Medium Chain Acyl CoA Dehydrogenase Deficiency

**(MCAD)**

#### Learning Objectives

* Understand the mechanism of oxidation of fatty acids, specially medium chain fatty acids
* Understand the significance of alpha-fetoprotein in maternal plasma as diagnostic tool of fetal development.
* Learn about methods for testing this deficiency in utero or following birth.
* Understand how mutations of critical amino acids can affect enzymatic activity
* Understand the relationship between MCAD and SIDS (sudden infant death syndrome)
* Understand the role of diet and ketone bodies in ameliorating MACD deficiency

# Resources

Harper’s Illustrated Biochemistry. pp180-189

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Amendt, B. A. and Rhead, W. (1985). Journal of Clinical Investigation. Vol. 76, pp. 963-969

MCAD deficiency is the most common genetic disorder of mitochondrial beta-oxidation of fatty acids in humans. This deficiency is as common as PKU affecting ~ 1 in 10,000 live births. Undiagnosed, MCAD deficiency has mortality rate of 20% and 10-15% are left severely handicapped. Early treatment is cheap, simple, and effective.

1. A boy, the fifth child of first cousins, presented at 6 days with lethargy, poor feeding and hypoglycemia. At age two months, he developed hypertrophy cardiomyopathy, hepatomegaly and severe hypotonia (loss of muscle tonicity). He had a sibling who had died of cardiomyopathy at an early age. The boy’s three distance cousins had also died at an early age from similar symptoms. The karyotype in this patient was normal, 46XY. Since one of the siblings had died at an early age, pregnancy was monitored carefully and showed elevated amounts of alpha-fetoprotein in maternal plasma and in amniotic fluid. Acyl carnitine profile in amniocytes as determined by tandem mass spectrometry was abnormal. The deficiency was diagnosed as MCAD. The parents were counseled as to the prognosis and possible outcomes of treatments and decided to go through pregnancy. Following birth, plasma acyl carnitines levels and fatty acid composition of plasma and urine were monitored. Plasma showed high of octanoyl carnitine and plasma and urine showed high levels of dicarboxylic fatty acids.

* 1. Explain the mechanism of fatty acid oxidation.
  2. What is the rationale for elevated level of alpha-fetoprotein?
  3. What is the significance of carnitine in fatty acid oxidation and why is acyl carnitine metabolites elevated in plasma?
  4. Are dicarboxylic acids are commonly present in plasma? If not, why is the concentration of dicarboxylic acids elevated.
  5. How might the possible clinical abnormality of MCAD explain SIDS?

1. Sonicated skin fibroblasts of this patient were monitored for activity of MCAD using 100 uM [3H 2,3] Octanoyl CoA as substrate (Km for Octanoyl CoA 10 uM). The fibroblasts from this patient showed 50% of the activity compared to controls. However, when the activity was measured in the presence of added FAD, phenazine methosulfate or pure ETF (electron transferring flavoprotein as electron acceptors) MCAD deficient fibroblasts showed only 5% of the control activity.
   1. What is the advantage of using [3H 2,3] octanoyl CoA as a substrate and how is this activity measured?
   2. What is the significance of adding external electron acceptors in the assay?
   3. What is the significance of these data in describing possible mutation in MCAD?
2. As a child, the boy was put on fat restricted diet along with glycine and L-carnitine and flavin and developed relatively normally. However, at age two, he started to have difficulty walking. Two weeks later, he could not walk or talk, was bedridden and had spastic quadriplegia with complete loss of the use of all four limbs. The patient was started on sodium-DL-beta-hydroxybutyrate at 900-mg/Kg weight. There was gradual clinical recovery to a point where after 16 months treatment, the boy could crawl, talk and ride a tricycle.
   1. What was the rational in putting on the patient on fat restricted diet and what kind of fatty acid may be appropriate in the diet?
   2. Why would beta-hydroxybutyrate in his diet bring about such dramatic change in clinical symptoms?

**Faculty Handout**

1. MCAD is an aberration of fatty acid oxidation (Fig. 1). Although fatty acid oxidation (FAO) is a normal physiological process, the activity of this pathway is greatly increased in a starved state, in diabetes and during periods of strenuous exercise. The process is described as follows:

a. Fatty acids (FA) are mobilized from the adipose tissue according to the process shown in Fig. 2. FAs circulate in plasma bound to albumin and enter the cells (muscle or cardiac etc) when it is necessary to use FA oxidation for energy production. FAs are activated to fattyacyl CoAs either by the enzyme located on ER of cells and/or on the outer membrane of mitochondria (fig. 3). The acyl CoAs are then converted to acyl carnitines in the intermembrane space (see Figs. 4,5). The acyl carnitines are then translocated to the matrix of mitochondria where they are reconverted back to acyl CoAs. The acyls CoAs then undergo beta-oxidation to produce two-carbon compound, acetyl CoA, and FADH2 and NADH (Fig. 6). These products can then be used to produce energy through TCA cycle and oxidative phosphorylation.

The first step in FA beta-oxidation is catalyzed by fattyacyl CoA dehydrogenases with an FAD covalently linked to the enzyme (called prosthetic group). There are three dehydrogenases (long chain for >12 carbon FAs, medium chain for 6-12 carbon FAs and short chain for 4-6 carbon FAs. All three dehydrogenases are required for complete oxidation of fatty acids. This dehydrogenation reaction is directly linked to oxidative phosphorylation via a flavoprotein called ETF (electron transfer flavoprotein) according to the following designation.

Fattyacyl CoA E-FADH2 ET-FMN

Trans Δ2 enoyl CoA E-FAD ET-FMNH2

b. Alpha fetoprotein is fetal counterpart of adult albumin synthesized in the liver. Its function in adult and in fetal development is not known (at least to me). This is an observation, which may be related to fetal liver damage. Its concentration is somewhat elevated in the amniotic fluid and in maternal plasma during abnormal fetal skeletal development e.g. in spina bifida

c. Carnitine is required to transport fatty acids from intermembrane space across inner mitochondrial membrane in the form of acyl carnitines (see Fig. 4,5). If acyl CoAs cannot be oxidized in mitochondria, these are released as acyl carnitines from the cells into plasma.

d. Dicarboxylic acids are not commonly present in plasma. However, if fattyacyl CoAs cannot be oxidized by beta-oxidation because of MCAD deficiency, these acyl CoAs are oxidized by omega oxidation to give alcohol derivatives, which are further, oxidized to acids e.g.

CH3-(CH2)6-COOH ---🡪 HO-CH2-(CH2)6-COOH-🡪 CHO—(CH2)6—COOH

---COOH-(CH2)-COOH

The first step in this sequence is catalyzed by microsomal mixed function oxidase which requires cytochrome P450, O2 and NADPH. The second and third steps are catalyzed by alcohol dehydrogenase and aldehyde dehydrogenase respectively.

e. SIDS, sudden infant death syndrome, is most likely related to deficiencies of one of the fattyacyl CoA dehydrogenases. Body tends to use fatty acid oxidation under hypoglycemic conditions and lack of complete fatty acid oxidation in MCAD deficiency could cause acidosis leading to coma and sudden death.

2.

a. Using [3H]2,3 Octanoyl CoA as substrate, the released products are a proton and a hydride ion which exchange with water. The reaction mixture can be placed on an anion exchange column. The acyl CoA would bind to the column and the tritium released in water is determined by scintillation counting.

b. From the data given it appears that sonicated skin fibroblasts from affected and normal individuals were deficient in natural electron acceptors and therefore activity measurements were not a true reflection of active enzyme. Addition of general electron acceptors or purified ETF resulted in true expression of total activity, which, in case of affected individuals, was only 5% of those from the unaffected individuals.

c. These data suggest that affected individuals have a loss of function of MCAD rather than mutations, which result in poor binding affinity of substrate or loss of function of ETF in affected individuals.

3.

a. The patient was placed on fat restricted diet because of possible mutations and loss of function of MCAD. Fatty acids with C4 and C6 triacylglycerols would be more appropriate because the affected individual would have short chain acyl CoA dehydrogenase activity (SCAD), which would be able to metabolize these fatty acids for energy.

b. Beta-hydroxybutyrate is a ketone body produced in mitochondria which is used by both brain and heart tissues for energy. It is not processed through beta-oxidation but instead is converted to acetoacetate and to acetoacetyl CoA by a reaction with succinyl CoA catalyzed by acetoacetate: succinyl CoA transferase (Fig. 7). Acetoacetyl CoA is then converted by beta-ketothiolase to acetyl CoA that enters TCA cycle and oxidative phosphorylation.