

**MATERNAL METABOLIC
DISORDERS IDENTIFIED
THROUGH NEWBORN SCREENING**

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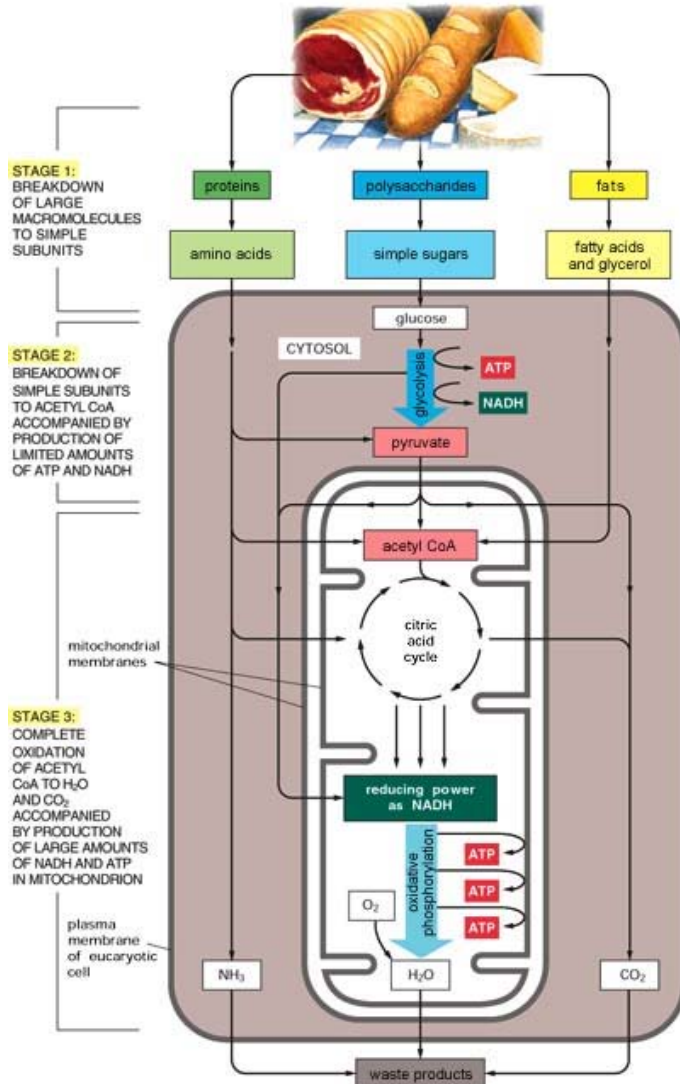
Park City, Utah

1 – 5 March 2010

OBJECTIVES

- **Discuss metabolic disorders and newborn screening**
- **Review maternal diseases identified by newborn screening**
- **Discuss clinical implications**

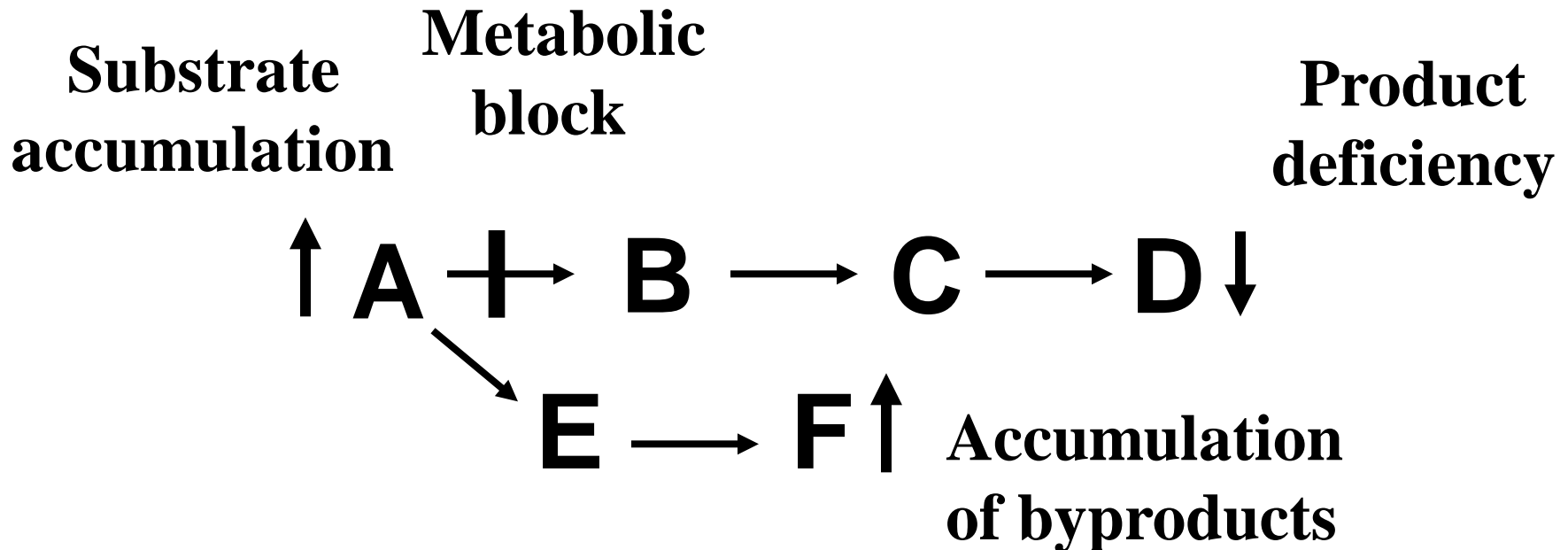
NORMAL METABOLISM



- Our body assimilates and transforms nutrients for growing and producing energy
- Metabolic disorders affect the transformation of nutrients to obtain energy and other products
- In most cases, these disorders are caused by mutations in enzymes which control the rate at which chemical reactions occur

METABOLIC DISORDERS

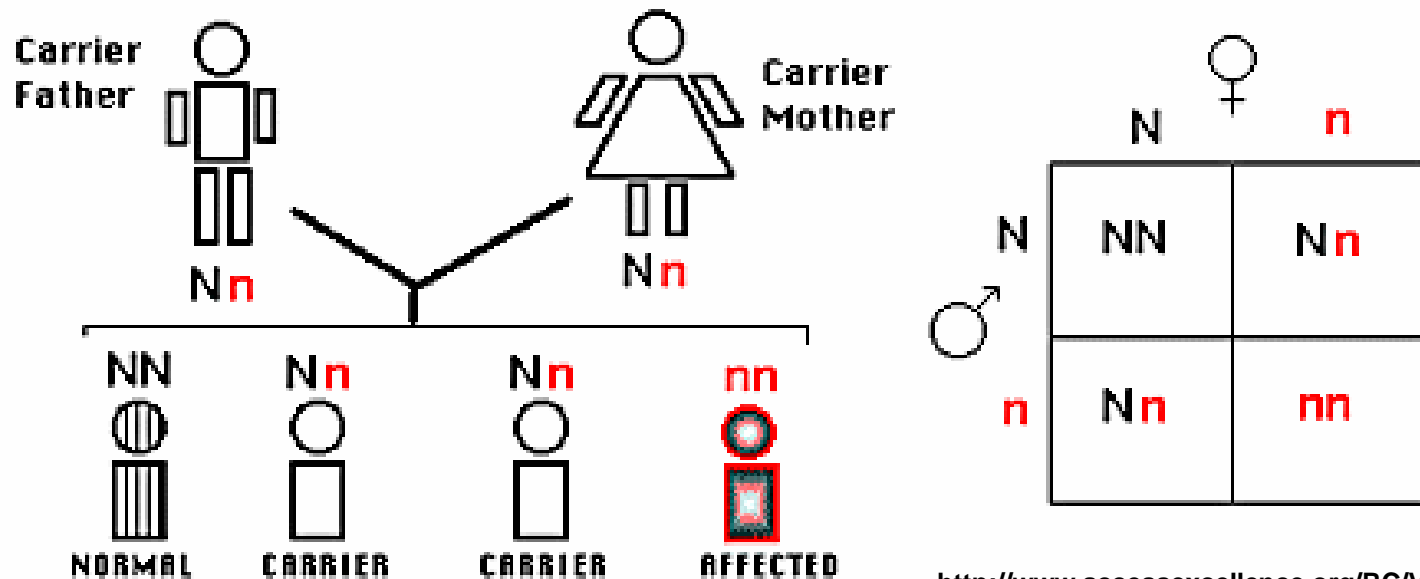
- The lack of an enzyme causes the accumulation of toxic metabolites proximal to the metabolic block, byproducts not normally present, and the lack of required products
- This results in morbidity and mortality characteristic of each disease



METABOLIC DISORDERS

- Most metabolic disorders are inherited as recessive traits
- Heterozygotes do not show any clinical manifestations

Recessive inheritance



FREQUENCY OF INHERITED METABOLIC DISORDERS

While the frequency of individual metabolic disorders is rare, their cumulative frequency is high (more than 1:3,000).

PKU (phenylketonuria)	1:12,000
MCAD deficiency	1:12,000
Glutaric Acidemia Type 1	1:30,000
Primary Carnitine Deficiency	1:40,000
Propionic Acidemia	1:50,000
Biotinidase deficiency	1:60,000
Galactosemia	1:60,000
Tyrosinemia Type I	1:100,000
Methylmalonic acidemia	1:100,000
Homocystinuria	1:120,000
Maple Syrup Urine Disease	1:180,000

INHERITED DISORDERS OF METABOLISM AND SCREENING

Amino acids:

**Phenylketonuria, Maple Syrup Urine Disease, Homocystinuria,
Citrullinemia, Argininosuccinic aciduria, Tyrosinemia Type I**

Fatty acids oxidation:

**MCAD, VLCAD, SCAD, MADD, CPT-2 deficiency, CACT deficiency,
LCHAD/TFP deficiency**

Organic acids:

**Glutaric acidemia Type I, Propionic acidemia, Methylmalonic acidemia,
Isovaleric acidemia, 3-hydroxy-3-methyl glutaryl CoA lyase, 3-
methylcrotonyl CoA carboxylase deficiency**

Sugars:

Galactosemia

PRESENTATION OF METABOLIC DISORDERS

- Some, such as phenylketonuria, affect primarily the brain, others such as urea cycle defects, cause protein-induced vomiting, neurologic dysfunction, and hyperammonemia with acute presentation usually in the newborn period.**
- Disorders of fatty acid oxidation, such as MCAD deficiency, can be completely silent until the body requires energy from fat, such as during infections, fever, and fasting.**
- These disorders can present in children and adults and can be easily confused with other more common problems.**

DIAGNOSIS OF METABOLIC DISORDERS

- **Metabolic disorders can be suspected from clinical presentation and routine laboratory testing: metabolic acidosis, hyperammonemia, hypoglycemia, ketonuria**
- **Metabolic disorders require specific “routine” tests: plasma amino acids, urine organic acids, plasma carnitine, plasma acylcarnitine profile, urine acylglycine analysis**
- **The diagnosis is usually confirmed by DNA testing or enzyme/transporter/receptor assay**

CURRENT THERAPY OF METABOLIC DISORDERS

- **Restriction of toxic substrates (examples Phe in PKU, Phe+Tyr in tyrosinemia, milk in galactosemia, etc.)**
- **Provision of products (arginine in urea cycle defects, tyrosine in PKU)**
- **Inhibitors of the formation of toxic products/byproducts (NTBC in tyrosinemia type 1, allopurinol in gout, statins in hypercholesterolemia)**
- **Drugs to bypass or reduce the effects of the metabolic block (phenylbutyrate/benzoate in urea cycle defects, carnitine in fatty acid oxidation defects, glycine in isovaleric acidemia, etc)**
- **Pharmacologic amounts of vitamins to stabilize or bypass mutant enzymes (Thiamine in MSUD, B12 in MMA, biotin in multiple carboxylase deficiency, B6 in homocystinuria, biotin in biotinidase deficiency, etc.)**

PRE-SYMPTOMATIC DIAGNOSIS OF METABOLIC DISORDERS: NEWBORN SCREENING

- **Therapy of metabolic disorders does not reverse brain damage**
- **Important to treat before symptoms appear**
- **Abnormal metabolites are present and can be identified in the newborn period**
- **Newborn screening can identify infants with a metabolic disorder**

NEWBORN SCREENING

- **Newborn screening is a public health activity aimed at the early identification of conditions for which timely intervention can lead to the elimination or reduction of mortality, morbidity, and disabilities associated with these conditions.**

NEWBORN SCREENING TODAY

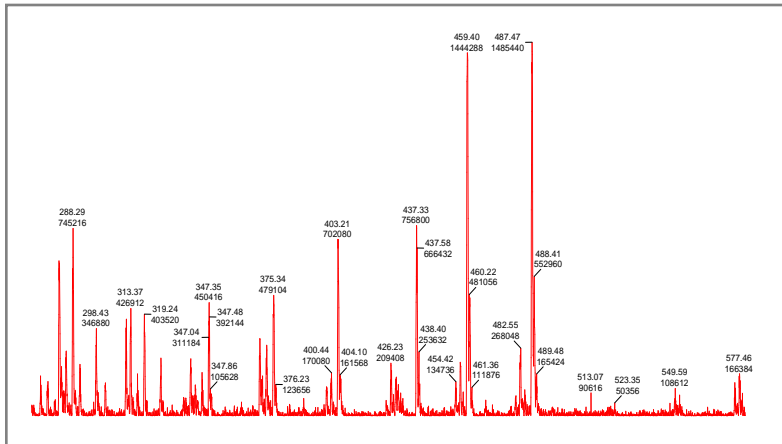
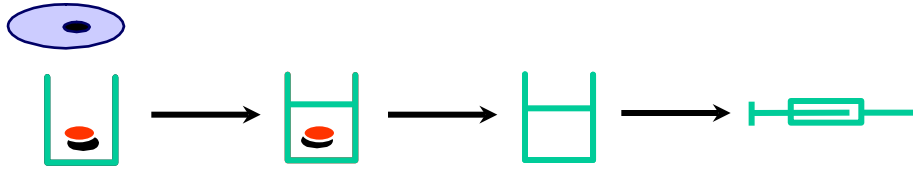
- **Mandated in all states in the United States**
- **Primarily performed by state public health laboratories**
 - **Some contract with private laboratories**
 - **Some contract with other states**
 - **Some states are combined into regional programs**
- **Newborn screening is the largest genetic testing effort in the nation**

HOW IS NEWBORN SCREENING DONE?

Blood is collected from each newborn at time of discharge from the hospital by heel stick and spotted on filter paper. Blood spots are sent to a centralized laboratory for analysis. Positive or suspicious results are followed up with a repeat newborn screen or with more definitive tests.



NEWBORN SCREENING



Multiple analytes

Many diseases

NBS TESTS RECOMMENDED BY ACMG AND MARCH OF DIMES

TANDEM MASS SPECTROMETRY (MS/MS)			Traditional methods (EIA, HPLC, etc.)	
<i>Acylcarnitines</i>		<i>Amino acids</i>		
FAO (5)	OA (9)	AA (6)	Hematology (3)	Others (6)
<i>MCAD</i>	<i>IVA</i>	<i>PKU</i>	<i>Hb SS</i>	<i>CH</i>
<i>VLCAD</i>	<i>GA-I</i>	<i>MSUD</i>	<i>Hb S/βThal</i>	<i>BIOT</i>
<i>LCHAD</i>	<i>HMG</i>	<i>HCY</i>	<i>Hb S/C</i>	<i>CAH</i>
<i>TFP</i>	<i>MCD</i>	<i>TYR I</i>		<i>GALT</i>
<i>CUD</i>	<i>MUT</i>	<i>ASA</i>		<i>HEAR</i>
	<i>Cbl A,B</i>	<i>CIT</i>		<i>CF</i>
	<i>3MCC</i>			
	<i>PROP</i>			
	<i>BKT</i>			

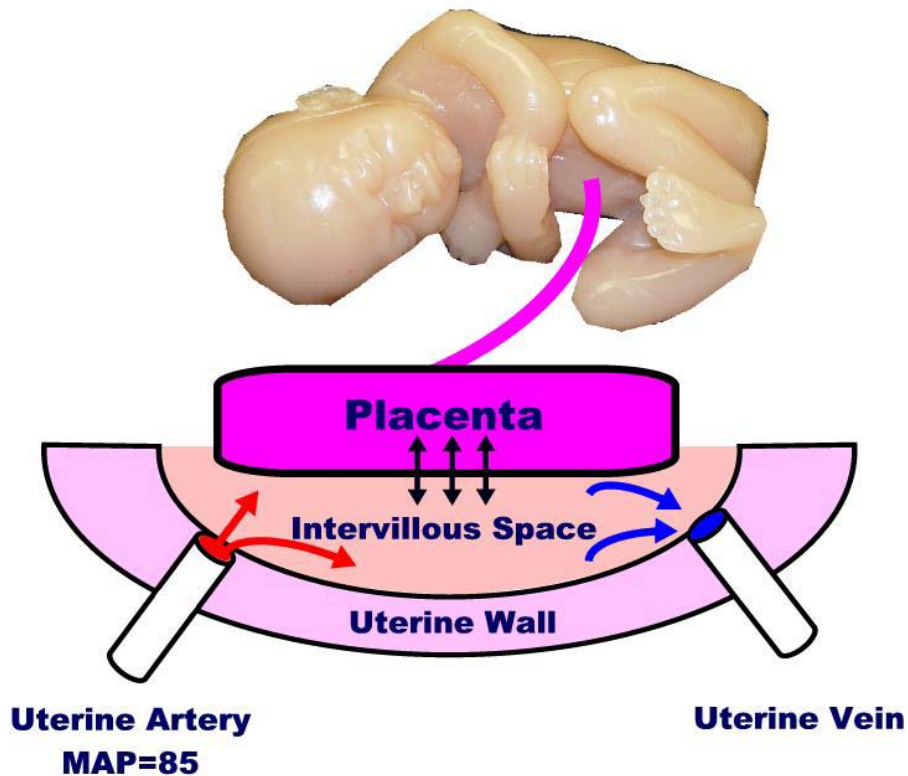
MS/MS screening in Utah: 2006-2008

- Total screens: 165,702
 - ***Total metabolic disorders: 73 1:2,270***
 - 17 PKU + 9 Hyperphenylalaninemia
 - 21 MCAD deficiency
 - 7 3-Methylcrotonylglycinuria
 - 5 VLCAD deficiency
 - 3 Glutaric acidemia type I
 - ***11 Maternal disorders***
 - 5 Primary carnitine deficiency (CUD)
 - 4 Vitamin B12 deficiency
 - 2 3-Methylcrotonylglycinuria

MOTHERS WITH METABOLIC DISORDERS

- **Several infants identified through newborn screening with a possible metabolic disorder, had completely normal confirmatory test results.**
- **This led to laboratory investigation of the mothers of these infants and their subsequent diagnosis.**

MATERNAL/FETAL CIRCULATION

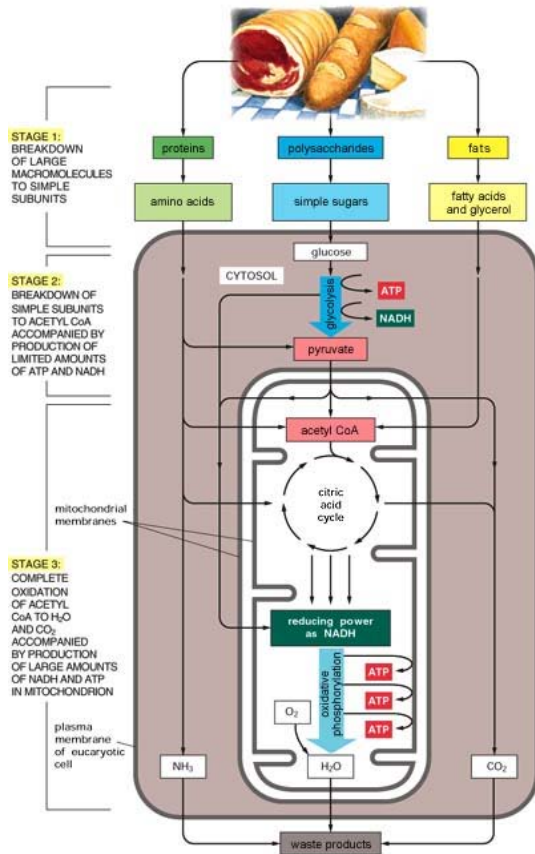


http://www.brooksidepress.org/Products/Military_OBGYN/

- Maternal blood is carried in the intervillous space by the uterine arteries through the uterine wall. The placenta floats on top of the IVS, with the chorionic villi dipping into it. Exchange of oxygen, carbon dioxide, nutrients and waste products occurs through the villous membrane.
- Abnormal metabolites accumulated in the mother will reach the fetus and will be still present at birth and for a certain period of time after birth.

DISORDERS OF METABOLISM

Amino acids/Fatty acids



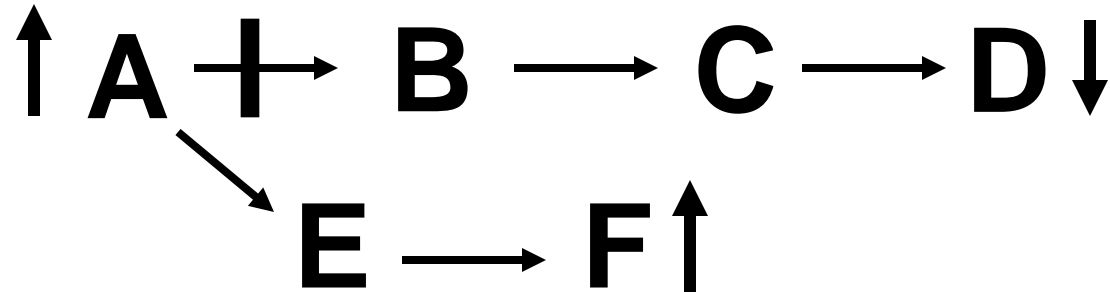
~~↓~~
~~↓~~
Short, medium, long chain organic acids/fatty acids

Carnitine ↓

~~↓~~
~~↓~~
Short, medium, long chain acylcarnitines ↑

Energy production

MARKERS OF MATERNAL DISEASE



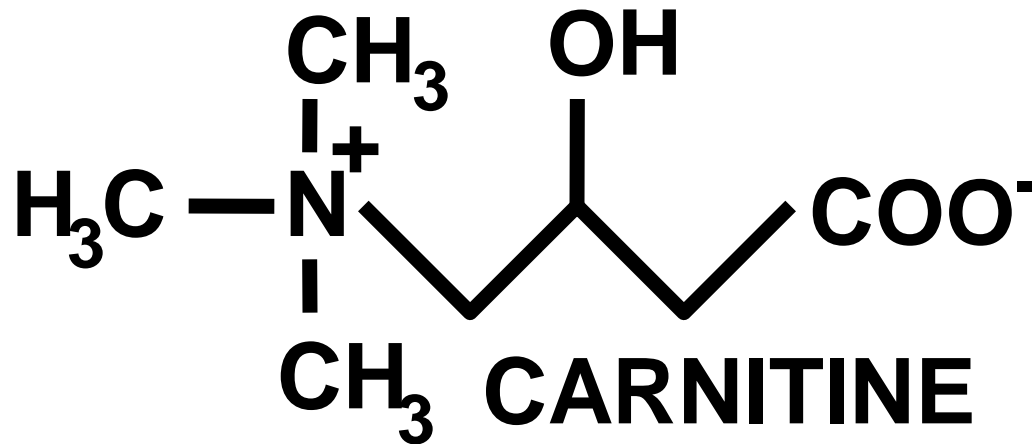
- **Primary markers:**
 - Primary analyte, immediately upstream of the metabolic block (amino acids, acylcarnitines)
- **Secondary marker:**
 - Carnitine (low concentrations resulting from chronic depletion due to undiagnosed condition)

PRIMARY MARKERS of MATERNAL DISEASES

- **Amino acids: usually patients have already been identified**
 - **Phenylalanine (PKU); Citrulline (Citrullinemia)**
- **Acylcarnitines: the most frequently identified maternal conditions are the following**
 - **C5OH-carnitine: 3-methylcrotonylglycinuria, 3-methylglutaconic aciduria**
 - **C3-carnitine: vitamin B12 deficiency**

SECONDARY MARKER of MATERNAL DISEASES: CARNITINE

- Carnitine (3-hydroxy-4-N-trimethylammonium butyrate) is essential for the transfer of fatty acids across the inner mitochondrial membrane.



SOURCES OF CARNITINE

Synthesized by liver and kidneys, but not in heart or skeletal muscle, which depend on carnitine transport for fatty acid oxidation.

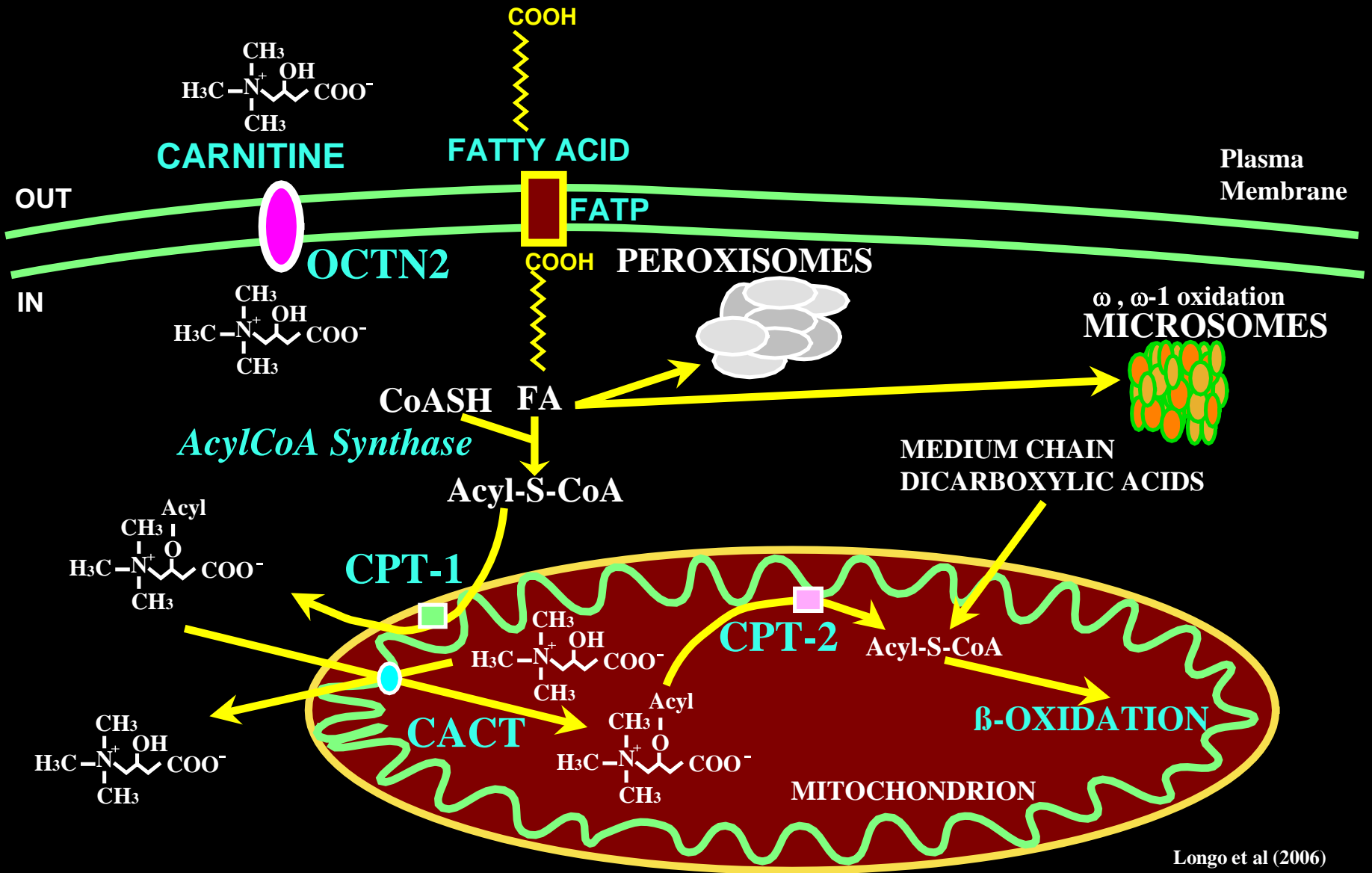
In the diet, most carnitine is supplied by red meat and dairy products, while fruits and vegetables contain insignificant amounts. About 75% of carnitine is provided by the diet in normal adults.

Carnitine is lost in the urine and secreted in the bile. Acute renal failure can result in high levels of plasma carnitine (100-300 μM). By contrast, chronic renal failure and dialysis can cause carnitine deficiency.

CARNITINE AND ACYLCARNITINES

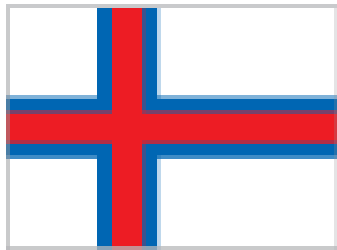
- **Carnitine is conjugated with: 1) long-chain fatty acyl-CoAs to facilitate their transfer in the mitochondria; 2) acyl-CoAs derived from intermediary metabolism to facilitate their excretion.**
- **Causes of low carnitine:**
 - **Reduced intake**
 - **Increased urinary losses**
 - **Primary impairment of the carnitine transporter**
 - **Increased conjugation with acyl-CoAs accumulated because of a metabolic disorder**

THE CARNITINE CYCLE IN FATTY ACID OXIDATION



CARNITINE UPTAKE DEFECT (PRIMARY CARNITINE DEFICIENCY)

- **Carnitine derives from diet and endogenous synthesis**
- **Frequency 1:40,000 (1% are carriers)**
- **Cause: Carnitine transporter (OCTN2)**
- **Pathogenesis: Loss of carnitine in urine reduces availability of carnitine in liver, muscle and heart, impairing FAO**
- **Presentation: Reye syndrome, sudden death, cardiomyopathy**
- **Diagnosis: Plasma carnitine levels (very low, usually <5 uM), confirmed by transport studies in fibroblasts**
- **Therapy: carnitine 100-300 mg/kg per day PO div. TID**
- **Prognosis: excellent (with treatment)**



FAROE ISLANDS



Small archipelago in the North Atlantic located between Scotland and Iceland. The Faroe Islands are a part of the Kingdom of Denmark, along with Denmark proper and Greenland. Population about 50,000 with another 20,000 living abroad, mostly in Denmark. They have a very high incidence of several IEMs.

Carnitine uptake defect: 1:1,300

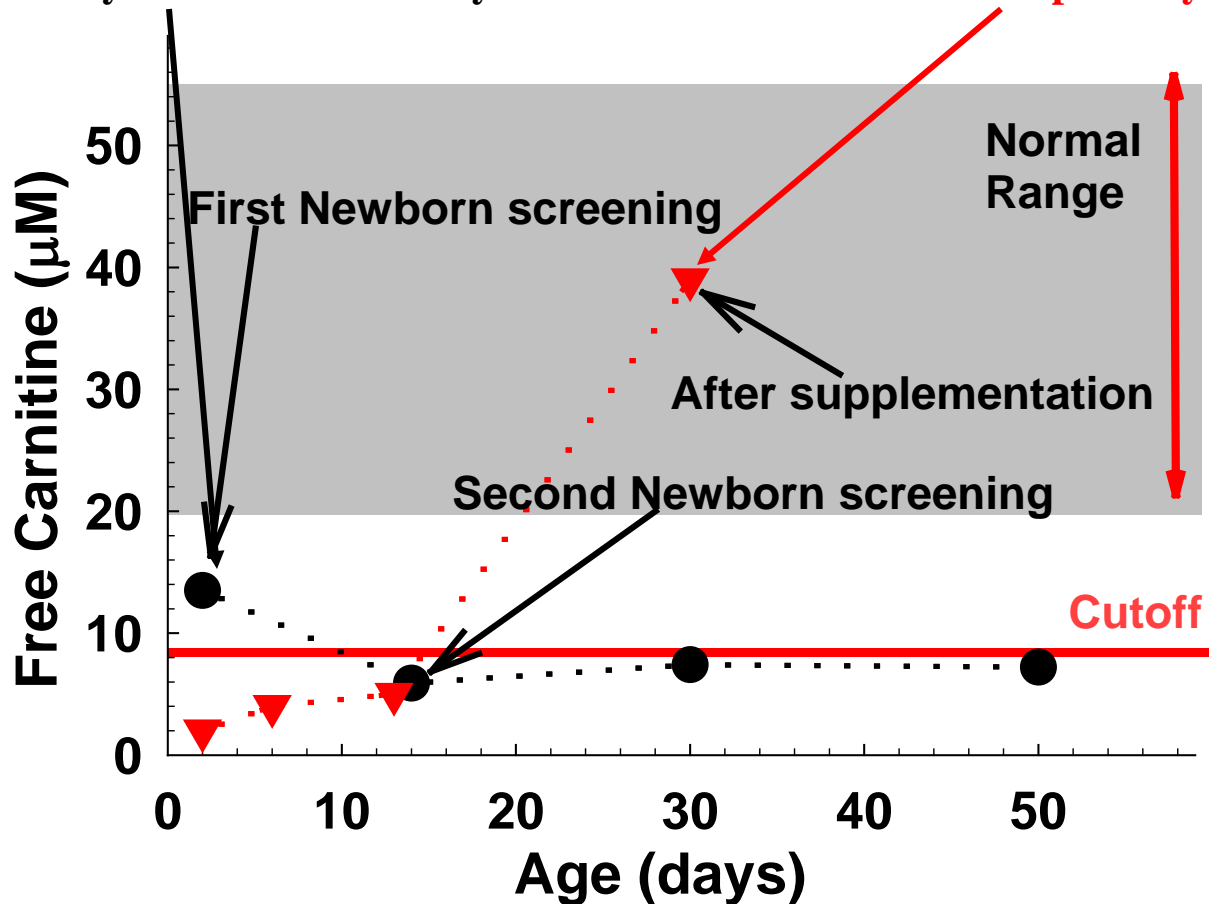
Founder mutation: N32S

Young adults (age 25-30 years) died from ventricular fibrillation (no cardiomyopathy). Some complain of lassitude and weakness which improves with carnitine supplementation.

NEWBORN SCREENING FOR PRIMARY CARNITINE DEFICIENCY

Infant with primary carnitine deficiency

Infant of mother with primary carnitine deficiency



NEWBORN SCREENING FOR PRIMARY CARNITINE DEFICIENCY

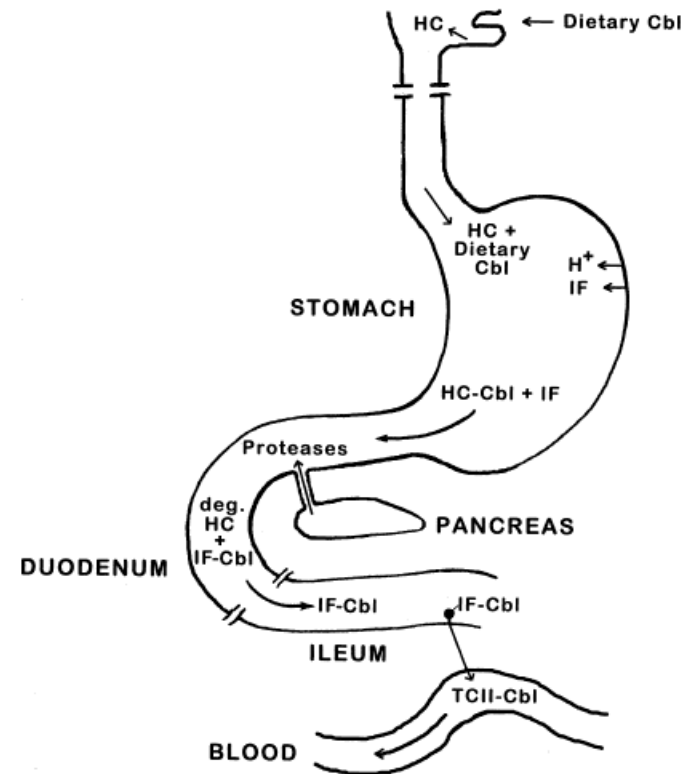
- **Carnitine is transferred from the mother to the fetus during pregnancy.**
- **Babies with primary carnitine deficiency usually do not have extremely low concentrations of carnitine at birth; however their carnitine concentration decreases with time.**
- **Often, a second screen at 7-28 days of life identifies infants with primary carnitine deficiency.**

NEWBORN SCREENING FOR PRIMARY CARNITINE DEFICIENCY

- **Extremely low concentrations of free carnitine in the first screen (24-48 hours of life) of an infant are often associated with a maternal disease:**
 - **Maternal primary carnitine deficiency**
 - **Maternal Glutaric acidemia type I**
 - **Maternal MCAD deficiency**
 - **Maternal 3-methylcrotonylglycinuria**
- **Often, low carnitine is the only abnormal finding in the newborn screen of an infant with a mother with undiagnosed metabolic disorder.**
- **Appropriate follow-up includes evaluation of plasma free and total carnitine, plasma acylcarnitine profile and/or urine organic acids in both, mother and infant.**

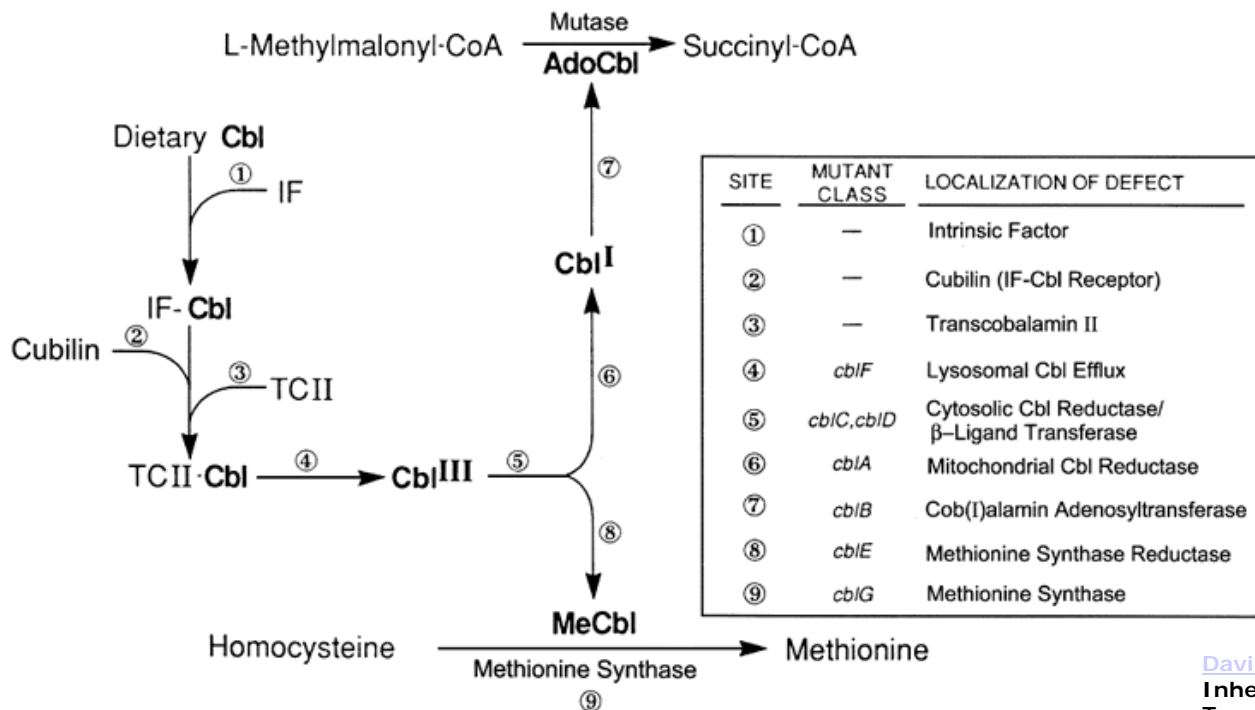
COBALAMIN (VITAMIN B12)

- Vitamin B₁₂ is acquired mostly from animal sources (meat and dairy products).
- Several steps are involved in the absorption of vitamin B12:
 - Proteolytic release from its associated proteins
 - Binding to intrinsic factor (IF), a gastric secretory protein
 - Recognition of the complex IF-Cbl by cubilin (a receptor on ileal mucosal cells)
 - Transport across the mucosal cells
 - Release into portal circulation bound to transcobalamin II (TC II), the serum protein that carries newly absorbed Cbl throughout the body



COBALAMIN (VITAMIN B12)

- Higher animals convert the vitamin into the two required coenzyme forms, adenosylcobalamin (AdoCbl) and methylcobalamin (MeCbl).
- AdoCbl and MeCbl are co-factor of, respectively, methylmalonyl-CoA mutase (elevated methylmalonic acid) and methionine synthase (elevated homocysteine).



MATERNAL VITAMIN B12 DEFICIENCY

- **One of the most frequent maternal diseases identified is vitamin B12 deficiency.**
- **Infants of mothers with vitamin B12 deficiency show elevated C3-(propionyl-) carnitine in the newborn screen.**
- **Confirmatory tests performed on these infants (urine organic acids, plasma amino acids, total plasma homocysteine) will show elevated excretion of methylmalonic acid and possibly elevated total plasma homocysteine.**
- **After administration of vitamin B12, usually, laboratory tests normalize in these infants.**

CLINICAL CASE

The patient is a 5-month-old infant who was admitted to the Children's Hospital with a hematocrit of 18. The patient's mother reported that he has always looked pale, but this has gotten worse in the last two weeks. On the day of admission he went to his primary care physician who checked a CBC which was significant for a hematocrit of 18 as well as a lipid layer when it was spun.

Normal birth and previous history. Eating and growing well, normal development.

WBC	5.5-17.0	7.4
RBC	3.70-5.30	1.48 L
HGB	10.5-13.5	
Hct	33.0-39.0	* 13.5 L
MCV	70.0-86.0	86.5
PLTS	150-400	104 L

B12 DEFICIENCY

URINE ORGANIC ACIDS

ABNORMAL: Methylmalonic aciduria. This can be due to a defect in B12 metabolism, B12 deficiency or methylmalonyl-CoA mutase deficiency. Would evaluate plasma amino acids and repeat this study. Ketonuria was also detected suggesting either catabolic state or impaired activity of the Krebs' cycle due to methylmalonic aciduria.

Organic acid quantitation in mmol/mol creatinine:

Analyte Result		1 mo-12 yrs
Lactic acid	95	<160
Pyruvic acid	109	<30
Succinic acid	29	<80
Fumaric acid	52	<10
2-Ketoglutaric	276	<120
Methylmalonic acid	2074	<5
3-OH-butyric acid	216	<4
Acetoacetic acid	34	<4
2-Keto-3-methylvaleric	1	<10
2-Keto-isocaproic	2	<4
2-Keto-isovaleric	ND	<4
Ethylmalonic acid	11	<15
Adipic acid	101	<35
Suberic acid	30	<10
Sebacic acid	1	<3
4-OH-phenylacetic acid	12	<100
4-OH-phenyllactic acid	3	<4
4-OH-phenylpyruvic acid	4	<2

PLASMA AMINO ACIDS

Abnormal. Low methionine and presence of free homocystine indicating a defect in homocystine remethylation (B12 deficiency, defects in B12 metabolism). Would evaluate B12 levels, urine organic acids and total plasma homocystine. Immediate genetic evaluation is recommended.

Amino Acids, Plasma Quant. [Show more...](#)

Test Status	Last Ref. Range	Units	1/14/2004	1/7/2004
			5:45	22:15
Amino Acid Interp., Ur			Final	Final
			* SEE NOTE	* SEE NOTE
Alanine	200-600	umol/L	244	288
Arginine	20-160	umol/L	35	20
Aspartic Acid	0-40	umol/L	5	6
Citrulline	Jun-60	umol/L	11	6
Cystine	Jul-70	umol/L	28	18
Glutamate	10-190	umol/L	80	54
Glutamine	410-960	umol/L	819	703
Glycine	220-520	umol/L	210 L	165 L
Histidine	40-120	umol/L	59	61
Homocystine	NDT	umol/L	* NOT DET*	PRESENT
Hydroxyproline	Jun-90	umol/L	19	8
Isoleucine	20-130	umol/L	27	35
Allo-Isoleucine	NDT	umol/L	* NOT DET*	NOT DET
Leucine	40-230	umol/L	38 L	75
Lysine	60-250	umol/L	109	105
Methionine	Oct-60	umol/L	11	3 L
Ornithine	20-135	umol/L	60	36
Phenylalanine	30-100	umol/L	13 L	35
Proline	110-500	umol/L	164	204

B12

193-982

pg/mL

974

<100L

141L

SUMMARY

- **Newborn screening for metabolic disorders can identify effectively infants with a metabolic condition and, often, even mothers with undiagnosed diseases.**
- **The most frequent maternal diseases identified by newborn screening are primary carnitine deficiency (carnitine uptake defect) and vitamin B12 deficiency.**
- **Appropriate follow-up of abnormal newborn screen results may include evaluation of the mother.**