

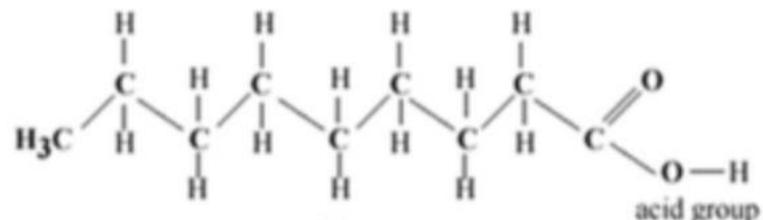
# Inborn Errors & Nitrogen Metabolism

Dr. Spicer

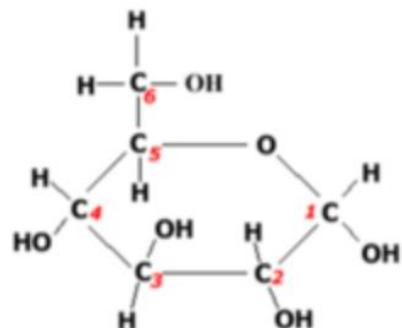
[dspicer@une.edu](mailto:dspicer@une.edu)

# Objectives

- Describe the role of glutamine and alanine in the transport of nitrogen from peripheral tissues to the liver
- Describe conditions that would lead to a positive or negative nitrogen balance
- Describe the importance of nitrogen transport mechanisms in extrahepatic tissues (e.g. muscle, brain, kidney).
- Define essential and conditionally-essential amino acids, and describe changes in requirements during growth and metabolic stress (e.g. burns, infections, healing)
- Define nitrogen balance and explain how it is affected by dietary intake, growth, stress and disease.
- Describe the products and regulation of the fructose and galactose metabolic pathways
- Distinguish the following disease states associated with Inborn Errors of Metabolism, including the enzyme deficiency, relation of the deficiency to the buildup of secondary metabolites, and clinically relevant information related to the disease state: Phenylketonuria, Maple Syrup Urine Disease, Essential Fructosuria, Hereditary Fructose Intolerance, and Classical and Nonclassical Galactosemia.



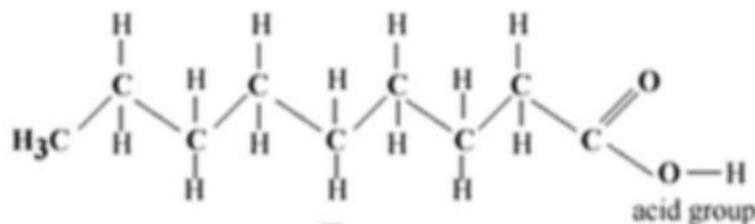
## Fatty Acids



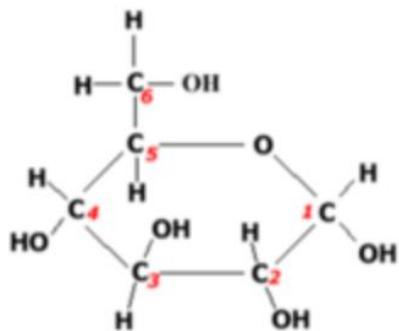
## Glucose



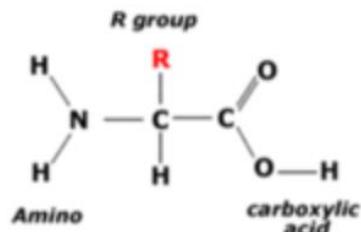
## Amino acids



## Fatty Acids

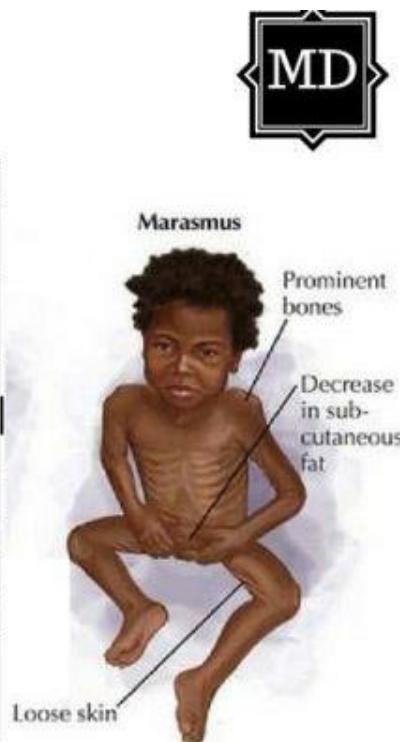


## Glucose



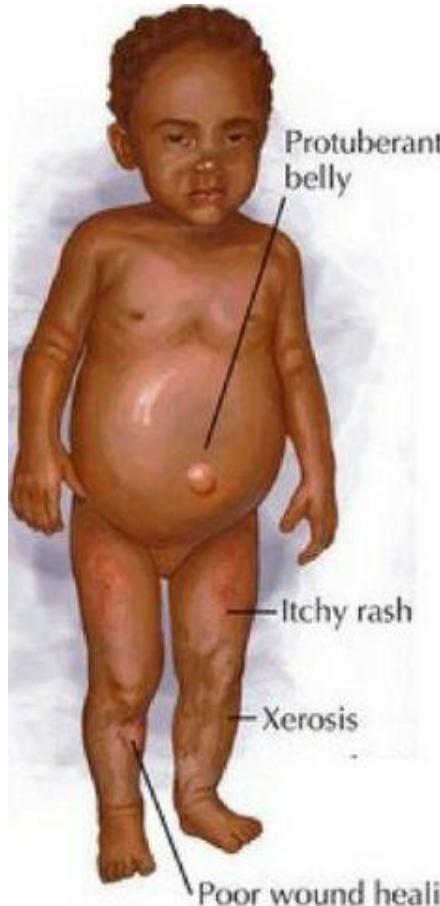
### Amino acids

<b>Marasmus</b>
It is due to deficiency of proteins and calories.
It is common in infants under 1 year of age.
Subcutaneous fat is not preserved.
Oedema is absent
No fatty liver.
Ribs become very prominent.
Alert and irritable.
Severe muscle wasting
Voracious feeder.
The person suffering from Marasmus needs adequate amount of protein, fats and carbohydrates.



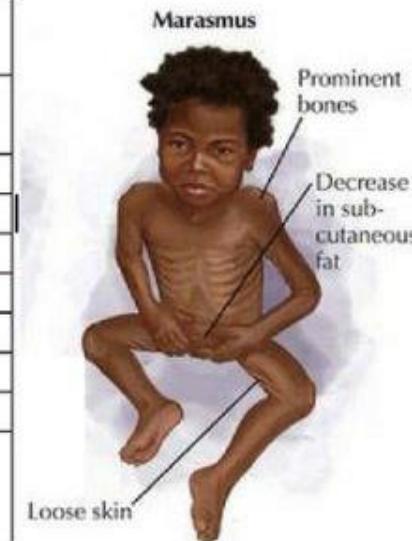
# Marasmus

A 1-year-old-male is brought to the physician by his mother because of concerns of weight loss, edema of the abdomen, and upper and lower extremities. The child is lethargic and irritable when aroused. The mother says that after weaning the child from breastfeeding, she had placed the child on the BRAT diet consisting of bananas, white rice, applesauce and toast as a result of concern for food allergies.



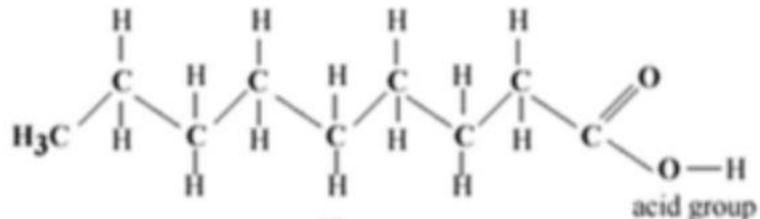
Comparison Table

Kwashiorkor	Marasmus
It develops in children whose diets are deficient of protein.	It is due to deficiency of proteins and calories.
It occurs in children between 6 months and 3 years of age.	It is common in infants under 1 year of age.
Subcutaneous fat is preserved.	Subcutaneous fat is not preserved.
Oedema is present.	Oedema is absent
Enlarged fatty liver.	No fatty liver.
Ribs are not very prominent.	Ribs become very prominent.
Lethargic	Alert and irritable.
Muscle wasting mild or absent.	Severe muscle wasting
Poor appetite.	Voracious feeder.
The person suffering from Kwashiorkor needs adequate amounts of proteins.	The person suffering from Marasmus needs adequate amount of protein, fats and carbohydrates.

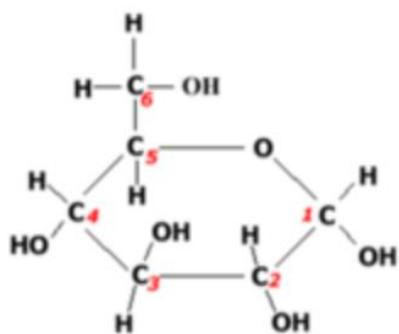


## Kwashiorkor vs Marasmus

# Amino Acid Pool



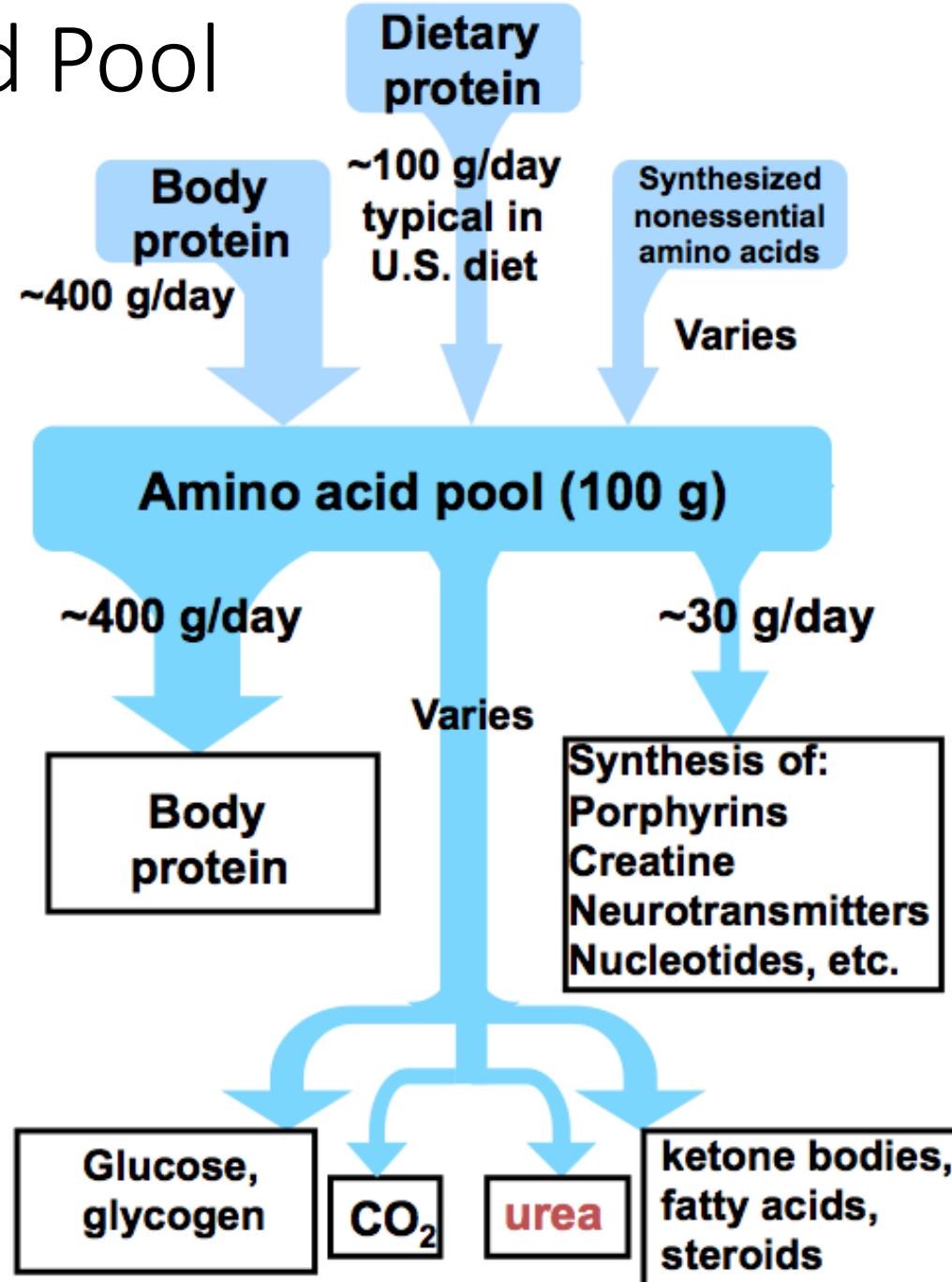
## Fatty Acids



## Glucose



## Amino acids



A 15-year-old girl, accompanied by her mother, presents to her primary care physician complaining of fatigue and sleeplessness for 6 months' duration. The doctor notes the patient is quite petite and is wearing an oversized, baggy dress. There are no physical findings. She is found to be 88% of the minimum weight requirements for her age and height. Her mother is concerned as her daughter has been eating little and exercising daily. Which metabolic flux is most prevalent in this patient?

- A. Glucose → fatty acids
- B. Glucose → glycogen
- C. Fatty acids → triacylglycerol
- D. Protein → amino acids

# Nitrogen Balance

Positive balance



Protein in > Protein out

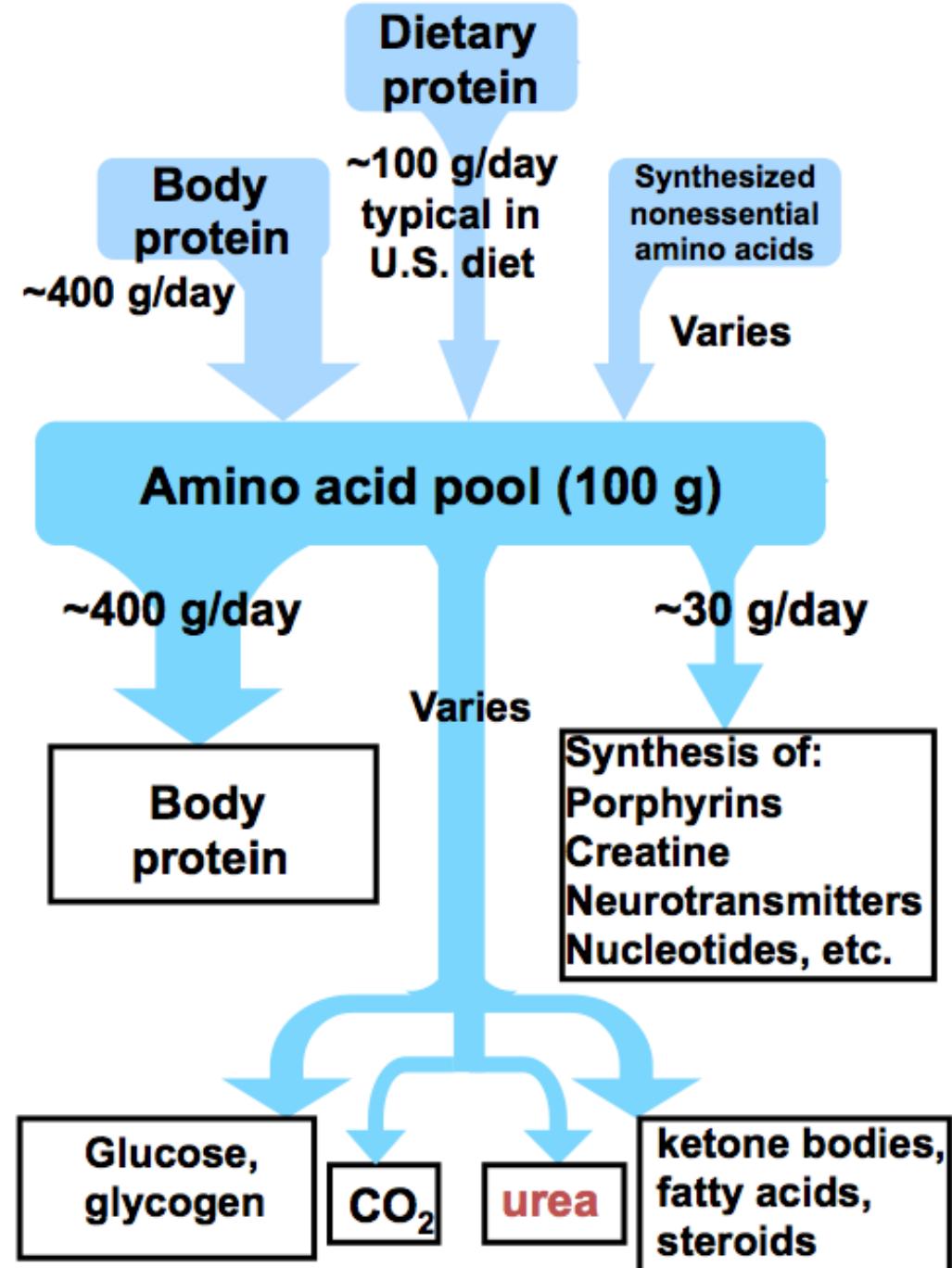
- Pregnancy
- Growth
- Muscle building
- Recovery from illness

Negative balance

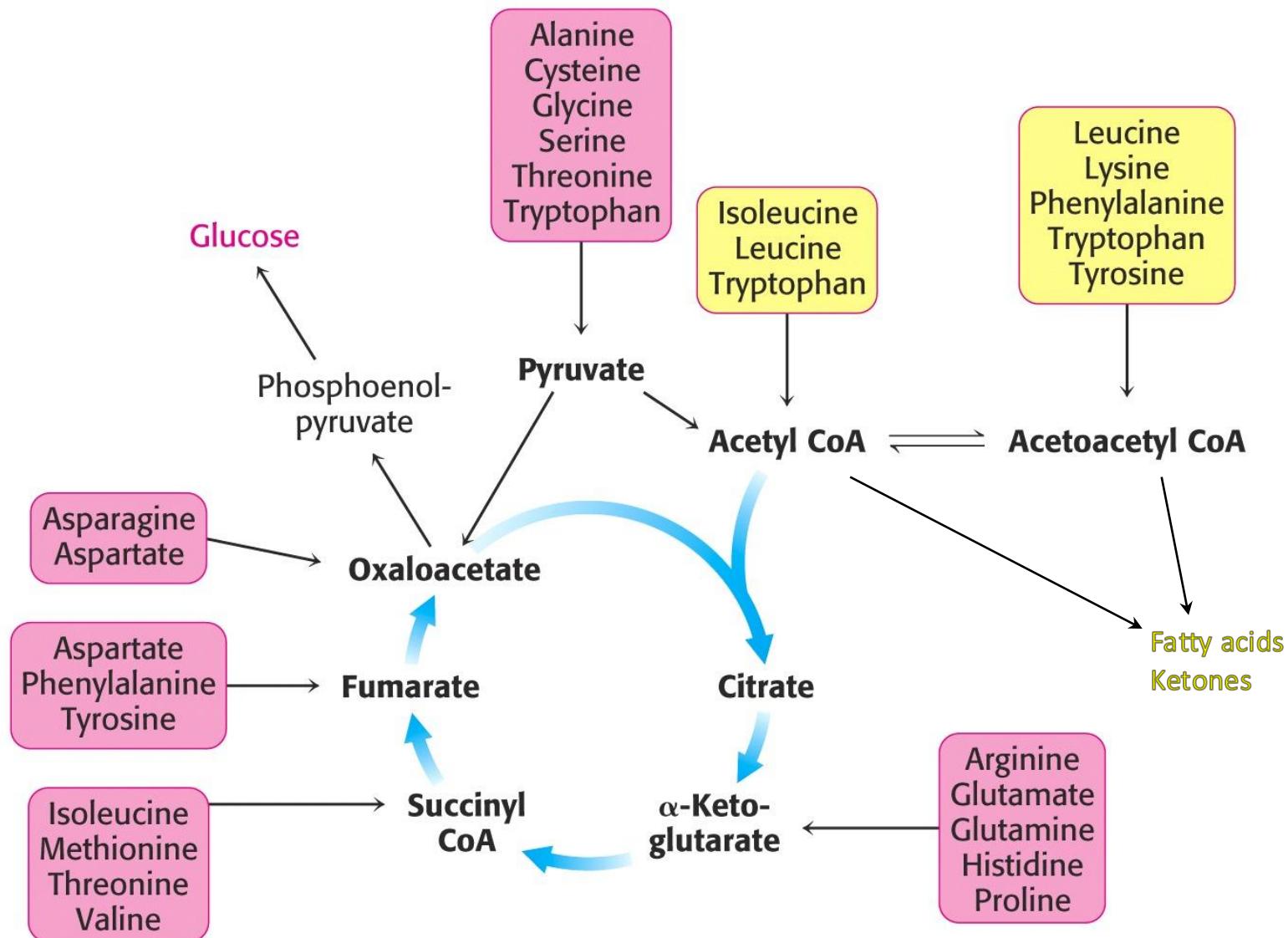


Protein in < Protein out

- Protein insufficiency in diet
- Starvation
- Stress
- Hypercatabolic state
  - Infection
  - Fever
  - Surgery
  - cachexia



# Glucogenic and Ketogenic Amino Acids



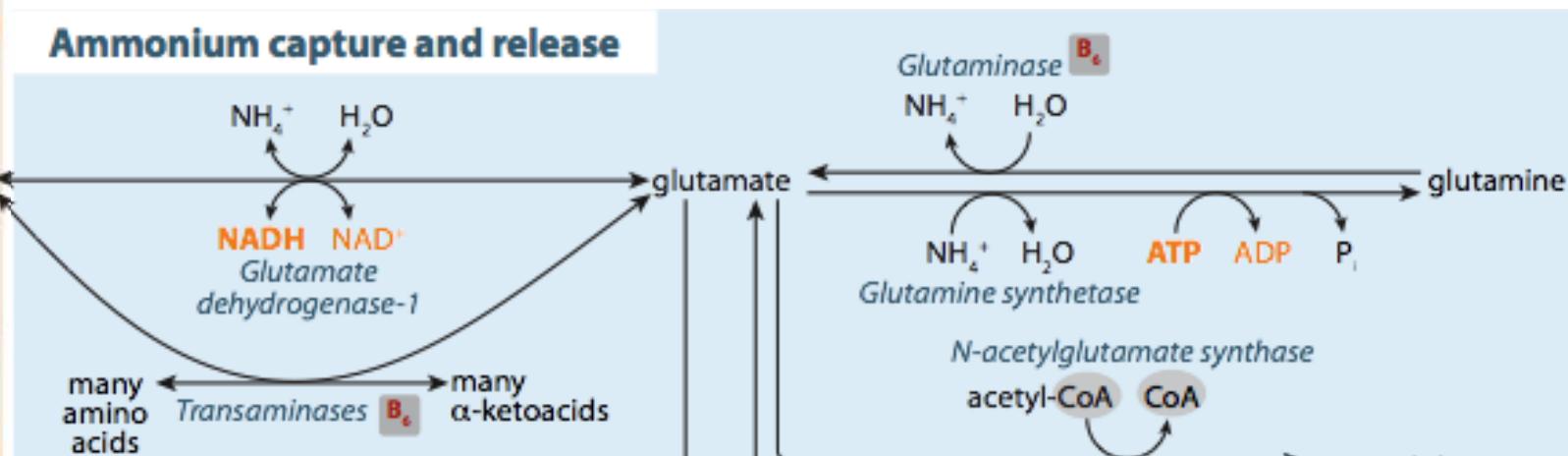
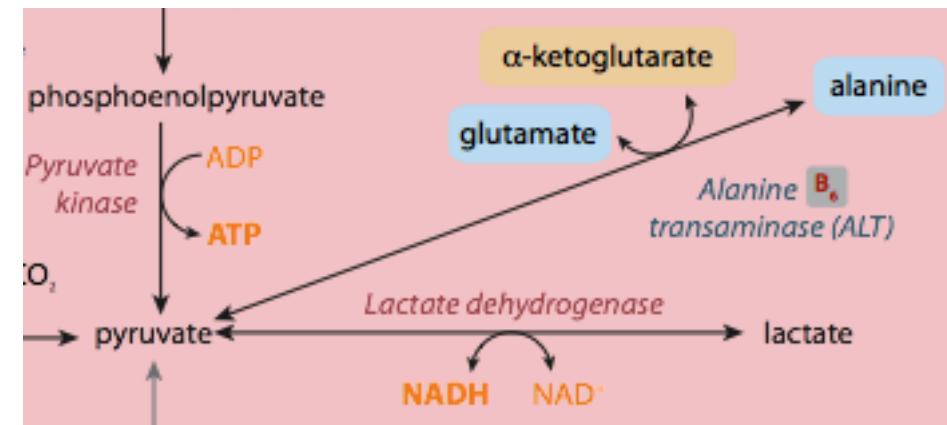
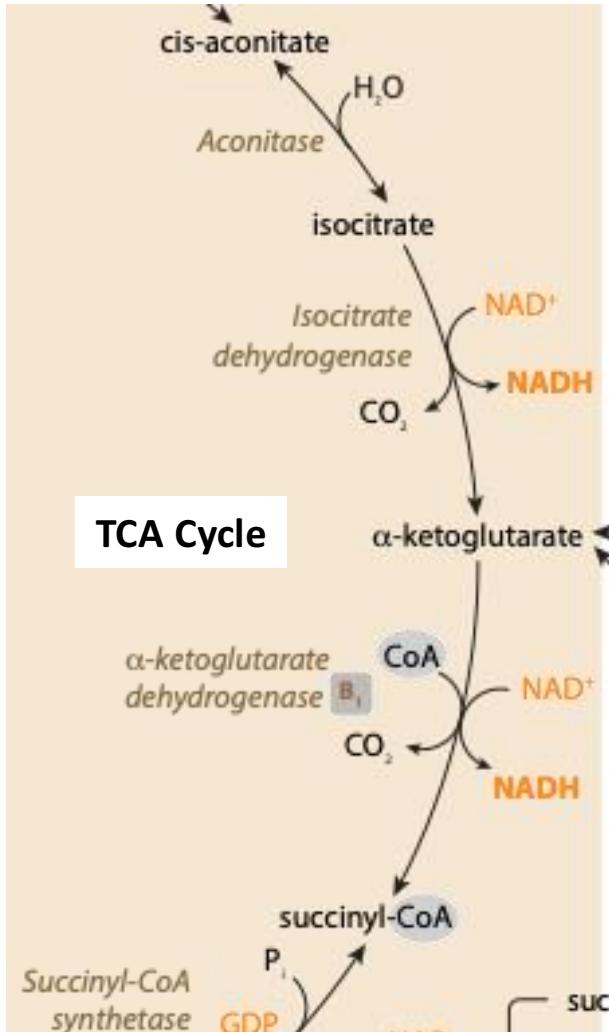
**Table 6.1****The Mighty Twenty**

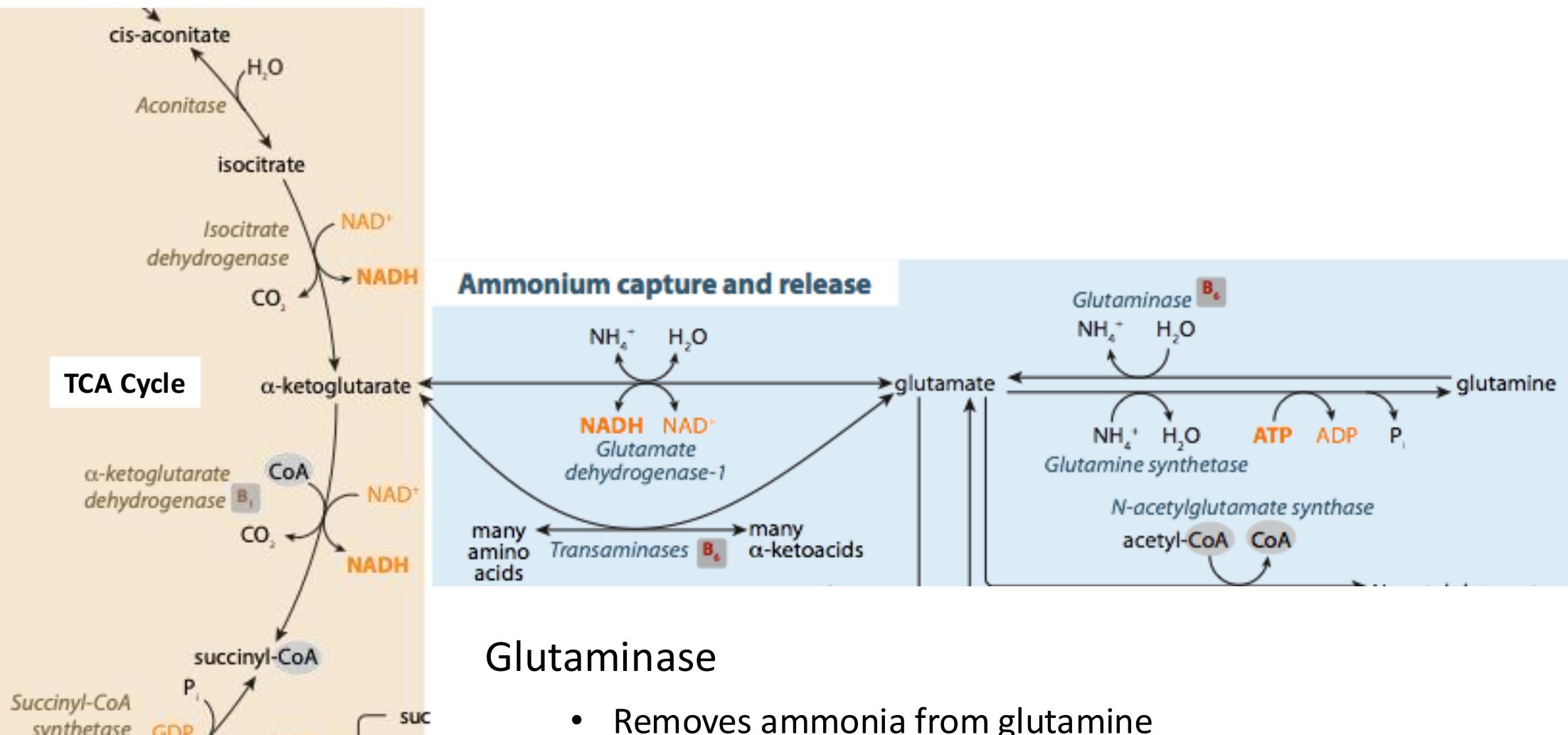
Essential Amino Acids	Nonessential Amino Acids
Histidine (His) <sup>a</sup>	Alanine (Ala)
Isoleucine (Ile)	Arginine (Arg) <sup>b</sup>
Leucine (Leu)	Asparagine (Asn)
Lysine (Lys)	Aspartic acid (Asp)
Methionine (Met)	Cysteine (Cys) <sup>b</sup>
Phenylalanine (Phe)	Glutamic acid (Glu)
Threonine (Thr)	Glutamine (Gln) <sup>b</sup>
Tryptophan (Trp)	Glycine (Gly) <sup>b</sup>
Valine (Val)	Proline (Pro) <sup>b</sup>
	Serine (Ser)
	Tyrosine (Tyr) <sup>b</sup>

<sup>a</sup> Histidine was once thought to be essential only for infants. It is now known that small amounts are also needed for adults.

<sup>b</sup> These amino acids can be “conditionally essential” if there are either inadequate precursors or inadequate enzymes available to create these in the body.

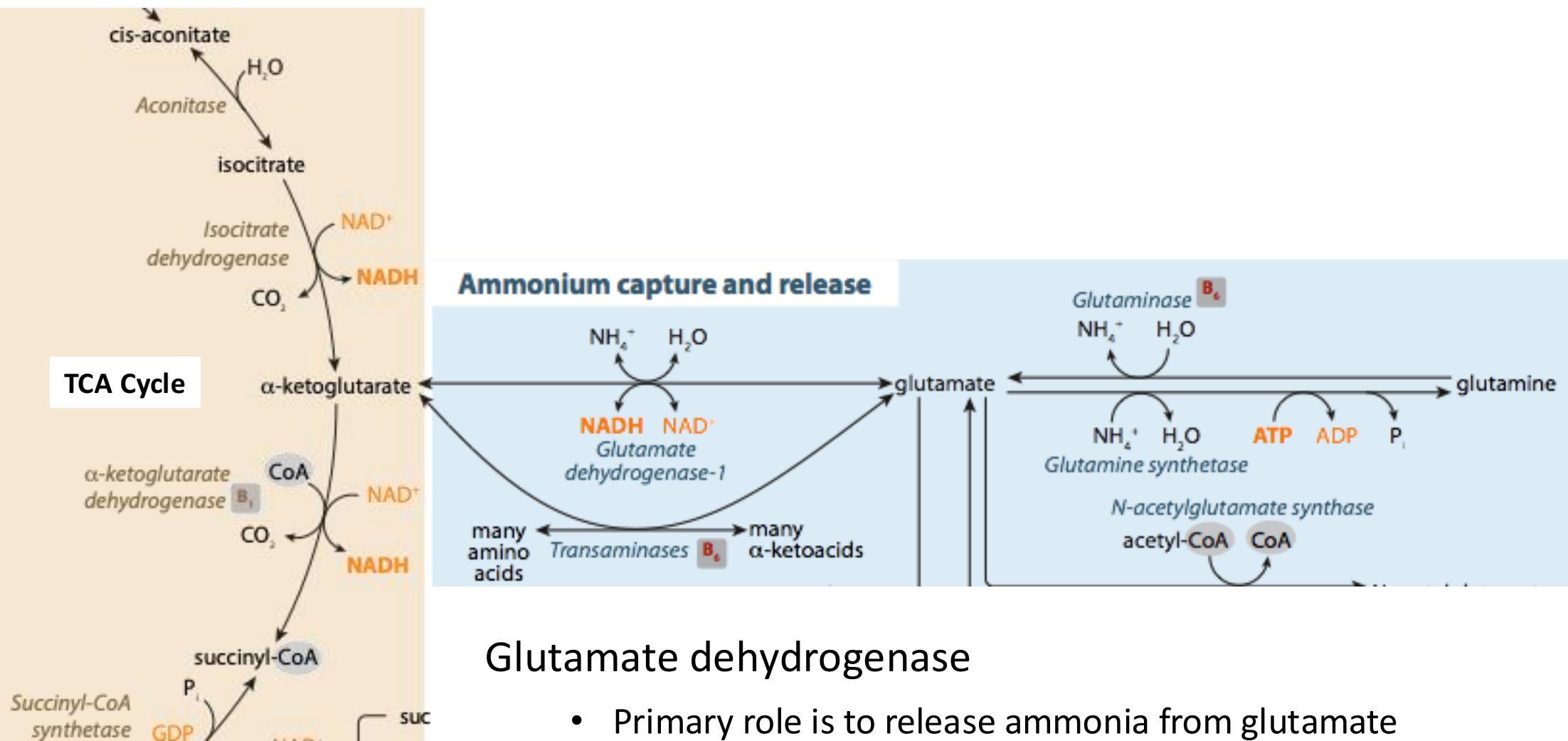
Glutamine and alanine are used for transporting nitrogen





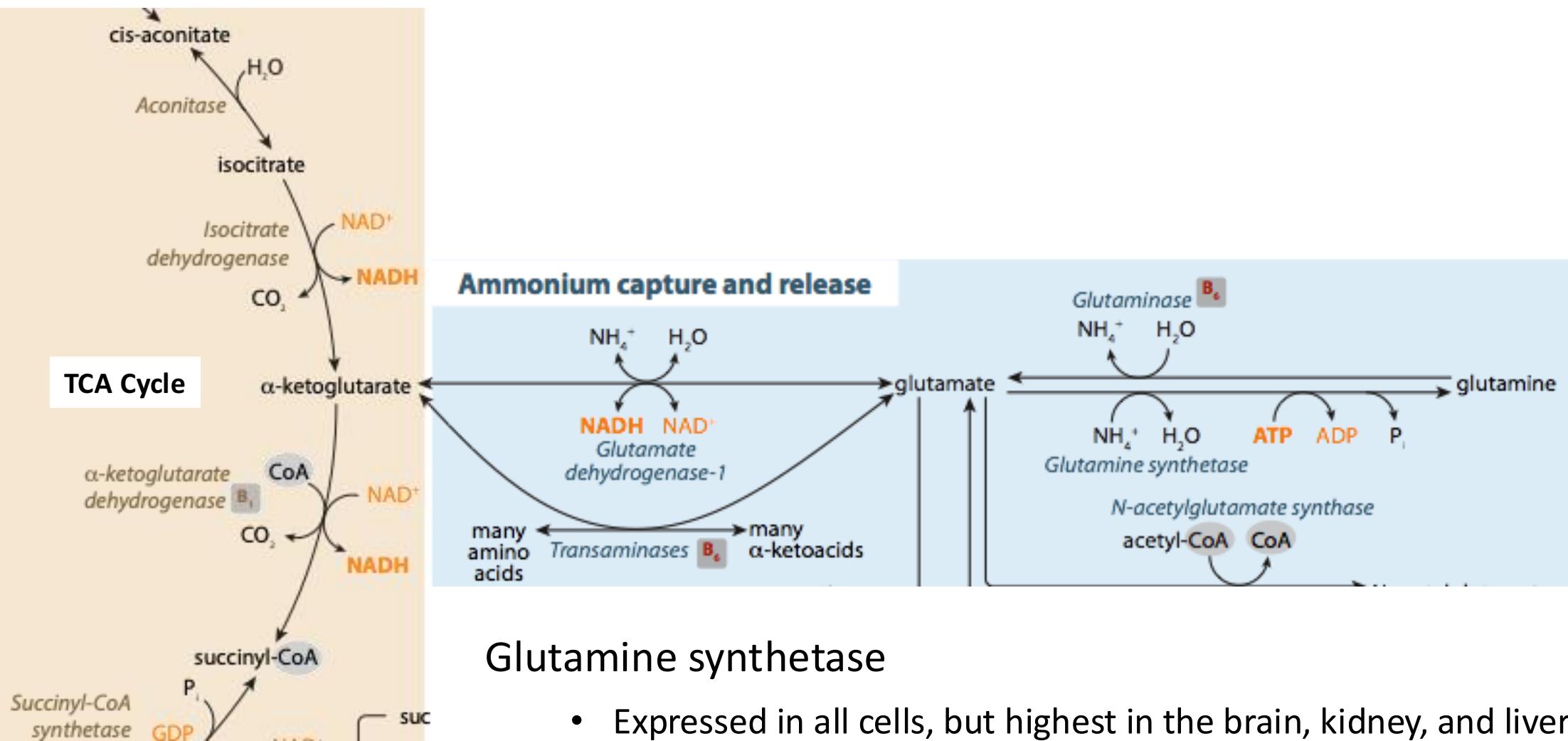
## Glutaminase

- Removes ammonia from glutamine
- Highest expression in liver, kidney, brain, and small intestine



## Glutamate dehydrogenase

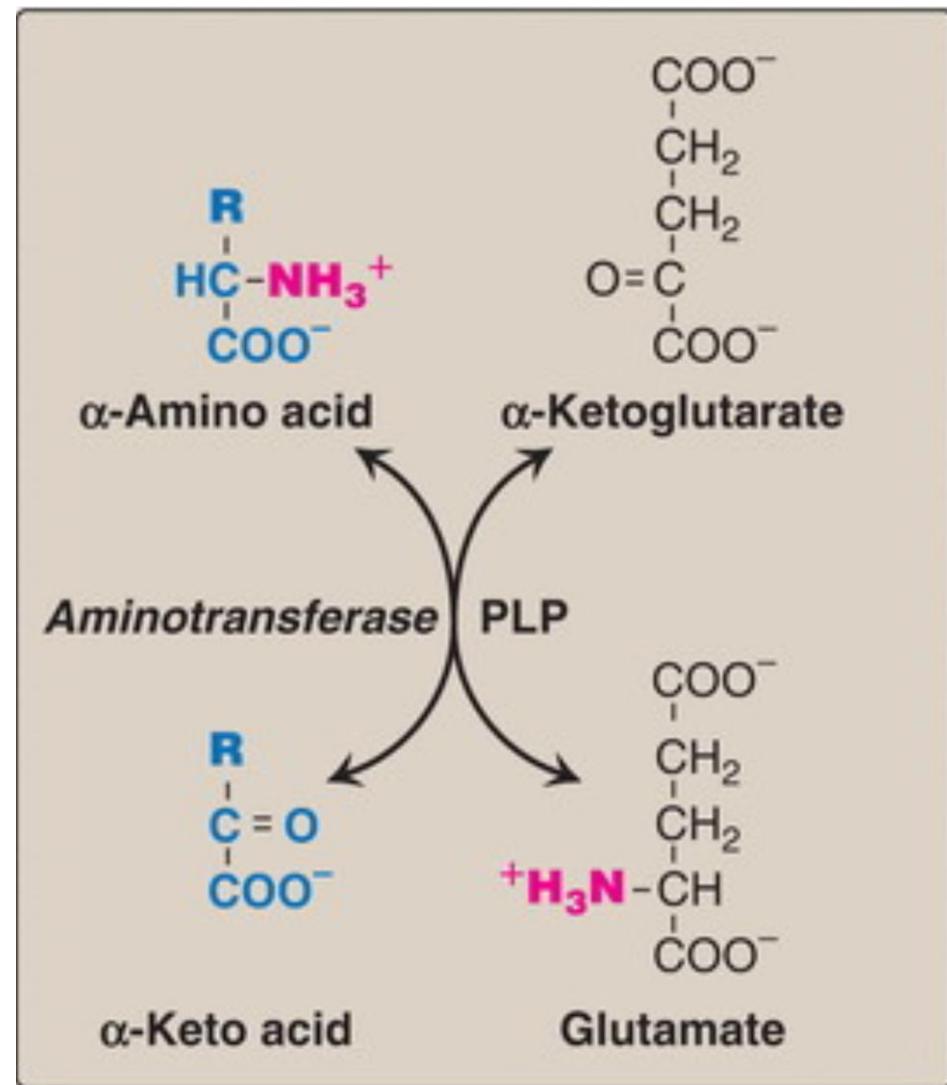
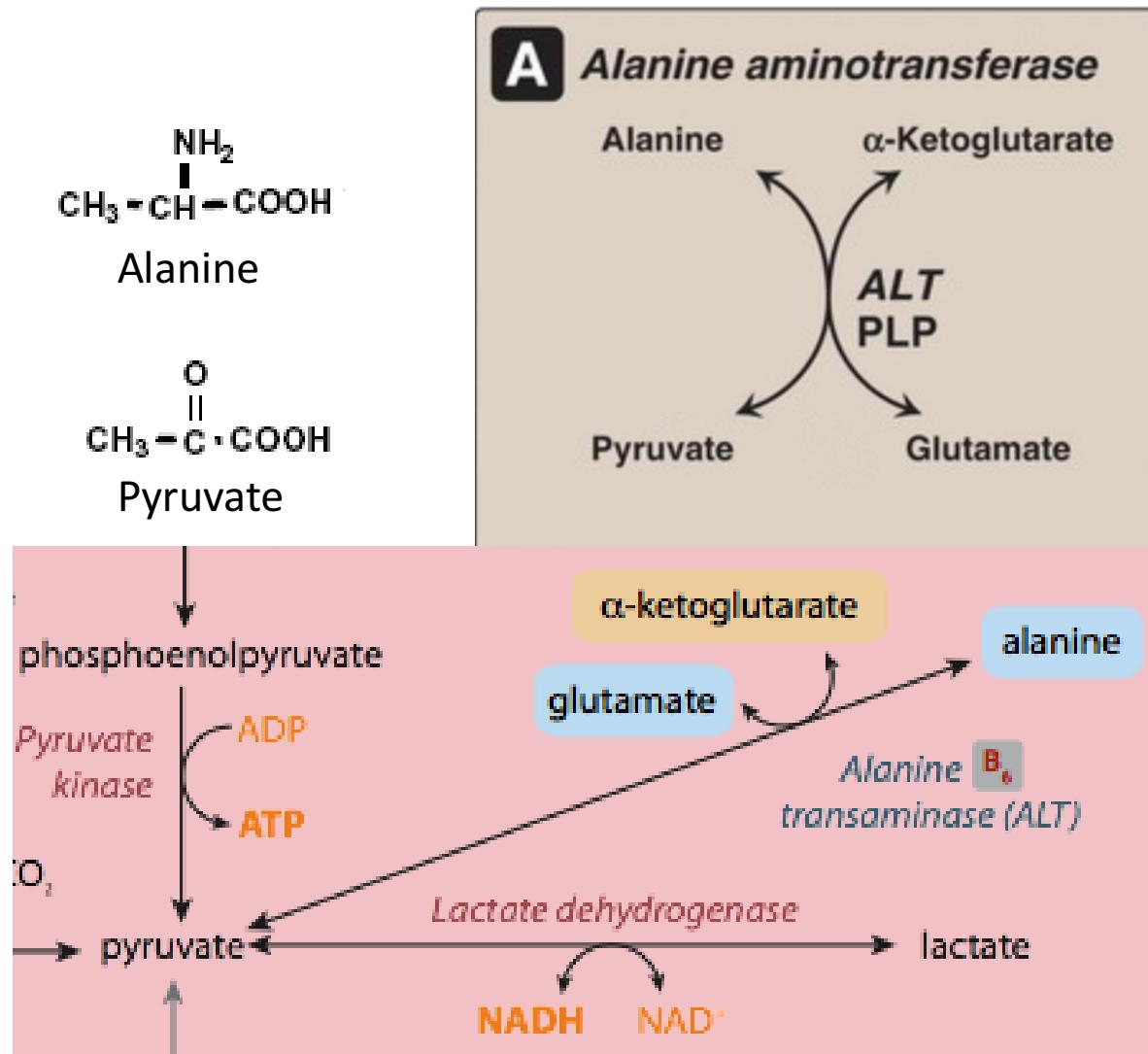
- Primary role is to release ammonia from glutamate
- Highest expression in liver, kidney, and small intestine, but present in most cells
- Can fix free ammonia to form glutamate



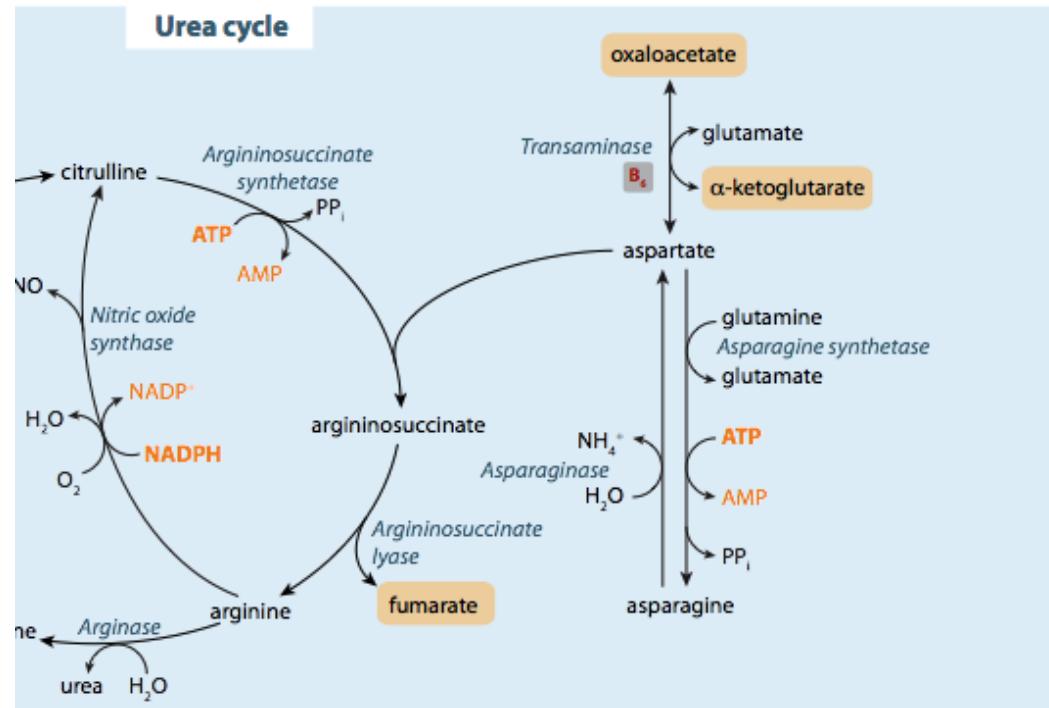
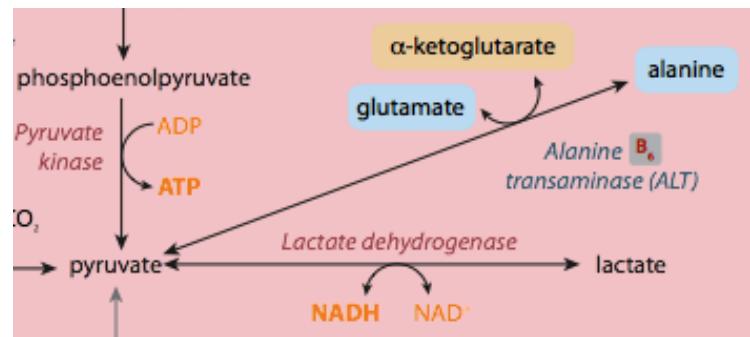
## Glutamine synthetase

- Expressed in all cells, but highest in the brain, kidney, and liver
- Generates glutamine from glutamate by fixing free ammonia
- Uses ATP
- Inhibited by high AMP

# AST and ALT



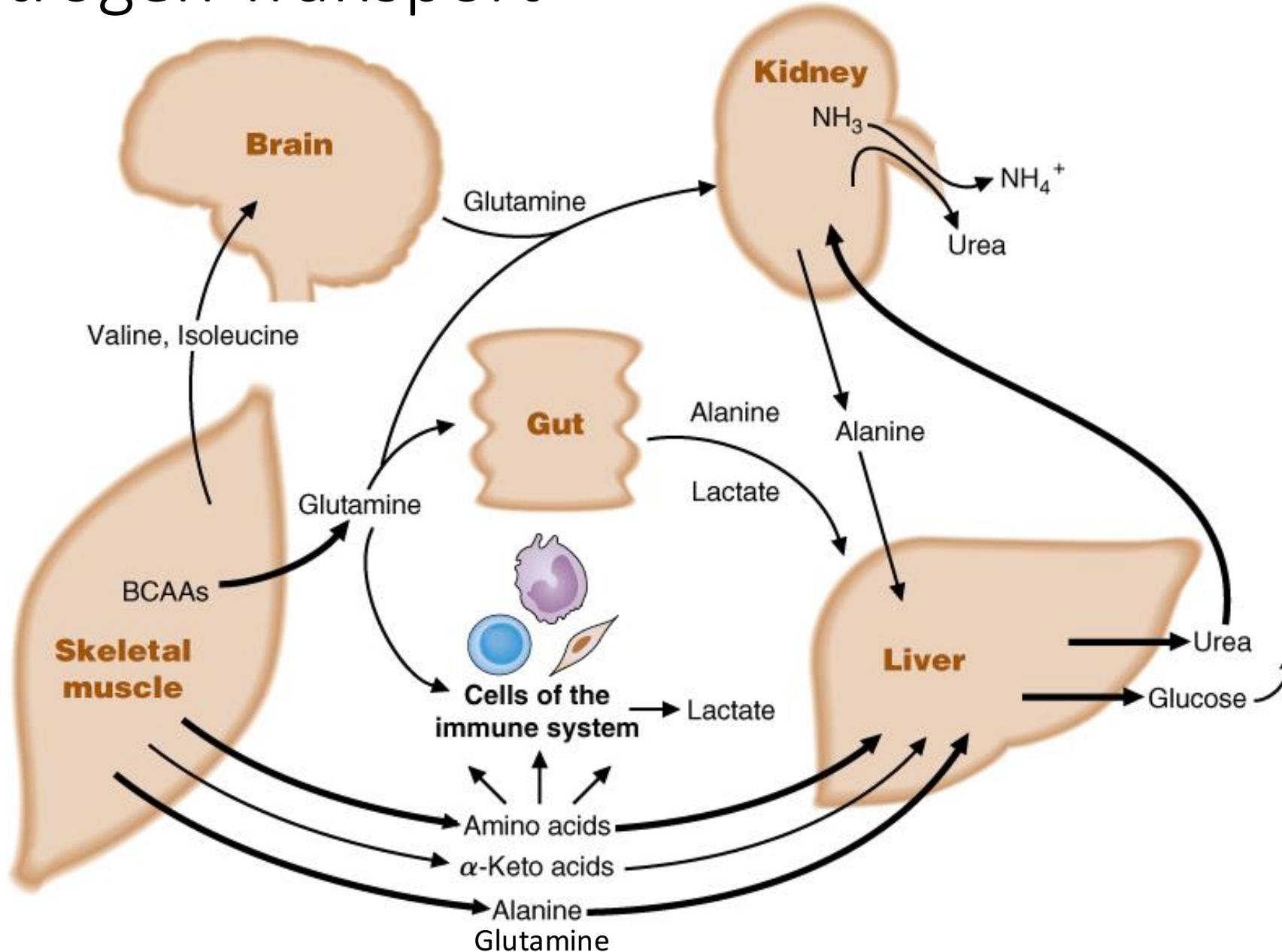
# AST and ALT



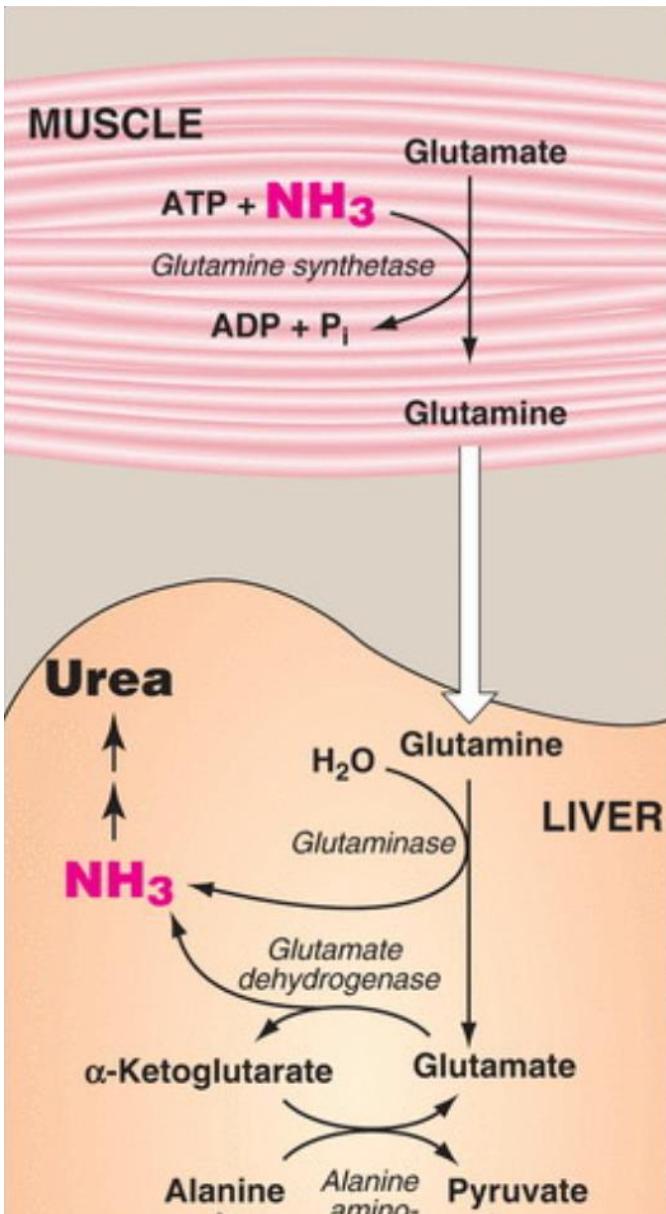
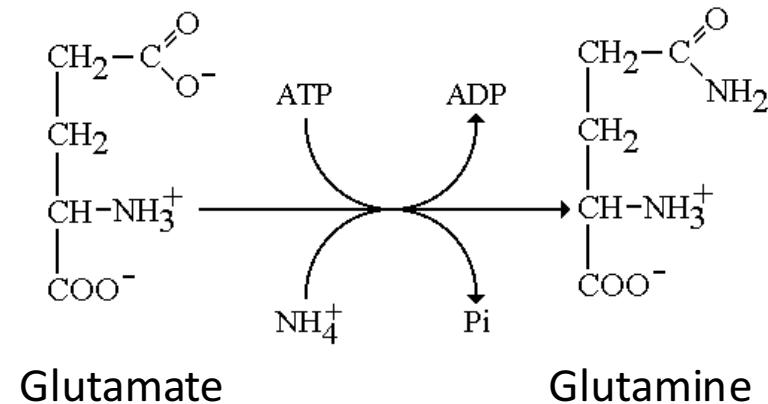
## De Ritis Ratio Decision Limit

Condition	AST/ALT Ratio	<1.0	1.0 to <1.5	1.5 to <2.0	≥ 2.0
<b>Healthy</b>		Women (up to 1.7)			
		Men (up to 1.3)		Children	Neonate
<b>Acute Viral Hepatitis</b>		Resolving		Worsening	Fulminant
<b>Alcoholic Hepatitis</b>		Resolving		Alcohol Abuse	Acute Hepatitis
<b>Chronic Liver Disease</b>		Stable	Fibrosis risk		Other Causes
<b>Muscle Disease</b>		Chronic	Resolving		Acute

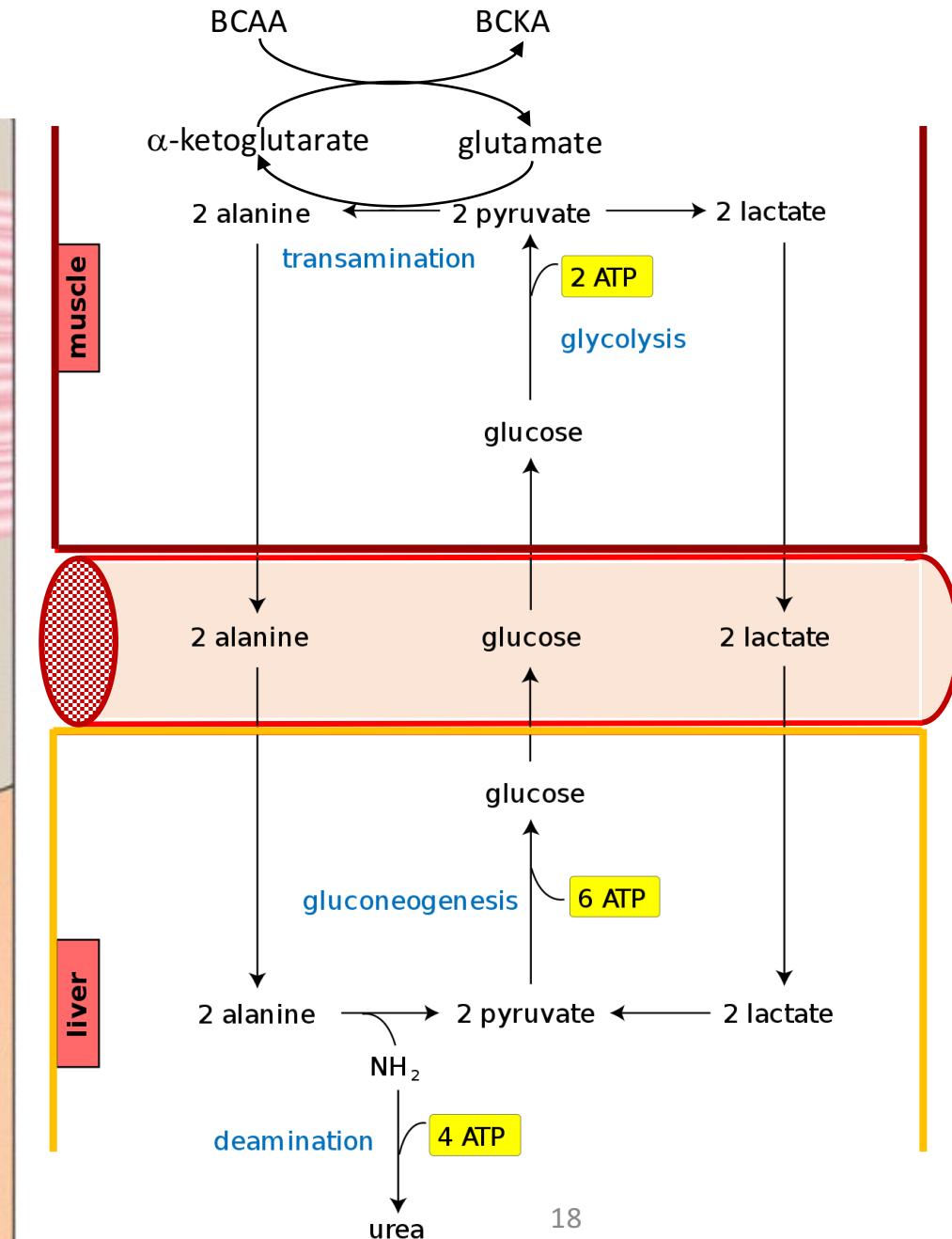
# Nitrogen Transport



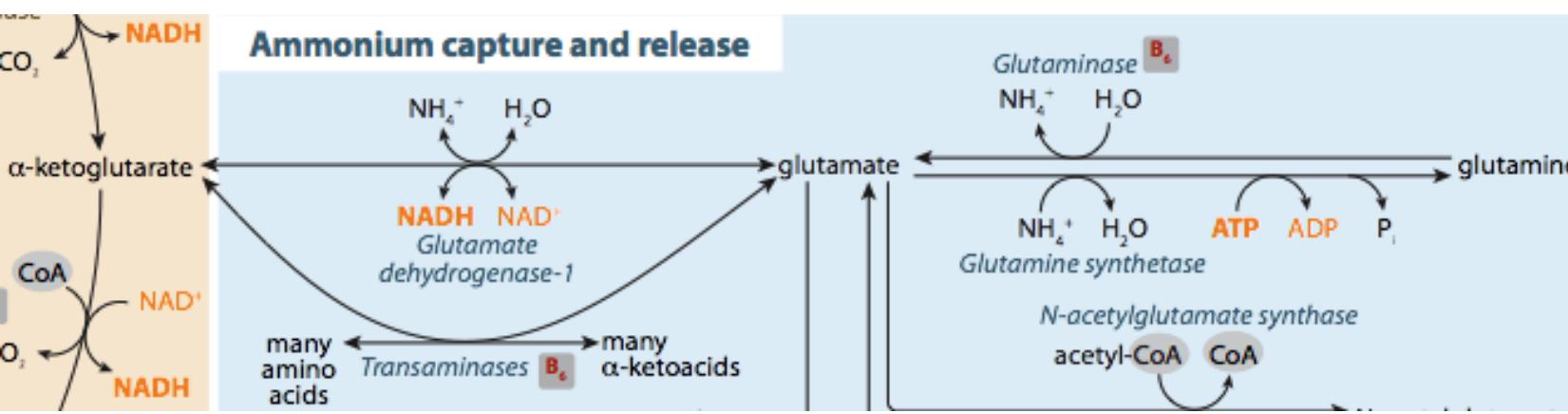
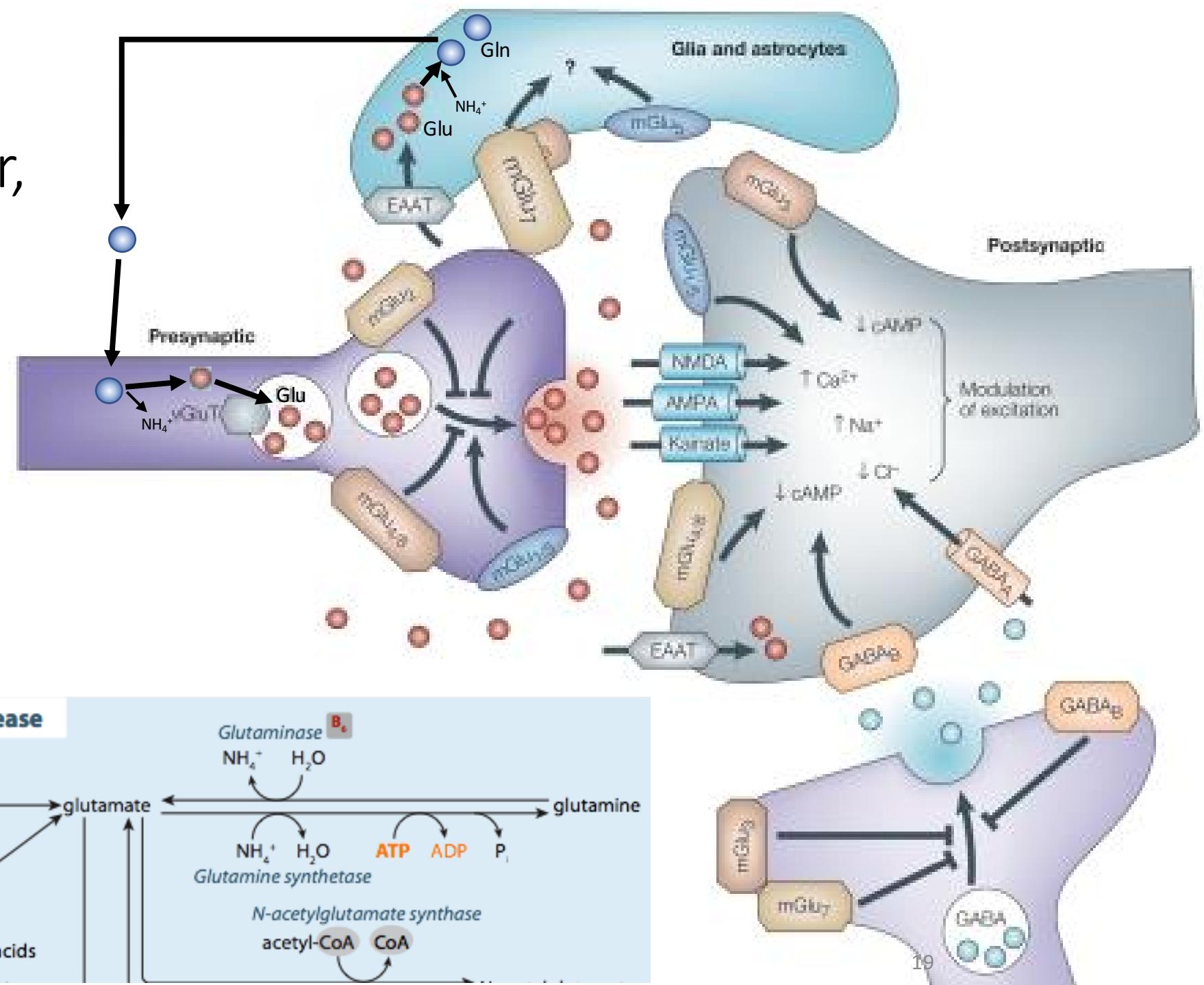
# Inactive muscle and other tissues



# Active muscle



Glutamate is a neurotransmitter,  
so glutamine is  
used as a  
nitrogen  
transporter

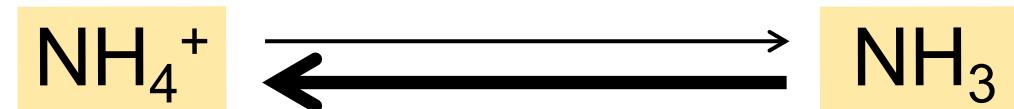


A 15-year-old girl, accompanied by her mother, presents to her primary care physician complaining of fatigue and sleeplessness for 6 months' duration. The doctor notes the patient is quite petite and is wearing an oversized, baggy dress. She is found to be 88% of the minimum weight requirements for her age and height. Her mother is concerned as her daughter has been eating little and exercising daily. Which amino acids would be found in the highest concentration in the serum?

- A. Branched chain amino acids
- B. Alanine and Glutamine
- C. Arginine and Ornithine
- D. Glutamate and Aspartate

Ammonium Ion

Ammonia



Physiological pH (approximate)

$$7.4 = 9.25 + \log \frac{[\text{NH}_3]}{[\text{NH}_4^+]}$$

pKa of ammonia (approximate)

Therefore...

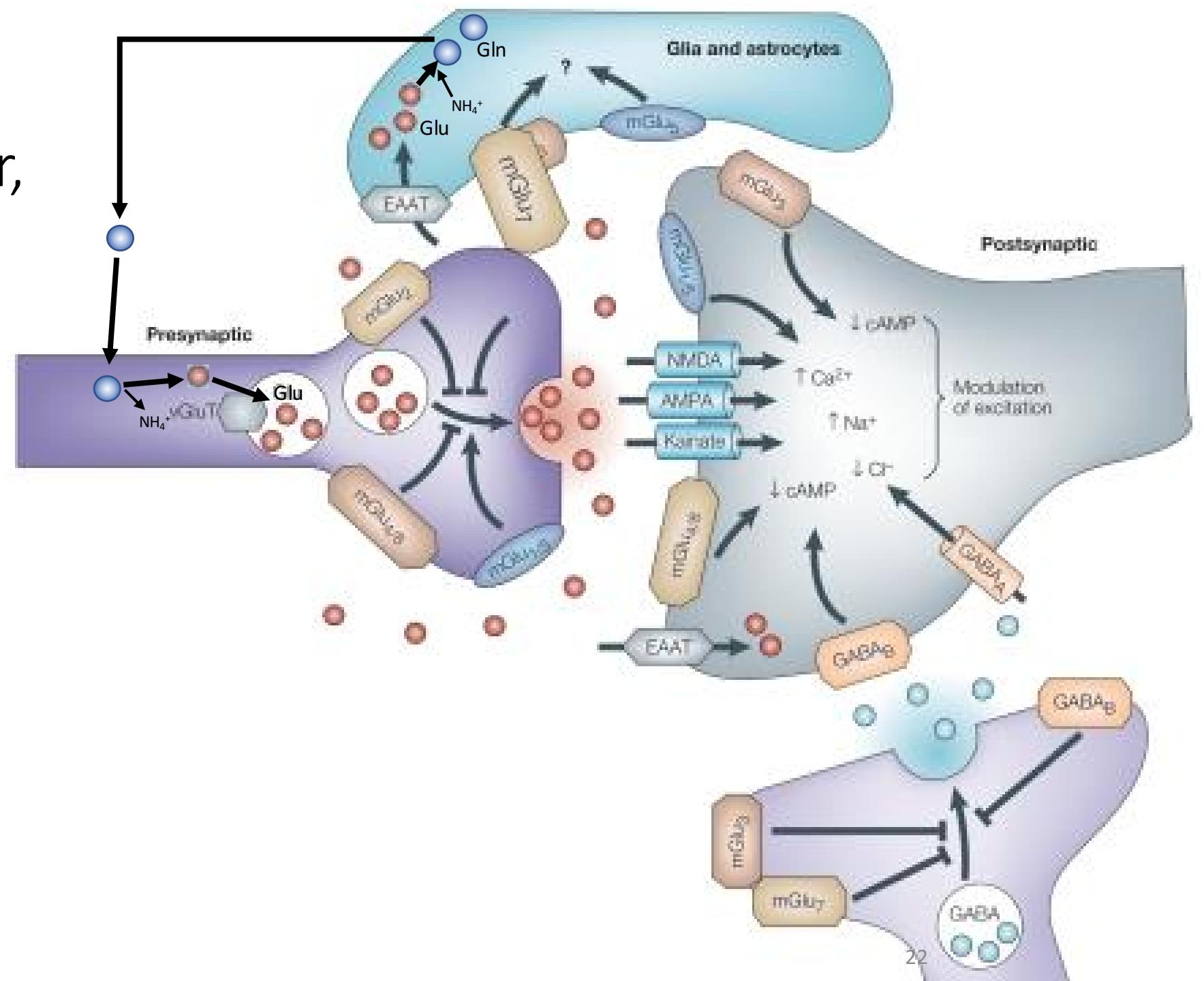
$$[10^{-1.85}] = \frac{[\text{NH}_3]}{[\text{NH}_4^+]} = \sim .01413$$

and

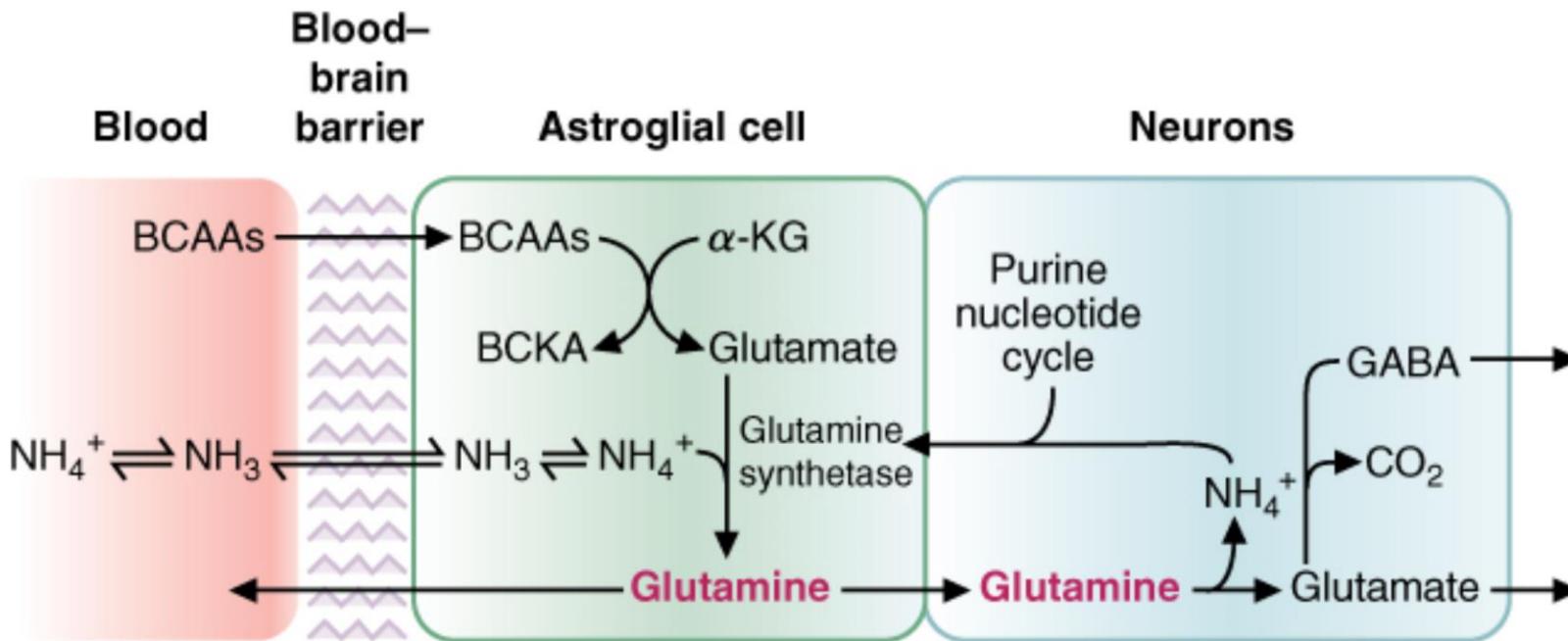
$$\sim .01413 = \frac{[\text{NH}_3]}{[\text{NH}_4^+]} = \sim \frac{[14.13]}{[1000]}$$

\*\*at physiologic pH (7.4)  
98.6% will be  $\text{NH}_4^+$  and  
1.4% will be  $\text{NH}_3$

Glutamate is a neurotransmitter,  
so glutamine is used as a  
nitrogen  
transporter



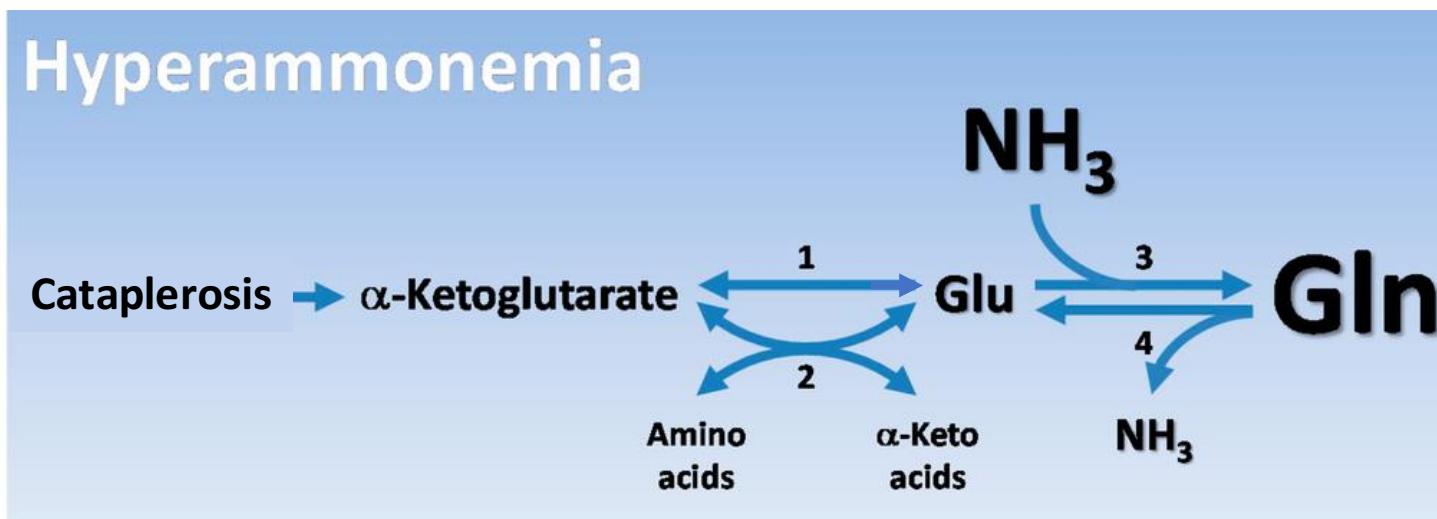
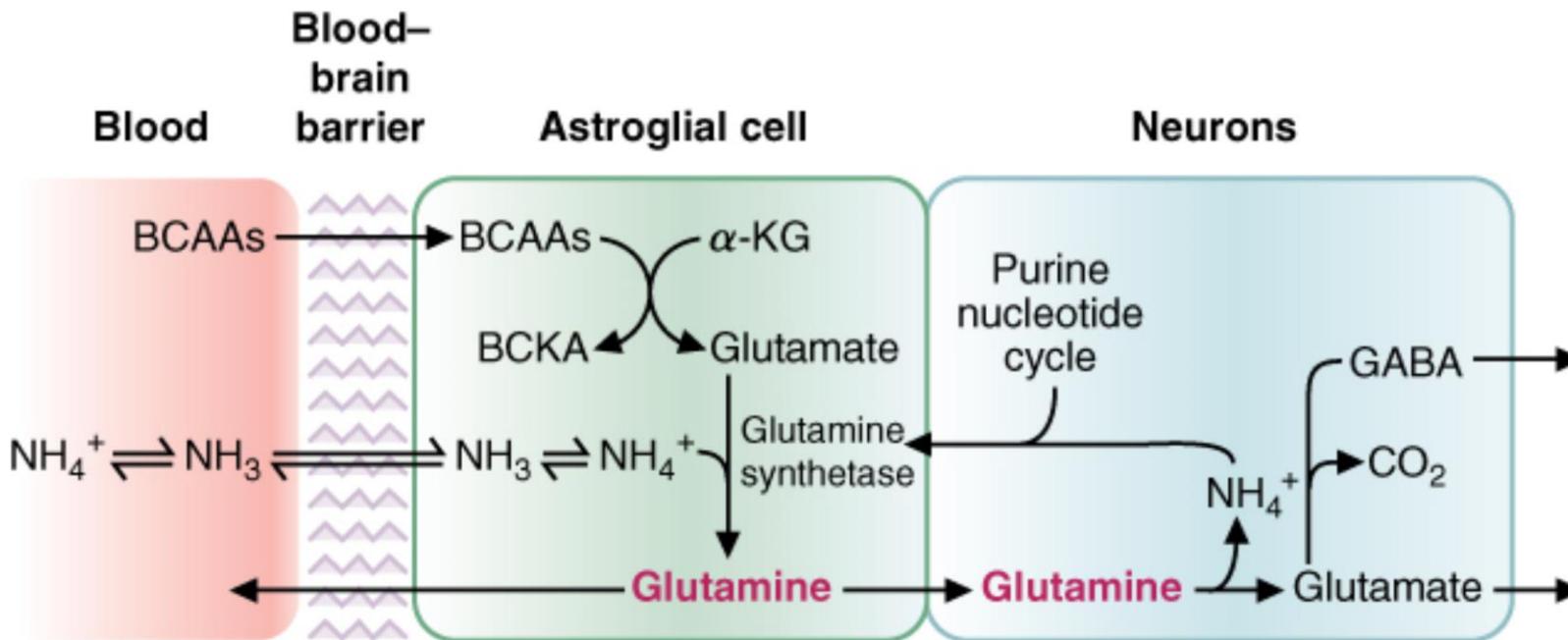
# Role of glutamine in the brain



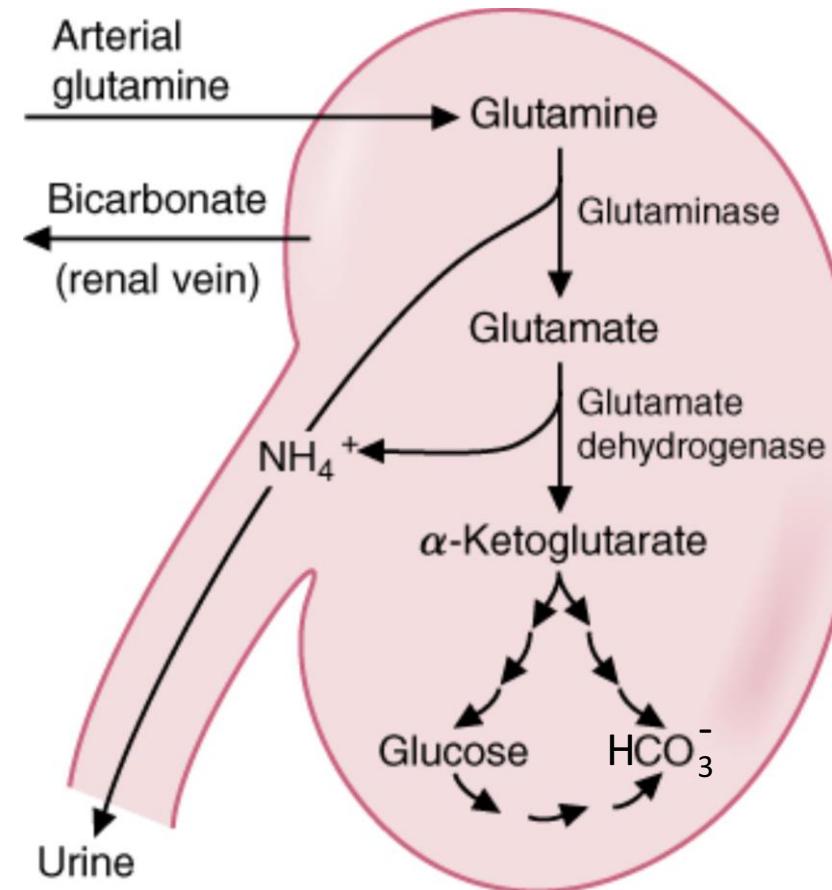
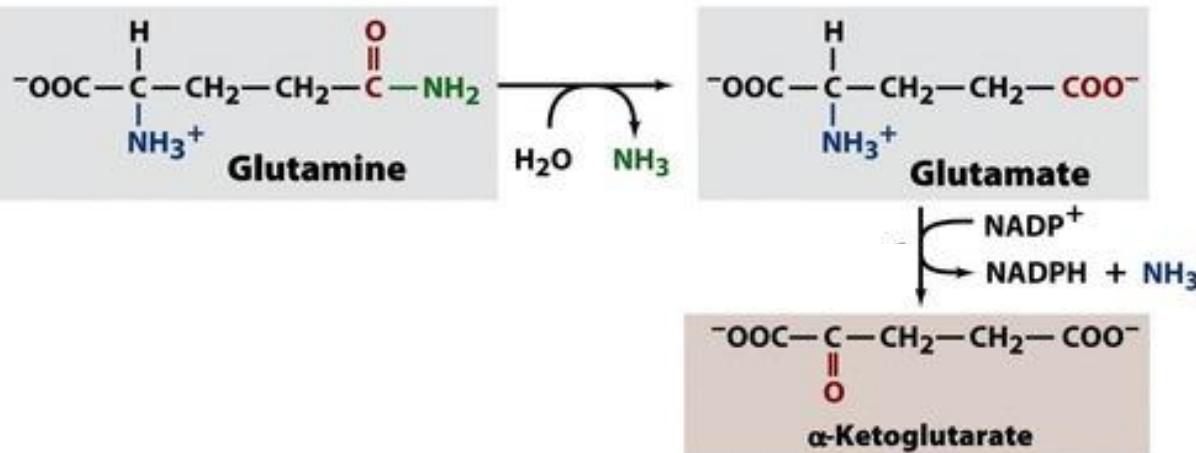
Role of glutamine in the brain. Glutamine serves as a nitrogen transporter in the brain for the synthesis of many different neurotransmitters. Different neurons convert glutamine to  $\gamma$ -aminobutyric acid (GABA) or to glutamate. Glutamine also transports excess  $\text{NH}_4^+$  from the brain into the blood.

$\alpha$ -KG,  $\alpha$ -ketoglutarate; BCAAs, branched-chain amino acids; BCKA, branched-chain  $\alpha$ -keto acids.

# Role of glutamine in the brain



# Role of glutaminase in the kidney during acidosis



Renal tubule cells preferentially oxidize glutamine. During metabolic acidosis, it is the major fuel for the kidney. Conversion of glutamine to  $\alpha$ -ketoglutarate generates NH<sub>4</sub><sup>+</sup>, and  $\alpha$ -ketoglutarate can form 2 bicarbonate molecules. Ammonium ion excretion helps to buffer systemic acidemia.

# Inborn Errors of Metabolism

For the inborn errors listed below write out the following;

- A) Genetic defect
- B) Most common age of presentation
- C) Fed or fasting presentation (if fed, what food does it correlate with)
- D) Presenting symptoms

Explain why these symptoms have occurred

- E) Diagnostic lab findings
- F) Dietary treatment

- 1) MCAD deficiency
- 2) Primary carnitine deficiency
- 3) Refsum disease
- 4) Hereditary fructose intolerance
- 5) Essential fructosuria
- 6) Classic galactosemia
- 7) Galactokinase deficiency
- 8) Phenylketonuria (PKU)
- 9) Maple syrup urine disease
- 10) Urea cycle defects

# RECOMMENDED UNIFORM SCREENING PANEL - 2021



## Amino acid disorders

Phenylketonuria  
Homocystinuria  
Maple syrup urine disease  
Tyrosinemia type I  
Citrullinemia type I  
Argininosuccinic aciduria

## Other disorders

Congenital hypothyroidism  
Congenital adrenal hyperplasia  
Hemoglobinopathies (3)  
Cystic fibrosis  
Severe combined immunodeficiencies

## Organic acid disorders

Propionic acidemia  
Methylmalonic acidemia (MMA, mutase)  
MMA (cobalamin disorders)  
Isovaleric acidemia  
Beta-ketothiolase deficiency  
Holocarboxylase synthetase def'y  
Glutaric acidemia type I  
3-MC carboxylase deficiency  
Biotinidase deficiency  
HMG CoA lyase deficiency

Critical congenital heart disease  
Hearing loss  
Spinal muscular atrophy

## Fatty acid oxidation defects

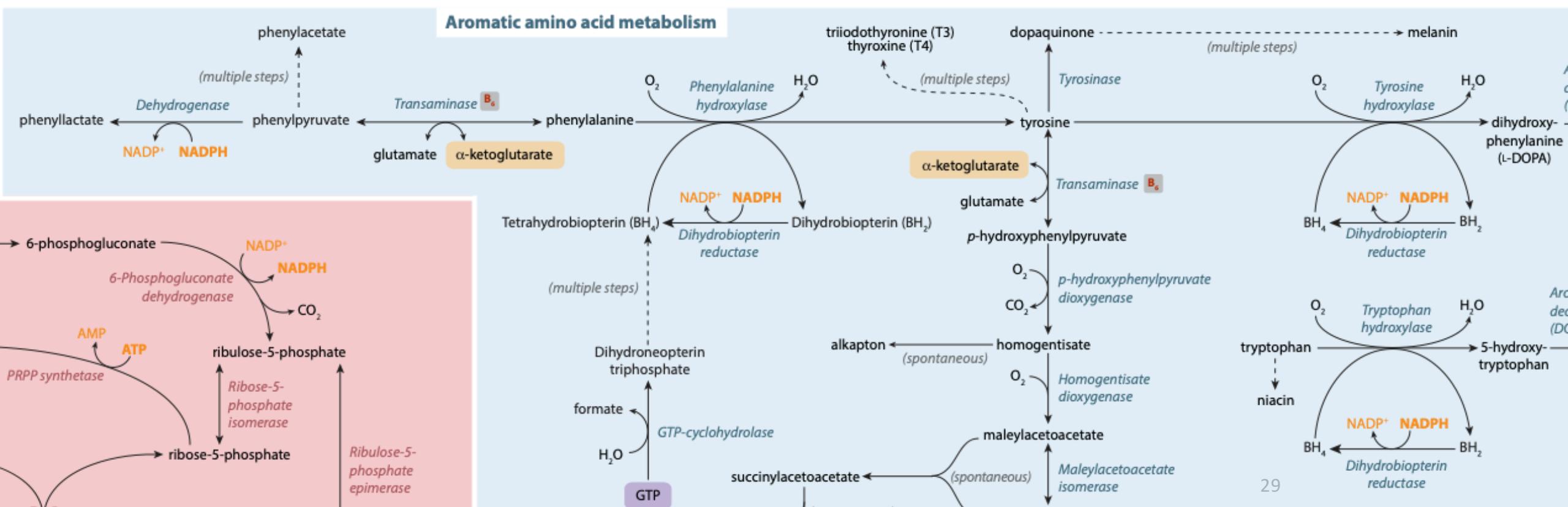
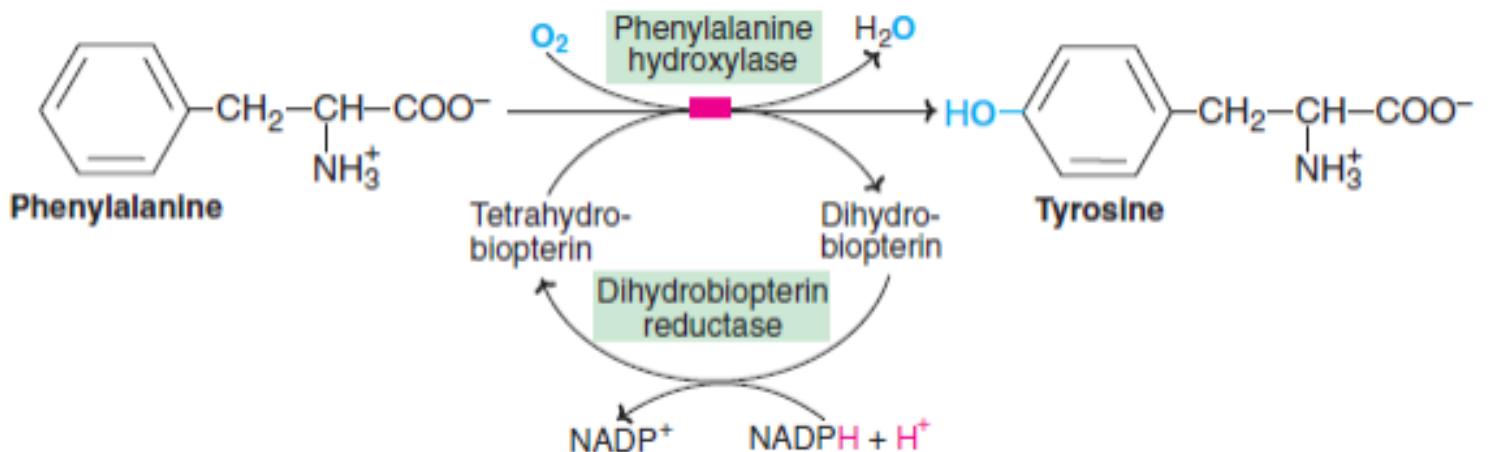
MCAD deficiency  
VLCAD deficiency  
LCHAD deficiency  
Trifunctional protein deficiency  
Carnitine uptake defect

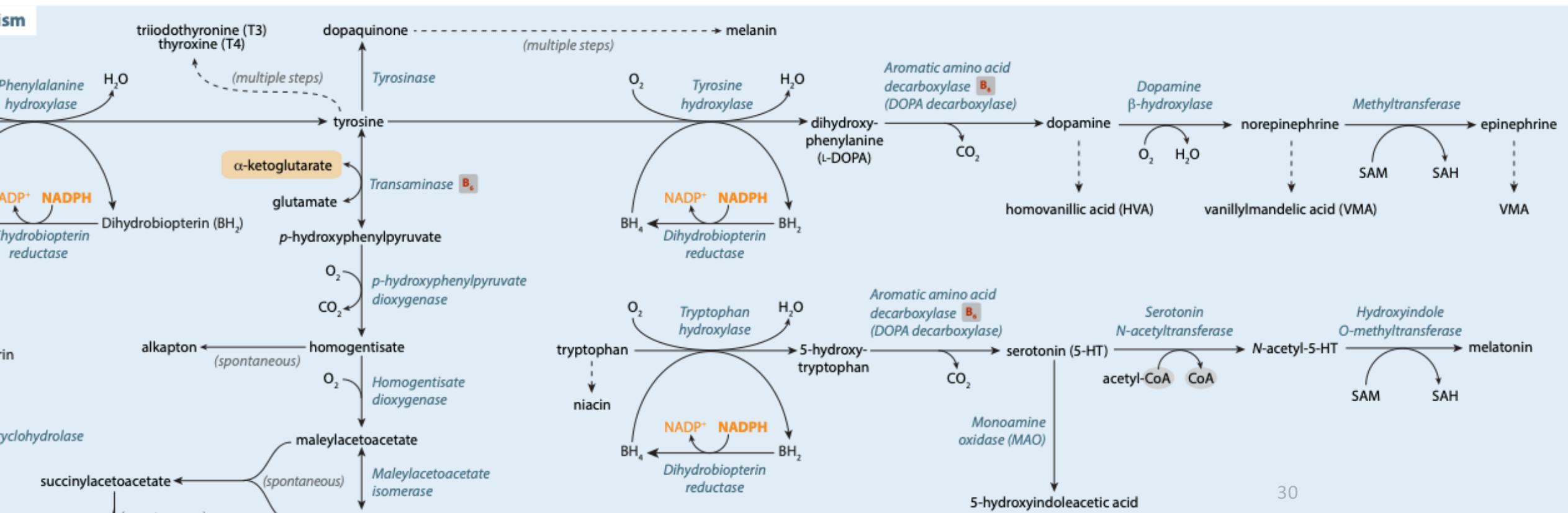
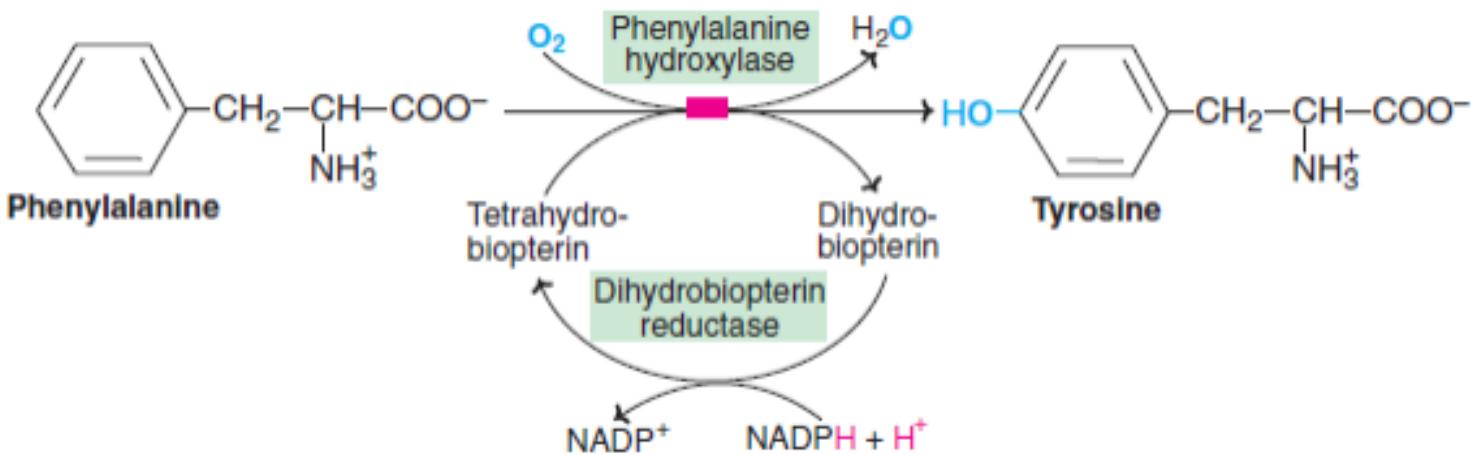
## Other Metabolic disorders

Galactosemia  
Pompe disease  
Hurler disease (MPS I)  
X-linked adrenoleukodystrophy

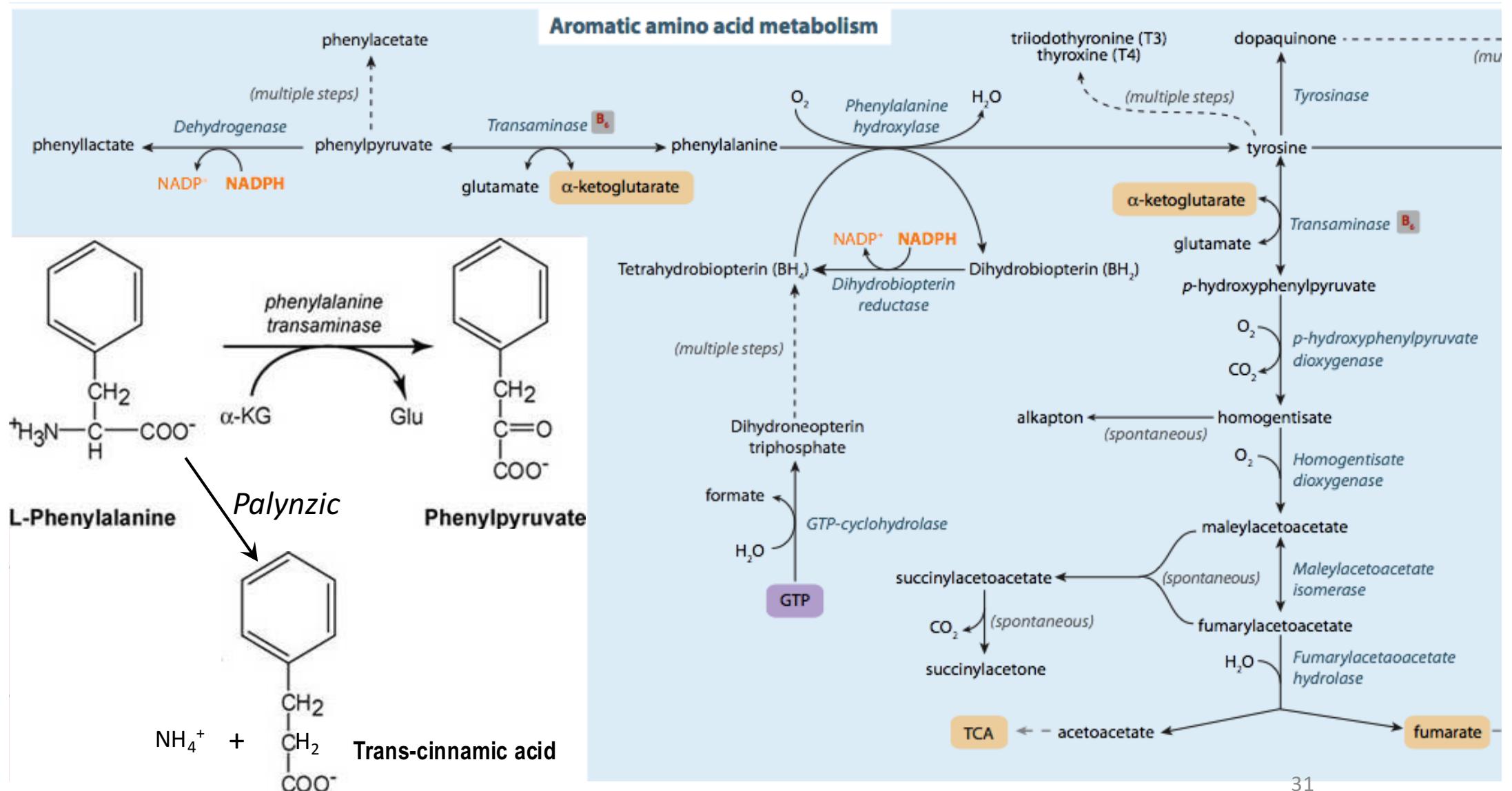
# Urine odors associated with IEMs

Odor	Disorder
Acid like	Methylmalonic acidemia (MMA)
Cabbage like	Tyrosinemia, type 1
	Methionine malabsorption
Cat urine	3-Methylcrotonylglycinuria (MCC)
	Multiple carboxylase deficiency (MCD)
Curry	Maple syrup disease (MSD)
Fish market	Trimethylaminuria
Musty, mousy	Classical phenylketonuria (PKU)
Maple syrup or burnt sugar	Maple syrup disease (MSD)
Rancid butter	Tyrosinemia, type 1
Sulphurous like	Cystinuria
Sweaty feet	Isovaleric acidemia (IVA)
	Multiple acyl-CoA dehydrogenase deficiency (MADD or glutaric acidemia, type II, GAI)

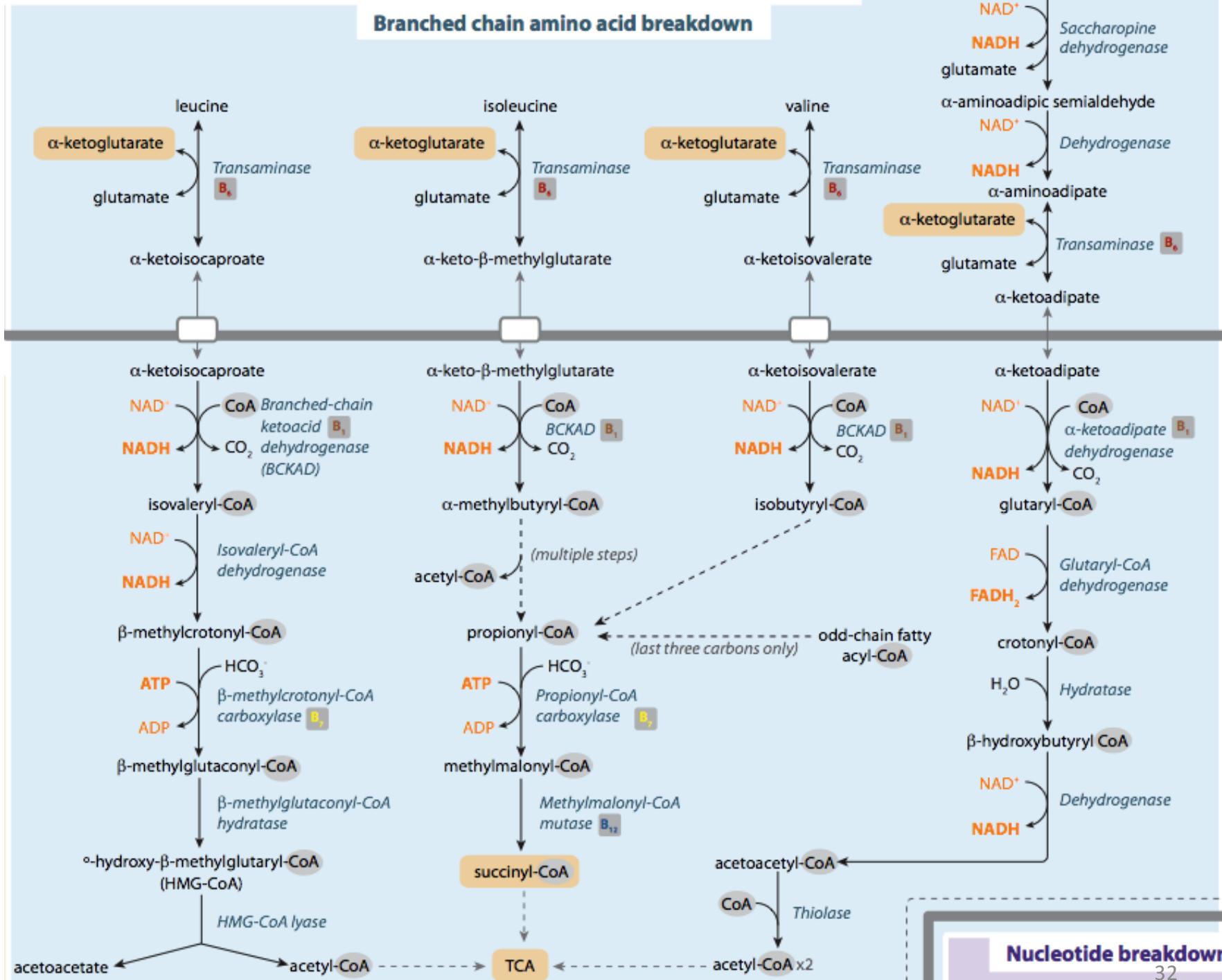




# PKU Treatment



## Branched chain amino acid breakdown



Maltose (glucose + glucose)



Lactose (glucose + galactose)



Sucrose (glucose + fructose)



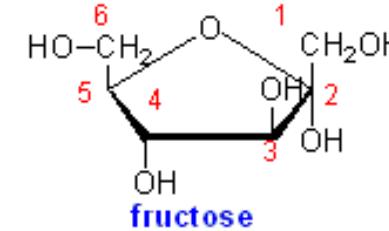
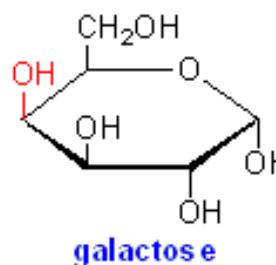
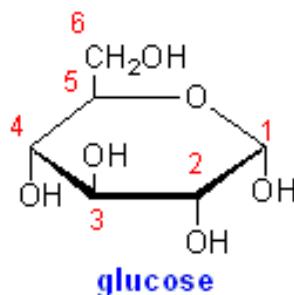
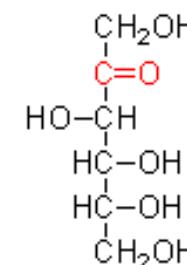
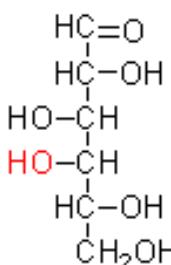
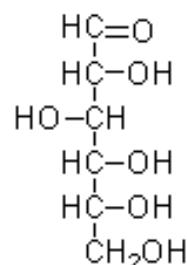
**Malt**  
(Glucose)



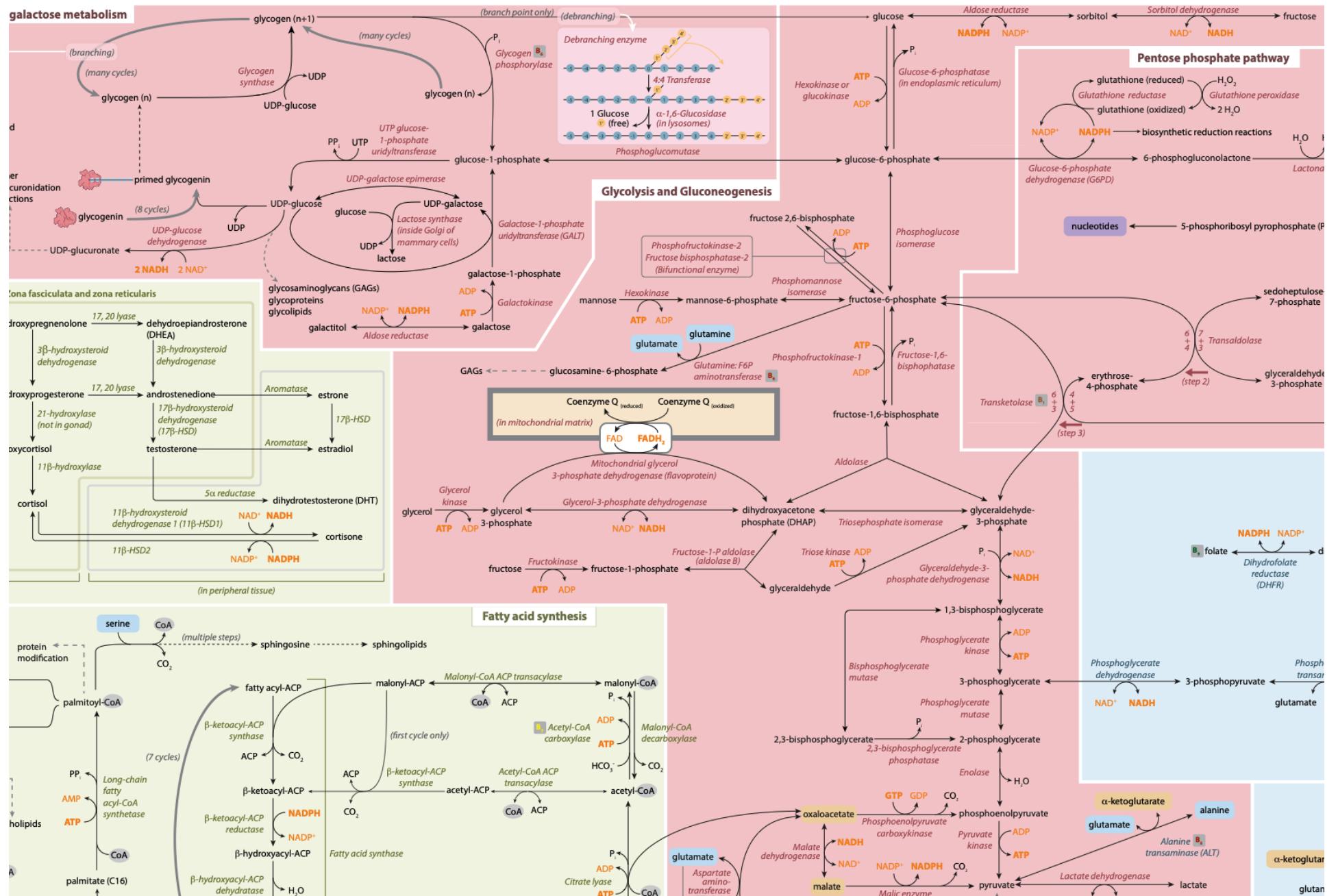
**Yogurt**  
(Galactose)



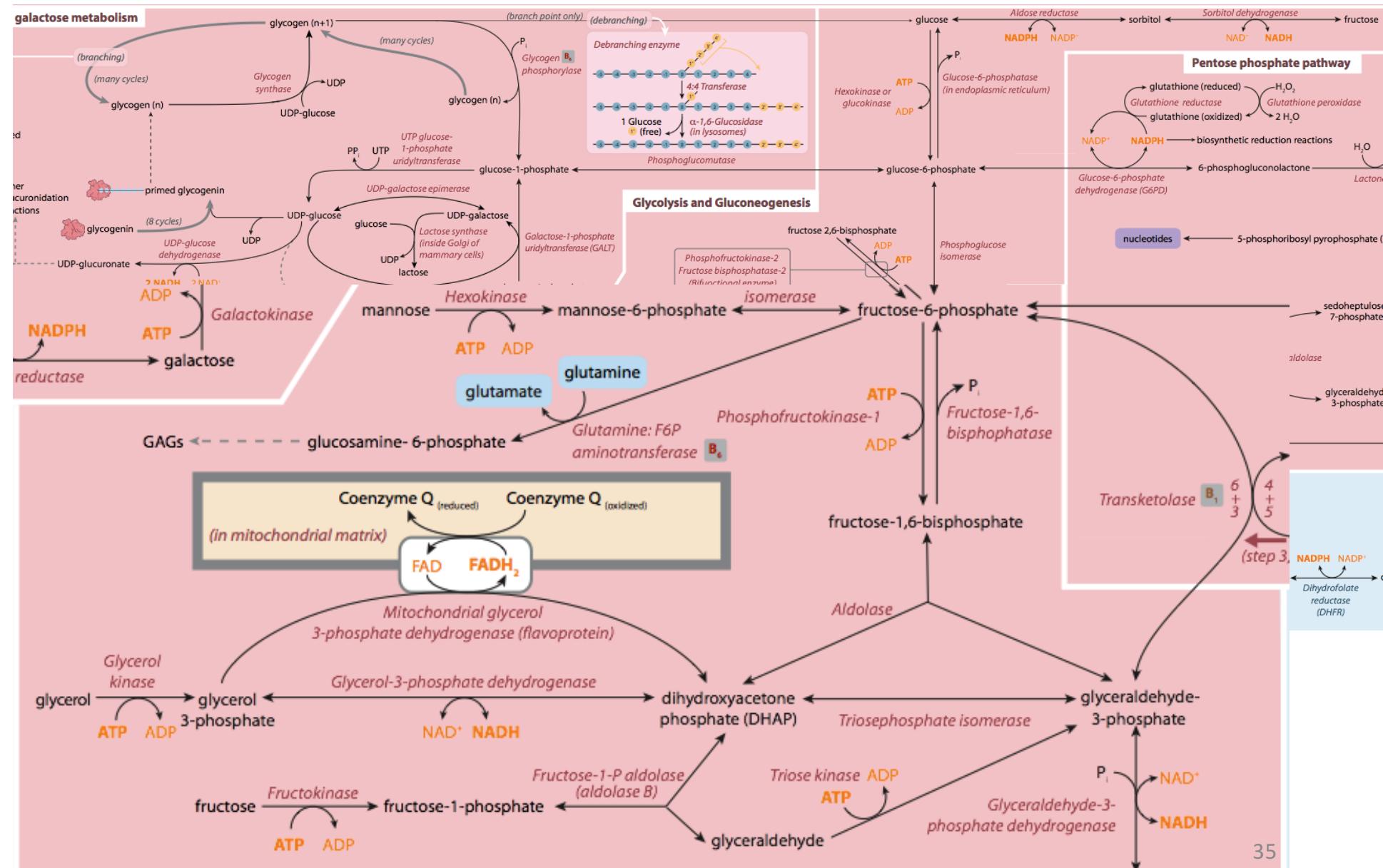
**Cherries**  
(Fructose)



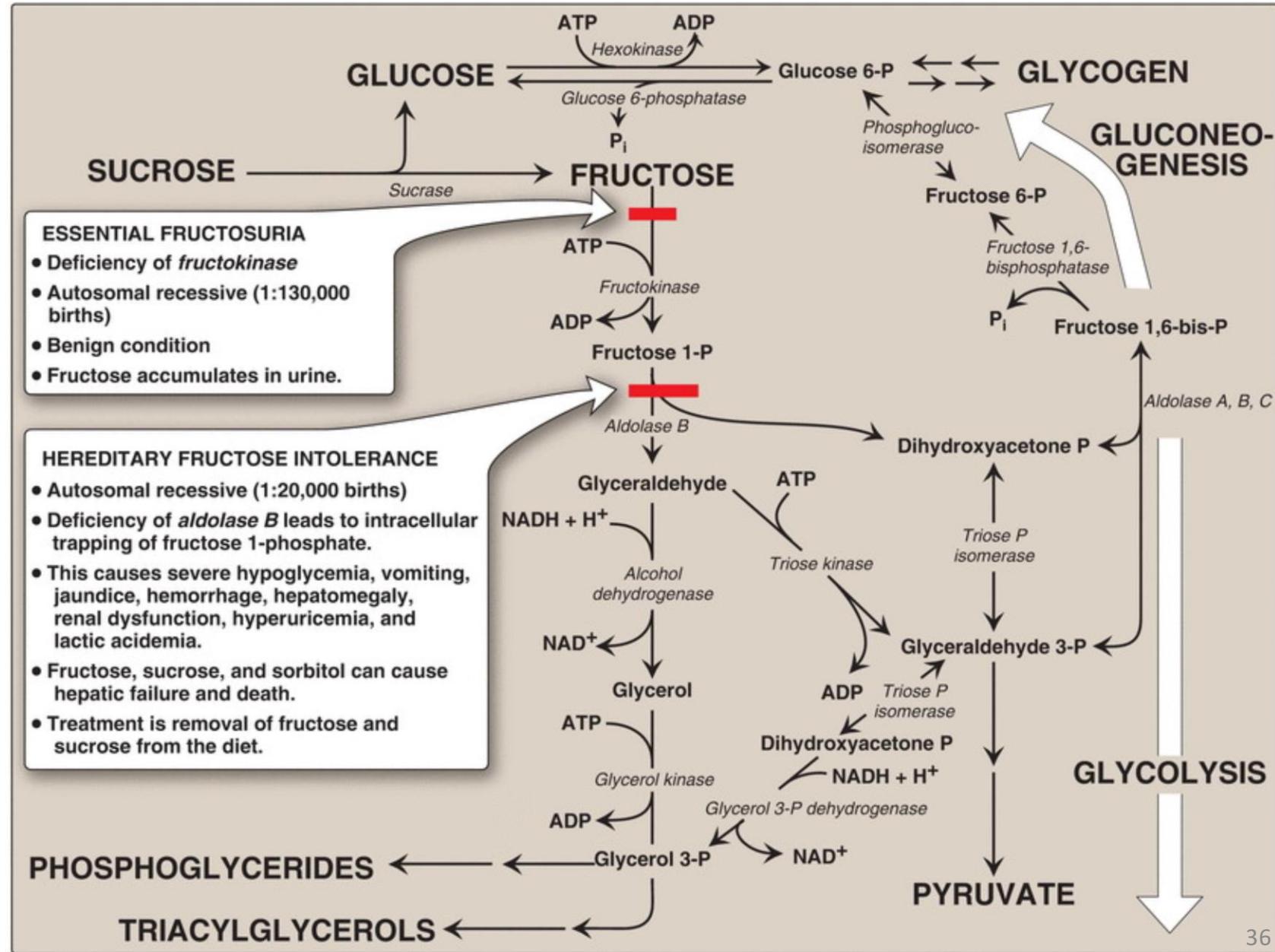
# Sugar Metabolism



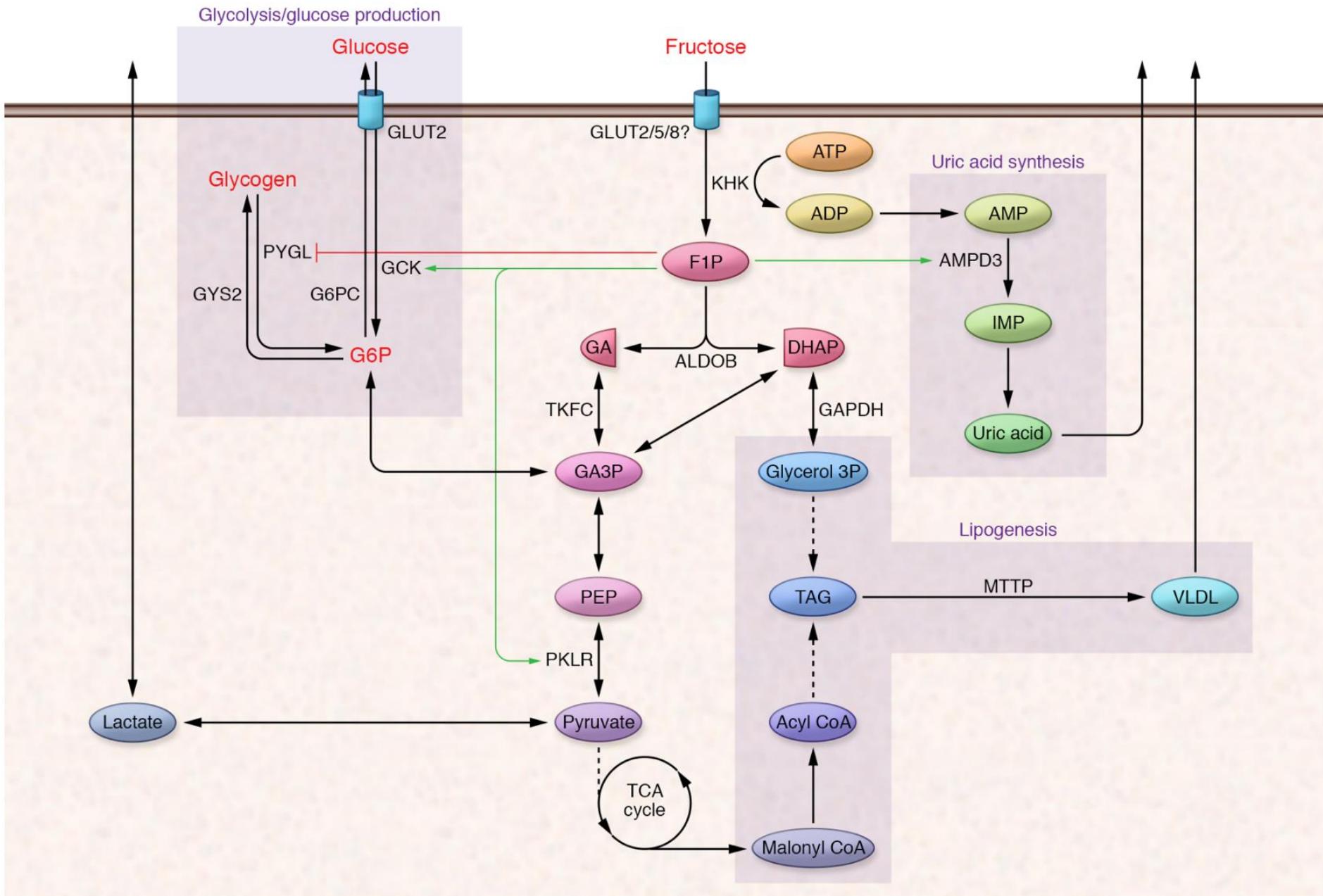
# Fructose Metabolism



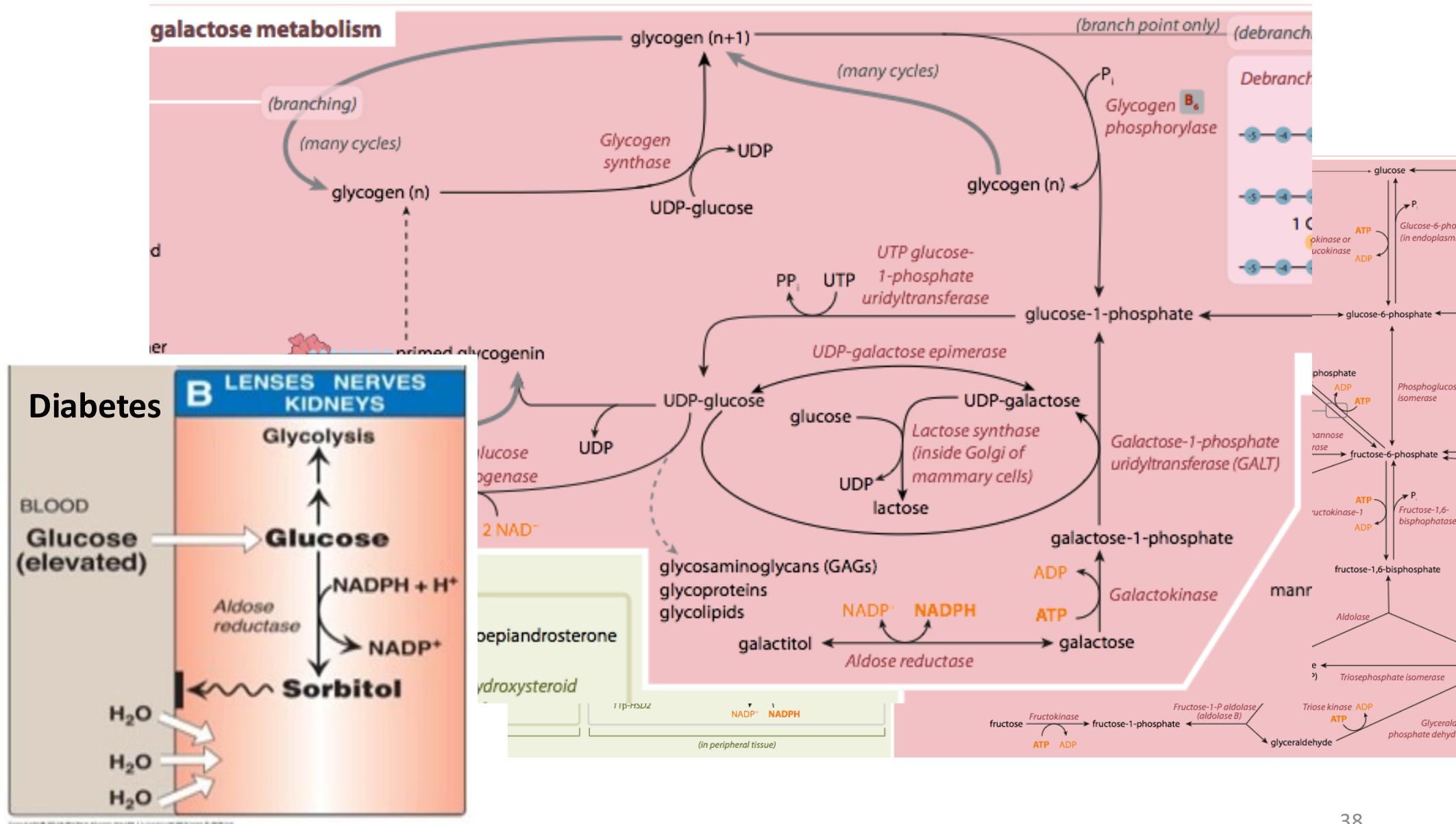
# Fructose metabolism



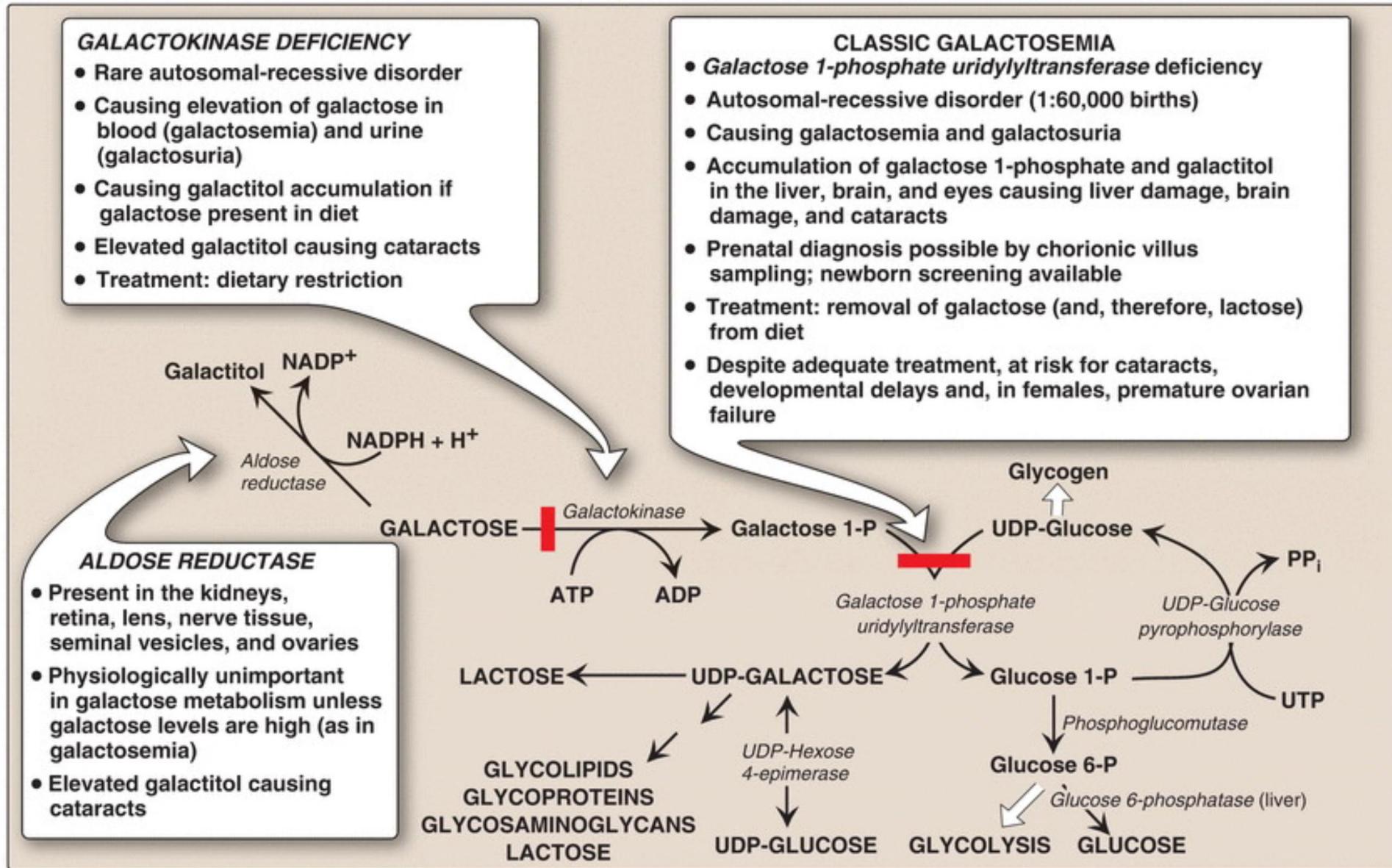
# Fructose Metabolism



# Galactose metabolism



# Galactose metabolism



A 4-day-old Hispanic female presents with respiratory distress, vomiting and refusal to feed. She is lethargic and hypotonic and appears dehydrated. The family history is unremarkable. The patient's urine has the smell of burnt sugar.

### Principle Laboratory Findings:

Test	Result	Reference Interval
pH	7.10	7.35 - 7.45
pCO <sub>2</sub>	21 (2.79)	34 – 50 mm/Hg (4.52 – 6.65 kPa)
HCO <sub>3</sub>	6	16 – 24 mmol/L
Sodium	151	139 – 146 mmol/L
Chloride	116	96 – 106 mmol/L
AGAP	28	5 – 14

## Diagnostic laboratory findings:

- Metabolic workup:

Test	Result	Reference Interval
------	--------	--------------------

### *Amino acid*

#### *analysis:*

-Leucine	4375 $\mu$ mol/L	47 – 160
-Isoleucine	588 $\mu$ mol/L	26 – 91
-Valine	1155 $\mu$ mol/L	64 – 336
-Alloisoleucine (abnormal metabolite)		

### *Organic acid*

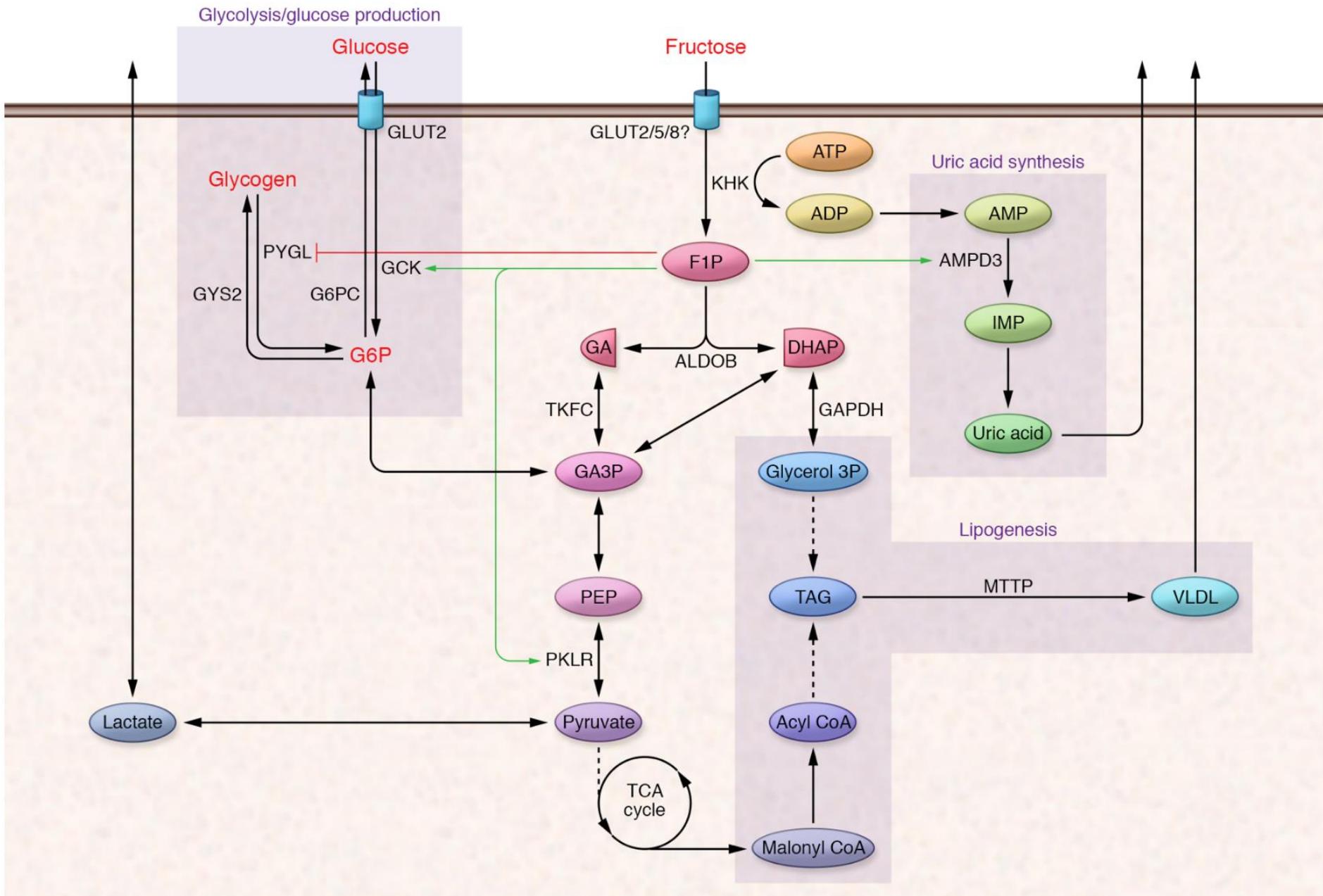
#### *analysis:*

- Presence of: 2-hydroxy-isovaleric acid, 2-hydroxy-isocaproic acid and 2-hydroxy-3-methylvaleric acid
- A. Classic Galactosemia
- B. Dihydrobiopterin Reductase Deficiency
- C. Essential Fructosuria
- D. Galactokinase Deficiency (non-classic galactosemia)
- E. Hereditary Fructose Intolerance
- F. Maple Syrup Urine Disease
- G. Phenylketonuria

An 8-month-old first born girl is evaluated by her pediatrician in the emergency department for an abrupt change in her mental status after several episodes of vomiting and lethargy. The parents report that she has become listless over the past several weeks. Her growth and development have been normal over the first 6 months. Now, however, as her diet is advanced to soft solids and juice in the past 6 weeks, the parents note these changes in behavior. Laboratory testing reveals a serum glucose level of 30 mg/dl (normal >80 mg/dL) and urinalysis is positive for reducing sugar but negative for glucose. What is the likely diagnosis?

- A. Classic Galactosemia
- B. Dihydrobiopterin Reductase Deficiency
- C. Essential Fructosuria
- D. Galactokinase Deficiency (non-classic galactosemia)
- E. Hereditary Fructose Intolerance
- F. Maple Syrup Urine Disease
- G. Phenylketonuria

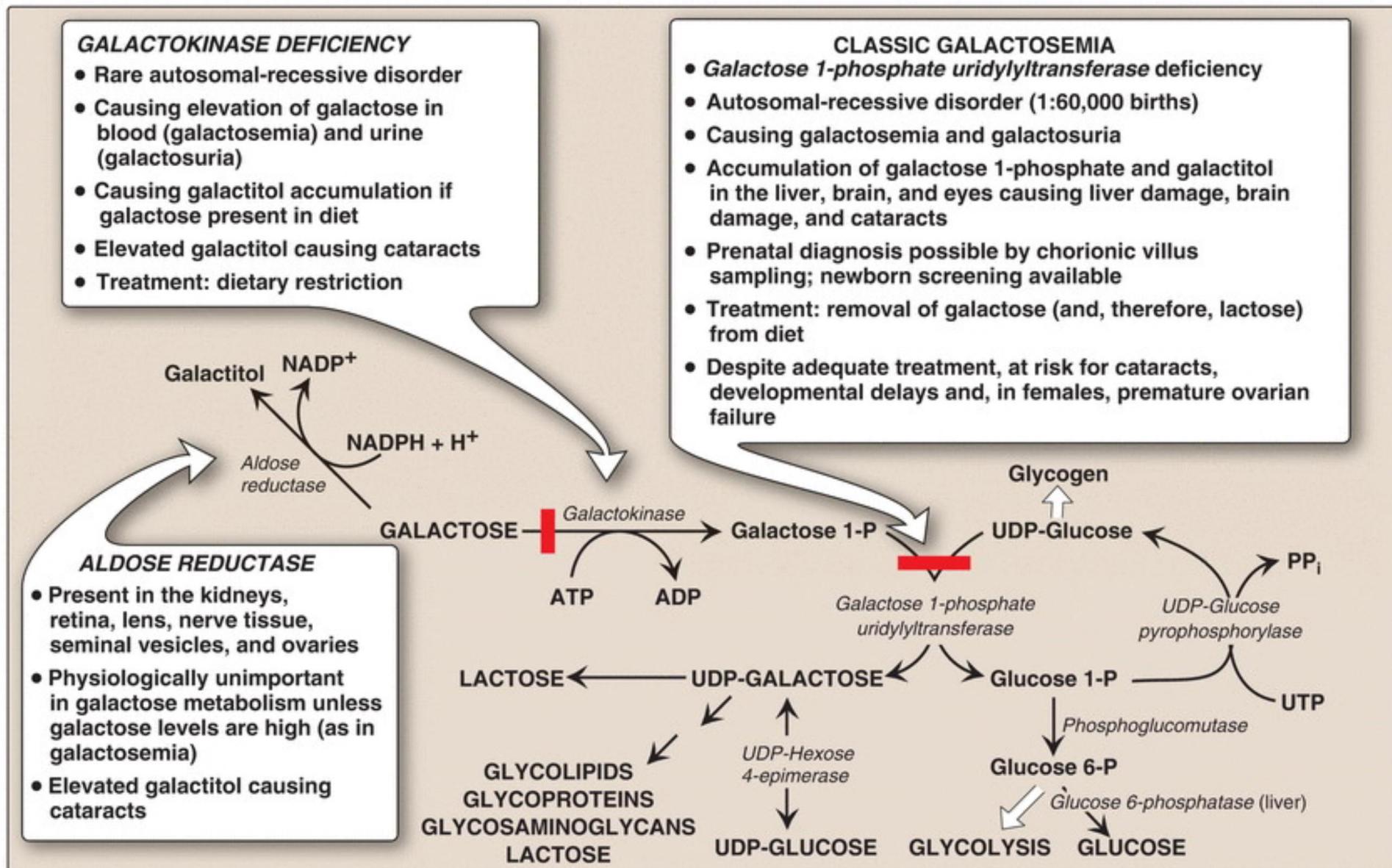
# Fructose Metabolism



A 3-month-old girl is developing cataracts. Other than not having a social smile or being able to track objects visually, all other aspects of the girl's examination are normal. Tests on the baby's urine are positive for reducing sugar but negative for glucose. What is the likely diagnosis?

- A. Classic Galactosemia
- B. Dihydrobiopterin Reductase Deficiency
- C. Essential Fructosuria
- D. Galactokinase Deficiency (non-classic galactosemia)
- E. Hereditary Fructose Intolerance
- F. Maple Syrup Urine Disease
- G. Phenylketonuria

# Galactose metabolism



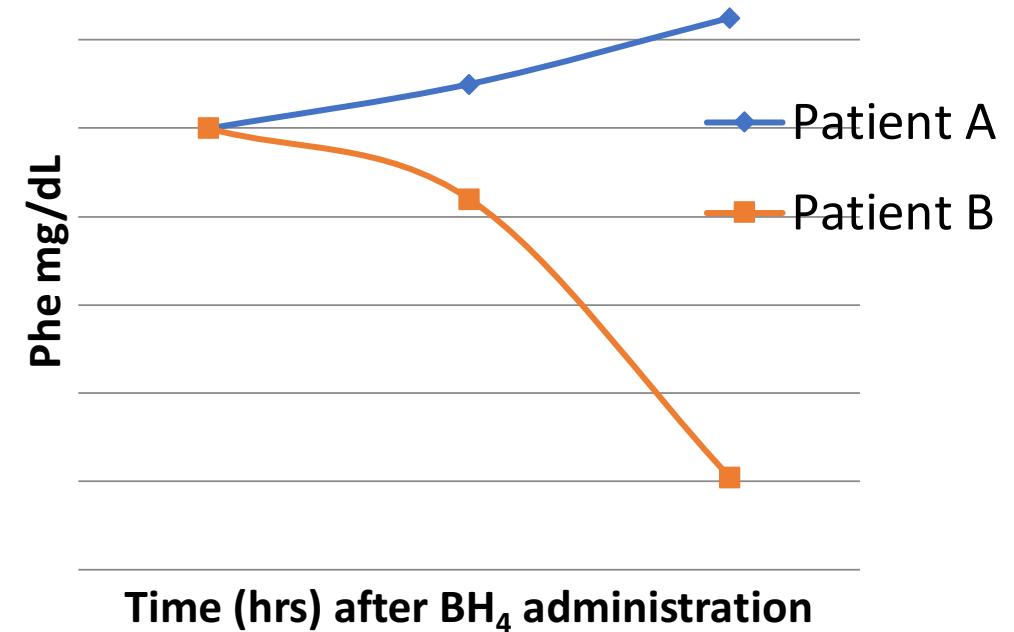
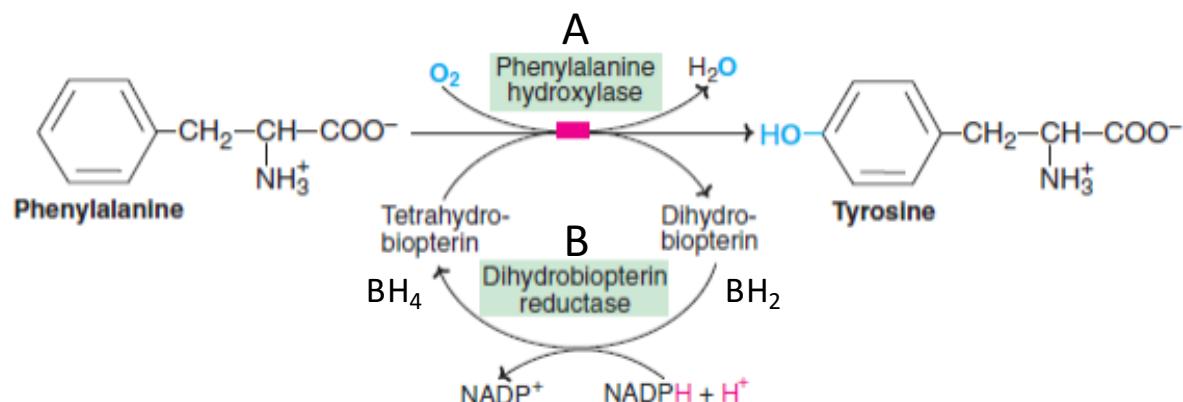
A 1-year-old girl is brought to her pediatrician's office with concerns about her development. She had an uncomplicated birth outside of the United States at term. The mother reports that the baby is not achieving the normal milestones for a baby of her age. She also reports an unusual odor to her urine and some areas of hypopigmentation on her skin and hair. The urine collected is found to have a "mousy" odor.

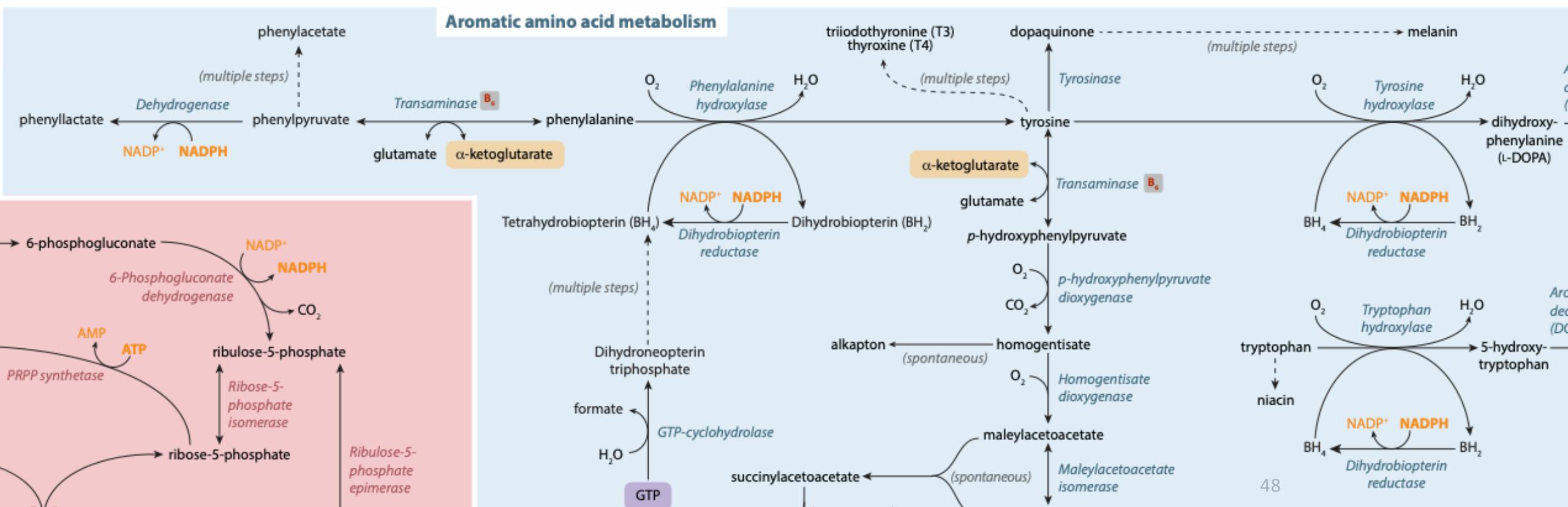
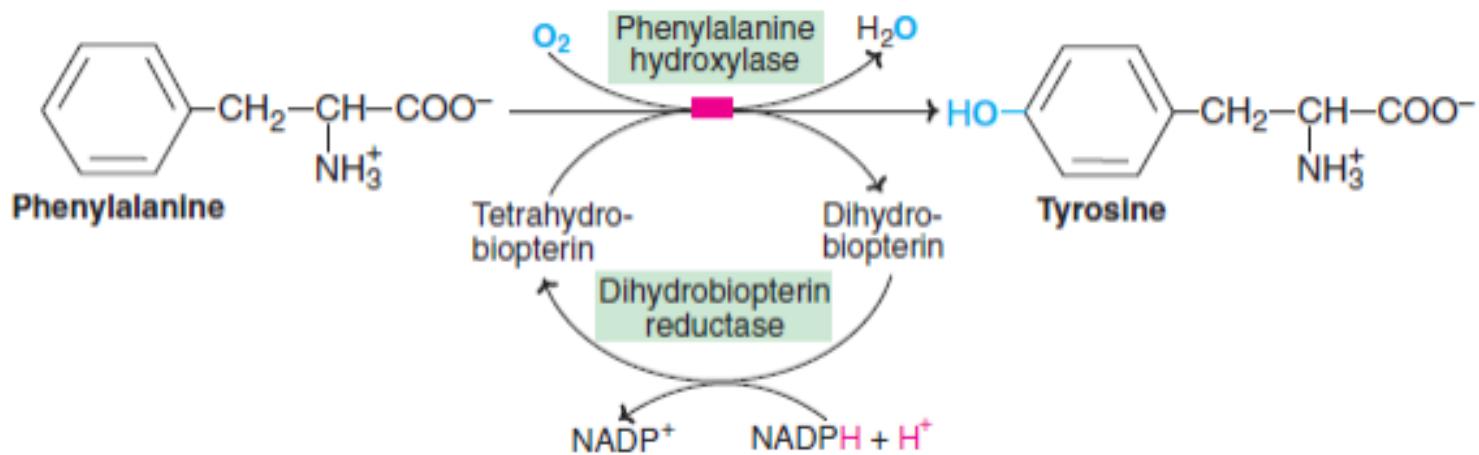
What is the most likely diagnosis?

- A. Classic Galactosemia
- B. Dihydrobiopterin Reductase Deficiency
- C. Essential Fructosuria
- D. Galactokinase Deficiency (non-classic galactosemia)
- E. Hereditary Fructose Intolerance
- F. Maple Syrup Urine Disease
- G. Phenylketonuria

Two infants, patient A and B, are being evaluated for suspected PKU after newborn screening results demonstrated phenylalanine (Phe) level of 6.6 and 6.8 mg/dL (normal=1-2mg/dL), respectively. BH<sub>4</sub> loading tests are performed on both patients and the results are given below. For the loading test, BH<sub>4</sub> tablets are given 30 minutes before a meal. Blood is drawn before, and 4 and 8 hours after BH<sub>4</sub> loading and Phe levels are determined. Which patient likely has a deficiency in phenylalanine hydroxylase?

- A. Patient A
- B. Patient B





A previously healthy 2-month-old female presents with jittery spells several hours after breastfeeding. Her mother reports she has not given the baby anything to eat other than breastmilk since birth. The baby has only recently been sleeping for more than 2 hours between feedings and that's when the mother reports these symptoms started. Laboratory results are consistent with hypoglycemia (low blood glucose) but are otherwise unremarkable. Epinephrine is administered and there is no increase in blood glucose but there is an increase in blood lactate (Hint: think of what pathways would be activated by this hormone).

Physical exam reveals a liver edge 4 cm below the right costal margin. Percussion of the right chest and abdomen confirms hepatomegaly (enlarged liver).

Blood glucose levels increase after breastfeeding but she cannot maintain blood glucose levels within a normal range during fasting.

Based on the information above, which of the following is the most likely diagnosis of this patient?

*Hereditary fructose intolerance*

*Galactosemia*

*Glycogen storage disease Type 1a*