

## Opinion



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### Correspondence to

**Han-Wook Yoo**

Department of Pediatrics and Medical Genetics & Genomics, Asan Medical Center, University of Ulsan College of Medicine, 88 Olympic-ro 43-gil, Songpa-gu, Seoul 05505, Korea.

E-mail: hwyoo@amc.seoul.kr

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### ORCID iDs

Han-Wook Yoo

<https://orcid.org/0000-0001-8615-186X>

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# Inborn Errors of Mitochondrial Fatty Acid Oxidation: Overview from a Clinical Perspective

**Han-Wook Yoo**

Department of Pediatrics and Medical Genetics & Genomics, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

## ABSTRACT

Mitochondrial fatty acid  $\beta$ -oxidation (mFAO), which is the major pathway for the degradation of fatty acids and is critical for maintaining energy homeostasis in the human body, consists of carnitine transport, the carnitine shuttle, and fatty acid  $\beta$ -oxidation. Inherited metabolic defects of mFAO result in more than 15 distinct mFAO disorders (mFAODs) with varying clinical manifestations. The common elements of the clinical presentation of mFAODs are hypoketotic hypoglycemia, (cardio)myopathy, arrhythmia, and rhabdomyolysis, indicating the importance of FAO during fasting or stressful situations. The management of all mFAODs includes avoidance of fasting, aggressive treatment during illness, and supplementation of carnitine or appropriate nutritional support, if necessary. Through the introduction of newborn screening using tandem mass spectrometry, early identification of mFAODs became feasible, leading to an early initiation of treatment with improved outcomes. However, many unmet needs remain with regard to the long-term management of patients with mFAODs.

**Keywords:** Mitochondrial fatty acid  $\beta$ -oxidation (mFAO); Metabolic defects of mFAO

## INTRODUCTION

Fat is a crucial source of energy and principal fuel store. Fatty acids are preferentially utilized in skeletal muscles, particularly in cardiac muscle, rather than glucose. They are the main energy source for skeletal muscles during sustained exercise. Most organs mobilize energy from fatty acids during prolonged fasting in order to spare glucose for the central nervous system. Fatty acid oxidation in the liver generates acetyl-CoA for the synthesis of ketone bodies, which the brain is able to spend as an energy source. Fatty acid  $\beta$ -oxidation is a major source of energy in the mitochondria, producing the reducing agents flavin adenine dinucleotide (FADH<sub>2</sub>) and nicotinamide adenine dinucleotide (NADH) that function as electron donors to the respiratory chain complex for oxidative phosphorylation and ATP generation.<sup>1</sup>

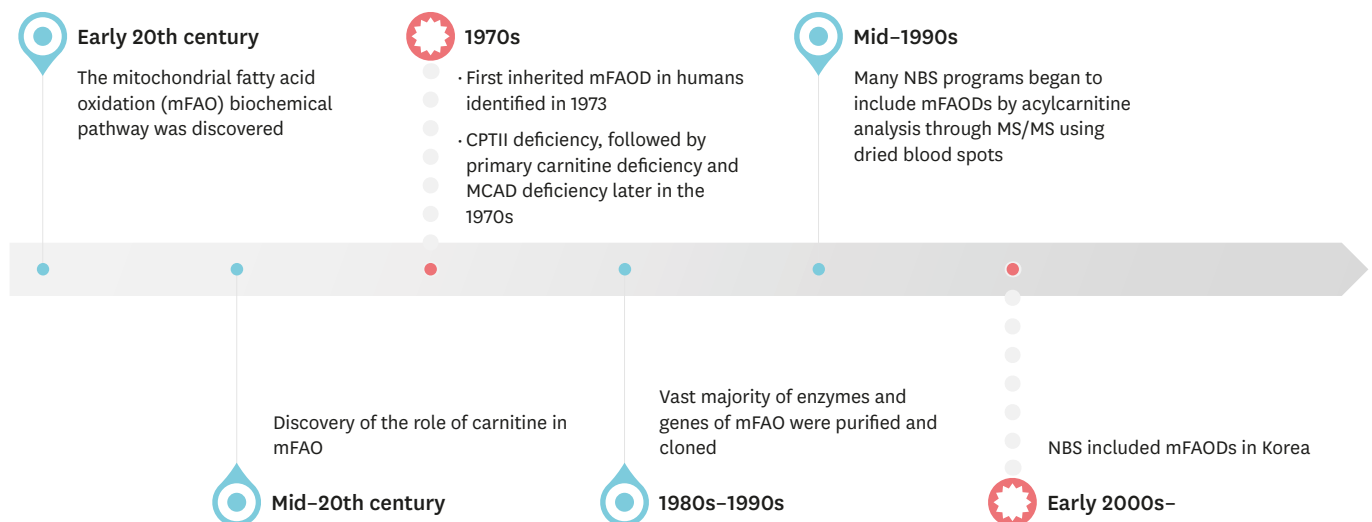
## HISTORY

The mitochondrial fatty acid oxidation (mFAO) biochemical pathway was discovered in the early 20th century. Fatty acid metabolism proceeds by the sequential elimination of 2-carbon

fragments. The residual fatty acid chain carries a carboxylic acid group on the  $\beta$  carbon atom. Another epoch-making event was the discovery of the role of carnitine in mFAO in the mid-20th century. The first inherited mFAO disorder (mFAOD) in humans, carnitine palmitoyltransferase II (CPTII) deficiency, was reported in 1973, followed by primary carnitine deficiency and medium-chain acyl-coenzyme A (CoA) dehydrogenase (MCAD) deficiency later in the 1970s. The vast majority of enzymes and genes involved in mFAO were purified and cloned through the 1980s and 1990s. Most mFAODs are identified through rapid acylcarnitine profile analysis by flow injection electrospray ionization tandem mass spectrometry (MS/MS). These disorders are treatable and preventable by simple avoidance of fasting or dietary modification in most cases, whereas acute metabolic crashes are often fatal in affected individuals not suspected to have these conditions. Therefore, many newborn screening (NBS) programs began to include mFAODs through acylcarnitine analysis by MS/MS using dried blood spots since the mid-1990s. In Korea, NBS has included mFAODs since the early 2000s (**Fig. 1**).<sup>2</sup>

## BIOCHEMISTRY OF mFAO

mFAO includes 3 principal processes; carnitine uptake (transport), the carnitine shuttle, and fatty acid  $\beta$ -oxidation. Fatty acids need to be transported across the plasma membrane through an unknown mechanism. Carnitine uptake occurs through plasma membrane via the organic cation carnitine transporter (CT). The mitochondrial membrane is not permeable to acyl-CoAs; therefore, the carnitine shuttle imports acyl-CoAs into mitochondria. Long-chain fatty acids are activated to CoA esters in the cytoplasm. However, in order to cross the mitochondrial outer and inner membrane, acyl-CoA needs to be transferred to carnitine, by carnitine palmitoyltransferase I (CPT I), yielding acylcarnitine; this is the main step involved in the regulation of fatty acid oxidation. Fatty acids are transferred back to CoA esters within mitochondria via carnitine acylcarnitine translocase (CACT) and CPTII, with carnitine shuttled back to the mitochondrial membrane. Therefore, carnitine is a crucial component for long-chain fatty acid oxidation. Medium- and short-chain fatty acids are able to enter into the



**Fig. 1.** Historical timeline: inborn errors of mitochondrial fatty acid oxidation. mFAO, mitochondrial fatty acid oxidation; mFAOD, mitochondrial fatty acid  $\beta$ -oxidation disorder; CPTII, carnitine palmitoyltransferase II; MCAD, medium-chain acyl-coenzyme A dehydrogenase; NBS, newborn screening; MS/MS, tandem mass spectrometry.

mitochondrial matrix independently of carnitine once they are directly activated to CoA esters. Second, in  $\beta$ -oxidation via a cascade pathway, each turn of the biochemical pathway shortens the acyl-CoA by the removal of 2 carbons in 4 steps, including 2 dehydrogenation reactions associated with flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide (NAD<sup>+</sup>). Chain-length specific enzymes catalyze reactions in the mFAO pathway. The enzymes for long-chain fatty acids are mitochondrial membrane-bound, and these reactions are catalyzed by very-long-chain acyl-CoA dehydrogenase (VLCAD) and mitochondrial trifunctional protein (MTP) including long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD). The enzymes for medium- and short-chain substrates are located in the mitochondrial matrix, including MCAD and short-chain acyl-CoA dehydrogenase (SCAD).<sup>2,3</sup>

## INBORN ERRORS OF mFAO AND CLINICAL PRESENTATIONS

Inherited defects of mFAO in human (inborn errors of mFAO) have been described for almost all enzymes, and transporters involved in mFAO, with at least 12 disorders. All these defects are inherited in an autosomal recessive manner. They result from mutations in *CPT1A* (carnitine palmitoyltransferase IA [liver]; *CPT1A*), *CPT2* (carnitine palmitoyltransferase type II; *CPT2*), *SLC25A20* (*CACT*), *SLC22A5* (organic cation/CT), *ACADVL* (VLCAD), *ACAD9* (acyl-CoA dehydrogenase 9), *ACADM* (MCAD), *ACADS* (SCAD), *HADHA* (mitochondrial trifunctional protein,  $\alpha$  subunit), *HADHB* (mitochondrial trifunctional protein,  $\beta$  subunit), *ECHS1* (short-chain enoyl-CoA hydratase), *HADH* (short-chain (S)-3-hydroxyacyl-CoA dehydrogenase), and *ACAT1* (acetoacetyl-CoA thiolase). Different types of enzyme deficiencies can affect MTP. Mutations in *HADHA* can lead to a deficiency of all 3 enzymatic functions of MTP or to an isolated defect in LCHAD; the latter is more frequent. Similarly, mutations in *HADHB* may cause complete MTP deficiency or an isolated long-chain 3-ketoacyl-CoA thiolase defect; the latter is extremely rare. Mutations in *SLC22A5* lead to primary carnitine deficiency, mainly due to renal carnitine wasting. As a result, tissue carnitine levels are low, resulting in impairment of the carnitine cycle function and thus mFAO. Although SCAD deficiency leads to an aberrant pattern of specific metabolites consistent with a mFAOD, it is currently regarded as a benign biochemical phenotype, rather than a clinically significant inborn error of metabolism, and no treatment is necessary. Multiple acyl-CoA dehydrogenase deficiency (MADD) (also known as glutaric acidemia type 2) is a complex inborn error of metabolism due to a combined defect of multiple acyl-CoA dehydrogenases involved in fatty acid metabolism. The electron transfer flavoprotein (ETF) is composed of two subunits: ETF<sub>A</sub> and ETF<sub>B</sub>. Mutations in the genes *ETF<sub>A</sub>*, *ETF<sub>B</sub>*, and electron transfer flavoprotein dehydrogenase (*ETFDH*) impair the electron transfer from ETF to ETFDH and then ultimately to coenzyme Q10 in complex III of the mitochondrial electron transport chain. Human inborn errors of some enzymes in mFAO have not been described, such as carnitine palmitoyltransferase IB (muscle), long-chain acyl-CoA dehydrogenase, and the auxiliary enzymes.

The general clinical presentation of mFAODs is diverse, with a varying onset of symptoms depending on individual enzyme's residual activity. Most mFAODs are identified through NBS by MS/MS, although the details are different from country to country. For instance, MCAD deficiency (MCADD) is the most common (incidence of 1/20,000 newborns) in Caucasians because its founder mutation (c.985A>G [p.K304E] of the *ACADM* gene) is found in approximately 70% of mutant alleles in affected patients of Northern European descent. There are 3 common major clinical features shared in most mFAODs. First, acute hypoketotic

hypoglycemia is often accompanied by enlargement of the liver and its dysfunction, particularly during infancy and early childhood. This acute episode is usually precipitated by prolonged fasting or an infection with poor feeding. The second is cardiac presentation with hypertrophic cardiomyopathy, pericardial effusion, or arrhythmia. The third is myopathy with elevated creatinine kinase levels, muscle weakness, or recurrent rhabdomyolysis in adolescence or adulthood. Many patients are clinically asymptomatic until precipitating environmental factors such as fasting, infection, and strenuous physical exercise cause the symptoms to manifest.

In addition, mFAOD-specific phenotype diversity is present. MCADD may present in infancy and in toddlers accompanied by infection with vomiting and poor oral intake, leading to dehydration, altered consciousness, and hypoketotic hypoglycemia. Liver dysfunction mimicking Reye syndrome can occur. Progression of the disease can often be fatal because of hyperammonemia and encephalopathy. VLCAD deficiency (VLCADD) is the most common mFAOD in Korea, and patients present with severe cardiomyopathy, pericardial effusion, and arrhythmia or suffer from recurrent hypoketotic hypoglycemia, or recurrent myopathy and/or rhabdomyolysis in adolescence. The most critical presentation of neonatal LCHAD deficiency (LCHADD) and MTP deficiency is a relentlessly progressive cardiomyopathy and profound lactic acidosis. Infantile presentations may include recurrent hypoketotic hypoglycemia and liver dysfunction with coagulopathy, cardiomyopathy, myopathy, and rhabdomyolysis during acute illness. Pigmentary retinopathy and long-term aggravating skeletal myopathy with peripheral neuropathy are characteristics of LCHADD and require cautious monitoring. Heterozygous mothers pregnant with a fetus affected by LCHADD have a risk of developing hemolysis, elevated liver enzymes, and low platelets syndrome. CPTII deficiency is one of the most common etiologies of metabolic myopathy diagnosed in adolescence or adulthood after strenuous physical exercise, and affected patients often experience recurrent episodes of rhabdomyolysis with a high risk of renal function impairment. CPTIA deficiency may lead to hypoketotic hypoglycemia, liver dysfunction, and rapid progression to liver failure in early childhood. However, hypoglycemia is rare in the neonatal period and myopathy is infrequent even in adulthood. Complete CACT deficiency presents with severe neonatal cardiomyopathy, ventricular dysrhythmias, hypoglycemia, hyperammonemia, and sudden death. Patients with CT defect (CTD) usually develop hypoketotic hypoglycemia, hyperammonemia, liver dysfunction, cardiomyopathy, and hypotonia in childhood. Patients with MADD demonstrate a considerably wide range of clinical presentations, from neonatal lethal forms to mild late-onset forms. Neonates may or may not have congenital anomalies (such as enlarged polycystic kidneys, rocker-bottom feet, inferior abdominal musculature defects, hypospadias, cerebral cortical dysplasia, and gliosis). Dysmorphic facial features may include macrocephaly, a large anterior fontanel, malformed ears, a high forehead, and flat nasal bridge). Neonatal-onset MADD is usually lethal, and these patients often die from severe acidosis, nonketotic hypoglycemia, and hypertrophic cardiomyopathy during the neonatal period despite early diagnosis by NBS and early initiation of treatment. Later-onset MADD, which is more likely to be riboflavin-responsive, does not involve congenital malformations. However, affected individuals have a persistent risk of acute intermittent episodes of metabolic decompensation, usually triggered by metabolic stress. Most patients with late-onset MADD benefit from treatment with riboflavin. This clinical phenotype is called riboflavin-responsive MADD (RR-MADD). Most patients with RR-MADD present with fluctuating muscle weakness, exercise intolerance, myalgia, and dramatic riboflavin-responsiveness. Cardiac or gastrointestinal symptoms are occasionally observed in some patients. RR-MADD has been reported in Western countries. However, patients with RR-

MADD are preponderantly found in Southeast and East Asian countries, including China, Taiwan, Hong Kong, Japan, Thailand, and Korea, with a shared common mutation. Almost all cases of RR-MADD are caused by the *ETFDH* gene.<sup>1,37</sup>

## NEWBORN SCREENING AND DIAGNOSIS OF mFAODs

The collective incidence of mFAODs based on many NBS programs is 1 in 10,000–15,000 newborns, although the individual prevalence varies significantly.<sup>8</sup> In Korea, the overall incidence of mFAODs is 1/110,000 newborns (**Table 1**).<sup>9</sup> VLCADD is the most common. Based on high-risk screening, the prevalence of mFAODs is 1/15,800, mostly long-chain fatty acid oxidation disorders (LCFAODs).<sup>10</sup> Most FAODs can be detected by NBS utilizing MS/MS, although the situation varies from country to country. For example, MCADD was the first mFAOD introduced to NBS programs, particularly in Western countries where this is the most common mFAOD. Patients with MCADD detected by NBS are less likely to suffer from severe metabolic decompensations and/or fatal outcomes, with the risk reduced by 74%.<sup>11,12</sup> Subjects who test positive need further retesting before a confirmatory diagnosis by an acylcarnitine profile assay, including free carnitine levels, and DNA testing. Evaluating the ratios of different acylcarnitines enhances the diagnostic specificity. An assay of urine organic acids or urine acylglycines can be helpful, as these patients show a typical urinary excretion pattern of dicarboxylic acids or acylglycines without ketonuria. However, an abnormal plasma acylcarnitine profile and urine organic acid analysis results are not found in some mFAODs and in mild cases with high residual enzyme activity levels. In addition, it is very difficult to differentiate certain individual mFAODs only based on the plasma acylcarnitine profile. Therefore, DNA testing is required to confirm the specific diagnosis. Variants of uncertain significance necessitate further investigational enzymatic assays using fibroblasts, leukocytes, or liver tissue. Patients with LCFAODs need a cautious cardiac evaluation using chest radiography, electrocardiography, or echocardiography on a regular basis.<sup>13</sup>

## MANAGEMENT OF mFAODs

The 3 mainstays of nutritional management for all mFAODs are avoidance of fasting, aggressive treatment during illness, and supplementation of carnitine if deficient, except in patients with LCFAODs. However, there are some differences in the nutritional management of specific mFAODs. It is important for patients with MCADD to avoid medium-chain triglyceride (MCT) oil. LCFAODs differ by requiring a fat-restricted diet, potentially higher protein intake, and supplementation of MCT. These steps may resolve cardiomyopathy. Carnitine supplementation is effective in CTD patients, but the beneficial effects of carnitine

**Table 1.** Current status of mFAODs in South Korea<sup>9,10</sup>

Incidence of mFAODs and the most common mFAOD		
Newborn screening	1/110,000 newborns	VLCADD
High-risk group screening	1/15,800 patients	LCFAODs
Availability of biochemical assay and confirmatory DNA testing for mFAODs		
Acylcarnitine profiling	Available at clinical laboratories	
Organic acid analysis	Available at clinical laboratories	
Enzymatic assay	Not usually available (research laboratory only)	
Genetic testing	Available at clinical laboratories	

mFAOD, mitochondrial fatty acid oxidation disorder; VLCADD, very-long-chain acyl-coenzyme A dehydrogenase deficiency; LCFAOD, long-chain fatty acid oxidation disorder.

therapy on other mFAODs are controversial. Carnitine therapy is even harmful in patients with LCFAODs because it raises the concentration of long-chain acylcarnitine, which may cause arrhythmia. Riboflavin is the drug of choice for patients with RR-MADD, which is common in Southeast and East Asia. Bezafibrate has been tried for myopathy from CPTII deficiency and VLCADD by drug repurposing. It augments the expression of mFAO enzymes by activating peroxisome proliferator-activated receptor alpha and delta receptors. However, its effectiveness remains unproven. Recently, a clinical trial using triheptanoin (Ultragenyx Pharmaceutical Inc., Novato, CA, USA), a triglyceride made up of three 7-carbon fatty acids, has been underway for LCFAODs, with promising results. It provides energy in patients with mFAODs as an alternative fuel source. Once they survive the initial acute metabolic decompensation, most patients with mFAODs experience a long-term clinical course. Therefore, meticulous long-term management and monitoring are critical for preventing recurrent rhabdomyolysis or episodes of metabolic decompensation. Since mFAODs are inherited in an autosomal recessive fashion, prenatal genetic counseling is needed in at-risk families.<sup>1,4,5</sup>

## CONCLUSION

Although NBS programs have made progress in reducing mortality and morbidity caused by mFAODs, there are still many unmet needs for treatment, particularly for severe neonatal-onset mFAODs. Some forms of mFAODs are curable with medication, such as CTD and CPTI deficiency with carnitine, and RR-MADD with riboflavin. Early diagnosis of these actionable disorders is crucial for obtaining better outcomes. Recurrent episodes of rhabdomyolysis, hypertrophic cardiomyopathy, and arrhythmia often turn out to be fatal. A multisystemic approach and monitoring can ameliorate episodes of life-threatening events.

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