Follow-up for Metabolic Disorders: Fatty Acid Oxidation Disorders, Galactosemia & Biotinidase Deficiency

Dr. Kathy Grange, MD
Division of Genetics and Genomic Medicine
Department of Pediatrics
Washington University School of Medicine

Fatty Acid Oxidation Disorders

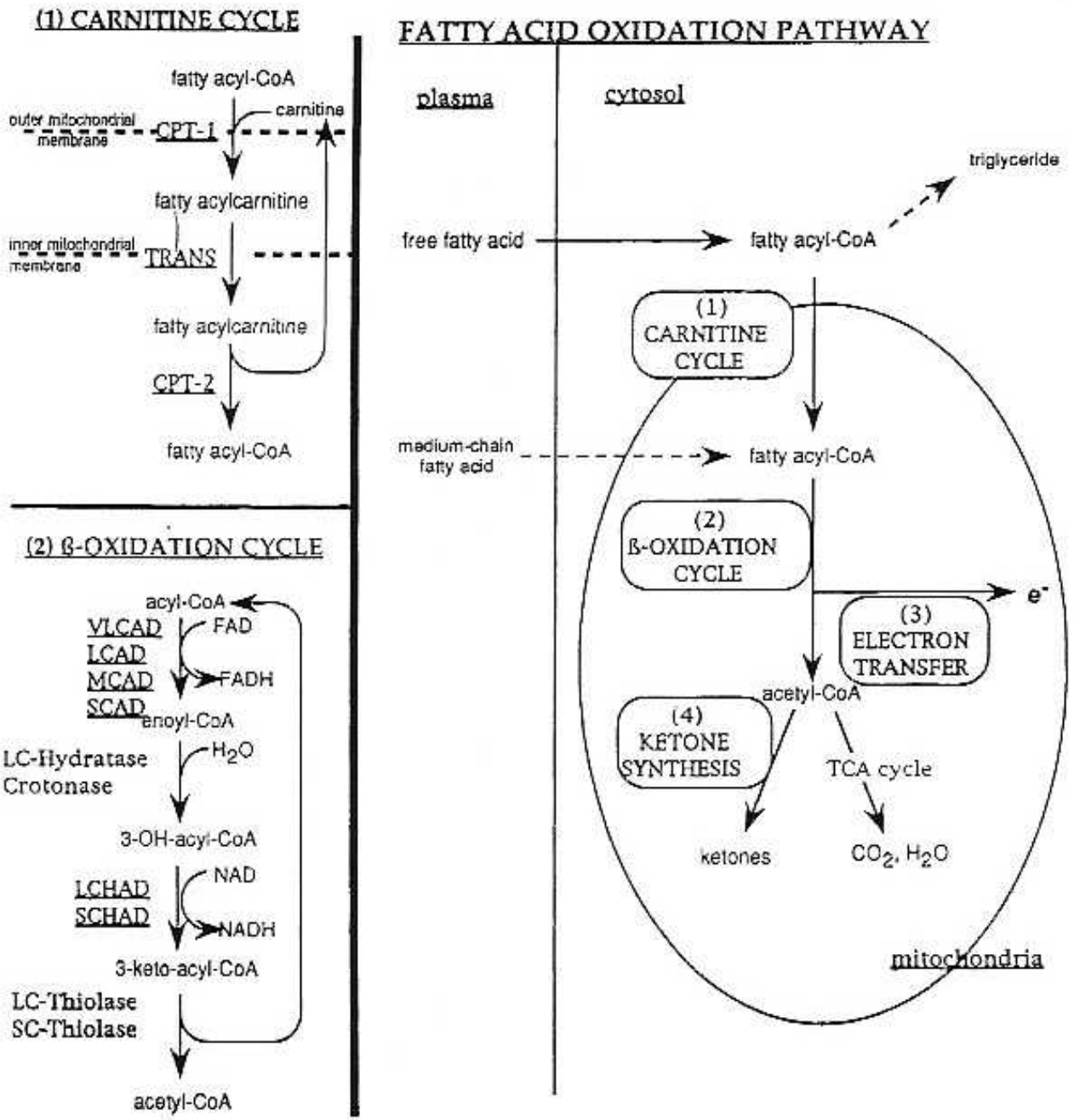
- Blood spots should be collected between 24 and 48 hours of age
- Samples collected too late may result in a false negative result
- Metabolites in FAO disorders can decrease after the first few days of life as the baby is fed

Fatty Acid Oxidation Disorders

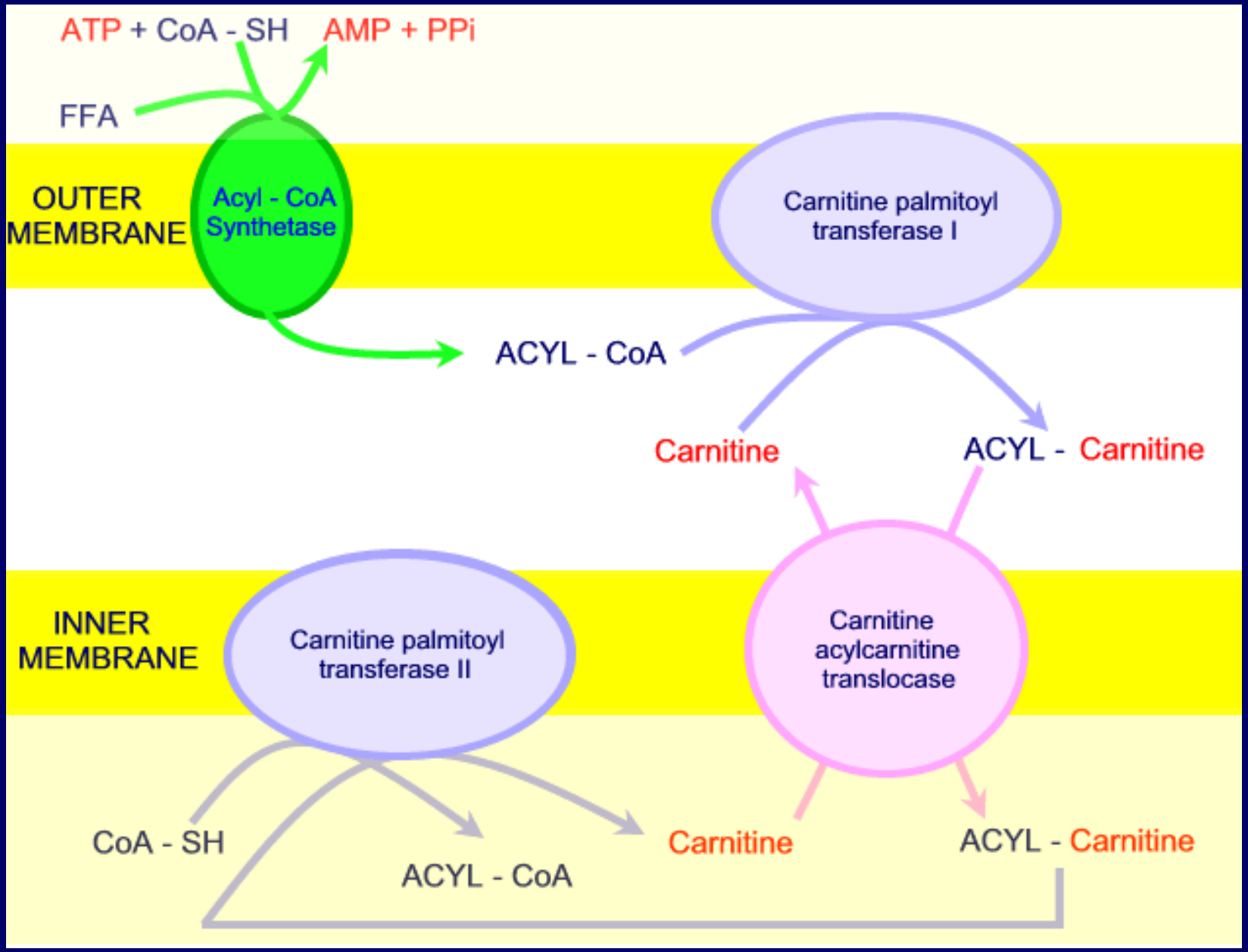
- Follow-up testing
  - Acylcarnitine profile
  - Total and free carnitine levels
  - Urine acylglycine profile
  - Urine organic acids
  - Comprehensive metabolic panel, uric acid, CK
- Counsel parents to feed infant frequently and seek medical care for any signs of illness
- For high risk cases (eg. high risk for VLCAD or LCHAD) may need immediate visit to geneticist and other evaluation such as echocardiogram

Fatty Acid Oxidation Disorders

- FAO disorders are caused by reduced or no activity of one of the enzymes necessary for fatty acid breakdown
- Breakdown, or oxidation, of fatty acids is necessary for energy production when glucose levels are low
- Each disorder has its own profile of acylcarnitines that rise in the infant's blood from the result of a disabled or missing enzyme in the fatty acid oxidation pathway

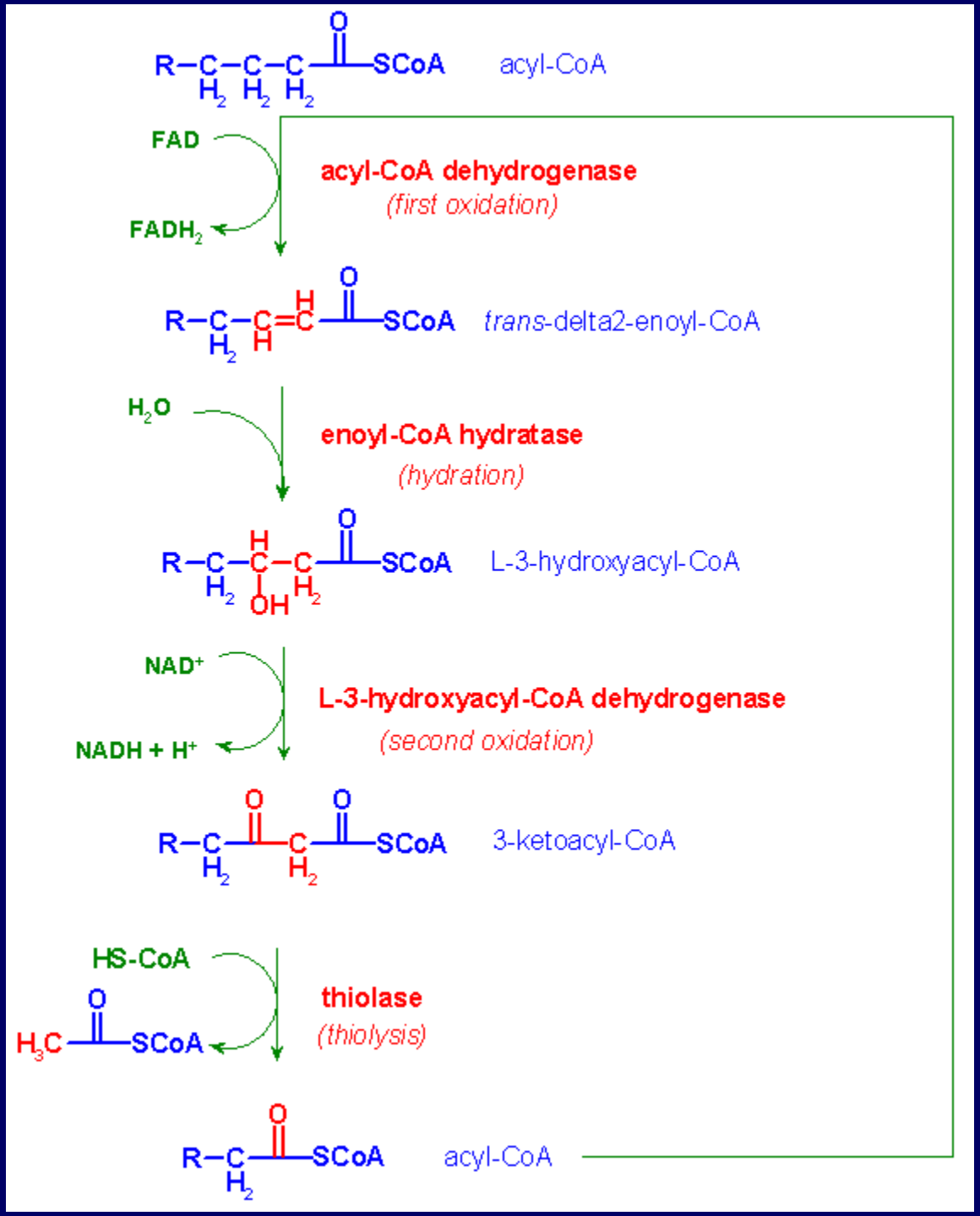

(1) CARNITINE CYCLE
fatty acyl-CoA
carnitine
outer mitochondrial membrane
CPT-1
fatty acylcarnitine
inner mitochondrial membrane
TRANS
fatty acylcarnitine
CPT-2
fatty acyl-CoA
(2) ß-OXIDATION CYCLE
acyl-CoA
VLCAD
LCAD
MCAD
SCAD
FAD
FADH
enoyl-CoA
LC-Hydratase
Crotonase
H2O
3-OH-acyl-CoA
LCHAD
SCHAD
NAD
NADH
3-keto-acyl-CoA
LC-Thiolase
SC-Thiolase
acetyl-CoA
FATTY ACID OXIDATION PATHWAY
plasma
cytosol
triglyceride
free fatty acid
fatty acyl-CoA
(1) CARNITINE CYCLE
medium-chain fatty acid
fatty acyl-CoA
(2) ß-OXIDATION CYCLE
e-
(3) ELECTRON TRANSFER
acetyl-CoA
(4) KETONE SYNTHESIS
TCA cycle
ketones
CO2, H2O
mitochondria

Transport of Fatty Acyl-CoA Esters into Mitochondria



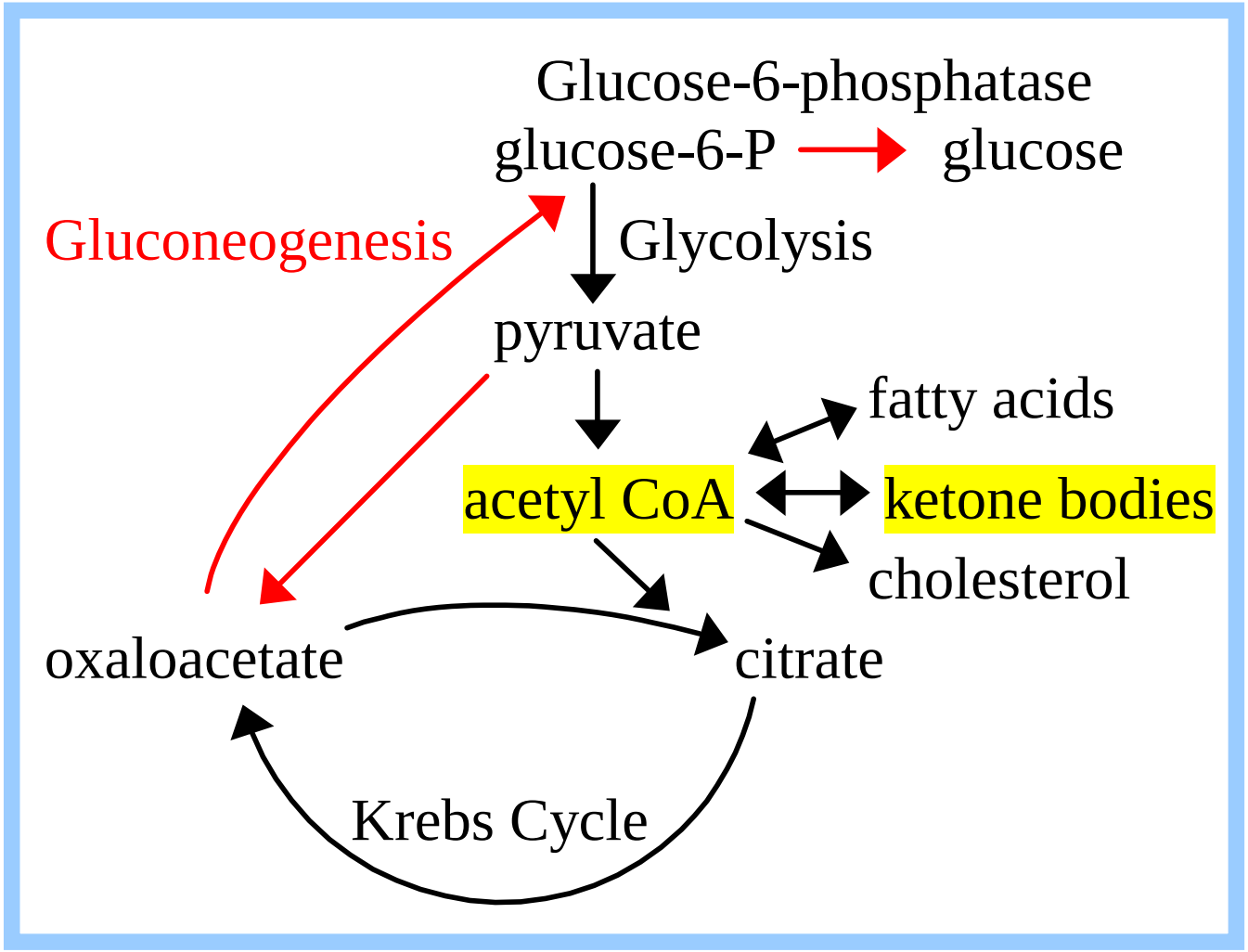
Fatty Acid Oxidation Pathway

Each cycle removes 2 carbons & produces acetyl-CoA



Metabolic Needs

- Brains must have glucose - no fat metabolism in brain
- Animals cannot make glucose from fats
- If the body is low on glucose, oxaloacetate is shunted away from the Krebs cycle to make glucose to feed the brain, and the Krebs cycle slows down, causing a loss of energy
- During severe starvation, the body makes ketone bodies from fatty acids (acetoacetic acid), this provides acetyl CoA for the brain
  - Glucose comes from protein degradation
  - Four carbon units are obtained from protein degradation



During carbohydrate starvation, oxaloacetate in liver is depleted due to gluconeogenesis. This impedes acetyl-CoA entry to Krebs cycle. Acetyl-CoA in liver mitochondria is converted then to ketone bodies, acetoacetate & β-hydroxybutyrate.

MCAD Deficiency

- Primary marker: C8
- Secondary markers: C8/C10 ratio, C6, C10:1
- Follow-up testing
  - Acylcarnitine profile
  - Total and free carnitine levels
  - DNA analysis for common A985G mutation (80-90% of alleles) or other mutations
- Avoidance of fasting
- IV fluids containing D10 during illnesses
- Monitor glucose during illness
- Possible carnitine supplementation

VLCAD Deficiency

- Primary marker: C14:1
- Secondary markers: C14:1/C12:1, C14, C14:2
- Follow-up testing
  - Acylcarnitine profile and total and free carnitine levels
  - DNA analysis and/or enzyme analysis on skin fibroblasts
- Avoidance of fasting
- IV fluids containing D10 during illness
- Monitor glucose, CMP, CK
- Echocardiogram

VLCAD Deficiency

- Lipistart or Portagen formula in infancy
- Low fat diet (30% of calories total with 10-15% calories from long-chain fats)
- MCT oil supplement to provide remainder of fat calorie requirement
- Essential fatty acid supplement with to provide minimum of 3% kcal from linoleic acid and 0.5% kcal from linolenic acid
- Possible low dose carnitine supplementation if deficient

LCHAD or TFP Deficiency

- Primary marker: C16-OH
- Secondary markers: C18:1-OH, C18-OH, C14:1
- Follow-up testing
  - Acylcarnitine profile
  - Total and free carnitine levels
  - CMP, uric acid, CK
  - DNA analysis for the common mutation (G1528C) and/or other mutations
- Avoidance of fasting
- IV fluids containing D10 during illnesses
- Monitor echocardiograms at least yearly
- Ophthalmology evaluations regularly for pigmentary retinopathy

LCHAD or TFP Deficiency

- Lipistart or Portagen formula in infancy
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- MCT oil supplement to provide remainder of fat calorie requirement
- Essential fatty acid supplement with to provide minimum of 3% kcal from linoleic acid and 0.5% kcal from linolenic acid
- Possible low dose carnitine supplementation if deficient

Multiple Acyl-CoA Dehydrogenase Deficiency (Glutaric Aciduria type II)

- Primary markers: C4, C5
- Secondary markers: C18:1, C8, C12, C14, C16
- Follow-up testing
  - Acylcarnitine profile
  - Total and free carnitine levels
  - Urine organic acid analysis---multiple compounds (ethylmalonic, adipic, glutaric, and lactic acids and others)
    - Distinguished from GA I by the presence of 2-OH-glutaric acid
- GA II can be confirmed with ETF/ETF-QO enzyme assay and gene sequencing

Multiple Acyl-CoA Dehydrogenase Deficiency (Glutaric Aciduria type II)

- Avoidance of fasting
- IV fluids with glucose during illnesses
- Low fat, low protein diet
- Carnitine and riboflavin supplementation
- Severe neonatal presentation can be associated with renal and other congenital anomalies and may be fatal

SCAD Deficiency

- Primary marker: C4
- Follow-up testing
  - Acylcarnitine profile
  - Total and free carnitine levels
  - Urine acylglycine profile
  - DNA analysis---common polymorphism
- Avoidance of fasting and IV fluids during illnesses
- Most people with SCAD deficiency are healthy without evidence of illness
- Severe forms with myopathy occur rarely

Carnitine Uptake Disorder

- Primary marker: C0
- Secondary marker: C2
- Follow-up testing
  - Blood total and free carnitine levels
  - Acylcarnitine profile
  - Urine carnitine levels
  - DNA analysis
- Test the mother as well
  - Many cases of maternal CUD have been diagnosed through newborn screening of their infant
- High dose carnitine supplementation
- Echocardiogram

Carnitine palmitoyltransferase 1 deficiency (CPT 1)

- Primary markers: C0 (elevated)
- Secondary markers: C0/C16, C0/C18
- Follow-up testing:
  - Acylcarnitine profile
  - Serum total and free carnitine levels---normal or elevated in CPT1
  - CPT 1 DNA analysis
- Avoid fasting, heavy exercise and catabolic condition
- Watch for hypoketotic hypoglycemia, lethargy, hepatomegaly
- Monitor cardiac status with EKG and echocardiogram---risk for cardiomyopathy and arrhythmias

CPT2 and CACT Deficiencies

- Primary marker: C16
- Secondary markers: C18:1, C18:2, C14
- Follow-up:
  - Acylcarnitine profile
  - Serum total and free carnitine levels
  - DNA testing
- Avoid fasting, heavy exercise and catabolic condition
- Carnitine supplementation if levels low
- MCT oil supplement and low long-chain fat diet may help

CPT2 and CACT Deficiencies

- Monitor cardiac status with EKG and echocardiogram
  - High risk for cardiomyopathy and arrhythmias
- Perinatal form of CPT 2 deficiency is almost invariably fatal
- High mortality rate (24% survival) for CACT deficiency

Galactosemia

- Incidence is 1 in 45,000 newborns for classic galactosemia
- Autosomal recessive disorder
- Deficiency of galactose-1-phosphate uridyltransferase (GALT) enzyme
- Inability to convert galactose-1-PO4 to glucose-1-PO4

Metabolism of Galactose

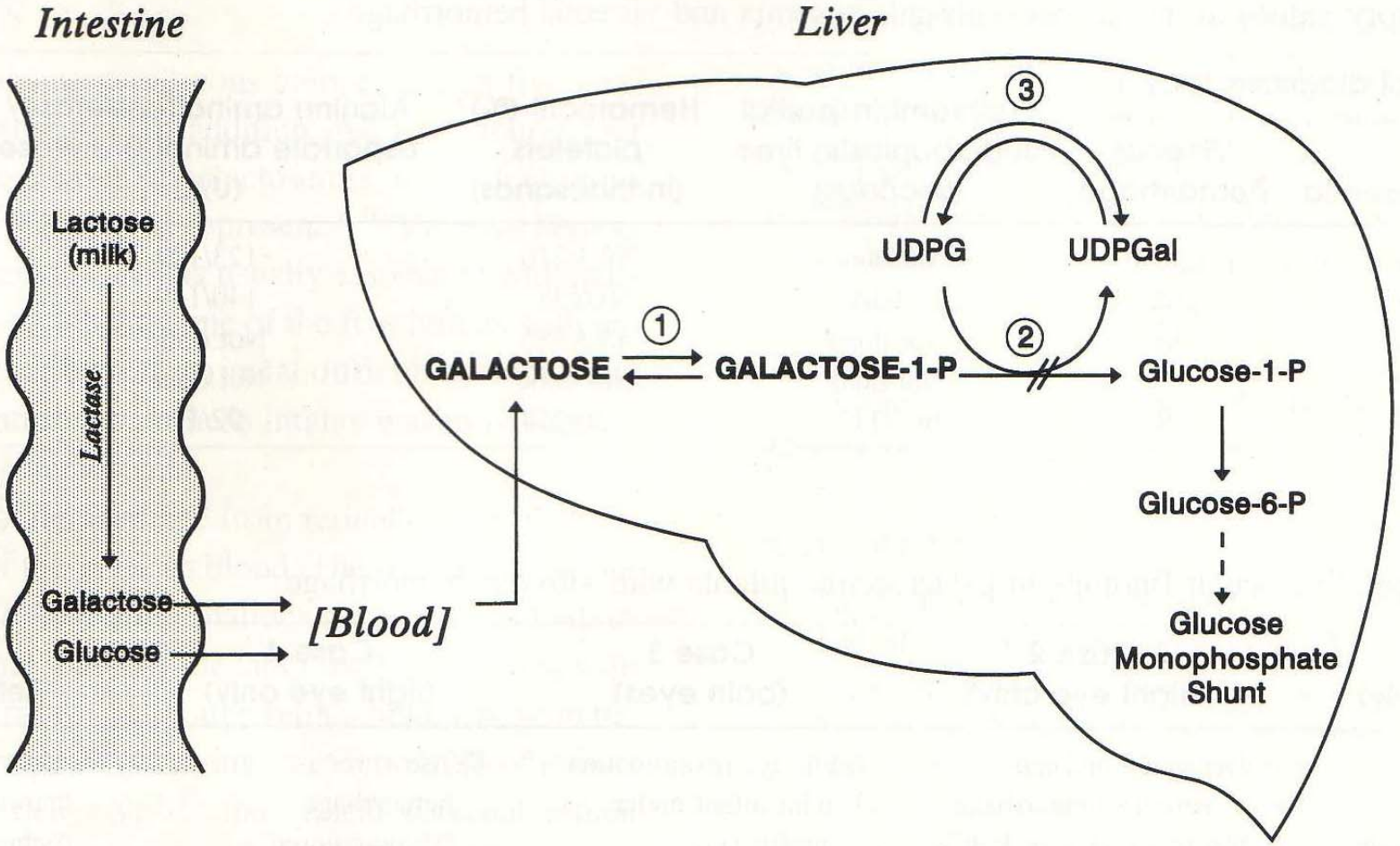


Figure. Liberation of galactose from lactose in the intestine and its metabolism. The enzymes required for galactose metabolism are galactokinase *(1)*, galactose l-phosphate uridyltransferase *(2)*, and uridine diphosphate-4-epimerase *(3)*. In galactosemia, the defective enzyme is galactose 1-phosphate uridyltransferase.

Classic Galactosemia

- Babies appear normal at birth
- Symptoms begin in 1st week of life if lactose is given:
  - Vomiting and diarrhea
  - Failure to thrive or weight loss
  - Hepatomegaly, jaundice, coagulopathy
  - Kidney dysfunction
  - Increased intracranial pressure/cerebral edema
  - Cataracts ("oil droplet")
  - E. coli sepsis

Classic Galactosemia

- Laboratory abnormalities include:
  - Galactosemia
  - Galactosuria (urine positive for reducing substances)
  - Hyperbilirubinemia
  - Hypokalemia
  - Hyperchloremia
  - Hypoglycemia (+/-)
  - Hyperammonemia
  - Elevated PT/PTT and liver enzymes

Galactosemia

- Borderline Risk (3.1 – 4.0 U/gHb)
  - Variant galactosemia (Duarte-galactosemia or others)
  - Carrier for galactosemia
  - Improperly handled sample (heat damage or transit delay)
- High Risk (≤ 3.0 U/gHb)
  - Classical galactosemia (usually <1.5)
  - Variant galactosemia or carrier (often 1.6-3.0)

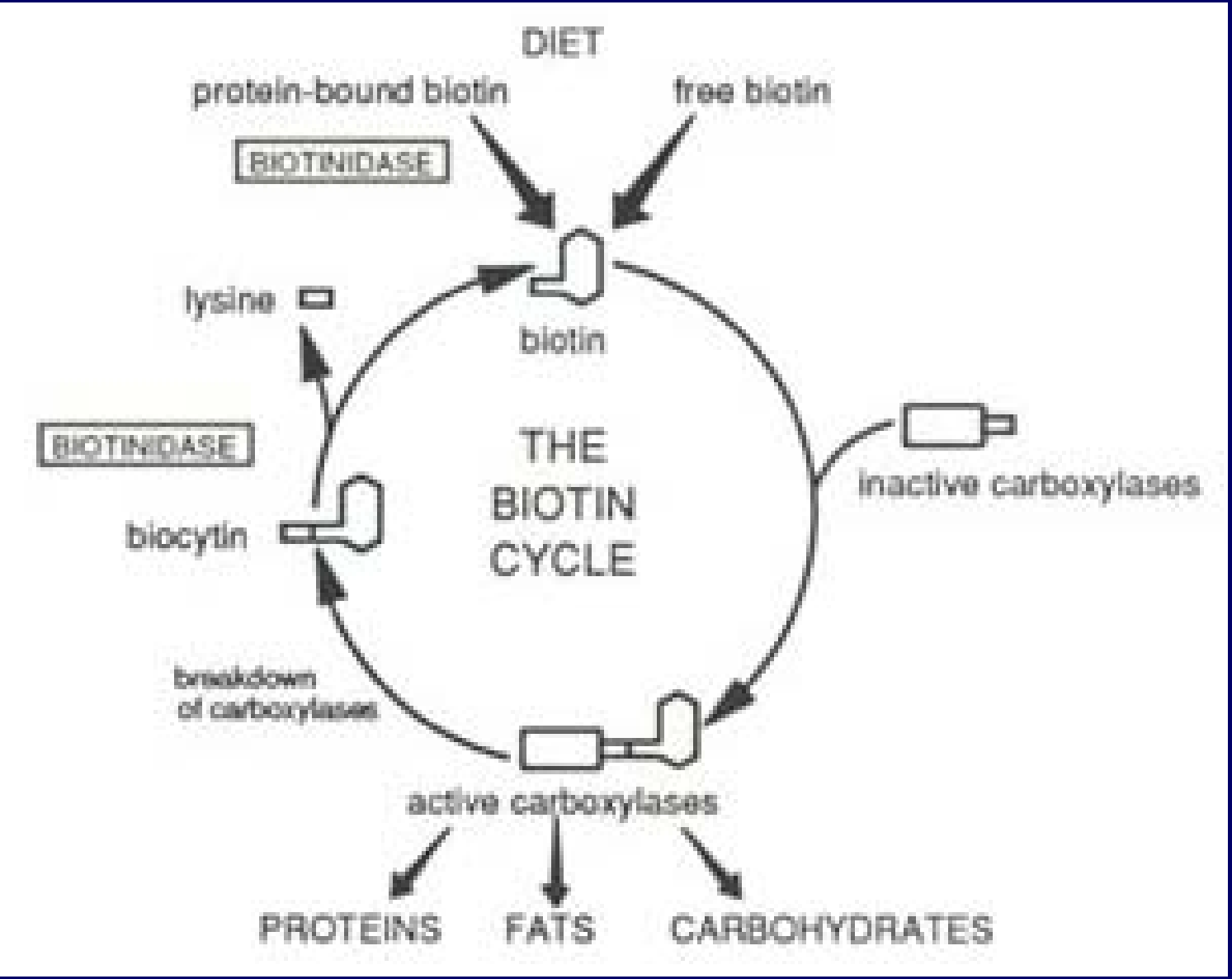
Galactosemia

- Follow-up testing
  - Quantitative galactose-1-phosphate uridyltransferase (GALT) enzyme assay
  - Red blood cell galactose-1-PO4 level
  - DNA analysis on the GALT gene
    - Common mutations analyzed first
    - Sequencing of GALT gene if still unclear
- Change to a soy formula while awaiting test results

Biotinidase Deficiency

- Enzyme deficiency affects biotin availability
- Lab test gives qualitative result (eg, 1+ to 4+)
  - Reported as normal or abnormal to provider
- Newborns are usually asymptomatic
- Episodic hypoglycemia, lethargy, hypotonia, and mild developmental delay can occur at any time from the neonatal period through childhood
- Untreated biotinidase deficiency leads to more severe developmental delay, seizures, alopecia, and hearing deficits
- Biotin treatment is highly effective

Biotin Cycle



Biotinidase Deficiency

- Follow-up of abnormal newborn screen requires quantitative biotinidase enzyme assay
  - Affected individuals will have low or absent activity
  - "Partial" (mild) defects may occur
- DNA testing for the common mutations is often helpful
- C5-OH acylcarnitine may be high but lack of an abnormal acylcarnitine profile does not rule out biotinidase deficiency
- Treatment is with 5-10 mg of biotin/day

Who Should Get a Repeat NBS?

- Unsatisfactory sample on the first filter paper
- Premature infants
- Infants who are ill and in the NICU
- Borderline abnormal results on the first screen
- Suspicion for metabolic disease

Missouri Guidelines for NBS Specimens from Premature, Low Birth Weight, or Sick NICU Infants

- CORE CATEGORY = All NICU infants (unless transfused)
  - 1st specimen at 24-48 hours of age
  - 2nd specimen 10-14 days of age or at discharge
- RBC transfusion <24 hrs of age
  - 1st specimen collected before transfusion
  - 2nd specimen 24-48 hours after transfusion
  - 3rd specimen 30 days after transfusion
- NOT collected prior to RBC transfusion
  - 1st specimen 24-48 hours after transfusion
  - 2nd specimen 30 days after transfusion
  - 3rd specimen 90 days after transfusion
- Infants on TPN should follow Core Category unless transfused; may need another specimen after off TPN