 patients with VLCADD and myopathy (5/13), all had their symptoms in the course of intercurrent illnesses and following exercise. Among the 11 patients with TFP disorders (including LCHADD) and myopathy (11/17), 11 presented with myopathy in the course of a febrile illness and 9 following exercise. Neuropathic symptoms and retinopathy were not induced by external triggers.

Table 5 Supplementation of essential fatty acids with a fat-modified diet and additional carbohydrates or tube feeding as a measure to delay lipolysis

	VLCADD	LCHADD	LKATD	TFPD	CPTIID
Data available	25/32	18/27	1/1	6/10	3/5
Supplementation of essential fatty acids	20/25	14/18	0/1	5/6[a]	1/3
As breast milk or infant formula	4/20			2/5	
As walnut, soy or wheatgerm oil	12/20	12/14		1/5	1/1
As other oil	4/20	2/14		2/5	
Amount (g/kg per day)	0.5–1 (mean: 0.65)	0.45–0.6 (mean: 0.53)		0.3–0.95	0.3
Data available	22/32	14/27	1/1	4/10	3/5
Additional carbohydrates	9/22	11/14	0/1	4/4	2/3
As glucose polymer	4/22	7/14		4/4	2/2
As uncooked cornstarch	5/22	4/14		0/4	0/2
Continuous nasogastric tube feeding overnight	3/22	2/14	0/1	2/4	0/3

[a] In 2 out of 5 patients, temporary supplementation of essential fatty acids.

Discussion

With fatty acid oxidation defects included in newborn screening programmes in many countries around the world, there is increasing need for the establishment of uniform treatment strategies. A group of metabolic experts from 18 metabolic centres in Central Europe collected data on 75 patients with disorders of long-chain fatty acid oxidation in order to characterize their clinical presentation and outcome, assess the potential benefit of detection by newborn screening, and compare current treatment modalities. These data were compiled in order to arrive at some consensus guidelines. However, these guidelines are expert opinion and are not based on any evidence that they improve clinical outcome. Reviewing current data and gathering a consensus is the necessary first step towards prospective multicentre treatment studies to be initiated in order to generate more reliable evidence for treatment recommendations.

The majority of patients in this study presented with VLCADD and disorders of the mitochondrial trifunctional protein; accordingly, characterization of current treatment strategies and outcome was possible only for these disorders. However, these disorders represent the whole spectrum of symptoms observed in long-chain fatty acid oxidation defects (Gregersen et al. 2004).

Many patients with VLCADD remain asymptomatic under preventive measures, which may be due to a high proportion of patients with higher residual enzyme activity and hence milder disease. For some of these individuals it may well be that, even without identification by screening and preventive measures, they would have remained asymptomatic throughout life. These asymptomatic or milder phenotypes are detected with increasing prevalence since the implementation of newborn screening for VLCADD, which has greatly changed the approach to the disease particularly with respect to disease-specific treatment.

Collection of long-term follow-up data is essential for evaluation of treatment. However, follow-up in subjects identified by newborn screening is limited. Earlier reports indicated that initially asymptomatic VLCAD-deficient patients may develop muscle weakness and muscle pain associated with exercise or illness in later life despite treatment (Spiekerkoetter 2007), suggesting that disease phenotype is also dependent on environmental factors and disease triggers. In our study, all asymptomatic individuals with VLCADD identified by newborn screening remained asymptomatic during the observation period up to 7 years of age. In the symptomatic VLCADD patients, treatment reversed the initial clinical symptoms, but up to 38% still suffered from intermittent muscular symptoms or rhabdomyolysis despite treatment. For disorders of the TFP-complex, in addition to these episodic myopathic symptoms, chronic, progressive neuropathic symptoms occurred in about 21% of cases. Forty-three per cent of patients had irreversible retinopathy. Overall, prevalence of peripheral neuropathy in our cohort of TFP-complex patients was lower than previously reported (Ibdah et al. 1998; Sander et al. 2005). The lower incidence of retinopathy and peripheral neuropathy in our study may be related to the fact that patients in our cohort are younger than patients in previously published surveys. Since also initially asymptomatic individuals develop these neuropathic symptoms even if they remain free of acute, life-threatening clinical events, dietary long-term treatment has to be further evaluated in order to define predictive markers for the development of neuropathy and retinopathy. According to a recent survey from Gillingham and colleagues (Gillingham et al. 2005), optimal dietary therapy as indicated by low plasma 3-hydroxyacylcarnitine and high plasma DHA concentrations was associated with retention of retinal function and visual acuity in children with LCHADD or TFPD.

Different mechanisms are responsible for the diverse clinical symptoms. Episodic muscular symptoms such as rhabdomyolysis are most likely due to energy deficiency. Supply of sufficient energy from carbohydrates or medium-chain fat prevents and completely reverses these symptoms (Gillingham et al. 2006). There is published evidence that exercise-induced rhabdomyolysis may be prevented with sufficient MCT intake directly prior to exercise (Gillingham et al. 2005; Spiekerkoetter 2007). In contrast, muscular symptoms and weakness due to peripheral neuropathy are not reversible and cannot be prevented with short-term therapeutic interventions (den Boer et al. 2003), suggesting that neuropathy and retinopathy in long-chain fatty acid oxidation disorders are most likely due to toxic effects of accumulating acylcarnitines or acyl-CoA esters. As these neuropathic changes occur only in disorders of the TFP-complex, long-chain 3-hydroxyacyl compounds appear to be more toxic than other acylcarnitines and acyl-CoA esters accumulating in other long-chain fatty acid oxidation defects. In view of the high incidence of irreversible neuropathic symptoms in patients with disorders of the TFP-complex, dietary long-chain fat intake in our cohort was more restricted in these patients than in VLCAD-deficient patients. A recent study from Roe and co-workers in fibroblasts suggests that acylcarnitine production may also be

dependent on the spectrum of fatty acids in the diet and may be modified by decreasing saturated fat and increasing polyunsaturated fat in the diet. However, this has to be demonstrated *in vivo* (Roe et al. 2007).

Fifty-four per cent of patients received l-carnitine in doses varying from 5 to 100 mg/kg per day. In half of them, blood free carnitine was decreased prior to treatment. About half of the patients had normal blood carnitine concentrations before start of l-carnitine treatment. Half of all patients receiving l-carnitine were supplemented only intermittently when free carnitine in blood was decreased. None of the VLCADD patients identified by neonatal screening was started on l-carnitine at the time of diagnosis. In some patients not supplemented with l-carnitine, blood free carnitine was intermittently decreased and endogenously replenished without l-carnitine supplementation. This is in line with observations in VLCAD-deficient mice (Liebig et al. 2006; Primassin et al. 2008). Furthermore, in the mouse model it has been shown that l-carnitine supplementation induced the production of long-chain acylcarnitines (Liebig et al. 2006; Primassin et al. 2008), raising the possibility that for disorders of the TFP-complex, supplementation of l-carnitine may enhance production of potentially toxic 3-hydroxyacylcarnitines. Overall, supplementation of l-carnitine in fatty acid oxidation defects remains a controversial topic. Potential adverse effects are well recognized by metabolic specialists.

Conclusions

Dietary regimens differ markedly between VLCADD and disorders of the mitochondrial trifunctional protein. In VLCADD, a great number of individuals with presumably mild clinical phenotypes are identified by newborn screening. Restriction of dietary fat is no longer a mainstay of long-term treatment in these individuals. However, MCT seems to prevent episodic myopathic symptoms triggered by exercise and is therefore supplemented long term or in some patients only in situations of increased energy demand. In LCHADD and TFPD the suggested toxic effects of long-chain 3-hydroxyacylcarnitines and CoA-esters determine that long-chain fat intake has to be maximally restricted in all disorders of the trifunctional protein. The exact factors determining the development of irreversible neuropathic long-term complications including retinopathy have not yet been identified.

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