

## Workshop report

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### 1. Introduction

The first ENMC workshop on disorders of muscle lipid metabolism hosted a group of experts comprising clinicians and basic scientists. It was attended by 16 participants from seven countries (Denmark, France, Germany, Italy, The Netherlands, UK and USA). The workshop had the following objectives: (1) to state the diagnostic strategies (clinical, biochemical and molecular) to be used for the diagnosis of disorders of muscle lipid metabolism in adults, (2) to share experience on cases of undiagnosed metabolic myopathies presenting as muscle lipidosis, and to describe diagnostic strategies for such patients, and (3) to state on the current treatments and to plan multicenter treatment trials for these disorders.

Clinical phenotypes of the main fatty-acid oxidation (FAO) disorders were presented, and the diagnostic strategy, integrating enzymatic and genetic studies, was discussed at the end of the workshop. Other presentations emphasized the phenotype, pathophysiology and genetic analysis of neutral lipid storage diseases (NLS). Therapeutic approaches were also discussed, in particular dietary supplementations and uses of agonists of peroxisome proliferator-activated receptors (PPARs), which are potent pharmaceutical tools stimulating FAO. Plans were made to collaborate on assessment of biological tools and future multicenter therapeutic trials.

### 2. Background

Disorders of lipid metabolism or fatty-acid oxidation (FAO) are rare inborn errors of metabolism, which occur in adults either as progressive muscle weakness of the limbs or as exercise intolerance

with exercise-induced muscle stiffness and pain, often accompanied by recurrent episodes of rhabdomyolysis. Although rare, these diseases are increasingly recognized due to the increased use of tandem mass spectrometry (MS/MS) screening method, allowing the detection of accumulated acylcarnitines in blood samples. Currently, the most prevalent FAO disorders are carnitine palmitoyl transferase (CPT II), very-long-chain acyl-CoA dehydrogenase (VLCAD) and multiple acyl-CoA dehydrogenase (MAD) deficiencies. More rare diseases, such as primary carnitine transporter deficiency (PCD) or mutations in *PNPLA2* gene, cause muscle lipidosis. However, some patients with recurrent rhabdomyolysis attacks or muscle lipidosis, remain undiagnosed even after thorough biochemical investigations.

Current treatments for most FAO deficiencies are based on frequent carbohydrate-rich meals and a diet low in long-chain fat, and in some cases supplements of medium-chain triglycerides (MCT). These approaches, along with avoidance of fasting, seem more efficient in children and adolescent, than in adults. Carnitine supplementation and riboflavine treatment frequently improve the clinical condition of patients with primary carnitine or MCAD deficiencies. New therapeutic approaches have also recently been developed for CPT II and VLCAD deficiencies: (1) dietary supplementation with triheptanoin, a seven-carbon medium-chain fatty acid, stimulating the anaplerotic pathway, and (2) use of agonists of peroxisome proliferator-activated receptors (PPARs), that are potent pharmaceutical tools stimulating FAO in a wide range of cells.

There is still a need for a better delineation of the clinical phenotype and severity of these disorders, in particular in adults who present mainly with muscular symptoms. The multiplicity of biological techniques used for a diagnostic purpose needs also to be better defined, to find the most useful markers for diagnosis. The new therapeutic perspectives for these disorders should be followed up by multicenter trials in these very rare diseases.

### 3. Overview of muscle lipidosis and FAO disorders involving muscle

#### 3.1. Biochemistry of FAO

C. Vianey-Saban presented an overview of biochemistry of mitochondrial FAO, which has a major role in energy production

*Abbreviations:* CPT, carnitine palmitoyl transferase; ETF, electron transfer flavoprotein; ETF-QO, electron transfer flavoprotein ubiquinone-oxidoreductase; LCEH, long-chain-2-enoyl-CoA hydratase; LCHAD, long-chain-3-hydroxyacyl-CoA dehydrogenase; LCKAT, long-chain-3-ketoacyl-CoA thiolase; MAD, multiple acyl-CoA dehydrogenase; MCAD, medium-chain acyl-CoA dehydrogenase; MTP, mitochondrial trifunctional protein, NLS, neutral lipid storage diseases; PCD, primary carnitine deficiency (or carnitine transporter deficiency); VLCAD, very-long-chain acyl-CoA dehydrogenase.

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and is the main pathway for FAO. However, other pathways of FAO are peroxisomal  $\beta$ -oxidation and microsomal  $\omega$  and  $\omega$ -1 oxidation, but these pathways are poor alternatives to ATP production, utilizing other substrates. Heart and kidney are using preferentially fatty acids as energetic substrates, whereas brain can only use glucose or eventually lactate and ketone bodies. Skeletal muscle uses circulating fatty acids in postprandial period, or during fasting and exercise. Mitochondrial long-chain FAO requires carnitine, which has a double origin: liver synthesis (approximately 25% of requirements) and feeding (mainly present in meat) [1]. Carnitine transport through the plasma membrane requires OCTN2, a sodium dependent transporter of high affinity, present in nearly all tissues, except liver. The carnitine cycle is a key step for the metabolism of FA, allowing the translocation of long-chain fatty acids (LCFA) into the mitochondrial matrix. Carnitine palmitoyl transferase I (CPT I), carnitine acylcarnitine translocase (CACT) and CPT II are the sequential following steps allowing transfer of esterified LCFA inside the mitochondria as acyl-CoAs. Medium-chain and short-chain fatty acids enter mitochondria by diffusion. The mitochondrial  $\beta$ -oxidation itself depends on enzymes located either in the inner membrane or in the matrix of the mitochondria. Oxidation of long-chain fatty acids (LCFA) depends on membrane-bound enzymes: VLCAD and MTP (mitochondrial trifunctional protein composed of LCEH (LC-2-enoyl-CoA hydratase), LCHAD (LC-3-hydroxyacyl-CoA dehydrogenase) and LCKAT (LC-3-ketoacyl-CoA thiolase). LCAD (long-chain acyl-CoA dehydrogenase) is a matrix soluble enzyme specific of long-chain 2-methylacyl-CoA. The oxidation of medium-chain and short-chain fatty acids depends on five matrix enzymes: MCAD (medium-chain acyl-CoA dehydrogenase), SCAD (short-chain acyl-CoA dehydrogenase), SCEH (SC-enoyl-CoA hydratase, also called crotonase), SCHAD (SC-3-hydroxyacyl-CoA dehydrogenase), and MCKAT (MC-3-ketoacyl-CoA thiolase). All these enzymes have specific chain length specificity with some overlapping activity allowing to somewhat overpass the metabolic block when one of them is deficient.

The last step of mitochondrial FAO is the electron transport to the respiratory chain (from VLCAD, LCAD, MCAD, SCAD and other mitochondrial FAD dependent acyl-CoA dehydrogenases), which is mediated by two electron transporter: electron transfer flavoprotein (ETF) localized in mitochondrial matrix and membrane-bound electron transfer flavoprotein ubiquinone-oxidoreductase (ETF-QO).

The regulation of FAO in muscle is mediated by a key enzyme, AMPK (AMP-activated protein kinase), and a key metabolite, malonyl-CoA. Large inter-individual variations in FA utilization occur during exercise, which are influenced by diet, muscle glycogen content, exercise intensity, training status and muscle fibre composition. Muscle contraction activates AMPK and malonyl-CoA decarboxylase (leading to increased FA transport into mitochondria and intramyocellular triglyceride hydrolysis), stimulates insulin-dependent uptake of glucose, inhibits glycogen synthesis (via inhibition of glycogen synthase) and therefore stimulates glucose oxidation. Pathophysiology of FAO disorders relies on energy deficit, accumulation of toxic compounds (long-chain acylcarnitines leading to cardiac arrhythmias), medium-chain fatty acids with neurotoxic effect, and long-chain 3-hydroxyacyl-CoAs which inhibit the respiratory chain), and sequestration of vital compounds (acyl-CoAs and carnitine, which is used to detoxify mitochondria from accumulated acyl-CoAs).

### 3.2. Non-mitochondrial muscle fatty-acid metabolism and triglyceride metabolism

C. Angelini presented the biological and clinical characteristics of two diseases associated with muscle triglyceride accumulation, also named neutral lipid storage diseases (NLS) [2]. The energetic reservoir for lipid metabolism is the triglyceride droplets stored in the

cytoplasm of muscle fibres, which are endogenous sources of free fatty acids. Fatty acids are mobilized from adipose tissue stores and transported into the circulation primarily bound to albumin. After their entry into the cell by a specific membrane transporter, fatty acids can be deposited in lipid droplets (LD) or undergo  $\omega$ -oxidation by microsomes or  $\beta$ -oxidation in mitochondria. Hormone sensitive lipase has for long been designated as the main lipase in LD. However, novel mechanisms have been identified recently for the lipolytic breakdown of cellular LD. Adipose triglyceride lipase (ATGL) catalyses the first step of hydrolysis of tri-acyl-glycerol, generating free fatty acids and di-acyl-glycerol. ATGL requires the activator protein called “Comparative Gene Identification 58” (CGI-58), a new protein of the esterase-lipase subfamily located on the surface of cytoplasmic lipid droplets. Lefèvre et al. [3] observed that mutations in CGI-58, the gene encoding a new protein of the esterase-lipase subfamily, caused Chanarin–Dorfman syndrome, a multi-systemic lipid storage disease with massive triglyceride storage in many tissues including muscle, and ichthyosis. Mutations in the adipose triglyceride lipase gene (*PNPLA2*) were found subsequently [4] in patients with lipid storage and myopathy, but without ichthyosis. An important question, which remains unsolved, is why such an important lipid accumulation occurs despite normal oxidative machinery and normal carnitine.

## 4. Diagnostic evaluation of FAO disorders in adults

### 4.1. Metabolite analyses: diagnostic value, pitfalls (C. Vianey-Saban)

The five main FAO disorders with muscle involvement in adults are deficiencies in OCTN2 (carnitine transporter), CPT II, VLCAD, MTP, and MAD deficiencies. Main metabolite analyses are assessments of free and total carnitine, urinary organic acid profile and plasma/blood acylcarnitine profile. Plasma total and free carnitine levels are frequently lowered in FAO disorders, but levels are always dramatically decreased in carnitine transporter deficiency (also called primary carnitine deficiency, PCD). Urinary organic acid profile is most often normal or shows non-specific abnormalities in adult FAO diseases, but one: in MADD an abnormal excretion of 2-hydroxyglutaric acid is observed, as well as sometimes acylglycine derivatives. The acylcarnitine profile is the most sensitive analysis, and should be performed either on plasma or in blood spotted on Guthrie cards. Acylcarnitine profile theoretically can detect all FAO disorders, but accumulation of abnormal compounds may be detected only after metabolic stress, such as after a prolonged fast (>12 h in adults) or during an acute decompensation. Therefore, when the patient (infant or adult) is in good clinical condition, a normal profile does not exclude a mitochondrial FAO defect. Using acylcarnitine profile as the first diagnostic test in suspected defects of FAO has provided the opportunity to bypass time-consuming enzyme assays, and move directly to molecular genetic testing, even though only few disorders of FAO are associated with hotspot mutations.

### 4.2. Muscle morphology in FAO disorders

N. Streichenberger presented the basic techniques for assessment of lipid storage in muscle. Out of 1600 muscle biopsies performed in Lyon (France), approximately 10% exhibited a lipid accumulation. However, a FAO could be identified only in a few cases. The findings indicate that mild lipidosis may be associated with a variety of diseases unrelated to primary disorders of FAO, such as drug toxicity, inflammatory diseases, or mitochondrial disorders. Conversely, it is important to know that some FAO defects are rarely associated with a muscle lipidosis (CPT II, and VLCAD deficiency).

#### 4.3. *In vitro* diagnosis of mitochondrial FAO disorders using fibroblasts

C. Roe presented data on *in vitro* diagnosis of mitochondrial FAO disorders, using fibroblasts as an alternative to direct enzyme assay. Probing fibroblasts with deuterated precursors of metabolic pathways and measuring labelled acylcarnitines provides a useful alternative to direct enzyme assay for disorders of mitochondrial fat oxidation. All enzyme defects can be detected, except for PCD and CPT I deficiency [5].

#### 4.4. Enzymatic assessment (C. Vianey-Saban)

Until recently, the confirmation of diagnosis relied on overall assays of FAO (lymphocytes and cultured fibroblasts) by measurement of  $^{14}\text{CO}_2$  and  $^3\text{H}_2\text{O}$  production, and specific assays for enzyme activities (lymphocytes, fibroblasts, muscle, and liver). However, overall assays can be normal in adult patients with FAO defects, and enzyme assays (except for the assay of carnitine uptake and CPT II activity) require poorly available substrates. Therefore, despite the fact that frequent mutations are only observed in few disorders of FAO (mainly CPT II deficiency), it is often advisable to proceed to mutation analysis first if the acylcarnitine profile is suggestive of a particular defect, and only afterwards to enzyme assay if the genotype is not completely elucidated.

#### 4.5. Molecular analysis of FAO defects (B. Andresen)

Mutation analysis is possible for all known enzyme deficiencies of FAO. Some disorders are associated with hotspot mutations, but generally there is a high level of genetic heterogeneity. MCAD deficiency is the most frequent defect of FAO, but adult muscular disease is rare with only a few anecdotal reports. Many asymptomatic adults are known (parents), who do not report muscular symptoms. A MS/MS-based newborn screening allows detection of this frequent enzyme defect with a prevalent mutation: c.985A > G (p.Lys304Glu). It is still unknown whether some patients with MCAD deficiency identified by MS/MS will experience muscular symptoms. For VLCAD deficiency there is no “prevalent” mutation in the *ACADVL* gene, with more than 150 different mutations described in 200 families (c.848C > T relatively frequent). In CPT II deficiency, p.Ser113Leu mutation is associated with the adult muscular form (leading to high residual enzyme activity), whereas more “severe” mutations are detected in infantile/childhood severe forms with no or very little residual CPT II activity [6]. MAD deficiency (due to mutations of *ETFA*, *ETFB* or *ETDH* genes) may be subdivided into milder and riboflavin-responsive forms associated with residual enzyme activity, and a severe form (congenital) with more “severe” mutations and no residual enzyme activity. Genotype–phenotype correlations are found in long-chain defects of FAO (VLCAD, CPT II, and MAD), allowing to establish a prognosis when the genotype is known. Conversely, there is no genotype–phenotype correlation in medium- and short-chain defects of FAO (MCAD and SCAD).

#### 4.6. RNA-based analysis in emetine-treated fibroblasts

A rapid approach for mutation detection in long-chain fatty-acid oxidation disorders has been described by M. Brivet. Defects of mitochondrial trifunctional protein (MTP) have been chosen to illustrate the usefulness of RNA-based sequencing. MTP is a heteropolymeric enzyme encoded by two large *HADHA* and *HADHB* genes. RT-PCR and cDNAs sequencing of *HADHA* and *HADHB* is less time consuming than exon by exon sequencing. However mutations may result in premature termination codons, which lead to rapid degradation of the mutant messenger RNA by the nonsense-mediated mRNA decay pathway (NMD). Emetine that pharmacologi-

cally inhibit the NMD pathway in cultured fibroblasts, allows to circumvent this limitation [7].

#### 4.7. Respiratory chain analysis with emphasis on secondary respiratory chain (RC) defects (R Horvath)

The tight connection between mitochondrial FAO and the RC could theoretically lead to secondary RC deficiencies. Decreased RC activities have been detected in some patients with PCD, MAD deficiency (C. Vianey-Saban, unpublished results), LCHAD deficiency [8,9], and ethylmalonic encephalopathy (*ETHE1* mutations) [10]. No data is published about decreased RC in other FAO and lipid catabolism defects. This could be related to the lack of systematic investigation of RC activity in these disorders. The only firm link between RC and lipid metabolism disorders is MAD/myopathic Q10 deficiency. In patients with mutations in *ETFDH* gene and secondary RC defect, CoQ10 may rescue the RC defect, and thus dramatically improve the symptoms of the disease (these patients were initially considered as having primary CoQ10 deficiency) [11]. A few reports also mentioned the association of impaired FAO and RC, but without gene mutation and no possibility to identify the primary defect. However there might be novel genes leading to specific phenotypes affecting both pathways. Conversely, lipid metabolism may show non-specific abnormalities in primary RC disorders, and in case of non-specific changes in acylcarnitine profile, primary RC deficiency should be considered.

#### 4.8. Turnover studies of fat during exercise (M. Ørngreen)

Stable isotope technique, using  $^{13}\text{C}$ -labelled palmitate as the tracer, provides a non-invasive way to assess fat oxidation. M. Ørngreen described such findings in patients with VLCAD and CPT II deficiencies. In adult onset cases of VLCAD and CPT II deficiencies, fat oxidation rates at rest are normal in accordance with the asymptomatic state of these patients under basal conditions. During exercise, however, patients had an abolished or severely impaired increase in fat oxidation, thus explaining the exercise-induced character of symptom-induction [12,13]. Using the same stable isotope technique, it was also shown that symptomatic carriers of single *CPT2* gene mutations have a severely impaired fat oxidation during exercise, thus verifying the existence of symptomatic carriers in CPT II deficiency [13]. This could relate to the tetrameric structure of the functional CPT II molecule, with a dominant negative effect of mutant proteins on the complex. Stable isotope techniques will be a central methodology to monitor future treatment effects on exercise-induced increments in fat oxidation.

### 5. Clinical features and natural history of late-onset FAO disorders

#### 5.1. Primary carnitine deficiency (C. Angelini)

Primary carnitine deficiency should be differentiated from other causes of secondary carnitine deficiency, including organic acidemias, and FAO defects. The condition is defined by a decrease of intracellular carnitine that impairs fatty-acid oxidation, and that is not associated with another identifiable systemic illness that might deplete tissue carnitine stores. Criteria for diagnosis are: (1) a severe reduction of plasma (<10% of normal) or tissue carnitine levels, (2) absence of other causes of carnitine depletion (FAO defects, disorders of organic acid metabolism, respiratory chain defects, renal Fanconi syndromes, and treatments with valproate, pivampicillin or hemodialysis), (3) evidence that the low carnitine levels impair fatty-acid oxidation, and (4) correction of the disorder when carnitine levels are restored. Primary carnitine

deficiency is a treatable autosomal recessive disorder due to mutation in the *OCTN2* gene (organic cation transporter 2) [14]. Affected patients may have a predominant metabolic or cardio-muscular presentation. Clinical signs encompass hypotonia, hypoketotic hypoglycemia, Reye-like syndrome, hyperammonemia (potentially lethal severe forms with onset between 3 months and 2.5 years), dilated cardiomyopathy and lipid storage myopathy (myopathic form, onset 1–7 years). Muscle and liver biopsies show a massive lipidosis. Biochemical studies reveal normal urinary organic acid and plasma free fatty-acid profiles, low levels of plasma free and total carnitine (<10% of controls), no accumulation of carnitine esters (normal free carnitine:acylcarnitine ratio and no acylcarnitine), and abnormal carnitine uptake in lymphocytes and fibroblasts (reduced below 10% of controls). Over 50 different mutations widespread along the high-affinity human plasma membrane carnitine transporter gene *OCTN2* have been reported. Most mutations are null mutations, with some “leaky” mutations. No clear genotype–phenotype relationship has been established [15]. Despite excellent response of cardiomyopathy to oral carnitine supplementation, along with repletion of carnitine levels in plasma, muscle, and liver, these patients need a careful, long-term cardiac follow-up as the first reported case died from unexplained arrhythmia [2].

Muscle carnitine deficiency is associated with a defect of carnitine level in muscle, but only mildly reduced plasma carnitine levels, in contrast to systemic carnitine deficiency described above. Pathogenesis is due to a low affinity transport defect and excessive loss of carnitine from muscle. Patients respond to dietary carnitine supplementation, and the long-term prognosis is favourable as long as children remain on carnitine supplementation.

### 5.2. CPT II deficiency: clinical features and metabolic aspects (J. Vissing, F. Taroni)

CPTII deficiency was the first reported disorder of muscle FAO, and it is one of the most common causes of episodic myoglobinuria [16]. Three clinical phenotypes are described: (1) a neonatal form (hypotonia, hypoketotic hypoglycemia, metabolic acidosis, hepato- and cardiomegaly, respiratory distress, developmental abnormalities, lethal), (2) an infantile form (hypoketotic hypoglycemia, hepatomegaly, lethargy, cardiac arrhythmias) and (3) a juvenile-adult form (recurrent myoglobinuria, muscle aching and stiffness on exercise, impaired exercise endurance). Myopathic symptoms most often encountered in adults include acute symptoms (myoglobinuria, rhabdomyolysis, muscle stiffness and aching, renal failure), and intolerance to long-term exercise. Triggers for symptoms and myoglobinuria may be exercise of long duration, fever, cold-shivering, emotional stress, and fasting. In 20% of cases, there is no apparent trigger for acute manifestations. CK levels are normal outside episodes of muscle injury. Studies of exercise on cycle showed a near normal  $VO_{2max}$ , no second wind phenomenon, and fat oxidation during exercise is impaired [13]. Acylcarnitine profile may show increased long-chain acylcarnitines (mainly  $C_{16}$ ,  $C_{18:1}$ , and  $C_{18}$ ).

A clear phenotype–genotype relationship exists for CPT II deficiency. p.Ser113Leu is the prevalent mutation in the myopathic form (this mutation is present in 50% of alleles and 90% of patients), and one third of mutations are null mutation. The myopathic phenotype is generally secondary to the association of p.Ser113Leu and a null mutation, while lethal neonatal phenotype is associated with two null mutations [6,15].

### 5.3. VLCAD deficiency (P. Laforêt)

VLCAD deficiency has many similarities with CPT II deficiency. Three phenotypes have been described according to the age at onset

of clinical symptoms: (1) a severe infantile form presenting in the neonatal period with hypertrophic cardiomyopathy and liver failure; (2) a childhood onset between ages 1 and 13 years with hypoketotic hypoglycemia as the main presenting feature; and (3) a juvenile or adult-onset muscular form characterized by recurrent episodes of rhabdomyolysis triggered by prolonged exercise or fasting [17,18]. Myoglobinuria episodes in adults are generally triggered by strenuous exercise, fasting, cold or fever [19]. Life-threatening general manifestations before the age of 3 years may also occur in patients who later develop a milder, myopathic form. Muscle biopsy may show a mild lipidosis, but is normal in the majority of cases. The diagnosis of VLCAD deficiency relies on tandem mass spectrometry (MS/MS), allowing the detection of abnormal long-chain acylcarnitines (with  $C_{14:1}$  as the predominant species) in blood samples. Analysis of the *ACADVL* gene shows a wide mutational spectrum, most of the mutations being private [20,21].

### 5.4. Myopathic form of multiple acyl-CoA dehydrogenase deficiency caused by mutations in *ETFDH* gene (R. Horvath)

Myopathic form of MADD has been recently described in patients who had been initially reported as isolated myopathy associated with Coenzyme Q10 deficiency [11,22]. These patients presented with fluctuating proximal and axial myopathy, and exercise intolerance. Episodic encephalopathy, hepatopathy and periodic vomiting often triggered by metabolic stress were also observed (R. Horvath, UK). Some patients experienced rhabdomyolysis and a few died of coma or sudden unexplained death. Triggering factors could be infections, metabolic stress, pregnancy, surgery, psychic stress, and drugs. Biological analysis showed increased CK and lactate levels, with low carnitine levels. Muscle biopsies exhibited mild to severe vacuolar changes, increased lipid content, ragged-red fibres, focal or diffuse SDH deficiency, COX negative fibres, combined complex I/II + III deficiency, and decreased level of CoQ10 in muscle (50% of low normal). Diagnosis was made with tandem mass spectrometry (TMS) showing in all cases an acylcarnitine profile suggestive of multiple acyl-CoA dehydrogenase deficiency. Mutations were found in *ETFDH* gene in all patients. CoQ10 or riboflavine supplementation was generally followed by clinical improvement, and normalization of CK and lactate levels.

### 5.5. Mitochondrial trifunctional protein (MTP) deficiency (F. Taroni)

MTP deficiency type I or LCHAD deficiency is associated with severely reduced long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) activity, and mild decrease of LC-enoyl-CoA hydratase (LCEH) and LC-3-ketoacyl-CoA thiolase (LCKAT) activities. Clinical features are mainly cardiomyopathy, skeletal myopathy, retinopathy, or acute fatty liver of pregnancy (AFLP). Biochemical analyses show hypoketotic hypoglycemia, and 3-OH-dicarboxylic aciduria may be detected on GC/MS urine organic acid profile. GC/MS profile of plasma total fatty acids may exhibit an increase of 3-hydroxy LCFA and tandem MS profile of blood acylcarnitines an increase of long-chain hydroxyacylcarnitines. A prevalent mutation (c.1528G > C) has been identified which accounts for approximately 90% of mutant alleles. Combined deficiency or MTP Deficiency Type 2 is associated with a strong decrease in LCEH and LCKAT activities, and milder decrease in hydratase activity. Peripheral neuropathy and retinopathy, associated with cardiomyopathy or recurrent myoglobinuria, are two important diagnostic clues.

### 5.6. Triglyceride storage disease/Adipose triglyceride lipase deficiency (C Angelini, C Bruno)

Neutral lipid storage disease with ichthyosis (NLSDI), or Chanarin–Dorfman syndrome (CDS), was initially described by Chanarin

et al. [23]. This disease has been reported in around 60 cases from all over the world. It is a rare autosomal recessive disorder of neutral lipid metabolism, characterized by ichthyosiform, non-bullous erythroderma, often associated with mild myopathy and hepatomegaly. Additional, but inconsistent features, are ocular symptoms (cataract, nystagmus, and strabismus), hearing loss, mental retardation, short stature, microcephaly, and intestinal involvement [24]. CDS is associated with excessive triglyceride (TG) accumulation in most tissues. The most common laboratory abnormalities are May–Grunwald–Giemsa negative lipid droplets (Jordan's bodies) in otherwise normal neutro- and eosinophils from peripheral blood. Muscle involvement is frequent (around 60% of cases) with slowly progressive weakness of proximal limb muscles sparing axial muscles, raised serum muscle enzymes and electromyographic evidence of myopathy. There is a striking accumulation of lipid droplets in both type 1 and 2 muscle fibres. Electron microscopy shows multiple none-membrane-bound vacuoles in the cytoplasm, while mitochondria appear normal in number and size. Mutations in *CGI-58* are not always identified in clinically typical patients, suggesting that mutations in other genes may mimic the disease. Neutral lipid storage disease with myopathy and without ichthyosis related to mutations in *ATGL* gene has been identified more recently [4], and so far only 10 cases from eight families have been reported. A distal muscle weakness was noticed in five patients. Cardiac hypertrophy was present in six cases, with heart failure leading to death in two cases. Short stature, chronic pancreatitis, and hepatopathy were other inconstant features.

## 6. Current and future treatments

### 6.1. Dietary treatments of FAO disorders (M. Ørngreen)

A mainstay in the treatment of disorders of muscle fat metabolism is to provide a diet that will keep high levels of liver and muscle glycogen, on which patients are heavily dependent during exercise. Thus, patients with CPT II deficiency had a higher exercise tolerance when on a high carbohydrate diet [25]. Another key issue is to refrain from fasting, which will progressively decrease glycogen stores. There is general consensus that acute episodes should be treated with intravenous infusion of glucose. The effect of supplementation with medium-chain-triglycerides (MCT) in defects of long-chain fatty acids is controversial in adult-onset patients [26]. Oral riboflavin (B<sub>2</sub>) supplementation in doses between 100 and 400 mg daily often has a dramatic effect on patients with MADD [27].

### 6.2. Triheptanoin diet (C. Roe)

The rationale for triheptanoin diet therapy is the stimulation of anaplerosis that is refilling the pools of catalytic intermediates of the citric acid cycle (CAC), and therefore the enhancement of ATP production via the respiratory chain. The critical substrates for the CAC are acetyl-CoA and oxaloacetate. Even-carbon fatty acids (MCT, palmitate, etc.) provide only acetyl-CoA, and are not anaplerotic, while odd-carbon numbered fatty acids such as heptanoate are both anaplerotic and gluconeogenic, since they provide both acetyl-CoA and propionyl-CoA (precursor of oxaloacetate in the CAC) as an external dietary source. Successful therapy with triheptanoin has been described in children with VLCAD deficiency and cardiomyopathy [28]. Mortality was reduced from 75% with the even-carbon MCT conventional diet to 4% with the odd-carbon triglyceride (triheptanoin) diet. Seven patients with CPT II deficiency, ranging in age from 10 to 55 years, were studied from 4 to 58 months on the triheptanoin diet [29]. All except one had been hospitalized and had muscular pain on exertion sufficient to restrict exercise before starting the diet. The daily dose was 1–2 g/

kg body weight for adults and adolescents. For children younger than 12 years, the daily dose was usually 3–4 g/kg body weight. Benefits, extending to 58 months, included reduced muscle pain, and no episodes of rhabdomyolysis or hospitalizations in any of the seven reported. Improvement in muscle endurance was noted as early as 4 days after start of treatment, and continuing for up to nearly 5 years. Exercise restriction was eliminated and all patients returned to normal physical activities including strenuous sports activities in two patients. Previously abnormal Health Survey SF-36 physical composite scores returned to normal in all five symptomatic patients in 1–2 months, and remained normal for the duration of the therapy. The anaplerotic effect from oxidation of triheptanoin, also successfully corrected the energy deficit, and seems like an effective therapy for CPT II deficiency.

### 6.3. Pharmacological treatments (R. Lachmann)

The aim of treatment in disorders of FAO is to restore energy production and to remove potentially toxic metabolites. Drugs could have a role in conjugation and excretion of acylcarnitines, alternative energy sources and enhancement of residual enzyme activity. The most spectacular clinical improvements were observed after carnitine supplementation in patients with PCD, which was the first report of a treatable cause of Reye's syndrome [30]. Myopathy and cardiomyopathy respond well to carnitine treatment, and it has now been proven that early treatment and strict compliance prevents disease development [31]. It is also commonly thought that other FAO disorders could benefit from carnitine treatment, because of frequent occurrence of secondary carnitine deficiency. However, recent fundamental studies in VLCAD deficient mice demonstrated that carnitine supplementation may induce long-chain acylcarnitines [32], and mice treated with long-term carnitine supplementation develop a dilated cardiomyopathy. Therefore, even apparently harmless treatments should be prescribed with rigour, and need scientific proof of efficacy before being recommended.

### 6.4. Potential role of fibrates and drugs targeting AMP-activated protein kinase (AMPK) for treatment of FAO disorders (P. Laforêt, S. Jorgensen)

In recent years, PPARs (peroxisomal proliferator-activated receptors) have been identified as potential targets for pharmacological therapy of CPT II and VLCAD deficiencies. PPAR $\alpha$  is a transcription factor, belonging to the super-family of steroid-thyroid hormone receptors, which is able to modify CPT II and VLCAD gene expression. The "fibrate" class of hypolipidaemic drugs (clofibrate, bezafibrate, and gemfibrozil) are specific ligands of PPAR $\alpha$  and the interaction with PPAR $\alpha$  forms the molecular basis of therapeutic effects of these drugs [33]. Recent studies showed a marked increase in FAO capacities in cultured cells from patients with CPT II and VLCAD deficiencies [34,35]. A pilot clinical trial assessing the effects of bezafibrate in six adults with CPT II deficiency has been performed in France, showing an improvement of muscular symptoms, along with an up-regulation of CPT II mRNA and protein levels assessed on muscle biopsies [36]. However the major endpoints of this study were mainly biochemical analysis, and a randomised double-blind, placebo-controlled crossover clinical trial is currently in progress in order to evaluate the effect of bezafibrate on metabolism during exercise in adult patients affected with CPT II or VLCAD deficiencies. This trial will be conducted in two centres (Paris and Copenhagen), and the primary outcome of this study will be the assessment of fat oxidation in vivo on cycle ergometry using stable isotope techniques.

Another potential target for improving FAO oxidation is AMP-activated protein kinase (AMPK). Metabolic actions of AMPK in

skeletal muscle are directed towards glucose uptake, glycogen synthesis, protein synthesis, palmitate oxidation and mitochondrial biogenesis. Exercise (ATP consumption), starvation, and some drugs (metformine, rosiglitazone, AICAR) are already known activators of AMPK in skeletal muscle [37]. Leptin and adiponectin also activate AMPK activity and increase FAO in muscle [38,39]. The development of new drugs modulating the activity of AMPK could open new avenues for the treatment of FAO disorders.

#### 6.5. Role of exercise in patients with exercise intolerance (R. Haller)

The rationale for exercise training as therapy for muscle energy defects is based on the fact that deconditioning due to habitual inactivity may reduce muscle energy reserves and the capacity to metabolise alternative fuels, and thus increase exercise intolerance. Benefits of regular exercise training has already been demonstrated in patients with mitochondrial myopathy due to heteroplasmic mtDNA mutations (increased peak  $\text{VO}_2$  achieved by an increase in functional wild type mtDNA) [40,41] and in patients with McArdle disease (increased peak  $\text{VO}_2$  achieved by increased circulatory capacity and increased mitochondrial mass) [42]. Possible benefit and safety of exercise training in adult FAO defects could be foreseen by increasing mitochondrial biogenesis, and available data have shown that exercise at 70–80% of maximal oxidative capacity is powered predominantly by carbohydrate oxidation and appears to be well tolerated by patients with adult FAO defects.

### 7. Flow chart: biochemical and molecular diagnosis of FAO disorders in patients with unexplained rhabdomyolysis or muscle lipidosis

The workshop group suggests a diagnostic flow chart for patients with recurrent myoglobinuria  $\pm$  weakness/neuropathy/retinopathy, or exercise-induced stiffness and pain, or unexplained

muscle weakness (Fig. 1). After cardiac assessments (echocardiography and ECG) and routine blood samples (CK, lactate, TSH and myoglobin), the assessment of acylcarnitines is a pivotal starting point for the diagnosis of patients with disorders of FAO.

If an acylcarnitine profile is abnormal and suggestive of a specific FAO defect, the molecular genetic analysis should be performed first (direct gene mutation analysis for *CPT II*, *VLCAD* and *ETFDH*), and then enzyme activity (but not mandatory). Enzyme activity may be assessed only after identification of gene mutation in order to correlate the genotype with the phenotype.

If an abnormal acylcarnitine profile is not typical of known defects of FAO, one should consider drug-induced symptoms or non-metabolic diseases.

Normal acylcarnitine profiles should be repeated after at least 12 h of fasting. If it is still normal, a functional testing of carbohydrate metabolism should be performed either by forearm exercise or cycle testing, before a muscle biopsy is performed.

The acylcarnitine profile is a cornerstone in the diagnostic workup of patients suspected of defects of FAO, but the exact sensitivity of the assay in adult-onset patients with defects of FAO is unknown. The participants of the workshop therefore decided to prospectively collect acylcarnitine profiles of genetically verified patients with disorders of muscle FA metabolism in the fed and fasted condition.

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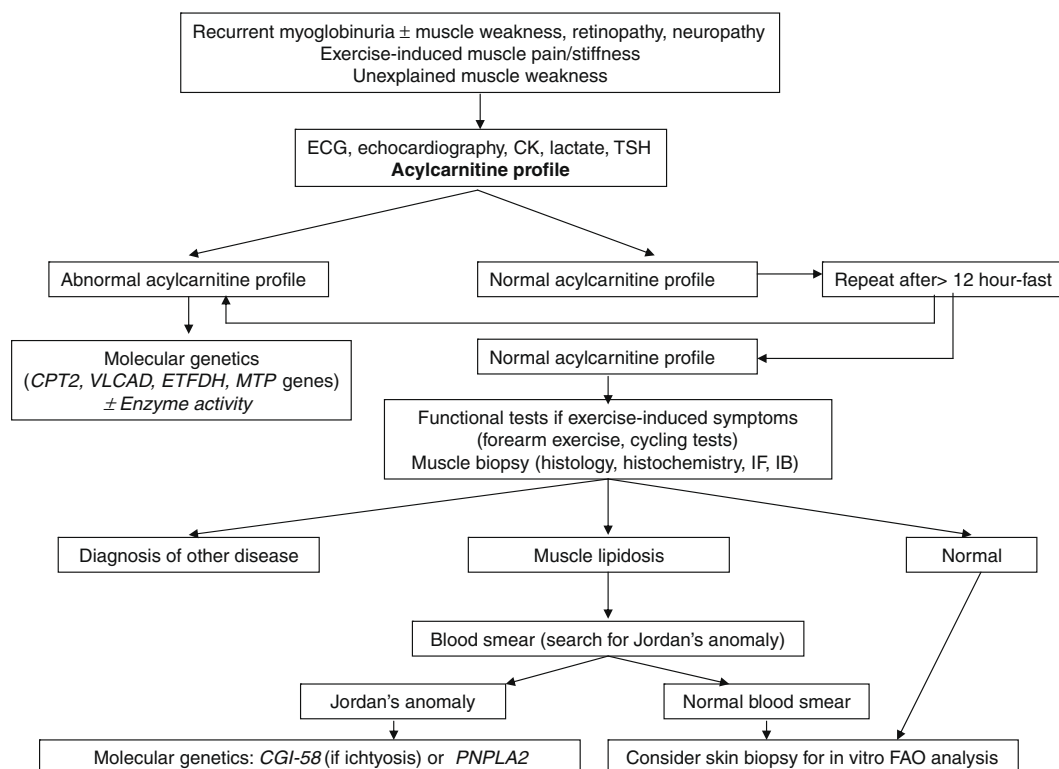


Fig. 1. Flowchart for detection of lipid storage disorders and FAO defects.

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