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# Fetal Fatty Acid Oxidation Disorders, Their Effect on Maternal Health and Neonatal Outcome: Impact of Expanded Newborn Screening on Their Diagnosis and Management

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## ABSTRACT

Mitochondrial fatty acid oxidation disorders (FAOD) are recessively inherited errors of metabolism. Newborns with FAOD typically present with hypoketotic hypoglycemia, metabolic acidosis, hepatic failure, and cardiomyopathy. Late presentations include episodic myopathy, neuropathy, retinopathy, and arrhythmias. Sudden unexpected death can occur at any age and can be confused with sudden infant death syndrome. Some FAOD are associated with intrauterine growth restriction, prematurity, and pregnancy complications in the heterozygous mother, such as severe preeclampsia, acute fatty liver of pregnancy (AFLP), or hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome. Maternal pregnancy complications occur primarily in mothers carrying a fetus with long-chain L-3-hydroxyacyl CoA dehydrogenase deficiency or general trifunctional protein deficiencies. FAOD as a group represent the most common inborn errors of metabolism, and presymptomatic diagnosis of FAOD is the key to reduce morbidity and avoid mortality. The application of tandem mass spectrometry to newborn screening provides an effective means to identify most FAOD patients presymptomatically. At the beginning of 2005, 36 state newborn screening programs have mandated or adopted this technology resulting in a marked increase in the number of asymptomatic neonates with FAOD diagnosed. To ensure the long-term benefits of such screening programs, pediatricians and other health care providers must be educated about these disorders and their treatment. (***Pediatr Res* 57: 78R–86R, 2005**)

**Abbreviations**

**AFLP,** acute fatty liver of pregnancy
**FAO,** fatty acid oxidation
**FAOD,** fatty acid oxidation disorders
**HELLP,** hemolysis, elevated liver enzymes, and low platelets syndrome
**HMGCL,** 3-hydroxy-3-methylglutaryl-CoA lyase
**HMGCS2,** 3-hydroxy-3-methylglutaryl-CoA synthetase
**LCAD,** long-chain acyl CoA dehydrogenase
**LCHAD,** long-chain L-3-hydroxyacyl CoA dehydrogenase
**LKAT,** long-chain 3-ketoacyl-CoA thiolase
**MCAD,** medium-chain acyl CoA dehydrogenase
**MS/MS,** tandem mass spectrometry
**MTP,** mitochondrial trifunctional protein
**OA,** organic acidemias
**SCHAD,** short-chain L-3-hydroxyacyl CoA dehydrogenase
**SIDS,** sudden infant death syndrome
**VLCAD,** very-long-chain acyl CoA dehydrogenase

Fatty acids constitute the largest energy reserve in the body and play a crucial role in supplying energy-yielding substrates during periods of fasting and stress through the $\beta$-oxidation pathway (1). FAO provides nearly 80% of energy to organs like heart, liver, and skeletal muscles, especially during fasting when tissue glycogen stores become depleted. The $\beta$-oxidation pathway also generates ketone bodies, which are used by peripheral tissues and brain (2). This metabolic pathway is critical for the neonate who has limited glycogen reserve and a high metabolic rate leading to rapid metabolic decompensation if there is any perturbation of individual enzymes (3). FAOD are potentially fatal autosomal recessive disorders and are now diagnosed frequently in the perinatal and infantile periods. Mothers heterozygous for a FAOD and pregnant with an affected fetus may develop severe preeclampsia, AFLP, and the HELLP syndrome, and may deliver a premature, intrauterine growth-restricted (IUGR) infant (4).

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The first genetic defect in fat oxidation was described in 1973, involving a patient with what is now known as carnitine palmitoyltransferase II (CPT II) deficiency (5,6). Since that time, there has been a steady increase in discovery of newer FAOD and an exponential rise in patients diagnosed with these disorders (6). Advances in this field have recently been facilitated by the availability of MS/MS technology with which a single analysis provides a clue as to the type of FAOD the patient may have. Here we review this seemingly complex subject, and discuss its likely impact on health care of newborn infants and their mothers in the near future.

## THE MITOCHONDRIAL β-OXIDATION PATHWAY

Figure 1 represents a schematic of the mitochondrial β-oxidation pathway starting with uptake of fatty acids and carnitine into the cell, transfer of fatty acid from the cytosol into mitochondria, and entry into the β-oxidation spiral. Medium- and short-chain fatty acids are transported directly into the cytosol and mitochondria, but long-chain fatty acids and carnitine are transported by specific plasma membrane transporters like fatty acid transporter (FAT), and fatty acid-binding protein (FABP) (7–9). Carnitine a key factor in facilitating entry of long-chain fatty acids into the mitochondria is transported into the cell through its transporter OCTN2 (10). Activated fatty acyl-CoA are converted to carnitine esters by carnitine palmitoyltransferase I (CPT I), transferred across the mitochondrial membranes by carnitine-acylcarnitine translocase (CACT), and fatty acyl-CoA reconstituted by CPT II (11).

The initial step in the FAO spiral is the acyl-CoA dehydrogenase reaction catalyzed by the homologous flavoprotein-linked (FAD) enzymes MCAD, LCAD, and VLCAD and leads to formation of a 2,3-enoyl-acyl-CoA (12). The second step is the conversion of a 2,3-enoyl-acyl-CoA to a 3-hydroxyacyl-CoA catalyzed by 2,3-enoyl-CoA hydratase. The third step of the spiral is the conversion of 3-hydroxyacyl-CoA to 3-ketoacyl-CoA catalyzed by the two homologous enzymes SCHAD and LCHAD, and, in the final step, one acetyl-CoA molecule is removed from the 3-ketoacyl-CoA by the two homologous enzymes, short-chain 3-keto-acyl-CoA thiolase (SKAT) and long-chain 3-keto-acyl-CoA thiolase (LKAT), respectively. For longer chain fatty acids, the latter three steps of this pathway are catalyzed by the membrane-bound MTP, a hetero-octameric complex in which the α-subunit contains the LCHAD and hydratase activities and the β-subunit contains the long-chain 3-ketoacyl-CoA thiolase activity. The end result of each cycle of β-oxidation is production of a shortened acyl-CoA that reenters the β-oxidation spiral within the mitochondrial matrix and one molecule of acetyl-CoA. Acetyl-CoA may be used for steroidogenesis, enter the TCA cycle, or become transformed into ketone bodies in the liver by the action of 3-hydroxy-3-methylglutaryl-CoA synthetase (HMGCS2) and 3-hydroxy-3-methylglutaryl-CoA lyase (HMGCL) (13). The FAD-linked dehydrogenases (MCAD, LCAD, and VLCAD) generate electrons which are transferred to ubiquinone *via* the electron transfer flavoproteins (ETF) and ETF dehydrogenase (ETFDH). Electrons from NADH-linked dehydrogenation (SCHAD and LCHAD) are shifted to complex 1 in the respiratory chain eventually leading to production of energy as ATP.

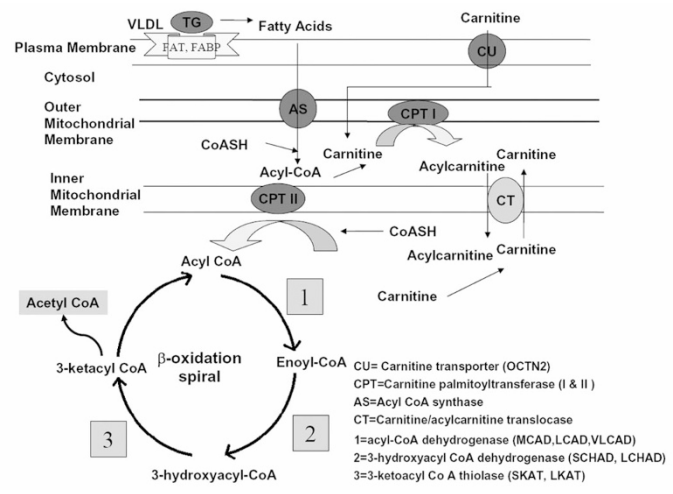


**Figure 1.** The mitochondrial fatty acid β-oxidation pathway. This schematic shows the various enzymes involved in cellular uptake of fatty acids and carnitine, followed by their transport into the mitochondria and subsequent β-oxidation. *Reproduced with permission from Am J Physiol Endocrinol Metab 284:E1098–E1105. Copyright © 2003 the American Physiological Society.*

Unsaturated fatty acids with *cis* double bonds are also degraded by mitochondrial β-oxidation and require two auxiliary enzymes such as enoyl-CoA isomerase and dienoyl-CoA reductase (13). Our current knowledge of cellular uptake mechanisms, intracellular trafficking, degradation, and utilization of long-chain fatty acids is incomplete. The fact that there are many patients with clinical presentations indicative of a FAOD in whom FAO enzymatic activity analyses and molecular studies of all known enzymes fail to reveal any abnormalities, suggests that additional enzymes remain undiscovered. The opposite situation of known or newly detected fatty acyl-CoA dehydrogenases with yet-to-be-defined clinical deficiency states further underscores the current lack of understanding of this complex metabolic pathway (14,15).

## THE MITOCHONDRIAL FATTY ACID β-OXIDATION DISORDERS

More than 20 defects in fatty acid transport and mitochondrial β-oxidation are known and all are inherited as autosomal recessive disorders (1). The genes encoding most of the known enzymes are known and mutations have been discovered in affected patients. The clinical presentations of FAOD vary from a neonatal onset with severe metabolic acidosis, hypoglycemia associated with absent or inadequate ketone production, hyperammonemia, cardiomyopathy, liver failure, and sudden death, to a late onset with episodic myopathy, neuropathy, and retinopathy. FAOD become apparent during periods of increased energy demands, such as prolonged fasting, febrile illness, or any other stressful situation during which the inability to use fatty acids causes metabolic decompensation. FAOD have been implicated as the cause of death in 5–8% cases of sudden unexpected death in infancy based on metabolic studies

of surviving siblings, "near-miss" SIDS cases, and postmortem studies (16).

The genetic defects and clinical and biochemical features of major FAOD are summarized in Table 1. Incidences of individual FAOD may vary from 1:8000 to 1:100,000; but, as a group, FAOD represent the most common metabolic disorder with severe consequences for affected individuals. More detailed descriptions of individual FAOD are available in recently published reviews and book chapters (1–3,17,18).

## PREECLAMPSIA, AFLP, AND HELLP SYNDROME

Preeclampsia is characterized by pregnancy-induced hypertension, edema, and proteinuria and occurs in up to 5–8% of all pregnancies (19). In a small percentage of cases, preeclampsia progresses to severe eclampsia with marked hypertension, encephalopathy, and seizures. Severe preeclampsia in some cases is associated with HELLP syndrome, with an incidence of 1–6 cases per 1000 deliveries (20,21). AFLP is another severe condition of pregnancy with a prevalence ranging from 1 in 10,000 to 1 in 15,000 pregnancies and carries a high mortality (21). Preeclampsia, HELLP syndrome, and AFLP have been suggested to represent a spectrum of the same pathologic process (21–24).

HELLP syndrome is characterized by microangiopathic hemolytic anemia, elevated liver enzymes, and thrombocytopenia. Typically, patients with HELLP syndrome present in their third trimester (28–34 wk gestation) with nausea, vomiting, headache, hypertension, proteinuria, and right upper quadrant pain, and disseminated intravascular coagulation in advanced cases (22). Women with HELLP syndrome have a higher incidence of fetal distress and cesarean section and often give birth to a preterm, IUGR infant with lower Apgar scores in about a third of cases (25). AFLP is the rarest of the three maternal pregnancy complications. The potentially fatal clinical presentation of AFLP is similar to HELLP syndrome but jaundice is frequently seen in these patients. Fatty liver has been demonstrated in some cases where a biopsy was performed. In one recent analysis of multiple studies, AFLP was associated with maternal hypoglycemia in nearly 50% of patients, disseminated intravascular coagulopathy (DIC) in 88%, encephalopathy in 38%, and death in 6% (21). AFLP led to a high perinatal mortality (15%) and 70% of infants were born preterm (26,27). AFLP and HELLP have a similar clinical presentation with elevated liver enzymes and ultrasound findings of increased echogenicity, and only histologic investigation of a liver biopsy allows an unequivocal diagnosis.

## ASSOCIATION OF FETAL FAOD AND MATERNAL PREECLAMPSIA, AFLP AND HELLP SYNDROME

During pregnancy, increased activity of hormone-sensitive lipase in combination with gestational insulin resistance causes an increase in the levels of FFA in maternal blood. The maternal liver responds to these metabolic changes by synthesizing triglycerides, which are secreted as VLDL and LDL that are taken up by placenta. In the last trimester, greater metabolic demands of the fetus shift maternal metabolism toward ketogenesis and the fetus uses maternal ketone bodies for lipogenesis as well as for energy production. Therefore, defects in FAO in the fetoplacental unit become clinically evident at this stage of gestation (28). These associations of placental complications and poor fetal and neonatal outcomes have been described with defects in fatty acid transport across mitochondrial membranes and in enzymes involved in mitochondrial FAO (Fig. 2).

Genetic defects in FAO within the mitochondria have been shown to be associated with maternal, placental and fetal complications. The first report of this association was published by Wilcken *et al.* (29) in 1993, in which 11 pregnancies in five mothers resulted in six babies with LCHAD deficiency. Each of these pregnancies was complicated by maternal fatty liver, HELLP syndrome, and preterm delivery. Wilcken and colleagues based their diagnosis of LCHAD deficiency on 3-hydroxydicarboxylic aciduria. In 1995, Sims *et al.* (30) first defined a molecular basis of this association in three families using DNA analysis. In another large case series, Strauss and co-workers (31) found that 62% of mothers carrying an affected fetus with LCHAD deficiency developed AFLP or the HELLP syndrome during their pregnancies. Since that time, similar associations have been described for defects in other enzymes in the FAO pathway. In isolated LCHAD (24,30–34) or general trifunctional protein (TFP) (24,31,32,35) deficiency, maternal liver disease is common, occurring in approximately 20–70% of affected pregnancies. MCAD (36), short-chain acyl CoA dehydrogenase (SCAD) (37,38), and CPT-1 (39) deficiencies have been associated with maternal liver disease in single case reports.

## ROLE OF FAO IN THE PLACENTA AND FETUS DURING GESTATION

Although studies in humans with genetic defects in FAO have steadily generated evidence for an essential role of fatty acid oxidation in the fetoplacental unit, recent basic science work has demonstrated that human placenta indeed, expresses six key enzymes of the $\beta$-oxidation pathway. Crude human placental extracts showed high activity of these enzymes, comparable to skeletal muscle; and isolated placental trophoblast cells are able to use labeled long-chain fatty acids, palmitate, and myristate in substantial quantities (40–42). As shown in Figure 2, when a heterozygous mother is pregnant with an affected fetus, the placenta and the fetus are unable to optimally oxidize fatty acids, potentially leading to transfer of metabolic intermediates to the maternal circulation. These compounds have been postulated to cause maternal preeclampsia, HELLP syndrome, and AFLP. In a recent study of 33 preeclamptic mothers, maternal plasma had significantly higher long and very-long chain acylcarnitines compared with controls (43). Although the etiology of preeclampsia remains unknown, this indirect evidence suggests that perturbation of mitochondrial FAO may be partially responsible for this condition.

Several other elegant animal studies have produced unequivocal data demonstrating that fatty acid metabolism is critical for placental function and fetal development. Ablation of genes encoding enzymes involved in FAO such LCAD, VLCAD, and

**Table 1.** *Mitochondrial fatty acid oxidation disorders—clinical and biochemical features*

| Enzyme deficiency | Gene | Clinical phenotype | Laboratory findings |
|---|---|---|---|
| Carnitine transporter | OCTN2 | Cardiomyopathy, skeletal myopathy, liver disease, sudden death, endocardial fibroelastosis, prenatal and NB screening diagnosis reported | ↓ Total and free carnitine, normal acylcarnitines, acylglycine, and organic acids |
| Long-chain fatty acid transporter | FATP1-6 | Rare, acute liver failure in childhood requiring liver transplantation | Reduced intracellular $C_{14}$–$C_{18}$ fatty acids, reduced fatty acid oxidation |
| Carnitine palmitoyl transferase-I | CPT-I | Liver failure, skeletal myopathy, renal tubulopathy and sudden death. Prenatal and NB screening diagnosis reported, maternal preeclampsia, HELLP syndrome association described in a few patients. | Normal or ↑ free carnitine, normal acylcarnitines, acylglycine, and organic acids |
| Carnitine translocase | CACT | Chronic progressive liver failure, persistent ↑ $NH_3$, hypertrophic cardiomyopathy | Normal or ↓ free carnitine, abnormal acylcarnitine profile |
| Carnitine palmitoyl transferase-II | CPT-II | Early and late onset types. Liver failure, encephalopathy, cardiomyopathy, renal cystic changes, NB screening diagnosis reported. | Normal or ↓ free carnitine, abnormal acylcarnitine profile |
| Short-chain acyl CoA dehydrogenase | SCAD | Benign to a severe presentation, to include encephalopathic disease to progressive myopathy. NB screening diagnosis possible, maternal preeclampsia, HELLP syndrome association described in a few patients. | Normal or ↓ free carnitine, elevated urine ethylmalonic acid, inconsistently abnormal acylcarnitine profile |
| Medium-chain acyl CoA dehydrogenase | MCAD | Hypoglycemia, hepatic encephalopathy, sudden death. NB screening diagnosis possible, maternal preeclampsia, HELLP syndrome association described rarely. | Normal or ↓ free carnitine, ↑ plasma acylglycine, plasma $C_6$–$C_{10}$ free fatty acids, ↑ $C_8$–$C_{10}$ acyl-carnitine |
| Very long-chain acyl CoA dehydrogenase | VLCAD | Dilated cardiomyopathy, arrhythmias, hypoglycemia, and hepatic steatosis. Late-onset, stress-induced rhabdomyolysis, episodic myopathy. Prenatal and NB screening diagnosis possible. | Normal or ↓ free carnitine, ↑ plasma $C_{14:1}$, $C_{14}$ acylcarnitine, ↑ plasma C10–$C_{16}$ free fatty acids |
| ETF dehydrogenase* | ETF-DH | Nonketotic fasting hypoglycemia, congenital anomalies, milder forms of liver disease, cardiomyopathy, and skeletal myopathy | Normal or ↓ free carnitine, increased ratio of acyl to free carnitine, ↑ acyl-carnitine, urine organic acid and acylglycines |
| Electron transport flavoprotein-$\alpha$* | $\alpha$-ETF | Nonketotic fasting hypoglycemia, congenital anomalies, liver disease, cardiomyopathy and skeletal myopathy also described | Normal or ↓ free carnitine, increased ratio of acyl to free carnitine, ↑ acyl-carnitine, urine organic acid and acylglycines |
| Electron transport flavoprotein-$\beta$* | $\beta$-ETF | Fasting hypoglycemia, congenital anomalies, liver disease, cardiomyopathy and skeletal myopathy also described | Normal or ↓ free carnitine, increased ratio of acyl to free carnitine, ↑ acyl-carnitine, urine organic acid and acylglycines |
| Short-chain L-3-hydroxyacyl CoA dehydrogenase | SCHAD | Hypoglycemia, hyperinsulinemia, cardiomyopathy, myopathy. NB screening diagnosis possible | Normal or ↓ free carnitine, elevated free fatty acids, inconsistently abnormal urine organic acid and plasma acylcarnitines |
| Long-chain L-3-hydroxyacyl CoA dehydrogenase | LCHAD | NB screening diagnosis possible, maternal preeclampsia, HELLP syndrome and AFLP association described frequently | Normal or ↓ free carnitine, increased ratio of acyl to free carnitine, ↑ free fatty acids, ↑ $C_{16}$-OH and $C_{18}$-OH carnitines |
| Mitochondrial trifunctional protein | MTP | Severe cardiac and skeletal myopathy, hypoglycemia, acidosis, hyper $NH_3$, sudden death, elevated liver enzymes, retinopathy. Maternal preeclampsia, HELLP syndrome, and AFLP association described frequently. | Normal or ↓ free carnitine, increased ratio of acyl to free carnitine, ↑ free fatty acids, ↑ $C_{16}$-OH and $C_{18}$-OH carnitines |
| Long-chain 3-ketoacyl-CoA thiolase | LKAT | Severe neonatal presentation, hypoglycemia, acidosis, ↑ creatine kinase, cardiomyopathy, neuropathy and early death. Late onset presents with myopathy. Maternal preeclampsia, HELLP syndrome association frequent. | Normal or ↓ free carnitine, increased ratio of acyl to free carnitine, ↑ free fatty acids, [↑ 2-trans, 4-cis decadienoylcarnitine |
| 2,4-Dienoyl-CoA reductase | DECR1 | Only one patient described, hypotonia in the newborn, mainly severe skeletal myopathy, and respiratory failure. Hypoglycemia rare. | Normal or ↓ free carnitine, ↑ acyl to free carnitine ratio, normal urine organic acids and acyl-glycines |
| HMG-CoA synthetase | HMGCS2 | Hypoketosis and hypoglycemia, rarely myopathy | Elevated total plasma fatty acids, enzymes studies in fibroblasts may be diagnostic |
| HMG-CoA lyase | HMGCL | Hypoketosis and hypoglycemia and rarely myopathy | Normal free carnitine, ↑ $C_5$-OH, and methylglutaryl-carnitine, enzymes studies in fibroblasts may be diagnostic |

* Also known as glutaric acidemia type II.

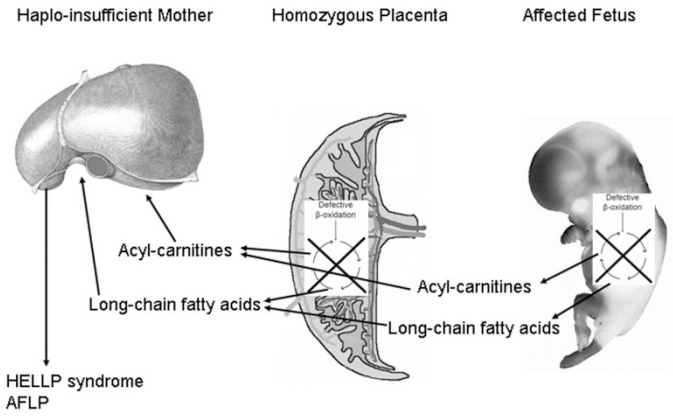


**Figure 2.** Schematic depicting the transfer of long-chain fatty acids and acylcarnitines from the affected fetus and placenta to the haplo-insufficient mother. This mechanism has been postulated to cause liver disease during pregnancy, which includes HELLP syndrome and AFLP.

TFP is associated with increased *in utero* fetal demise, reduced fertility, and fetal growth restriction (44–46). Similarly, ablation of the genes coding for the transcription factors peroxisome proliferator-activated receptors $\beta/\delta$ and $\gamma$ (PPAR$\beta/\delta$ and PPAR$\gamma$) and the co-activator of PPAR$\gamma$ (PGC-1) that are the master regulators of fatty acid metabolism, results in embryonic lethality. More importantly, inactivation of these transcription factors leads to specific placental phenotypes in which the syncytiotrophoblast, the functional unit of the placenta, fails to develop and sustain pregnancy (47–49). Ablation of the gene encoding fatty acid synthase also leads to fetal demise (50). Thus, fatty acids play a critical role in placental development and function and in fetal well being.

## LABORATORY INVESTIGATION OF FAOD

The biochemical features of FAOD commonly seen in clinical situations include hypoglycemia without evidence of appropriate ketone body production, lactic acidosis, and elevated liver enzymes like AST and ALT. Along with clinical features listed earlier, these laboratory abnormalities should prompt immediate work up for a FAOD, including at the least urine organic acids, and plasma or blood acylcarnitine analysis. Additional biochemical investigations, such as urine acylglycine and plasma FFA profiling, as well as determination of total fatty acids, and free and total carnitine in plasma should also be considered (1–3). Measurement of free and total carnitine in urine is only recommended in a patient at risk of primary carnitine uptake defect and only before L-carnitine supplementation. Samples for analysis should preferably be procured early during the acute manifestation because successful reversal of catabolism may normalize the biochemical phenotype of several FAOD. If the patient is doing well, samples should be collected before a meal. Fasting tests, which are potentially life threatening, can be avoided in most patients and should only be conducted in controlled settings and with i.v. access. Biochemical investigations during a metabolic crisis are usually suggestive, if not diagnostic, of a particular FAOD, and allow for initiation of appropriate treatment.

Definitive diagnosis of individual FAOD is accomplished by measurement of individual enzyme activities and FAO substrate utilization rates in fibroblasts derived from a small skin biopsy. Molecular genetic analysis of genomic DNA can often define the exact defect and is particularly helpful when an FAOD is caused by an enzyme not expressed in fibroblasts. Once a FAOD has been diagnosed, the above mentioned analyses can aid in determination of treatment efficacy. For example, fatty acid profiling can be used to avoid nutritional deficiencies of essential fatty acids by overt dietary fat restriction (51,52).

The metabolic work-up of patients who suffered a sudden unexpected death ("metabolic autopsy") should include the investigation for a FAOD because they have been identified as the underlying cause of death in 5–8% of cases (53). This is facilitated by acylcarnitine analysis that can be performed most efficiently on small samples of blood and bile collected postmortem and dried on filter paper (Table 2) (54).

To identify and initiate treatment of patients with an FAOD before the development of symptoms, acylcarnitine analysis by MS/MS can also be performed on newborn screening blood spots. The ongoing introduction of MS/MS into newborn screening laboratories since the early 1990s has markedly increased the number of diagnosed and successfully treated patients with a FAOD (55).

Once a diagnosis has been made, family investigations and genetic counseling should be pursued. Prenatal diagnosis of most FAOD is possible by molecular genetic, enzyme, or metabolite analyses using chorionic villi, amniotic fluid, or amniocyte cultures (56). Although FAOD are associated with high morbidity and mortality, it must be remembered that some FAOD, like MCAD deficiency, which have an excellent prog-

**Table 2.** *Summary of studies of the occurrence of FAOD in cases of sudden unexpected death*

| Study (reference) | No. | Sample | Comments |
|---|---|---|---|
| Boles *et al.* (16) Maryland and Connecticut | 418 SIDS | Retrospective study of SIDS (313), accident or abuse (34), 45 cases with infections | All cases of accident or abuse tested negative, 14 profiles were diagnostic for FAOD or OA (4.5%). FAOD diagnosed were MCAD, VLCAD, LCHAD, and carnitine uptake defect |
| Chace *et al.* (77) US and Canada | 7058 | Retrospective study on filer paper blood spots from infants who died due to unknown cause | 66 specimens (0.93%) tested positive, 34.8% cases had MCAD deficiency, 13.6% had VLCAD, 9% had CPT-II and CACT and 6% LCHAD deficiency |
| Wilcox *et al.* (78) Oregon | 247 SUDI | Retrospective postmortem study on newborn screening Guthrie card of SUDI cases over 5 y | MS/MS analysis found that 1.2% cases of SUDI were due to FAOD |

SUDI, sudden unexpected death in infancy.

**Table 3.** *Summary of reports of prospective newborn screening using MS/MS*

| Study (reference) | N | Sample | Comments |
|---|---|---|---|
| Millington and Koeberl 69<br>North Carolina | 237,774 | Summary of 2-year experience of prospective newborn screening between 04/1999–04/2001 | Overall incidence of inborn errors of metabolism detected by MS/MS was 1 in 4,400 newborns with MCAD deficiency being the most common (1:13,600) |
| Wilcken et al 55<br>NSW, Australia | 362,000 | Prospective newborn screening study between 04/1998 and 03/2002 | Rates of 31 disorders compared to 16 previous years. Rate of diagnosis of MCAD and other FAOD increased significantly. Cost of each screen was $0.70, cost of confirmatory test was $217 & cost of detecting one case $2519. |
| Hoffman et al 70<br>Southern Germany | 382,247 | Prospective newborn screening 1999–2000, compared to high-risk screening of symptomatic patients | Overall frequency of FAOD & OA was 1:8000 using MS/MS, 10 FAOD & OA more common than PKU. MS/MS technique three times more sensitive in diagnosis. MCAD accounted for 63% cases, other FAOD for 8.7% cases. |
| Insinga et al 74<br>Wisconsin | — | Estimates study using historical data | Wisconsin state screens for 14 diseases by MS/MS. Adding MCAD diagnosis alone makes this screen cost effective. Cost per assay was $3.99 per sample with a cost-effectiveness ratio of $41,862/quality of life year gained. |
| Shigematsu et al 79<br>Japan | 102,200 | Prospective newborn screening between 04/1997 and 07/2001, compared to high-risk screening of symptomatic patients | Pilot newborn screening study of MS/MS comparing it to a selective screening study. Positive screen found in 1:8527 patients, overall recall rate was 0.58% and a false positive case rate of 0.39%. |
| Andresen et al 80<br>Pennsylvania, Ohio, Florida, North Carolina | 930,078 | Prospective study determining incidence and genotype of MCAD deficient patients detected by a private newborn screening laboratory (NeoGen Screening, Inc., Bridgeville, PA) | MCAD deficiency found in 1/15,001 samples screened, positive samples confirmed by mutation analysis. |
| Carpenter et al 81<br>Australia | 275,653 | Prospective newborn screening for MCAD and analysis of clinically diagnosed patients | Most patients with symptomatic MCAD deficiency could be detected by newborn screening. 90% patients with MCAD deficiency were homozygous for the common AG985 mutation with a carrier frequency of 1:86. |
| Zytkovicz et al 82<br>New England | 164,000 | Summary of 2-year experience of prospective newborn screening between 1999 and 2001 by a regional laboratory. | 22 infants with AA disorders, 20 infants with FAOD were detected. 3% of samples were flagged as abnormal. Half of abnormal flagged infants required neonatal intensive care or had low birth weight. |

NB = newborn.

nosis when treatment consisting of avoidance of fasting is initiated at birth, are questionable candidates for invasive prenatal diagnostic procedures or pregnancy termination.

## MS/MS

Mass spectrometry was first introduced in clinical medicine in the 1960s (57) to diagnose organic acidemia by gas chromatography-mass spectrometry (GC-MS). In the 1980s, MS/MS was applied to the diagnosis of inborn errors of metabolism by acylcarnitine analysis (58). Due to the development of affordable, computer-driven, and relatively user-friendly instruments, MS/MS as a highly sensitive and versatile analytical technology is becoming an integral part of biochemical genetics, clinical chemistry, and newborn screening laboratories, as attested by several excellent reviews published in recent years (59–62). This analytical technique measures the weight of ions derived from a neutral compound after ionization. Most instruments currently used in clinical laboratories consist of two triple-quadrupole mass spectrometers in series separated by a collision cell. The liquid samples are introduced into the first MS after ionization that occurs in the ion source. The quadrupoles consist of four rods that separate the ions by their mass-to-charge ratio (m/z). When passing through the collision cell, the ions are fragmented by collision with an inert gas (*i.e.* nitrogen) and the resulting ion fragments' weights are determined by the second MS. Analysis, data acquisition, and generation of a mass spectrum are completed in minutes. Due to the unique fragmentation pattern of molecules, only limited sample clean-up and preparations are typically necessary (61).

## MS/MS IN NEWBORN SCREENING FOR FAOD

Due to the high sensitivity of MS/MS instruments and the availability of isotopically labeled internal standards, researchers at Duke University Medical Center (Durham, NC) in the early 1990s began to study the possibility to use MS/MS for the analysis of acylcarnitines and amino acids in dried blood spots collected for newborn screening. After a short sample preparation, which consists of the extraction of analytes (acylcar-

nitines and amino acids) from small (typically ⅛–3⁄16 in) dried blood spot discs punched from the screening card, the addition of isotopically labeled internal standards, and esterification with butanol-HCl, Chace and colleagues (63) first demonstrated the feasibility of MS/MS-based screening for phenylketonuria. The first FAOD that was demonstrated to be detectable by MS/MS in newborn screening blood spots was MCAD deficiency (64). Newborn screening for MCAD deficiency by MS/MS is now included in 34 states in the United States (http://genes-r-us.uthscsa.edu/) and many other parts of the developed world (65). Twenty-eight of these 34 states also mandate the inclusion of a variable number of additional FAOD, organic acidemias, and amino acidemias into their panels because MS/MS analysis allows the simultaneous detection of at least 30 different inborn errors fatty acid and amino acid metabolism in the same sample (http://genes-r-us.uthscsa.edu/). Table 3 summarizes recent reports about individual programs' experiences with MS/MS. MCAD deficiency is the most frequently detected FAOD by these programs that screen primarily white populations. Surprisingly, LCHAD deficiency appears unexpectedly rare among the detected FAOD. The reason for this is not entirely clear as several publications were able to demonstrate pathologic acyl-carnitine profiles when retrospectively analyzing the original newborn screening samples of patients who were diagnosed either during an acute episode or postmortem (66–68). Part of the problem may be the analytical platform represented by MS/MS, which currently does not provide "plug-and-play" characteristics and is fundamentally different to the traditional single-analyte screening assays, in particular the original Guthrie test, a bacterial inhibition assay. MS/MS not only requires experience with this technology but also with the interpretation of metabolite profiles generated for acylcarnitines and amino acids.

The first pilot studies using MS/MS for newborn screening were conducted in North Carolina, and the first report of North Carolina's state newborn screening program's experience included 237,774 infants screened over a 2-y period. The incidence of MCAD was found to be 1 in 13,600 live births, with an overall incidence of inborn errors of fatty acid and amino acid metabolism detected by MS/MS of 1 in 4,400 (69). The newborn screening program of New South Wales, Australia, reported their findings in 362,000 newborns over a 4-y period. Analysis of data showed that rate of detection of MCAD and other FAOD increased significantly compared with the time before expanded newborn screening (55). MS/MS screening of 382,247 subjects from Bavaria, Germany, compared with 844,575 subjects among whom diagnosis or ascertainment was done after symptomatic presentation of FAO showed a much higher yield of diagnosis when MS/MS technology was used (70).

## LOGISTICAL, FINANCIAL, AND ETHICAL CONSIDERATIONS FOR THE INCLUSION OF FAOD IN NEWBORN SCREENING PROGRAMS

Screening of newborn infants for genetic diseases has been in existence for 40 y with the objective of identifying effectively treatable disorders in the presymptomatic stage to avoid long-term morbidity and mortality by early initiation of treatment (71). Selection of the disorders to be screened has been at the discretion of each state. However, with the advent of MS/MS technology and its ability to identify more than 30 different inborn errors of metabolism simultaneously in a small blood spot sample, the discrepancies between states become more pronounced. The US Department of Health and Human Services has therefore established the Secretary's Advisory Committee on Heritable Disorders and Genetic Diseases in Newborns and Children. This committee has recently submitted their recommendations for a universal newborn screening panel listing 30 disorders (http://www.modimes.org/professionals/681_1200.asp).

Newborn screening by MS/MS by individual state screening programs involves the purchase and maintenance of the necessary equipment, training of personnel, and establishment of a system of follow-up by experts for diagnosed or suspected patients. Although this suggests increased costs to states' health-care systems, a recent study showed that the incremental cost of MS/MS screening over and above traditional newborn screening assays used in state laboratories is only about $0.70 per sample (55). Moreover, thorough cost-benefit analysis studies demonstrate definite benefits of early diagnosis by avoidance of long-term care costs (55,72–74) for FAOD patients who suffer from neurologic sequelae of their disease manifestation. Newborn screening clearly prevents death and disability in most patients. A recent study from Boston has reviewed this aspect of screening and found that proactive newborn screening identified significantly more disorders and reduced the rate of mental retardation and parental anxiety among screened population (75).

Expanded MS/MS-based newborn screening, despite the modest extra costs involved and some limitations, has revolutionized our ability to diagnose FAOD disorders early in life. The benefits to society of expanded newborn screening are evident, and this screening should be adopted as a standard of care. The often-voiced concern about a lack of specialists available for follow-up of patients identified by newborn screening seems unwarranted. However, the benefits of newborn screening for the individuals, their families, and society can only be achieved in a newborn screening system characterized by effective communication between the public health department that must provide the framework, the screening laboratories that must provide reliable results, the primary care providers, and the metabolic specialists (76). At the same time, there is an urgent need to impart extensive education to health-care professionals about these disorders hitherto considered rare, their outcomes, and the appropriate follow-up and preventive management. Expanded newborn screening with MS/MS will bring new challenges to our health-care systems, and there is need for open communication between all involved in the screening process to formulate a rational approach to FAOD diagnosis and management.

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