Inborn Errors of Metabolism with Myopathy

Defects of Fatty Acid Oxidation and the Carnitine Shuttle System

Areeg El-Gharbawy, MD[a,b], Jerry Vockley, MD, PhD[a,*]

KEYWORDS

• Fatty acid oxidation defects • Carnitine shuttling defects • Cardiomyopathy
• Rhabdomyolysis

KEY POINTS

- Inherited metabolic myopathies should be considered in the differential diagnosis of any individual with muscle pain, fatigue, and recurrent rhabdomyolysis, particularly when triggered by physiologic stress, such as strenuous exercise, intercurrent illnesses, or prolonged fasting.
- Metabolic myopathies, including fatty acid oxidation disorders (FAODs) and carnitine shuttle defects, are heterogeneous disorders that are mostly detected by newborn screening. Because of wide phenotypic variability, diagnostic and treatment challenges remain.
- Referral to a metabolic specialist allows establishing the diagnosis in a timely, cost-effective manner.
- Early recognition of inherited metabolic myopathies allows appropriate choice of therapies conditions and the opportunity to provide genetic counseling to families.

INTRODUCTION

Muscle tissue (heart and skeletal) has a high energy demand to perform essential functions such as ionic homeostasis and contractility. Metabolic fuels for the generation of adenosine triphosphate (ATP) come from different sources, including glucose, free fatty acids, pyruvate, lactate, and ketone body metabolism, and to a lesser extent from amino acids.[1,2] Fatty acids are used as an alternative energy source when glucose is not available. In fetal heart and immediately after birth,

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[a] Department of Pediatrics, Division of Medical Genetics, University of Pittsburgh School of Medicine, Children's Hospital of Pittsburgh, 4401 Penn Avenue, Pittsburgh, PA 15224, USA;
[b] Cairo University, Kasr Al-Aini, Cairo, Egypt
* Corresponding author.
E-mail address: Gerard.vockley@chp.edu

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acetyl-CoA derived from pyruvate metabolism and glycolysis provides reducing equivalents for energy generation. In adult hearts, the main source of ATP is oxidative phosphorylation, with 50% to 70% of the reducing equivalents coming from fatty acid oxidation (FAO).[3] The remaining ATP in the heart is derived from glycolysis and the tricarboxylic acid (TCA) cycle. In skeletal muscle, red muscle fibers rich in mitochondria are used for slow and prolonged contractions, whereas white skeletal muscle fibers depend on anaerobic glycolysis for "fast and short twitch" movements. During rest, glycolysis and oxidative phosphorylation using reducing equivalents from a low basal rate of FAO are the main source of ATP production in skeletal muscle. During fasting or physiologic stress, FAO is upregulated and becomes a major source of energy.[2–4] FAO is regulated by the availability of competing substrates (eg, glucose, lactate, ketones, and amino acids), hormonal influences, contractility, blood supply, and restrictions in oxygen supply. FAO rates are ultimately modulated by transcriptional control of the genes for enzymes involved in fatty acid metabolism and mitochondrial biogenesis.[3,5]

Long-chain fatty acyl-CoAs cross the inner mitochondrial membrane via the carnitine shuttle. Acyl-CoA molecules are first conjugated to carnitine by carnitine-palmitoyl transferase I (CPT1). Acylcarnitines are then transported across the highly impermeable inner mitochondrial membrane by the carnitine-acylcarnitine translocase (CACT). Free acyl-CoAs are then released into the mitochondrial matrix via the action of carnitine-palmitoyl transferase 2 (CPT2) with transport of free carnitine back to the cytoplasm (**Fig. 1**).[3–5] Medium- and short-chain acyl-CoAs enter mitochondria

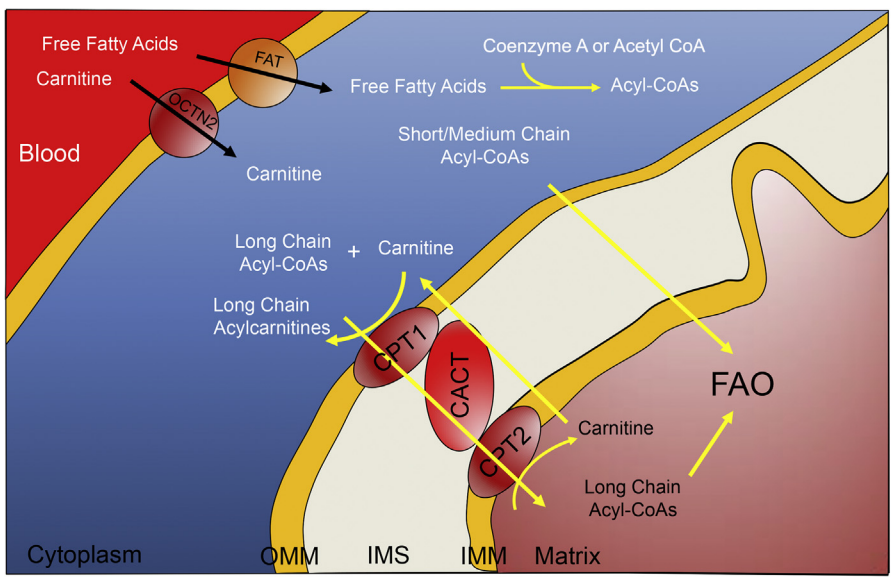

Fig. 1. Fatty acid transport and carnitine shuttle. Carnitine is imported into cells by the carnitine transporter (OCTN2). Free fatty acids enter the cell through dedicated transferases (FAT). Carnitine acyltransferases reversibly transfer an acyl group from an acyl-CoA to carnitine for long-chain substrates. The carnitine shuttle system facilitates the transport of long-chain fatty acids from the cytosol into the mitochondrial matrix, where FAO takes place. This system is made up of CPT1 on the outer mitochondrial membrane (OMM), CACT an inner-mitochondrial membrane space (IMS) protein, and CPT2 on the inner membrane of the mitochondria (IMM).

directly. Oxidation of acyl-CoAs occurs through sequential metabolism of acyl-CoAs by 4 enzymatic steps catalyzed by enzymes varying in chain length specificity: acyl-CoA dehydrogenases (ACADs), enoyl-CoA hydratases, L-3-hydroxyacyl-CoA dehydrogenases, and 3-ketoacyl-CoA thiolases.[3–5] Three ACADs are primarily used for energy production in muscle and heart: very long-chain, medium-chain, and short-chain acyl-CoA dehydrogenases (VLCAD, MCAD, and SCAD). The subsequent 3 steps for long-chain substrates are catalyzed by trifunctional protein (TFP), a heterooctomer encompassing all 3 remaining enzymatic activities. Each cycle of oxidation shortens the carbon chain length by 2, generating 1 molecule of acetyl-CoA, reduced flavin adenine dinucleotide ($FADH_2$), and reduced nicotinamide adenine dinucleotide ($NADH^+$). Reducing equivalents from the flavoenzyme ACADs are channeled to complex III of the respiratory chain by sequential redox reactions with electron transfer flavoprotein (ETF) and ETF:CoQ oxidoreductase (ETF:CoQO; also known as ETF dehydrogenase, ETFDH).[6–8] $NADH^+$ from the 3-hydroxyacyl-CoA dehydrogenase reaction serves as substrate for complex I of the respiratory chain.

During times of high energy demand, acetyl-CoA in the mitochondrial matrix is channeled into the TCA cycle, leading to the production of additional redox equivalents ($FADH_2$, $NADH^+$) that enter the respiratory chain. Proton pumping by respiratory chain complexes I, III, and IV leads to the establishment of an electrochemical proton gradient across the inner mitochondrial membrane that can subsequently be used to synthesize ATP via the mitochondrial ATP synthase (complex V) or drive transmembrane transport processes directly.[2,9] Insufficient fuel reserves are associated with the risk of developing cardiomyopathy and/or rhabdomyolysis during periods of physiologic stress and illness.[2,10–12]

Multiple inborn errors of metabolism (IEMs) have been associated with variable forms of myopathy and/or cardiomyopathy. **Table 1** includes a nonexhaustive list of more frequently encountered disorders. Metabolic myopathies associated with energy defects involve primarily defects in FAO, glycogenolysis, glycolysis, oxidative phosphorylation, and mitochondrial disorders. Long-chain FAODs, including CPT2 deficiency, VLCAD deficiency, long-chain hydroxyacyl-CoA dehydrogenases (LCHAD) deficiency and TFP deficiency, and glycogen metabolism disorders, including glycogen storage diseases (GSDs) types V, VII, and IXd, are associated with an increased risk of rhabdomyolysis induced by exercise. Rhabdomyolysis is less likely to occur in mitochondrial disorders, including oxidative phosphorylation defects, or in GSD types II, IIIa, and IV.[12] Other disorders speculated to cause primarily toxic accumulation of metabolites, and possibly secondary energy defects, and carnitine depletion if not supplemented include organic acidemias as propionic and methylmalonic acidemias where low muscle tone and motor developmental delay may be noted. Posttranslational defects such as those detected in congenital disorders of glycosylation (N-, O-linked and combined) constitute an expanding group of disorders that cause multiple forms of muscular dystrophy. Myopathy is common in O-mannosylation defects leading to muscle eye brain disease, and limb girdle muscular dystrophy.[13] Only defects of FAO and the carnitine shuttle system are discussed in this review.

CARNITINE SHUTTLE DEFECTS AND FATTY ACID OXIDATION DISORDERS

General Concepts

Mitochondrial FAO is essential for energy supply in all tissues. In skeletal muscle, FAO is active during sustained periods of low-intensity exercise, prolonged fasting, and times of physiologic stress, such as intercurrent illness. Cardiac muscle preferentially

Table 1
Inborn errors of metabolism associated with myopathy

IEMs Associated with Myopathy	Inheritance	Clinical Phenotype
Defects in energy metabolism		
Carnitine shuttle defects		
Primary systemic carnitine deficiency	AR	HCM, hypotonia, muscle weakness, fatigue
Carnitine palmitoyl transferase deficiency type 2 (CPT2) deficiency[b]	AR	Muscle weakness, rhabdomyolysis, exercise intolerance (isolated muscle phenotype), CM, hepatomegaly, hypoglycemia, seizures, cystic kidneys (severe infantile)
Carnitine acylcarnitine translocase (CACT) deficiency	AR	CM, arrhythmias, muscle damage, hepatomegaly, hypoglycemia
FAODs		
VLCAD deficiency[b]	AR	HCM, arrhythmias, sudden death, muscle weakness, exercise intolerance, recurrent rhabdomyolysis, hypoketotic hypoglycemia, "Reye-like" hepatic syndrome
LCHAD deficiency[b]	AR	Sudden death, "Reye-like" hepatic syndrome, hypoketotic hypoglycemia, myopathy, recurrent rhabdomyolysis, CM, retinopathy
TFP deficiency[b]	AR	Sudden death, "Reye-like" hepatic syndrome, hypoketotic hypoglycemia, CM, recurrent, rhabdomyolysis, peripheral neuropathy
MAD deficiency	AR	Muscle weakness, CM, hypoglycemia, hepatopathy, respiratory dysfunction, encephalopathy, acidosis
Mitochondrial respiratory chain defects		
Respiratory chain complexes I–V	AR	Myopathy, CM, hepatopathy, Leigh syndrome, epilepsy, developmental delay ± lactic acidosis
Coenzyme Q deficiency		Myopathy, proteinuria, ataxia, low tissue Coenzyme Q, corrected by Coenzyme Q supplementation
Mitochondrial disorders with mt DNA mutations		
Mitochondrial encephalomyopathy with lactic acidosis and strokelike episodes (MELAS)	Mitochondrial	MELAS
Myoclonic epilepsy with ragged-red fibers (MERRF)		MERRF
Neurogenic muscular weakness, ataxia, retinitis pigmentosa (NARP)		NARP
Kearns-Sayre syndrome		CHB, muscle weakness, ataxia, ophthalmoplegia

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Table 1
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IEMs Associated with Myopathy	Inheritance	Clinical Phenotype
Disorders of glycogen metabolism		
Glycogen storage disease type 3a (Cori disease; debrancher deficiency)	AR	Hepatomegaly, ketotic hypoglycemia, muscle weakness, CM, growth retardation, liver cirrhosis, hepatocellular carcinoma (adulthood)
Glycogen storage disease type 5 (McArdle disease; myophosphorylase deficiency)[b]	AR	Exercise intolerance (2nd wind phenomena), muscle weakness, exercise-induced rhabdomyolysis
Glycogen storage disease type 7 (Tarui disease; phosphofructokinase deficiency)[b]	AR	Exercise intolerance (out of wind), muscle weakness, rhabdomyolysis, infantile CM, mild macrocytic anemia
Glycogen storage disease type 9d (muscle phosphorylase kinase deficiency)[b]	XL	Exercise intolerance, muscle weakness and atrophy, hepatomegaly
IEMs with possible secondary energy defect, carnitine deficiency		
Propionic aciduria	AR	DCM, long QT, abnormal respiratory complex in cardiac and skeletal muscle, lactic acidosis, hyperammonemia, DD, low carnitine if not supplemented, hypotonia
Methylmalonic aciduria	AR	Hypotonia, lactic acidosis, hyperammonemia, DD, low carnitine if not supplemented
Other IEMs with myopathy		
GSD II (Pompe disease; acid maltase deficiency)[a]	AR	Infantile DCM, myopathy, atrophy, diaphragmatic weakness (lysosomal storage defect)
GSD IV (Anderson disease; brancher deficiency)	AR	Hepatomegaly, CM, muscle weakness, atrophy, neuromuscular disease, adult isolated myopathy
Danon disease (*LAMP2*-related)[a]	XD	HCM, DCM, short PR, WPW, isolated cardiac variants, proximal muscle weakness (85%)
Congenital disorders of glycosylation N- and O-linked disorders	AR	Multisystem disorder, including brain muscle eye disease, CM, limb girdle muscular dystrophy (O-mannosylation defects), hypotonia, liver disease, (N-linked)

Abbreviations: AR, autosomal recessive; CHB, congenital heart block; CHF, congestive heart failure; CM, cardiomyopathy; DCM, dilated cardiomyopathy; DD, developmental delay; HCM, hypertrophic cardiomyopathy; RCM, restrictive cardiomyopathy; WPW, Wolff-Parkinson-White; XD, X-linked dominant; XL, X-linked recessive.

[a] Classified also as a lysosomal storage disease.

[b] Conditions associated with the risk of recurrent rhabdomyolysis.

oxidizes fatty acids for energy generation even under nonstress conditions and has a limited ability to rely completely on glucose during periods of stress.[10,11] Carnitine is a trimethylated amino acid derived from the diet (especially red meat, fish, and dairy products) and is biosynthesized from lysine and methionine in the liver, kidney, and brain. It is ubiquitously distributed in tissues, but is especially in high concentration in muscle.[14] Importantly, carnitine is necessary for import of long-chain fatty acyls-CoAs into mitochondria as acylcarnitines for FAO. It also facilitates oxidation of branched-chain ketoacids, transports acyl moieties from degraded fatty acids out of peroxisomes, and modulates intramitochondrial acyl CoA/CoA sulfhydryl ratio.[15] Enzymatic defects in FAO and the carnitine shuttling pathway are associated with impaired energy production during times of increased demand. Fatty acid oxidation disorders (FAODs) are collectively one of the most common groups of disorders identified through newborn screening.[6,7] Carnitine shuttle defects and mitochondrial long-chain FAODs have similar clinical findings and are among the most frequent IEMs associated with myopathy and/or cardiomyopathy (**Table 2**). In this review, the authors focus primarily on long-chain FAODs and carnitine shuttle defects associated with myopathy.

There likely are multiple mechanisms of pathogenesis in FAO and carnitine shuttle defects. An insufficient ATP supply to meet energetic demands of heart and muscle

Table 2
Carnitine shuttle and fatty acid oxidation disorders

Disorder (Prevalence)	Acylcarnitine Profile	Cardiac and Muscle Disease
Carnitine shuttle defects		
Primary systemic carnitine deficiency; carnitine transporter defect (1:200,000)	↓ C0, C2	HCM, DCM, CHF, arrhythmias, sudden death hypotonia, muscle weakness
Carnitine palmitoyl transferase deficiency type 2 (1:50–100,000)	↑ C16, C18, C18:1	Cardiomyopathy (infantile form), CHF, muscle weakness, rhabdomyolysis, exercise intolerance
Carnitine acylcarnitine translocase deficiency	↑ C16, C18, C18:1	Cardiomyopathy, CHF arrhythmias, muscle damage
Fatty acid oxidation pathway defects		
VLCAD deficiency (1:40–80,000)	↑ C14:1, C14:2, C14, C12:1	HCM, DCM, CHF arrhythmias, sudden death, muscle weakness, exercise intolerance, rhabdomyolysis
LCAD deficiency (1:80,000)	↑ OH-C16, OH-C18:1, OH-C18:2	CM, CHF myopathy, muscle weakness, retinitis pigmentosa, exercise intolerance, rhabdomyolysis
TFP deficiency (1:200,000)	↑ OH-C16, OH-C18:1, OH-C18:2	CM, DCM, CHF, muscle weakness, exercise intolerance, peripheral neuropathy-myopathy, rhabdomyolysis
MAD deficiency (1:200,000)	Complex ↑ in chains of variable lengths	CM, muscle weakness, rhabdomyolysis
MCAD deficiency (1:10–15,000)	↑ C6, C8, C10, C12	Muscle weakness, exercise intolerance, rhabdomyolysis

cells has clear adverse effects. Although all cellular functions are susceptible to the reduced availability of ATP, its hydrolysis by myosin is essential for muscle cell–specific sarcomere contraction. Thus, defects in any enzyme involved in energy generation and homeostasis will affect cardiac and skeletal muscle, especially during times of physiologic stress, such as fasting and acute illness.[2,8] Depletion of TCA cycle intermediates has been postulated to exacerbate the primary enzyme deficiency.[16,17] Accumulated toxic metabolites from compromised FAO (long-chain CoA-esters, or their free long-chain fatty acids) may cause adverse cellular effects because of altered pH (acid accumulation), inhibition of intermediary metabolism (acyl-CoA deficit), or cell damage due to free radical production.[2,8]

Depending on the severity of the underlying enzymatic defect, clinical manifestations vary from one disorder to another and are clinically heterogeneous within each disorder. In infants, FAODs typically present during periods of acute illness or when oral intake is poor. Hypoglycemia, liver disease, and cardiomyopathy occur in more severe infantile forms of the disease, whereas exercise intolerance and rhabdomyolysis may manifest later in toddlers or older children.[10,11,18] Exercise is the most common trigger of rhabdomyolysis in late-onset CPT2 deficiency, the most common inherited metabolic cause of rhabdomyolysis in adults.[12] Symptoms associated with these defects typically appear after prolonged, moderate-intensity exercise, such as jogging or swimming. Viral infections, fasting, cold, general anesthesia, and sleep deprivation are also trigger factors for metabolic decompensation.[7,11,12,18]

Most FAODs and carnitine shuttle defects are detected by newborn screening using tandem mass spectrometry of blood spots. Morbidity and mortality can be reduced in most of these conditions when identified early and treated before symptoms appear.[19] However, newborn screening has not been successful in reducing the poor prognosis associated with some severe phenotypes such as neonatal/severe infantile forms of CPT2 and CACT deficiencies, or preventing the development of neuropathy and retinal complications in severe mitochondrial TFP deficiency.[7,19,20]

Positive newborn screening results require follow-up studies, including a blood acylcarnitine profile (see **Table 2**), urine organic acids, functional/enzyme testing, or molecular analysis.[6,7,11,15] In the absence of newborn screening, these disorders pose a diagnostic challenge because of the intermittent nature of clinical symptoms and biochemical abnormalities, many of which are present only during times of physiologic distress and catabolism. In this setting, analysis of blood and urine samples obtained during an acute illness or episode of decompensation is critical to unmask the biochemical defect.

Outcome is variable from one disorder to the other, typically depending on the severity of the underlying metabolic defect and residual enzymatic activity. Affected individuals with more severe defects presenting early in the neonatal period, infancy, or early childhood have a poorer prognosis, especially if diagnosed in a symptomatic child rather than through newborn screening. Milder forms presenting later in life may still be life threatening, especially if cardiomyopathy is present.[7,19,20]

Specific Disorders

The following FAODs will be discussed in detail: systemic primary carnitine deficiency (carnitine transporter deficiency; CTD), CACT deficiency, CPT2 deficiency, VLCAD deficiency, MCAD deficiency, mitochondrial TFP deficiency, LCHAD deficiency, and multiple acyl-CoA dehydrogenase (MAD) deficiency (glutaric aciduria type 2; GA II).

Systemic primary carnitine deficiency (carnitine transporter deficiency)

Free carnitine is freely filtered by renal glomeruli, and 95% is reabsorbed by the renal tubules by a high-affinity carnitine transporter in the cellular plasma membrane, whereas most esterified carnitine is excreted in the urine. Carnitine is not catabolized in humans, and its only metabolic conversion is through ester formation.[15,21] Active carnitine transport from blood into cells is mediated by the same transporter that functions in the kidney. Active transport of carnitine into tissue takes place against a concentration gradient, permitting tissue carnitine concentrations to be 20- to 50-fold higher than plasma levels.[22] The carnitine transporter OCTN2 is encoded by the *SLC22A5* gene on chromosome 5q31.2-3 and transports carnitine in a sodium-dependent manner.[22,23] CTD (OMIM 212120) is inherited as an autosomal recessive trait. As a result of its deficiency, carnitine is not reabsorbed in the kidney, leading to urinary loss and depletion of blood and tissue levels.

Clinical manifestations and complications Loss of carnitine in the kidney results in very low concentration in other tissues, resulting in severe impairment of long-chain FAO, which leads to hypoketotic hypoglycemia with fasting and stress. Age of presentation may range from infancy to adulthood, but neonatal hypoglycemia and sudden death may occur.[24,25] Clinical manifestations in early-onset disease include chronic or acute skeletal and cardiomyopathy, typically exacerbated by metabolic decompensation. Untreated, cardiac disease proceeds to dilated cardiomyopathy with reduced left ventricular ejection fraction or restrictive mild interventricular septal hypertrophy. Electrocardiogram findings include abnormal T waves, ventricular hypertrophy, and atrial arrhythmias.[25–27] Life-threatening arrhythmias can also occur, including nonsustained ventricular tachycardia with periods of sinus rhythm and ventricular premature beats, even in the presence of only borderline left ventricular hypertrophy.[27] During episodes of metabolic decompensation, glucose and ketone bodies are inappropriately low. Transaminases and ammonia may be moderately elevated, and metabolic acidosis, prolonged prothrombin time, and elevated creatine kinase (CK) can occur.[25,28] Later-onset disorders can present with milder skeletal muscle manifestations, including hypotonia, myopathy, and exercise intolerance. A founder mutation has led to an extremely high incidence of CTD in the Faroe Islands, often manifesting as sudden death in adults due to previously undetected disease.[29] Secondary systemic carnitine deficiency can be caused by lack of dietary intake usually in strict vegans, prolonged total parenteral nutrition without carnitine supplementation, defective intestinal uptake, or renal loss due to a more general renal tubulopathy.[30] It can also be seen in FAODs or organic acidurias, and can be iatrogenically induced by valproate intake leading to carnitine depletion.[31]

Diagnosis CTD deficiency should be suspected by the finding of very low free plasma carnitine concentrations (<10 μmol/L) accompanied by increased fractional excretion of carnitine in urine. Mutation analysis of the *SLC22A5* gene confirms the diagnosis, but fibroblast carnitine uptake can also be measured if a functional assay is needed. Maternal carnitine deficiency has been identified through newborn screening of an unaffected baby, emphasizing the need to check a plasma carnitine level in mothers of newborns with a critically low free carnitine level.

Treatment Carnitine supplementation should be provided at a dose of 200 to 300 milligrams per kilogram body weight divided throughout the day.[2,26] Affected individuals can develop a “fishlike” body odor due to bacterial metabolism of excess carnitine in sweat or urine, but no serious adverse effects are described. This side effect can be minimized by intermittent treatment with metronidazole.

Carnitine-acylcarnitine translocase deficiency

CACT, located in the inner mitochondrial membrane, facilitates transfer of long-chain acylcarnitine species from CPT1 to CPT2. Mutations in the *SLC25A20* on chromosome 3p21.31 are responsible for CACT deficiency (OMIM 212138).

Clinical manifestations and complications Because neonates depend largely on metabolism of long-chain fatty acids for energy, neonatal presentation is typically severe, with hypoketotic hypoglycemia, hyperammonemia, hypertrophic cardiomyopathy and/or arrhythmia, apnea, hepatic dysfunction, skeletal muscle weakness, and encephalopathy. Unexpected death has also been reported.[32] Children with severe CACT deficiency have a poor prognosis, with most dying before 1 year of age, although longer-term survival is now being reported.[17]

Diagnosis Free carnitine is low in blood, with marked elevations of C16, C18, and C18:1 carnitine species. This acylcarnitine profile is identical to that seen in CPT2 deficiency, and genetic or enzymatic testing is needed to differentiate the 2 disorders.[33] Urine organic acids may show dicarboxylic aciduria. Newborn screening by tandem mass spectrometry will identify CACT deficiency in most cases.

Treatment Avoidance of fasting with continuous feeds for neonates (or every 2–3 hours during the day and continuous at night) is the only available treatment of CACT deficiency. Formula should have reduced long-chain fat plus medium-chain triglyceride (MCT) supplementation. Triheptanoin, an odd chain, MCT with anaplerotic properties, has been reported to successfully treat cardiomyopathy in a limited number of affected individuals.[17] Carnitine supplementation remains controversial because of a theoretic risk of accumulation of long-chain acylcarnitine species, although no proof of toxicity has been reported. Regardless, it is probably not useful unless carnitine levels are low.[34] During an acute episode, intravenous glucose should be administered at a rate of 8 to 12 mg/kg/min in order to inhibit lipolysis and promote anabolism.

Carnitine palmitoyl tansferase 2 deficiency

CPT2 is located on the inner surface of the inner mitochondrial membrane and catalyzes conversion of long-chain acylcarnitines back into long-chain acyl-CoA species with return of carnitine to the cytoplasm. The *CPT2* gene is located on chromosome 1p32.[35]

Clinical manifestations and complications Individuals with CPT2 deficiency (OMIM 600650) present with heterogeneous clinical symptoms based on the severity of the underlying enzymatic defect and are really represent a nearly continuous spectrum.[28] Missense mutations that allow production of some functional enzyme activity are usually associated with milder phenotypes, whereas complete inactivating and protein-truncating mutations produce the more severe forms.[28,36,37] A severe neonatal form presents in the first few days after birth with cardiomyopathy, hypoketotic hypoglycemia, multiorgan dysfunction and failure (including liver and heart), neuronal migration defects, and cystic kidneys. Later-onset, infantile disease is characterized by liver failure, cardiomyopathy, myopathy, and ketotic hypoglycemia in the first year of life.[37] Partial deficiency of CPT2 activity typically leads to episodes of recurrent rhabdomyolysis in adolescence or adulthood, the most common phenotype in this disorder. Affected individuals present with exercise intolerance and recurrent attacks of rhabdomyolysis triggered by fasting, rigorous exercise, cold, and acute illness. Cardiomyopathy and liver disease are not seen.[38–41] Prognosis in neonatal or infantile onset disease is poor[37] with near uniform mortality. Longevity is not

affected in late-onset disease, and affected individuals are usually well or minimally symptomatic between acute episodes.

Diagnosis The plasma acylcarnitine profile shows elevated C16, C18:1, and C18:2 carnitine species. CK levels are high during rhabdomyolysis but may return to normal or be only mildly elevated when affected individuals are well. Carnitine levels are usually normal.[38,39] Persistent elevation of serum CK level is observed in approximately 10% of affected individuals.[38] Diagnosis is confirmed by DNA mutation analysis that detects mutations in roughly 80% of affected individuals. A c.338C>T (p.Ser113Leu) mutation is found in 60% to 75% of mutant alleles and is associated with late-onset disease.[40,41] This mutation leads to a thermolabile protein in cells, likely resulting in degradation of the protein during fever or muscular exercise accompanied by elevated body temperature.[41,42] Enzyme analysis of fibroblasts or muscle tissue is possible.

Treatment Individuals with CPT2 deficiency should be instructed to avoid prolonged fasting (>10 hours) and sustained, intensive exercise. Carbohydrate intake before and during exercise may prevent attacks.[12,43] Dietary supplementation with MCT provides an alternative substrate for FAO.[43] General measures to treat acute rhabdomyolysis include intravenous hydration, alkalization of the urine, and close monitoring of CK, kidney function, and electrolytes. Treatment of electrolyte imbalances and electrocardiogram monitoring is important to reduce the risk of arrhythmias. Hemodialysis and hemofiltration may be indicated to prevent progressive renal failure.[44] Carnitine supplementation is probably not useful but may be given if levels are persistently low.

Very long-chain acyl-CoA dehydrogenase deficiency

VLCAD is bound to the inner mitochondrial membrane and catalyzes the first intramitochondrial step of the long-chain FAO spiral.[45] It is encoded by the *ACADVL* gene on chromosome 17p13. VLCAD deficiency (OMIM 201475) is inherited as an autosomal recessive condition.

Clinical manifestations and complications The clinical presentation of VLCAD deficiency is a spectrum from severe neonatal symptoms to late-onset muscle disease and probably relates to residual enzyme activity.[46–48] Early, severe, infantile disease presents shortly after birth with hypertrophic or dilated cardiomyopathy, arrhythmias, pericardial effusion, hypoglycemia, and liver failure. Early childhood disease may manifest with hypoketotic hypoglycemia, hyperammonemia, lactic acidosis, and elevated transaminases. Regardless of age of onset, affected individuals typically transition to muscular symptoms later in childhood as seen in affected individuals with late onset, characterized by exercise intolerance, and muscle cramps and recurrent episodes of rhabdomyolysis triggered by prolonged exercise or fasting. Hypoglycemia is unusual beyond the first few years of life, but the risk remains. Genotype-phenotype correlations have been reported but are imperfect.[46–49] VLCAD deficiency may be asymptomatic at birth, and thus, newborn screening is critical to identify affected infants. Abnormal newborn screening results should be followed by confirmatory functional and molecular testing.[50,51]

Diagnosis Plasma acylcarnitine profile shows characteristic elevation of C14:1, C14:2, C14, and C12:1 species.[49–51] Urine organic acids are notable for extremely reduced or absent ketones, with elevated long-chain carboxylic and dicarboxylic acids. Diagnostic abnormalities may disappear when affected individuals are well, making analysis of samples obtained during acute episodes critical. Individuals diagnosed with VLCAD deficiency require baseline and follow-up measurements of blood CK, liver

transaminases, echocardiography, and an electrocardiogram. In the setting of acute disease, measurement of blood glucose concentration, lactic acid, and blood ammonia concentration are indicated. Molecular testing with gene sequencing is currently the least invasive and easiest confirmatory test.[46,47,49] If 2 known deleterious *ACADVL* mutations are identified, a presumptive diagnosis of VLCAD deficiency is confirmed. A c.848T>C mutation (V283L) represents ~20% of all mutant alleles in infants detected by newborn screening and is predictive of milder disease.[49–51] Measurement of VLCAD enzyme activity in leukocytes and cultured fibroblasts is available. Flux through the FAO pathway can be demonstrated in cultured fibroblasts by supplementing the growth medium with stable isotope-labeled palmitic acid (C16) and analyzing acylcarnitines in the medium. An abnormal profile is diagnostic and can distinguish VLCAD deficiency from other FAODs.[2,11,49] In addition, the pattern of metabolites provides some insight into clinical phenotype, with excess tetradecanoyl (C14) carnitine correlating with more severe disease, and dodecanoyl (C12) carnitine correlating with milder disease.

Treatment Individuals with VLCAD deficiency should avoid fasting and receive high caloric glucose containing fluids during acute illness to prevent catabolism. A glucose infusion rate of 8 to 12 mg/kg/min is recommended to prevent lipolysis and reverse catabolism.[52,53] General measures for treatment of rhabdomyolysis should be initiated as indicated, but alkalization of the urine and dialysis are usually not necessary. Cardiac dysfunction is usually reversible with early, intensive supportive care, pharmacologic treatment, and diet modification. Frequent, small meals with a snack before bed and with activity may provide greater metabolic stability. Infant formulas optimized for long-chain fatty acid disorders are available. Supplemental fat calories provided through MCT (15%–18% of total calories) provide a fat source that bypasses long-chain FAO.[52–54] MCT oil (0.5 g/kg lean body weight) has been demonstrated to improve exercise tolerance in individuals with long-chain FAODs if administered 20 minutes before exercise.[43,52–54] Use of an odd chain, MCT, triheptanoin, has been reported to improve exercise tolerance and heart function and reduce the frequency and severity of episodes of metabolic decompensation.[16,17] Dietary restriction of long-chain fats in asymptomatic and mild cases and the use of carnitine supplementation are controversial.[53,55,56] Affected individuals with low carnitine levels and myopathic symptoms may benefit from low-dose carnitine supplementation, but concern has been raised (unsupported by clinical data) about the arrhythmogenic potential of long-chain acylcarnitines.

Medium-chain acyl CoA dehydrogenase deficiency

MCAD is the first enzyme in mitochondrial FAO of CoA esters of medium-chain fatty acids. MCAD deficiency (OMIM 201450) is an autosomal recessive condition and is the most common FAO disorder detected by newborn screening.[57]

Clinical manifestations and complications Presentation can occur at any age. Neonates may present with “Reye-like” hepatic syndrome, hypoglycemia, or sudden infant death syndrome (more frequent in breast-fed than bottle-fed infants). Infants with MCAD deficiency usually have normal development but present with hypoglycemia, lethargy, and seizures, during physiologic stress due to intercurrent illness or fasting. Sudden infant death may also occur. Prepubertal children show a tendency toward obesity. Symptoms in adults usually occur after prolonged fasting or alcohol intoxication; sudden death may be the first presentation in undiagnosed cases. Affected individuals have reported complaints of fatigue, exercise intolerance, and muscle aches; elevated CK and rhabdomyolysis have also been reported.[57–60]

Accumulation of toxic metabolites and impaired gluconeogenesis during acute metabolic decompensation result in hypoketotic hypoglycemia. Lactic acidosis and hyperammonemia may also occur.

Diagnosis Newborn screening using tandem mass spectrometry effectively identifies MCAD deficiency, showing elevated C6-C12 species. The diagnosis may be confirmed by molecular analysis, cellular function studies, or plasma acylcarnitine. Urine organic acid analysis may show dicarboxylic aciduria, but may also be normal when an affected individual is well.[11] Some medications and supplements such as valproate and formulas containing MCT oil may falsely elevate medium-chain species.[57] Urine acylglycine analysis is the preferred test in persons who are clinically asymptomatic showing urinary hexanoylglycine, 3-phenylpropionylglycine, and suberylglycine.[61] A c.985A>G mutation is the most common mutation identified in individuals of Northern European decent, followed by the c.233T>C mutation.[57]

Treatment Prevention is the mainstay of therapy and includes educating the family about avoidance of fasting and seeking medical care during acute illness or poor oral intake. Frequent feeding is recommended in infants, starting at every 4 hours until 6 months of age and then increasing to 8 hours after 1 year of age. A low-fat diet (eg, 30% of total energy from fat) may be beneficial.[57] All affected individuals should have an updated "emergency" letter that includes a detailed explanation of the management of acute metabolic decompensation, emphasizing the importance of intravenous glucose infusion and hospitalization, even if glucose level is normal because hypoglycemia is an end-stage event in this condition. Treatment of symptomatic individuals entails reversing catabolism by provision of carbohydrate orally or intravenously. If intravenous fluids are necessary, they should contain at least 10% dextrose with appropriate electrolytes beginning at a rate of 10 to 12 mg glucose per kg/min, with adjustment based on the affected individual's age and needs to maintain normoglycemia.[56,57] The use of L-carnitine supplementation is controversial. Carnitine supplementation (50 mg/kg/d in 2 or 3 divided doses) is not harmful and may be administered when carnitine levels are low, but its need is not proven.

Mitochondrial trifunctional protein deficiency and long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency

TFP is an enzyme that catalyzes the second through fourth steps of FAO for substrates with chain lengths of C12 to C18. TFP enzyme activities include 2-enoyl-CoA hydratase, LCHAD, and 3-ketoacyl-CoA thiolase. TFP is a hetero-octamer, made up of 4 α-subunits encoded by the nuclear gene *HADHA* containing the LCHAD and 2-enoyl-CoA hydratase domains, and 4 β-subunits encoded by *HADHB* containing 3-ketoacyl-CoA thiolase activity. Both genes are located in tandem in opposite directions relative to gene transcription on chromosome 2p23. Isolated LCHAD deficiency (OMIM 609015) is more common than TFP deficiency (OMIM 609016).[62–64]

Clinical manifestations and complications Clinical symptoms in TFP deficiency are usually more severe than in isolated LCHAD deficiency, with earlier onset and a higher risk for mortality.[63,64] However, in either disorder, presentation is variable. Neonates and infants many present with sudden death, hepatopathy (Reye-like disease), hypoketotic hypoglycemia, rhabdomyolysis, myopathy, cardiomyopathy, and pulmonary edema. Long-term complications, such as cardiomyopathy, peripheral neuropathy, and pigmentary retinopathy, and retinal degeneration leading to progressive visual loss also occur.[64–66] A late-onset neuromyopathic form is characterized by progressive peripheral neuropathy and intermittent exercise-induced myoglobinuria. Although

individuals with isolated LCHAD deficiency usually lose deep tendon reflexes in the first few years of life, progressive neuropathy is predominantly seen in individuals with TFP deficiency. Retinopathy is typically more severe in LCHAD deficiency. Individuals with complete TFP deficiency often do not survive the second decade of life.

Diagnosis The blood acylcarnitine profile is abnormal but does not distinguish LCHAD from TFP deficiency; long-chain hydroxyl acylcarnitines (OH-C16, OH-C18:1, and OH-C18:2) are elevated in both. These abnormalities can usually be identified at birth by newborn screening of dried blood spots with tandem mass spectrometry. Urine organic acids under stress are notable for minimal ketones and the presence of dicarboxylic acids. Enzyme analysis, fibroblast FAO flux studies, and gene sequencing will differentiate LCHAD from TFP deficiency.[62–64] Affected individuals require routine monitoring of blood CK, liver transaminases, electrocardiography, and echocardiography. In acute decompensation, measurement of blood glucose concentration, lactic acid, and blood ammonia concentration is also indicated. Mutations in the *HADHA* gene usually cause isolated LCHAD deficiency, and a common mutation in *HADHA* (c.1528G>C; E474Q) accounts for ~80% of the mutant alleles in LCHAD deficiency. Defects in the *HADHB* gene invariably affect all 3 enzymatic activities causing complete TFP deficiency. Molecular studies in individuals with TFP deficiency show a wide range of "private" mutations in both genes. *HADHB* RNA level and the rate of thiolase degradation correlate with the severity of clinical manifestations.[62–64,66]

Treatment Therapy is similar to VLCAD deficiency and includes avoiding the physiologic triggers of fasting and illness. Diet should be modified to decrease long-chain fat intake along with supplementation of the diet with MCT oil and essential fatty acids. Docosahexaenoic acid is recommended at a dose of 60 mg/d in children weighing less than 20 kg and a dose of 120 mg per day in children greater than 20 kg body weight in an attempt to delay or prevent retinal disease.[43,52,53] Carnitine supplementation remains controversial, but low doses do not cause harm. Intravenous supplementation of carnitine in high doses during decompensation is not recommended.[52]

Multiple acyl-CoA dehydrogenase deficiency (glutaric aciduria type 2)

MAD deficiency (OMIM 231680) is an autosomal recessive combined disorder of fatty acid, amino acid, and choline metabolism. It results from deficiency of one of the subunits of ETF (ETFA and ETFB), or ETF;CoQO (ETFDH), located on chromosomes 15q23-q25, 19q13.3, and 4q32-qter, respectively.[66,67] The broad effect of these defects is due to a global inability to reoxidize all of the primary mitochondrial flavin adenine dinucleotide (FAD)-dependent dehydrogenases, which are involved in multiple catabolic pathways. The clinical picture is variable, based on the severity of the underlying enzymatic defect. In its most severe form, affected individuals have congenital anomalies, including cystic dysplastic kidneys and abnormal brain findings, and die in the newborn period of hypoglycemia, hyperammonemia, and metabolic acidosis. Individuals with less severe disease show less dramatic hypoglycemia, encephalopathy, muscle weakness, or cardiomyopathy.[68] Respiratory dysfunction may be present.[69] Some affected individuals may present with only late-onset myopathy. There is significant genetic heterogeneity in MAD deficiency with some genotype-phenotype correlations.[68] Specific mutations in ETFDH have been associated with riboflavin-responsive symptoms as well as a myopathic form related to secondary CoQ10 deficiency.[68,70–72] Furthermore, disorders of FAD synthesis and transport have been described, with overlapping clinical and laboratory findings to classical MAD deficiency.

Diagnosis A diagnosis of MAD deficiency can be made through blood acylcarnitine profiling and characterization of urine organic acids. The secondary deficiency of all primary mitochondrial FAD-dependent dehydrogenases leads to a complex and variable accumulation of metabolites, including glutaric acid (thus the alternative name of GA II), ethylmalonic, butyric, isobutyric, 2-methylbutyric, and isovaleric acids. Lactic acid and ammonia may be secondarily elevated, and hypoglycemia may be present.[2,68] Confirmation of diagnosis is accomplished by direct DNA sequence of the *ETFA*, *ETFB*, and *ETFDH* genes. If variants of unknown significance are found, functional assays, including enzyme activity and acylcarnitine profiling, can be performed on fibroblasts.[2,68,70–73] Newborn screening will identify many, but not all cases.

Treatment During an episode of acute metabolic decompensation, affected individuals should receive a high-glucose infusion rate, similar to other FAODs. Chronically, treatment of severe MAD deficiency is difficult because of multiple affected metabolic pathways. Avoidance of fasting and conjugation of toxic metabolites with L-carnitine and glycine are indicated, and a low-fat diet may be helpful.[68] A general restriction of protein may be helpful but is difficult because of the large number of amino acids whose metabolism is affected. MCT oil should be avoided, because oxidation of all chain length fats is impaired. D,L-3-hydroxybutyrate has been shown to be of benefit in a limited number of case reports, especially in treating cardiomyopathy.[74] For riboflavin-responsive *ETFDH* mutations, resolution of symptoms occurs with riboflavin supplementation (up 150 mg daily). Coenzyme Q_{10} supplementation may also be of some benefit in some individuals with riboflavin-responsive MAD deficiency and may augment riboflavin response.[68,70–73]

THERAPIES UNDER INVESTIGATION FOR LONG-CHAIN FATTY ACID OXIDATION DISORDERS

Triheptanoin is a source of 7-carbon fatty acids proposed to be superior to MCT because its metabolism provides an anaplerotic 3-carbon product (propionyl-CoA).[75] Studies to date suggest an improvement in glucose homeostasis and cardiomyopathy along with a residual but reduced risk for rhabdomyolysis.[16,17,75] The drug is currently in a US Food and Drug Administration approval trial.

Bezafibrate, a PPAR pan-agonist, has been shown to increase CPT2 and VLCAD enzyme activity in cultured fibroblasts from some individuals with missense mutations in this gene.[76,77] However, one clinical trial in individuals with CPT2 or VLCAD deficiency failed to demonstrate efficacy, and thus, further study is needed.[78]

SUMMARY

Disorders of carnitine transport and long-chain FAO are a heterogenous group of disorders with a common end pathophysiology related to reduced mitochondrial energy production. Identification through newborn screening is possible for most of the disorders, but in those regions where it is not performed, a high index of clinical suspicion is necessary because diagnostic metabolites may normalize when affected individuals are well. Prognosis in general is good with early diagnosis and treatment, and new therapies currently in clinical trials are likely to improve therapeutic options in the near future.

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