Task force guidelines handbook: EFNS guidelines on diagnosis and management of fatty acid mitochondrial disorders

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Guidelines in the diagnosis and current dietary treatment of long-chain fatty acid (LCFA) defects have been collected according to evidence-based medicine. Since the identification of carnitine and carnitine palmitoyltransferase deficiency more than 25 years ago, nearly every enzymatic step required for \(\beta\)-oxidation has been associated with an inherited metabolic disorder. These disorders effectively preclude the use of body fat as an energy source. Clinical consequences can range from no symptoms to severe manifestations including cardiomyopathy, hypoglycaemia, peripheral neuropathy and sudden death. A diet high in carbohydrates, diet with medium-chain triglycerides and reduced amount of LCFA has a beneficial effect (class IV evidence) and in appropriate deficiency states carnitine and riboflavin are used (good practice points).

Background

Lipid storage myopathies (LSM) represent various disease entities whose biochemical defects are heterogeneous [1]. These disorders might be due to either defects of the carnitine membrane carrier or enzymatic defects in beta-oxidation energy supply. L-Carnitine, carnitine palmitoyltransferase I (CPT I), carnitine acyltranslocase and CPT II provide a mechanism whereby long-chain fatty acyl-CoA molecules are transferred from cytosol across the outer and inner mitochondrial membrane to the matrix where they undergo beta-oxidation [2]. A series of enzymes bound to the mitochondrial inner membrane or dissolved in the cytosol transform fatty acyl-CoA into acetyl-CoA (Fig. 1). A mitochondrial trifunctional protein associated with the inner mitochondrial membrane has been identified that performs three different enzymatic activities during long-chain fatty acid (LCFA) oxidation. The description of the disorders has been organized according to the pathway of LCFA transfer and oxidation.

Search strategy

The task force for metabolic disorders systematically searched the MEDLINE database using key words, and examined textbooks and existing guidelines. According to the guidance for the preparation of neurological management by EFNS task force [3] articles were included if they contained data, which could be rated according to grades of recommendation for treatment classified in terms of evidence-based medicine. Most guideline recommendations derived in this document are from case reports (class IV evidence), as no large trials have been conducted in fatty acid disorders. These guidelines reflect consensus opinions of experts in the field (good practice points). The consensus was reached analysing series of treated patients and in discussing pre-existing guidelines.

Results

Carnitine palmitoyltransferase II deficiency

In the most typical presentations CPT II deficiency is seen in young adults (Table 1) experiencing episodes of muscle pain and rhabdomyolysis triggered by prolonged exercise or cold. The disease is autosomal recessive and is mostly seen in males, but intolerance to exercise might be observed also in carriers of CPT II mutations suggesting a dominant negative effect of this tetrameric protein [4]. The rhabdomyolytic attacks are triggered by fasting or cold and consist of pain, stiffness without cramps and highly elevated creatine kinase levels (up to 50 000 U) reflecting muscle necrosis. This may lead to acute renal failure.

Recommendations

Preventing myoglobinuric episodes is important and this can be achieved by avoiding strenuous exercise...
During fasting or cold. During an attack 5% of glucose solution is used as an alternative metabolic fuel. According to published guidelines a standard treatment protocol for myoglobinuria [5] is intravenous infusion of hypotonic sodium chloride and sodium bicarbonate (sodium chloride 110 mmol/l and bicarbonate 40 mmol/l) in 5% of glucose solution to which 10 g of mannitol per litre is added in a 20% of solution. The solution should be infused into a young adult of 75 kg weight at the rate of 12 l/day in order to obtain a diuresis of 8 l/day and keep pH above 6.5. This therapeutic regimen will control both hyperkalemia and acidosis and therefore might prevent acute renal failure.
Carnitine transport defects

Primary L-carnitine deficiency syndromes are rare biochemical disorders and can be classified on the basis of clinical and biochemical criteria into muscle carnitine deficiency and systemic carnitine deficiency. A carnitine deficiency syndrome can be suspected in a patient with LSM when the following symptoms are present: hypoglycaemia, with or without ketoacidosis with a Reye-like syndrome, myalgias, weakness, abnormal fatiguability and cardiomyopathy with left axis deviation [6,7]. Primary systemic carnitine deficiency is a well-recognized treatable entity of childhood (Table 2) characterized by progressive cardiomyopathy, LSM and attacks of hypoglycaemia, hepatomegaly with Reye-like syndrome that may lead to permanent brain damage [8].

Diagnosis

In several cases a defect of carnitine ‘high-affinity’ transport organic cation transporter 2 (OCTN2) gene has been demonstrated in cultured fibroblasts and genomic DNA can be screened for mutations [9,10].

Guidelines for therapy

Carnitine supplementation corrects cardiomyopathy and other clinical signs [6]. In some cases this treatment might avoid cardiac transplant. The L-carnitine dose may vary from 100 to 600 mg/kg/day on the basis of the calculated carnitine depletion from muscle, liver, heart and kidney. Individually adjusted dosing may require plasma level measurement. No side-effects are noted for L-carnitine supplementation except occasional diarrhoea or fishy body odour. In some cases a medium-chain triglyceride (MCT) diet may be added (class IV evidence).

Muscle carnitine deficiency

In primary muscle carnitine deficiency the clinical syndrome is confined to skeletal muscle [11,12]; the clinical features are episodes of fluctuating muscle weakness, affecting mostly limb and neck muscles and severe myalgia.

Table 2 Primary systemic carnitine deficiency

| Inheritance: autosomal recessive |
| Gene: OCTN2 organic cation transporter |
| Clinical presentation |
| Progressive cardiomyopathy |
| Muscle weakness |
| Fasting hypoglycaemia |
| Urine: normal organic acid pattern |
| Low total carnitine in plasma, urine and muscle |
| Normal ratio carnitine/acyl-carnitines |
| Molecular biology: several point mutations reported |

OCTN2, organic cation transporter 2.

Defects of beta-oxidation

Defects of fatty acids oxidation may affect muscle alone or in conjunction with signs in other tissues, i.e. liver and heart (Table 3). For most of the different enzyme deficiencies the clinical features are similar. In some patients this is reflected by exercise-induced muscle pain and rhabdomyolysis. The diagnosis is often suggested by characteristic patterns of organic acids excreted in the urine, which are specific for various enzymatic blocks.

Enzymatic and immunochemical analysis performed in fibroblasts and/or in muscle and liver mitochondria will confirm the diagnosis. Inborn errors of beta-oxidation are:

1. Very long-chain acyl-CoA deficiency (VLCAD)
2. Medium-chain acyl-CoA deficiency (MCAD)
3. Short-chain acyl-CoA deficiency (SCAD)
4. Riboflavin-responsive disorders of beta-oxidation

Guidelines for laboratory diagnosis of fatty acid oxidation defects

Dicarboxylic aciduria is a distinct finding associated with a metabolic block of beta-oxidation. The substrates are converted to dicarboxylic acids by the combined action of omega-oxidation in the endoplasmic reticulum and by peroxisomal beta-oxidation.

The metabolic intermediates derived from the enzymatic block can be detected in the urine and blood.
Often they are formed only during a metabolic crisis. The qualitative and quantitative study of the organic acids produced in the patients is indicated by gas chromatography-mass spectrometry (GC-MS) analysis. Acyl-carnitines can be revealed in patients with organic aciduria due to the activity of acyl-carnitine-transferase and their pattern of appearance in plasma and urine is a useful diagnostic test [2]. They are especially important in diagnosis of β-oxidation block such as VCLAD or MCAD deficiency. Other secondary metabolites, produced by enzymatic reactions that free CoA from acyl residues, can be detected in patients’ urine. Glycine derivatives like hexanoyl-glycine or phenylpropionyl-glycine are pathognomonic of MCAD deficiency. The presence of glycine or acyl-carnitine derivatives in the urine indicates an increased accumulation of acyl-CoA in the mitochondria. Glutaric aciduria type 2 is pathognomonic of riboflavin-responsive LSM. Fat accumulation in the muscle biopsy depends upon diet and activity level. Analysis of metabolites is a crucial investigation and can be combined with a study of labelled fatty acid oxidation and appropriate enzyme studies in fibroblasts.

**Very long-chain acyl-CoA-dehydrogenase deficiency**

Very long-chain acyl-CoA deficiency has mostly been described in children [13]. The patients reported so far can be grouped according to their clinical course: the first group has onset in the first few months of life and shows a high mortality; the second group is characterized by recurrent episodes of coma after fasting, but presents no cardiomyopathy; the third group presents with late-onset rhabdomyolysis and myalgia after muscle exercise.

Deficient patients cannot oxidize C18 to C16 fatty acids, whereas they can normally utilize shorter fatty acids (shorter than C14). Exercise-induced myoglobinuria is a possible presentation [14,15]. Cardiac involvement is frequent. Other distinctive laboratory findings include hypoglycaemia, hypoketonuria, high serum ammonia, and slight elevation of serum aminotransferases. Low ketones during severe hypoglycaemia strongly suggest a specific defect of fatty acid oxidation. Liver biopsy, when performed, reveals an increase in both macro- and micro-vesicular fat and mitochondrial abnormalities.

**Trifunctional enzyme deficiency**

The disease is inherited as an autosomal recessive trait. The common mutation for long-chain β-hydroxy acyl-CoA-dehydrogenase (LCHAD) deficiency is 1538 G > C. Onset of symptoms is in the first year of life, characterized by intermittent hypoglycaemia, lethargy, and coma. The typical presentation is a progressive lethargy, evolving into coma during a fasting or during a febrile episode associated with vomiting and diarrhoea that induces a catabolic state. Hepatomegaly, cardiomyopathy and muscle weakness are usually observed.

Three adult patients from a family with recurrent rhabdomyolysis and peripheral neuropathy were reported [16]. A low-fat/high-carbohydrate diet was beneficial in one patient reducing the frequency of rhabdomyolytic episodes.

**Medium-chain acyl-CoA-dehydrogenase deficiency**

Medium-chain acyl-CoA deficiency (OMIM number 22274) is the most common error of fatty oxidation found in the US, UK, and Northern Europe. It is manifested by a recurrent syndrome of somnolence, vomiting, coma, hypoglycaemia, fatty infiltration of the liver, and dicarboxylic aciduria. The crises are often precipitated by intercurrent infections. Patients cannot oxidize the medium-chain fatty acids (C12 to C6). The disorder becomes life threatening during episodes of stress or fasting (Table 4), which result in decreased caloric intake or increased catabolism.
Medium-chain acyl-CoA deficiency has been found in cases of Reye-like syndrome and in some cases of sudden infant death syndrome. The first episodes of the disorder occur in the first 12–18 months of life. Incidence in both the sexes is similar. The mortality rate is 25% but can reach 60% in cases with later onset (second year of life). In half of the families there was a high incidence of death in infancy. Hepatomegaly because of fatty liver has been described in some cases. Seizures have been reported, but patients may have normal development and growth, and no clinical sign of cardiomyopathy or myopathy. During the crisis, all patients develop hypoketotic hypoglycaemia, with increased ratio of free fatty acids (FFA)-to-ketone bodies elevated serum aminotransferases, and mild hyperammonemia, probably because of increased proteolysis. Plasma and tissue carnitine is low (25% of control in liver and muscle), with increased acyl/free carnitine ratio. The secondary carnitine insufficiency observed in MCAD-deficient patients is due to not only increased excretion of acyl-carnitines, with depletion of tissue carnitine, but also defective reabsorption in the kidney.

Molecular biology
Several laboratories have identified the molecular aetiology of MCAD deficiency as a common point mutation in the locus lp3 (chromosome 1). The mutation, an A to G transition at nucleotide 985, leads to a substitution of lysine with glutamic acid of the mature protein dehydrogenase. It has been observed that patients with MCAD synthesize a normal-size MCAD precursor, which is usually targeted to the mitochondria. A small group (10%) of mutation carriers is completely asymptomatic.

Treatment recommendation
The treatment is similar in LCHAD and MCAD deficiency: fasting and long intervals between meals should be avoided; a high-carbohydrate, low-fat diet should be administered and L-carnitine supplementation can be useful in preventing secondary carnitine insufficiency (type IV evidence). Prevention is important, considering the high incidence of the disease (1/8930 in a newborn screening programme in Pennsylvania) and the good prognosis in patients under adequate dietary control. The best prevention is the identification of the patients during their asymptomatic period, possibly at birth. Screening of all newborns can be achieved by searching for the typical metabolites in the urine. In Pennsylvania [17], a dry blood spot test on Guthrie cards of newborn babies has been proposed analysing blood acyl-carnitines using GC or MS. On peripheral blood DNA the identification of the A to G mutation, present in 90% of the patients, is obtained by restriction analysis (NcoI) of the relevant sequence amplified by the polymerase chain reaction. Data obtained after the initial screening indicate that there is a high prevalence of the mutated allele in babies of German and British heritage, whereas this mutation has been rarely found in newborns of the Mediterranean area. These data suggest that the mutation occurred in a single progenitor of a Germanic tribe. Prenatal diagnosis is possible using the same molecular analysis.

Table 4 Medium-chain acyl-CoA-dehydrogenase deficiency

| Children | Reye-like syndrome | Fasting hypoglycaemia, non-ketotic | Episodes of coma | Low total plasma carnitine | Decreased tissue carnitine | Decreased octanoic oxidation in fibroblasts | Medium-chain dicarboxylic aciduria | Chromosome lp3 | Common mutation 329 lysine to glutamic acid 90% of cases (986 A > G, K304E) |

Short-chain acyl-CoA-dehydrogenase deficiency
Few patients with SCAD deficiency have been described. In SCAD deficiency the dicarboxylic aciduria is not striking. Many shorter-chain length fatty acid residues are seen, such as ethylmalonic, butyric and methylsuccinic acids. In these patients the oxidation of C4 to C6 fatty acids is compromised. As MCAD catalyses 50% of C4 dehydrogenation, the diagnosis may be difficult and may require inhibition of MCAD with specific antisera. SCAD deficiency is associated with different clinical phenotypes: a severe infantile form [18] and a late-onset myopathic picture.

Riboflavin-Responsive Multiple Acyl-CoA-Dehydrogenase defects
This is a relatively common LSM presenting in adult life with fluctuating episodes of profound weakness, associated with carnitine insufficiency and glutaric aciduria and usually underdiagnosed, that responds dramatically to riboflavin [19–21].

Both SCAD and MCAD activity are low in skeletal muscle and mitochondria of these patients that present with a LSM [19,20], therefore this entity is called riboflavin-responsive multiple acyl-CoA-dehydrogenase (RR-MAD) deficiency (Table 5).

It is difficult to explain the improvement of patients and the enzyme changes observed during riboflavin treatment. RR-MAD deficiency may be due to different
mechanism(s). Riboflavin enters as a coenzyme not only in acyl-CoA-dehydrogenase but also in complex I and complex II of respiratory chain. Possible mechanisms of riboflavin deficiency include (i) decreased cellular riboflavin uptake and decreased flavin adenine dinucleotide (FAD) synthesis; (ii) decreased FAD transport into mitochondria; (iii) abnormal binding of FAD to apo-enzymes; (iv) increased catabolism of FAD for increased FADPase. The biochemical study in mitochondria and muscle of FAD and flavin mononucleotide (FMN) levels reveals different mechanism(s) in patients with riboflavin deficiency [21].

Treatment recommendations
It is important to recognize these patients as they improve after riboflavin treatment (100–200 mg/day). Several cases of LSM-associated beta-oxidation defects have been reported, because of multiple acyl-CoA-dehydrogenase deficiency, that was riboflavin responsive (class IV evidence).

Evidence that a biochemical defect involving the oxidation of short-chain fatty acids causes a deficiency of both SCAD, MCAD and FAD and FMN cofactor depletion in biopsied muscle mitochondria should be sought in most cases, especially in those who are riboflavin responsive [21].

Good practice points for treatment of fatty acid disorders
The main caution in defects of mitochondrial β-oxidation is the avoidance of fasting (class IV evidence). By not allowing patients in such disorders to become dependent for energy needs on β-oxidation, the accumulation of toxic intermediate metabolites is avoided and development of most critical symptoms is minimized. Fat consumption should be restricted to 25% of total calories and have reduced amount of LCFA (class IV evidence). Increased caloric intake from carbohydrates may be necessary during intercurrent illness because of increased metabolic demands on the body. A low-fat/high-carbohydrate diet is beneficial in reducing rhabdomyolytic episodes in several disorders of fatty acid metabolism including CPT II deficiency [22] and trifunctional enzyme deficiency [16]. The current dietary treatment of LCFAs defects (high carbohydrate with medium-even-chain triglycerid and reduced long-chain fats) is based on evidence provided by expert opinion alone or by descriptive case series without controls. It is difficult to perform double-blind studies to prevent cardiomyopathy, rhabdomyolysis and muscle weakness. A possible alternative diet has been proposed by replacing dietary medium-even-chain fatty acids by medium-odd-chain fatty acids [23], or by precursors of acetyl-CoA such as by the anaplerotic effect of propionyl-CoA* to restore energy production and improve cardiac and skeletal muscle function.

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References

*The anaplerotic capacity of propionyl-CoA refers to its capacity to provide the formation of methylmalonil-CoA and energy to the Krebs cycle, because it is a precursor of succinyl-CoA, a citric acid cycle intermediate.