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Review

Disorders of muscle lipid metabolism: Diagnostic and therapeutic challenges

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ABSTRACT

Disorders of muscle lipid metabolism may involve intramyocellular triglyceride degradation, carnitine uptake, long-chain fatty acids mitochondrial transport, or fatty acid β-oxidation. Three main diseases leading to permanent muscle weakness are associated with severe increased muscle lipid content (lipid storage myopathies): primary carnitine deficiency, neutral lipid storage disease and multiple acyl-CoA dehydrogenase deficiency. A moderate lipidosis may be observed in fatty acid oxidation disorders revealed by rhabdomyolysis episodes such as carnitine palmitoyl transferase II, very-long-chain acyl-CoA dehydrogenase, mitochondrial trifunctional protein deficiencies, and in recently described phosphatidic acid phosphatase deficiency. Respiratory chain disorders and congenital myasthenic syndromes may also be misdiagnosed as fatty acid oxidation disorders due to the presence of secondary muscle lipidosis. The main biochemical tests giving clues for the diagnosis of these various disorders are measurements of blood carnitine and acylcarnitines, urinary organic acid profile, and search for intracytoplasmic lipid on peripheral blood smear (Jordan's anomaly). Genetic analysis orientated by the results of biochemical investigation allows establishing a firm diagnosis. Primary carnitine deficiency and multiple acyl-CoA dehydrogenase deficiency may be treated after supplementation with carnitine, riboflavine and coenzyme Q10. New therapeutic approaches for fatty acid oxidation disorders are currently developed, based on pharmacological treatment with bezafibrate, and specific diets enriched in medium-chain triglycerides or triheptanoin.

1. Introduction

Disorders of lipid metabolism affecting muscle may involve endocellular triglyceride degradation, carnitine uptake, long-chain fatty acids mitochondrial transport, or β-oxidation. The pathological hallmark of some of these diseases is an increased neutral lipid content, which may be observed on muscle biopsies specimen with the specific staining of Sudan black or oil red O techniques by optic microscopy. In a normal muscle, lipid content takes the aspect of small droplets which concentration and size are usually higher in type 1 fibres than in type 2 fibres [1]. The average lipid fraction of the fibre volume is estimated to less than 0.2% [2], but most pathologists evaluate this lipid content subjectively, making therefore difficult to determine a clear-cut level of lipid accumulation which could be considered accurately as "pathological". The term of lipid storage myopathies is often used when the accumulation of lipid droplets in muscle fibres is uppermost, and associated with a vacuolated appearance on routine histological stains such as hematoxylin and eosin or Gomori trichrome. Conversely, lipid metabolism disorders are inconstantly leading to a muscle lipidosis, and therefore awareness of their clinical features and main biological anomalies are essential for establishing accurate diagnosis. Muscle lipid metabolism having been comprehensively described previously [2–6], we provide here a scheme of the major enzymatic pathways involved in currently known metabolic myopathies involving lipid metabolism (Figs. 1 and 2). In this paper, we describe the main muscle disorders to consider in this context, according to the severity of pathological findings. Although some of these disorders are extremely rare, their diagnostic approach may be considerably improved considering the clinical features, the importance of lipid accumulation, and results of routine biochemical analysis such as plasma carnitine and acylcarnitine profile.

Abbreviations: CPT, carnitine palmitoyl transferase; ETF, electron transfer flavoprotein; FAO, fatty acid oxidation; LCHAD, long-chain 3-hydroxyacyl-CoA dehydrogenase; MAD, multiple acyl-CoA dehydrogenase; MCAD, medium-chain acyl-CoA dehydrogenase; MTP, mitochondrial trifunctional protein; NLSD, neutral lipid storage disease; SCAD, short-chain acyl-CoA dehydrogenase; VLCAD, very-long-chain acyl-CoA dehydrogenase.

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2. Diagnoses to consider in patients with massive lipidosis

2.1. Primary carnitine deficiency (PCD)

PCD (also called carnitine uptake defect or systemic carnitine deficiency) is the most classical cause of lipid storage myopathy

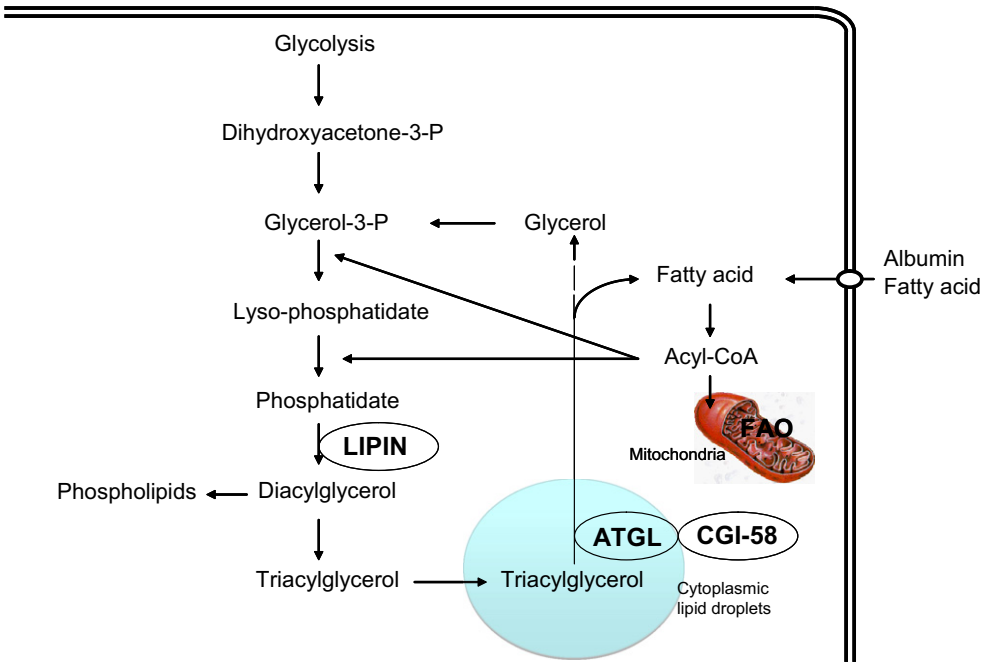

Fig. 1. Simplified scheme of lipid metabolism. ATGL: adipose triglyceride lipase; CGI-58: activator of ATGL; FAO: fatty acid oxidation; LIPIN: phosphatidic acid phosphatase.

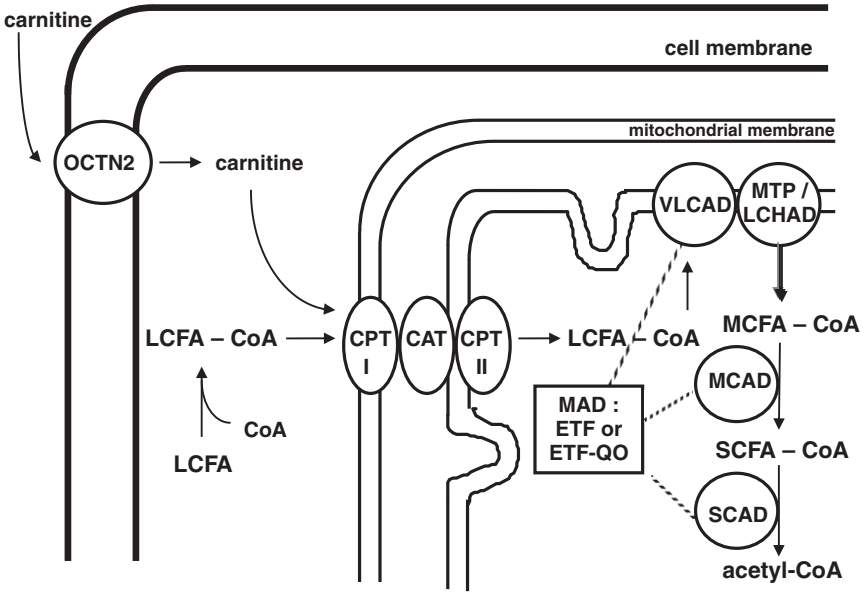

Fig. 2. Simplified scheme of mitochondrial fatty acid oxidation. CAT: carnitine acylcarnitine translocase; CPT I: carnitine palmitoyl transferase I; CPT II: carnitine palmitoyl transferase II; LCFA: long-chain fatty acid; LCHAD: long-chain 3-hydroxyacyl-CoA dehydrogenase; MAD: multiple acyl-CoA dehydrogenase; MCAD: medium-chain acyl-CoA dehydrogenase; MCFA: medium-chain fatty acid; MTP: mitochondrial trifunctional protein; OCTN2: plasma membrane sodium-dependent carnitine transporter; SCAD: short-chain acyl-CoA dehydrogenase; SCFA: short-chain fatty acid; VLCAD: very-long-chain acyl-CoA dehydrogenase.

but remains exceptional [7]. This disease is caused by a defect in the high-affinity plasma membrane sodium-dependent carnitine transporter (OCTN2) in several tissues, including, muscle, heart, and kidney, but not liver. This induces increased loss of carnitine in urine and decreased concentration in plasma, heart and skeletal muscle. The most common phenotype is characterized by generalized muscle weakness, and progressive hypertrophic or dilated cardiomyopathy leading to cardiac failure, occurring before the age of 10 years. Severe fasting hypoglycaemia leading to coma is sometimes observed in infants. PCD has also been diagnosed in asymptomatic adult women, whose unaffected infants were identified with low carnitine levels by newborn screening using tandem mass spectrometry [8]. A massive lipid storage may be observed in skeletal muscle, heart and liver. Lipid vacuoles in skeletal muscle are predominantly observed in type 1 fibres, with often type 2 fibre atrophy [9]. Biochemical investigations show a generalized reduction of carnitine content in all tissues (heart, muscle, liver) and in plasma. Plasma total and free carnitine are less than 10% of controls, but carnitine esters are not increased (no acylcarnitines), and total carnitine is reduced to less than 5% of controls in muscle [10]. Diagnosis may be confirmed by demonstrating reduced carnitine uptake in lymphocytes or skin fibro-

blasts [11], and mutations have been found in *SLC22A5*, the gene coding for OCTN2 [12].

2.2. Neutral lipid storage diseases (NLSD)

NLSD (initially named multisystem triglyceride storage disorder) are disorders of endogenous triglyceride catabolism due to deficiencies of hormone-sensitive lipases which normally hydrolyze triglycerides, diglycerides and monoglycerides. One of these enzymes, adipose triglyceride lipase (ATGL) specifically catalyzes the first step in the hydrolysis of triacylglycerol, generating free fatty acids and diacylglycerol. This enzyme requires the activator protein CGI-58 (protein of the esterase/lipase/thioesterase subfamily), located on the surface of cytoplasmic lipid droplets. Mutations in the gene coding for CGI-58 (*ABHD5*) are responsible for neutral lipid storage disease with ichthyosis (NLSDI) also called Chanarin–Dorfman syndrome [13,14]. NLSDI is a multisystem triglyceride storage disease occurring in childhood, characterized by the presence of non-bullous congenital ichthyosiform erythroderma, mild proximal myopathy (around 60% of cases), and hepatomegaly. Several additional clinical features may also be present, including microcephaly, mental retardation, hearing loss, cataract, nystagmus, and intestinal involvement. The most salient laboratory abnormality is the occurrence of intracytoplasmic lipid droplets in leukocytes, visible on peripheral blood smear (Jordan's anomaly). A massive accumulation of lipid droplets is also present in type 1 and 2 muscle fibres, even in patients without clinical myopathy [15], and lipid vacuoles are also observed in keratinocytes of epidermidis.

Neutral lipid storage disease with myopathy (NLSDM) is caused by mutations of the gene coding for ATGL (*PNPLA2*) [16]. Patients may present initially walking delay and sport activities impairment during childhood, but clinical investigations are generally undertaken because of a slowly progressive muscle weakness occurring between the second or third decade. Proximal and distal limb muscles may be involved, with a predominant distal weakness in some patients [16,17]. Dilated cardiomyopathy, leading to heart failure and severe arrhythmia, has also been reported [16–18]. In peripheral blood smears, neutral lipid vacuoles are always observed in leukocytes. Muscle biopsy shows a massive lipidosis, and rimmed autophagic vacuoles have been recently reported as a salient feature in a Japanese patient [17]. Biochemical analyses do not detect any defect in cholesterol, triglycerides, blood carnitine, mitochondrial FAO or respiratory chain activity. Mutations have been reported so far in less than 10 patients involving mainly the hydrophobic lipid-binding domain which is essential for its association with lipid droplets and for its interaction with CGI-58 leading to enzyme activation. However one mutation has also been reported in the catalytic site (patatin domain) of the enzyme [19].

2.3. Multiple acyl-CoA dehydrogenase (MAD) deficiency

MAD deficiency, also known as glutaric aciduria type II (GAII), results in abnormal fatty acid, amino acid, and choline metabolism. The biochemical abnormalities are explained by deficiency of one of the two electron transfer flavoproteins which transfer electrons from acyl-CoA dehydrogenases to the respiratory chain: ETF (Electron Transfer Flavoprotein, coded by *ETFA* and *ETFB* genes) and ETF-QO (ETF-ubiQuinone Oxidoreductase coded by *ETFDH* gene). MAD deficiency has a wide range of clinical presentations, the most severely affected patients dying in the newborn period of congenital anomalies such as cystic renal dysplasia. Milder cases present later in childhood with hypoglycaemia, encephalopathy, muscle weakness or cardiomyopathy. Less severely affected patients might present with progressive muscle weakness or rhabdomyolysis episodes at adult age, and some of these patients show a dramatic response to riboflavin treatment.

It has been recently demonstrated that riboflavin-responsive MAD deficiency is associated with mutations in *ETFDH* gene, at least in a large proportion of cases [20]. Major symptoms observed in these patients were cyclical vomiting during childhood, episodes of acute encephalopathy generally precipitated by an infection, and rapidly evolving muscle weakness with myoglobinuria in one case. The pattern of muscle involvement was characterized by a severe proximal and axial weakness (predilection for neck involvement), dysphagia and respiratory insufficiency in some patients. Muscle histology showed a muscle lipidosis, except in one patient. Urine organic acids were abnormal in all patients at the time of presentation, typically with C_5–C_{10} dicarboxylic aciduria and acylglycine derivatives. Blood acylcarnitine analysis showed raised concentrations of all chain lengths acylcarnitines (C_4–$C_{18:1}$) whether the patient was stable or acutely unwell. Plasma free carnitine levels were lowered. In addition, probably secondary impairment of respiratory chain enzymes deficiencies (RC) have been found in patients for whom such studies have been possible, and the decrease was more pronounced for complex I and ETF/ETF:QO-dependent activities.

ETFDH pathogenic mutations have also been identified in seven patients who carried the diagnosis of myopathic form of coenzyme Q10 deficiency [21]. All patients presented with fluctuating proximal myopathy, premature fatigue and high CK levels. Triggering factors could be infections, fasting, pregnancy or surgery. The muscle biopsies from these patients showed excessive lipid droplets with small vacuoles, predominantly in type 1 fibres, a few cytochrome oxidase (COX) negative fibres in all cases, and ragged red fibres in two cases. A focal or diffuse decrease of succinate dehydrogenase (SDH) staining was noticed in two cases. The activity of respiratory chain complexes I, II + III, and IV, and muscle CoQ10 level were decreased in all patients. Tandem mass spectrometry detected a combined elevation of all chain length acylcarnitines (C_4–$C_{14:1}$). Free carnitine was constantly diminished. A dramatic clinical improvement was noticed in all these patients after CoQ10 and riboflavin supplementation.

These data, and recent reports of teen and adult patients with *ETFDH* mutations [22–24] indicate that most of myopathic forms of MAD deficiency are probably related to mutations in *ETFDH* gene, and demonstrate also the difficulty to distinguish between mitochondrial FAO and respiratory chain disorders either on morphological or on biochemical basis when an enzyme deficiency has potential repercussions on both pathways.

3. Diagnoses to consider in patients with mild or inconstant muscle lipidosis

3.1. Carnitine palmitoyl transferase II (CPT II) deficiency

CPT II was the first inherited defect of FAO to be identified [25]. Three different clinical phenotypes are associated with a defect in CPT II according to the age of onset, but muscular symptoms (recurrent myoglobinuria, muscle aching and stiffness on long-term exercise) occur mainly in the juvenile-adult onset form. This myopathic form is probably the most frequent cause of recurrent myoglobinuria in young adults. Episodes of myalgias and rhabdomyolysis are triggered by prolonged exercise, infections, fasting, cold or emotional stress. Permanent muscle weakness is very uncommon, and heart is not affected. CK levels are normal outside episodes of muscle injury. A mildly increased lipid content in muscle biopsy is observed in 20% of cases [26]. Acylcarnitine profile may show increased long-chain acylcarnitines (C_{16}, $C_{18:1}$, C_{18}), and CPT II activity may be assessed in leukocytes, cultured fibroblasts or muscle biopsies. A prevalent mutation (c.338C > T,

p.Ser113Leu) in the *CPT2* gene has been identified in more than 60% of mutant alleles in the myopathic form [27,28].

3.2. Very-long-chain acyl-CoA dehydrogenase (VLCAD) deficiency

Very-long-chain acyl-CoA dehydrogenase (VLCAD) deficiency most often occurs in childhood with cardiac or liver involvement, but rhabdomyolysis attacks have been increasingly reported in adults [29,30]. The juvenile or adult-onset myopathic form is very similar to CPT II deficiency, characterized by recurrent episodes of rhabdomyolysis triggered by prolonged exercise, fever, cold or fasting. However, in contrast to adult CPT II deficiency in which the clinical features are limited to skeletal muscle, early-onset extramuscular symptoms may be observed in some patients with VLCAD deficiency. Pathologic findings in muscle biopsies are a moderate lipid storage in approximately one third of cases, predominating in type 1 fibres. A mild mitochondrial proliferation, associated with a decrease of respiratory chain activity and coenzyme Q levels has been observed in one patient [30]. The diagnosis of VLCAD deficiency relies on the measurement of plasma or blood spotted onto filter paper acylcarnitines by tandem mass spectrometry (MS/MS), allowing the detection of abnormal long-chain acylcarnitines with tetradecenoylcarnitine ($C_{14:1}$) as predominant species. Immunohistochemical analysis using an antibody to VLCAD on muscle seems to be another efficient diagnostic method, but comparative studies with results of acylcarnitine screening are needed [31]. Analysis of the *ACADVL* gene shows a wide mutational spectrum, most of the mutations being private [32], with a clear correlation of genotype with disease phenotype [33].

3.3. Mitochondrial trifunctional protein (MTP) deficiency

Patients with MTP deficiency can be classified into two groups: long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) and MTP (combined enzyme) deficiencies. The majority of MTP-deficient patients have an isolated deficiency of LCHAD activity due to mutations in LCHAD domain of the α-subunit (*HADHA* gene). Clinical manifestations are heterogeneous, but similar in LCHAD and MTP deficiencies [34]. Severe encephalopathy and hepatopathy may occur in infancy often leading to death. A late-onset form may occur later in childhood, which predominant manifestations are rhabdomyolysis episodes, cardiomyopathy, pigmentary retinopathy and progressive sensorimotor axonal peripheral neuropathy. Rhabdomyolysis episodes may be induced by exercise, illness or fasting, and are accompanied by life-threatening respiratory failure in 45% of patients [35]. The sensorimotor neuropathy is a distinguishing feature which has not been reported in patients with other FAO defects, and Spiekerkoetter et al. [35] reported the combination of episodic rhabdomyolysis and peripheral neuropathy in 10/11 patients with first clinical symptom occurring in childhood. Muscle biopsy reveals evidence of denervation with atrophic fibres and a predominance of type 1 fibres, but lipidosis is rare, and was observed in only one patient in this series. Skeletal muscle from patients with LCHAD deficiency sometimes show impaired respiratory chain function (decreased activity of complex I and often of complex II, III) and, on trichrome preparation some fibres resemble ragged red fibres [36,37].

During stable clinical conditions, a normal blood acylcarnitine profile may be observed, mainly in adult patients, whereas during episodes of rhabdomyolysis long-chain hydroxyacylcarnitines as well as 3-hydroxydicarboxylic aciduria are significantly elevated. A prevalent missense mutation in the LCHAD domain of the α-subunit (c.1528G > C; p.Glu510Gln) has been identified in approximately 90% of mutant alleles in LCHAD deficiency [38]. Unlike LCHAD deficiency, the molecular basis of MTP deficiency is heterogeneous with mutations identified in both *HADHA* and *HADHB*, the genes coding for the α- and β-subunits, respectively [39].

3.4. Phosphatidic acid phosphatase (LIPIN) deficiency

Mutations in *LPIN1* gene is a newly identified cause of recurrent episodes of acute myoglobinuria in childhood [40]. The *LPIN1* gene encodes the muscle-specific phosphatidic acid phosphatase (LIPIN), a key enzyme in triglyceride and membrane phospholipids biosynthesis, which catalyzes the conversion of phosphatidate to diacylglycerol in the triacylglycerol pathway. Seven patients were reported by Zeharia et al. [40], all suffering from recurrent episodes of myoglobinuria precipitated by febrile illnesses. Age at first episode varied between 15 months and 7 years, with peak CK levels varying from 20,000 to 450,000 U/l. All the following tests gave normal results: total and free carnitine, blood acylcarnitine profile, urinary organic acids, and FAO studies in fibroblasts or lymphocytes. Muscle biopsy findings showed a moderate lipid accumulation in two patients, but a normal lipid content in two other patients.

3.5. Medium-chain acyl-CoA dehydrogenase deficiency (MCAD)

MCAD deficiency is one of the most frequent diseases of mitochondrial FAO. Typical symptoms include hypoketotic hypoglycaemia, Reye-like syndrome, and coma beginning in the second year of life [2]. Skeletal muscle disease is very rare, but it seems that some patients may develop a mild myopathy with lipid excess in muscle, or rhabdomyolysis episodes after strenuous exercise or alcohol ingestion [41,42]. Increased octanoylcarnitine (C_8) and decenoylcarnitine ($C_{10:1}$) as well as their corresponding free acids (octanoic and cis-4-decenoic acids) are the most useful diagnostic markers in blood, and are also detectable in high amounts in urines. Patients may have dicarboxylic aciduria, with abnormal acylglycine excretion (hexanoyl-, suberyl- and phenylpropionylglycine). Plasma carnitine levels are usually lowered. Genetic studies showed that most symptomatic MCAD-deficient patients carry a homozygous c.985A > G (p.Lys304Glu) point mutation [43].

3.6. Short-chain acyl-CoA dehydrogenase deficiency (SCAD)

Patients in whom SCAD deficiency has been detected present a wide range of clinical findings including acute acidosis, developmental delay, hypotonia, seizures, and more subtle later onset progressive myopathy [44]. Hypoglycaemia is not usual in this disorder. A variant phenotype characterized by progressive external ophthalmoplegia with ptosis, cardiomyopathy, and scoliosis has also been described. Muscle histology may reveal either lipid storage myopathy or multiminicore features [45,46]. The major biochemical hallmark is the presence of increased concentrations of ethylmalonic acid (EMA) in urine. The spectrum of variations in the *ACADS* gene is remarkable, compared to other FAO defects, since no patients with null mutation on both alleles have been identified: it must be considered that they are incompatible with life [47]. There are difficulties to differentiate causative mutations, benign variants and disease susceptibility alleles in this disease. The majority of reported patients with apparent SCAD deficiency carry only the common variant genotypes (c.625G > A/c.625G > A, c.511C > T/c.511C > T and c.625G > A/c.511C > T) or a genotype defined by common variant in one allele and rare mutation in the other. However these variations are present in a high percentage of the general population in homozygous or heterozygous form, and it has been hypothesized that the c.625G > A (p.Gly209Ser) and c.511C > T (p.Arg171Trp) variations, may in combination with other genetic and/or environmental factors like fever, during foetal development or early life, trigger disease in some individuals. Therefore the relevance of *ACADS* gene mutations to clinical problems remains unclear in several cases.

3.7. Other diseases with lipid accumulation in muscle

Although the most important lipid accumulations are usually due to deficiencies of lipid metabolism, various conditions may be also associated to lipid accumulation, such as obesity, Cushing disease, alcoholism, or drug-induced myopathies [48]. A relationship between insulin resistance and fat content of the skeletal muscle has been demonstrated in animal tissue [49]. Human studies assessing intramyocellular lipid content with dual energy X-ray absorptiometry (DEXA), computed tomography, or ^{1}H-magnetic resonance spectroscopy showed that increased fat content was an important marker of insulin resistance in obesity [50].

Congenital myasthenic syndromes (CMS) are a group of diseases caused by genetic defects impairing neuromuscular transmission. In our personal experience CMS has been misdiagnosed in several patients who presented moderately fluctuating limb-girdle weakness more suggestive of a congenital myopathy, and an increased lipid content on muscle biopsy leading to a diagnosis of metabolic myopathy. Ben Ammar et al. [51], recently described the phenotype–genotype analysis of 15 patients with CMS due to mutations in *DOK7* gene, and found a high frequency of lipidosis in muscle biopsy (approximately half of the patients). A reduced palmitate oxidation rate was also detected in the cultured myoblasts from one of these patients, arguing in favour of a secondary FAO defect.

4. Diagnostic strategy of muscle lipidosis

Although there is no clear-cut definition of a muscle lipidosis due to the subjective analysis, pathologists are generally able to determine if a biopsy shows a massive or a moderate lipidosis (Fig. 3). When a massive lipidosis is present, three lipid storage myopathies should be envisaged: primary carnitine deficiency, multiple acyl-CoA dehydrogenase deficiency, and neutral lipid storage disease (with or without ichthyosis). The main biochemical exams allowing orientation towards a precise diagnosis are measurements of plasma/blood carnitine and acylcarnitines, urinary organic acid profile and search for Jordan's anomaly in blood smear. Genetic analysis will be performed in a second step, searching for mutations in *SLC22A5*, *ETFDH*, *ETFA*, *ETFB*, *ABHD5*, or *PNPLA2* genes, according to the results of these exams (Table 1).

If muscle biopsy shows a moderate lipidosis, in a context of exercise intolerance or rhabdomyolysis episodes, a FAO disorder should be systematically suspected and blood acylcarnitine profile is now the key biochemical analysis. A diagnostic flowchart has been recently proposed, in which it is proposed to directly perform genetic analysis when results of acylcarnitine profile are characteristic of CPT II, VLCAD, or MTP deficiencies [6]. However a biochemical analysis of CPT II activity or a direct search for mutation(s) in *CPT2* gene should be performed systematically even if the acylcarnitine profile is non conclusive, in the initial investigations of patients with recurrent rhabdomyolysis episodes. Possibility of mutations in *LPIN1* gene should also be evoked in children with recurrent rhabdomyolysis without acylcarnitine profile abnormality and normal CPT II activity.

5. Treatment

Proposed treatment strategies for lipid metabolism disorders include: (1) avoidance of exacerbating factors, (2) carnitine supplementation, (3) riboflavin treatment and (4) dietary modifications (medium-chain triglycerides and triheptanoin).

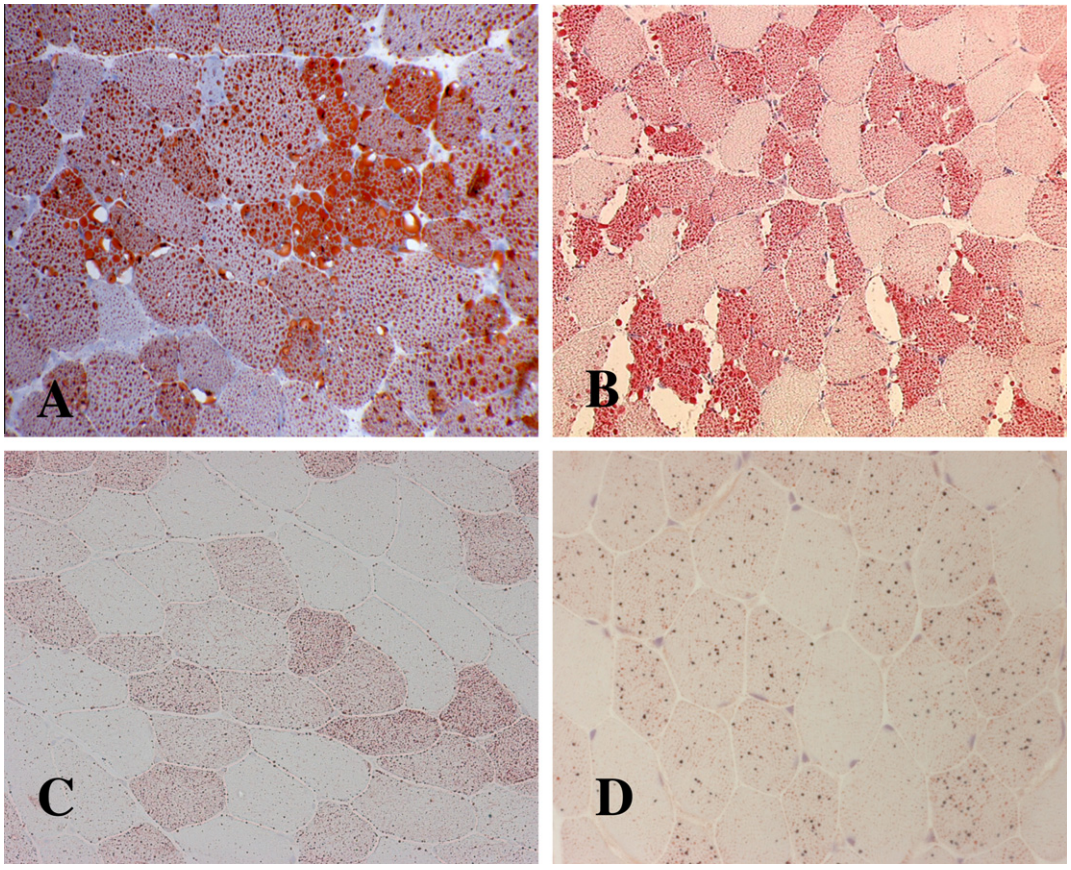

Fig. 3. Muscle pathology showing the variability of lipid accumulation in patients with lipid metabolism disorders (oil red O staining). Neutral lipid storage disease (A), multiple acyl-CoA dehydrogenase deficiency (B), very-long-chain acyl-CoA dehydrogenase deficiency (C), LIPIN deficiency (D).

Table 1
Main clinical neuromuscular and biological features of metabolic disorders with muscle lipidosis.

Disorder	Main neuromuscular symptoms [a]	Increase in muscle lipid droplets	Laboratory features[b]	Gene
Primary carnitine deficiency	Proximal muscle weakness, cardiomyopathy	+++	**Very low plasma and muscle carnitine** No acylcarnitines	*SLC22A5*
Neutral lipid storage disease (NLSD)	Proximal or distal muscle weakness, cardiomyopathy	+++	Normal plasma carnitine Normal acylcarnitine profile **Lipid vacuoles in leukocytes**	*ABHD5* *PNPLA2*
Multiple acyl-CoA dehydrogenase (MAD) deficiency	Proximal and axial weakness Rhabdomyolysis (rarely)	++ to +++	Low plasma carnitine **Increased acylcarnitines of all chain lengths** **2-Hydroxyglutaric aciduria ± acylglycines**	*ETFDH*
Carnitine palmitoyl transferase II (CPT II) deficiency	Rhabdomyolysis episodes	0 to +	Normal or moderately reduced plasma carnitine **Increased long-chain acylcarnitines (C_{16}, $C_{18:1}$, C_{18})**	*CPT2* (p.Ser113Leu prevalent mutation)
Very-long-chain acyl-CoA dehydrogenase (VLCAD) deficiency	Rhabdomyolysis episodes Cardiomyopathy	0 to +	Normal or moderately reduced plasma carnitine **Increased long-chain acylcarnitines ($C_{14:1}$ as main species)** Dicarboxylic aciduria	*ACADVL*
Mitochondrial trifunctional protein (MTP) deficiency	Rhabdomyolysis episodes Cardiomyopathy Axonal peripheral neuropathy	0 to +	**Increased long-chain 3-hydroxyacylcarnitines** Dicarboxylic and 3-hydroxydicarboxylic aciduria	*HADHA* *HADHB*
Phosphatidic acid phosphatase deficiency	Rhabdomyolysis episodes	0 to +	Normal plasma carnitine Normal acylcarnitine profile Normal urinary organic acids	*LPIN1*
Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency	*Muscle weakness* *Rhabdomyolysis episodes*	0 to +	Low plasma carnitine **Increased medium-chain acylcarnitines (C_6, C_8, C_{10}, $C_{10:1}$)** Dicarboxylic aciduria + **acylglycines**	*ACADM* (p.Lys304Glu prevalent mutation)
Short-chain acyl-CoA dehydrogenase (SCAD) deficiency	*Muscle weakness*	0 to +	**Increased butyrylcarnitine (C_4)** **Ethylmalonic aciduria**	*ACADS* (p.Gly209Ser or p.Arg171Trp prevalent variations)

Quantification of lipid accumulation: none = 0, mild = +, important = ++, massive = +++.
[a] Uncommon symptoms are in italics.
[b] Key biochemical analyses for diagnosis are in bold.

Avoidance of exacerbation factors still plays a large part in the management of these diseases. In children with FAO defects, fasting and infections are the major causes of metabolic decompensation and rhabdomyolysis. Patients need to avoid fasting and maintain a regular carbohydrate intake during infections to minimise lipolysis. Similar strategies are necessary in adults, with avoidance of intense or prolonged exercise, fast and alcohol ingestion.

PCD is one of the rare treatable aetiologies of metabolic cardiomyopathies. When PCD is suspected, there is a need to start treatment very early, even before receiving the results of blood sampling if suspicion is high, because rapid improvement of ventricular function can be obtained. Literature data recommend intravenous therapy of 100–400 mg/kg per day of carnitine during life-threatening events, and 100–300 mg/kg per day of oral carnitine for chronic treatment. This treatment should be continued during all life with high risk of sudden death in case of interruption. This supplementation restores plasma and liver carnitine levels to normal, whereas muscle carnitine levels remain low [52].

Riboflavin treatment (100–400 mg/day) may induce within a few days, a dramatic improvement of muscle symptoms and encephalopathy in some patients with riboflavin-responsive MAD deficiency [20]. A significant improvement of muscle weakness has also been observed after a few months of CoQ10 supplementation in patients with secondary coenzyme Q10 deficiency, but some of them improved even more with combined CoQ10 and riboflavin therapy [21]. These authors suggest that patients with ETF-QO deficiency should be kept on both CoQ10 and riboflavin in the long term in order to maintain a good muscle function. Because of the secondary carnitine deficiency, carnitine supplementation was repeatedly tried, but never resulted in clear improvement, with possible worsening of symptoms in some patients.

Children with long-chain FAO defects are generally treated with a low long-chain fat diet and supplements of MCT, because medium-chain acyl-CoA esters bypass the carnitine shuttle and are converted to ketone bodies which may be protective by suppressing the production of long-chain esters. Benefit is less pronounced in patients whose problems are recurrent rhabdomyolysis [53,54]. A remarkable improvement of cardiac and muscular symptoms also occurred in three children with VLCAD deficiency and in seven patients with CPT II deficiency after dietary supplementation with triheptanoin, a seven-carbon medium-chain fatty acid triglyceride, which supposed mechanisms are the production of C_5 ketone bodies and propionyl-CoA, allowing to replenish the pool of catalytic intermediates of the citric acid cycle [55,56]. Further clinical trials and prolonged clinical follow-up are needed to confirm the benefit of these treatments.

A recently tested alternative way to treat FAO disorders are agonists of peroxisome proliferators-activated receptors (PPARs), that are potent pharmaceutical tools stimulating FAO enzymes in a wide variety of cells. Recent data showed *in vivo* correction of CPT II and VLCAD deficiency in cultured patients' fibroblasts, with bezafibrate a widely prescribed hypolipidemic drug [57,58]. The potential for bezafibrate to correct inborn FAO disorders, has already conducted to the achievement of a pilot clinical trial in 6 adults with CPT II deficiency showing a clear improvement of FAO in muscle [59].

6. Conclusion and perspectives

Many patients in whom muscle biopsy shows lipidosis remain without diagnosis despite thorough investigations [9]. This low rate of diagnosis of muscle lipidosis could be explained by the following possibilities: (1) the physiological and inter-individual variability of lipid accumulation within muscle fibres limiting the accuracy of the pathological diagnosis; (2) the possibility of still unknown metabolic diseases; and (3) secondary increase of lipid in muscle due to other diseases without primary enzymatic defect.

Diagnosis of a metabolic myopathy can be difficult, particularly in late-onset case, as there may be high residual enzyme activity with few detectable biological abnormalities at rest or at distance of acute manifestations. In addition, some of these biochemical defects, such as NLSD, are only expressed in muscle without possibility to detect biochemical anomaly in blood analysis. Nevertheless, many of patients with a metabolic disorder show abnormal blood acylcarnitines when analysed by tandem mass spectrometry which should be undertaken in all case of unexplained muscle lipidosis. For many disorders it is also now possible to identify the causative genes, thus improving the diagnosis and genetic counselling.

We finally emphasize on the tight connections between FAO disorders and respiratory chain. In particular, secondary respiratory chain or coenzyme Q10 deficiencies may be observed, beside SDH or COX staining abnormalities, in FAO disorders such as MAD deficiency. Conversely secondary FAO defects may be also observed in respiratory chain disorders, with abnormal organic acid [60,61] or plasma acylcarnitine profiles (increase of C_8 and C_{10}). Moreover *in vitro* FAO studies may not discriminate defects of these two groups of disorders [62,63]. Therefore the diagnosis of mitochondrial myopathy versus FAO disorder may be difficult to establish in some cases, in the absence of a genetically confirmed enzyme deficiency. A narrow collaboration between physicians, pathologists, biochemists and geneticists is essential in order to elucidate undiagnosed cases of muscle lipidosis or recurrent rhabdomyolysis episodes, which are often revealing lipid metabolism disorders.

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References

[1] Dubowitz V, Sewry CA. Muscle biopsy. A practical approach. Philadelphia: Saunders Elsevier; 2007.
[2] Di Donato S, Taroni F. Disorders of lipid metabolism. In: Engel A, editor. Myology. New York: McGraw-Hill Professional Publishing; 2004. p. 1587–615.
[3] Vockley J, Singh RH, Whiteman DA. Diagnosis and management of defects of mitochondrial beta-oxidation. Curr Opin Clin Nutr Metab Care 2002;5: 601–9.
[4] Angelini C. Disorders of lipid metabolism. In: Mastaglia FL, Hilton-Jones D, editors. Handbook of clinical neurology, vol. 46. Elsevier; 2007. p. 182–91.
[5] Bruno C, DiMauro S. Lipid storage myopathies. Curr Opin Neurol 2008;21:601–6.
[6] Laforêt P, Vianey-Saban C, Vissing J. 162nd ENMC international workshop: disorders of muscle lipid metabolism in adults 28–30 November 2008, Bussum, The Netherlands. Neuromuscul Disord 2010;20:283–9.
[7] Engel AC, Angelini C. Carnitine deficiency of human skeletal muscle with associated lipid storage myopathy. A new syndrome. Science 1973;179:899–902.
[8] Schimmenti LA, Crombez EA, Schwahn BC, et al. Expanded newborn screening identifies maternal primary carnitine deficiency. Mol Genet Metab 2007;90:441–5.
[9] Ohkuma A, Noguchi S, Sugie H, et al. Clinical and genetic analysis of lipid storage myopathies. Muscle Nerve 2009;39:33–342.
[10] Treem WR, Stanley CA, Finegold DN, Hale DE, Coates PM. Primary carnitine deficiency due to a failure of carnitine transport in kidney, muscle, and fibroblasts. N Engl J Med 1988;319:1331–6.
[11] Tein I, DeVivo DC, Bierman F, et al. Impaired skin fibroblast carnitine uptake in primary systemic carnitine deficiency manifested by childhood carnitine-responsive cardiomyopathy. Pediatr Res 1990;28:247–55.
[12] Nezu J, Tamai I, Oku A, et al. Primary systemic carnitine deficiency is caused by mutations in a gene encoding sodium ion-dependent carnitine transporter. Nat Genet 1999;21:91–4.
[13] Chanarin I, Patel A, Slavin G, Wills EJ, Andrews TM, Stewart G. Neutral-lipid storage disease: a new disorder of lipid metabolism. Br Med J 1975;1:553–5.
[14] Lefèvre C, Jobard F, Caux F, et al. Mutations in CGI-58, the gene encoding a new protein of the esterase/lipase/thioesterase subfamily, in Chanarin–Dorfman syndrome. Am J Hum Genet 2001;69:1002–12.
[15] Bruno C, Bertini E, Di Rocco M, et al. Clinical et genetic characterization of Chanarin–Dorfman syndrome. Biochem Biophys Res Commun 2008;369: 1125–8.
[16] Fischer J, Lefevre C, Morava E, et al. The gene encoding adipose triglyceride lipase (PNPLA2) is mutated in neutral lipid storage disease with myopathy. Nat Genet 2007;39:28–30.
[17] Ohkuma A, Nonaka I, Malicdan MCV, et al. Distal lipid storage myopathy due to PNPLA2 mutation. Neuromuscul Disord 2008;18:671–4.
[18] Campagna F, Nanni L, Quagliarini F, et al. Novel mutations in the adipose triglyceride lipase gene causing neutral lipid storage disease with myopathy. Biochem Biophys Res Commun 2008;377:843–6.
[19] Akiyama M, Sakai K, Ogawa M, McMillan JR, Sawamura D, Shimizu H. Novel duplication mutation in the patatin domain of adipose triglyceride lipase (PNPLA2) in neutral lipid storage disease with severe myopathy. Muscle Nerve 2007;36:856–9.
[20] Olsen RK, Olpin SE, Andresen BS, et al. ETFDH mutations as a major cause of riboflavin-responsive multiple acyl-CoA dehydrogenation deficiency. Brain 2007;130:2045–54.
[21] Gempel K, Topaloglu H, Talim B, et al. The myopathic form of coenzyme Q deficiency is caused by mutations in the electron-transferring flavoprotein dehydrogenase (ETFDH) gene. Brain 2007;130:2037–44.
[22] Liang W-C, Ohkuma A, Hayashi YK, et al. *ETFDH* mutations, CoQ10 levels, and respiratory chain activities in patients with riboflavin-responsive multiple acyl-CoA dehydrogenase deficiency. Neuromuscul Disord 2009;19: 212–6.
[23] Wen B, Dai T, Li W, et al. Riboflavin responsive lipid storage myopathy caused by ETFDH gene mutations. J Neurol Neurosurg Psychiatry 2010;81:231–6.
[24] Maillard E, Acquaviva-Bourdain C, Rigal O, et al. Multiple acyl-CoA dehydrogenase deficiency (MADD): a curable cause of genetic muscular lipdisosis. Rev Neurol 2010;166:289–94.
[25] DiMauro S, Melis-DiMauro PM. Muscle carnitine palmitoyltransferase deficiency and myoglobinuria. Science 1973;182:929–31.
[26] DiMauro S, Papadimitriou A. Carnitine palmitoyl transferase deficiency. In: Engel AG, Banker BQ, editors. Myology. New York: McGraw-Hill; 1986. p. 1687–708.
[27] Thuillier L, Rostane H, Droin V, et al. Correlation between genotype, metabolic data, and clinical presentation in carnitine palmitoyltransferase 2 deficiency. Hum Mutat 2003;5:493–501.
[28] Corti S, Bordoni A, Ronchi D, et al. Clinical features and new molecular findings in carnitine palmitoyltransferase II (CPTII) deficiency. J Neurol Sci 2008;266: 97–103.
[29] Vianey-Saban C, Divry P, Brivet M, et al. Mitochondrial very-long-chain acyl-coenzyme A dehydrogenase deficiency: clinical characteristics and diagnostic considerations in 30 patients. Clin Chim Acta 1998;269:43–62.
[30] Laforêt P, Acquaviva-Bourdain C, Rigal O, et al. Diagnostic assessment and long-term follow-up of 13 patients with very long-chain acyl-coenzyme A dehydrogenase (VLCAD) deficiency. Neuromuscul Disord 2009;19:324–9.
[31] Ohashi Y, Hasegawa Y, Murayama K, et al. A new diagnostic test for VLCAD deficiency using immunohistochemistry. Neurology 2004;62:2209–13.
[32] Andresen BS, Vianey-Saban C, Bross P, et al. The mutational spectrum in very long-chain acyl-CoA dehydrogenase deficiency. J Inherit Metab Dis 1996;19:169–72.
[33] Andresen BS, Olpin S, Poorthuis BJHM, et al. Clear correlation of genotype with disease phenotype in very-long-chain acyl-CoA dehydrogenase deficiency. Am J Hum Genet 1999;64:478–94.
[34] Olpin SE, Clark S, Andresen BS, Bischoff C, et al. Biochemical, clinical and molecular findings in LCHAD and general mitochondrial trifunctional protein deficiency. J Inherit Metab Dis 2005;28:533–44.
[35] Spiekerkoetter U, Bennett MJ, Ben-Zeev B, Strauss AW, Tein I. Peripheral neuropathy, episodic myoglobinuria, and respiratory failure in deficiency of the mitochondrial trifunctional protein. Muscle Nerve 2004;29:66–72.
[36] Das AM, Fingerhut R, Wanders RJ, Ullrich K. Secondary respiratory chain defect in a boy with long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: possible diagnostic pitfalls. Eur J Pediatr 2000;159:243–6.
[37] Tyni T, Majander A, Kalimo H, Rapola J, Pihko H. Pathology of skeletal muscle and impaired respiratory chain function in long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency with the G1528C mutation. Neuromuscul Disord 1996;6:327–37.
[38] Wanders RJ, Vreken P, den Boer ME, et al. Disorders of mitochondrial fatty acyl-CoA beta-oxidation. J Inherit Metab Dis 1999;22:442–87.
[39] Brivet M, Tubiana S, Boutron A, et al. Transcriptional analysis of HADHA and HADHB genes encoding the mitochondrial trifunctional protein. J Inher Metab Dis 2008;31:35.
[40] Zeharia A, Shaag A, Houtkooper RH, et al. Mutations in LPIN1 cause recurrent acute myoglobinuria in childhood. Am J Hum Genet 2008;83:489–94.
[41] Ruitenbeek W, Poels PJ, Turnbull DM, et al. Rhabdomyolysis and acute encephalopathy in late onset medium chain acyl-CoA dehydrogenase deficiency. J Neurol Neurosurg Psychiatry 1995;58:209–14.
[42] Lang TF. Adult presentations of medium-chain acyl-CoA dehydrogenase deficiency (MCADD). J Inherit Metab Dis 2009;32:675–83.
[43] Gregersen N, Andresen BS, Pedersen CB, Olsen RK, Corydon TJ, Bross P. Mitochondrial fatty acid oxidation defects-remaining challenges. J Inherit Metab Dis 2008;31:643–57.

[44] van Maldegem BT, Duran M, Wanders RJ, Niezen-Koning KE, Hoveveen M, Ijlst L, et al. Clinical, biochemical, and genetic heterogeneity in short-chain acyl-coenzyme A dehydrogenase deficiency. JAMA 2006;296:943–52.
[45] Tein I, Elpeleg O, Ben-Zeev B, et al. Short-chain acyl-CoA dehydrogenase gene mutation (c.319C > T) presents with clinical heterogeneity and is candidate founder mutation in individuals of Ashkenazi Jewish origin. Mol Genet Metab 2008;93:179–89.
[46] Baerlocher KE, Steinmann B, Aguzzi A, Krähenbühl S, Roe CR, Vianey-Saban C. Short-chain acyl-CoA dehydrogenase deficiency in a 16-year-old girl with severe muscle wasting and scoliosis. J Inher Metab Dis 1997;20:427–31.
[47] Pedersen CB, Kølvraa S, Kølvraa A, et al. The ACADS gene variation spectrum in 114 patients with short-chain acyl-CoA dehydrogenase (SCAD) deficiency is dominated by missense variations leading to protein misfolding at the cellular level. Hum Genet 2008;124:43–56.
[48] Skeletal muscle pathology. In: Mastaglia FL, Lord Walton of Detchant J, editors. New York: Churchill Livingston; 1992.
[49] Storline L, Jenkins A, Chisholm D, Pascoe W, Khouri S, Kraegen E. Influence of diary fat composition on development of insulin resistance in rats: relationship to muscle triglyceride and w3 fatty acids in muscle phospholipids. Diabetes 1991;40:280–9.
[50] Goodpaster BH, Thaete FL, Simoneau JA, Kelley DE. Subcutaneous abdominal fat and thigh muscle composition predict insulin sensitivity independently of visceral fat. Diabetes 1997;46:1579–85.
[51] Ben Ammar A, Petit F, Alexandri N, et al. Phenotype genotype analysis in 15 patients presenting congenital myasthenic syndrome due to mutation in DOK7. J Neurol 2010;257:754–66.
[52] Scholte HR, Rodrigues Pereira R, de Jonge PC, et al. Primary carnitine deficiency. J Clin Chem Clin Biochem 1990;28:351–7.
[53] Ørngreen MC, Nørgaard MG, van Engelen BG, Vistisen B, Vissing J. Effects of IV glucose and oral medium-chain triglyceride in patients with VLCAD deficiency. Neurology 2007;69:313–5.
[54] Vissing J, Di Donato S, Taroni F. Metabolic myopathies. In: Karpati G, Hilton-Jones D, Bushby K, Griggs RC, editors. Disorders of voluntary muscles. UK: Cambridge University Press; 2009. p. 390–407.
[55] Roe CR, Sweetman L, Roe DS, David F, Brunengraber H. Treatment of cardiomyopathy and rhabdomyolysis in long-chain fat oxidation disorders using an anaplerotic odd-chain triglyceride. J Clin Invest 2002;110:259–69.
[56] Roe CR, Yang BZ, Brunengraber H, Roe DS, Wallace M, Garritson BK. Carnitine palmitoyltransferase II deficiency: successful anaplerotic diet therapy. Neurology 2008;71:260–4.
[57] Djouadi F, Aubey F, Schlemmer D, Bastin J. Peroxisome proliferator activated receptor delta (PPARdelta) agonist but not PPARalpha corrects carnitine palmitoyl transferase 2 deficiency in human muscle cells. J Clin Endocrinol Metab 2005;90:1791–7.
[58] Djouadi F, Aubey F, Schlemmer D, et al. Bezafibrate increases very long chain acyl-CoA dehydrogenase protein and mRNA expression in deficient fibroblats and is a potential therapy for fatty acid oxidation disorders. Hum Mol Genet 2005;18:2695–703.
[59] Bonnefont JP, Bastin J, Behin A, Djouadi F. Bezafibrate for an inborn mitochondrial beta-oxidation defect. N Engl J Med 2009;360:838–40.
[60] Christensen E, Brandt NJ, Schmalbruch H, Kamieniecka Z, Hertz B, Ruitenbeek W. Muscle cytochrome *c* oxidase deficiency accompanied by a urinary organic acid pattern mimicking multiple acyl-CoA dehydrogenase deficiency. J Inherit Metab Dis 1993;16:553–6.
[61] Enns GM, Bennett MJ, Hoppel CL, et al. Mitochondrial respiratory chain complex I deficiency with clinical and biochemical features of long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency. J Pediatr 2000;136:251–4.
[62] Venizelos N, von Döbeln U, Hagenfeldt L. Fatty acid oxidation in fibroblasts from patients with defects in beta-oxidation and in the respiratory chain. J Inherit Metab Dis 1998;21:409–15.
[63] Sim KG, Carpenter K, Hammond J, Christodoulou J, Wilcken B. Acylcarnitine profiles in fibroblasts from patients with respiratory chain defects can resemble those from patients with mitochondrial fatty acid beta-oxidation disorders. Metabolism 2002;51:366–71.