MEDICAL BIOCHEMICAL GENETICS
CLINICAL CORE
SEMINAR SERIES

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University of Colorado
Anschutz Medical Campus

Fatty Acid Oxidation, Carnitine, Ketone disorders

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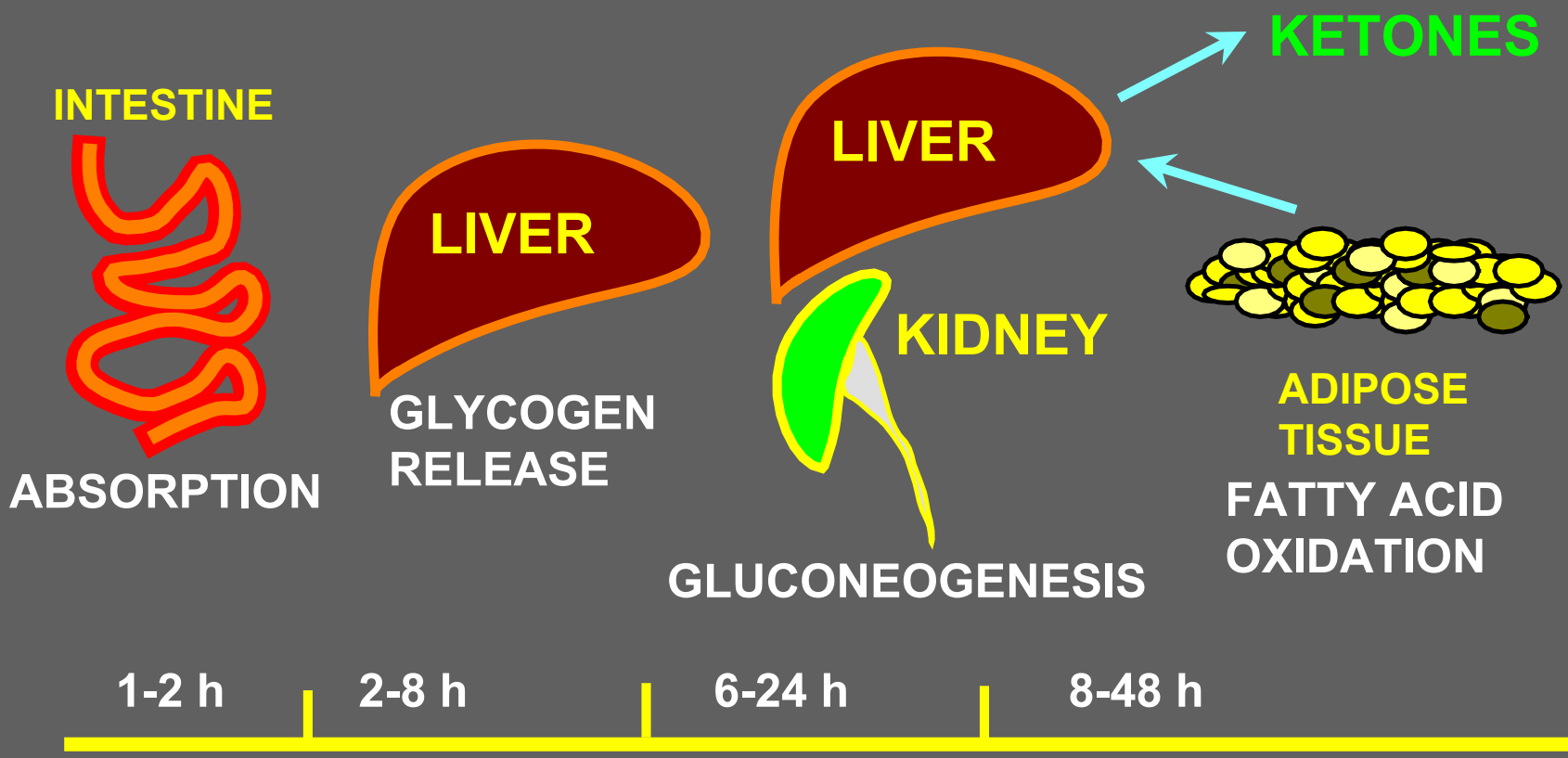
DISORDERS OF FATTY ACID OXIDATION

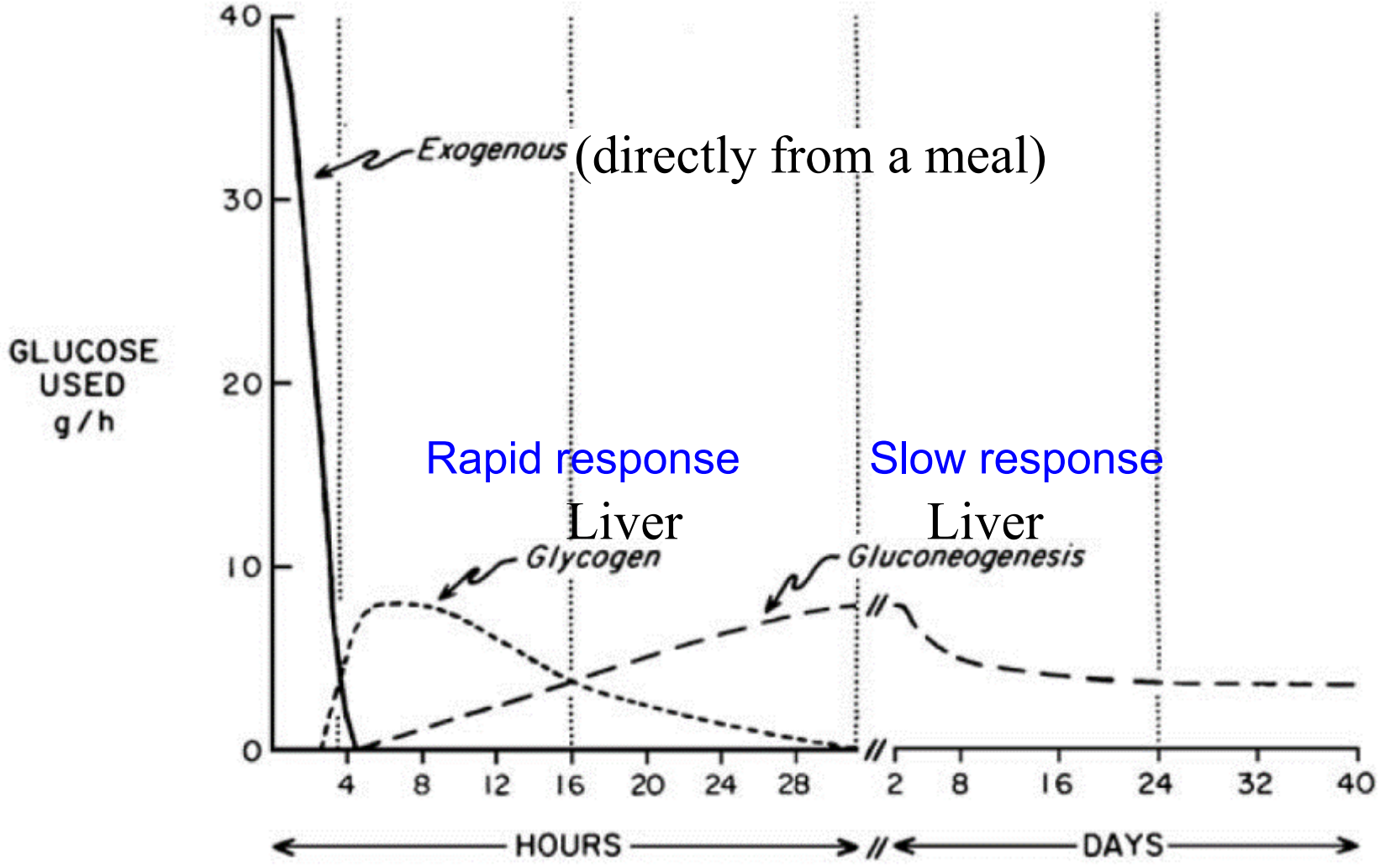
Disorders of fatty acid oxidation

Objectives:
Define role of fatty acid oxidation in fasting
Recognize the role of carnitine in fatty acid oxidation
Define principles of treatment of fatty acid oxidation defects

GLUCOSE HOMEOSTASIS



Phases of blood glucose regulation



Major fuel of the brain:

Glucose

Glucose, Ketone bodies

Ketone bodies Glucose,

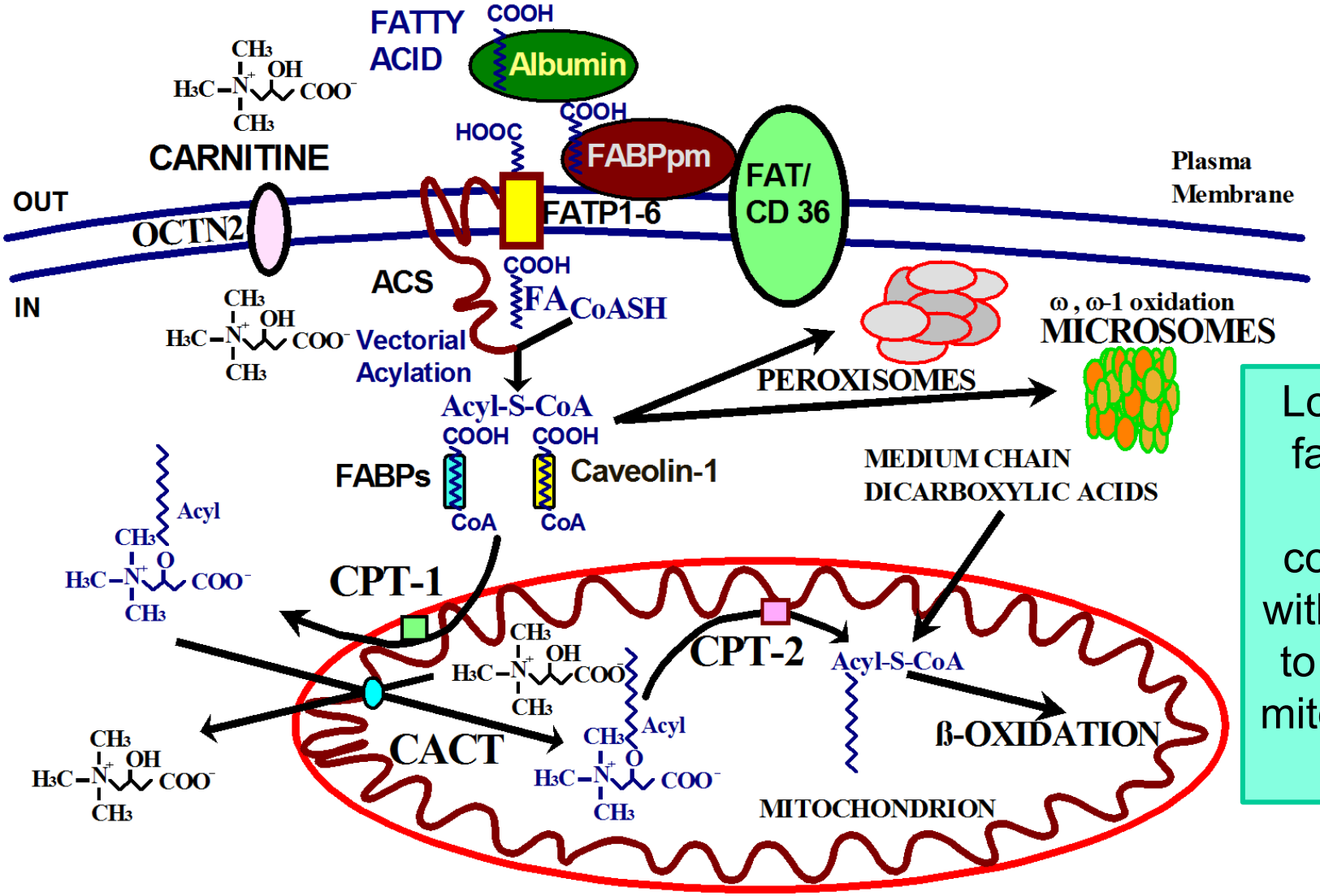
Cahill (2006) Annu. Rev. Nutr. 26:1-22.

Disorders of the carnitine cycle and of fatty acid oxidation

Fatty acid oxidation plays a major role in energy production during fasting. It requires at least 20 individual steps, some of which catalyzed by enzymes with overlapping chain-length specificities.
Carnitine carries fatty acids inside mitochondria and the beta oxidation cycle can extract energy from them.
All known fatty acid oxidation defects are transmitted as autosomal recessive traits.

Longo N, et al. Biochim Biophys Acta. 2016;1863(10):2422-35

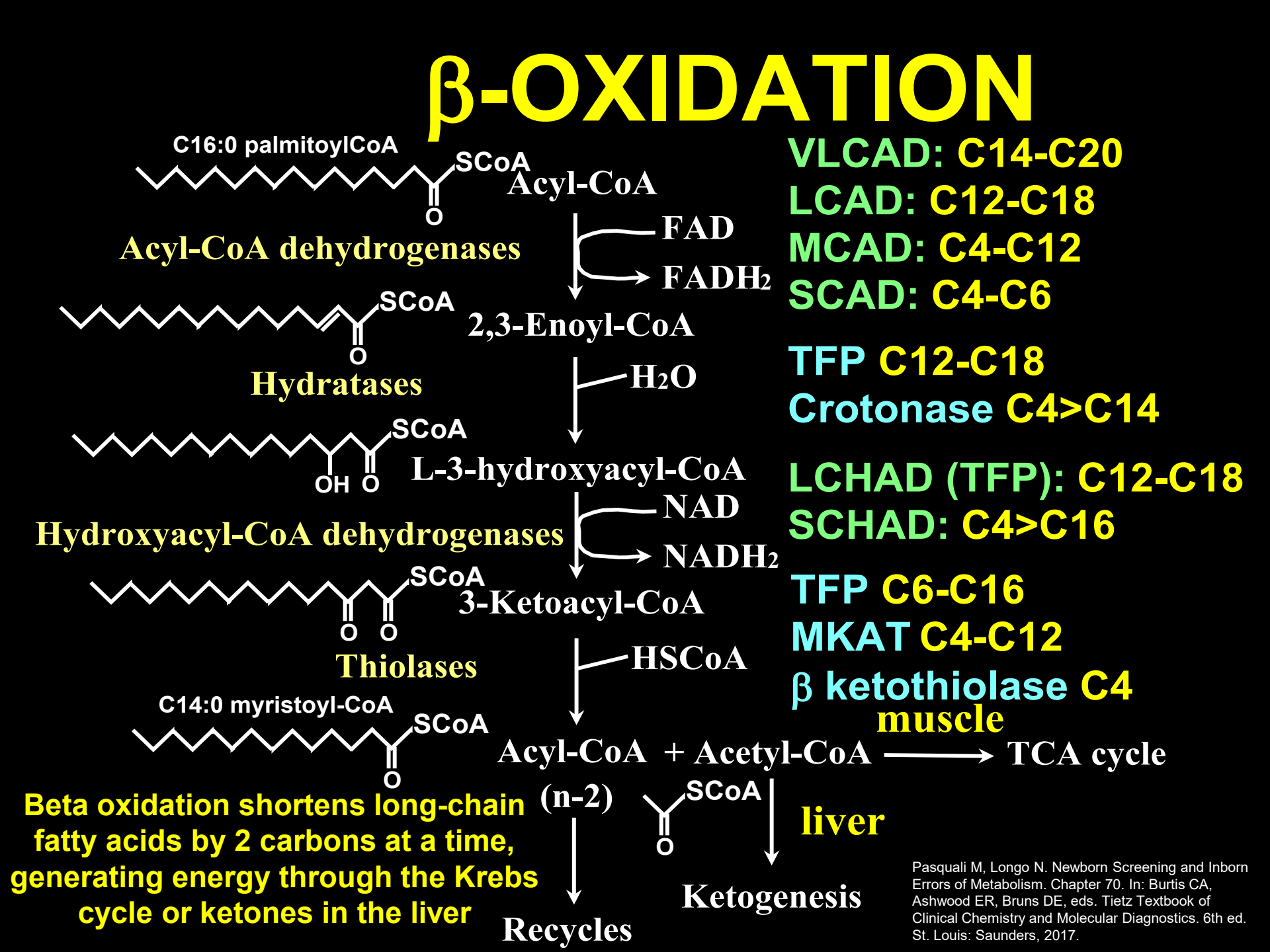
THE CARNITINE CYCLE IN FATTY ACID OXIDATION



Long-chain fatty acids are conjugated with carnitine to enter the mitochondrial matrix

Modified from: Longo N, Amat di San Filippo C, Pasquali M (2006) Disorders of carnitine transport and the carnitine cycle. **Am J Med Genet C Semin Med Genet** 142(2):77-85

β-OXIDATION



VLCAD: C14-C20
LCAD: C12-C18
MCAD: C4-C12
SCAD: C4-C6

TFP C12-C18
Crotonase C4>C14

LCHAD (TFP): C12-C18
SCHAD: C4>C16

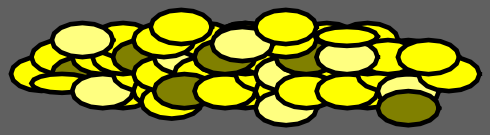
TFP C6-C16
MKAT C4-C12
β ketothiolase C4

Beta oxidation shortens long-chain fatty acids by 2 carbons at a time, generating energy through the Krebs cycle or ketones in the liver

Pasquali M, Longo N. Newborn Screening and Inborn Errors of Metabolism. Chapter 70. In: Burtis CA, Ashwood ER, Bruns DE, eds. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 6th ed. St. Louis: Saunders, 2017.

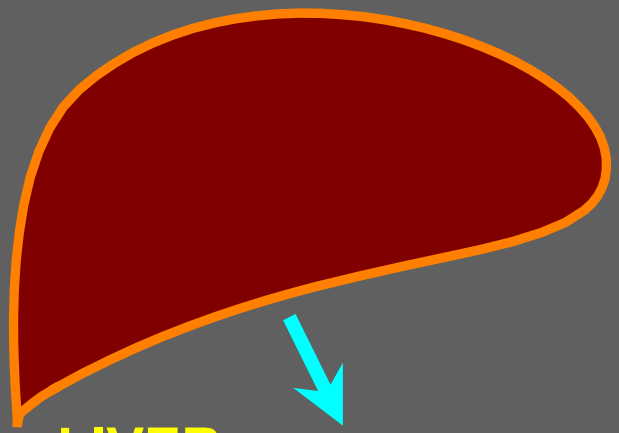
FATTY ACID OXIDATION DURING FASTING

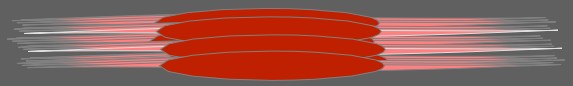
ADIPOSE TISSUE



FATTY ACIDS

HEART





SKELETAL MUSCLE

LIVER

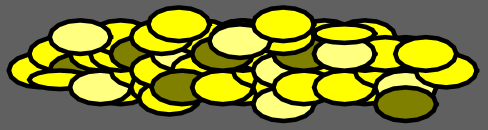
KETONES

ß-hydroxybutyrate
acetoacetate

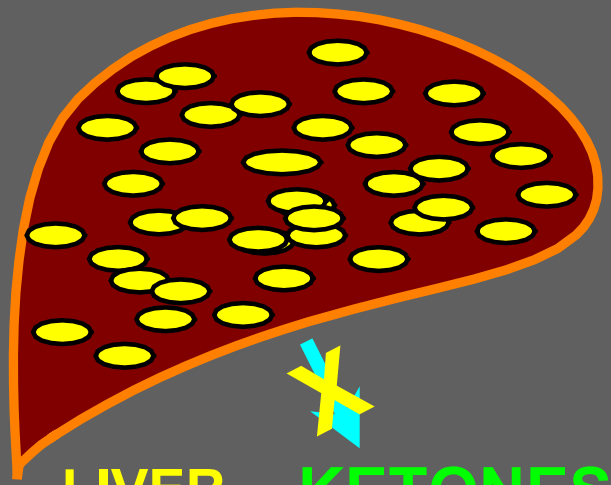
BRAIN

DEFECTIVE FATTY ACID OXIDATION

ADIPOSE TISSUE



FATTY ACIDS



LIVER

STEATOSIS

KETONES

HEART

CARDIOMYOPATHY
ARRHYTHMIA

SKELETAL MUSCLE

MYOPATHY
HYPOTONIA
MYOGLOBINURIA

BRAIN

LOSS OF CONSCIOUSNESS

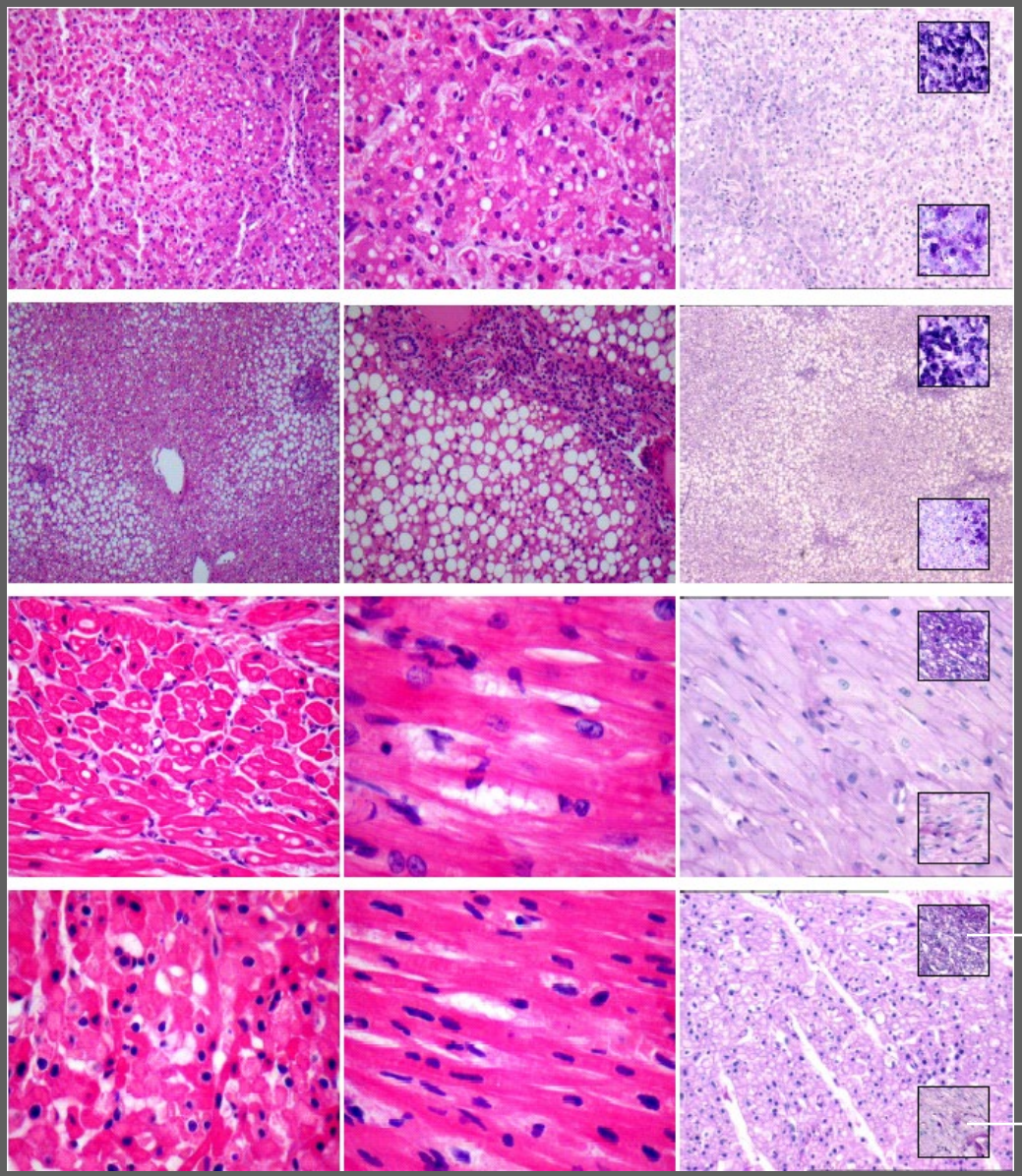
PATHOLOGY IN FATTY ACID OXIDATION DEFECTS

LIVER

Lipid deposition in peripheral areas of lobules

HEART

Focal lipid deposition exp. in subendocardium. Fiber/nuclei size variability (hypertrophy)



Exhaustion of glycogen reserve

control

Control calory-deprived

Hematoxylin-eosin

PAS

Modified from: Melegh et al. (2004) Am J Med Genet A. 2004 Dec 1;131(2):121-6.

TRIGGERS OF FATTY ACID OXIDATION DISORDERS

Most fatty acid oxidation defects are episodic and clinically silent when fat is not utilized.

Triggering conditions include fever, infections, gastroenteritis, reduced caloric intake.

Therefore children present shortly after birth (initiation of breastfeeding) or at any age during an illness causing catabolism.

FATTY ACID OXIDATION DEFECTS

Cause: More than 20 enzymes/transporters are involved in FAO. They are all autosomal recessive

Epidemiology: Most frequent is MCAD deficiency (1:10,000) All others are much rarer (1:30,000-1:1,000,000)

Pathogenesis: Accumulation of fat and toxic metabolites, Lack of energy, Cell death. On autopsy, fat infiltration of all tissues.

Presentation: Fasting-induced hypoketotic hypoglycemia, liver failure, hyperammonemia (Reye syndrome), cardiomyopathy, myopathy, hypotonia, neuropathy, arrhythmia, sudden death, rhabdomyolysis

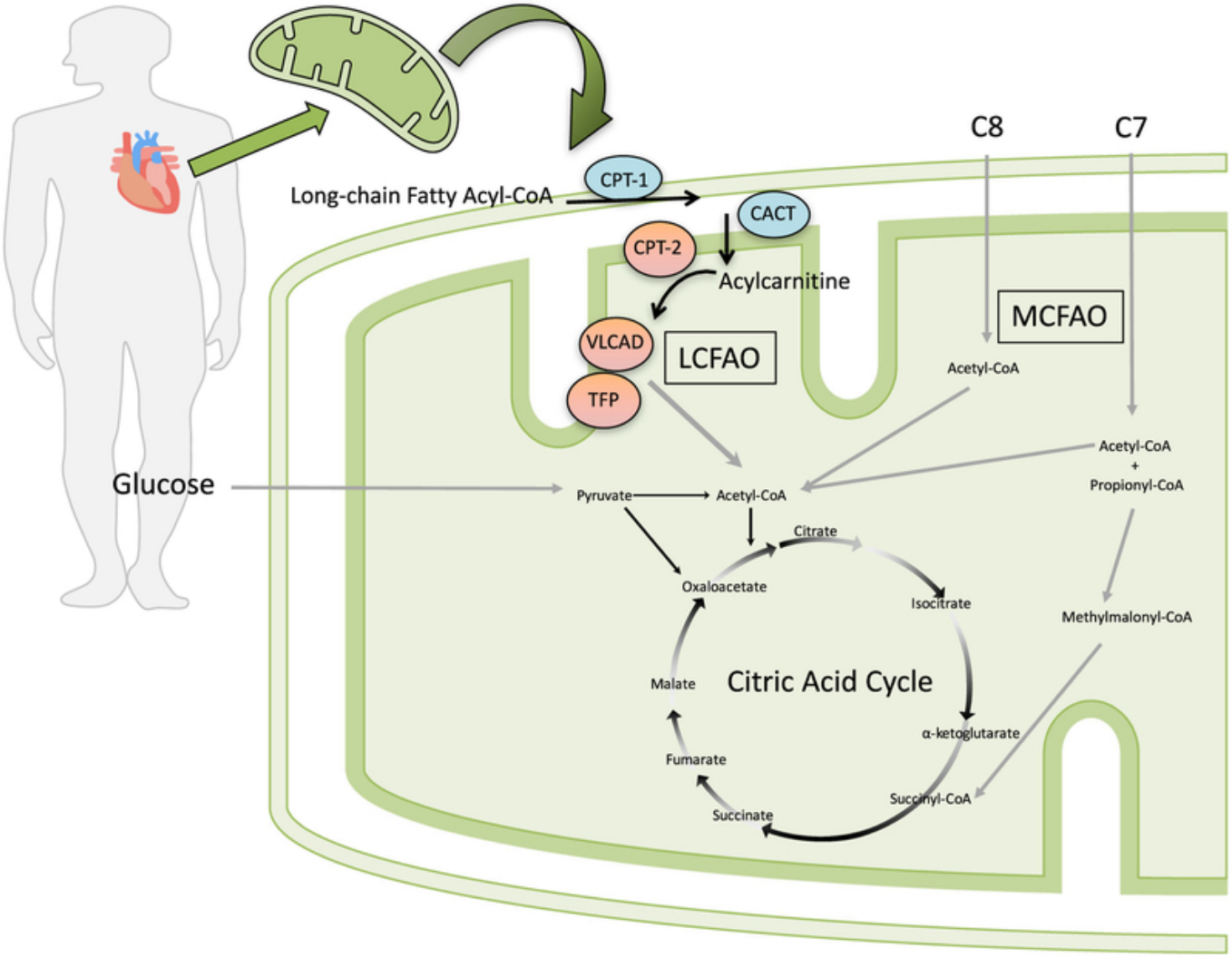
Diagnosis: Plasma carnitine and acylcarnitine profile, urine organic acids during acute attack, free fatty acids, DNA studies, in vitro probes, fibroblast enzyme/transport assay.

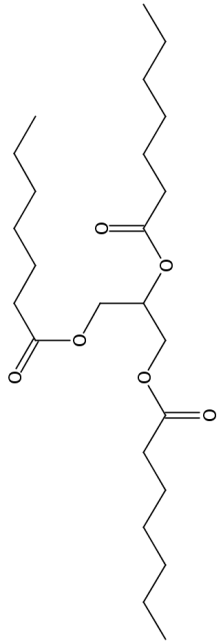
Therapy: Fasting avoidance, prompt treatment of infections, low fat diet, MCT oil/triheptanoin (in some), carnitine, essential fatty acids, ketones

Pasquali M, Longo N. Newborn Screening and Inborn Errors of Metabolism. Chapter 70. In: Burtis CA, Ashwood ER, Bruns DE, eds. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 6th ed. St. Louis: Saunders, 2017.

MCT, medium-chain triglyceride.

MEDIUM CHAIN TRIGLYCERIDES (C8) AND TRIHEPTANOIN (C7) IN LONG-CHAIN FATTY AXID OXIDATION DISORDERS





Gillingham MB, Heitner SB, Martin J, Rose S, Goldstein A, El-Gharbawy AH, Deward S, Lasarev MR, Pollaro J, DeLany JP, Burchill LJ, Goodpaster B, Shoemaker J, Matern D, Harding CO, Vockley J. Triheptanoin versus trioctanoin for long-chain fatty acid oxidation disorders: a double blinded, randomized controlled trial. J Inherit Metab Dis. 2017 Nov;40(6):831-843. doi: 10.1007/s10545-017-0085-8. Epub 2017 Sep 4. PMID: 28871440

EMERGENCY PROTOCOL FOR PATIENTS WITH FATTY ACID OXIDATION DEFECTS

If unable to eat, give IV Fluids to provide calories:

D10 (10% glucose) + 75-150 mEq/L NaCl + 20 mEq/L KCl at 150 ml/kg per day

Labs: Electrolytes, liver function tests, CK (Creatine Kinase), plasma ammonia, urine analysis.

Labs/Imaging to identify cause of problems, mostly infections (cultures/X-Rays)

Start enteral feeds as soon as tolerated

CHILDHOOD HYPOGLYCEMIA

Eight months old boy with history of frequent infections and vomiting presented with low oral intake and lethargy prompting hospital admission.
Exam: hepatomegaly, lethargy
Labs: nonketotic hypoglycemia (glucose 35 mg/dL), hyperammonemia, and elevated liver function tests (Reye syndrome). Urine organic acids; mild dicarboxylic aciduria, Normal plasma amino acids.
Therapy: he improved with intravenous fluids and glucose.

Table I: Plasma carnitine

	Total carnitine µM	Free carnitine µM	Acyl-carnitine µM
Proband	1	1	0
Mother	21	16	5
Father	24	20	4
Controls	30-70	24-56	6-14

Scaglia et al (1998)
Genet Med 1: 34-39

Carnitine transporter deficiency (Primary carnitine deficiency MIM 212140)

- **Frequency** 1:142,336 (USA), 1:127,678 (Utah), 1:300 (Faroes)
- **Cause**: Carnitine transporter (OCTN2) defect (*SLC22A5* gene)
- **Pathogenesis**: Loss of carnitine in urine reduces availability of carnitine in liver, muscle and heart, impairing FAO
- **Presentation**: Hepatic encephalopathy, hypoglycemia, cardiomyopathy in childhood, arrhythmia and/or exertional rhabdomyolysis in adults, sudden death in children and adults
- **Diagnosis**: very low plasma carnitine (usually C0<5 μM, can be higher in newborns), decreased urinary carnitine reabsorption, confirmed by DNA testing or transport studies in fibroblasts. Can be detected by newborn screening.
- **Therapy**: Carnitine 100-150 mg/kg up to 3 g per day PO divided into 3-4 daily doses
- **Monitoring:** Plasma carnitine free and total
- **Prognosis**: Excellent (with treatment)

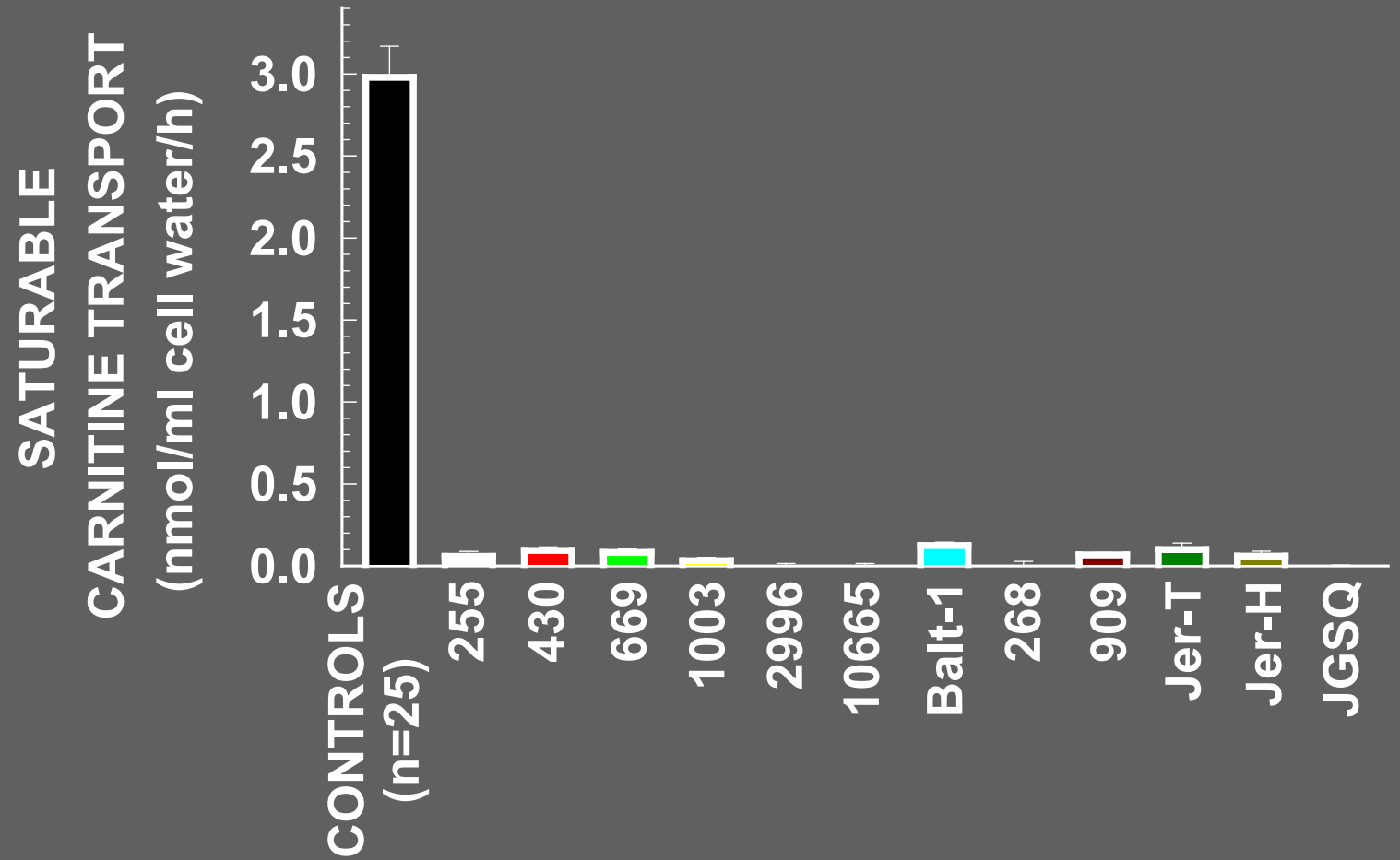
LOW CARNITINE LEVELS IN PRIMARY CARNITINE DEFICIENCY

Free and total carnitine are reduced and remain low with treatment. Goal is to keep free carnitine >15 μmol/L (*SLC22A5* gene: homozygous p.Arg227His).

Carnitine, Free & Total (umol/L)

	Ref. Range	04/07/15 13:23*	03/06/15 15:55*	02/09/15 17:40*	08/04/14 17:20*	02/03/14 12:48	08/03/13 12:39	03/11/13 15:04	02/25/13 12:18	02/13/13 16:55
Carnitine, Free	25-55	24 L	10 L	11 L	9 L	19 L	33	39	9 L	3 L
Carnitine, Total	35-90	35	17 L	15 L	19 L	27 L	61	65	12 L	10 L
Carnitine, Esterified	4-36	11	7	4	10	8	28 H	26 H	3 L	7 L
Carnitine Ester/Free (Ratio)	0.1-0.8	0.5	0.7	0.4	1.1 H	0.4	0.8	0.7	0.3	0.4

DEFECTIVE CARNITINE TRANSPORT IN FIBROBLASTS FROM PATIENTS WITH PRIMARY CARNITINE DEFICIENCY

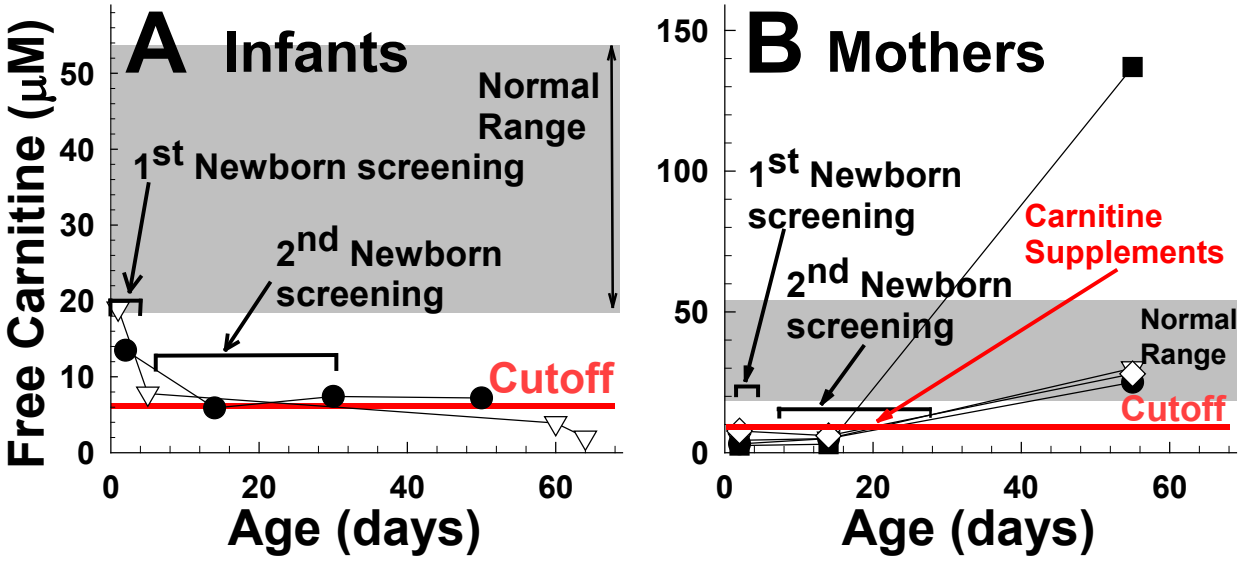


Wang et at (1999) *Proc Natl Acad Sci USA* 96: 2356-2360
Wang et al (2001) *Genet Med* 3: 387-392

Carnitine Transporter Deficiency: Newborn Screening

Carnitine is transferred from the mother to the fetus during pregnancy. Babies can have normal plasma carnitine levels at birth that decline with time.

In maternal primary carnitine deficiency, carnitine levels in the baby are very low at birth. Most mothers are asymptomatic, but at risk of sudden death.



Pasquali M, Longo N. Response to Chen et Al.: carnitine uptake defect (primary carnitine deficiency): risk in genotype-phenotype correlation. Hum Mutat. 2013 Apr;34(4):656

SUDDEN DEATH IN PRIMARY CARNITINE DEFICIENCY

In the Faroe Islands, children and young adults died from cardiac arrhythmia after presenting with altered mental status, psychotic behavior and stupor. In most cases, the episode was triggered by the use of pivalic acid containing antibiotics.

Long QT syndrome and cardiac arrhythmia can be seen in pregnancy in patients with the p.N32S mutation. This resolved with carnitine supplements.

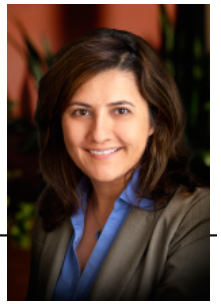
Rasmussen J, Dunø M, Lund AM, Steuerwald U, Hansen SH, Joensen HD, Køber L, Nielsen OW. Increased risk of sudden death in untreated primary carnitine deficiency. J Inherit Metab Dis. 2020 Mar;43(2):290-296. doi: 10.1002/jimd.12158. Epub 2019 Dec 15. PMID: 31373028

J Inherit Metab Dis (2013) 36:35–41
DOI 10.1007/s10545-012-9488-8

ORIGINAL ARTICLE

Primary carnitine deficiency and pivalic acid exposure causing encephalopathy and fatal cardiac events

Jan Rasmussen • Olav W. Nielsen • Allan M. Lund • Lars Køber • Høgni Djurhuus

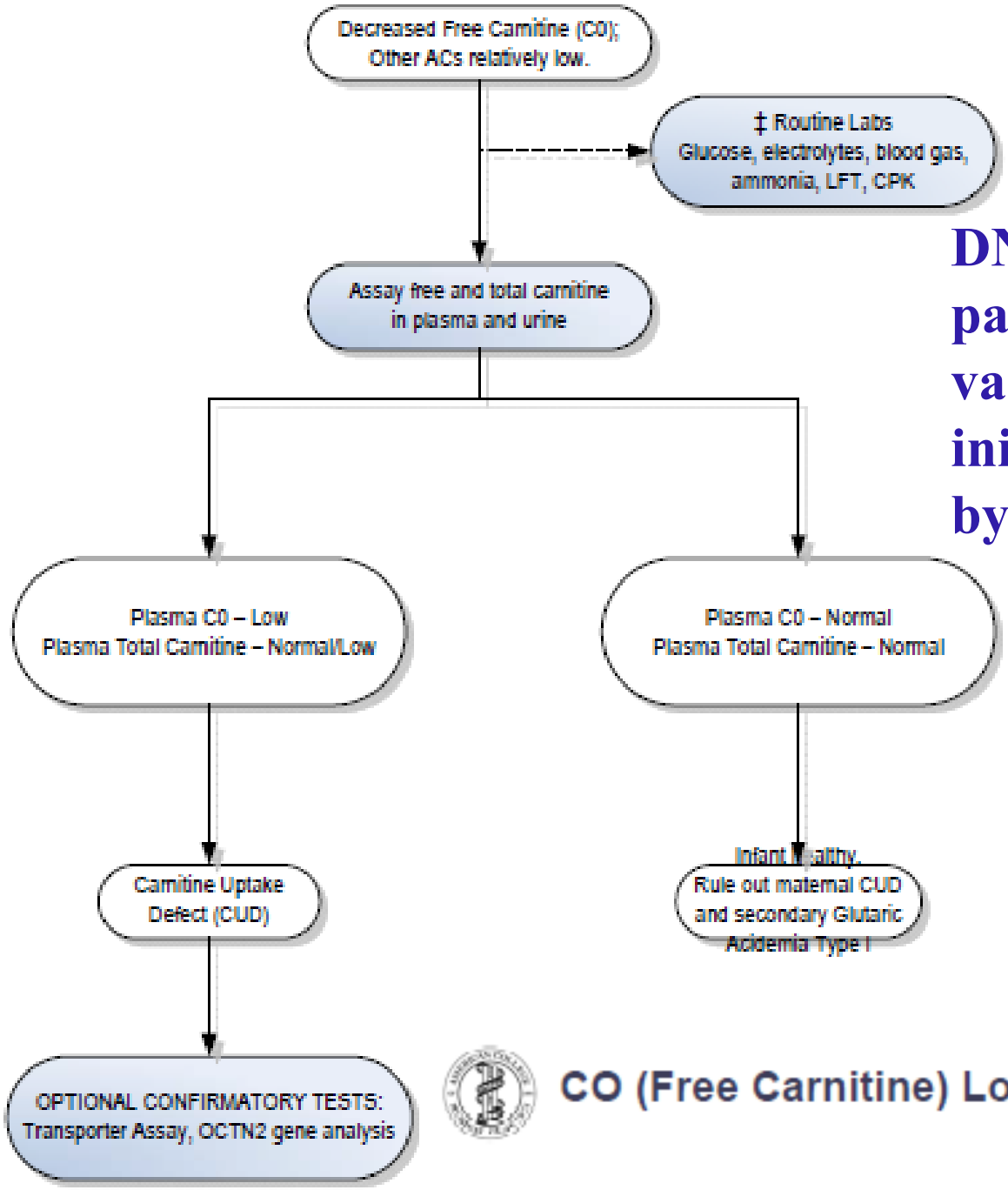


JIMD Reports
DOI 10.1007/8904_2011_52

CASE REPORT

Primary Carnitine Deficiency Presents Atypically with Long QT Syndrome: A Case Report

Irene De Biase • Neena Lorenzana Champaigne • Richard Schroer • Laura Malinda Pollard • Nicola Longo • Tim Wood



DNA testing: common pathogenic (about 20%) variant 5' to the ATG initiation site is missed by current sequencing

Ferdinandusse S, Te Brinke H, Ruiter JPN, Haasjes J, Oostheim W, van Lenthe H, IJlst L, Ebberink MS, Wanders RJA, Vaz FM, Waterham HR. A mutation creating an upstream translation initiation codon in SLC22A5 5'UTR is a frequent cause of primary carnitine deficiency. Hum Mutat. 2019 Oct;40(10):1899-1904. doi: 10.1002/humu.23839. Epub 2019 Jul 3. PMID: 31187905

CO (Free Carnitine) Low

Carnitine Palmitoyl Transferase-1A (CPT-1A) deficiency (OMIM 255120)

Frequency very rare, except in the Alaskan population (1.3:1,000, milder variant)

Cause/Pathogenesis: Deficiency of CPT-1A (liver) impairs synthesis of long-chain acylcarnitine preventing transfer of long-chain fatty acid inside mitochondria.

Presentation: fasting-induced hepatic encephalopathy, hypoglycemia, liver failure, failure to thrive

Diagnosis: Elevated carnitine levels with low C16, C18 (Increased C0/(C16+C18) in blood spots. Free carnitine can be normal or high in plasma. Confirmed by DNA testing. Can be detected by newborn screening.

Therapy: Avoidance of fasting, low-fat diet in which most derive from medium-chain triglycerides (C6-C10 fatty acids) or triheptanoin

Monitoring: liver function tests

Prognosis: not many data, better with treatment

Carnitine Palmitoyl Transferase-1A (CPT-1A) deficiency (OMIM 255120)

Severe (Classic) cases identified by newborn screening:

***CPT1A* gene: c.222C>A (p.Y74X)/c.222C>A (p.Y74X). C0: 240 on first screen, C0/(C16+C18)=574 (normal <100). 5.5 yo. History of failure to thrive, developmental delays, elevated transaminases while breastfeeding. Formula with MCT oil improved growth and development, normalized liver enzymes. Still mildly behind in development.**

***CPT1A* gene: p.A275T/p.R508X. C0: unknown on first screen (patient from other state). 8 yo. Serum C0= 51-61, total 56-72, very low C16-C18, usually reported as normal in plasma. AST/ALT occasionally elevated. Complications: Seizures, ADHD, unclear compliance with therapy.**

CPT1A DEFICIENCY

Carnitine levels can be normal in plasma, but remain high in whole blood.

Ratio esterified/free carnitine is in the low-normal range.

Carnitine, Free & Total (umol/L)

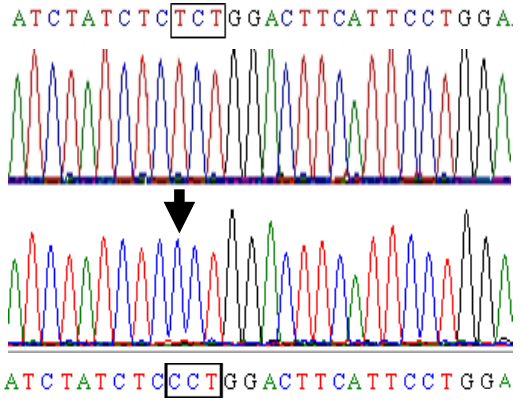
	Ref. Range	02/23/15 14:45*	08/04/14 11:17*	03/03/14 13:30	08/26/13 14:30
Carnitine, Free	22-63	48	61	56	51
Carnitine, Total	31-78	57	72	69	56
Carnitine, Esterified	3-38	9	11	13	5
Carnitine Ester/Free (Ratio)	0.1-0.9	0.2	0.2	0.2	0.1

Acylcarnitine Quantitation, (Plasma)

	Ref. Range	02/23/15 14:45*	08/04/14 11:17*	03/03/14 13:30	08/26/13 14:30
Interpretation:	umol/L	Normal	Normal	Normal	Normal
C2, Acetyl	3.74-16.56	7.53	8.44	6.73	4.94
C3, Propionyl	0.00-0.83	0.81	0.63	0.67	0.39
C4, Iso/Butyryl	0.00-0.45	0.24	0.27	0.34	0.15
C5, Isovaleryl/2Mebutyryl	0.00-0.30	0.18	0.12	0.14	0.10
C5-DC, Glutaryl	0.00-0.09	0.03	0.06	0.02	0.02
C6, Hexanoyl	0.00-0.12	0.05	0.00	0.05	0.05
C5-OH, 3-OH Isovaleryl	0.00-0.07	0.00	0.00	0.03	0.02
C8, Octanoyl	0.00-0.23	0.02	0.09	0.02	0.00
C8:1, Octenoyl	0.00-0.61	0.18	0.17	0.10	0.05
C10, Decanoyl	0.00-0.31	0.03	0.11	0.02	0.00
C10:1, Decenoyl	0.00-0.31	0.06	0.12	0.02	0.02
C12, Dodecanoyl	0.00-0.12	0.04	0.06	0.02	0.01
C12:1, Dodecenoyl	0.00-0.17	0.04	0.07	0.02	0.01
C12-OH, 3-OH Dodecanoyl	0.00-0.02	0.00	0.00	0.00	0.01
C14, Tetradecanoyl	0.00-0.05	0.01	0.01	0.01	0.00
C14:1, Tetradecenoyl	0.00-0.16	0.02	0.04	0.01	0.00
C14:2, Tetradecadienoyl	0.00-0.12	0.02	0.04	0.01	0.00
C14-OH, 3-OH-Tetradecanoyl	0.00-0.02	0.00	0.01	0.00	0.00
C14:1-OH, 3-OH-Tetradecenoyl	0.00-0.02	0.01	0.01	0.00	0.00
C16, Palmitoyl	0.00-0.10	0.00	0.01	0.00	0.00
C16:1, Palmitoleyl	0.00-0.04	0.00	0.01	0.00	0.00
C16-OH, 3-OH-Palmitoyl	0.00-0.01	0.00	0.00	0.00	0.00
C16:1-OH, 3-OH-Palmitoleyl	0.00-0.01	0.00	0.00	0.00	0.00
C18, Stearoyl	0.00-0.04	0.01	0.01	0.01	0.00
C18:1, Oleyl	0.00-0.17	0.01	0.01	0.00	0.00
C18:2, Linoleyl	0.00-0.10	0.00	0.01	0.00	0.00
C18-OH, 3-OH-Stearoyl	0.00-0.01	0.00	0.00	0.00	0.00
C18:1-OH, 3-OH-Oleyl	0.00-0.01	0.00	0.00	0.00	0.00

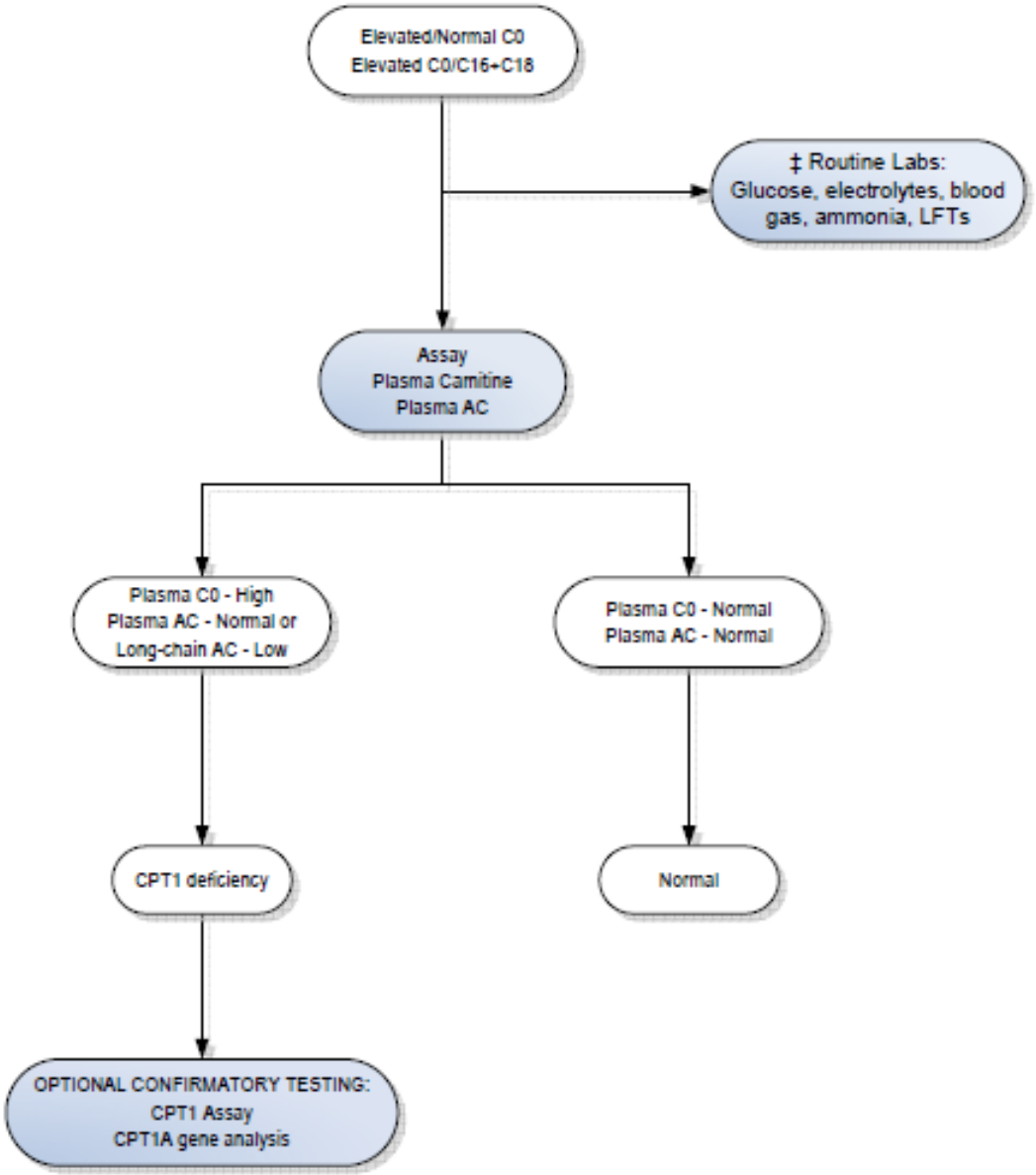
Carnitine Palmitoyl Transferase-1A (CPT-1A) deficiency (OMIM 255120)

Mild variants: Homozygous p.S34P (Marshall Islands). Carnitine high only in whole blood, not in plasma. Unclear if milder forms in Alaskan natives (p.P479L) or Pacific Islanders (p.S34P) need treatment.
Might increase risk for respiratory infections.



Collins SA, Sinclair G, McIntosh S, Bamforth F, Thompson R, Sobol I, Osborne G, Corriveau A, Santos M, Hanley B, Greenberg CR, Vallance H, Arbour L. Carnitine palmitoyltransferase 1A (CPT1A) P479L prevalence in live newborns in Yukon, Northwest Territories, and Nunavut. Mol Genet Metab. 2010 Oct-Nov;101(2-3):200-4. PMID: 20696606

C0 Elevated; C0/C16+C18 Elevated



Carnitine levels can be normal in plasma, but remain high in whole blood. Need DNA testing to exclude CPT1A deficiency

Carnitine Palmitoyl Transferase-1B (OMIM 601987)

Expressed in the heart and skeletal muscle.

Homozygous deletion of this gene is embryonically lethal in mice Ji S, You Y, Kerner J, Hoppel CL, Schoeb TR, Chick WS, Hamm DA, Sharer JD, Wood PA. Homozygous carnitine palmitoyltransferase 1b (muscle isoform) deficiency is lethal in the mouse. Mol Genet Metab. 2008 Mar;93(3):314-22. Epub 2007 Nov 19. PMID: 18023382

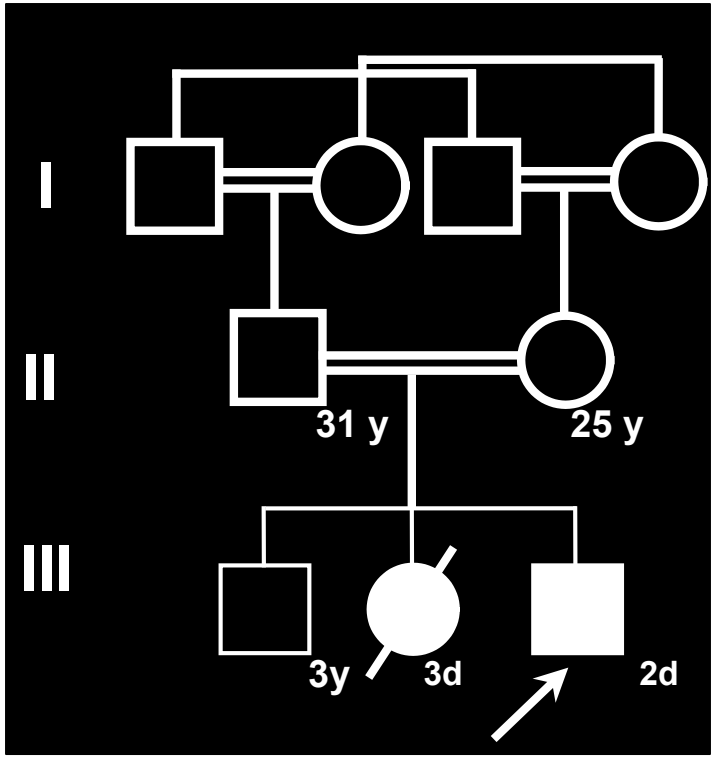
Carnitine Palmitoyl Transferase-1C (OMIM 255120)

Localizes to the endoplasmic reticulum of neuronal cells and might serve as a sensor for fats.

A mutation (p.R37C) in this gene causes autosomal dominant spastic paraplegia-73, possibly by affecting lipid composition in the brain.

NEONATAL CARDIAC ARREST

Term infant developed hypothermia, desaturations, low blood pressure and hypoglycemia (glucose 7 mg/dL) at 18 h of age. Intubated, developed tachy- and bradycardia. Cardiac ECHO: cardiomyopathy. Had cardiac arrest requiring 5 min of chest compressions. Had mild hyperammonemia with increased liver function tests (ALT/AST up to 400) and mildly increased CPK (up to 350).
Started on IV glucose with stabilization.



ALT, alanine transaminase;
AST, aspartate transaminase;
CPK, creatine phosphokinase;
ECHO, echocardiogram.

Iacobazzi V, Pasquali M, Singh R, Matern D, Rinaldo P, Amat di San Filippo C, Palmieri F, Longo N. Response to therapy in carnitine/acylcarnitine translocase (CACT) deficiency due to a novel missense mutation. Am J Med Genet A. 2004 Apr 15;126A(2):150-5. doi: 10.1002/ajmg.a.20573.PMID: 15057979

Carnitine acylcarnitine translocase (CACT) deficiency MIM 212138

Frequency: very rare

Cause/Pathogenesis: Deficiency of the acylcarnitine translocator impairs entry of long-chain acylcarnitines into mitochondria, resulting in the accumulation of long-chain acylcarnitine, long-chain fatty acids and defective energy production.

Presentation: Arrhythmia, cardiac arrest shortly after birth, hypoketotic hypoglycemia, cardiomyopathy

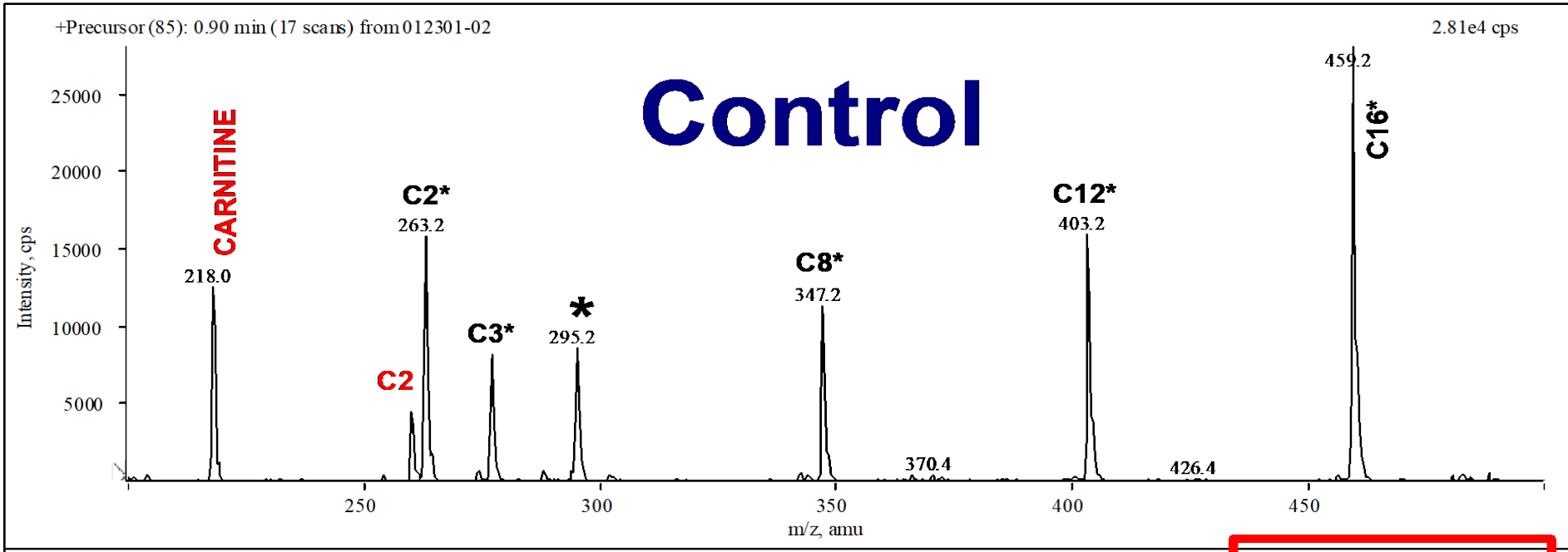
Diagnosis: increased C16, C18, C18:1, C18:2-carnitine, low C0 in plasma, abnormal organic acids (dicarboxylic aciduria), confirmed by DNA testing. Identified by newborn screening, but most infants present before newborn screening is obtained.

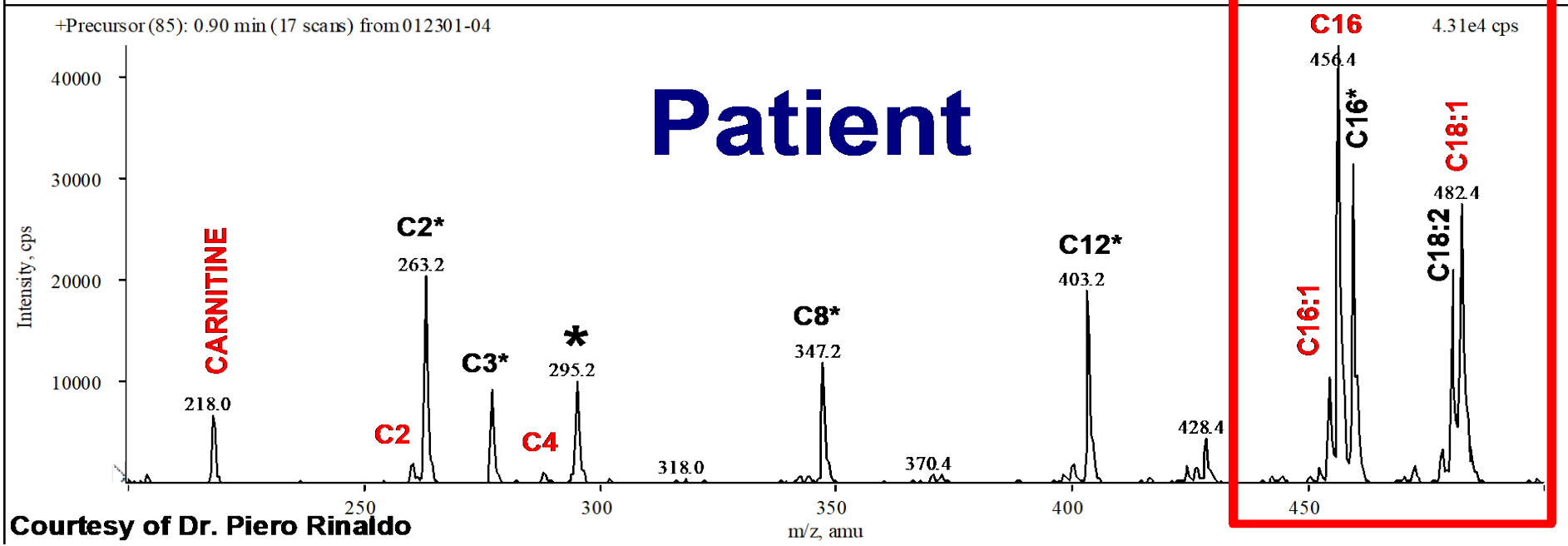
Therapy: fasting avoidance, low fat diet, MCT oil, triheptanoin, carnitine

Monitoring: acylcarnitine profile, carnitine F & T, CK, ALT, AST

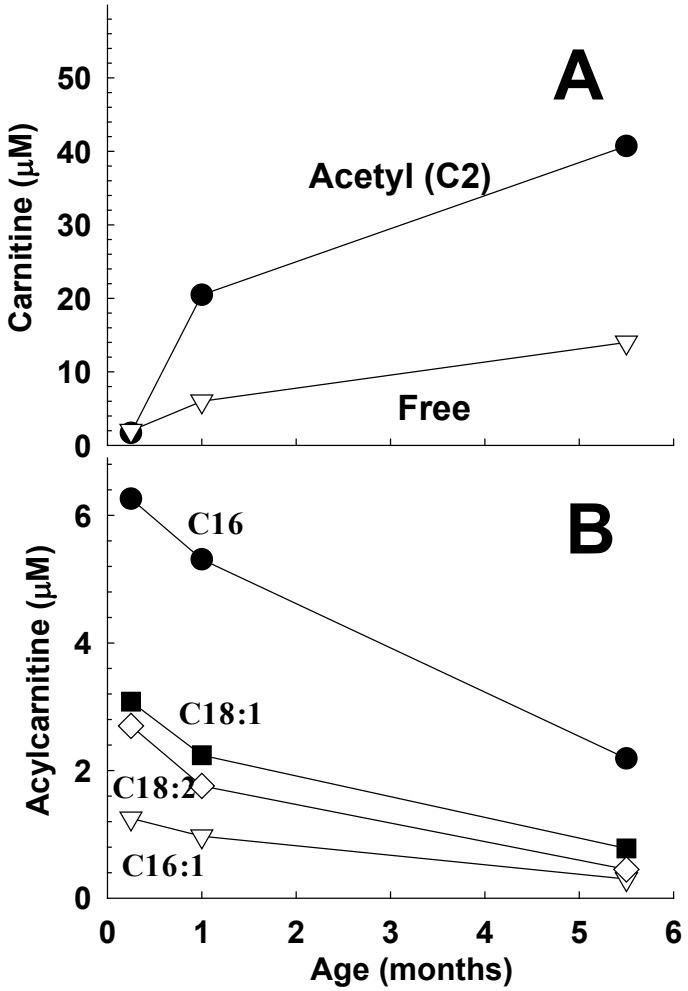
Prognosis: not always good, but there are teenagers with milder variants doing well with therapy.

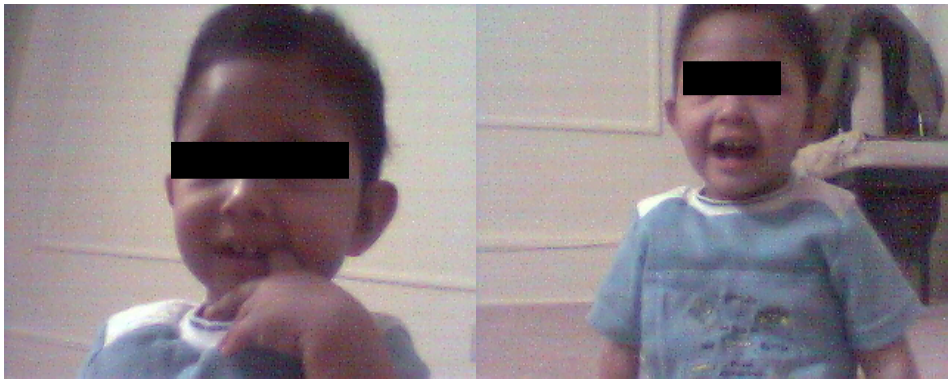
Plasma Acylcarnitine Profile



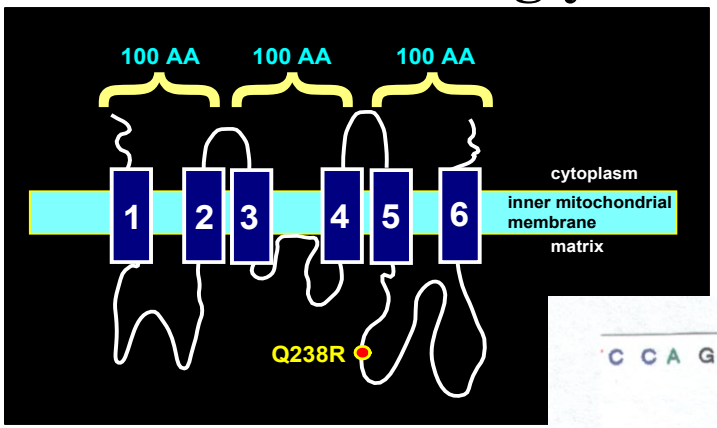


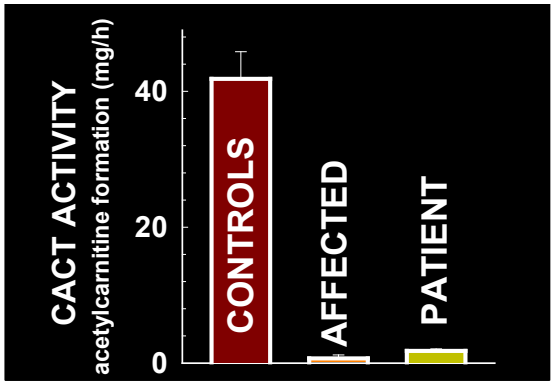
Courtesy of Dr. Piero Rinaldo



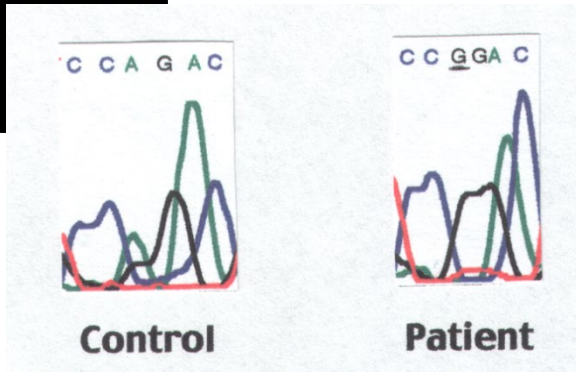


Progressive normalization of carnitine levels in a patient with CACT deficiency with carnitine and medium chain triglycerides.





Iacobazzi V, Pasquali M, Singh R, Matern D, Rinaldo P, Amat di San Filippo C, Palmieri F, Longo N. Response to therapy in carnitine/acylcarnitine translocase (CACT) deficiency due to a novel missense mutation. Am J Med Genet A. 2004 Apr 15;126A(2):150-5.



RHABDOMYOLYSIS

- 78-year-old man hospitalized for persistent muscle cramps and myoglobinuria
- Has not been able to run or participate in sustained physical exercise since he was a teenager
- Was in the military during 2 wars but was assigned to an office
- Now, he develops muscle pain and myoglobinuria even without exercise (P50H/unk)

	Last Ref. Range	08/06/07 10:30	12/18/06 12:25	11/09/06 12:00
C2, Acetyl		13.53	* 10.23	* 7.21
C3, Propionyl		0.53	* 0.77	* 0.68
C4, Iso/Butyryl		0.27	* 0.24	* 0.27
C5, Isovaleryl/2Mebutyryl		0.16	* 0.15	* 0.10
C5-DC, Glutaryl		0.04	* 0.03	* 0.08
C6, Hexanoyl		0.09	* 0.10	* 0.12
C8, Octanoyl		0.10	* 0.16	* 0.13
C8:1, Octenoyl		0.24	* 0.36	* 0.14
C10, Decanoyl		0.24	* 0.28	* 0.15
C10:1, Decenoyl		0.16	* 0.21	* 0.22
C12, Dodecanoyl		0.17 H	* 0.18 H	* 0.10
C12:1, Dodecenoyl		0.07	* 0.11	* 0.19 H
C12-OH, 3-OH Dodecanoyl		0.03 H	* 0.06 H	* 0.01
C14, Tetradecanoyl		0.14 H	* 0.10 H	* 0.11 H
C14:1, Tetradecenoyl		0.08	* 0.06	* 0.09
C14:2, Tetradecadienoyl		0.03	* 0.05	* 0.04
C14-OH, 3-OH-Tetradecanoyl		0.02	* 0.02	* 0.00
C14:1-OH, 3-OH-Tetradecenoyl		0.03 H	* 0.05 H	* 0.05 H
C16, Palmitoyl		0.76 H	* 0.46 H	* 0.70 H
C16:1, Palmitoleyl		0.05 H	* 0.06 H	* 0.08 H
C16-OH, 3-OH-Palmitoyl		0.01	* 0.01	* 0.03 H
C16:1-OH, 3-OH-Palmitoleyl		0.03 H	* 0.03 H	* 0.03 H
C18, Stearoyl		0.37 H	* 0.31 H	* 0.36 H
C18:1, Oleyl		0.49 H	* 0.41 H	* 0.82 H
C18:2, Linoleyl		0.25 H	* 0.21 H	* 0.20 H
C18-OH, 3-OH-Stearoyl		0.01	* 0.01	* 0.04 H
C18:1-OH, 3-OH-Oleyl		0.02 H	* 0.01	* 0.03 H
C18:2-OH, 3-OH-Linoleyl		0.02 H	* 0.01	* 0.02 H

Carnitine Palmitoyl Transferase-2 (CPT-2) deficiency

Frequency: Very rare, except for the myopathic form which is still rare, but with several reported cases (>300)

Cause/Pathogenesis: Deficiency of CPT-2 impairs the transfer of long-chain fatty acids from carnitine to CoA resulting in the accumulation of long-chain acylcarnitine, long-chain fatty acids and defective energy production.

Presentation: 1. Lethal Neonatal 608836: respiratory failure, liver failure, cardiomyopathy, arrhythmia, hypoglycemia; 2. severe infantile 600649: hypoglycemia, seizures, hepatomegaly, cardiomyopathy, and arrhythmia; 3. myopathic 255110: muscle pain with exercise.

Diagnosis: increased C16, C18, C18:1, C18:2-carnitine in plasma, confirmed by DNA testing. Can be identified by newborn screening, but infants with late-onset variant can have normal profile at birth.

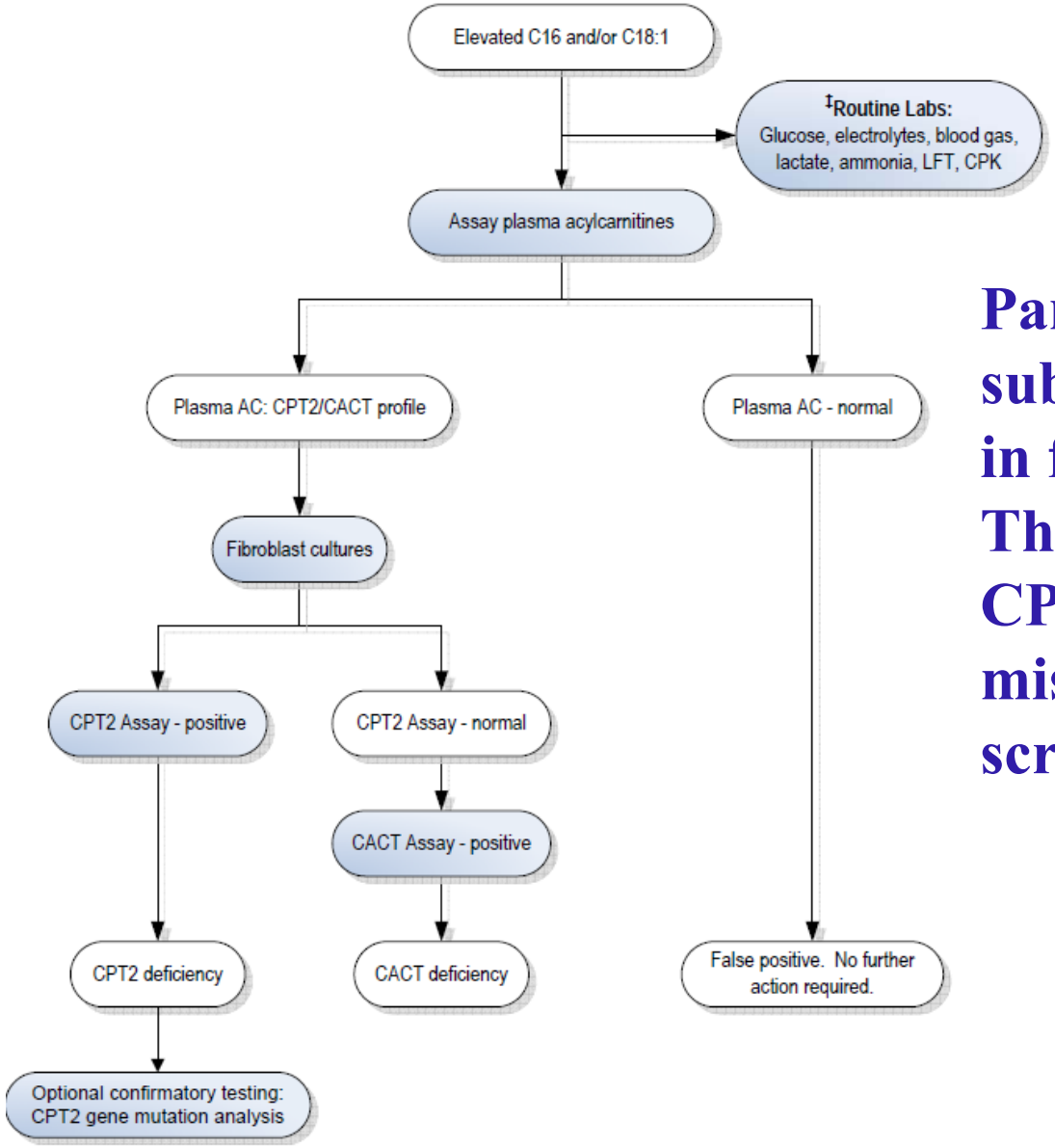
Therapy: Avoidance of fasting, MCT oil, triheptanoin, sugary drinks with exercise

Monitoring: ALT, AST, CK, acylcarnitines, carnitine free and total

Prognosis: myopathic form compatible with long life

Pasquali M, Longo N. Newborn Screening and Inborn Errors of Metabolism. Chapter 70. In: Burtis CA, Ashwood ER, Bruns DE, eds. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 6th ed. St. Louis: Saunders; 2017.

C16 and/or C18:1 Elevated



Panel DNA testing has substituted enzyme assay in fibroblasts.
The late-onset form of CPT2 deficiency is easily missed by newborn screening.

MUSCLE PAIN WITH EXERCISE
Differential Diagnosis

- **McArdle disease or other glycogen storage disorder**
- **CPT-2 deficiency**
- **Late-Onset MADD and VLCAD deficiency**
- **Other FAO/Carnitine disorders (LCHAD, carnitine deficiency)**
- **Myoadenylate deaminase deficiency (?)**
- **Mitochondrial disorders (cytochrome b, CoQ10 deficiency)**
- **Anesthetic-induced malignant hyperthermia**
- **Autosomal recessive LPIN1 mutations** (Mg(2+)-dependent phosphatidic acid (PA) phosphohydrolase)

Neonatal Hypoglycemia

Full term Caucasian male, first child of healthy, unrelated parents is discharged home from the hospital after uneventful pregnancy and delivery. The mother is breastfeeding. Shortly after arriving at home at 36 hours of life, the infant becomes lethargic and is brought to the emergency room. The infant appears dehydrated and is given normal saline. Blood monitoring indicates glucose of 15 mg/dL.

Medium Chain AcylCoA Dehydrogenase (MCAD) Deficiency MIM 201450

Most common fatty acid oxidation defect

Frequency: 1:17,759 (USA) 1:7,738 (Utah)

Cause: mutations in *ACADM* gene

Presentation: Fasting-induced hypoketotic hypoglycemia, coma, sudden death. Normal between episodes. Many cases remain asymptomatic until adult life and can still result in unexpected death. Some cases present at birth.

Diagnosis: plasma acylcarnitine profile: elevated C8, C6, C10:1, urine organic acids (hexanoylglycine), DNA testing (common p.K329E mutation)

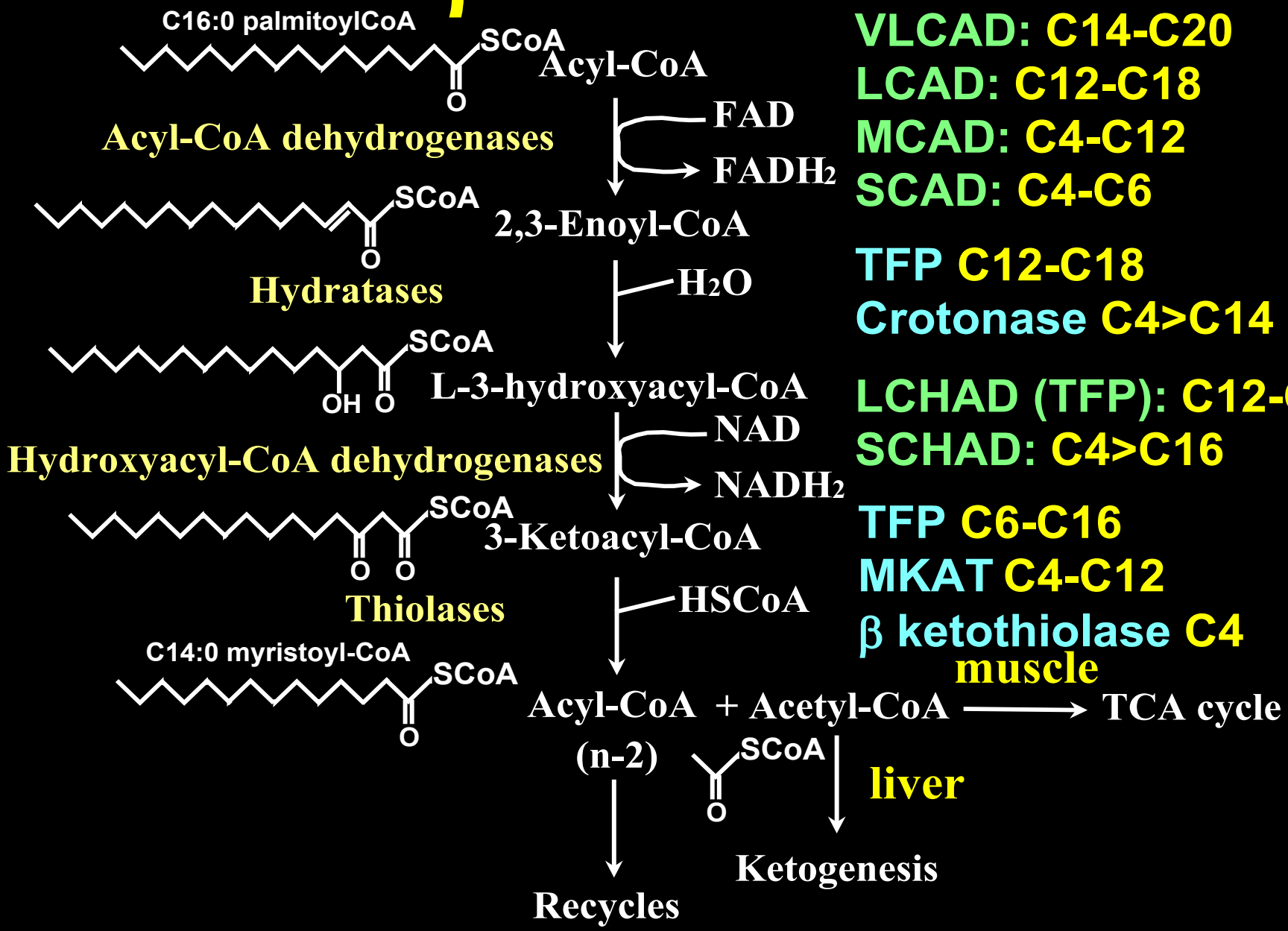
Therapy: avoidance of fasting, prompt treatment of infection, heart-healthy diet at age 1, carnitine supplements (unproven)

Monitoring: free and total carnitine, acylcarnitine profile

Prognosis: Excellent with treatment

Anderson DR, Viau K, Botto LD, Pasquali M, Longo N. Clinical and biochemical outcomes of patients with medium-chain acyl-CoA dehydrogenase deficiency. Mol Genet Metab. 2020 Jan;129(1):13-19. doi: 10.1016/j.ymgme.2019.11.006. Epub 2019 Nov 25. PMID: 31836396

β-OXIDATION



VLCAD: C14-C20
LCAD: C12-C18
MCAD: C4-C12
SCAD: C4-C6

TFP C12-C18
Crotonase C4>C14

LCHAD (TFP): C12-C18
SCHAD: C4>C16

TFP C6-C16
MKAT C4-C12
β ketothiolase C4

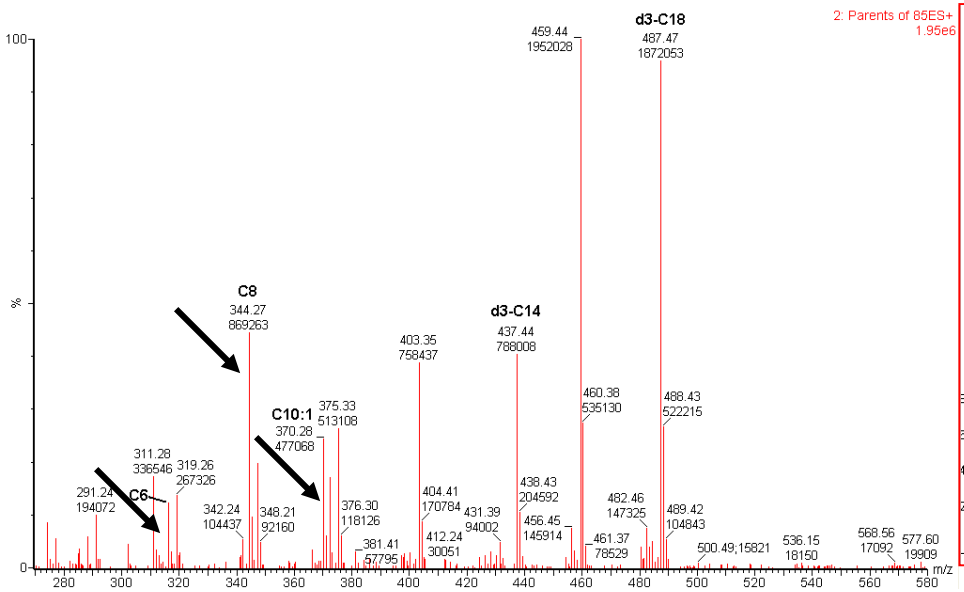
MCAD DEFICIENCY

Carnitine, Free	23-70	* 5 L
Carnitine, Total	26-81	* 10L

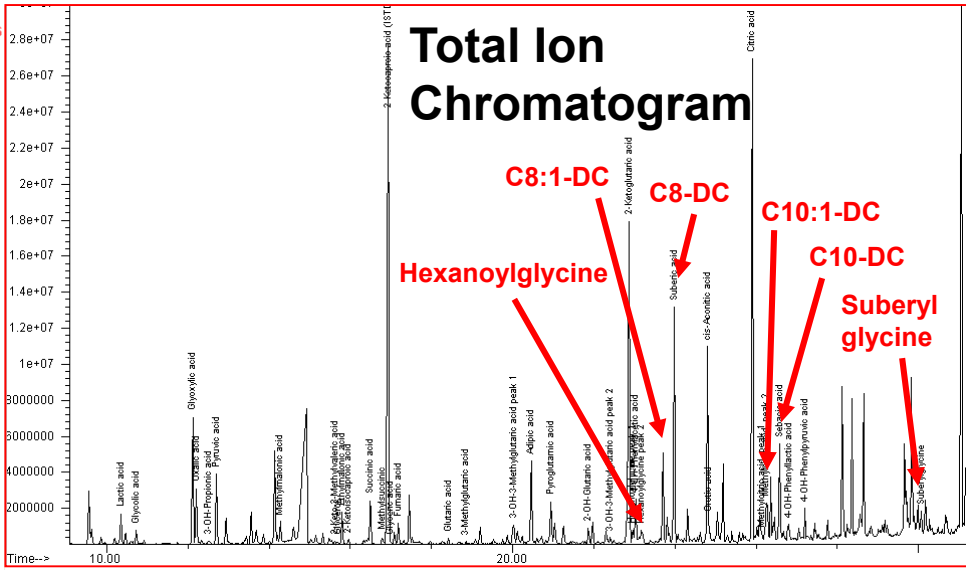
Prot	5.9-7.0	g/dL	5.3 L
Alb	3.1-3.9	g/dL	2.9 L
Bili, Total	0.2-1.3	mg/dL	0.2
Bili, Conj	0.0-0.3	mg/dL	0
Bili, Unconj	0.0-1.1	mg/dL	0.3
Alk Phos	145-320	U/L	112 L
ALT	May-45	U/L	457 H
AST	20-60	U/L	1241 H

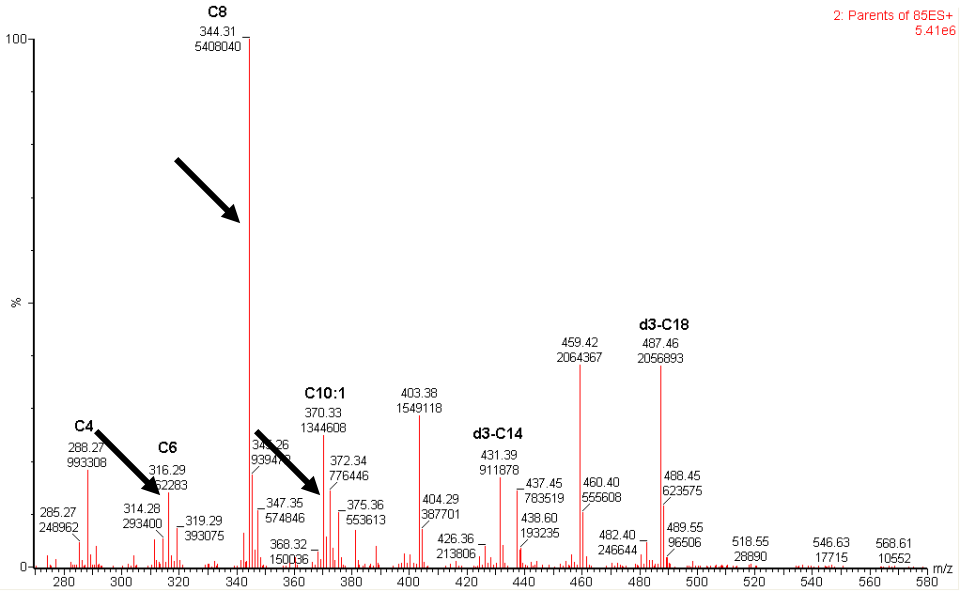
BIOCHEMICAL FINDINGS IN MCAD DEFICIENCY

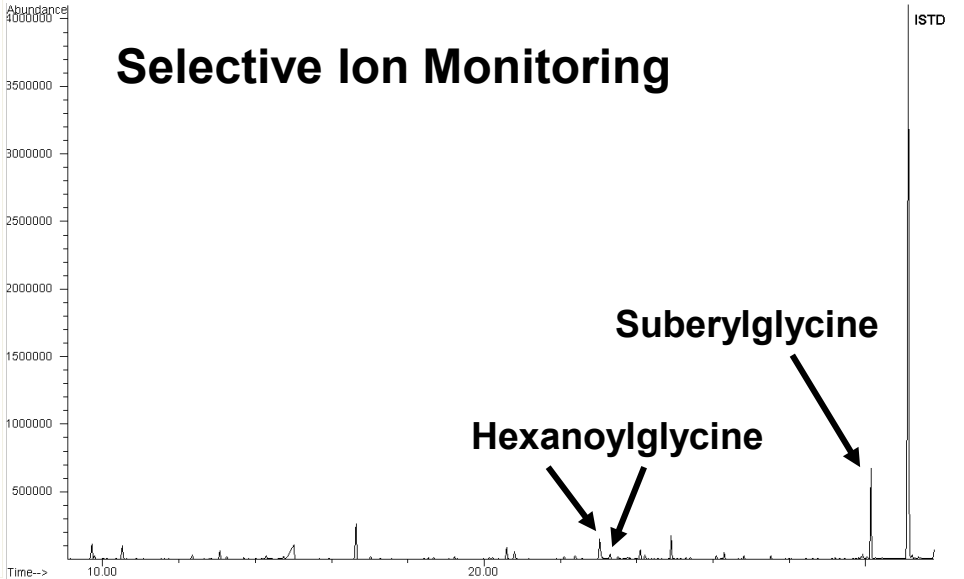
Plasma acylcarnitine profile by MS/MS



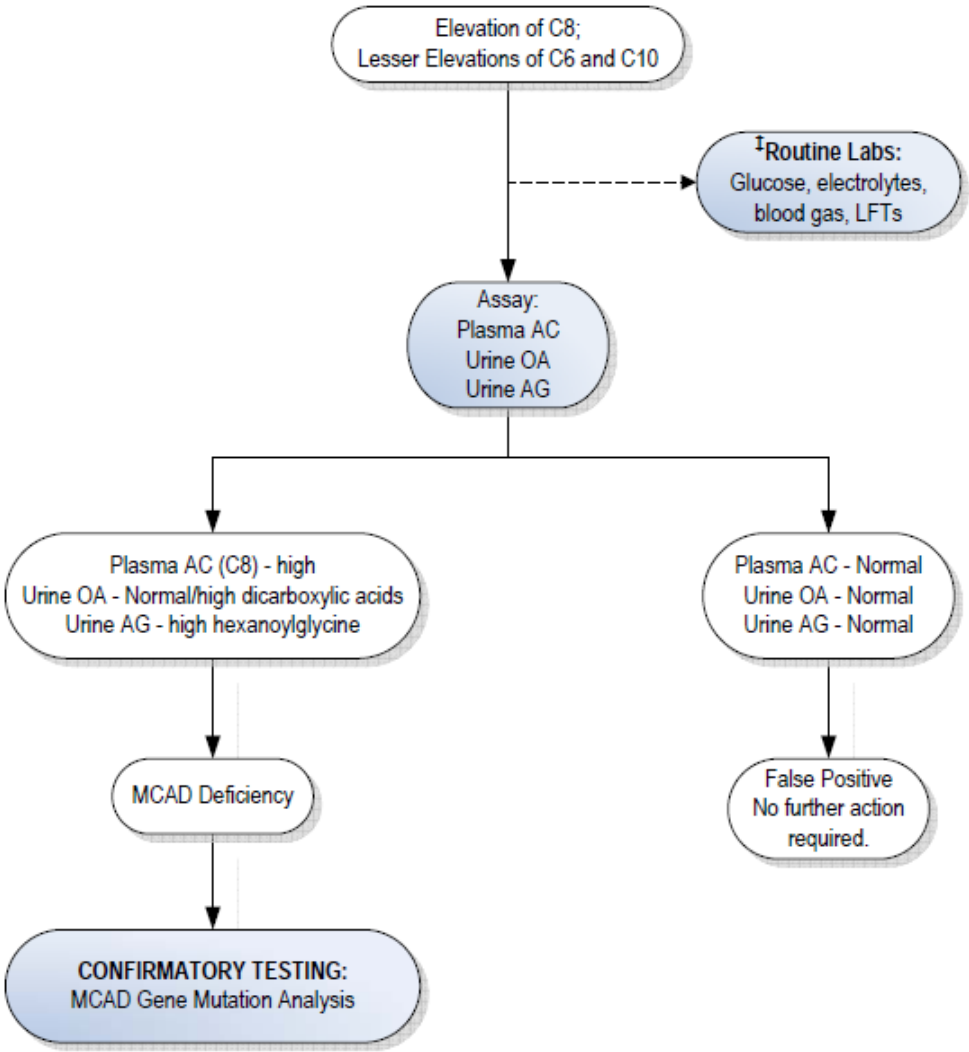
Urine organic acids by GC/MS







C8 Elevated + Lesser Elevations of C6 and C10



CARDIO MYOPATHY

8 yo female hospitalized after being unable to move or wake up completely. She woke up moaning, crying, unable to focus, drink or walk. She was admitted to Intensive Care 6 days and found to have cardiomyopathy with low cardiac ejection fraction, elevated CK, and cardiomegaly on chest X-ray.

	Last Ref. Range	11/12/07 17:25	05/21/07 12:16	12/18/06 12:00	10/23/06 13:15	08/30/06 15:20
C2, Acetyl		* 6.42	* 16.34	* 16.14	* 4.68	* 8.34
C3, Propionyl		* 0.44	* 0.51	* 0.52	* 0.37	* 1.33 H
C4, Iso/Butyryl		* 0.25	* 0.26	* 0.28	* 0.29	* 0.42
C5, Isovaleryl/2Mebutyryl		* 0.10	* 0.08	* 0.04	* 0.05	* 0.13
C5-DC, Glutaryl		* 0.02	* 0.03	* 0.03	* 0.10 H	* 0.04
C6, Hexanoyl		* 0.18 H	* 0.24 H	* 0.30 H	* 0.18 H	* 0.25 H
C8, Octanoyl		* 0.14	* 0.17	* 0.16	* 0.10	* 0.06
C8:1, Octenoyl		* 0.03	* 0.05	* 0.05	* 0.09	* 0.06
C10, Decanoyl		* 0.11	* 0.17	* 0.18	* 0.28	* 0.17
C10:1, Decenoyl		* 0.03	* 0.04	* 0.04	* 0.48 H	* 0.11
C12, Dodecanoyl		* 0.09	* 0.15 H	* 0.21 H	* 0.90 H	* 0.48 H
C12:1, Dodecenoyl		* 0.03	* 0.04	* 0.13	* 0.49 H	* 0.15
C12-OH, 3-OH Dodecanoyl		* 0.00	* 0.01	* 0.02	* 0.09 H	* 0.01
C14, Tetradecanoyl		* 0.37 H	* 0.52 H	* 0.77 H	* 1.90 H	* 0.77 H
C14:1, Tetradecenoyl		* 0.47 H	* 1.31 H	* 1.93 H	* 6.70 H	* 2.78 H
C14:2, Tetradecadienoyl		* 0.13 H	* 0.25 H	* 0.31 H	* 0.96 H	* 0.47 H
C14-OH, 3-OH-Tetradecanoyl		* 0.01	* 0.01	* 0.02	* 0.04 H	* 0.02
C14:1-OH, 3-OH-Tetradecenoyl		* 0.03 H	* 0.03 H	* 0.05 H	* 0.22 H	* 0.05 H
C16, Palmitoyl		* 0.36 H	* 0.46 H	* 0.70 H	* 3.33 H	* 0.99 H
C16:1, Palmitoleyl		* 0.10 H	* 0.22 H	* 0.39 H	* 2.14 H	* 0.50 H
C16-OH, 3-OH-Palmitoyl		* 0.00	* 0.02 H	* 0.02 H	* 0.03 H	* 0.01
C16:1-OH, 3-OH-Palmitoleyl		* 0.03 H	* 0.01	* 0.03 H	* 0.17 H	* 0.05 H
C18, Stearoyl		* 0.13 H	* 0.16 H	* 0.24 H	* 1.40 H	* 0.68 H
C18:1, Oleyl		* 0.14	* 0.25 H	* 0.47 H	* 3.33 H	* 1.07 H
C18:2, Linoleyl		* 0.05	* 0.09	* 0.18 H	* 0.95 H	* 0.29 H
C18-OH, 3-OH-Stearoyl		* 0.01	* 0.01	* 0.01	* 0.05 H	* 0.02 H
C18:1-OH, 3-OH-Oleyl		* 0.01	* 0.01	* 0.01	* 0.11 H	* 0.02 H
C18:2-OH, 3-OH-Linoleyl		* 0.00	* 0.02 H	* 0.01	* 0.11 H	* 0.03 H

Very Long Chain AcylCoA Dehydrogenase (VLCAD) Deficiency MIM 201450

Frequency: 1:63,481 (USA) 1:27,617 (Utah)

Cause: mutations in *ACADVL* gene

Presentation: 1. Early onset, hypertrophic cardiomyopathy, high morbidity and mortality; 2. Milder form with hypoketotic hypoglycemia, similar to MCAD deficiency with increased LFTs, elevated CPK; 3. Stress-induced rhabdomyolysis, like myopathic CPT2 deficiency.

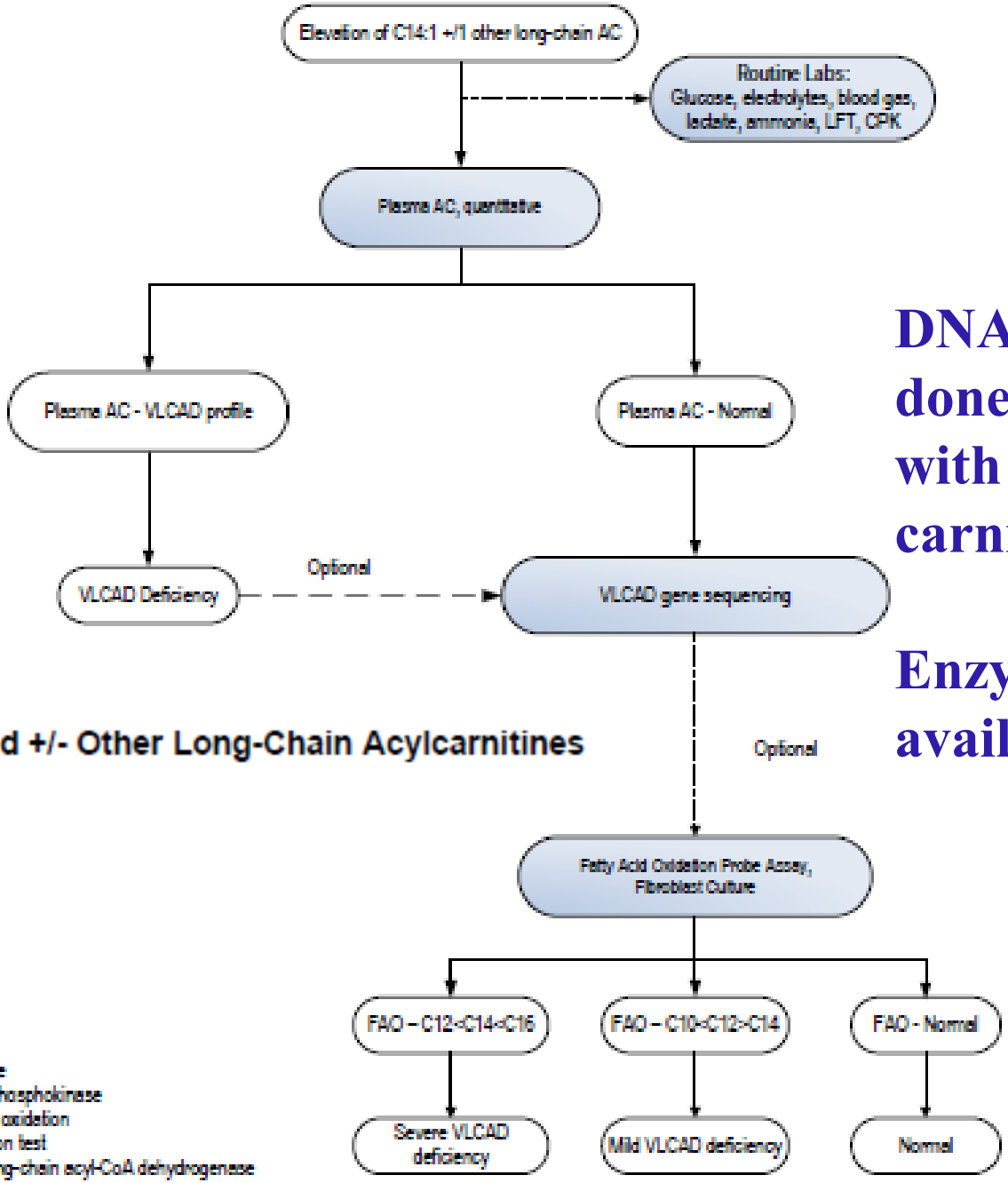
Diagnosis: plasma acylcarnitine profile (elevated C14:1, normalizes rapidly after stress), DNA testing (part of initial tests), FAO fluxes, VLCAD enzyme assay

Therapy: avoidance of fasting, prompt treatment of infection, MCT oil/triheptanoin with persistently abnormal acylcarnitines, low-fat diet, carnitine (25 mg/kg) with low plasma levels (unproven), MCT oil, triheptanoin, sugary drinks with exercise.

Monitoring: AST, ALT, CK, carnitine F & T, acylcarnitines, heart

Prognosis: Can be good with treatment

Rovelli V, Manzoni F, Viau K, Pasquali M, Longo N. Clinical and biochemical outcome of patients with very long-chain acyl-CoA dehydrogenase deficiency. Mol Genet Metab. 2019 May;127(1):64-73. doi: 10.1016/j.ymgme.2019.04.001. Epub 2019 Apr 16. PMID: 31031081



C14:1 Elevated +/- Other Long-Chain Acylcarnitines

Abbreviations:
AC = acylcarnitine
CPK = creatine phosphokinase
FAO = Fatty acid oxidation
LFT = liver function test
VLCAD = very long-chain acyl-CoA dehydrogenase

DNA testing must be done in all patients with elevated C14:1 carnitine

Enzyme assay is available in WBC

Long Chain 3-OH-AcylCoA Dehydrogenase (LCHAD) 609016 / Trifunctional Protein (TFP) 609105 Deficiency

LCHAD is part of a trifunctional protein (TFP). Mutations can abolish all 3 functions or only LCHAD activity

Frequency: 1:303,222 (USA) 1: 255,365 (Utah)

Cause: mutations in *HADHA* or *HADHB* gene

Presentation: IUGR, prematurity, fasting-induced vomiting and hypoglycemia, hypotonia, cardiomyopathy, liver dysfunction, sudden death. Rhabdomyolisis with stress/exercise/fasting. Retinitis pigmentosa with time. Neuropathy (more pronounced in TFP deficiency). Preeclampsia in mothers of infants with LCHAD deficiency

Diagnosis: High C16OH (C14OH, C18OH, C18:1OH) and other long-chain carnitines, DNA testing

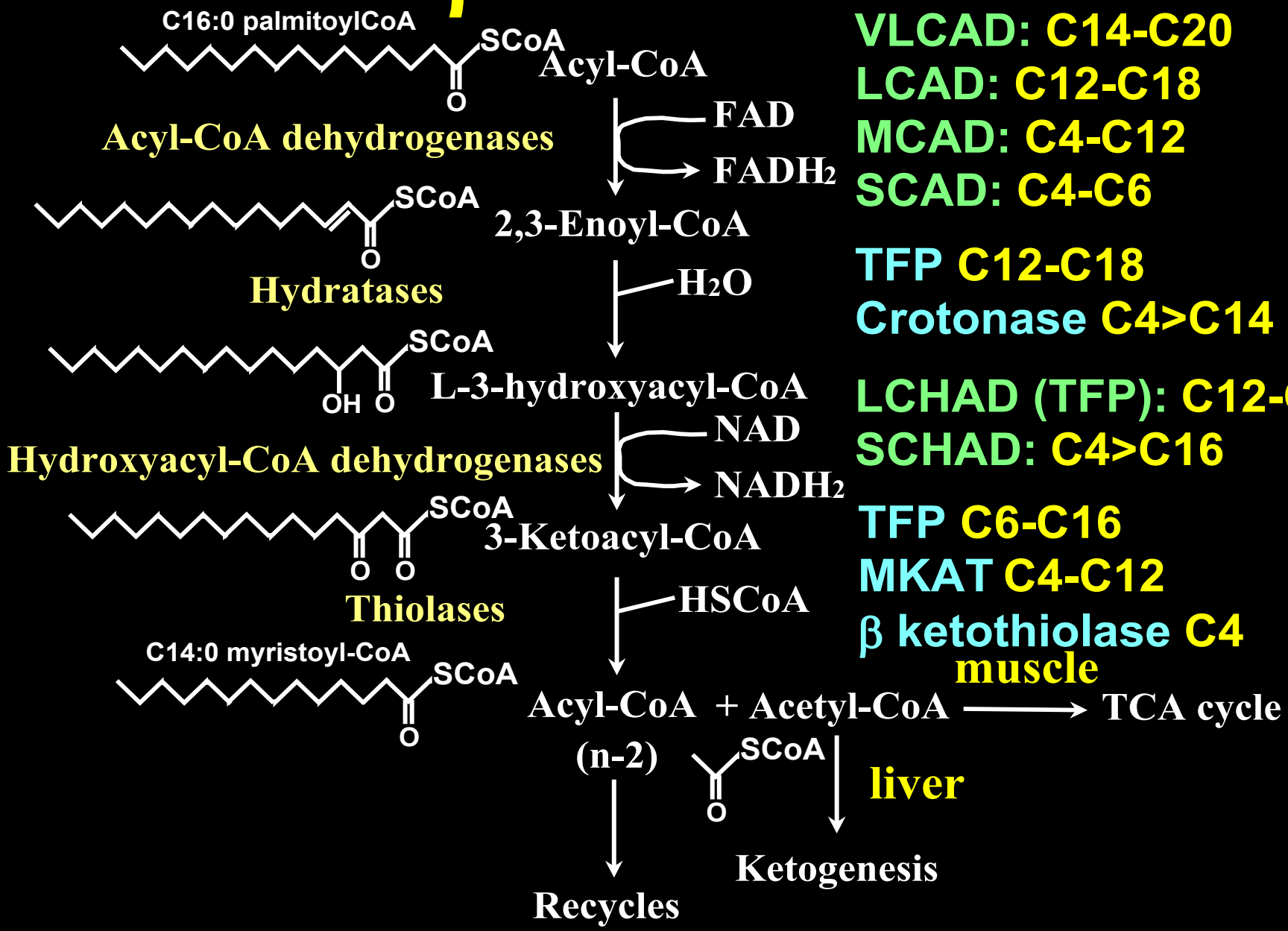
Therapy: avoidance of fasting, MCT oil, triheptanoin, low-fat diet, essential FA, Carnitine (25 mg/kg) with low plasma levels (unproven)

Monitoring: AST, ALT, CK, carnitine F & T, acylcarnitines, essential FA, eye, heart

Prognosis: bad without treatment, even with treatment there are problems (muscle pain, retinitis pigmentosa, neuropathy)

De Biase I, Viau KS, Liu A, Yuzyuk T, Botto LD, Pasquali M, Longo N. Diagnosis, Treatment, and Clinical Outcome of Patients with Mitochondrial Trifunctional Protein/Long-Chain 3-Hydroxy Acyl-CoA Dehydrogenase Deficiency. JIMD Rep. 2017;31:63-71. doi: 10.1007/8904_2016_558. Epub 2016 Apr 28. PMID: 27117294

β-OXIDATION



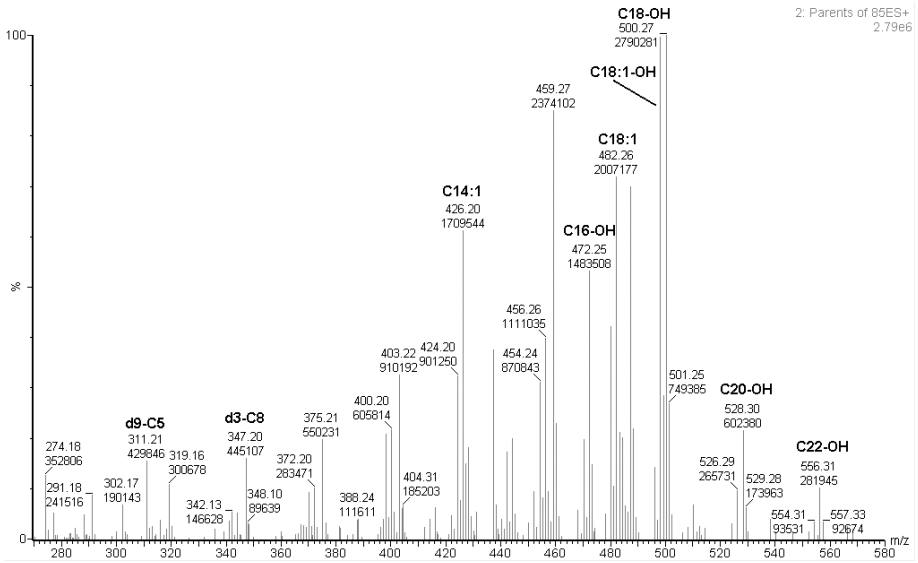
VLCAD: C14-C20
LCAD: C12-C18
MCAD: C4-C12
SCAD: C4-C6

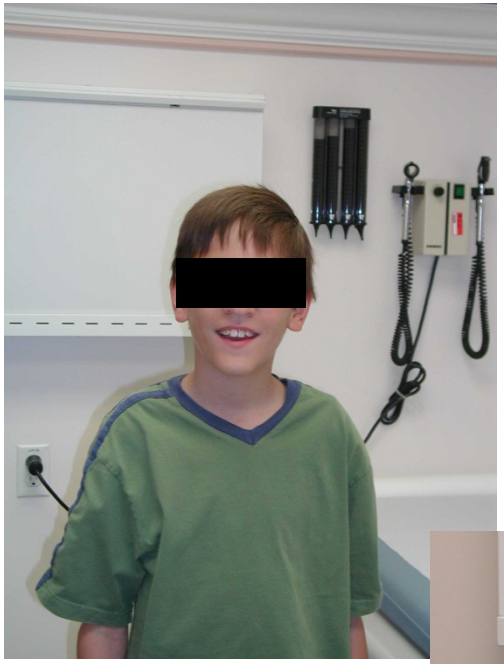
TFP C12-C18
Crotonase C4>C14

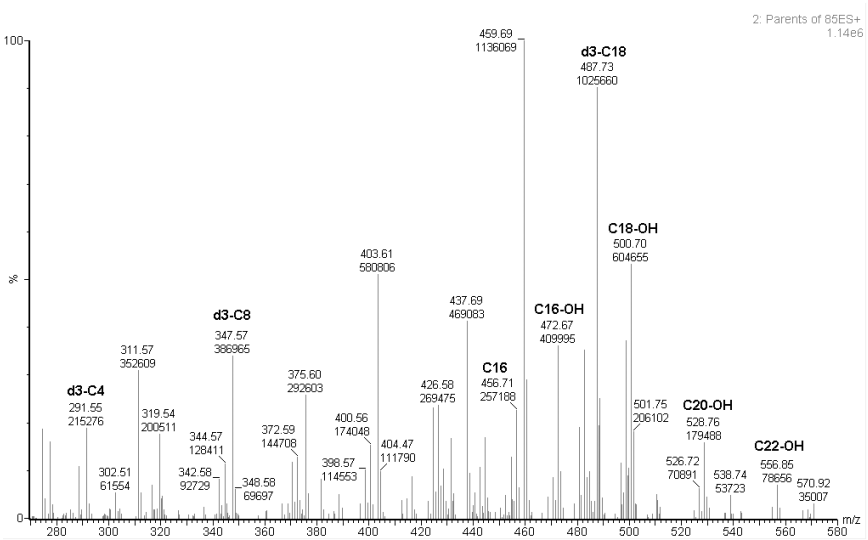
LCHAD (TFP): C12-C18
SCHAD: C4>C16

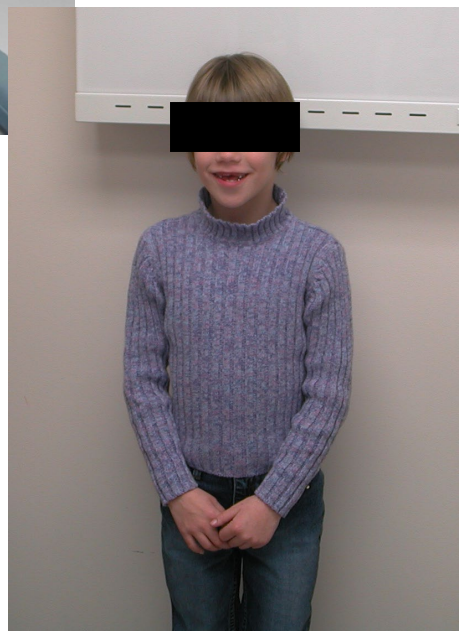
TFP C6-C16
MKAT C4-C12
β ketothiolase C4

Long Chain 3-OH-AcylCoA Dehydrogenase (LCHAD) Deficiency



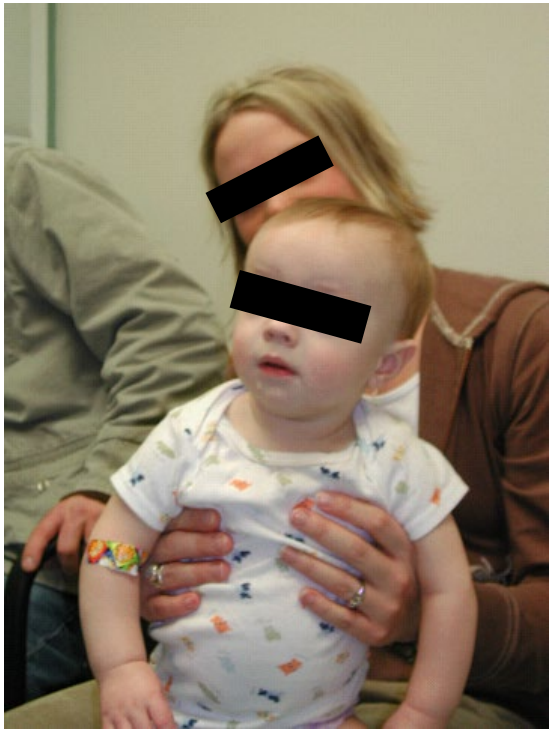




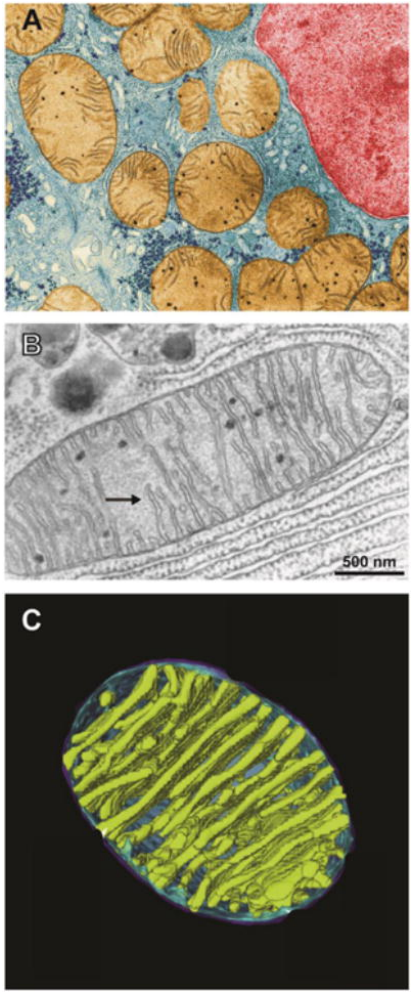


Long Chain 3-OH-AcylCoA Dehydrogenase Deficiency (LCHAD)

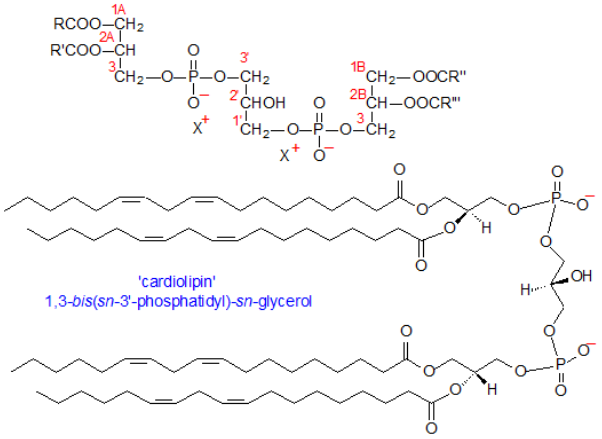
- **AFLP (acute fatty liver of pregnancy) syndrome or HELLP (hypertension, elevated liver functions, and low platelets) are frequent in mothers carrying a fetus with LCHAD deficiency. Patients do very well when treated, but can decompensate with fever, infections and require prompt hospital admission to receive intravenous glucose.**
  Mentality is normal.

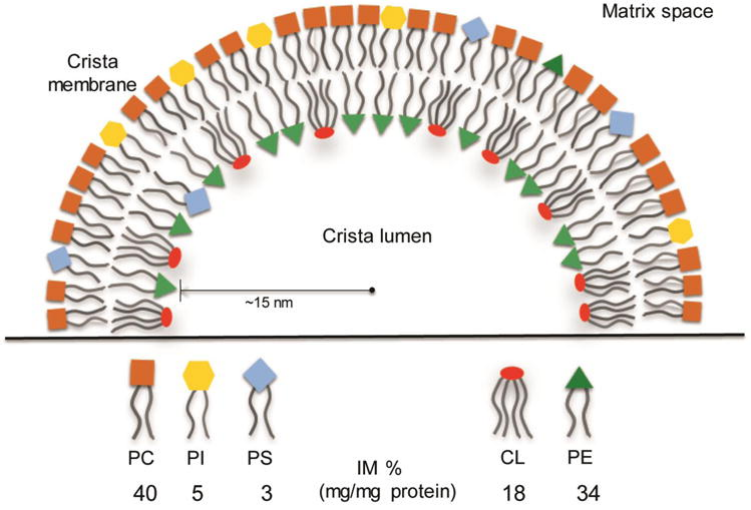


α–TFP MODIFIES CARDIOLIPIN



TFP has a fourth enzymatic activity: Monolysocardiolipin (MLCL) acyltransferase (MLCL AT)-1 activity. Cardiolipin constitutes about 20% of the total lipid composition of the inner mitochondrial membrane. Cardiolipin is essential for the formation of cristae.

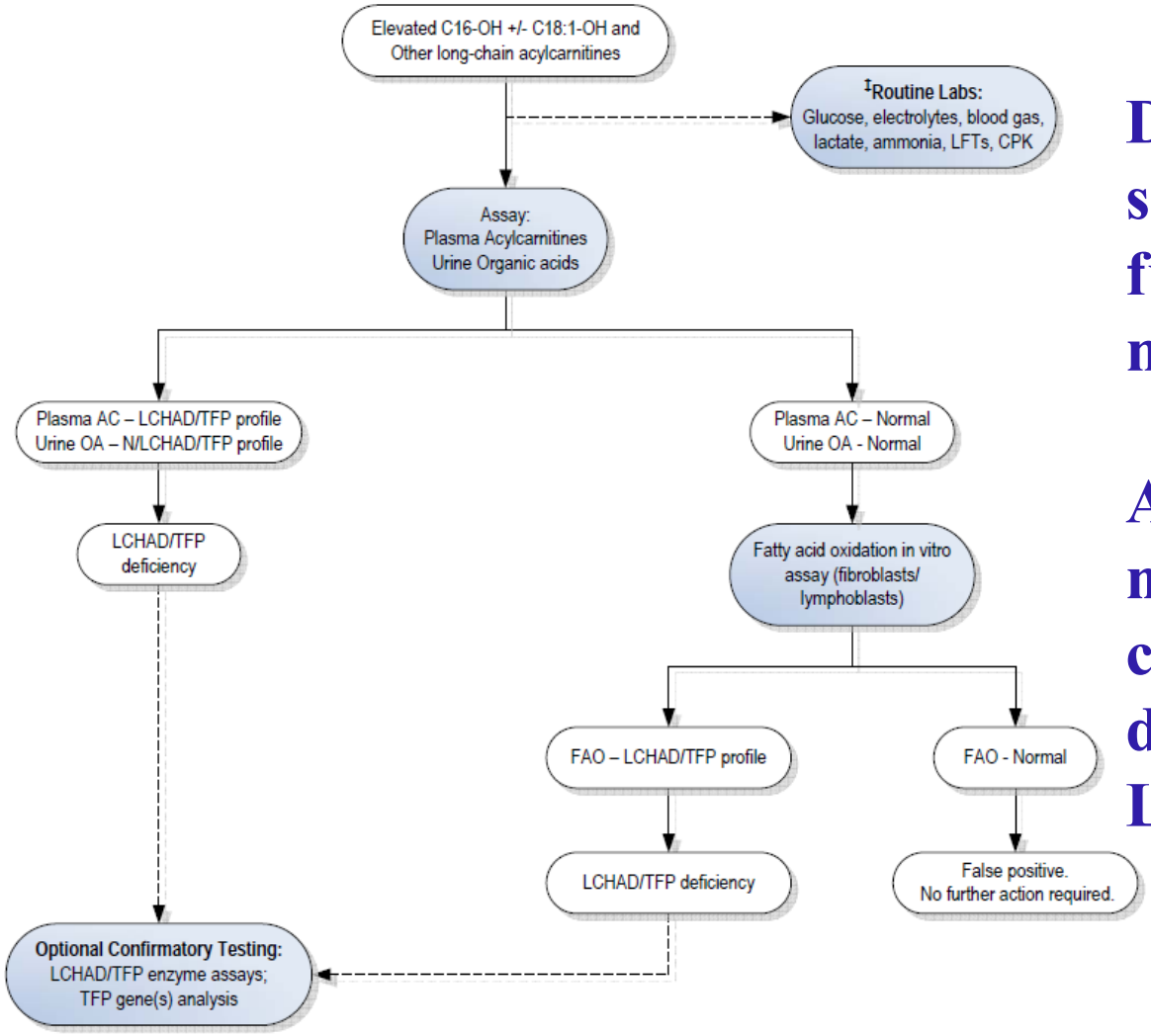




Ikon N, Ryan RO. Cardiolipin and mitochondrial cristae organization.Biochim Biophys Acta Biomembr. 2017 Jun;1859(6):1156-1163. PMID: 28336315

Taylor WA, Mejia EM, Mitchell RW, Choy PC, Sparagna GC, Hatch GM. Human trifunctional protein alpha links cardiolipin remodeling to beta-oxidation. PLoS One. 2012;7(11):e48628. doi: 10.1371/journal.pone.0048628. Epub 2012 Nov 9. PMID: 23152787

C16-OH Elevated +/- C18:1-OH and Other Long-Chain Acylcarnitines



DNA testing has substituted functional studies in most cases.

Acylcarnitines can normalize in some cases of TFP deficiency, not in LCHAD

Short-Chain Acyl-CoA Dehydrogenase (SCAD) deficiency (OMIM 201470)

Biochemical alteration of fatty acid oxidation with unclear clinical significance.

Frequency: 1:40,000-1:100,000. Polymorphisms in this gene are very frequent in the general population.

Cause: Mutations in *ACADS* gene 12q24.31

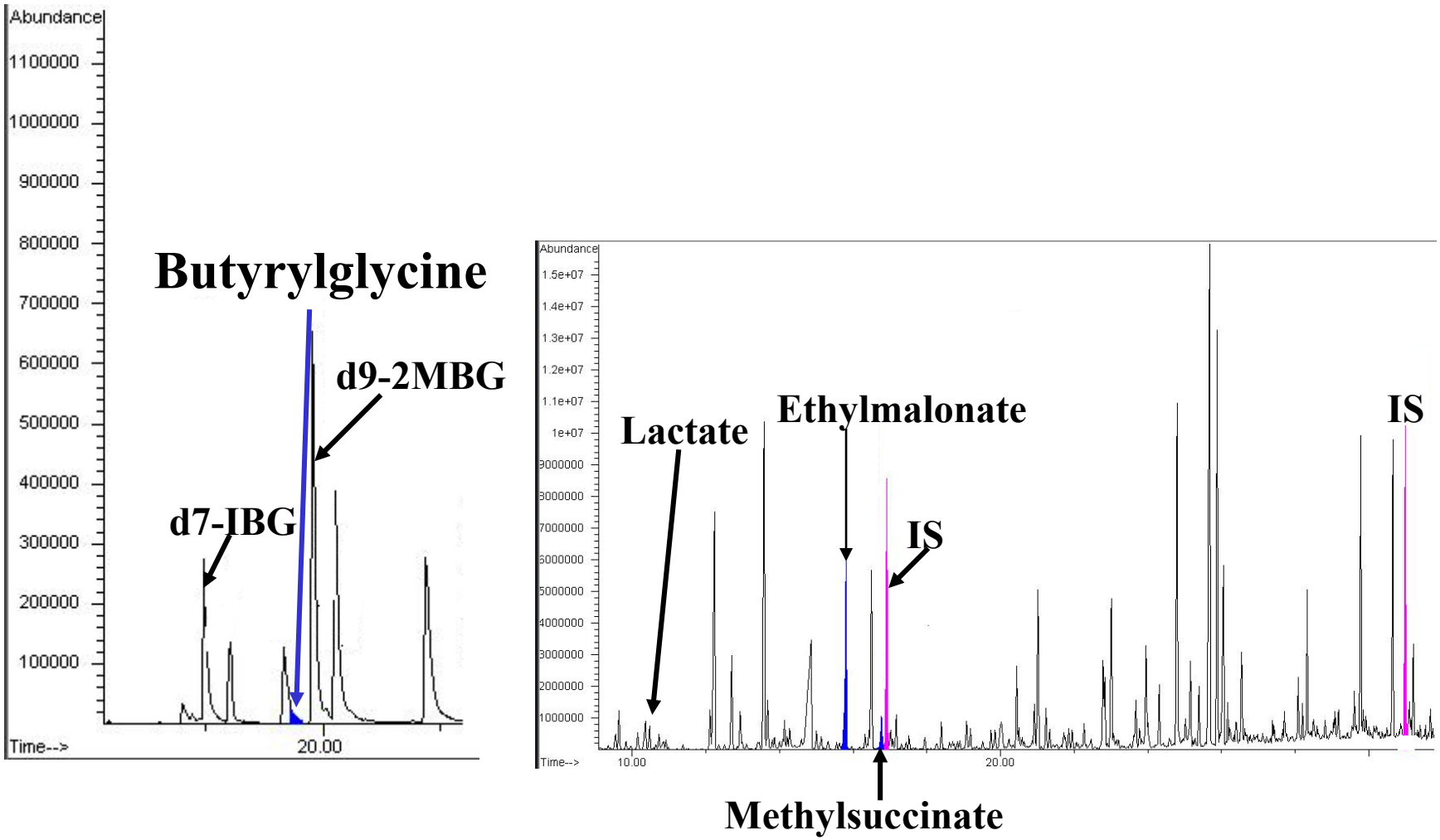
Presentation: hypotonia, myopathy, most likely no symptoms. This is a non-disease.

Diagnosis: Urinary organic acids: elevated ethylmalonic and methylsuccinic acids, n-butyrylglycine. Plasma acylcarnitine profile: increased C4 (butyrylcarnitine). Confirmed by DNA testing

Treatment: None. No therapy required.

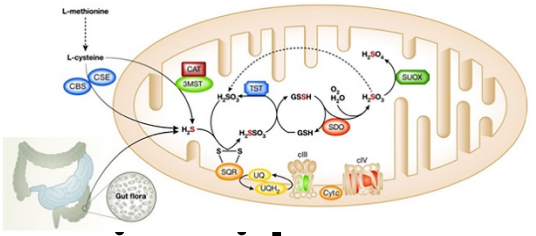
Need to exclude isobutyryl glycinuria (elevated isobutyryl glycine) and ethylmalonic encephalopathy (persistent lactic acidemia).

SCAD Deficiency: Urine acylglycines and urine organic acids



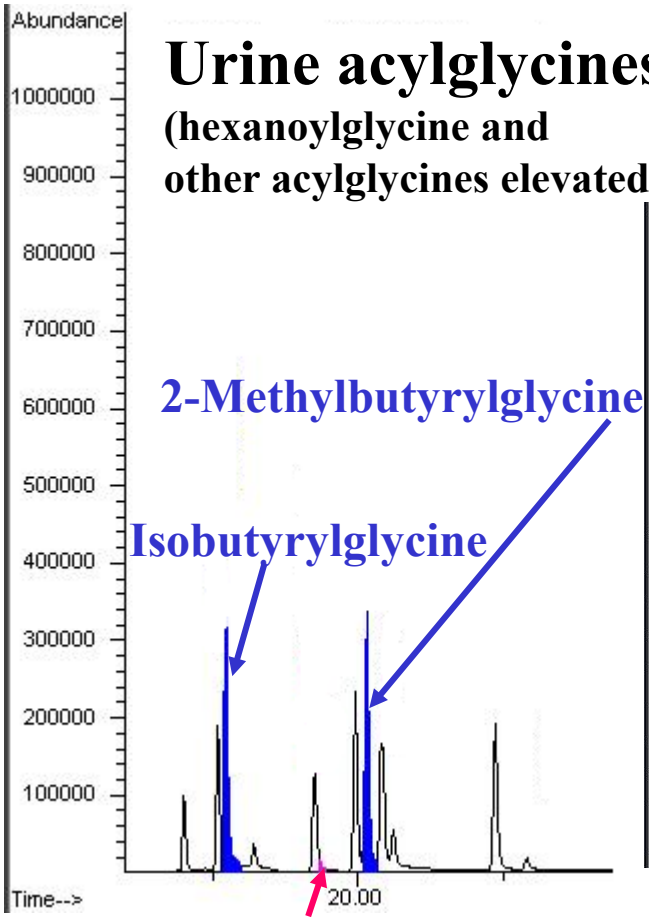
Ethylmalonic encephalopathy

Neurodegenerative disorder affecting the brain, GI tract, and peripheral vessels, caused by mutations in *ETHE1* gene encoding a mitochondrial matrix protein

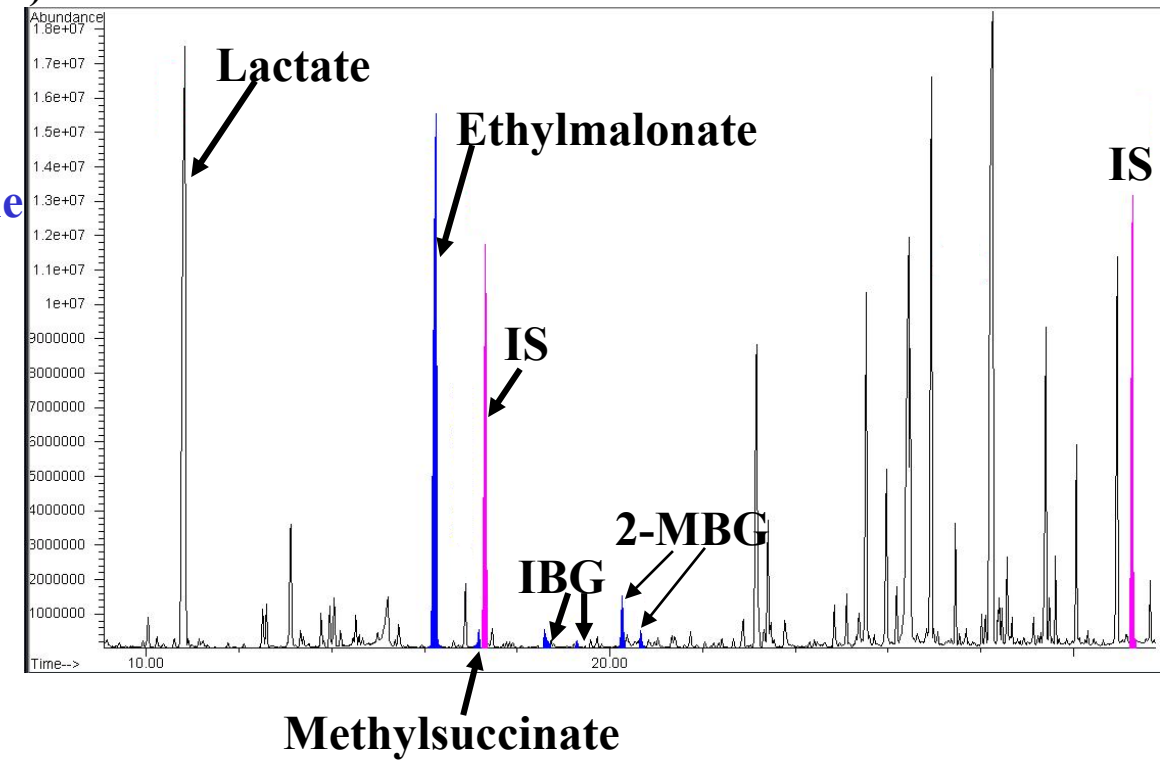


Urine acylglycines

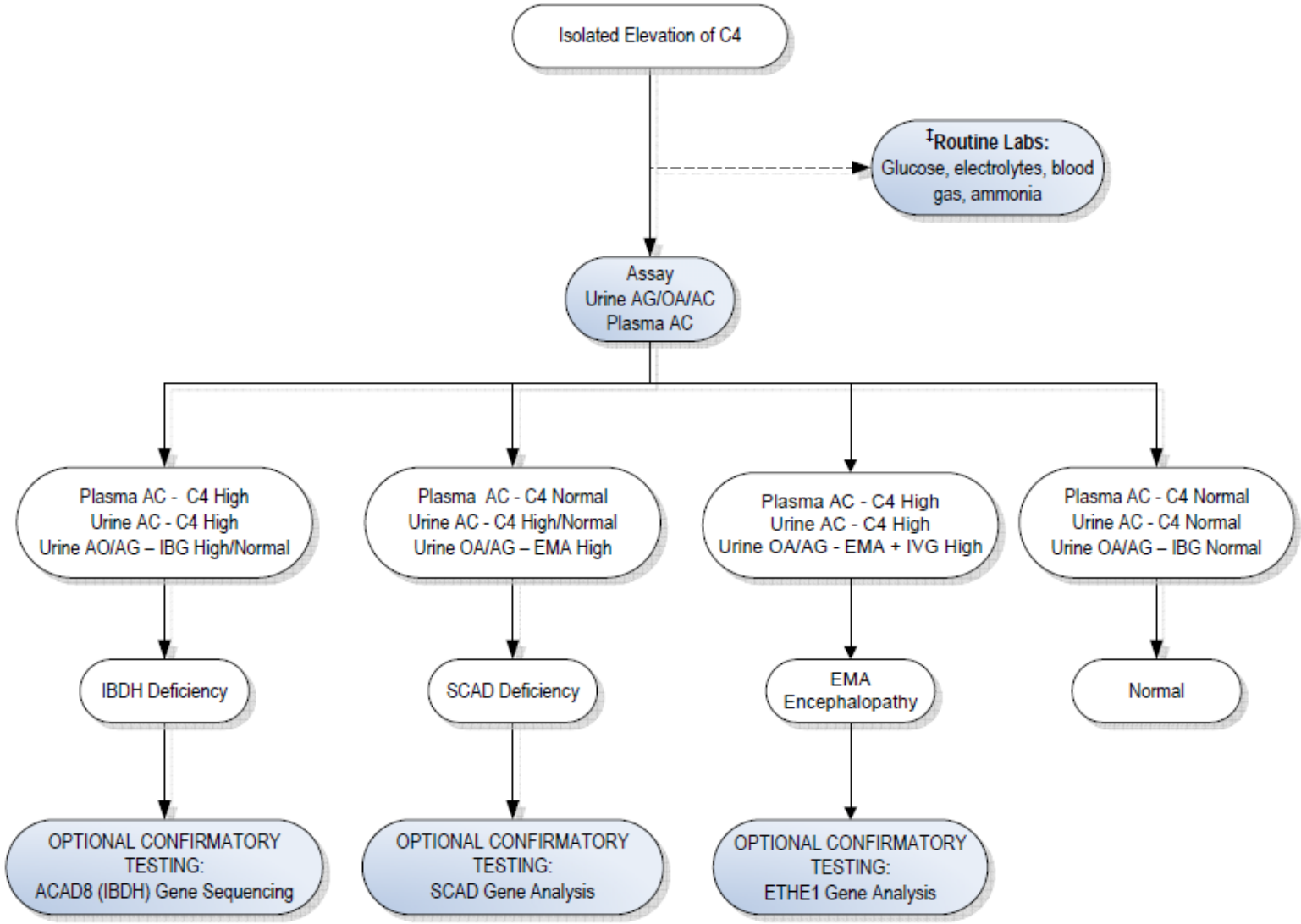
(hexanoylglycine and other acylglycines elevated)



Urine organic acids



C4 Elevated (Isolated)



Glutaric acidemia type 2 (GA-2)/Multiple Acyl-CoA Dehydrogenase Deficiency (MADD)

Disorder of mitochondrial fatty acid and organic acid metabolism

Frequency: 1:100,000

Cause: mutations impair the activity of the electron transfer flavoprotein (ETF) (*ETFA* and *ETFB* genes) or ETF ubiquinone oxidoreductase (ETFQO) (*ETFDH* gene) preventing electron transfer from multiple dehydrogenases. Riboflavin deficiency.

Presentation: neonatal-onset: with or without congenital anomalies (usually fatal): dysmorphic features with multiorgan abnormalities (if present), nonketotic hypoglycemia, metabolic acidosis, multisystem involvement, and excretion of large amounts of abnormal fatty acid and organic acid metabolites.

Late-onset: recurrent episodes of lethargy, vomiting, hypoglycemia, metabolic acidosis, and hepatomegaly often triggered by fever, infection or fasting. Some patients have predominant muscular involvement with pain, weakness, and lipid storage myopathy, neuropathy)

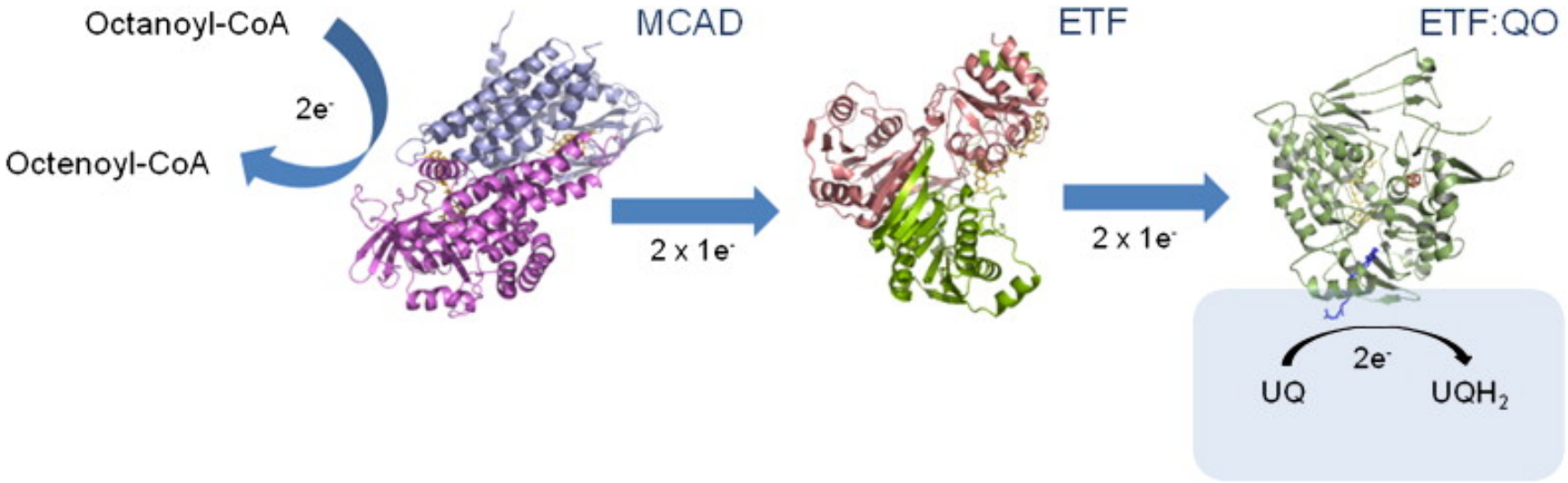
Glutaric acidemia type 2 (GA-2)/Multiple Acyl-CoA Dehydrogenase Deficiency (MADD)

Diagnosis: High C4, C5, C6<C8<C10, C12, C14, C14:1-carnitine, urine organic acids: 2-OH-glutaric, exclude riboflavin deficiency, DNA testing for the 3 genes (*ETFA, ETFB, ETFDH*). *ETFA* mutations are the most frequent followed by *ETFB*. *ETFDH* mutations many times respond to riboflavin

Therapy: avoidance of fasting, prompt treatment of infection, low-fat diet, ketones, riboflavin (100-400 mg/day), ubiquinol (100-400 mg/day) at age 1, carnitine (100 mg/kg), essential FA supplements

Monitoring: AST, ALT, CK, carnitine F & T, acylcarnitines, essential FA, heart

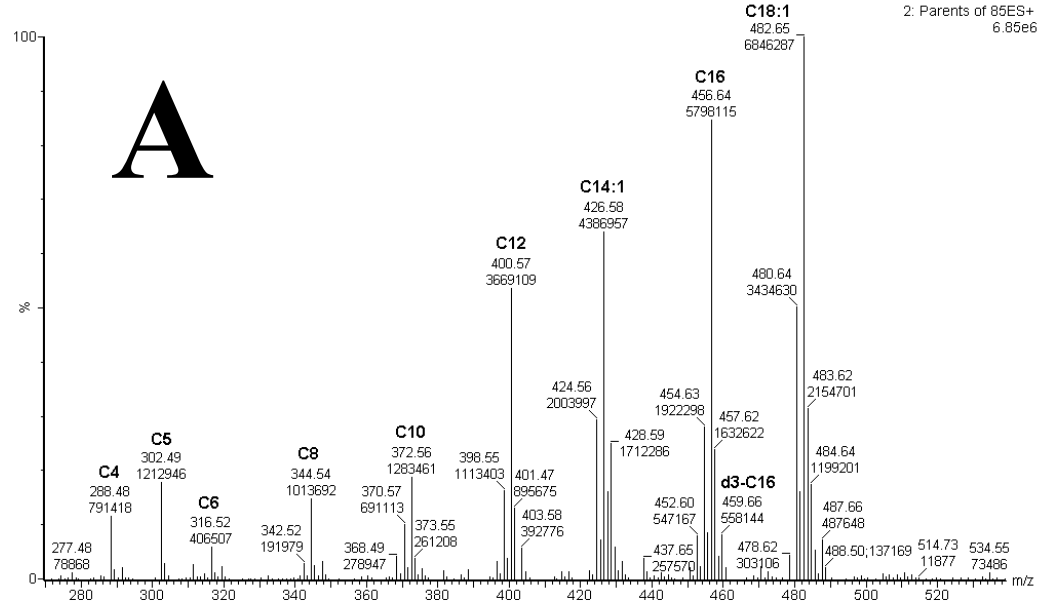
Prognosis: severe for neonatal forms; not well characterized for the others.

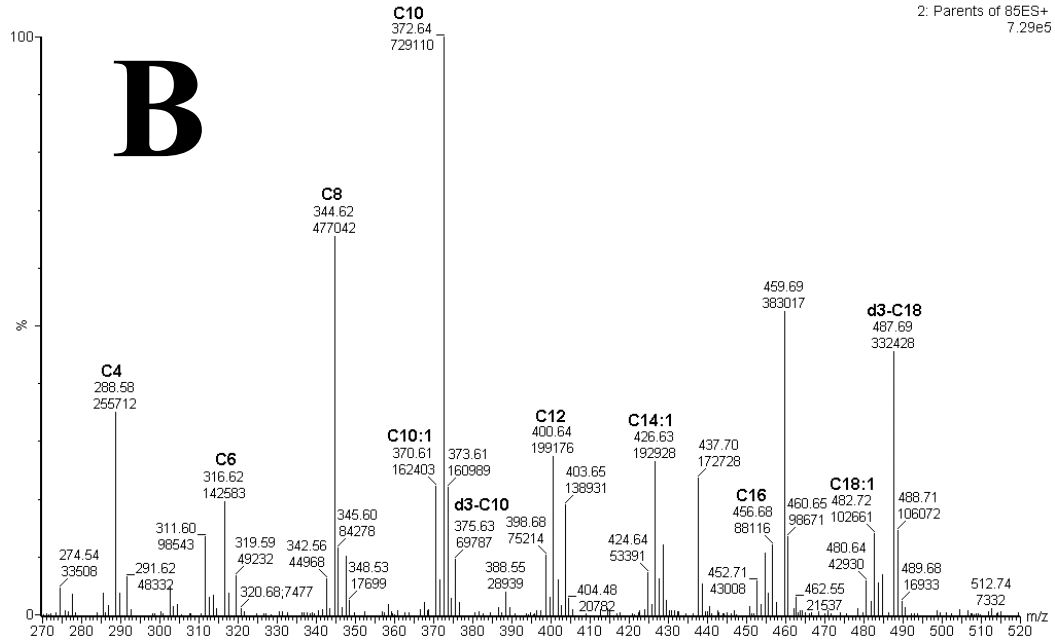


At least 11 different dehydrogenases involved in fatty acid oxidation or amino acid metabolism use flavin adenine nucleotide (FAD) to capture electrons in different reactions. These are transferred to the electron transfer flavoprotein (ETF) and then by the electron transfer flavoprotein oxidoreductase (ETF:QO) to ubiquinone that will carry them along the respiratory chain. A deficiency in this process will impair activity of multiple dehydrogenases (multiple acyl CoA dehydrogenase deficiency – MADD).

Watmough NJ, Frerman FE. The electron transfer flavoprotein: ubiquinone oxidoreductases. Biochim Biophys Acta. 2010 Dec;1797(12):1910-6.

Plasma acylcarnitine profile: MADD





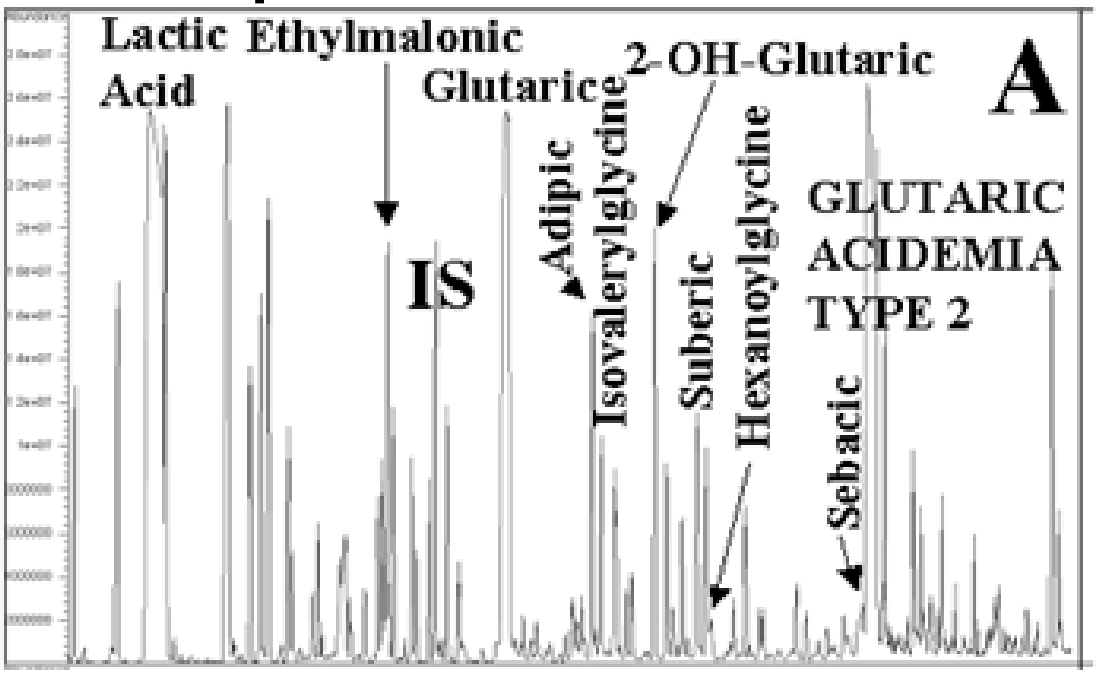
A. Symptomatic at diagnosis (2 days of age).
B. Identified by newborn screening. Similar profile in late-onset patients.

Courtesy of Dr. Marzia Pasquali, ARUP laboratories.

URINE ORGANIC ACIDS: MADD

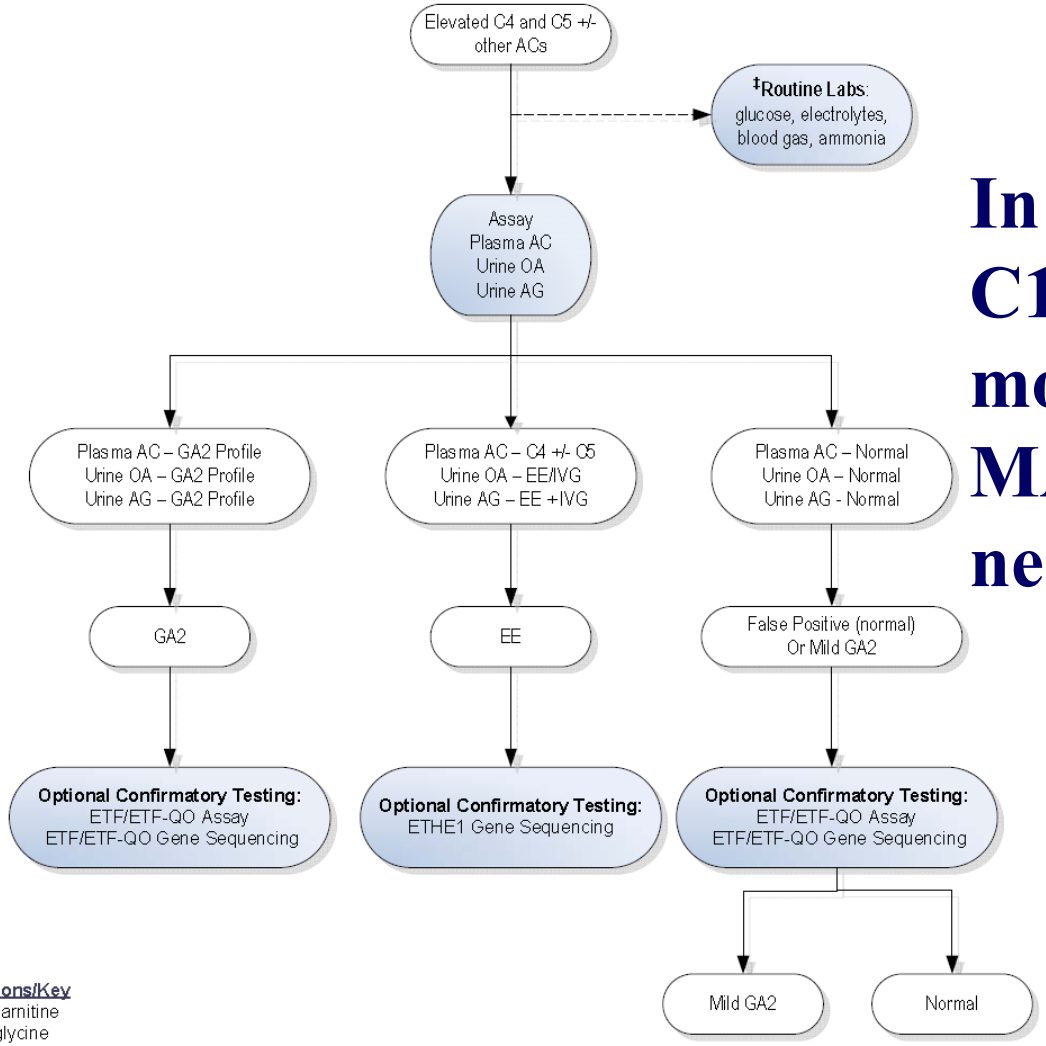
In addition to glutaric acid, isovaleric, lactic and pyruvic, ethylmalonic, 2-OH-glutaric, dicarboxylic acids are also elevated, reflecting impairment of multiple dehydrogenases.

Urine organic acid and urine acylglycines (elevated hexanoyl- and suberyl-glycine) can normalize when the patient is well compensated.



Hedlund GL, Longo N, Pasquali M. Glutaric acidemia type 1. Am J Med Genet C Semin Med Genet. 2006 May 15;142C(2):86-94

C4 and C5 +/-
Other Acylcarnitines Elevated



In reality, C10 and C12 carnitine are the most elevated in MADD (apart neonatal cases)

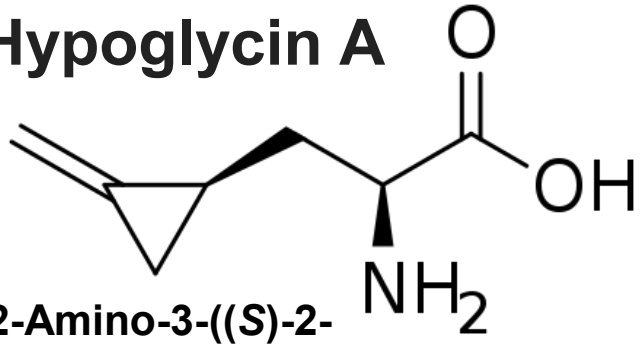
Abbreviations/Key
AC = Acylcarnitine
AG = Acylglycine
EE = Ethylmalonic encephalopthy 1
ETF = Electron transfer flavoprotein
GA2 = Glutaric aciduria Type
IVG = Isovaleryl glycine
OA = Organic acid

‡ = When the positive predictive value of screening is sufficiently high, some initiate diagnostic studies that are locally available at the same time as confirmation of the screening result is done.

MADD-like diseases

Jamaican vomiting sickness: caused by ingestion of unripe akee. Akee tree (Blighia sapida) originates in Western Africa and was brought to Jamaica in 18th century, with the slave ships. It was observed in Ohio with consumption of canned akee.

Hypoglycin A



(*S*)-2-Amino-3-((*S*)-2-methylenecyclopropyl)propanoic acid

Exposure to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and HPTP (the tetrahydropyridinyl analog of haloperidol).

McTague JA, Forney R Jr. Jamaican vomiting sickness in Toledo, Ohio Ann Emerg Med. 1994 May;23(5):1116-8

Mienie LJ, Bergh JJ, Van Staden E, Steyn SJ, Pond SM, Castagnoli N Jr, Van der Schyf CJ. Metabolic defects caused by treatment with the tetrahydropyridine analog of haloperidol (HPTP), in baboons. Life Sci. 1997;61(3):265-72.

SUMMARY

Inherited defects of the carnitine cycle and fatty acid oxidation can present at any age when energy from fat is needed (fasting, infections, fever).

Patients can appear perfectly normal between episodes, for which DNA testing is necessary to confirm or exclude the diagnosis.

Therapy requires fasting avoidance, low fat diet, carnitine, MCT oil/triheptanoin.

SUMMARY

Carnitine transporter deficiency causes low carnitine levels and presents with hepatic encephalopathy, cardiomyopathy and sudden death (Low C0).

CPT-1A deficiency causes high carnitine levels with low levels of long-chain acylcarnitine and can cause hypoglycemia and hepatic failure (High C0).

CACT deficiency can present even at birth with hypoglycemia and cardiac arrest (High C16, C18, C18:1, C18:2, Low C0).

The common form of CPT2 deficiency presents with exercise induced muscle pain and myoglobinuria (High C16, C18, C18:1, C18:2).

SUMMARY

MCAD deficiency is the most frequent FAOD and presents with fasting-induced arrest/hypoglycemia (High C8 (C6<C8>C10,C10:1)).

VLCAD deficiency causes a spectrum of phenotype with hypoglycemia, cardiomyopathy, cardiac arrest, exercise/fasting induced rhabdomyolysis (High C14:1, C14 (C14:1>C14, C16, C18, C18:1)).

LCHAD/TFP deficiency can present even at birth with hypoglycemia and cardiac arrest. Can cause cardiomyopathy, neuropathy retinitis pigmentosa (High C16OH (C14OH, C18OH, C18:1OH)).

SUMMARY

SCAD deficiency is a benign condition. Important to distinguish from isobutyrylglycinuria and ethylmalonic encephalopathy (High C4).

MADD deficiency causes a spectrum of phenotype with hypoglycemia, cardiomyopathy, cardiac arrest, exercise/fasting induced rhabdomyolysis. Can be mimicked by riboflavin deficiency (High C4, C5, C8 (C6<C8<C10, C12, C14, C14:1)).

DISORDERS OF KETONE BODIES SYNTHESIS AND UTILIZATION

Objectives

Understand why and where ketones are synthesized

Define enzymes involved in ketone synthesis and utilization

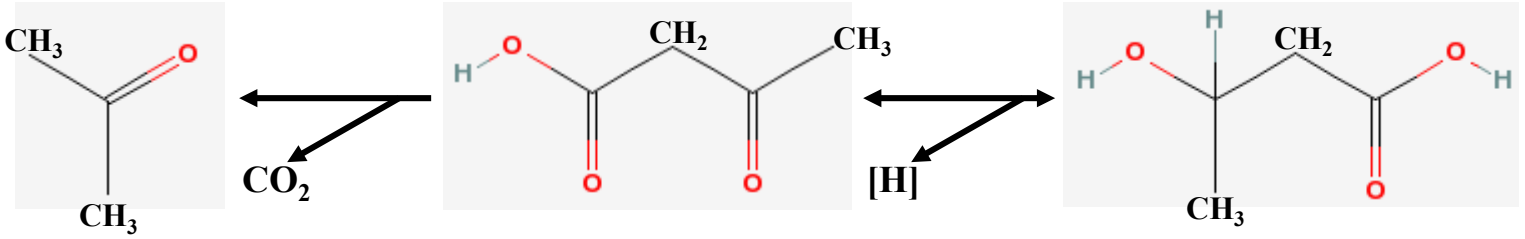
List therapies for disorders of ketone body synthesis and utilization

KETONE BODIES METABOLISM

- Ketone bodies are important in energy transfer during fasting or other lypolitic stresses.
- They derive from beta-oxidation of fatty acids and from ketogenic amino acid (leucine, lysine, isoleucine) catabolism.
- They are produced in liver mitochondria and are transported to extrahepatic tissues where they are utilized.
- Ketogenesis (hepatic ketone body formation) and ketolysis (extra hepatic ketone body utilization) are important processes, especially for the brain, to provide energy when glucose can not meet the metabolic need.
- Physiological levels of ketone bodies in plasma range from <0.1 mM (post-prandial) to 6 mM (prolonged fasting), they can reach 25 mM in diabetic ketoacidosis.
- Most of the ketone bodies are taken up by the extra hepatic tissues, 10-20% are lost in the urine during ketosis.

KETONE BODIES

- Three compounds are usually listed as “ketone bodies”: 3-hydroxybutyrate, acetoacetate, acetone.
- Acetoacetate is the main ketone body, acetone derives from its decarboxylation, while 3-hydroxybutyrate derives from its reduction.



Acetone **Acetoacetic acid** **3-Hydroxybutyric acid**

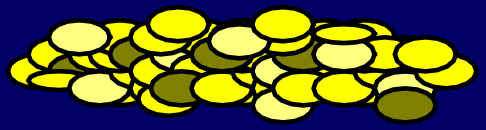
KETONE BODIES METABOLISM

- Rate of utilization of ketone bodies is proportional to their circulating levels.
- Heart and kidney have the greatest capacity for ketone utilization.
- The ketogenic pathway provides fat-derived fuel for the brain when glucose is low.
- Patients with defects in ketone synthesis or degradation are asymptomatic unless they are fasting:
  - **Defects of ketogenesis: hypoketotic hypoglycemia**
  - **Defects of ketolysis: ketoacidosis (severe) ± hypoglycemia**

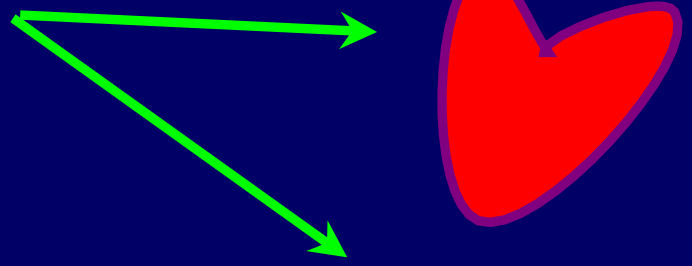
HSL (Hormone Sensitive Lipase) Releases Fatty Acids from adipocytes. Transcription of HSL is increased during fasting and suppressed by insulin and glucose.

FATTY ACID OXIDATION DURING FASTING

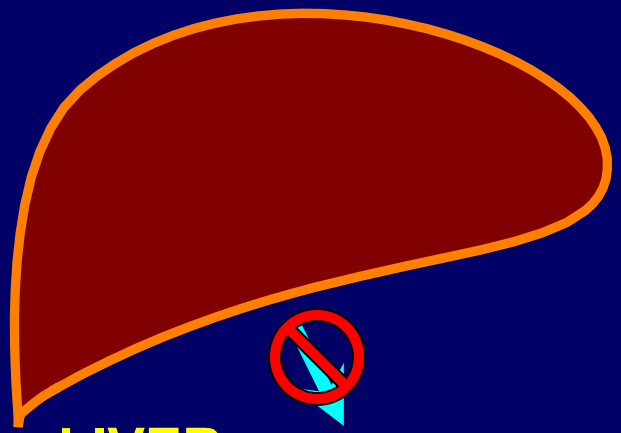
ADIPOSE TISSUE



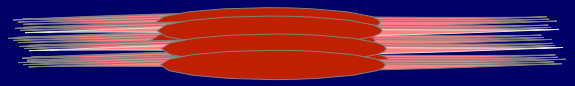
FATTY ACIDS



HEART



LIVER



SKELETAL MUSCLE

KETONES

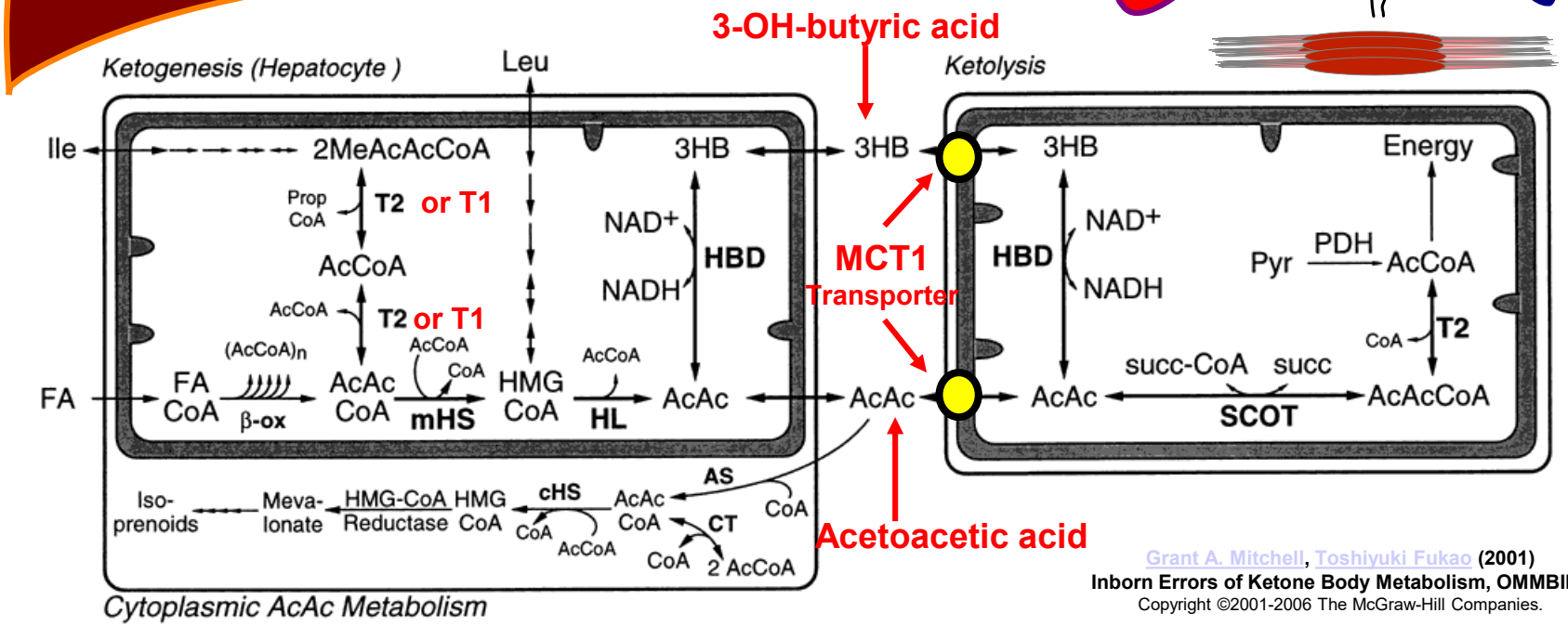
ß-hydroxybutyrate
acetoacetate

BRAIN

KETOGENESIS AND KETOLYSIS

- Ketogenesis is regulated by two hepatic mitochondrial enzymes:
  - **3-hydroxy-3-methylglutaryl-CoA synthase (mHS)**
  - **3-hydroxy-3-methylglutaryl-CoA lyase (HL)**
- Ketolysis in extra hepatic mitochondria is mediated by reversible reactions catalyzed by:
  - **The MCT1 transporter (*SLC16A1*): entry of ketones into tissues**
  - **SuccinylCoA:3-ketoacid(oxoacid) CoA transferase (SCOT)**
  - **Mitochondrial acetoacetyl-CoA thiolase (T2)(*ACAT1*)**
- Deficiencies of mHS or HL cause disorders of ketogenesis; deficiencies of MCT1, SCOT or T2 cause disorders of ketolysis.
- All are inherited as autosomal recessive traits

OGENESIS AND KETOL S



Acetoacetate is synthesized from acetylCoA by cytosolic acetoacetyl-CoA thiolase (*ACAT2* gene, T1). Acetoacetyl-CoA (AcAc-CoA) and acetyl-CoA via two enzymatic steps (mitochondrial Hydroxy Methyl Glutaryl CoA synthase (mHS), a highly regulated enzyme, and Hydroxy Methyl Glutaryl CoA lyase (HL)) form ketones. The liver has both T2 (*ACAT1*, mitochondrial) and T1 (*ACAT2,* cytosolic) thiolase. R-3-hydroxybutyrate dehydrogenase (3HBD) catalyzes the reduction of Acetoacetate to 3-OH-butyrate.

The MCT1 transporter mediates the uptake of ketones by peripheral tissues

HBD: 3-Hydroxy Butyrate Dehydrogenase

T1: *ACAT2*: cytosolic acetoacetyl-CoA thiolase

T2: *ACAT1*: mitochondrial acetoacetyl-CoA thiolase : MAT

DISORDERS OF KETOGENESIS

Mitochondrial 3-Hydroxy-3-Methyl-Glutaryl-CoA Synthase deficiency, mHS (OMIM 605911)

Frequency: rare

Presentation: hypoketotic hypoglycemia, metabolic acidosis, encephalopathy progressing to coma after fasting or infections, hepatomegaly. Can present without hypoglycemia.

Labs: Elevated serum free fatty acids and triglycerides at time of hypoglycemia, elevated acetylcarnitine, but acylcarnitines may be normal, dicarboxylic aciduria can be seen, 4-hydroxy-6-methyl-2-pyrone and 3-hydroxyglutarate can be present, ketones absent or barely present, normal lactate

Diagnosis: DNA testing: *HMGCS2* gene (1p13-p12)

Therapy: Fasting avoidance, cornstarch

DISORDERS OF KETOGENESIS

3-Hydroxy-3-Methyl-Glutaryl-CoA Lyase deficiency, HL (OMIM 246450)

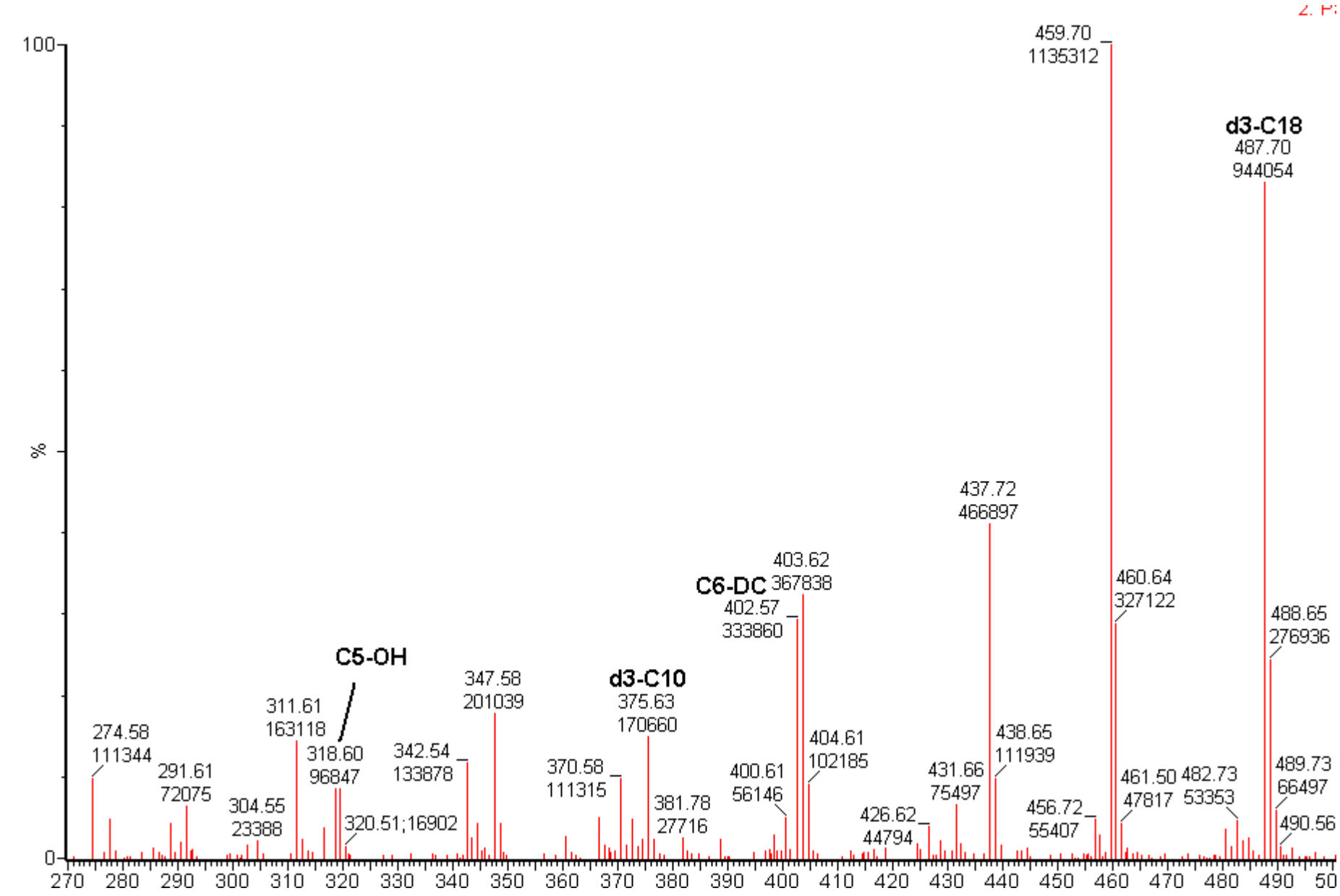
Presentation early in life with vomiting, seizures, unconsciousness, hepatomegaly. Labs: Hyperammonemia, acidosis, increased anion gap, elevated transaminases, hypoglycemia. Organic acids: Elevated excretion of 3-hydroxy-3-methylglutaric acid, 3-methylglutaconic acid, 3-methylglutaric acid, (3-hydroxyisovaleric acid, 3-methylcrotonylglycine); elevated 3-methylglutaryl (C6-DC) and 3-OH-isovaleryl- (C5OH) carnitine.

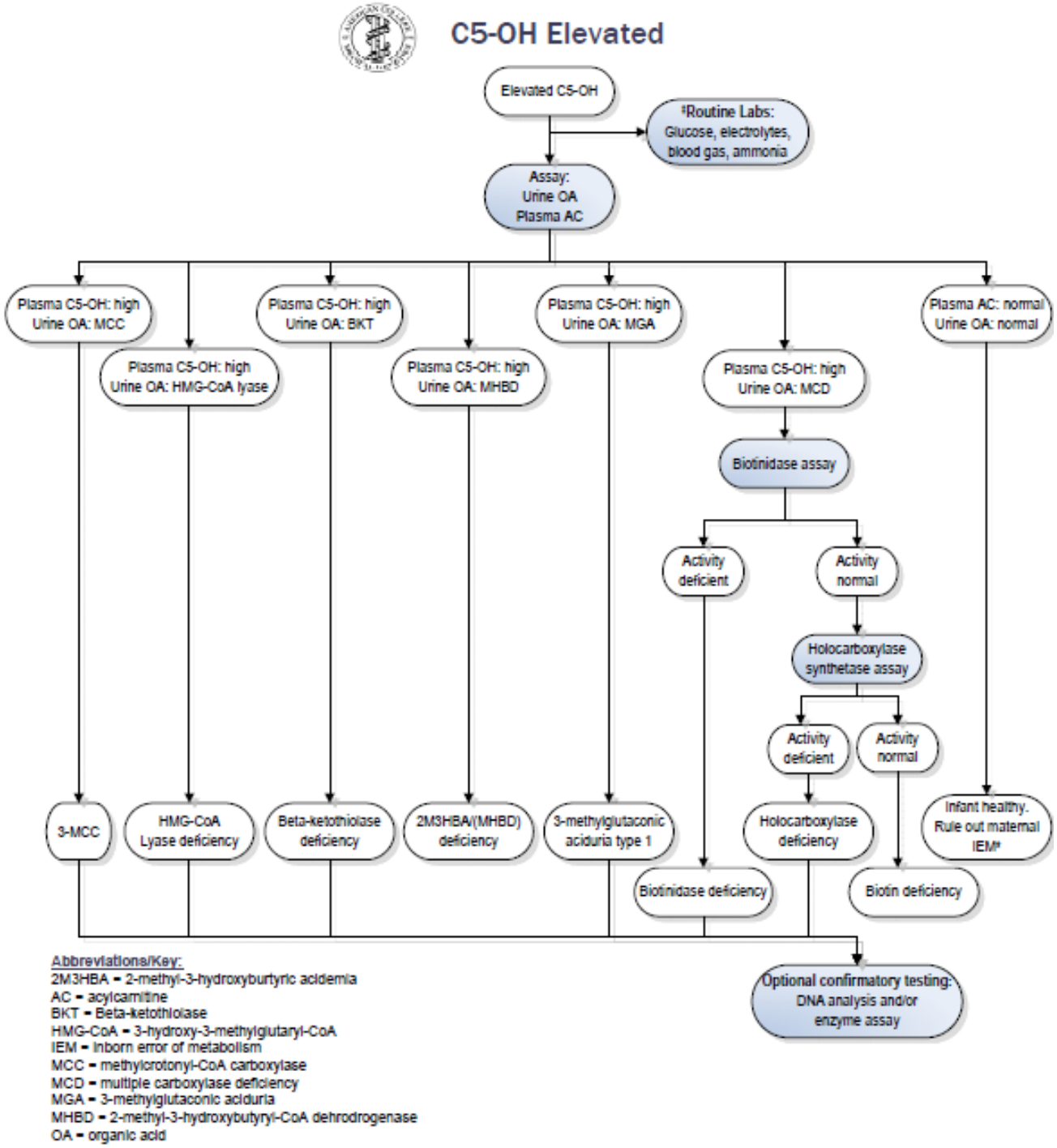
Diagnosis: DNA testing: *HMGCL* gene (1pter-p33)

Therapy: Fasting avoidance, carnitine, moderate protein restriction early in life, reduce fat calories to <30%, cornstarch supplements.

Grünert SC, Schlatter SM, Schmitt RN, Gemperle-Britschgi C, Mrázová L, Balcı MC, Bischof F, Çoker M, Das AM, Demirkol M, de Vries M, Gökçay G, Häberle J, Uçar SK, Lotz-Havla AS, Lücke T, Roland D, Rutsch F, Santer R, Schlune A, Staufner C, Schwab KO, Mitchell GA, Sass JO. 3-Hydroxy-3-methylglutaryl-coenzyme A lyase deficiency: Clinical presentation and outcome in a series of 37 patients. Mol Genet Metab. 2017 Jul;121(3):206-215. doi: 10.1016/j.ymgme.2017.05.014. Epub 2017 May 22. PMID: 28583327

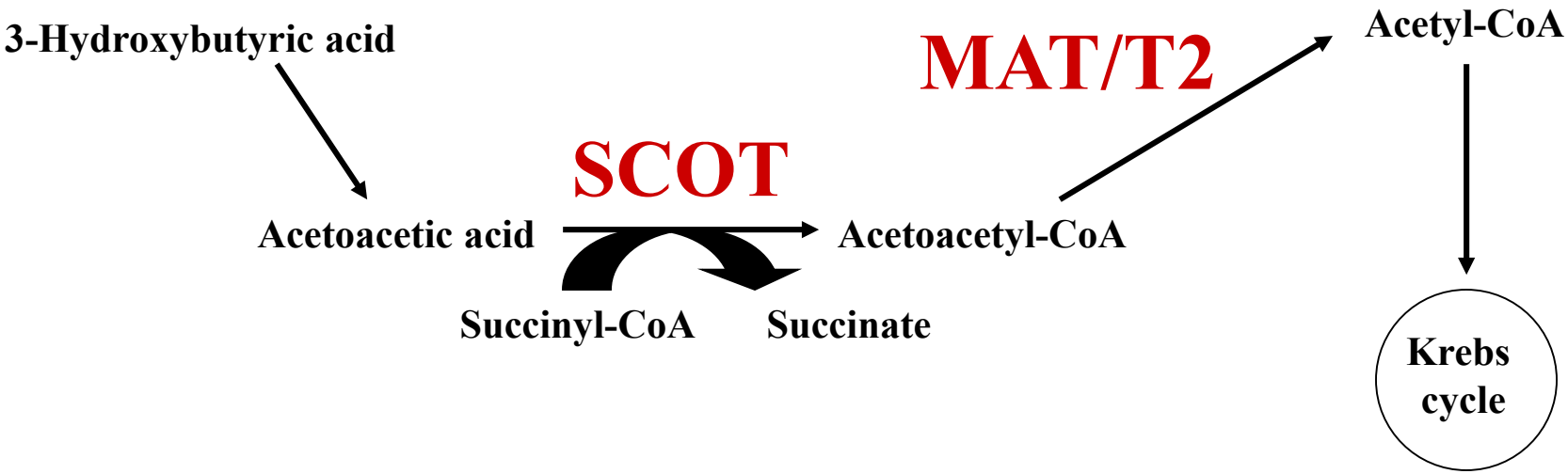
3-Hydroxy-3-Methyl-Glutaryl-CoA Lyase deficiency





3-methylglutaryl (C6-DC) can be elevated as well in HMG-CoA Lyase deficiency

BETA KETOTHIOLASE DEFICIENCY



Mitochondrial acetoacetyl-CoA thiolase, MAT/T2 (OMIM 203750): has a ketolytic role (converts acetoacetyl-CoA and CoA in two molecules of acetyl-CoA) and a ketogenic role (converts 2-methylacetoacetyl-CoA and CoA in acetyl-CoA and propionyl-CoA).

Presentation: ketoacidosis, therefore the ketolytic process is more dependent upon adequate function of MAT/T2: CAT/T1 might bypass the defect in ketone body synthesis.

METABOLIC ACIDOSIS

3-Year-old male with a 24-hour history of vomiting, lethargy, starting the day of admission. In the emergency department, he had a blood glucose of 15 with 3+ ketones in the urine, metabolic acidosis (pH 6.8), bicarbonate <5, and BMP glucose of 7. Head CT was normal. Described as poor eater, very active in his sleep. No previous hospitalizations or surgeries. Has speech delay.

		Units	07/30/07	05/22/07
Na	137-146	mmol/L	139	144
K	3.4-4.7	mmol/L	3.9	4.4
Cl	98-109	mmol/L	106	120 H
CO2	18-24	mmol/L	24	* <5 L
Anion Gap	3-16	mmol/L	9	19 H
Glucose	60-115	mg/dL	91	95
BUN	5-17	mg/dL	12	32 H
Creatinine	0.3-0.7	mg/dL	0.4	0.6
Ca	8.7-9.8	mg/dL	9.4	7.8 L
Prot	5.9-7.0	g/dL	7.5 H	6.1
Alb	3.1-3.9	g/dL	4.7 H	3.6
Bili, Total	0.2-1.3	mg/dL	0.2	<0.1 L
Alk Phos	145-320	U/L	200	235
ALT	5-45	U/L	16	52 H
AST	20-60	U/L	55	69 H
Ammonia	21-50	umol/L	21	* 54 H

METABOLIC ACIDOSIS

- Ketolytic enzymes, Fibroblasts:

Enzyme	Activity	Ref. range	Units
Beta-Ketothiolase	10.3	(8.9-20.6)	nmol/min/mg protein
Succinyl-CoA 3-ketotransferase	7.5	(2.6-8.6)	nmol/min/mg protein

- Interpretation: Beta-ketothiolase activity was in the low normal range, but not stimulated by potassium (normally K doubles enzyme activity).
- DNA *ACAT1* gene: c.T99A, p.Y33X; c.T155C, p.I52T
- Treatment: fasting avoidance, cornstarch and carnitine supplements.

BETA KETOTHIOLASE DEFICIENCY

Mitochondrial acetoacetyl-CoA thiolase deficiency

Presentation: intermittent ketoacidotic episodes during intercurrent illnesses, triggered by vomiting, fever.

Labs: Two groups of patients:

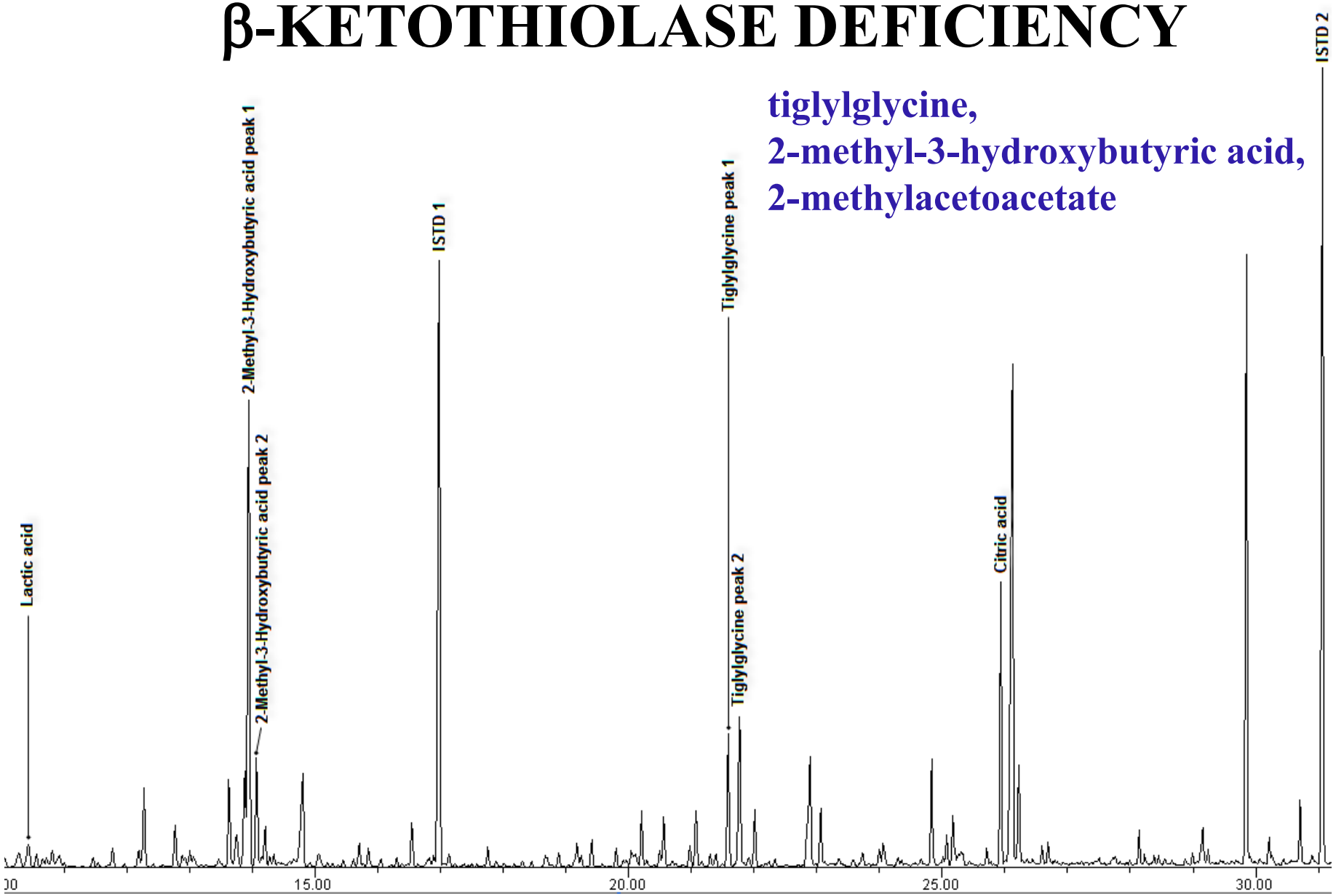
Group 1: no residual enzyme activity; urine organic acids ALWAYS show elevated tiglylglycine, 2-methyl-3-hydroxybutyric acid, 2-methylacetoacetate (unstable, rarely seen) with or without ketoacidosis; elevated tiglylcarnitine (C5:1) and 2-methyl-3-hydroxybutyrylcarnitine (C5OH).

Group 2: some residual enzyme activity; urine organic acids may be normal when stable; elevated tiglylcarnitine (C5:1) and 2-methyl-3-hydroxybutyrylcarnitine (C5OH). Newborn screening (and even acylcarnitine profile in plasma) can miss these patients .

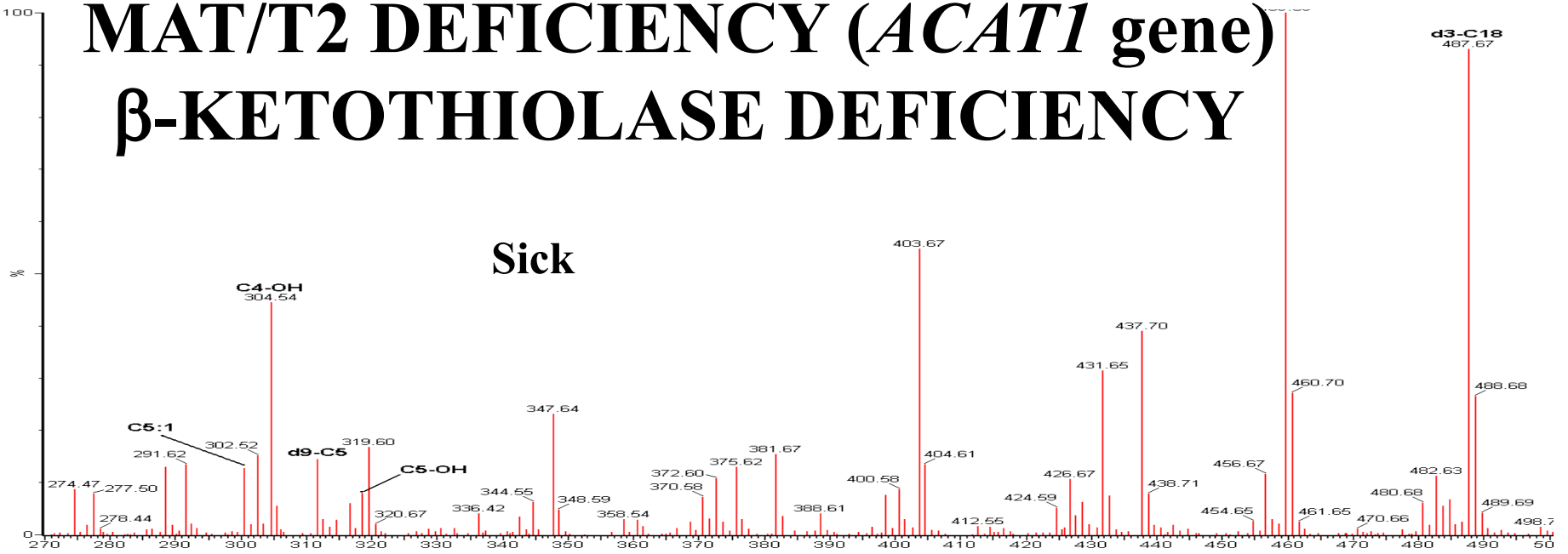
Diagnosis: DNA testing *ACAT1* gene (11q22.3-q23.1), enzyme assay

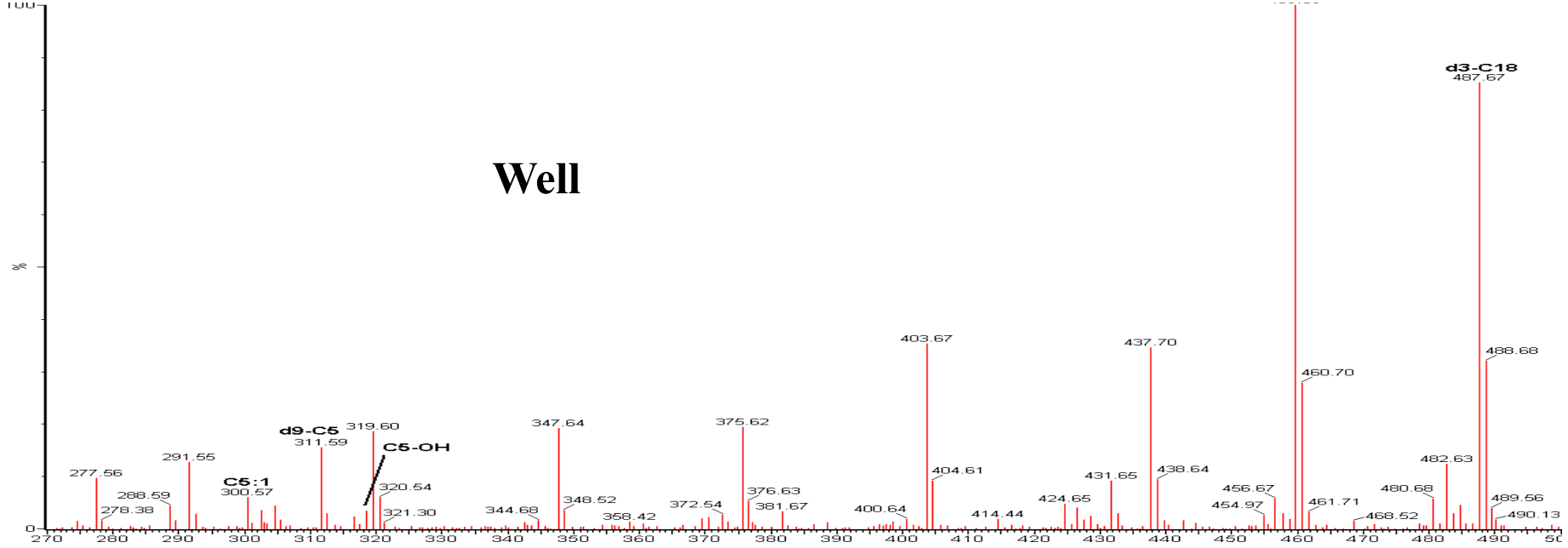
Therapy: Fasting avoidance, cornstarch, carnitine

MAT/T2 DEFICIENCY (*ACAT1* gene)
β-KETOTHIOLASE DEFICIENCY

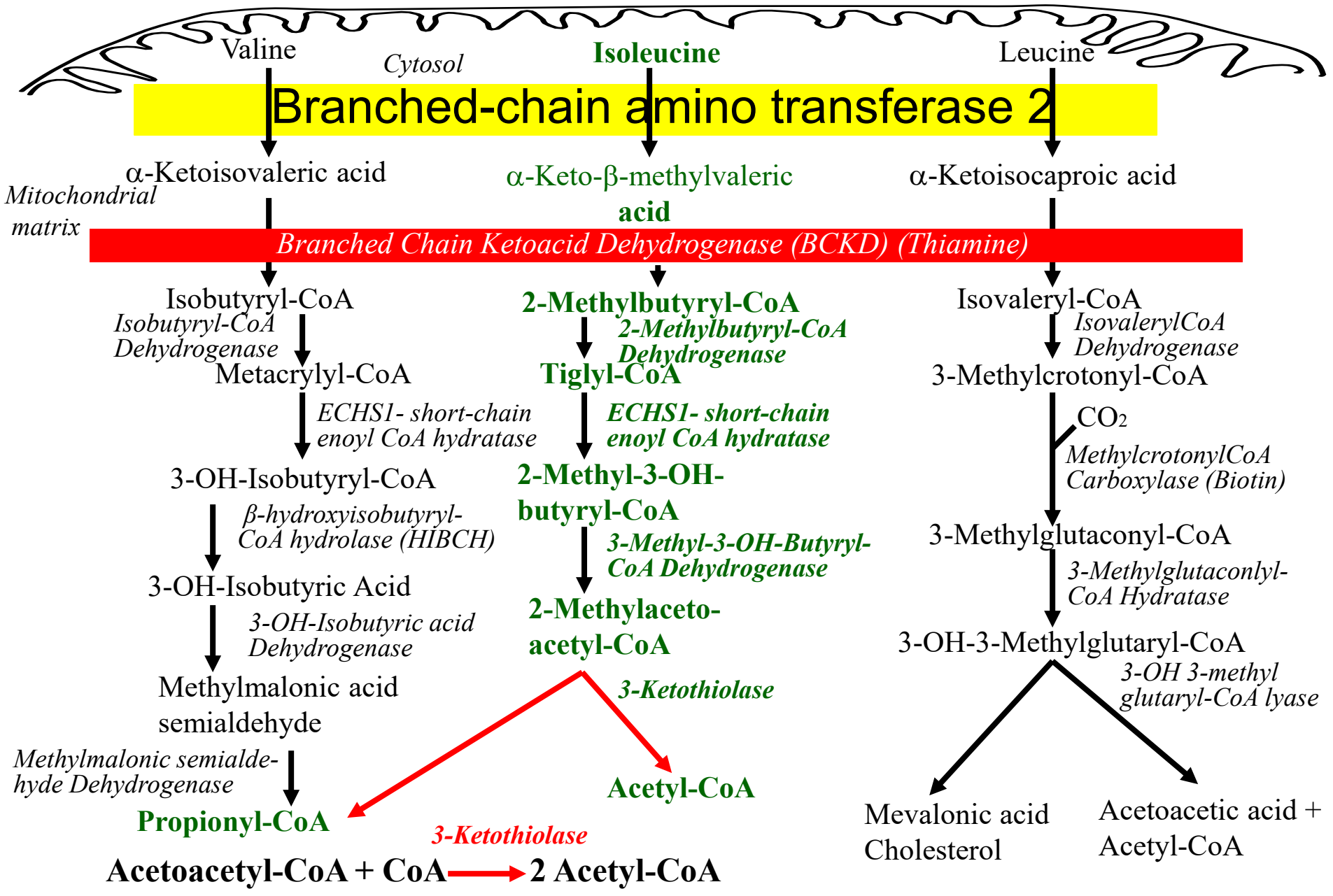


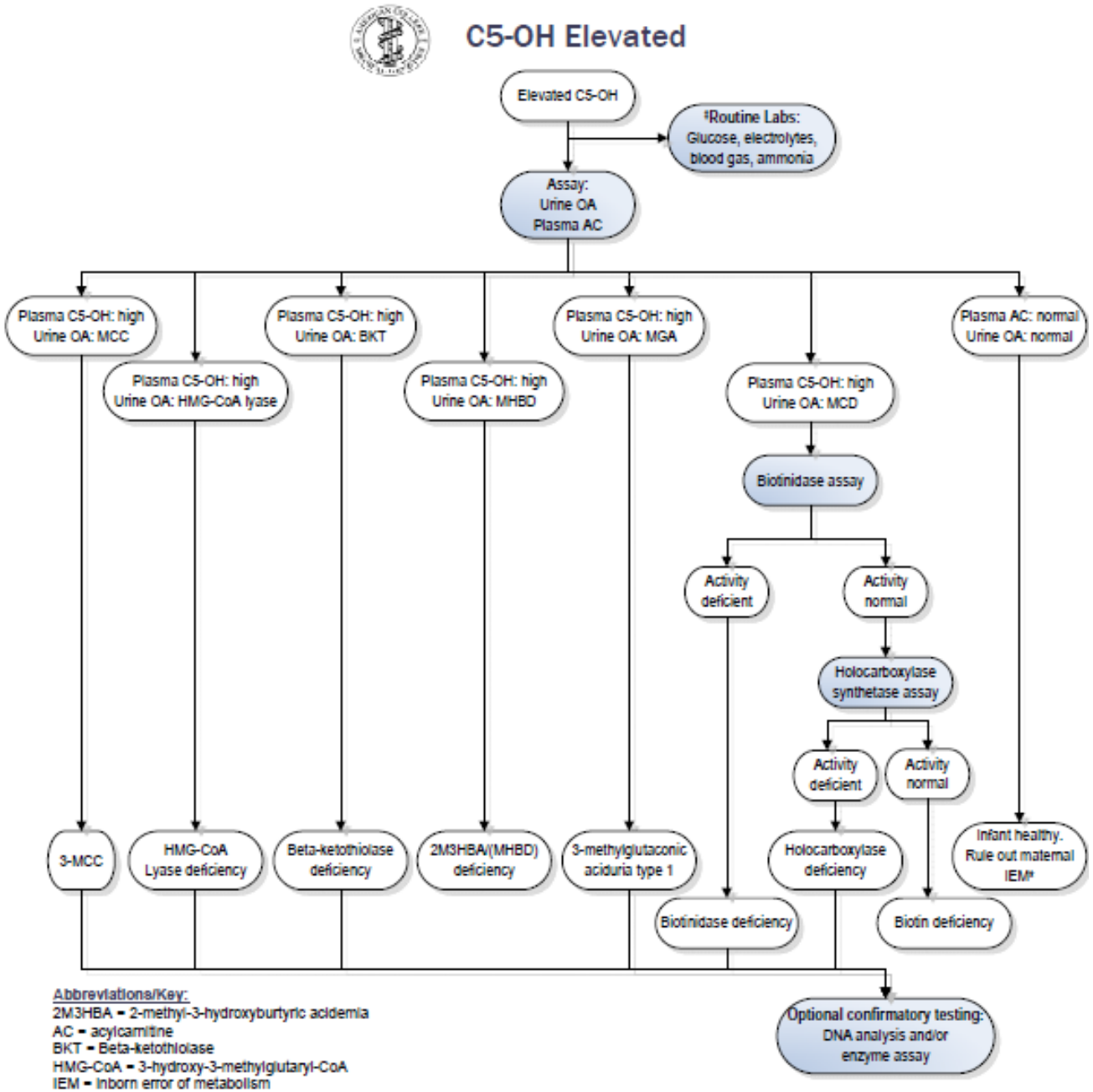
MAT/T2 DEFICIENCY (*ACAT1* gene)
β-KETOTHIOLASE DEFICIENCY





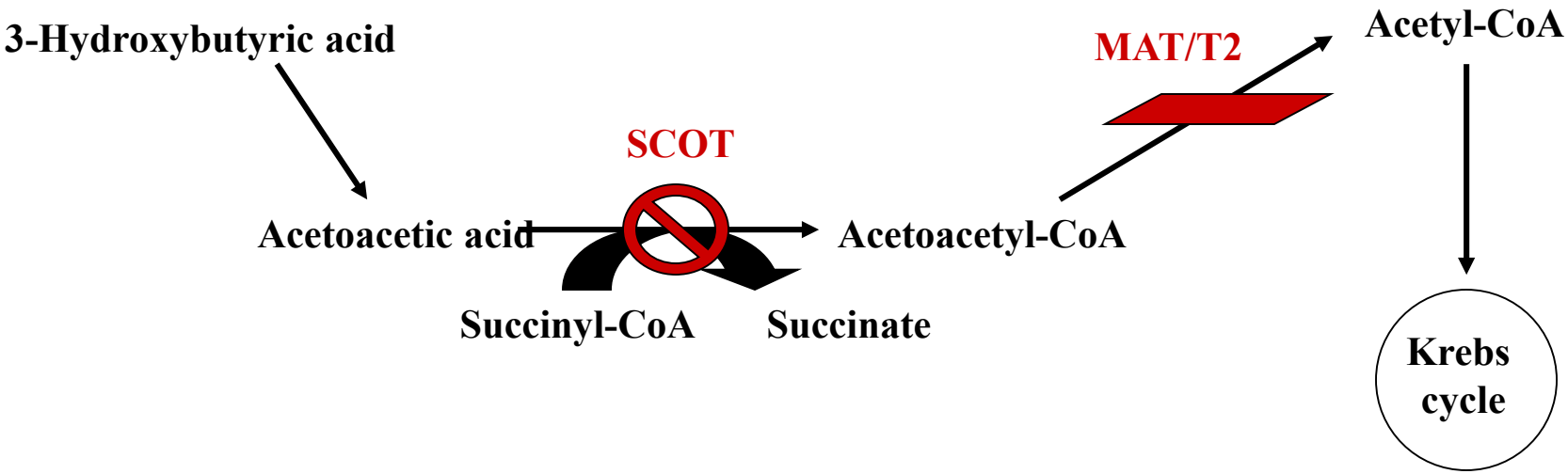
3-KETOTHIOLASE DEFICIENCY





C5-OH can be elevated, but we have seen combination of elevations of different species (C5:1, C4-OH) one at a time

SCOT deficiency



SCOT, Succinyl-CoA:3-ketoacid-CoA transferase (OMIM 245050) catalyzes the reversible rate-limiting step of ketolysis.

Cause: mutations in *OXCT* gene (5p12-p13).

OXCT gene not expressed in liver.

SCOT deficiency

Presentation: episodic, non-physiologic or exaggerated physiologic ketoacidosis: Tachypnea, lethargy, coma, severe ketoacidosis with elevated anion gap, persistent ketonemia/ketonuria even when stable or post-prandially, no diagnostic metabolites in urine or plasma. Present in fed state.

Diagnosis: Urine organic acids: increased Acetoacetate and 3-OH-Butyric acid, without abnormal urine organic acids. It is differentiated from physiological ketosis for the absence of adipic, suberic, and sebacic acids, usually seen during severe physiologic ketosis.

Confirmation: DNA testing *OXCT1* gene on 5p13.

Therapy: prevention of fasting, alkali to prevent acidosis, mild protein and fat restriction, cornstarch, carnitine.

METABOLIC ACIDOSIS

- Hispanic female, the first child of first cousin parents. Born prematurely with birth weight of 1.96 kg. Hospitalized for the first two months to achieve normal birth weight and for unspecified respiratory problems.
- At 8 months of age she had tachypnea, vomiting and lethargy following fever (39C). Severe metabolic acidosis with pH of 6.98, low CO2 (<5 mEq/L), an elevated anion gap (22-27 mEq/L), and hypokalemia (1.4-2 mEq/L). Glucose and ammonia were normal. Urine ketones were strongly positive.
- Acidosis was corrected by intravenous bicarbonate and peritoneal dialysis was initiated. Acidosis reappeared when dialysis was discontinued, for which she was kept on a regimen of daily dialysis.
- At 15 months of age, her growth and development were only mildly delayed. Hypoglycemia (glucose 1.22 mmol/L – 22 mg/dL) after overnight fasting but not during daytime was noted, with hypokalemia (2.5 mEq/L), normal bicarbonate and elevated anion gap (23.5 mEq/L). Urinary organic acid analysis showed excess ketone bodies without dicarboxylic aciduria or other abnormal metabolites.

LABORATORY FINDINGS

URINE ORGANIC ACIDS

ABNORMAL: Severe ketonuria suggesting severe catabolic state. No abnormal organic acids identified.

Organic acid quantitation in mmol/mol creatinine:

Analyte	Result	1 mo-12 yrs	
Lactic acid	676	<370	
Pyruvic acid		22	<34
Succinic acid	81	<80	
Fumaric acid		31	<10
2-Ketoglutaric	180	<150	
3-OH-butyric acid	10,563	<4	
Acetoacetic acid	17,704	<4	
2-Keto-3-methylvaleric	26	<10	
2-Keto-isocaproic	9	<4	
Ethylmalonic acid	8	<15	
Adipic acid	23	<100	
Suberic acid	14	<10	
Sebacic acid	0	<3	
4-OH-phenylacetic acid	81	<100	
4-OH-phenylpyruvic acid	8	<2	

SICK $CO_2 = 5$

URINE ORGANIC ACIDS

ABNORMAL: Severe ketonuria. Abnormal products of fatty acid oxidation are not present in this sample.

Organic acid quantitation in mmol/mol creatinine:

Analyte	Result	1 mo-12 yrs	
Lactic acid	349	<370	
Pyruvic acid		83	<34
Succinic acid		117	<80
Fumaric acid		33	<10
2-Ketoglutaric	577	<150	
3-OH-butyric acid	6,380	<4	
Acetoacetic acid	6,192	<4	
2-Keto-3-methylvaleric	23	<10	
2-Keto-isocaproic	8	<4	
Ethylmalonic acid	21	<15	
Adipic acid	28	<100	
Suberic acid	11	<10	
Sebacic acid	9	<3	
4-OH-phenylacetic acid	216	<100	
4-OH-phenylpyruvic acid	8	<2	

WELL $CO_2 = 27$

Normal plasma and urine amino acids

Plasma carnitine: excess acylcarnitines while on supplements.

LABORATORY FINDINGS

- **Ketolytic enzymes, Fibroblasts:**

Enzyme	Activity	Ref. range	Units
Beta-Ketothiolase	8.4	(5.6-15.9)	nmol/min/mg protein
Succinyl-CoA 3-ketotransferase	0.0	(4.1-8.1)	nmol/min/mg protein

- **Interpretation: SCOT deficiency.**
- **DNA OXCT gene: homozygous c.649C>T; p.R217X.**

MONOCARBOXYLIC TRANSPORTER 1 (MCT1) DEFICIENCY

Presentation: episodic, non-physiologic or exaggerated physiologic ketoacidosis: Tachypnea, lethargy, coma, severe ketoacidosis with elevated anion gap. Cyclic vomiting.
Diagnosis: Urine organic acids: increased Acetoacetate and 3-OH-Butyric acid, without abnormal urine organic acids. It is differentiated from physiological ketosis for the absence of adipic, suberic, and sebacic acids, usually seen during severe physiologic ketosis.
Confirmation: DNA testing *SLC16A1* gene on 1p13.2. Possible milder phenotype in heterozygotes with incomplete penetrance.
Therapy: prevention of fasting, alkali to prevent acidosis, mild protein and fat restriction, cornstarch, carnitine.

MJ, Duran K, Harakalova M, van der Zwaag B, Monavari AA, Okur I, Sharrard MJ, Cleary M, O'Connell N, Walker V, Rubio-Gozalbo ME, de Vries MC, Visser G, Houwen RH, van der Smagt JJ, Verhoeven-Duif NM, Wanders RJ, van Haaften G. Monocarboxylate transporter 1 deficiency and ketone utilization. van Hasselt PM, Ferdinandusse S, Monroe GR, Ruiter JP, Turkenburg M, Geerlings N Engl J Med. 2014 Nov 13;371(20):1900-7. doi: 10.1056/NEJMoa1407778. PMID: 25390740

SUMMARY

- **Fatty acids oxidation produces ketones (liver) that can be used by the body to produce energy.**
- **Disorders of ketogenesis (Mitochondrial 3-Hydroxy-3-Methyl-Glutaryl-CoA Synthase (mHs) and lyase (HL) deficiency) present as fatty acid oxidations defects with hypoketotic hypoglycemia.**
- **Disorders of ketolysis (MCT1, SCOT and MAT/T2 deficiency) present with acute metabolic acidosis during fasting.**
- **Urine organic acids and plasma acylcarnitine profile can identify abnormal metabolites in HL and MAT/T2 deficiency. No diagnostic metabolites are seen in mHS, SCOT and MCT1 deficiency that require DNA studies for diagnosis.**

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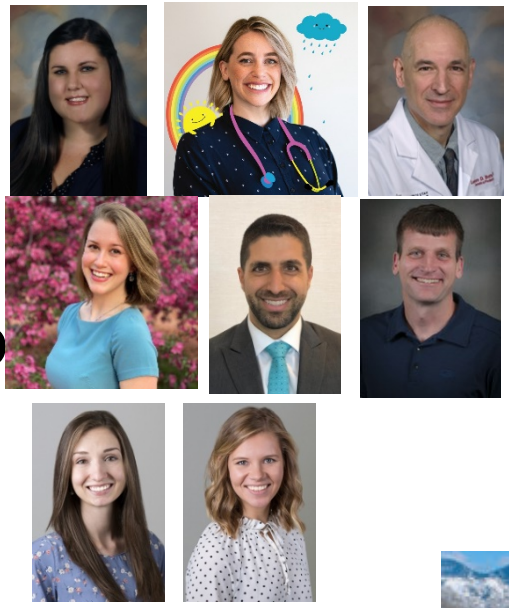

ARUP Laboratories

Marzia Pasquali PhD

Co-Author of all slides

All patients and their families.

MEDICAL BIOCHEMICAL GENETICS

CLINICAL CORE SEMINAR SERIES

Hosted by:

University of Colorado Anschutz Medical Campus

Thank you for attending!

Please leave feedback for this session using the QR code below or use this link.

Recordings of lectures (and handouts, if applicable) will be found later today at our dropbox until a permanent place is found.