# Congenital metabolic diseases

**Instructor:**

Annamarie Dalton, PhD
Assistant Professor, Dept. of Biochemistry and Molecular Biology
Basic Science Building Room 535E
Office telephone: 843-792-1495; daltonan@musc.edu

**Lecture Outline:**

**I. Overview of congenital metabolic diseases**
- A. Classification of congenital metabolic diseases
- B. Newborn metabolic screening in South Carolina
- C. List of Diseases in Newborn Screening Panels that we will discuss
- D. Metabolic screening panel

**II. Disorders of carbohydrate metabolism**
- A. Reintroduction to glucose utilization in the cell
- B. Galactose metabolic disorders
  - -classical versus non-classical galactosemia
- C. Glycogen storage disorders
- D. Pyruvate carboxylase deficiency
- E. Multiple carboxylase deficiency
  - i. Holocarboxylase synthetase deficiency
  - ii. Biotinidase deficiency

**III. Disorders of amino acid metabolism**
- A. Urea cycle disorders
  - i. Arginase deficiency
  - ii. Argininosuccinase deficiency
  - iii. Citrullenemia, Type I
  - iv. Ornithine transcarbamoylase deficiency
  - v. CPSI deficiency
  - vi. Hyperammonemia
- B. Disorders of amino acid catabolism
  - i. Degradation of phenylalanine and tyrosine
    - a. Phenylketonuria (PKU)
    - b.Tyrosinemia, Type I
  - ii. Branched-chain amino acids
    - -Maple syrup urine disease
  - iii. Methionine
    - -Homocystinuria

**IV. Disorders of organic acid metabolism**
- A. Propionic acidemia
- B. Methylmalonic acidemia

**V. Disorders of fatty acid β-oxidation**

A. Carnitine shuttle system

i. Primary carnitine deficiency
ii. CPT-I deficiency
iii. CPT-II deficiency
iv. Carnitine-acylcarnitine translocase deficiency

B. Fatty acid beta oxidation

-Mid-chain acyl CoA dehydrogenase deficiency

**VI. Disorders of peroxisomal functions**

A. Metabolism of very long-chain fatty acids

-X-linked adrenoleukodystrophy

B. Metabolism of branched-chain fatty acids

-Refsum disease

C. Peroxisome biogenesis

-Zellweger Spectrum Disorder

**VII. Lysosomal Storage Diseases**

Mucopolysaccharidoses

A. Hurler syndrome (Mucopolysaccharidosis type I)

B. Hunter syndrome (Mucopolysaccharidosis type II)

**Acknowledgment**: Special thanks to Drs. Yi-Te Hsu, Chris Davies and Hiroko Hama for providing contents of this syllabus.

**Learning objectives:**

1. State major classes of congenital metabolic disorders.
2. Describe the laboratory test used to screen for congenital metabolic disorders in newborns.
3. List diseases associated with the metabolism of galactose and specify the defective enzymes and key symptoms and signs.
4. Differentiate between classic and nonclassical galactosemia. State which carbohydrates must be avoided.
5. Specify the molecular basis of Pompe glycogen storage disorder and its clinical manifestations.
6. Explain the molecular basis of Leigh syndrome and why a keto diet is recommended for the afflicted individuals.
7. Detail the enzymatic reactions catalyzed by two proteins whose dysregulation can lead to multiple carboxylase deficiency.
8. Describe the mechanism of lactic acidosis in patients with multiple carboxylase deficiency.
9. Illustrate the urea cycle and name the enzymes catalyzing individual reactions.
10. List several metabolic defects involving the urea cycle and specify which intermediate will accumulate in each case, as well as the treatment strategies.
11. Distinguish between orotic aciduria caused by a defect in ornithine transcarbamoylase vs. UMP synthase.
12. Explain why urea cycle defects lead to hyperammonemia and describe the clinical manifestations of this condition.
13. Differentiate between the two forms of phenylketonuria and specify the key symptoms and signs and treatment strategies.
14. Describe the molecular basis of tyrosinemia I and specify the key symptoms and signs and treatment strategies.
15. Explain how branched-chain amino acid metabolism defects can lead to maple syrup urine disease and specify the key symptoms and signs and treatment strategies.
16. State four common causes of homocystinuria and specify the key symptoms and signs and treatment strategies. Distinguish between treatments based on distinct etiologies.
17. Specify the molecular basis of propionic and methylmalonic acidemia and the key symptoms and signs.
18. Describe the roles of OCTN2, CPT-I, CACT and CPT-II in long chain fatty acid oxidation.
19. Explain why hypoketotic hypoglycemia occurs in CPT-II and MCAD deficiency.
20. Describe where and how very long-chain fatty acids are broken down in cells and the diseases associated with deficiencies in this process.
21. Explain how branched-chain fatty acids such as phytanic acid are broken down in cells, the disease associated with the lack of this process, and the treatment strategy.
22. Define glycosaminoglycans; name two disorders associated with the metabolism of these mucopolysaccharides, list the respective defective enzyme, and specify the key symptoms and signs of each disorder.

## I. Overview of congenital metabolic diseases

### A. Classification of congenital metabolic diseases

A metabolic disorder is normally a rare genetic disorder that results from either a missing or defective enzyme in the body that normally catalyzes a specific biochemical reaction. So far, hundreds of metabolic disorders have been identified. The symptoms and signs of these disorders may vary widely. Presentation can range from mild to life threatening, depending on the extent to which the activity of the enzyme is affected. On the next page are some of the major classes of congenital metabolic disorders. They include disorders of carbohydrate, amino acid, organic acid, fatty acid, and purine/pyrimidine metabolism, as well as lysosome and peroxisome functions. We will be discussing a few of the disorders within each of these classes, particularly those covered in Step I and also by South Carolina newborn screening.

### B. Newborn metabolic screening in South Carolina

85 total disorders are screened for across the US, but there is no federally mandated list. Each state designs their own newborn screening panel; therefore, variety exists across the country. All infants born in South Carolina are required by law to participate in metabolic screening. The SC newborn screening panel includes 32 core metabolic and genetic conditions. The criteria for a core condition are as follows:

1. There is a specific and sensitive test available to detect the condition
2. The health outcomes of the condition are well understood
3. There is an available and effective treatment for the condition
4. Identification of the condition could affect future family planning decisions

In addition, the panel includes screening for 26 secondary metabolic and genetic conditions that may lead to severe consequences if not detected early in life.

## C. List of Diseases in Newborn Screening Panels that we will discuss

Of the diseases included on metabolic screening panels across the country, we will be covering the ones listed below. This link to an SC DHEC pdf shows the list of disorders screened for in South Carolina (https://dph.sc.gov/sites/scdph/files/2024-11/Newborn_Screening_Manual_Intro_20241107.pdf)

**Carbohydrate Metabolic Diseases**

- Galactosemia (GALT, GALK, and GALE)
- Pompe Disease (acid alpha-glucosidase deficiency)
- Pyruvate Carboxylase Deficiency (Leigh syndrome)
- Multiple Carboxylase Deficiency (Holocarboxylase synthetase and biotinidase deficiencies)

**Amino acid metabolism disorders**

**Urea Cycle Disorders**

- Arginase deficiency
- Argininosuccinic aciduria (Argininosuccinase [also called argininosuccinate lyase] deficiency)
- Carbamoyl phosphate synthetase I deficiency
- Citrullinemia, type I and II
- Ornithine transcarbamoylase deficiency

**Disorders of amino acid catabolism**

- Classic phenylketonuria
- Biopterin defect in cofactor biosynthesis / regeneration
- Non-PKU hyperphenylalaninemia
- Homocystinuria
- Hypermethioninemia
- Maple syrup urine disease
- Tyrosinemia type I (TYR II & III secondary)

**Disorders of organic acid metabolism**

- Propionic acidemia
- Methylmalonic acidemia

**Disorders of fatty acid β-oxidation**

- Primary carnitine deficiency
- CPT-I deficiency
- CPT-II deficiency
- Carnitine-acylcarnitine translocase deficiency
- Mid-chain acyl CoA dehydrogenase deficiency

**Disorders of peroxisomal functions**

- X-linked adrenoleukodystrophy
- Refsum disease
- Zellweger Spectrum Disorder

**Lysosomal Storage Diseases**

**Mucopolysaccharidoses**

- Hurler syndrome (Mucopolysaccharidosis type I)
- Hunter syndrome (Mucopolysaccharidosis type II)

## D. Metabolic screening panel

- Tandem mass spectrometry assesses levels of metabolites in newborn blood spot
- Metabolites include;
  - amino acids
  - urea cycle intermediates
  - fatty acids (acylcarnitines)
  - organic acids (e.g., C3= Propanoyl carnitine)

| **Amino acid disorders*** | **Analytes (Amino acids)**** |
|---|---|
| Phenylketonuria (PKU) or Hyperphenylalaninemia | Phenylalanine |
| Maple syrup urine disease (MSUD) | Leucine/Isoleucine, Valine |
| Homocystinuria (cystathionine synthase deficiency) or Hypermethioninemia | Methionine |
| Tyrosinemia, type 1 and possibly type II or III | Tyrosine - elevations may not be detectable on filter paper in first days of life |
| 5-oxoprolinuria (glutathione synthetase deficiency)* | 5-oxoproline |
| **Urea cycle disorders*** | |
| Citrullinemia | Citrulline |
| Argininosuccinic aciduria (ASA) | Citrulline, Argininosuccinic acid |
| Argininemia* | Arginine |
| **Fatty acid oxidation disorders*** | **Analytes (Acylcarnitines)**** |
| Short chain acyl-CoA dehydrogenase deficiency (SCAD) | **C4** |
| Isobutyryl-CoA dehydrogenase deficiency (IBCD) | **C4** |
| Glutaric aciduria, type 2 (GAII) or Multiple acyl-CoA dehydrogenase deficiency (MADD) | **C4, C5, C8:1**, C8, C12, C14, C16, C5-DC |
| Medium/Short chain L-3-hydroxyacyl-CoA dehydrogenase deficiency (M/SCHAD)* | **C4-OH** |
| Medium chain acyl-CoA dehydrogenase deficiency (MCAD) | C6, **C8**, C10, C10:1 |
| Long chain 3 hydroxyacyl-CoA dehydrogenase def. (LCHAD) | **C16-OH, C18:1-OH** |
| Trifunctional protein deficiency (TFPD)* | **C16-OH, C18:1-OH** |
| Very long chain acyl-CoA dehydrogenase deficiency (VLCAD) | **C14:1**, C14, C16 |
| Carnitine palmitoyl transferase deficiency, type 2 (CPTII)* | **C16, C18:1, C18** |
| Carnitine palmitoyl transferase deficiency, type 1A (CPT1A)* | **C0 elevated,** low C16, C18 |
| Carnitine/acylcarnitine translocase deficiency (CACT)* | **C16, C18:1, C18** |
| Carnitine uptake defect (CUD) | **Low C0** - may not be detected in first few days of life |
| **Organic acid disorders*** | **Analytes (Acylcarnitines)**** |
| Propionic acidemia (PA)* | **C3** |
| Methylmalonic acidemia (MMA)* | **C3** |
| Malonic aciduria (MA)* | **C3-DC** |
| Multiple carboxylase deficiency (MCD) | **C5-OH** |
| 3-hydroxy 3-methylglutaryl-CoA lyase deficiency (3HMG) | **C5-OH** |
| 3-methylcrotonyl-CoA carboxylase deficiency (3MCC) | **C5-OH** |
| 3-methylglutaconic aciduria (3MGA) | **C5-OH** |
| 2-methylbutyryl-CoA dehydrogenase deficiency (2MBD) | **C5** |
| Isovaleric acidemia (IVA) | **C5** |
| Glutaric acidemia, type 1 (GAI) | **C5-DC** |
| Beta-ketothiolase deficiency (BKT)* | **C5:1**, C5-OH |

**Notes**: *Some forms (genotypes) of these disorders may not be detected by neonatal screening, may not be detected in a newborn dried blood spot, or are extremely rare (1: >250,000).

**Primary MS/MS analyte(s) written in **bold** type.

MS/MS analytes are measured in micromoles per liter (uM/L).

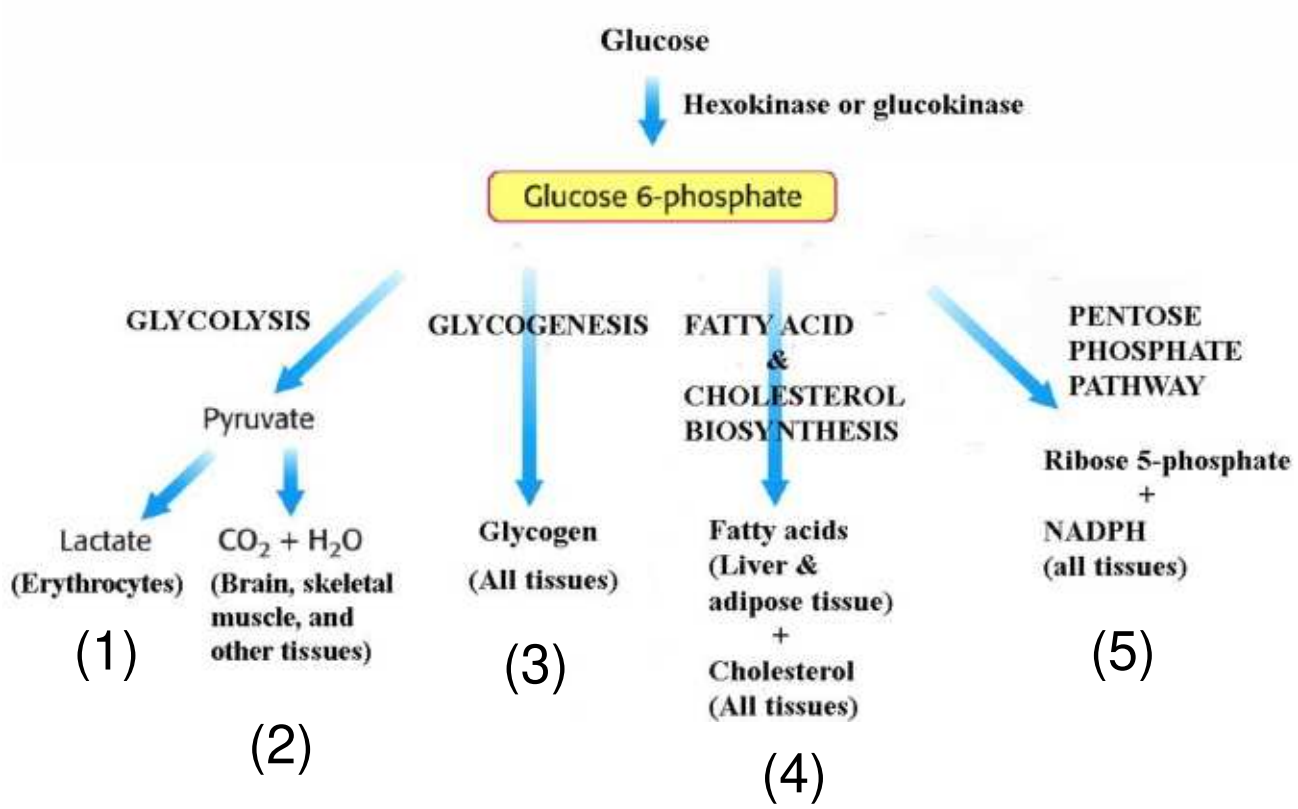
For substituted carnitines, a notation of (Cx) is used, in which (x) denotes the number of carbons in the fatty acid radical.

Hydroxylation is designated by (-OH), dicarboxylic acids are designated by (-DC), and unsaturation of the fatty acid chain is designated by (:1).

## II. Disorders of carbohydrate metabolism

### A. Reintroduction to glucose utilization in the cell

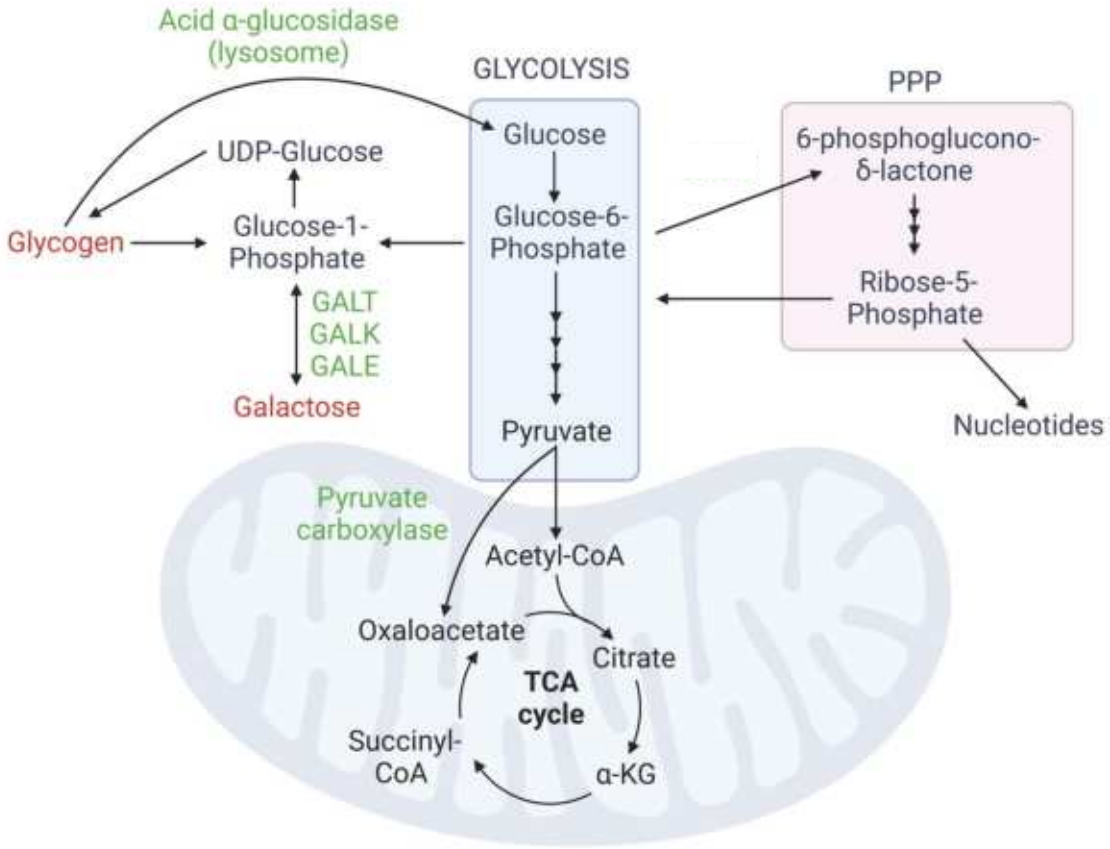
**Fates of dietary glucose**




Modified from Biochemistry 5th Ed. © 2002 W.H. Freeman and Company

Once taken inside cells, dietary glucose is phosphorylated by glucokinase (found in the liver and pancreatic beta cells) or by hexokinase (found in all other cell types) into glucose 6-phosphate. Phosphorylation of glucose traps it inside cells. Glucose 6-phosphate has many metabolic fates. It is converted into pyruvate by glycolysis. Pyruvate, under anaerobic condition or in red blood cells, will form lactate (1). Lactate that is formed will then be released and get picked up by the liver for reconversion back to glucose. This is known as the Cori cycle. On the other hand, under aerobic condition, pyruvate is oxidized to carbon dioxide and water via the TCA cycle and the electron transport chain to generate ATP (2). In all tissues, excess glucose 6-phosphate is stored as glycogen (3). In addition, excess glucose 6-phosphate is utilized to synthesize fatty acids in the liver and adipose tissue, and cholesterol for all tissues (4). Finally, glucose 6-phosphate can be shunted into the pentose phosphate pathway to form ribose 5-phosphate and NADPH (5). Ribose 5-phosphate is used for nucleotide biosynthesis and NADPH serves as a reducing molecule inside cells. NADPH can be utilized for the biosynthesis of fatty acids and cholesterol, and for the regeneration of reduced glutathione.

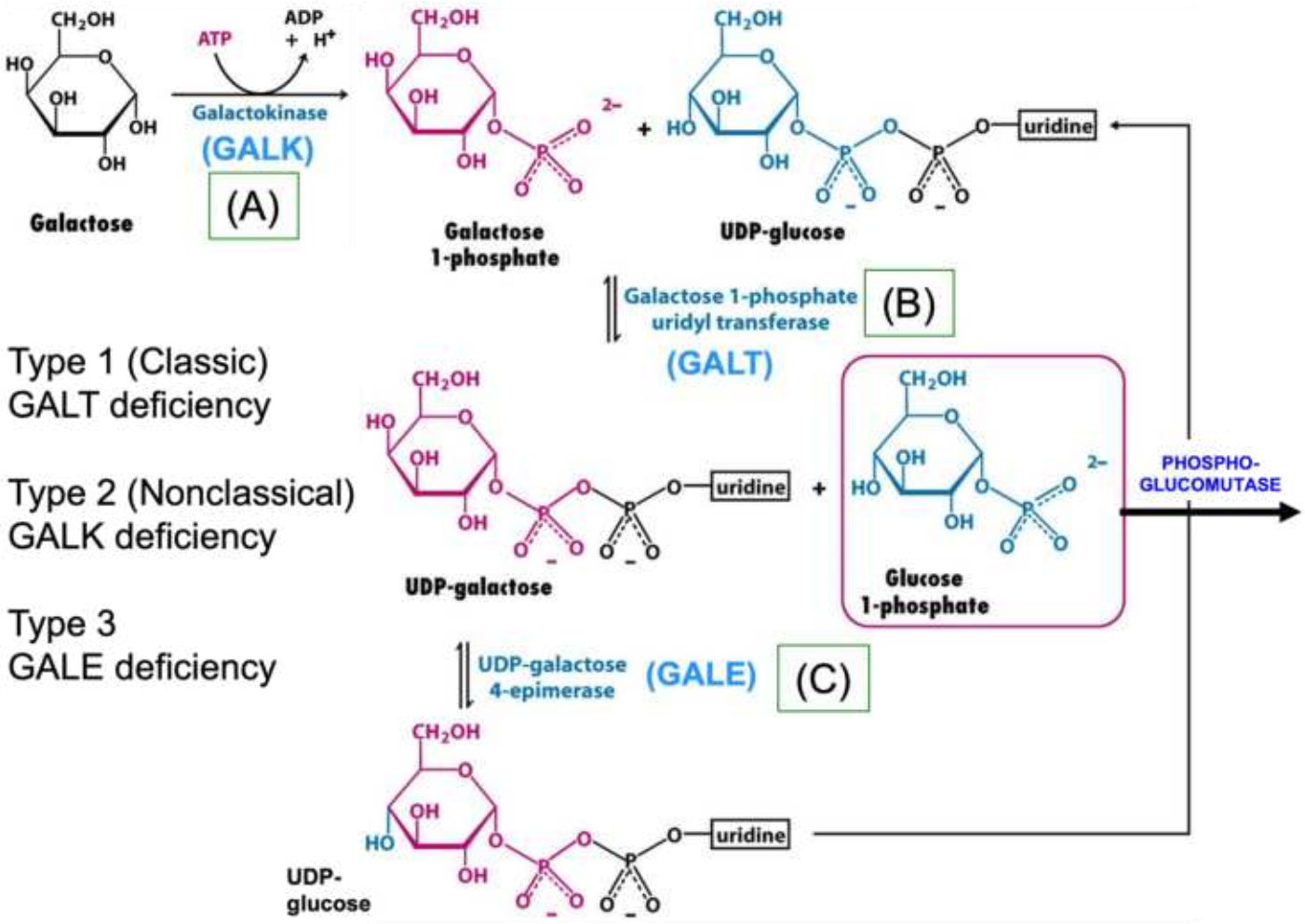
## Carbohydrate Metabolism Disorders




- Galactosemia (GALT, GALK, and GALE)
- Pompe Disease (acid alpha-glucosidase deficiency)
- Pyruvate Carboxylase Deficiency (Leigh syndrome)
- Multiple Carboxylase Deficiency (Holocarboxylase synthetase and biotinidase mutations)

Carbohydrate metabolism disorders highlighted in these lectures are shown in green in the figure above. Biotin cycle deficiencies are included here due to their effect on pyruvate carboxylase function, an enzyme that requires biotin as a cofactor.

**B. Galactose metabolism deficiencies**




Galactose derived from the breakdown of lactose can be metabolized by a number of tissues. Galactose is first phosphorylated to form galactose 1-phosphate by **galactokinase (A)**. Galactose 1-phosphate then reacts with UDP-glucose in a reaction catalyzed by **galactose 1-phosphate uridyl transferase (B)**. UDP-glucose becomes glucose 1-phosphate while galactose 1-phosphate turns into UDP-galactose. Glucose 1-phosphate is then isomerized by phosphoglucomutase to form glucose 6-phosphate which then enters the glycolytic pathway. UDP-galactose, on the other hand, is isomerized to UDP-glucose by **UDP-galactose 4-epimerase (C)**. UDP-glucose can then react with another molecule of galactose 1-phosphate (see above).

Defects in galactose metabolism can also give rise to diseases. **Nonclassical galactosemia** (type 2) is caused by defective **galactokinase (GALK) (A)**. On the other hand, **classic galactosemia** (type 1) is caused by a defect in **galactose 1-phosphate uridyl transferase (GALT) (B)**. **UDP-galactose 4-epimerase (GALE; more rare) (C)** deficiency causes galactosemia type 3.

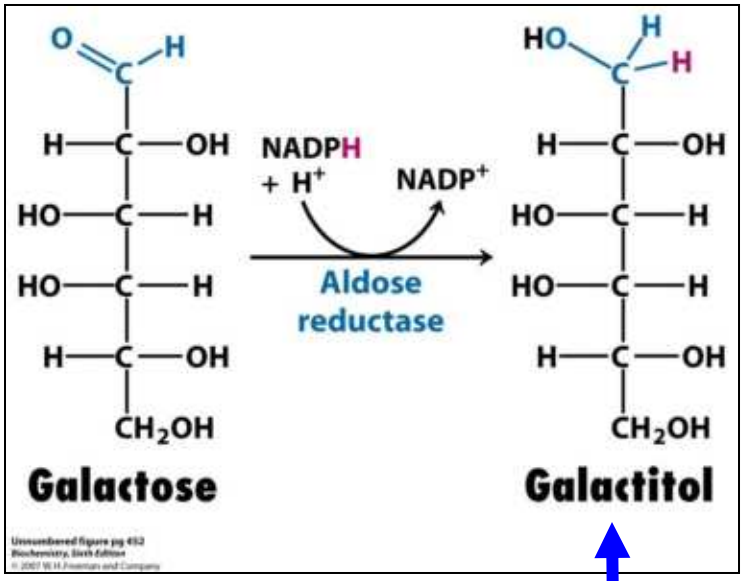
*i. Classic galactosemia vs. nonclassical galactosemia*

**Galactosemia**

**Nonclassical galactosemia (GALK)**
•Cataracts
•Increased risk of *E. coli* sepsis

**Classic galactosemia (GALT)**
•Growth delays
•Jaundice
•Eventual cataracts
•Lethargy
•Renal failure
•Intellectual developmental delays
•Increased risk of *E. coli* sepsis

**Treatment**
No galactose in diet
*i.e.,* no lactose (no milk)




**Causes cataracts**

When galactose accumulates, as in nonclassical galactosemia (GALK mutation), it gets into lens cells and is converted to galactitol by aldose reductase. Galactitol causes water diffusion into lens cells and leads to lens damage and cataract. For classic galactosemia, the accumulation of galactose 1-phosphate will cause enlargement of the liver, jaundice, lethargy, renal failure and irreversible intellectual developmental delays. Also, these patients will eventually get cataract, if left untreated. The affected infants cannot be breastfed and would need a special formula that has no lactose/galactose in it. Infants with galactosemia are highly susceptible to *E. coli* sepsis.

## C. Glycogen storage disorders

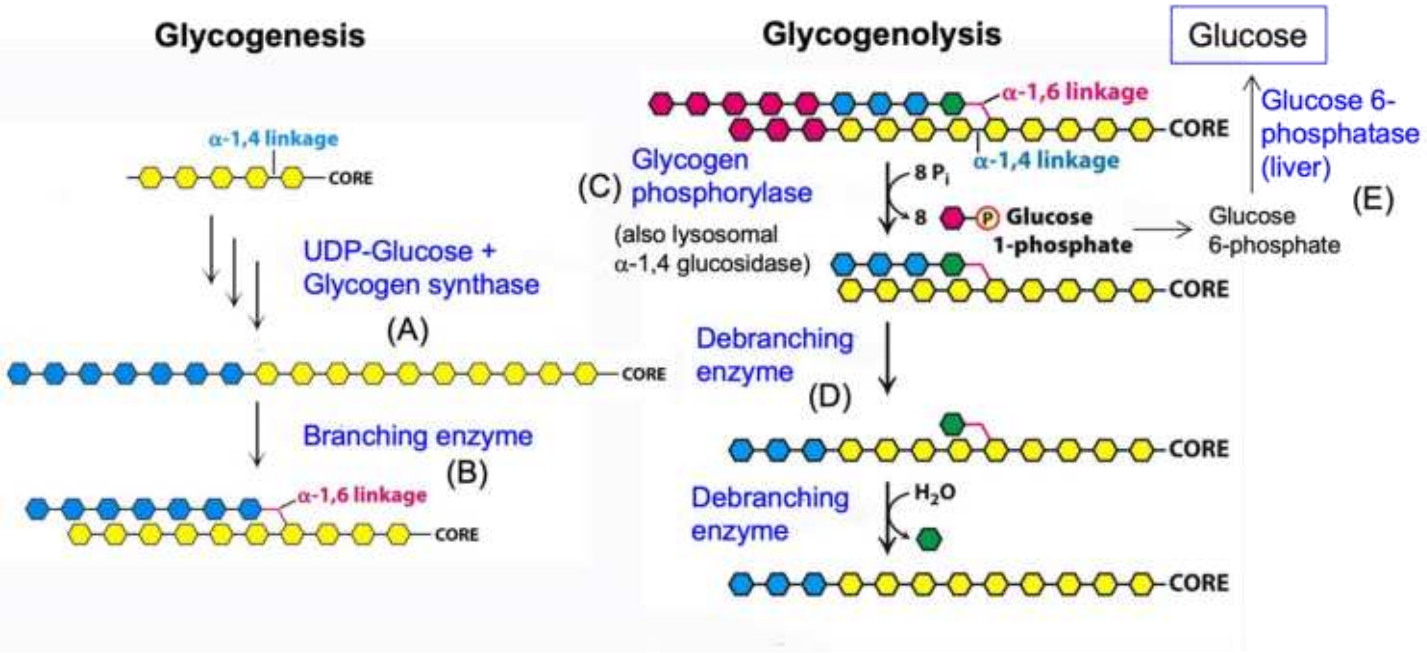
Excess glucose is stored as glycogen by glycogenesis. Glucose taken up by cells is first converted to glucose 6-phosphate by glucokinase (liver and pancreatic β cells) or hexokinase (all other cells). It is then isomerized by phosphoglucomutase to glucose 1-phosphate, activated as UDP-glucose, and lastly incorporated into glycogen by glycogen synthase (A). The branching enzyme then adds branches to the growing glycogen molecule (B). Glycogenesis is stimulated by insulin.

In times of need, glycogenolysis will occur to ultimately form glucose to maintain blood glucose homeostasis (in the liver) or glucose 6-phosphate for energy production (other cell types). This process is mediated by glycogen phosphorylase (C). A small amount of glycogen can also be broken down in lysosomes by lysosomal α-1,4 glucosidase, also known as acid α-1,4 glucosidase or acid maltase. When glycogen phosphorylase comes within 4 glucose residues away from the branch point, it will not proceed further. Instead, debranching enzyme is required to remove the short outer branches, also known as limit dextrin (D).

Glucose 1-phosphate produced by glycogenolysis is then isomerized to glucose 6-phosphate by phosphoglucomutase. In the liver, glucose 6-phosphate is subsequently acted on by glucose 6-phosphatase to form glucose (E). Free glucose is released into the bloodstream to maintain blood glucose homeostasis.

Defects in various enzymes involved in glycogen metabolism will give rise to glycogen storage disorders: von Gierke, Pompe, Cori, Andersen, and McArdle.

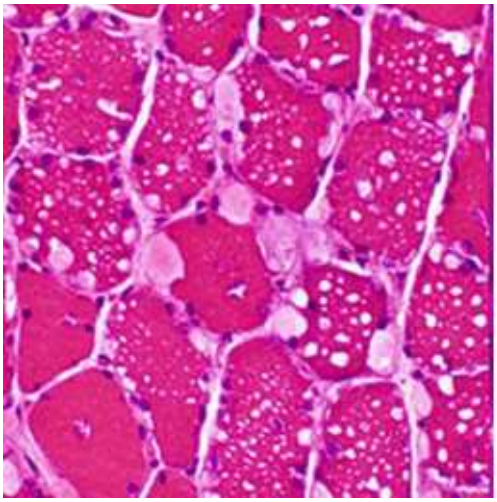
**Glycogen storage disorders**

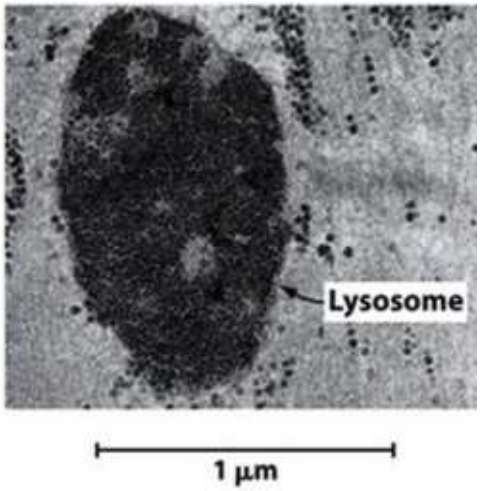



Type 1, von Gierke disease (Glucose 6-phosphatase deficiency)
Type 2, Pompe disease (Lysosomal acid α-1,4 glucosidase)
Type 3, Cori disease (Debranching enzyme)
Type 4, Andersen disease (Branching enzyme)
Type 5, McArdle disease (Muscle glycogen phosphorylase)

**Type II (Pompe disease)**

- Due to defective **lysosomal α-1,4-glucosidase** enzyme (**acid α-1,4-glucosidase or acid maltase**)
- Leads to accumulation of glycogen in lysosomes
- Affects all organs
- Key symptoms and signs:
  - Weak muscles, hypotonia, hepatomegaly, growth delays, and feeding problems
- Infantile form causes cardiorespiratory failure and early death





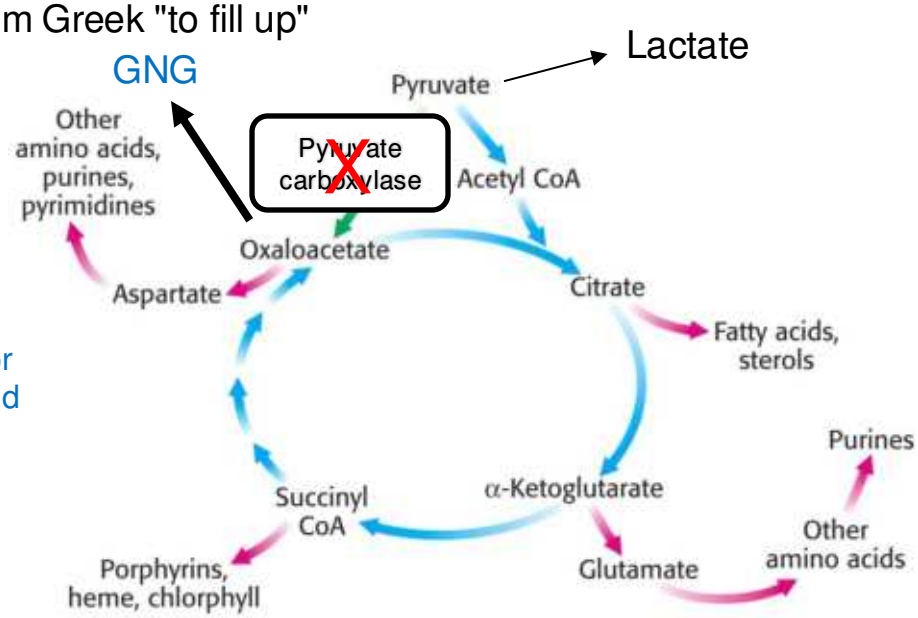

Pompe disease is the only glycogen storage disorder included in the newborn screening panels; however, the 5 listed on the previous page are important to know for Step 1.

**D. Pyruvate carboxylase deficiency (Leigh syndrome)**

**Pyruvate carboxylase converts pyruvate to OAA**

If TCA cycle intermediates are removed, they must be replenished.
Anaplerotic reactions - from Greek "to fill up"

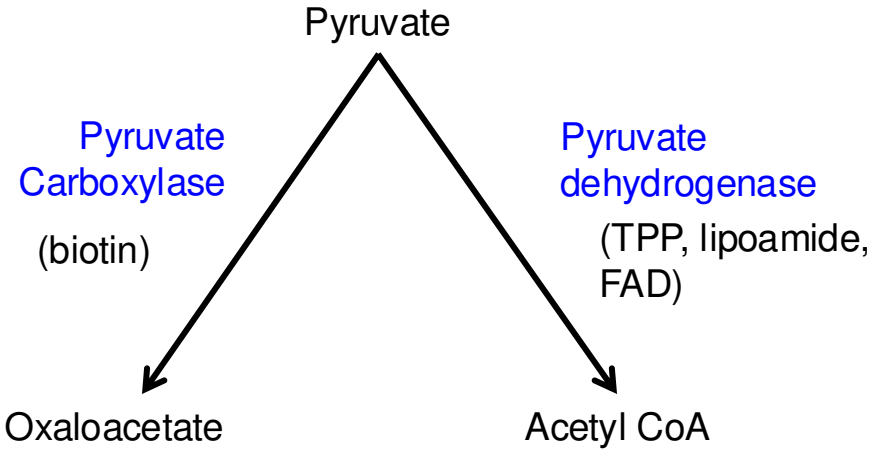
*e.g.* if oxaloacetate is used for gluconeogenesis or amino acid synthesis

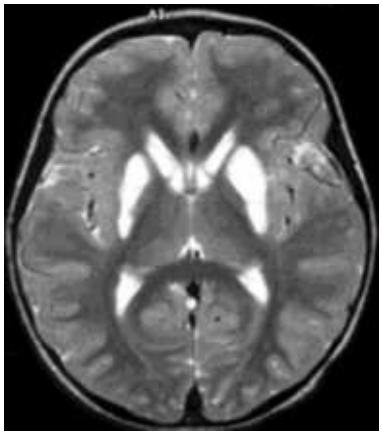


Leigh syndrome is the clinical manifestation of several metabolic deficiencies including mutations in pyruvate carboxylase, pyruvate dehydrogenase and the Fo subunit of ATP synthase. **Pyruvate carboxylase** transfers a carboxyl group onto pyruvate to generate oxaloacetate. The reaction requires biotin, which is a carrier of activated $CO_2$ to complete the transfer. **Pyruvate dehydrogenase complex** links glycolysis to the TCA cycle. It catalyzes the oxidative decarboxylation of pyruvate to acetyl CoA. Deficiencies in both pyruvate carboxylase and pyruvate dehydrogenase will lead to a build up of pyruvate. The buildup of pyruvate will lead to the formation of lactate by lactate dehydrogenase, resulting in metabolic acidosis. Without properly functioning pyruvate carboxylase or pyruvate dehydrogenase, gluconeogenesis and/ or the TCA cycle will be impaired, leading to the chronic lack of energy, causing motor and CNS problems. Leigh syndrome is characterized by visible necrotizing lesions on the brain, particularly in the midbrain and brainstem.

For treatment, the cofactors for the affected enzymes are typically supplied. Biotin is administered for patients with pyruvate carboxylase deficiencies to boost function of the present enzyme. Thiamine is often given to the patients to boost pyruvate dehydrogenase activity (thiamine pyrophosphate is a cofactor for this enzyme). In addition, a high-fat and low-carbohydrate diet is recommended. This diet would also preferentially contain ketogenic amino acids (**leucine and lysine**), instead of glucogenic amino acids, to avoid the formation of pyruvate (which is then converted to lactate). Oral sodium bicarbonate or sodium citrate may also be prescribed to manage lactate acidemia.

**Defects in pyruvate carboxylase, pyruvate dehydrogenase and the Fo subunit of ATP synthetase lead to Leigh syndrome**



- Pyruvate dehydrogenase or pyruvate carboxylase deficiency (X-linked) causes buildup of pyruvate => lactate => metabolic acidosis
- Characterized by necrotizing lesions on the brain, particularly in the midbrain and brainstem.
- Key symptoms and signs: loss of basic skills such as sucking, head control, walking and talking.
- Treatments: high thiamine and/or biotin, high fat and low carbohydrate diet, ketogenic amino acids (leucine and lysine), and oral supplementation of sodium bicarbonate or citrate.

## E. Multiple carboxylase deficiency

Pyruvate carboxylase is one of four carboxylase enzymes in the body that require biotin as a conjugated cofactor. Biotin is vitamin B7 and cannot be synthesized by any mammalian species; therefore, it is an essential vitamin for humans. When free biotin or a biotinylated protein enters our body through our diet, it must be processed through the biotin cycle to incorporate it into the carboxylase enzymes that require it for function.

Briefly, the biotin cycle covers the following reactions. Free biotin is covalently linked to lysine residues within the active site of the apo form of the carboxylase enzymes by holocarboxylase synthetase. When the "holocarboxylases," carboxylases with covalently linked biotin, have reached the end of their life cycle, the biotinylated proteins are degraded, and the biotin is released still covalently linked to the single lysine residue. This molecule of biotin linked to a lysine amino acid is called biocytin. The enzyme biotinidase removes the lysine residue from biocytin to free biotin for future incorporation into newly synthesized carboxylases.

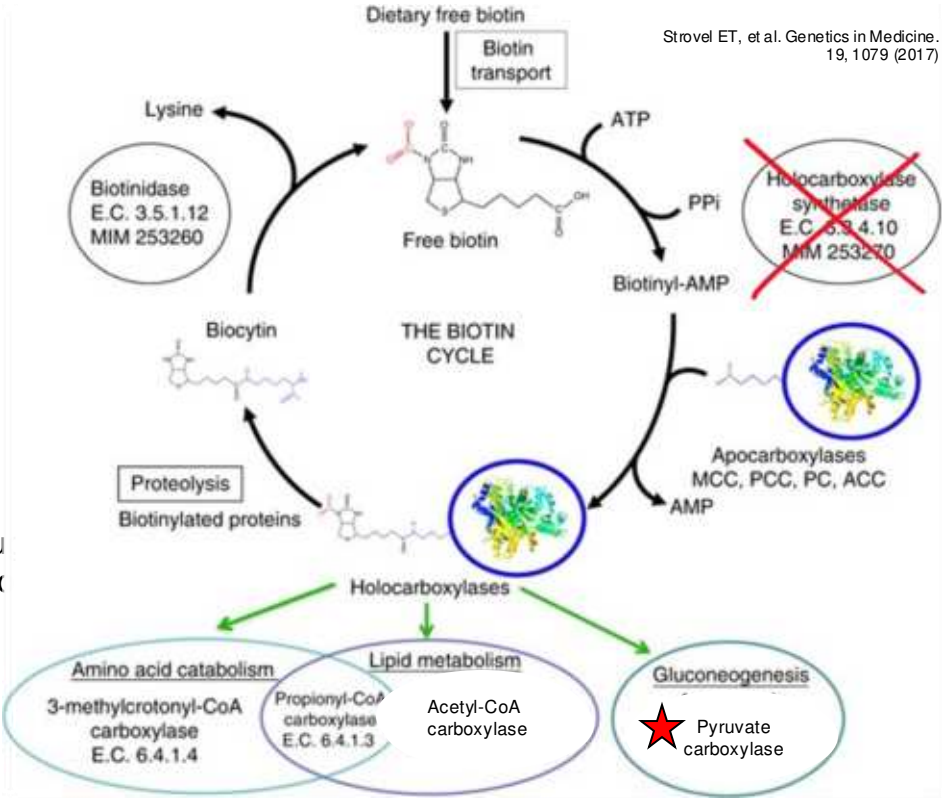
### *i. Holocarboxylase synthetase deficiency*

Holocarboxylase synthetase adds free biotin to the apo-forms of carboxylases in the "Biotin Cycle"

**Key signs and symptoms:** Metabolic acidosis, lethargy, hypotonia, seizures, and dermatitis

**Treatment**: Biotin

Leads to lactic acidosis throu
pyruvate carboxylase inhibitio




There are four carboxylases that require biotin; 3-methylcrotonyl-CoA carboxylase, propionyl-CoA carboxylase, acetyl-CoA carboxylase, and pyruvate carboxylase. These enzymes catalyze reactions within amino acid catabolism, lipid metabolism and gluconeogenesis. Therefore, mutations in biotin cycle enzymes will affect multiple areas of metabolism. One of the major signs and symptoms for **biotin cycle deficiencies, also called multiple carboxylase deficiencies**, is metabolic acidosis due to the buildup of pyruvate leading to excess lactate as a result of diminished pyruvate carboxylase function. Treatment is to supplement with biotin.

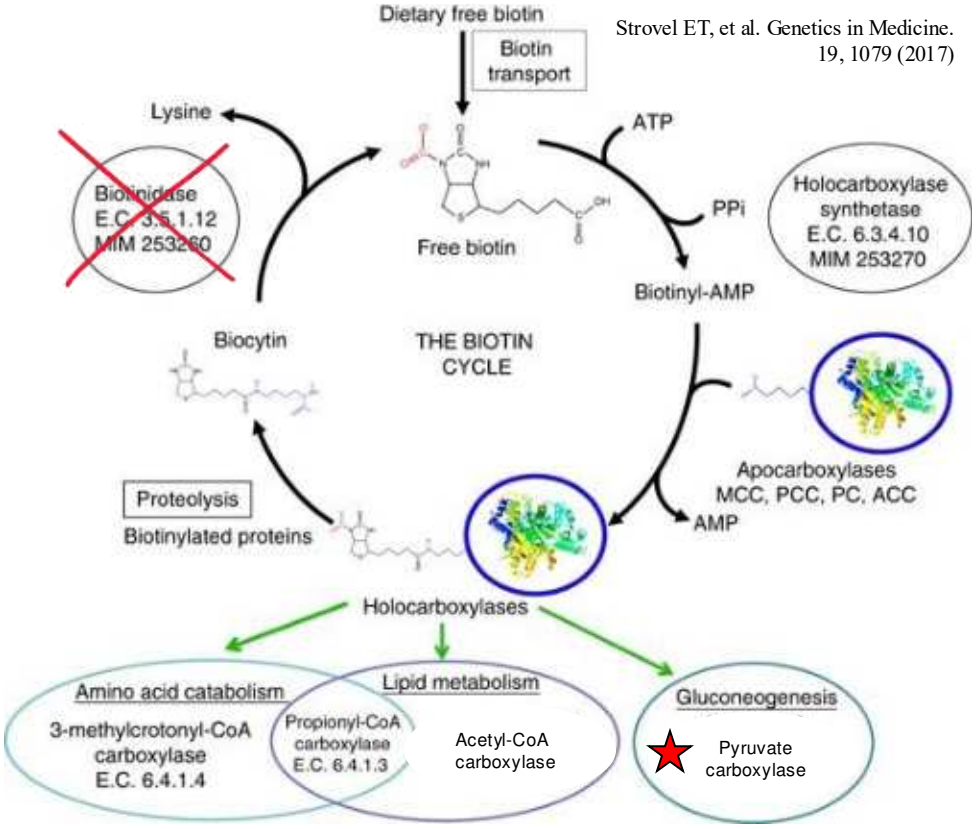
### *ii. Biotinidase deficiency*

Biotinidase deficiency leads to similar effects as seen with holocarboxylase synthetase but tends to be a later onset form. These deficiencies are sometimes grouped as “biotin-responsive multiple carboxylase deficiency.”

Biotinidase frees biotin in the “Biotin Cycle”

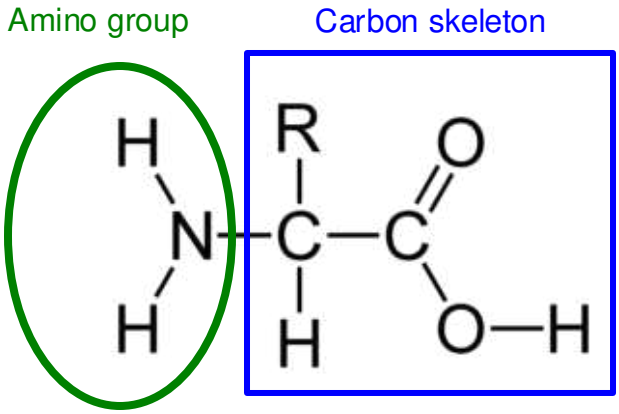
Without biotinidase, biotin is not available for the 4 carboxylases that require it.

Leads to lactic acidosis through pyruvate carboxylase inhibition



## III. Amino acid metabolism disorders

**Amino acid catabolism**




Amino group is removed via the urea cycle

Carbon skeleton is used for energy production

**Defects**

**Urea cycle disorders**

- Arginase deficiency
- Argininosuccinic aciduria (Argininosuccinase [also called argininosuccinate lyase] deficiency)
- Carbamoyl phosphate synthetase I deficiency
- Citrullinemia, type I and II
- Ornithine transcarbamoylase deficiency

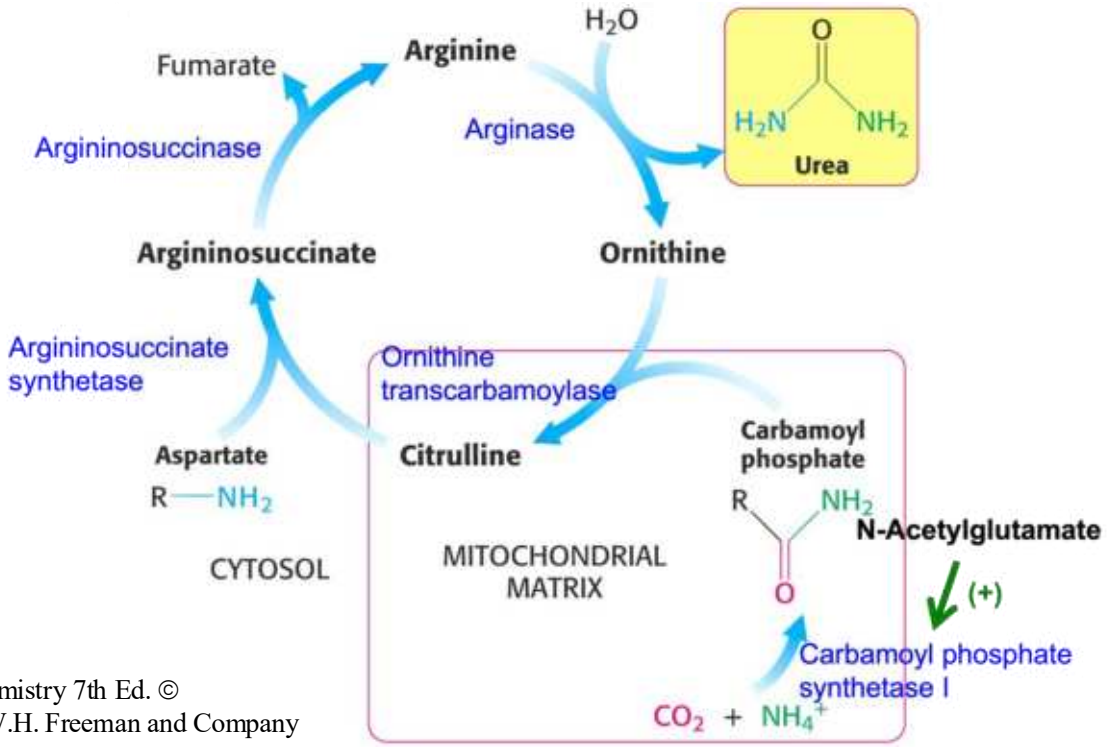
**Disorders of amino acid catabolism**

- Classic phenylketonuria
- Biopterin defect in cofactor biosynthesis / regeneration
- Non-PKU hyperphenylalaninemia
- Homocystinuria
- Maple syrup urine disease
- Tyrosinemia type I (TYR II & III secondary)

### A. The urea cycle

Amino acids in excess of the body's needs are degraded to specific metabolic intermediates. The major site of amino acid degradation in mammals is the liver. The degradation of amino acids has two parts: clearance of the amino group as urea and the catabolism of the carbon skeletons, which can be converted into major metabolic intermediates.

In the liver, transamination of various amino acids with $\alpha$-ketoglutarate will form glutamate. Glutamate will then undergo oxidative deamination to form ammonium. Inside the body, ammonium can be produced by a number of means. In addition, ammonium can be formed from deamination of the side chain amino groups of glutamine and asparagine and direct deamination of histidine, serine, and threonine. Moreover, purine nucleotide catabolism in various tissues and degradation of urea and amino acids by gut bacteria will generate ammonium. In the liver, ammonium is shunted into the urea cycle.




Biochemistry 7th Ed. ©
2012 W.H. Freeman and Company

The urea cycle involves both mitochondria and the cytoplasm. In the mitochondrial matrix, carbamoyl phosphate synthetase I condenses carbon dioxide or carbonic acid with ammonia to form carbamoyl phosphate. Carbamoyl phosphate then reacts with ornithine to form citrulline, in a reaction catalyzed by ornithine transcarbamoylase. Citrulline is then transported out of the mitochondria matrix into the cytoplasm where it reacts with aspartate to form argininosuccinate. This reaction is catalyzed by argininosuccinate synthetase. Argininosuccinate is then acted on by argininosuccinase to form fumarate and arginine. Fumarate can be recycled to reform aspartate while arginine is hydrolyzed by arginase to produce urea and regenerate ornithine. Urea is released from the liver into the blood and is picked up by the kidneys for excretion. Ornithine is transported from the cytoplasm back into mitochondria for another round of the urea cycle.

Urea removes two amino groups at a time, one from carbamoyl phosphate and the other from aspartate. The rate-limiting enzyme for the urea cycle is carbamoyl phosphate synthetase I (CPS I). N-Acetylglutamate is an allosteric activator of CPS I. It is synthesized from glutamate and acetyl CoA by N-acetylglutamate synthase (NAGS). Defects in NAGS can also lead to metabolic disease. A defect in any one of the enzymes involved in the urea cycle can give rise to urea cycle disorder and hyperammonemia. Liver transplant may be an option for all urea cycle disorders in circumstances where neurological symptoms are progressing despite implementation of less invasive therapies.

### *i. Arginase deficiency*

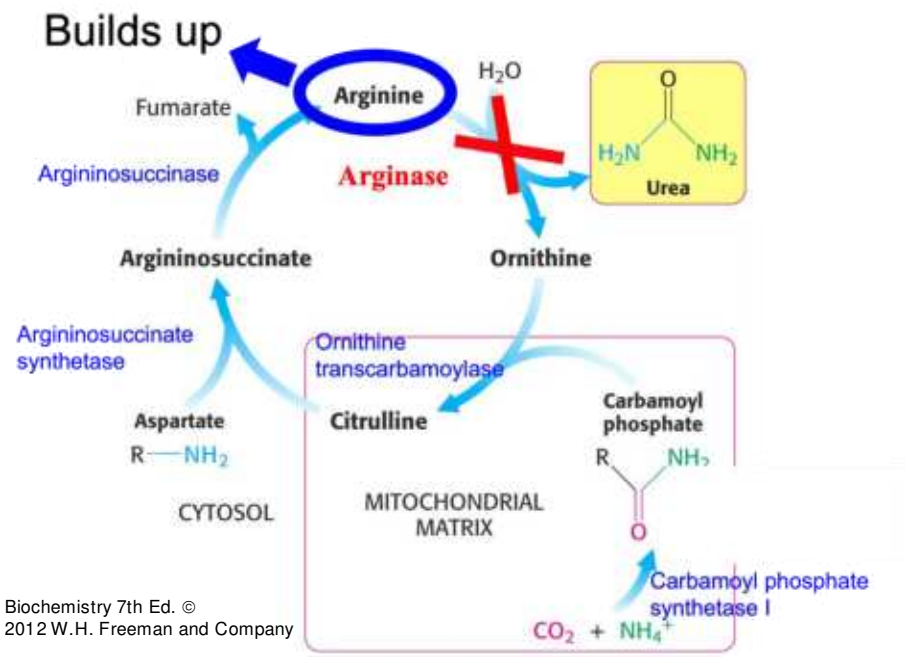
Arginase is one of the enzymes in the urea cycle. When it is defective, arginine will build up along with ammonia in some cases (see next page). High levels of arginine, and ammonia, can be particularly toxic to nerve cells and high arginine can cause brain damage. This is the only urea cycle disorder that is not treated with arginine supplementation for obvious reasons. Treatment is low arginine diet and administration of sodium phenylacetate-sodium benzoate which will conjugate with glycine and glutamine generating excretable products as a means to clear amino groups from the body. Sodium phenylacetate and sodium benzoate are called nitrogen scavengers.

## Arginase deficiency

**Screening Test:**
High arginine,
Sometimes high ammonia

**Signs and Symptoms:**
Hyperammonemia
Poor feeding, lethargy, vomiting, **spasticity/spastic paraplegia**, troubled breathing, seizures

**Treatment:**
sodium phenylacetate-sodium benzoate, low protein/ arginine diet supplemented with essential amino acids, hemodialysis for hyperammonemia, liver transplant (LT)



Biochemistry 7th Ed. © 2012 W.H. Freeman and Company

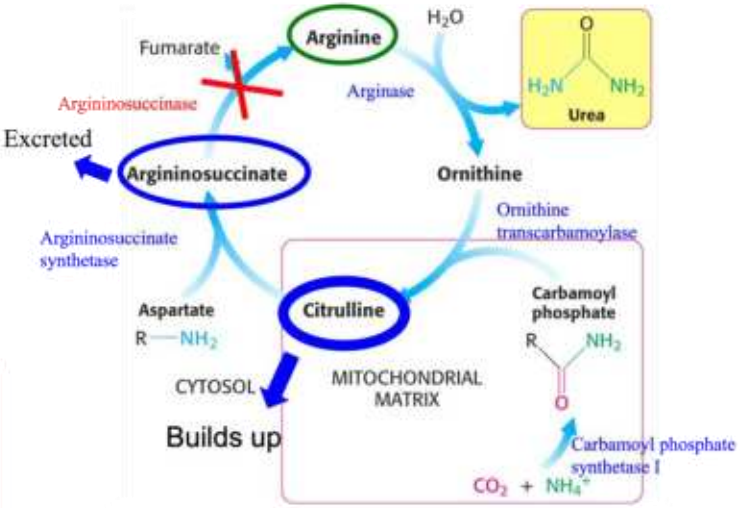
### *ii. Argininosuccinase deficiency*

## Argininosuccinase deficiency

**Screening Test:**
Increased citrulline +/- increased argininosuccinic acid

**Signs and Symptoms:**
Hyperammonemia
Poor feeding, lethargy, vomiting, spasticity, troubled breathing, seizures, coma

**Treatment:**
Arginine supplementation and sodium phenylacetate -sodium benzoate, low protein diet, hemodialysis for hyperammonemia , LT



Biochemistry 7th Ed. © 2012 W.H. Freeman and Company

For an individual with **argininosuccinase deficiency**, argininosuccinate and citrulline can build up. Argininosuccinate can be excreted. However, unless ornithine is being resupplied, the urea cycle will cease to operate. So, the therapeutic strategy in this case is to give the patient large amounts of arginine. Arginine is converted to urea by arginase and in the process, ornithine is generated. This supplemented ornithine can react with carbamoyl phosphate to make citrulline. Citrulline is then converted into argininosuccinate by reacting with aspartate and is then excreted. In effect, arginine replenishes the supply of ornithine needed for the removal of the amino groups from carbamoyl phosphate and aspartate. Nitrogen is removed in this case as argininosuccinate.

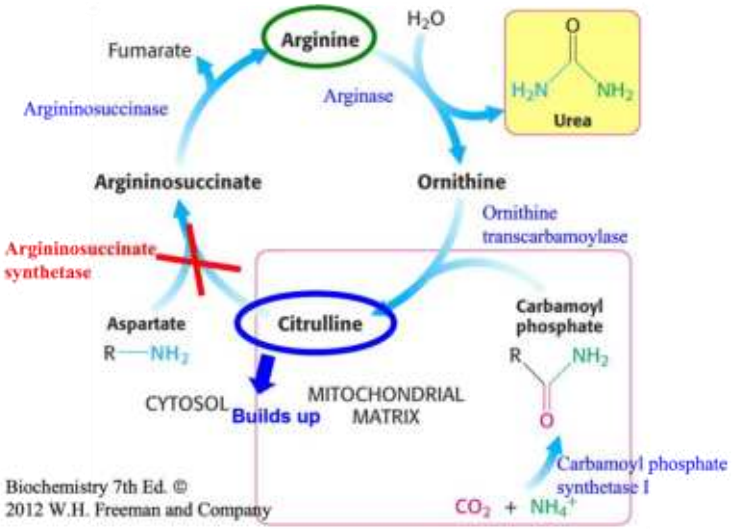
### *iii. Citrullenemia, type I (aka Argininosuccinate synthetase deficiency)*

**Citrullenemia type I**
**Argininosuccinate synthetase deficiency**

**Screening Test:**
Increased Citrulline

**Signs and Symptoms:**
Hyperammonemia
Poor feeding, lethargy, vomiting, spasticity, troubled breathing, seizures, coma

**Treatment:**
Arginine supplementation and sodium phenylacetate - sodium benzoate, low protein diet, hemodialysis for hyperammonemia, liver transplant




Deficiency in **argininosuccinate synthetase** causes **citrullinemia, type I**. Without an effective enzyme, these individual will have a build up of citrulline and ammonia. Treatment consists of arginine supplementation and sodium phenylacetate-sodium benzoate to help clear amino groups from the body and prevent significant damage to the brain and nerves.

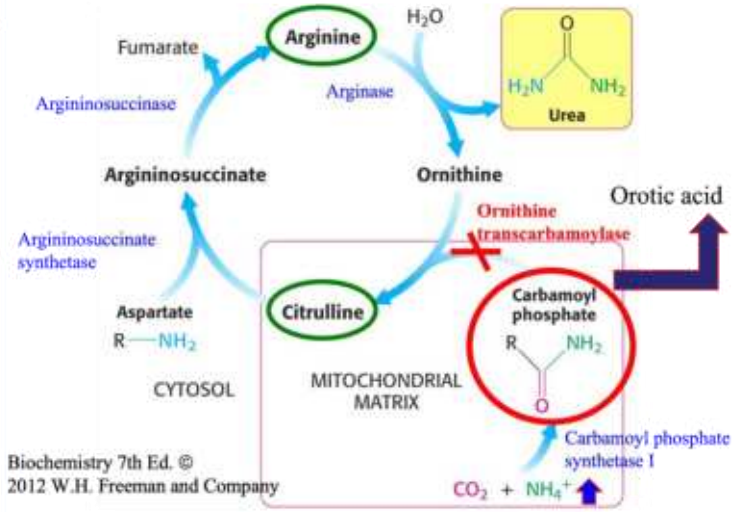
### *iv. Ornithine transcarbamoylase deficiency and orotic aciduria*

**Ornithine transcarbamoylase deficiency**
**and orotic aciduria**

**Screening Test:**
Low levels of citrulline

**Signs and Symptoms:**
Hyperammonemia, floppy arms and legs (hypotonia), poor feeding, lethargy, vomiting, troubled breathing, seizures

**Treatment:**
Arginine and citrulline supplementation and sodium phenylacetate - sodium benzoate, low protein diet, hemodialysis for hyperammonemia, liver transplant



Arginine therapy alone will not work in patients with either **ornithine transcarbamoylase** (on previous page) or **carbamoyl phosphate synthetase I** (below) deficiency. A deficiency in either one of these enzymes will compromise the formation of citrulline. OTC deficiency is treated with citrulline and arginine supplements along with sodium phenylacetate-sodium benzoate. Individuals with a deficiency in ornithine transcarbamoylase, the most common urea cycle disorder, will have high levels of carbamoyl phosphate in mitochondria. Carbamoyl phosphate can diffuse out of mitochondria and get utilized in the pyrimidine biosynthetic pathway in the cytoplasm to form orotic acid. High levels of orotic acid will give rise to **orotic aciduria**. Orotic aciduria can also arise if an individual has a deficiency in UMP synthase (hereditary orotic aciduria). However, individuals with UMP synthase deficiency will have megaloblastic anemia, normal blood urea nitrogen (BUN) and no hyperammonemia. On the other hand, individuals with ornithine transcarbamoylase deficiency will have hyperammonemia, low BUN, and no megaloblastic anemia.

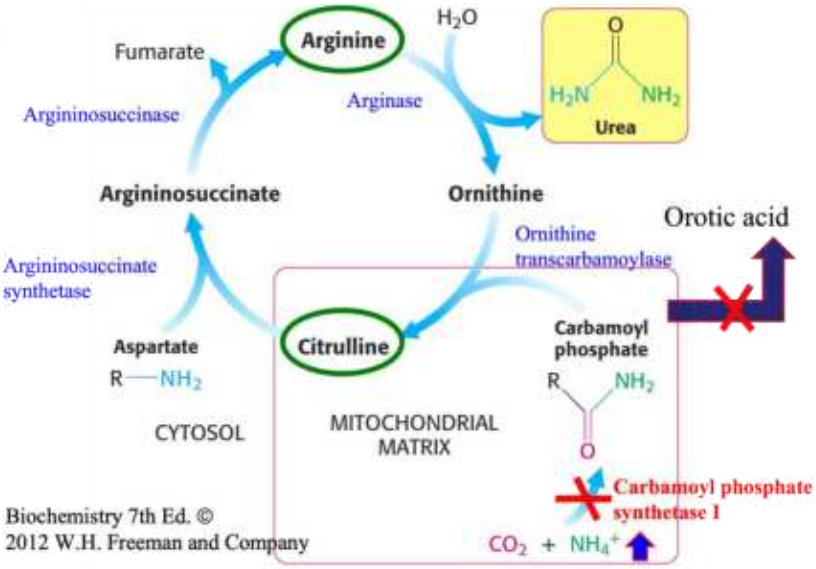
***v. Carbamoyl phosphate synthetase I deficiency (CPSI)***

## Carbamoyl phosphate synthetase I deficiency

**Screening Test:**
Low levels of citrulline

**Signs and Symptoms:**
Hyperammonemia, poor feeding, lethargy, vomiting, troubled breathing, seizures

**Treatment:**
Arginine and citrulline supplementation and sodium phenylacetate - sodium benzoate, low protein diet, hemodialysis for hyperammonemia, liver transplant



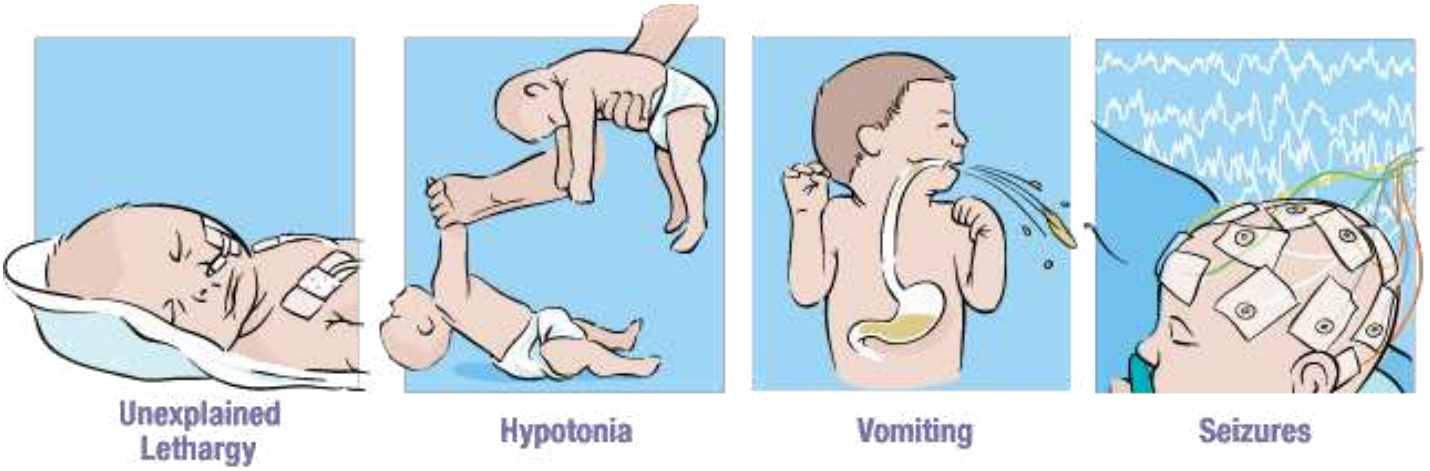

**CPSI** is the rate limiting enzyme for the urea cycle. If CPSI is defective, there will be a build up of ammonia and citrulline will not be generated. Citrulline and arginine supplements are administered along with sodium phenylacetate-sodium benzoate to circumvent CPSI deficiencies. Unlike OTC deficiencies, orotic acid build up is not observed because carbamoyl phosphate is not being synthesized.

## Urea cycle defects lead to hyperammonemia

- Defects in the urea cycle lead to elevated $NH_4^+$, a toxic substance and accumulation of urea cycle intermediates

- High $NH_4^+$ levels in the brain will deplete $\alpha$-ketoglutarate and promote glutamine synthesis, resulting in osmotic imbalance in astrocytes and brain damage

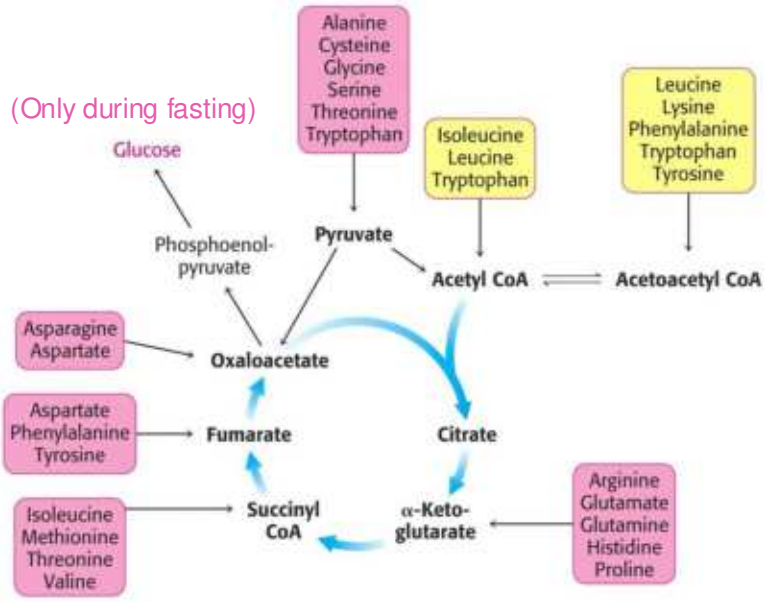
- Key symptoms and signs:
  Lethargy, hypotonia, vomiting, seizures, bilateral spastic paresis, choreoathetoid movements, and followed by coma and irreversible brain damage.




Liver damage or genetic defects in the urea cycle will result in **hyperammonemia**, or the accumulation of ammonium, a toxic substance. In the brain, high ammonium levels will deplete $\alpha$-ketoglutarate and promote glutamine synthesis. The buildup of glutamine will result in osmotic imbalance and brain damage. Signs and symptoms of hyperammonemia in neonates include lethargy, poor feeding, hypotonia, vomiting, hyperventilation, bilateral spastic paresis, choreoathetoid movements and seizures. If ammonium levels become too high, coma and death will result.

Adult onset urea cycle disorder may present with chronic, or acute psychiatric, neurological and digestive symptoms/signs and a penchant for protein avoidance. These individuals likely have milder forms of the disorder, and the defective enzymes likely still possess some functional activities. **Ornithine transcarbamoylase** deficiency is the most common urea cycle disorder.

## B. Disorders of amino acid catabolism




Biochemistry 5th Ed. ©
2002 W.H. Freeman and Company

**Phenylalanine**
- Classic phenylketonuria
- Non-PKU hyperphenylalaninemia
- Biopterin defect in cofactor regeneration

**Tyrosine**
- Tyrosinemia I (TYR II and III secondary)
- (Alkaptonuria)

**Branched-chain amino acids (Leu, Ile and Val)**
- Maple syrup urine disease

**Methionine**
- Homocystinuria
- Hypermethioninemia

Once the amino group of an amino acid is removed (primarily by transamination), the carbon skeleton is left. The carbon skeleton of amino acids without the primary amine are called α-ketoacids. The carbon skeletons of amino acids can be converted into major metabolic intermediates. Even though there are 20 amino acids, the carbon skeletons of these amino acids will yield only seven molecules: pyruvate, acetyl CoA, acetoacetyl CoA, α-ketoglutarate, succinyl CoA, fumarate, and oxaloacetate. Amino acids that yield acetyl CoA or acetoacetyl CoA are termed ketogenic, because they can be used to make ketone bodies. On the other hand, amino acids that form pyruvate, α-ketoglutarate, succinyl CoA, fumarate, and oxaloacetate are termed glucogenic because they can be used to make glucose. Only leucine and lysine are solely ketogenic. Phenylalanine, isoleucine, tyrosine, tryptophan and threonine (PITTT) are simultaneously ketogenic and glucogenic.

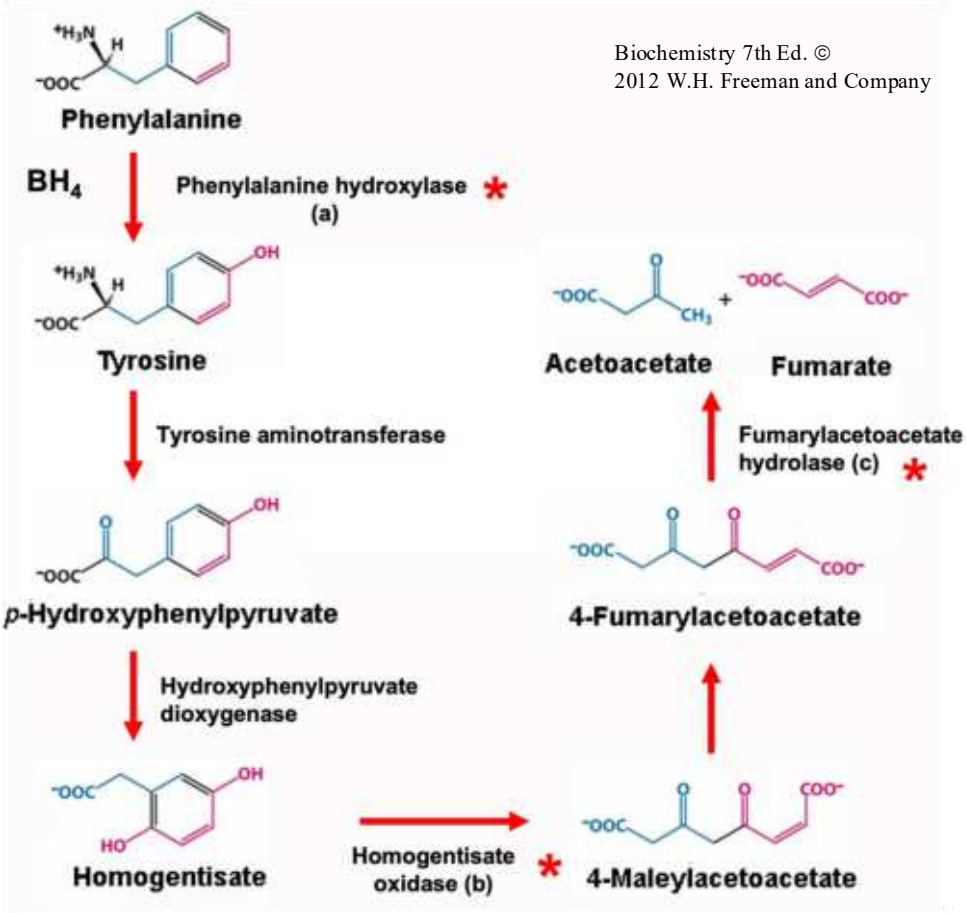
If there is a defect in an enzyme involved in the catabolism of the carbon skeletons of these amino acids, the buildup of metabolic intermediates can give rise to disease. Here, disorders associated with the catabolism of methionine, phenylalanine, tyrosine, and branched-chain amino acids (leucine, isoleucine, and valine) will be discussed.

### *i. Degradation of phenylalanine and tyrosine*

(a) Phenylketonuria

(b) Alkaptonuria

(c) Tyrosinemia I




Phenylalanine and tyrosine share a similar degradation pathway, yielding acetoacetate and fumarate. The degradation of phenylalanine begins with its hydroxylation by phenylalanine hydroxylase to form tyrosine. This reaction requires the cofactor tetrahydrobiopterin. Tetrahydrobiopterin is derived from guanosine triphosphate (GTP), instead of from a vitamin. In the next step, tyrosine is transaminated to form p-hydroxyphenylpyruvate which is then converted to homogentisate. Homogentisate is then acted on by homogentisate oxidase which cleaves its aromatic ring to form 4-maleylacetoacetate. 4-Maleylacetoacetate is subsequently isomerized to 4-fumarylacetoacetate. Finally, 4-fumarylacetoacetate is hydrolyzed by fumarylacetoacetate hydrolase to become acetoacetate and fumarate. Defects in this pathway can lead to phenylketonuria, alkaptonuria, and tyrosinemia I.

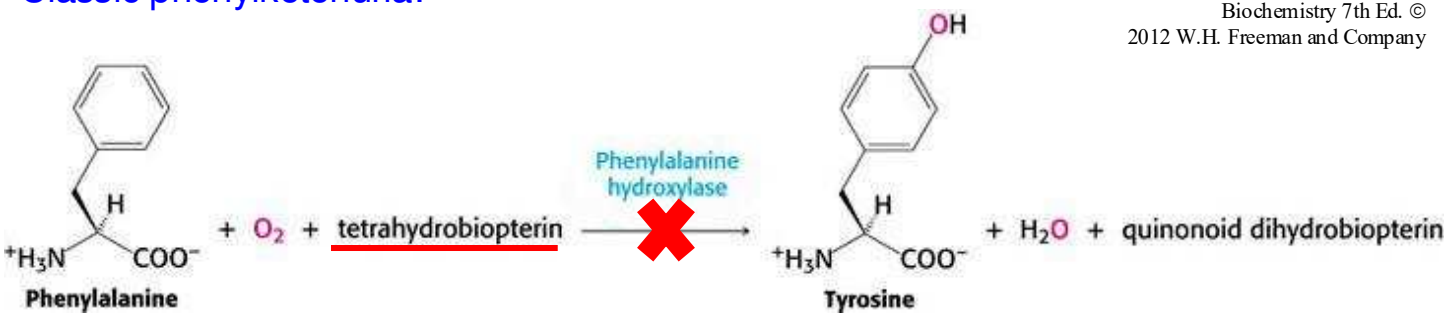
#### *a. Phenylketonuria*

The lack or deficiency of **phenylalanine hydroxylase**, the enzyme that converts phenylalanine to tyrosine, results in **phenylketonuria** or **PKU**. Because the major outflow pathway of phenylalanine is blocked in phenylketonuria, the blood level of phenylalanine may typically increase 20-fold compared to normal individuals.

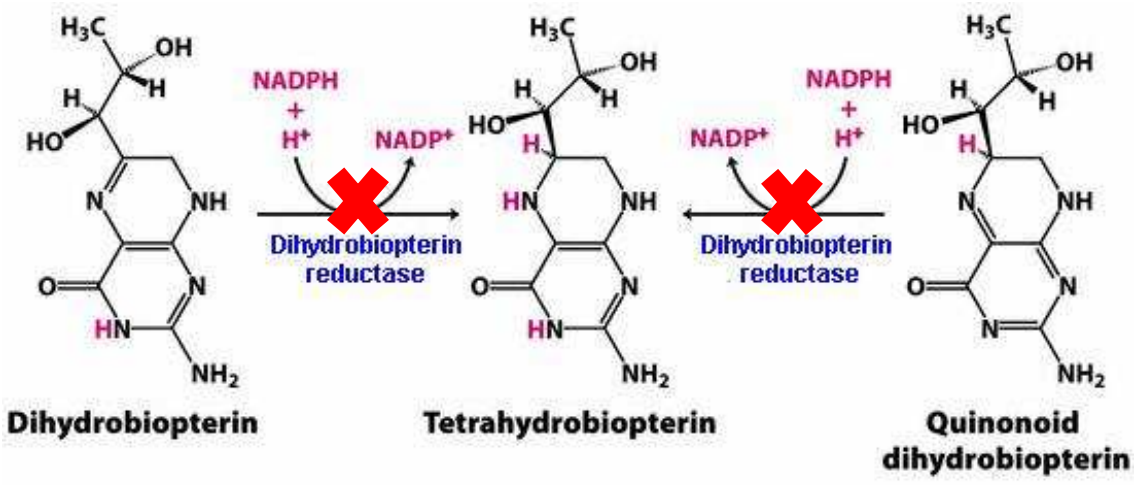
**Phenylalanine hydroxylase or dihydrobiopterin reductase defect leads to phenylketonuria**

## Classic phenylketonuria:






## Biopterin defect in cofactor regeneration:




A rarer form of phenylketonuria can be caused by a deficiency of **dihydrobiopterin** or **dihydrobiopterin reductase**. In this case, tetrahydrobiopterin cannot be regenerated, and therefore hydroxylation of phenylalanine cannot occur. This is a much more severe form of PKU, because tetrahydrobiopterin is involved in other hydroxylation reactions, such as the formation of dopamine, norepinephrine, epinephrine, serotonin, and melatonin.

Phenylketonuric infants appear normal at birth, but if PKU is not treated within the first month of life, the infants can develop irreversible cognitive impairment and other neurological disorders. Some key symptoms and signs of the disorder include delayed physical development, seizures, microcephaly, and hypopigmentation. The presence of high levels of phenylacetate in the baby's urine or sweat will give a "musty" or "mousy" odor. Phenylketonurics must decrease the intake of phenylalanine while at the same time increasing the intake of tyrosine. For individuals with dihydrobiopterin reductase deficiency, they must supplement their diet with tetrahydrobiopterin. For pregnant women with PKU, they must control their phenylalanine intake. Otherwise, the baby may suffer from developmental delay, microcephaly, and structural birth defects.

## Phenylketonuria

- **Positive test:** High levels of phenylalanine
- **Key symptoms and signs**: Buildup of phenylketones, severe intellectual and physical developmental delays, seizures, microcephaly, hypopigmentation of the skin, hair and certain regions of the brain (pallor of the substantia nigra, the locus ceruleus, and vagal nucleus dorsalis), dermatitis, musty body odor
- **Treatment**: Decreased phenylalanine intake and increased tyrosine intake from diet
  - Dietary supplementation with tetrahydrobiopterin for individuals with dihydrobiopterin reductase defect

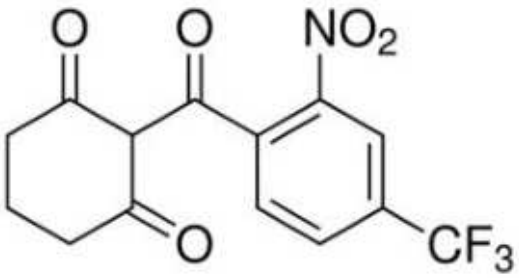
- Women who have PKU and become pregnant must control phenylalanine intake or risk harming the developing fetus
- Non-PKU hyperphenylalaninemia is listed as a separate newborn screening test, but it is simply less severe mutations in phenylalanine hydroxylase.

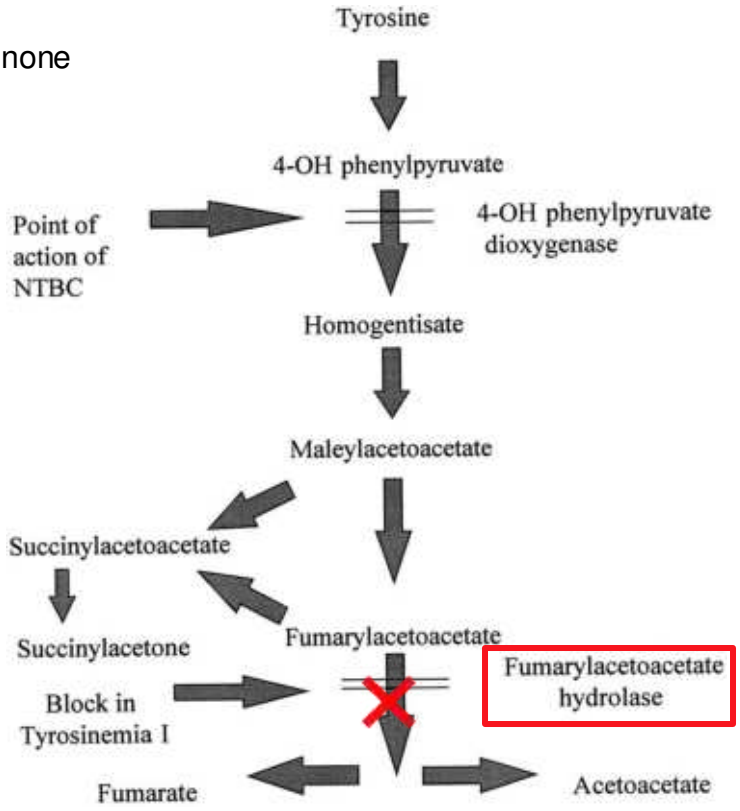
Metabolism of tyrosine will yield acetoacetate and fumarate. Defects in this metabolic pathway will give rise to alkaptonuria and tyrosinemia I. In addition, tyrosine is required for the synthesis of melanin. Defects in this pathway can give rise to albinism. Only tyrosinemia I is screened for on the newborn screening panel because of the severe potential consequences of delayed diagnosis.

*b. Tyrosinemia I*

**Tyrosinemia I**

- **Positive test:** High levels of tyrosine
- **Symptoms and signs:** Cabbage-like odor, heptomegaly, delayed growth, jaundice, ascites formation, and hemorrhage
- **Treatment:** Supplementation with nitisinone (NTBC; Orfadin)



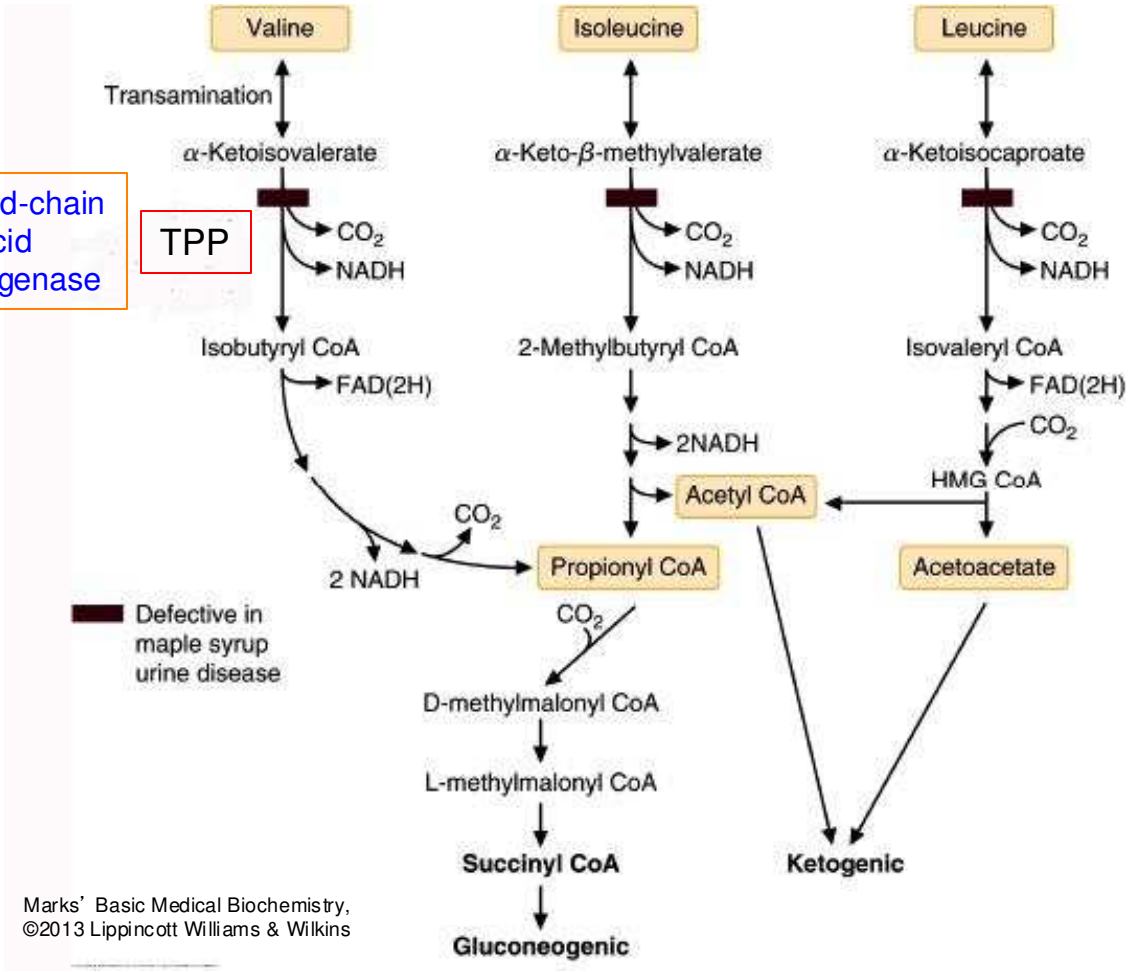




**Tyrosinemia I** is a genetic disorder caused by a deficiency of **fumarylacetoacetate hydrolase**, the enzyme that catalyzes the last step of tyrosine catabolism. This enzyme deficiency leads to the accumulation of maleylacetoacetate and fumarylacetoacetate. These compounds are reactive and can be converted to succinylacetone. Succinylacetone causes cellular damage to the liver and kidneys. The acute form of tyrosinemia I is associated with liver failure and death within the first year of life. Succinylacetone can be used as a marker for tyrosinemia I.

For individuals with tyrosinemia I, their skin and urine have a “cabbage-like” odor. Other symptoms and signs include delayed growth, hepatomegaly, jaundice, ascites formation and hemorrhage. They are treated with nitisinone (NTCB). This drug inhibits hydroxyphenylpyruvate dioxygenase to prevent the formation of homogentisate. Therefore, the downstream intermediates, maleylacetoacetate and fumarylacetoacetate, will not be formed. Hydroxyphenylpyruvate, in this case, can be excreted.

### *ii. Branched-chain amino acids*

**Defect in branched-chain α-ketoacid dehydrogenase leads to maple syrup urine disease**




The branched-chain amino acids leucine, isoleucine, and valine (**LIV**) serve as universal fuels for various tissues, with the muscle carrying out the highest level of utilization. The initial step in the degradation of branched-chain amino acids is the transamination of these amino acids to form α-ketoacids (α-ketoisovalerate, α-keto-β-methylvalerate, and α-ketoisocaproate). These α-ketoacids then undergo oxidative decarboxylation in a reaction catalyzed by branched-chain α-ketoacid dehydrogenase complex to form their respective CoA derivatives. This enzyme, like pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and transketolase, requires the coenzyme thiamine pyrophosphate, as well as CoA, $NAD^+$, $FAD^+$, and lipoamide, for its function. Eventually, valine and isoleucine catabolism will form propionyl CoA which is then converted to succinyl CoA. Isoleucine will also form acetyl CoA. The breakdown of leucine will form both acetyl CoA and acetoacetyl CoA. A defect in **branched-chain α-ketoacid dehydrogenase** will give rise to **maple syrup urine disease**.

## Maple syrup urine disease (MSUD)

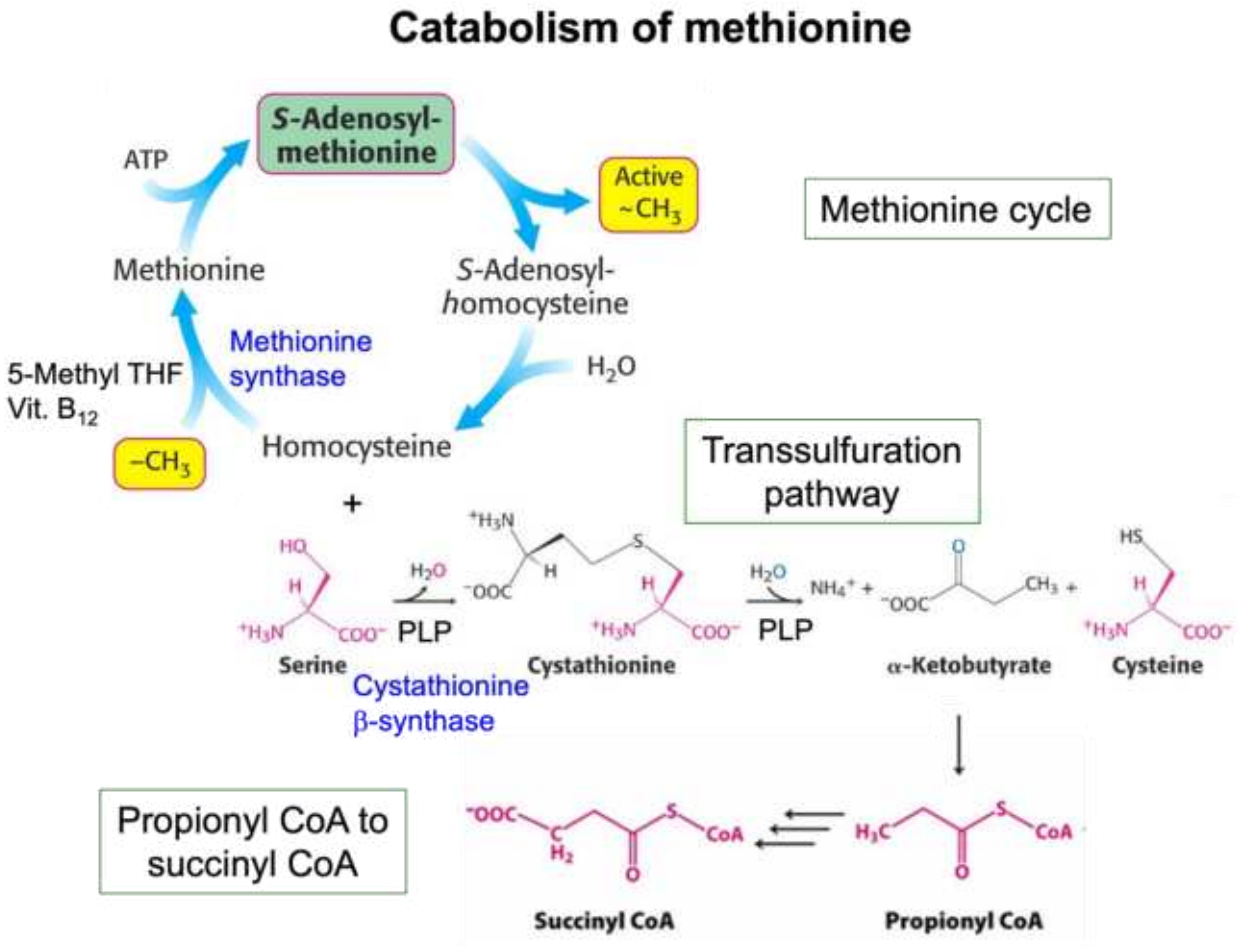
- Also known as branched-chain ketoaciduria
- **Positive test:** Elevated leucine/isoleucine, valine
- **Symptoms and signs:** Urine smells like maple syrup or burnt sugar, vomiting, convulsions, intellectual developmental delays, and early death if not treated
- **Treatment**: A diet low in valine, leucine, and isoleucine



Individuals with maple syrup urine disease have elevated levels of α-ketoacids and corresponding branched-chain amino acids in blood and urine samples. The urine of these patients has maple syrup or "burnt sugar" odor. If not treated, maple syrup urine disease will give rise to severe mental and physical delays, and it can also lead to early death.

Infants with this disease cannot be breastfed, and they are placed on a diet low in these three branched-chain amino acids (**l**eucine, **i**soleucine, and **v**aline). These amino acids cannot be excluded from the diet because they are essential amino acids. A diet with carefully controlled amounts of these 3 amino acids must be maintained to prevent their accumulation and avoid the subsequent buildup of α-ketoacid metabolites, which are toxic. Liver transplantation is another treatment option.

### *iii. Methionine*

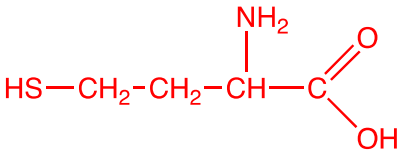



Degradation of methionine is rather complicated. It does not undergo the classic transamination reaction like glutamate or aspartate. Its degradation can be divided into 3 major stages. First, methionine is converted to homocysteine via the methionine cycle. Some homocysteine molecules will reenter the methionine cycle while others will condense with serine via the transsulfuration pathway to eventually form α-ketobutyrate and cysteine. Lastly, α–ketobutyrate is converted to propionyl CoA which subsequently forms succinyl CoA. A defect in enzymes involved in methionine metabolism will lead to the buildup of homocysteine, giving rise to homocystinuria.

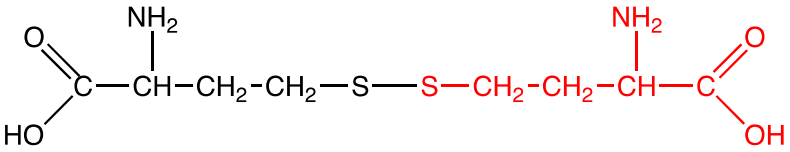
*a. Homocystinuria*

Homocysteine is derived from methionine via the methionine cycle. If it builds up in the body due to enzyme defects, it will oxidize and form the disulfide-linked homocystine. High levels of homocysteine/homocystine are found in the serum (> 100 mM) and urine of patients with this metabolic disorder. The buildup of homocyteine/homocystine leads to multisystemic disorders such as intellectual developmental delays, kyphosis, lens subluxation, and vascular thrombosis. Individuals with this disorder are prone to stroke and myocardial infarction.

**Defects in the clearance of homocysteine leads to homocystinuria**




Homocysteine




Homocystine

**Key symptoms and signs:**

Increased homocystine in urine
Cognitive impairment
Osteoporosis
Tall stature
Kyphosis
Lens subluxation
Atherosclerosis (prone to stroke and myocardial infarction)

The most common form of homocystinuria is due a deficiency in **cystathionine β-synthase** (see next page) which condenses homocysteine and serine to form cystathionine (part of the transsulfuration pathway). In this case, cysteine becomes an essential amino acid. Individuals with cystathionine β-synthase deficiency must decrease the intake of methionine and increase the intake of cysteine, vitamin $B_{12}$, and folic acid. Vitamin $B_{12}$ and folate promote the resynthesis of methionine from homocysteine.

Another form of homocystinuria involves **decreased affinity of cystathionine β-synthase for the cofactor pyridoxal phosphate**. These individuals respond well to high doses of vitamin $B_6$ (pyridoxine).

A third form of homocystinuria involves **methionine synthase** deficiency. Individuals with methionine synthase deficiency need to decrease the intake of methionine and take trimethylglycine (Betaine) supplement. Trimethylglycine methylates homocysteine to form methionine through a separate pathway in a folate-independent manner. For some individuals with mild methionine synthase deficiency, hyperhomocysteinemia may result (serum homocysteine levels > 15 mM but < 100 mM). These individuals are often asymptomatic.

A fourth form of homocystinuria involves a deficiency in **methylene tetrahydrofolate reductase**. Methylene-tetrahydrofolate reductase is essential for the regeneration of 5-methyl THF from 5,10-methylene THF. 5-Methyl THF is the methyl donor for the conversion of homocysteine to methionine. Supplementation with 5-methyl THF may be beneficial.

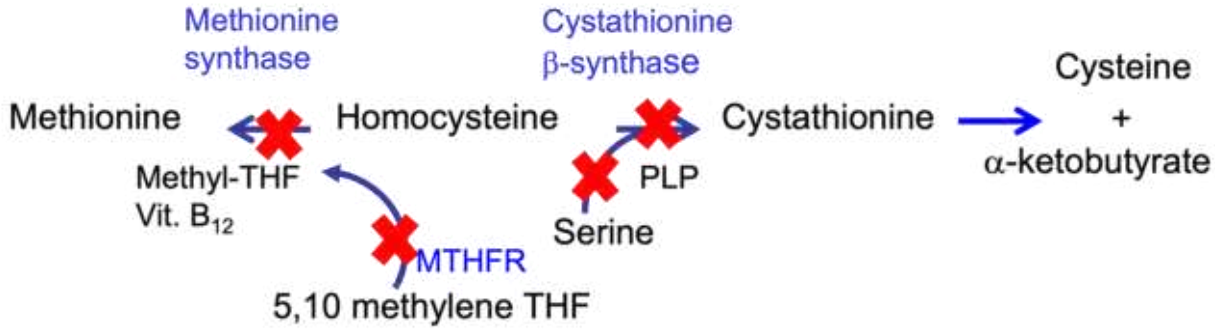
**Four forms of homocystinuria**

1. Cystathionine β-synthase deficiency
   Treatment: decreased methionine intake and increased cysteine, vitamin $B_{12}$, and folate intake in the diet

2. Decreased affinity of cystathionine β-synthase for PLP
   Treatment: increased vitamin $B_6$ in the diet

3. Methionine synthase deficiency
   Treatment: decreased methionine intake and supplementation with trimethylglycine (folate-independent methylation)

4. Methylene tetrahydrofolate reductase (MTHFR) deficiency
   Treatment: 5-methyl THF supplementation




## IV. Disorders of organic acid metabolism

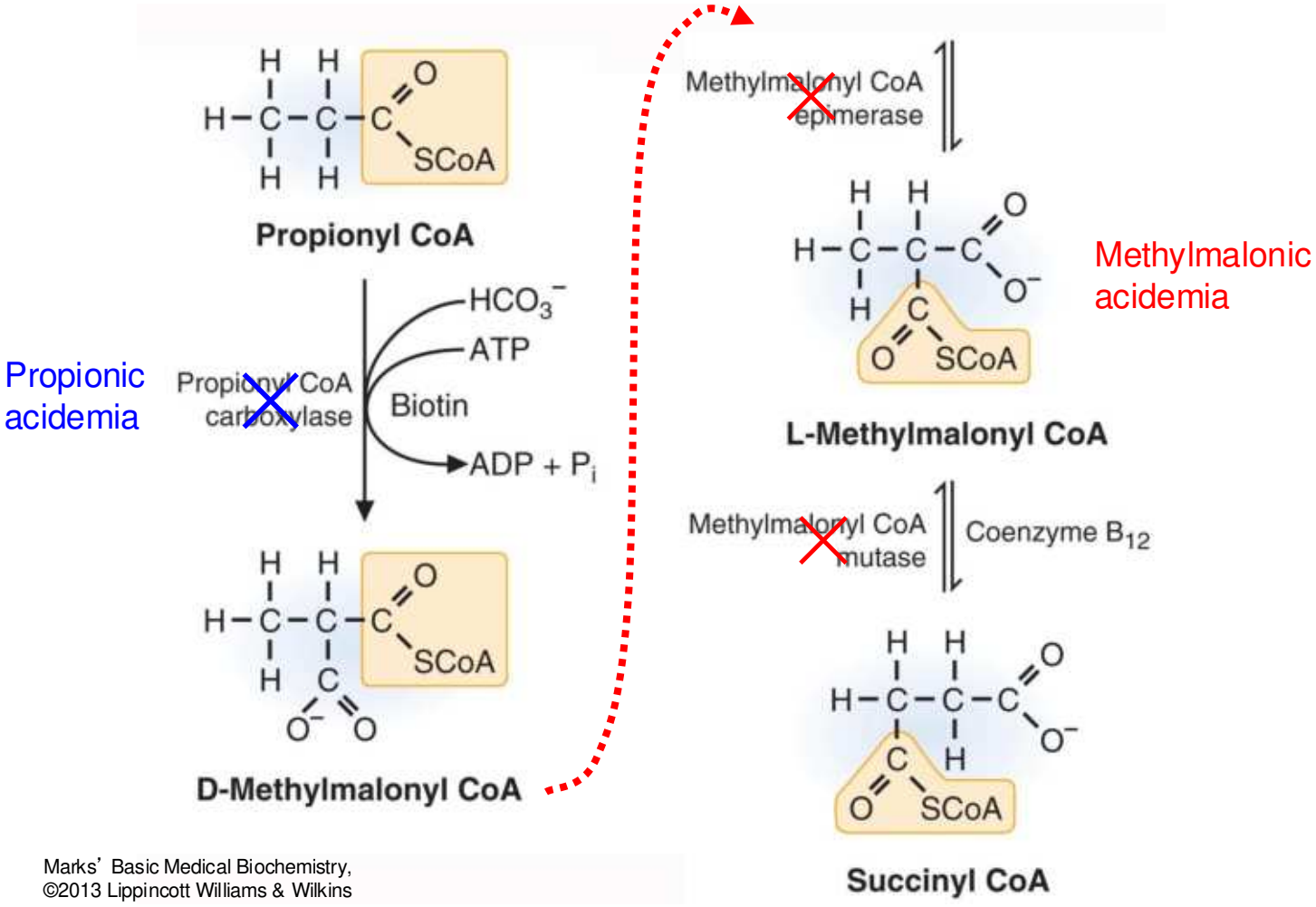
Organic acids are intermediate metabolites of amino acid and fatty acid catabolism. An organic acid disorder is caused by a deficiency in a specific enzyme necessary to this degradation process. O*rganic acids* can accumulate in body fluids and are also excreted in the urine. Excess buildup of organic acid can lead to *metabolic acidosis* and may become toxic to the body.

Here, we will look at organic acid metabolism disorders associated with the metabolism of isoleucine, methionine, threonine, and valine (IMTV) and branched chain fatty acids.

**Propionyl CoA to succinyl CoA**

Propionic acidemia
Methylmalonic acidemia (cobalamin disorders or methylmalonyl-CoA mutase)

**Organic acidemia associated with defects in the conversion of propionyl CoA to succinyl CoA**




Marks' Basic Medical Biochemistry,
©2013 Lippincott Williams & Wilkins

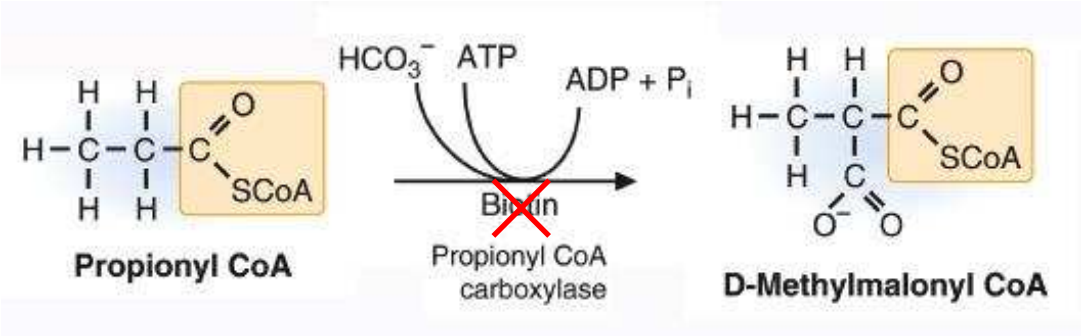
In the conversion of propionyl CoA to succinyl CoA, propionyl CoA is first carboxylated by propionyl CoA carboxylase (requires biotin) to form D-methylmalonyl CoA. D-Methylmalonyl CoA is then epimerized to L-methylmalonyl CoA by an epimerase. Lastly, L-methylmalonyl CoA is isomerized by methylmalonyl CoA mutase to form succinyl CoA. This reaction requires vitamin $B_{12}$. Thus, methylmalonic acid (MMA) is a marker for vitamin $B_{12}$ deficiency.

Organic aciduria can arise if there is a defect in **propionyl CoA carboxylase** (**propionic acidemia**), or in methylmalonyl CoA epimerase or more commonly **methylmalonyl CoA mutase** (**methylmalonic acidemia**).

Methylmalonic acidemias, as listed on the previous page, can have multiple genetic causes. **Methylmalonic acidemia (methylmalonyl-CoA mutase)** is caused by direct mutation in methylmalonyl-CoA mutase. **Methylmalonic acidemia (cobalamin disorders)** is caused by mutations that result in defective cobalamin processing enzymes, leading to a deficient methylmalonyl-CoA mutase due to a lack of available cobalamin ($B_{12}$). **Methylmalonic acidemia with homocystinuria** is caused by a separate set of cobalamin processing genes and likely also affects methionine synthetase (another enzyme requiring $B_{12}$) leading to homocystinuria.

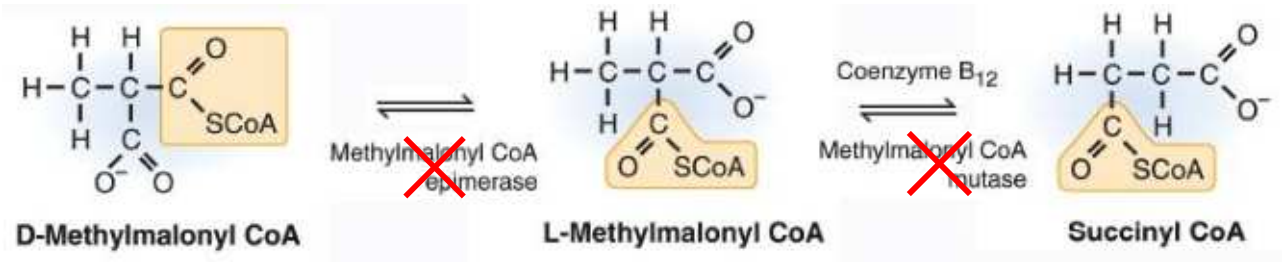
**A. Propionic acidemia**

- Due to the lack of functional propionyl CoA carboxylase (autosomal recessive inheritance pattern)
- Disorder appears in affected newborns (1:35,000)
- **Positive test:** Elevated levels of propionic acid and propionylcarnitine in the blood, but with normal biotin levels
- **Key symptoms and signs:** poor feeding, vomiting, dehydration, hypoglycemia, 2° hyperammonemia, hypotonia, lethargy, and seizures.
- Long-term complications may include intellectual disability, coma, and death if untreated
- **Treatment:** low-protein diet and carnitine supplementation




**B. Methylmalonic acidemia**

- Due to the lack of functional methylmalonyl CoA epimerase or methylmalonyl CoA mutase (autosomal recessive inheritance pattern)
- Disorder normally appears in early infancy (1:50,000-100,000)
- **Positive test:** Elevated levels of methylmalonic acid, methylmalonylcarnitine, propionic acid, and propionylcarnitine in the blood
- **Key symptoms and signs:** vomiting, dehydration, hypotonia, lethargy, hypoglycemia, 2° hyperammonemia, hepatomegaly and growth delays
- Long-term complications may include feeding difficulty, chronic kidney disease, intellectual disability, pancreatitis, and death if untreated
- **Treatment:** low-protein diet, carnitine, and vitamin $B_{12}$ supplementation



## V. Disorders of fatty acid β-oxidation

Most fatty acids are oxidized in the mitochondrial matrix via β-oxidation, with the exception of very long-chain fatty acids and branched-chain fatty acids, which are oxidized in peroxisomes. Prior to this β-oxidation step, fatty acids must be translocated into mitochondria via the carnitine shuttle system. Defects in either the β-oxidation of fatty acids or transport of fatty acids into mitochondria can give rise to diseases.

**A. Carnitine shuttle system**

i. Primary carnitine deficiency
ii. CPT-I deficiency
iii. CPT-II deficiency
iv. Carnitine-acylcarnitine translocase deficiency

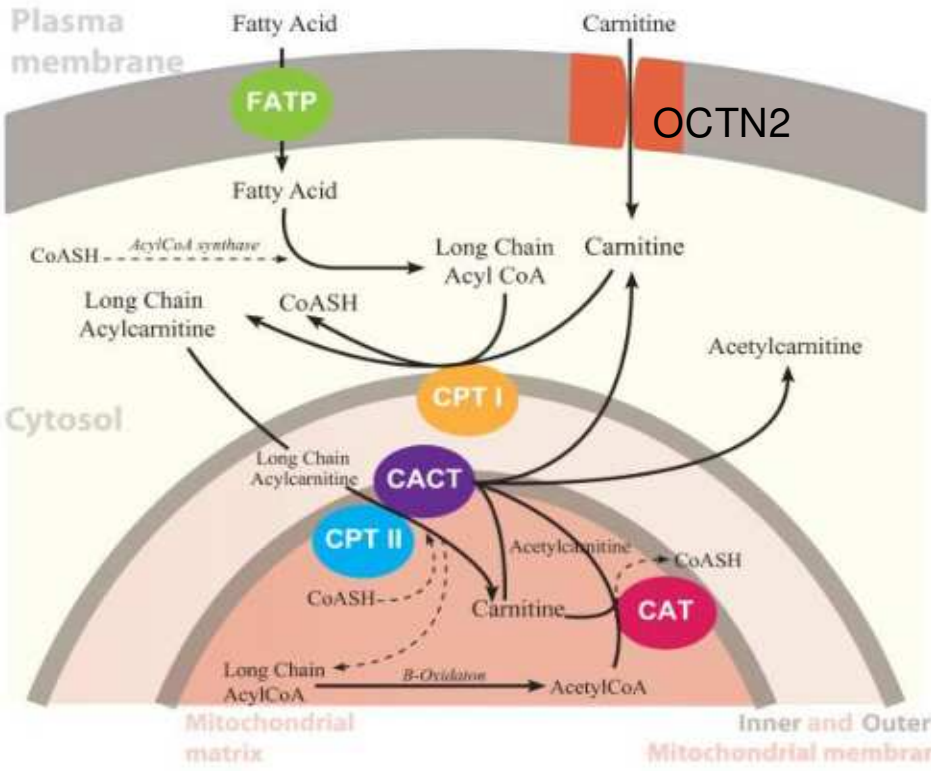
**B. Fatty acid beta oxidation**

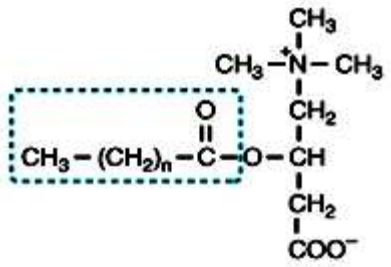
-Mid-chain acyl CoA dehydrogenase deficiency

### A. Carnitine shuttle system

Fatty acids must be conjugated to CoA to form fatty acyl CoA before they can be metabolized. Because of the large size of the CoA moiety of fatty acyl CoA, fatty acids have to be indirectly transported into mitochondria as acyl carnitines. Carnitine is first transported into the cytosol by the transporter **Organic Cation/Carnitine Transporter 2 (OCTN2)**. **Carnitine palmitoyltransferase I (CPT-I)** then exchanges the CoA of a fatty acyl CoA molecule for carnitine and the resulting acyl carnitine is taken into the mitochondrial matrix by the **carnitine-acylcarnitine translocase (CACT)**. Once inside the mitochondrial matrix, **CPT-II** converts acyl carnitine back to its fatty acyl CoA form. Thus, CPT-1 is the key regulatory enzyme for fatty acid β-oxidation. This enzyme is inhibited by malonyl CoA, an intermediate in fatty acid synthesis, to prevent simultaneous synthesis and degradation of fatty acids.

**The carnitine shuttle system**



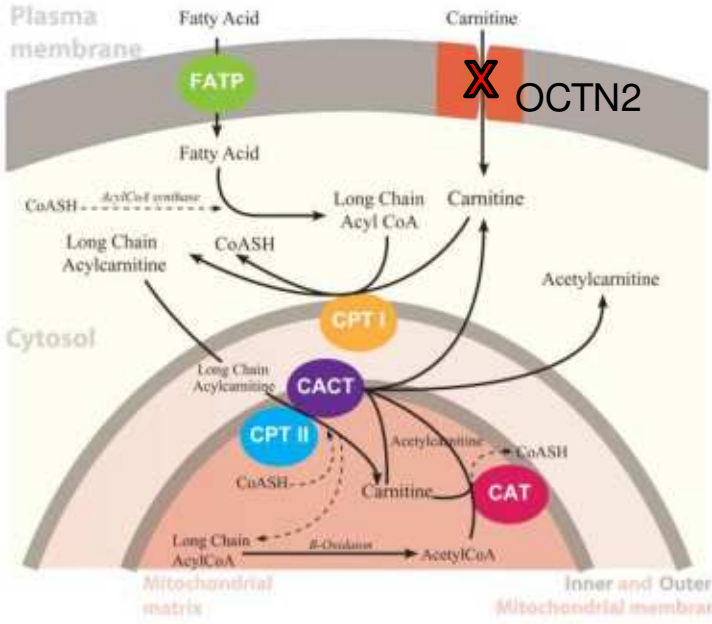

Acyl-carnitine

Flanagan, et al. Nutrition & Metabolism. Doi:10.1186/1743-7075-7-30

The carnitine shuttle system transports long chain fatty acids from the cytoplasm to the mitochondrial matrix where β-oxidation occurs.

### *i. Primary Carnitine Deficiency*

=OCTN2 deficiency; transporter that brings carnitine into the cells




**Positive test:** Decreased carnitine (C0) and other acylcarnitines

**Key symptoms and signs:**

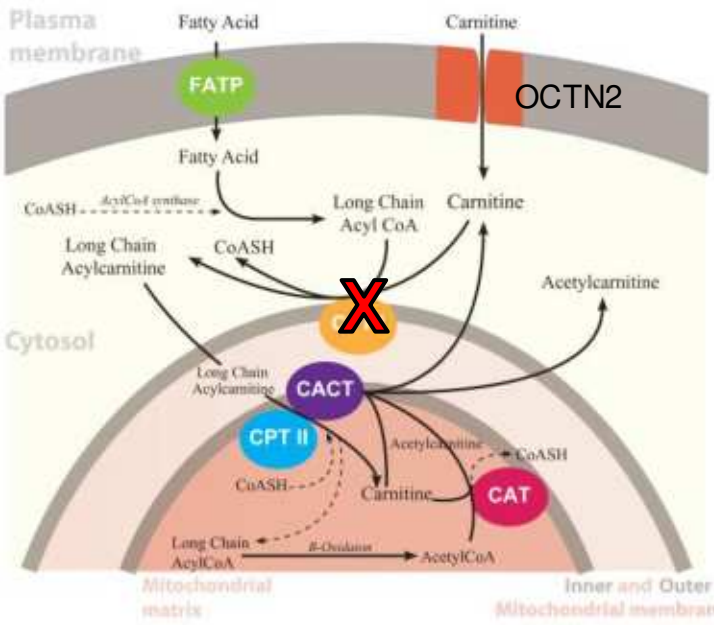
- Cardiomyopathy, hypoketotic hypoglycemia, hepatomegaly, poor feeding, lethargy, behavioral symptoms

**Treatment:**

- Regular and frequent meals and snacks
- Diet high in carbohydrates and low in fat
- L-carnitine supplements to help the body break down fats

Primary carnitine deficiency arises from mutations in OCTN2, the transporter that brings free carnitine into the cytoplasm. Without intracellular carnitine, fatty acid transport into the mitochondrial matrix and therefore β-oxidation is impaired. Carnitine left in circulation due to OCTN2 defects will be excreted in the urine, resulting in both low carnitine levels within cells and in the plasma. Carnitine supplements are given in an attempt to get as much carnitine into cells as possible.

### *ii. CPT-I deficiency*




**Positive test:** High free carnitine levels compared to the levels of C16:0 and C18 acylcarnitines

**Key symptoms and signs:**

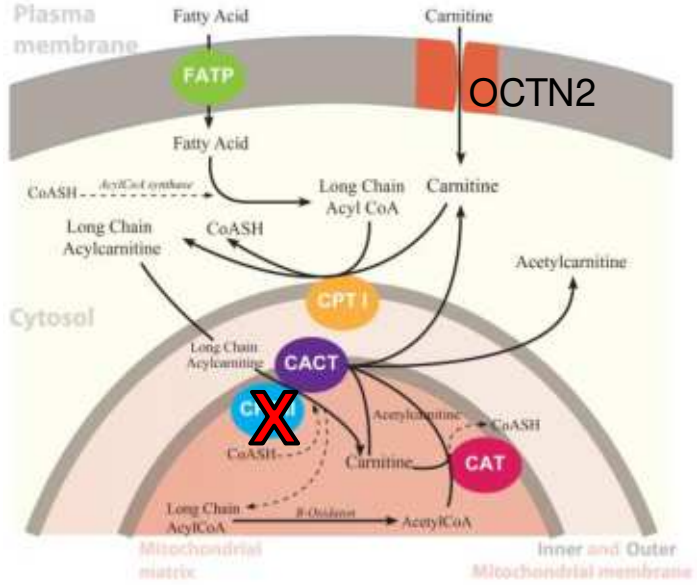
- Poor appetite, hepatomegaly, hypoketotic hypoglycemia, elevated blood carnitine levels, hypotonia, lethargy, breathing problems, vomiting, seizures, behavioral changes
- Signs often appear between 8-18 months, can be triggered by illness or fasting

**Treatment:**

- Regular and frequent meals and snacks
- Diet high in carbohydrates and low in fat
- Medium chain triglyceride (MCT) oil supplements to provide fats the body can break down

CPTI deficiencies prevent exchange of the CoA moiety for carnitine in the cytoplasm, precluding import of fatty acids into the mitochondrial matrix and a build up of carnitine compared to long chain fatty acyl-carnitines.

### *iii. CPT-II deficiency*




Most common form of CPT deficiencies
Caused by mutations in *CPT2*
Three forms are known:
Lethal neonatal form; Severe infantile hepatocardiomuscular form; Myopathic form (most common)

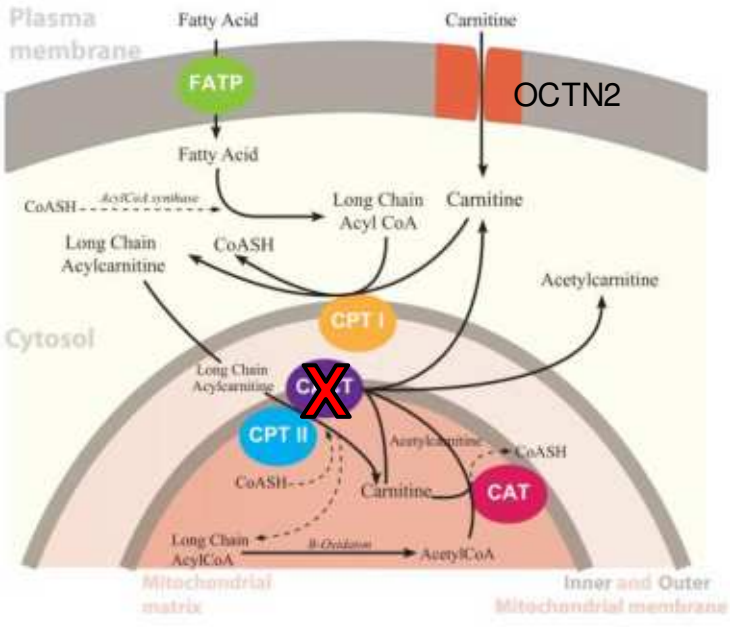
**Positive test:** High levels of C16 and C18:1 acylcarnitines

**Key symptoms and signs:**
In young children and newborn forms:
- cardiomyopathy, arrhythmia, liver failure, and sudden death
- hypotonia, hypoketotic hypoglycemia, hepatomegaly, lethargy, poor feeding, breathing problems, vomiting, seizures

**Treatment:**
•Regular/frequent meals and snacks, Diet high in carbs and low in fat
•Medium chain triglyceride (MCT) oil supplements to provide fats the body can break down
•L-carnitine supplements to help the body break down fats (in some cases)
•Avoiding cold weather (in some cases)

**CPT-II** deficiency is the most common form of carnitine shuttle deficiencies. There are 3 forms of CPT-II deficiency, with the myopathic form being the most common. Individuals with CPT-II deficiency may develop muscle pain and myoglobinuria after extended exercise, as well as hypoketotic hypoglycemia. In young children and newborns, this disorder may also lead to cardiomyopathy, liver failure, and sudden death.

### *iv. Carnitine-acylcarnitine translocase (CACT) deficiency*



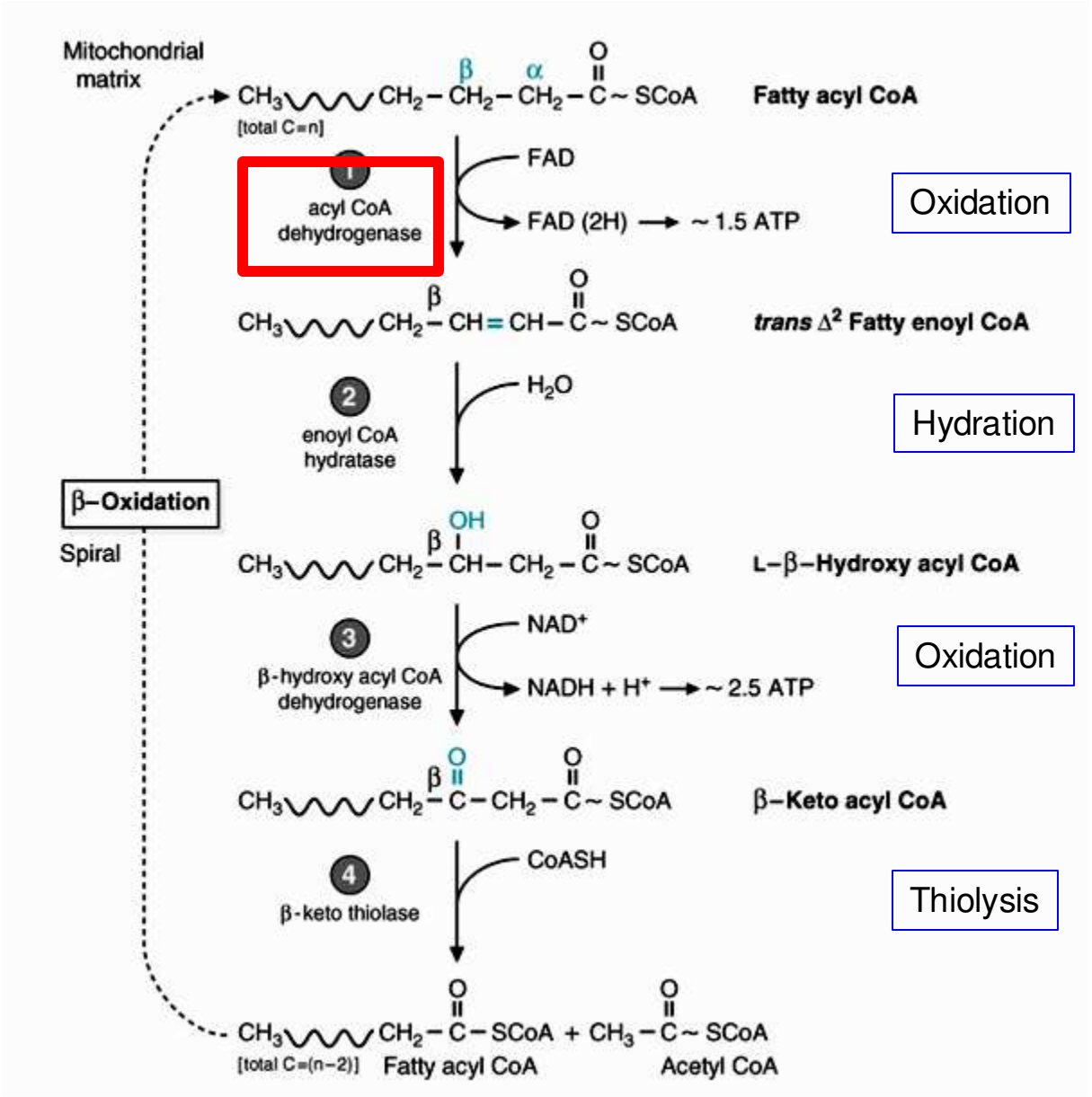

**Positive test:** High levels of C16 and C18:1 acylcarnitines

**Key symptoms and signs:**
In young children and newborn forms:
--Hyperammonemia, heart problems, hypotonia, hypoketotic hypoglycemia, hepatomegaly, lethargy, poor feeding, breathing problems, vomiting, seizures, behavior changes, coma

**Treatment:**
•Regular/frequent meals and snacks, Diet high in carbs and low in fat
•Medium chain triglyceride (MCT) oil supplements to provide fats the body can break down
•L-carnitine supplements to help the body break down fats (in some cases)

CACT deficiency prevents transport of acylcarnitines into the mitochondrial matrix, resulting in a build up of long-chain acylcarnitines, as can be observed in newborn metabolic screening panels.

## B. β-oxidation of fatty acids




Once in the mitochondrial matrix, fatty acyl CoA can then undergo repeated cycles of β-oxidation for energy production. It is called β-oxidation because oxidation occurs at the β-carbon of fatty acyl CoA. Each cycle of β-oxidation removes a two-carbon unit as acetyl CoA and this process involves four reactions: oxidation, hydration, oxidation, and thiolysis. In the two oxidation reactions, $FADH_2$ and NADH are formed. $FADH_2$ and NADH can be shunted to the electron transport chain for ATP synthesis. Acetyl CoA derived from fatty acid β-oxidation is oxidized by the TCA cycle to produce an additional 3 NADH, 1 $FADH_2$, and 1 GTP molecules. The family of acyl CoA dehydrogenases catalyze the initial oxidation step.

## Medium-chain acyl CoA dehydrogenase deficiency

Specific isoforms of acyl CoA dehydrogenase exist for fatty acids of different chain lengths. Of particular interest is the **medium/mid-chain acyl CoA dehydrogenase (MCAD)**. MCAD is essential for the oxidation of fatty acid with 8-10 carbons. People with **MCAD deficiency (MCADD)** cannot oxidize medium-chain fatty acids with 8-10 carbons, and therefore cannot obtain sufficient energy from fatty acids during fasting or an increased energy demand. Thus, like CPT-II deficiency, these individuals may develop hypoketotic hypoglycemia. In addition, they tend to have increased levels of plasma octanoylcarnitine and dicarboxylic acids (derived from medium-chain fatty acids). MCAD deficiency may lead to sudden unexpected death in infancy.

## MCAD deficiency

•Autosomal recessive
•Affects 1 in 15,000 live births in the United States
•May lead to sudden unexpected death in infancy (SUDI)

**Positive test:** Increased plasma octanoylcarnitine (C8 acylcarnitine)


**Symptoms and signs:**
Well until decompensation (i.e. following an excessive period of fasting/increased energy requirement)
- Vomiting, lethargy, seizures, hypoketotic hypoglycemia, and coma

**Treatment:**
•Regular and frequent meals and snacks
•Diet high in carbohydrates and low in fat
•L-carnitine supplements to help the body break down fats

## VI. Disorders of peroxisomal functions

Peroxisomes are small, membrane-enclosed organelles found in virtually all eukaryotic cells. They are involved in a number of degradative pathways, including the catabolism of very long-chain fatty acids, branched-chain fatty acids, D-amino acids, and polyamines and reduction of reactive oxygen species (hydrogen peroxide to water). Thus, defects in peroxisomal functions can lead to disorders.

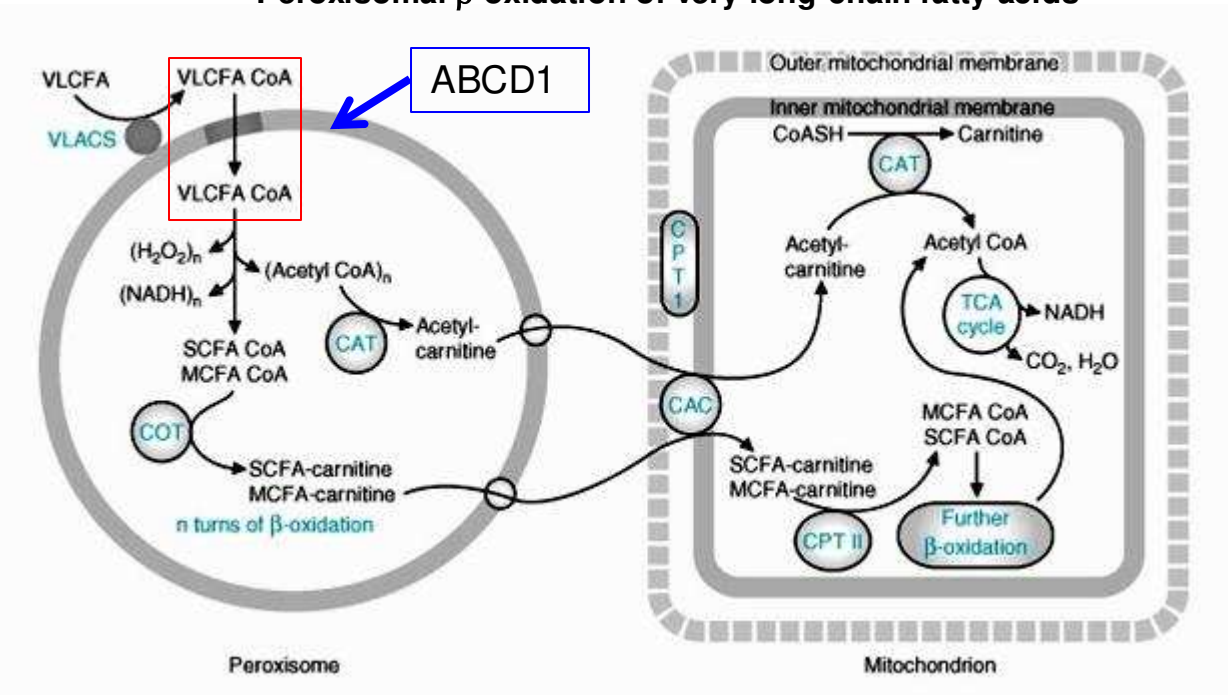
**A. Metabolism of very long-chain fatty acids**
-X-linked adrenoleukodystrophy

**B. Metabolism of branched-chain fatty acids**
-Refsum disease

**C. Peroxisome biogenesis**
-Zellweger Spectrum Disorder

### A. Metabolism of very long-chain fatty acids

**Peroxisomal β-oxidation of very long-chain fatty acids**



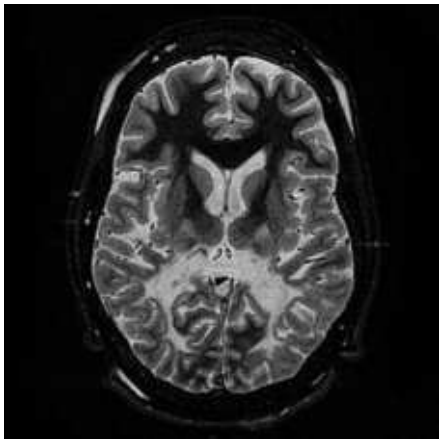

- Peroxisome imports very long-chain fatty acids (> 22 C) and catabolizes them into short-chain fatty acids which are then transported to mitochondria for further processing
- A deficiency of ABCD1, a peroxisomal membrane transporter of very long-chain fatty acids, leads to X-linked adrenoleukodystrophy

Very long-chain fatty acids are degraded in peroxisomes instead of the mitochondrial matrix. This involves first activating these fatty acids by fatty acyl CoA synthetase present in the peroxisomal membrane. The transporter, ABCD1, then imports these very long-chain fatty acyl CoAs into the peroxisome. These fatty acyl CoAs are then oxidized in a β-oxidation pathway similar to that of the mitochondrial pathway, until the chain length reaches 4-8 carbons. Short-chain and medium-chain acyl CoAs are then converted to acyl carnitines, exported out of peroxisomes, and transported to mitochondria for further oxidation.

## X-linked adrenoleukodystrophy (X-ALD)

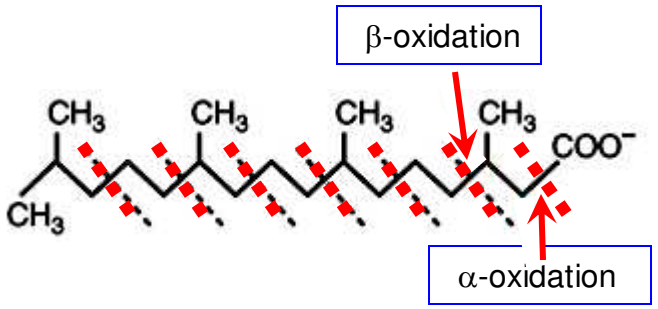
Defects in the degradative pathway of very long-chain fatty acids can lead to several serious diseases, such as **X-linked adrenoleukodystrophy (X-ALD)**. X-ALD is one of the most frequently seen genetic disorders of myelin. It is caused by defects in the **ABCD1**, the transporter involved in the import of very long-chain fatty acyl CoA into the peroxisome. This leads to the abnormal accumulation of very long-chain fatty acids in various tissues, including the brain, adrenal cortex, testes, and liver. Afflicted individuals may show adrenoinsufficiency, hyperactivity, emotional instability, and progressive demyelination. They may be treated with Lorenzo's oil (triacylglycerol forms of oleic acid and erucic acid), stem cell transplant, and gene therapy.

- Caused by mutations in the *ABCD1* gene (X-linked)
- The abnormal accumulation of VLCFAs in the brain, adrenal cortex, testes, and liver results in the clinical manifestations of this disorder
- There are several phenotypes of the disease
- **Positive test:** Increased C26:0 lysophosphatidylcholine
- **Symptoms and signs:**
  - Adrenoinsufficiency, hyperactivity, emotional instability, and progressive demyelination
- **Treatment:** Lorenzo's oil, stem cell transplant, and gene therapy



### B. Metabolism of branched-chain fatty acids

**Oxidation of branched-chain fatty acids in peroxisomes**



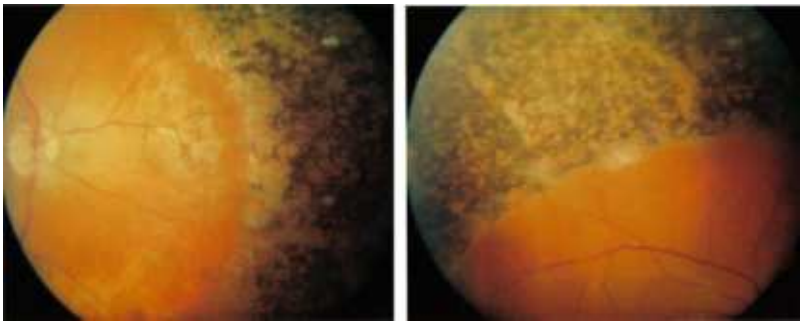

3,7,11,15-tetramethylhexadecanoic acid (phytanic acid)

- Phytanoyl CoA hydroxylase catalyzes the initial hydroxylation reaction in the α-oxidation of branched-chain fatty acids to form formyl CoA and pristanic acid
- Deficiency of phytanoyl CoA hydroxylase leads to Refsum disease
- Deficiency of other enzymes involved in phytanic acid metabolism or defects in peroxisome biogenesis can also give rise to Refsum disease

Another important function of peroxisomes is the degradation of branched-chain fatty acids via the α-oxidation pathway. This pathway oxidizes a common dietary branched-chain fatty acid, phytanic acid, which is a metabolite of chlorophyll. Significant quantities of phytanic acid can come from dairy products, beef, lamb, and some seafood. For phytanic acid, the initial oxidation of its β-carbon to the intermediate β-ketone is not possible due to the presence of a methyl group at this position. To overcome this problem, phytanoyl CoA hydroxylase introduces a hydroxyl group on the α-carbon. The subsequent α-oxidation process then forms formyl CoA and pristanic acid. Pristanic acid can then undergo the conventional β-oxidation, yielding acetyl CoA, propionyl CoA, and isobutyryl CoA.

**Refsum disease**

- Toxic levels of phytanic acid build up in the brain, blood, and other tissues
- Symptoms and signs:
  - Night blindness due to degeneration of the retina (retinitis pigmentosa), loss of the sense of smell (anosmia), cerebral degeneration, deafness, ataxia, peripheral neuropathy, dry and scaly skin (ichthyosis)
- Treatment: Avoid foods that contain phytanic acid (dairy products, beef, lamb, and fatty fish such as tuna and cod)



**Refsum disease** is a rare genetic disorder that results from mutations in the *PHYH* gene. The *PHYH* gene encodes **phytanoyl CoA α-hydroxylase**, and defects in this enzyme result in inability to oxidize phytanic acid. As a result, toxic levels of phytanic acid build up in the brain, blood, and other tissues, causing severe neurological problems including ataxia, peripheral neuropathy, blindness (retinitis pigmenosum), and deafness. Fortunately, it is the most treatable of the leukodystrophies because phytanic acid is not produced by the body. Individuals with Refsum disease must avoid foods that contain phytanic acid.

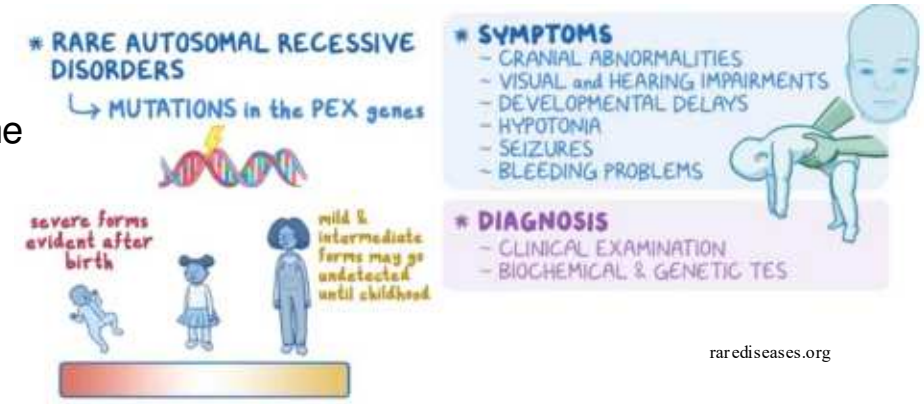
**C. Biogenesis of peroxisomes**

**Zellweger Spectrum Disorder**

Mutations in the PEX genes that encode for proper formation of peroxisomes.

**Positive test:** Increased C26:0 lysophosphatidylcholine

Treatment for symptoms only.




The *PEX* genes are required for genesis of peroxisomes within the cell. There are many different PEX genes that can potentially be mutated to give rise to Zellweger Spectrum Disorder with varying severity of signs and symptoms listed in the diagram above. Many processes will be disrupted by the malformation of peroxisomes including very long chain fatty acid oxidation. Build up of these very long chain fatty acids can be screened for by mass spectrometry on the newborn screening metabolic panel.

## VII. Lysosomal Storage Diseases

**Mucopolysaccharidoses**
(aka accumulation of heparan sulfate and dermatan sulfate)

A. Hurler syndrome (Mucopolysaccharidosis type I)
B. Hunter syndrome (Mucopolysaccharidosis type II)

**Sphingolipidoses**
(aka accumulation of various sphingolipids)

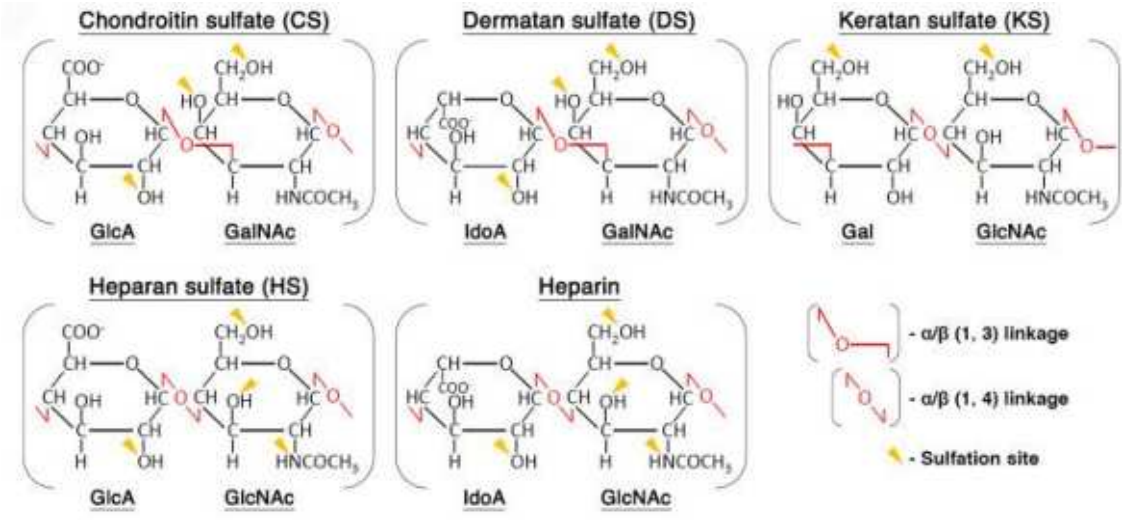
- Fabry disease
- Krabbe disease
- Gaucher disease
- Niemann-Pick disease
- (Also Tay-Sachs disease and metachromatic leukodystrophy, but these are not included in the newborn screening test)

These sphingolipidoses are important, but you will cover them in Block 10!

**Mucopolysaccharidoses** - **Accumulation of heparan sulfate and dermatan sulfate**
Glycosaminoglycans or mucopolysaccharides are unbranched polysaccharides consisting of repeating units of disaccharides made from modified amino sugars. Some of these amino sugars have a carboxylate or sulfate group attached to them, giving them a strong negative charge. Chondroitin 6-sulfate, dermatan sulfate, keratan sulfate, heparan sulfate, heparin, and hyaluronate are major glycosaminoglycans.

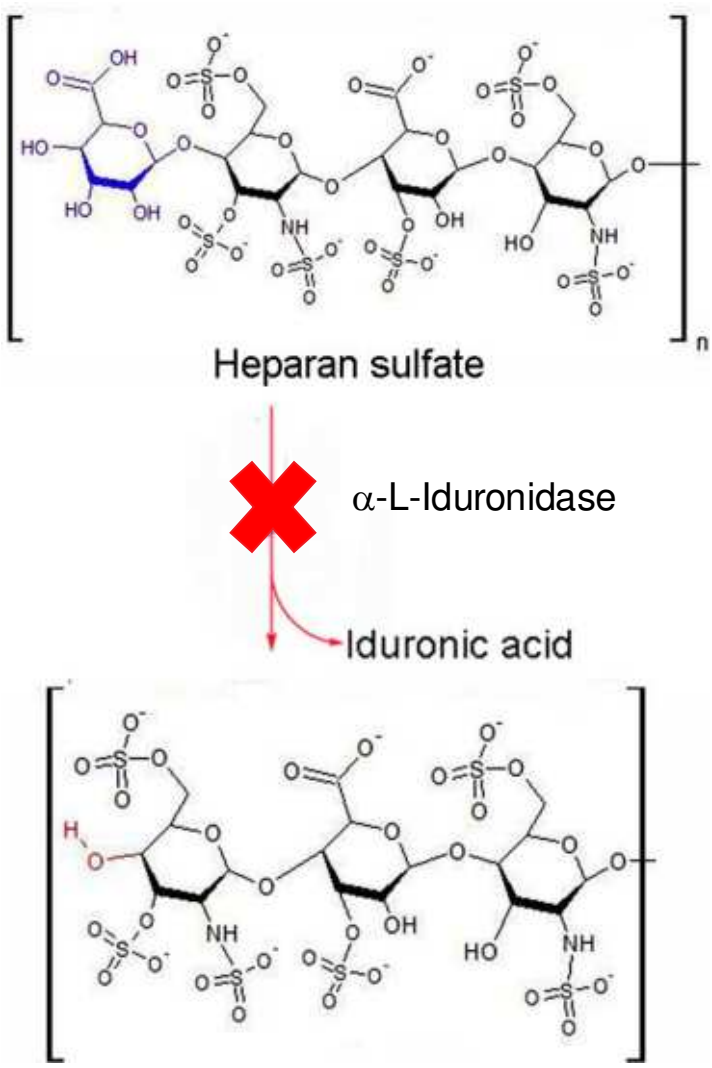
Glycosaminoglycans can attach to proteins to form proteoglycans. Some proteoglycans can have more than 95% of their masses made of carbohydrates. Proteoglycans serve a number of functions including lubrication, structural support in connective tissues, adhesion of cells to the extracellular matrix, and binding to factors that mediate cell proliferation. Abnormal buildup of glycosaminoglycans due to enzyme deficiencies will give rise to mucopolysaccharidoses.

**Glycosaminoglycans contain repeating disaccharide units of amino sugars**




- Glycosaminoglycans are generally associated with proteins found on cell surface and in the extracellular matrix

### *i. Hurler syndrome (mucopolysaccharidosis IH)*




- Deficiency of α-L-iduronidase
- Accumulation of heparan sulfate and dermatan sulfate
- **Symptoms and signs:**
  - Developmental delay, gargoylism/ coarse facial features, airway obstruction, corneal clouding, and hepatosplenomegaly



Hurler syndrome, also known as mucopolysaccharidosis type IH, is due to a deficiency in the lysosomal enzyme **α-L-iduronidase** which removes unsulfated iduronic acid residues during the recycling of heparan sulfate and dermatan sulfate. This leads to the buildup of these two types of glycosaminoglycan. This is an autosomal recessive disorder. Key symptoms and signs of this disorder include developmental delay, gargoylism, airway obstruction, corneal clouding, hepatosplenomegaly.

### *ii. Hunter syndrome (mucopolysaccharidosis II)*

- Deficiency of iduronate-2-sulfatase
- X-linked recessive
- Accumulation of heparan sulfate and dermatan sulfate
- Symptoms and signs:
    - Not apparent at birth, developmental decline, change in facial features, progressive loss of skills, hepatosplenomegaly, distinctive behavioral disturbances, and no corneal clouding
- Treatment: enzyme replacement therapy with idursulfase


**Hunter syndrome**, also known as mucopolysaccharidosis type II, is due to a deficiency in the lysosomal enzyme **iduronate-2-sulfatase**, which removes a sulfate group from sulfated iduronic acid residues during the recycling of heparan sulfate and dermatan sulfate. This leads to the buildup of these two types of glycosaminoglycan. This is an X-linked autosomal recessive disorder. Thus, this disorder affects primarily males. The symptoms and signs of this disorder are not apparent at birth. Eventually, the accumulation of these two types of glycosaminoglycan will lead to developmental decline, change in facial features, progressive loss of skills, hepatosplenomegaly, airway obstruction, and behavioral disturbances. Enzyme replacement therapy with idursulfase has been shown to improve symptoms and quality of life of these individuals.