CASE REPORT

HYPERGLYCINEMIA

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CASE

A 3-years-old girl

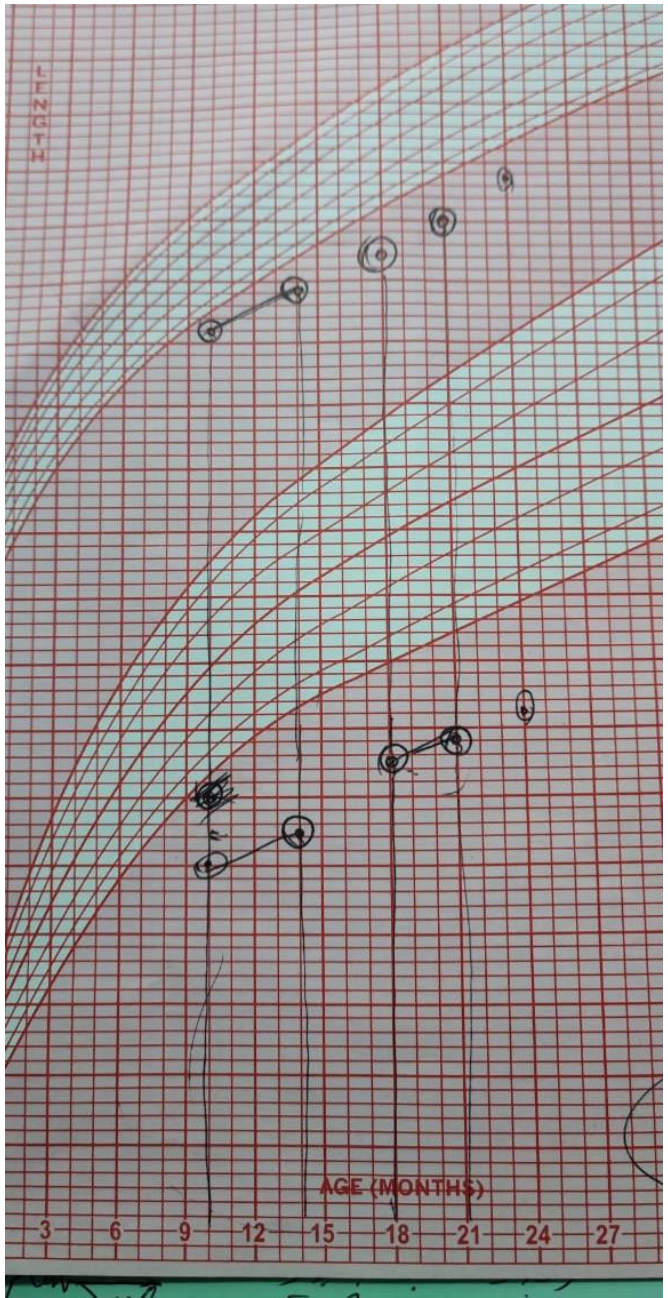
CC:

- Short stature
- Developmental delay
- Generalized edema

- Her signs & symptoms began at 5 months of age with:
  - Inability to hold her head up
  - Mild hypotonia
  - Regression of her developmental milestones.

PAST MEDICAL HISTORY

- Born by C/S due to maternal GDM
- GA:37 wk
- Birth weight: 3.550 kg.
- No history of asphyxia or any difficulty in the newborn period
- Her attention and verbal development were quite normal until the first year of age
- She was not able to stand up or walk until 2 years



Height and weight were – 3 SD
Poor catch-up growth

Family history

- First cousin
- Hypothyroidism and GDM in mother
- No CNS abnormality
- No history of abortion or still birth

Past medical history

- Newborn metabolic screening was normal
- Urine organic acid Normal
- Acylcarnitine Profile Normal

At the age of 1year

- Urine amino acid profile → Excessive amount of glycine 1712 (normal 105-403)
- Plasma amino acid chromatography → Normal.
- VBG → Normal

After one year

- Plasma Glycine Level → 581 (normal135-350) (× 1.6)
- Urine Glycine →1932 (normal 105-403) (× 4.6)
- Other amino acids were within the normal limit

High glycine → VBG

- Normal → Organic acidemia
- Acidosis:
  Non ketotic hyperglycinemia

Treatment for NKH

- Low glycine diet
- Folinic acid
- Sodium benzoate
- Referred for measurement of glycine in CSF
- Genetic study recommended

CSF →

Entry	Compound	Amount (umol/L)	<1Month	1-23 Month	2-17 Years	>18 Years
1	Aspartate	1	≤3	<1	<1	≤2
2	Glutamate	3	1-9	≤5	≤11	1-13
3	Serine	51	30-88	22-61	15-62	15220
4	Glutamine	534	525-1583	386-742	377-1738	361-1175
5	Histidine	12	8-32	4-25	7-25	7-22
6	Glycine	8	3-26	≤12	≤13	≤10
7	Threonine	21	23-104	10-55	8-85	12-64
8	Citrulline	3	1-4	≤3	1-2	≤2
9	Argenine	24	2-27	7-32	9-31	10-32
10	Alanine	29	13-50	8-48	5-62	1-107
11	Tyrosine	15	9-41	5-20	5-32	5-18
12	Methionine	2	2-14	1-7	≤9	1-8
13	Valine	18	11-31	8-19	2-37	7-42
14	Tryptophane	3	≤6	≤8	1-5	≤9
15	Phenylalanine	13	4-31	4-14	≤25	6-31
16	Isoleucine	4	3-11	3-7	2-13	3-10
17	Ornithine	3	≤26	≤5	≤5	≤14
18	Leucine	11	7-22	7-12	8-27	9-32
19	Lysine	20	6-38	3-29	9-58	19-60

One month later

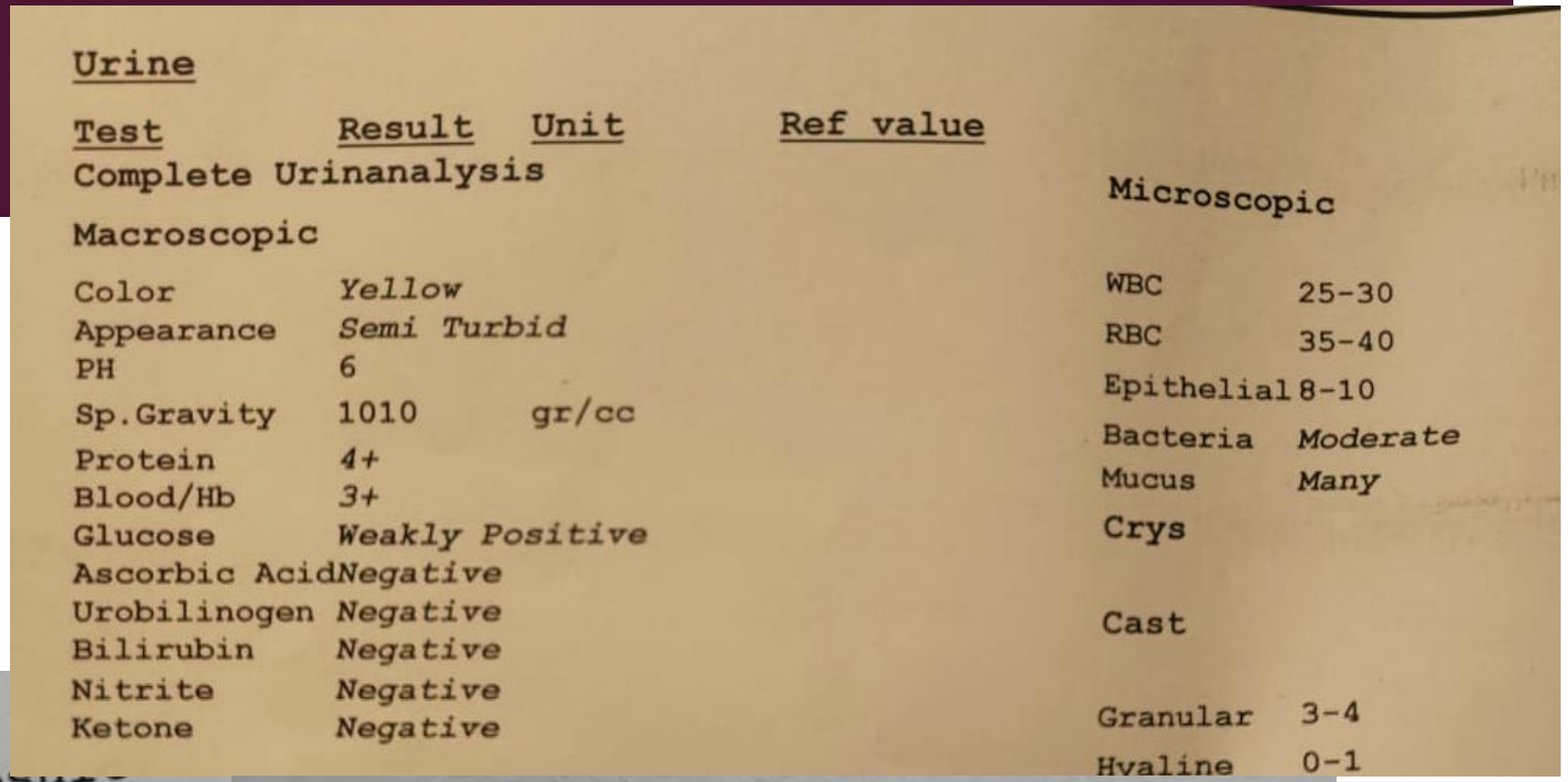
- Irritability
- Loss of concentration
- Bizarre movement of the hand
- One episode of seizure

Admitted to hospital and sodium benzoate discontinued

Phenobarbital for seizure

New syptomps

- Inability to stand
- Generalized edema
- Autism

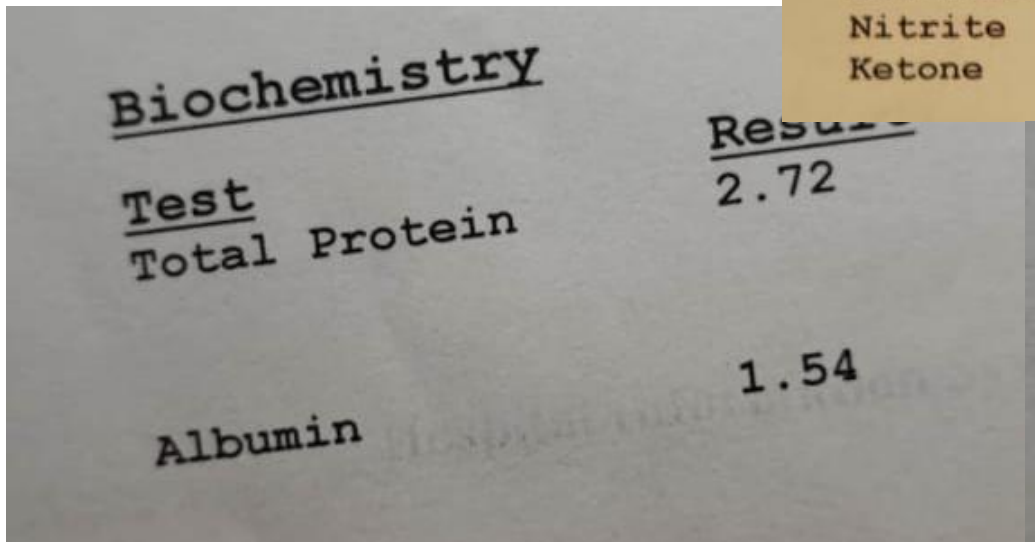


Urine

Test	Result	Unit	Ref value
Complete Urinanalysis			
Macroscopic			
Color	*Yellow*		
Appearance	*Semi Turbid*		
PH	6		
Sp.Gravity	1010	gr/cc	
Protein	*4+*		
Blood/Hb	*3+*		
Glucose	*Weakly Positive*		
Ascorbic Acid	*Negative*		
Urobilinogen	*Negative*		
Bilirubin	*Negative*		
Nitrite	*Negative*		
Ketone	*Negative*		

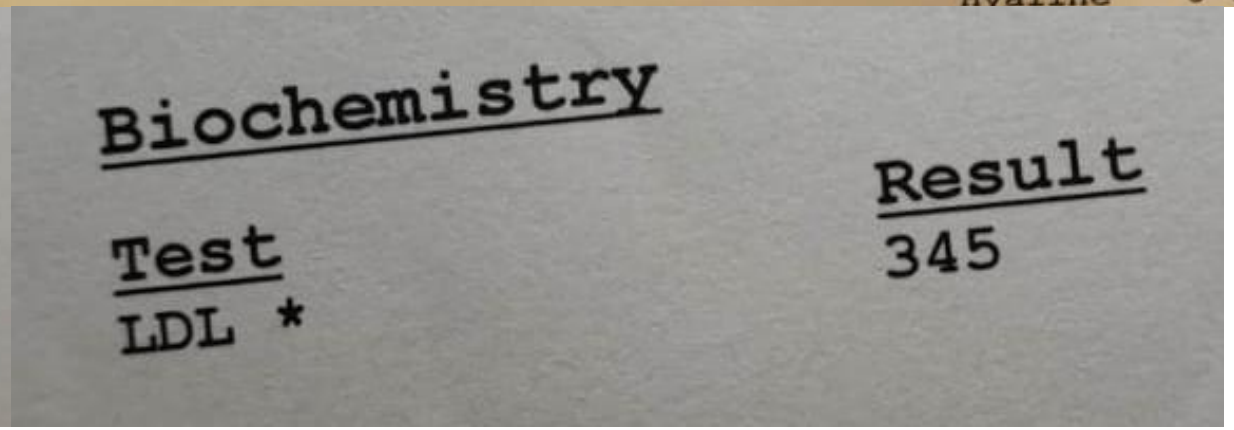
Microscopic

WBC	25-30
RBC	35-40
Epithelial	8-10
Bacteria	*Moderate*
Mucus	*Many*
Crys	
Cast	
Granular	3-4
Hyaline	0-1



Biochemistry

Test	Result
Total Protein	2.72
Albumin	1.54



Biochemistry

Test	Result
LDL *	345

Lipid profile

- Total cholesterol: 957 mg/dl
- Triglyceride: 2596 mg/dl
- HDL: 34 mg/dl
- LDL: 306 mg/dl

Nephrotic syndrome → prednisolone

Cyclosporine for 6 month

Genetic study

Results: Final ■ **Preliminary □** **Revised □**

Patient name& ID	Gene & transcript	Variant	Zygosity	ACMG Classification	Inheritance
Narges Nourmohamadi *****51067	***GLYAT*** NM_201648	**c.322C>T** p.Q108X	**Homozygous**	**VUS**	?
Masoud Nourmohamadi *****42702			**Heterozygous**		
Zahra Nourmohamadi *****26220			**Heterozygous**		

Interpretation: According to the results **Masoud Nourmohamadi** and **Zahra Nourmohamadi** are **Heterozygous** and **Narges Nourmohamadi** is **Homozygous** for variant **c.322C>T** in ***GLYAT*** gene. Mutation in this gene is associated with **Glycine N-acetyltransferase deficiency.** This variant has not been previously reported. The frequency of this variant in normal population is very low. With a CADD score of 40

Comment: For this family genetic counseling is recommended.

Glycine N-acyl transferase (GLYAT)

- GLYAT was first identified in bovine liver in 1953
- It was subsequently isolated and characterized from human liver in 1976
- Mawal and Qureshi (1994) characterized human GLYAT and its substrate specificity
- The monomeric enzyme had an apparent molecular mass of 30 kD.

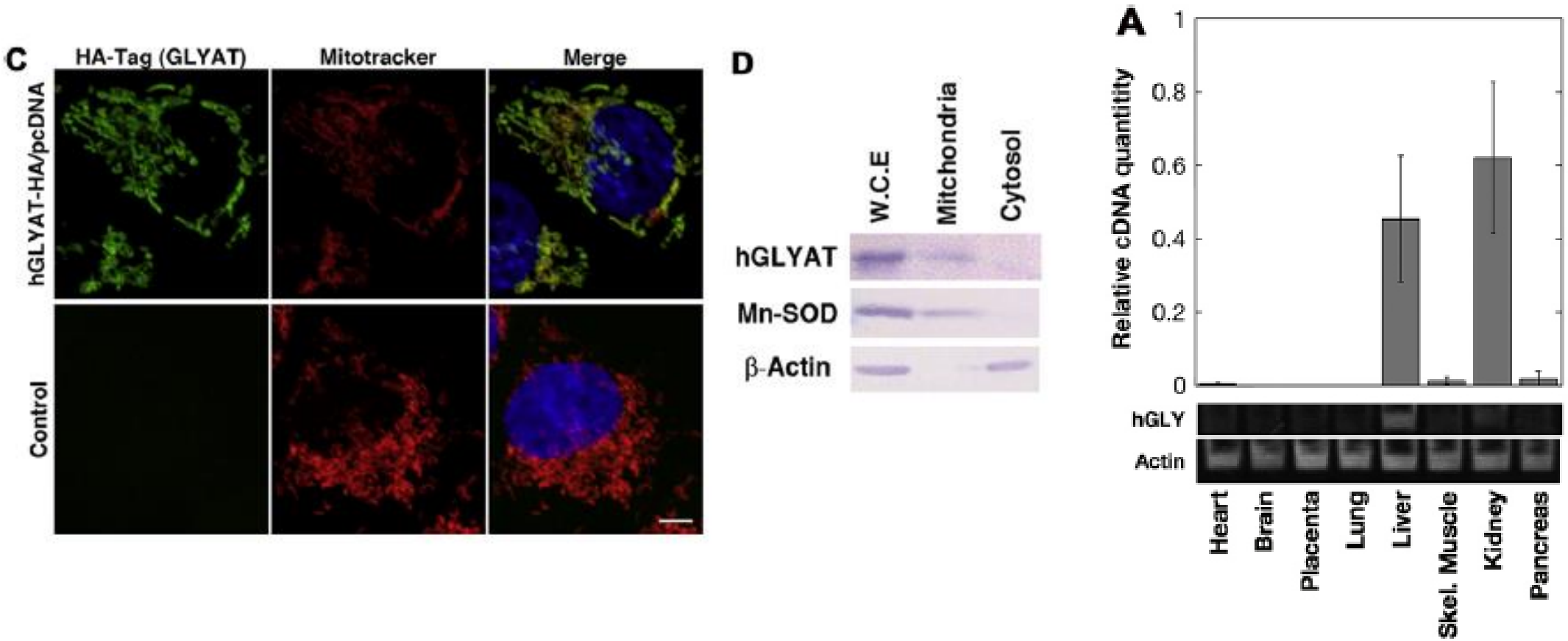
Substrate	Human ACGNAT	
	Km*	Vmax**
Benzoyl CoA	57.9	17.1
Salicyl CoA	83.7	10.1
Isovaleryl CoA	124	7.64
Octanoyl CoA	198	3.3

Mawal and Qureshi, *Biochem. Biophys. Res. Commun.* 1994; 205: 1373-1379.

GLYAT substrates

- Benzoate
  - ~83 - 90% of ingested benzoate is excreted as glycine conjugates
- Salicylate
  - ~75- 84% of ingested salicylate is excreted as glycine conjugates
- Isovaleric acid
- Octanoyl CoA
- Short chain fatty acids
- Phenyl acetate
- Indoleacetic acid
-

Tissue and cell distribution



Matsuo et a. *Biochem Biophys Res Commun*, 2012:420:4, P 901-906

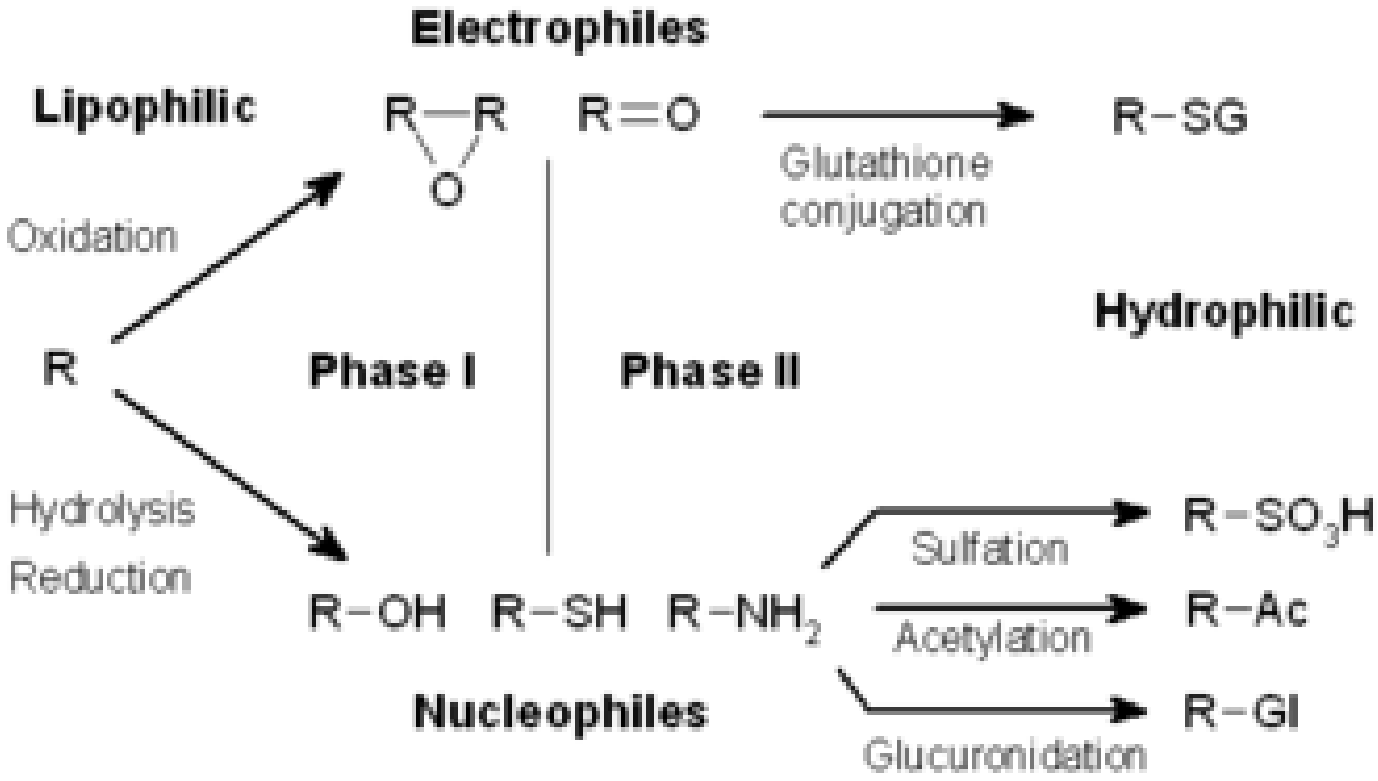
Metabolic processes of GLYAT

- Acyl-Co A metabolism
- Benzoyl-Co A metabolism
- Glycine metabolism
- Monocarboxylic acid metabolism
- Response to toxic substance; detoxification
- Xenobiotic metabolic process

UniProtKB - Q6IB77 (GLYAT_HUMAN)

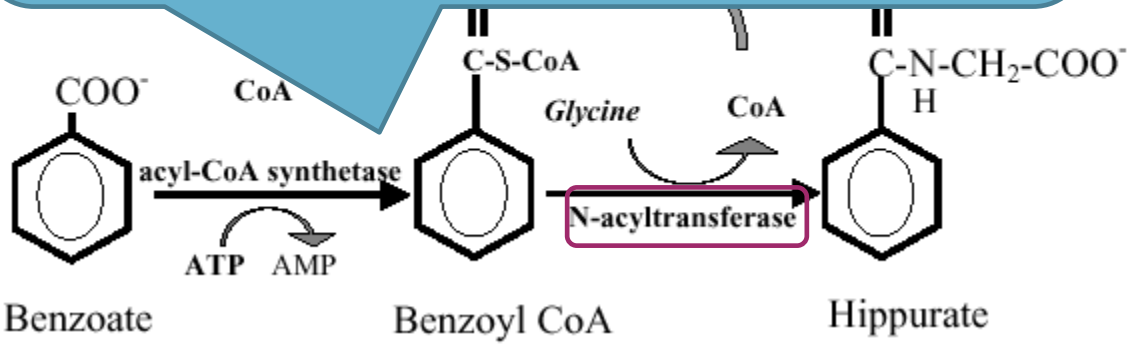
Different types of conjugation reactions

Conjugation	Group in xenobiotic
Glucuronidation	-OH, -COOH, -NH_2
Sulfatation	-OH, -NH_2, -SH
Methylation	-OH, -NH_2
Acetylation	-OH, -NH_2
By GSH	Ar-halogen
By amino acid	-COOH

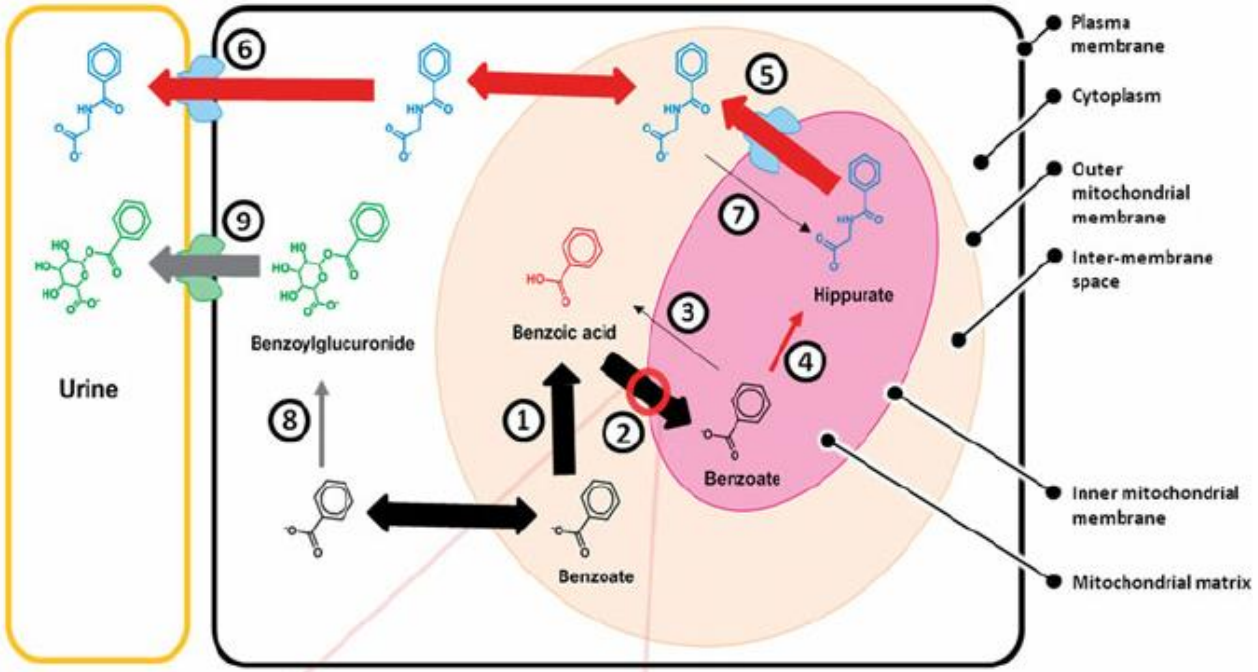


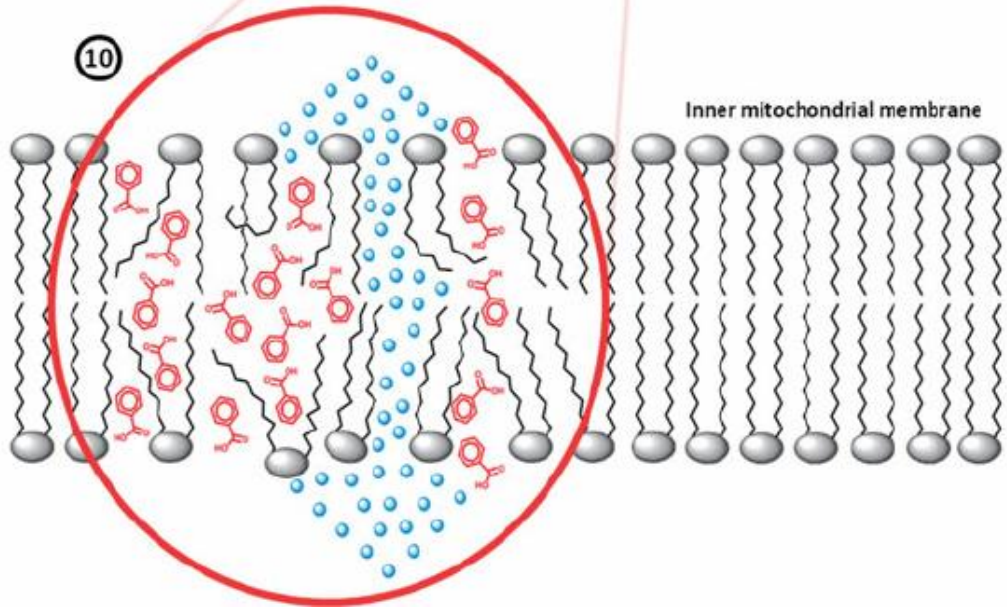
Detoxification of Benzoate

Most xenobiotics that undergo glycine conjugation are activated by the mitochondrial medium-chain ligases, which also activate C4-C12 acids for β-oxidation



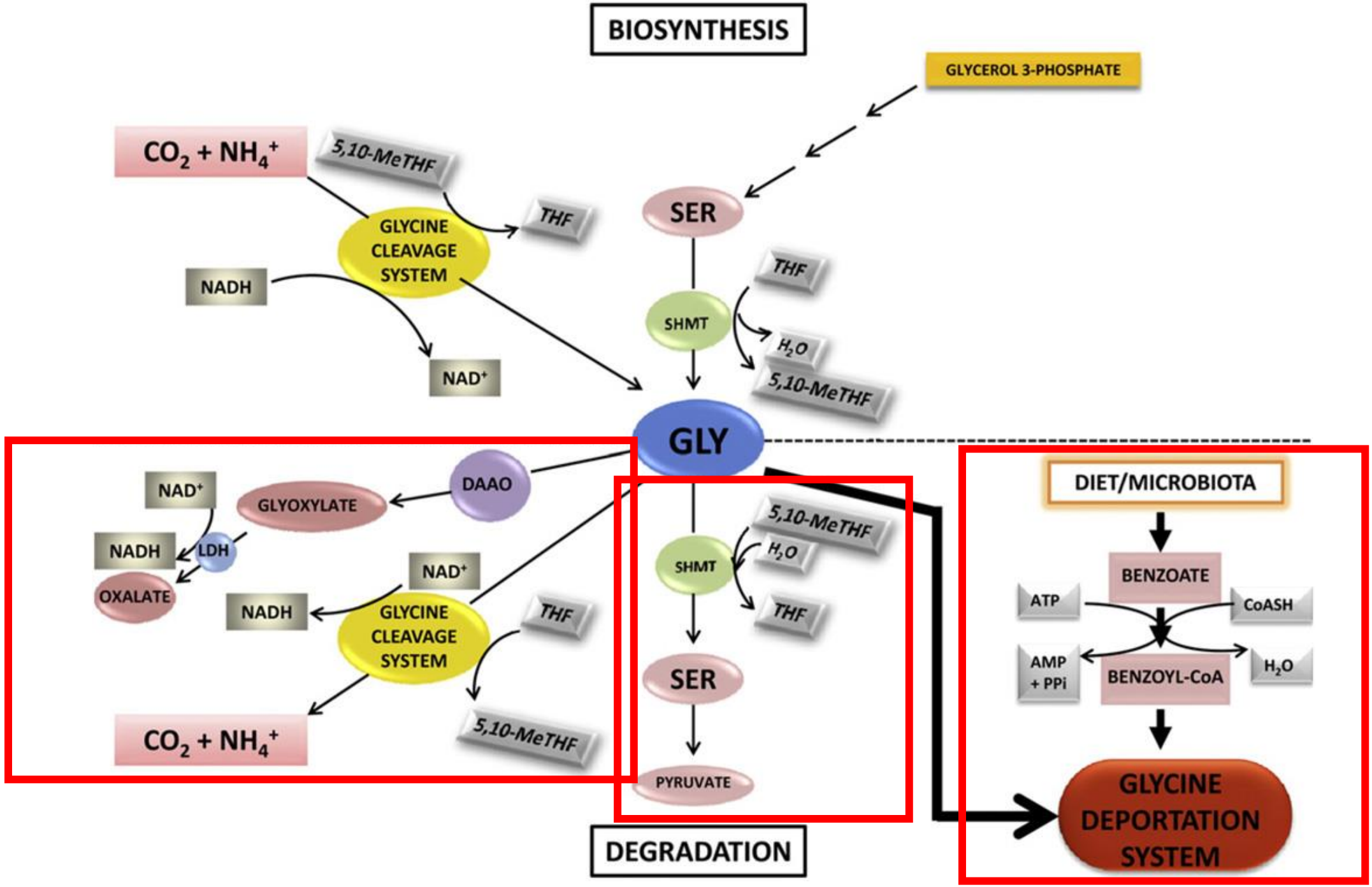
mitochondrial accumulation of xenobiotic acyl-CoA esters may interfere with b-oxidation and disturb mitochondrial metabolism



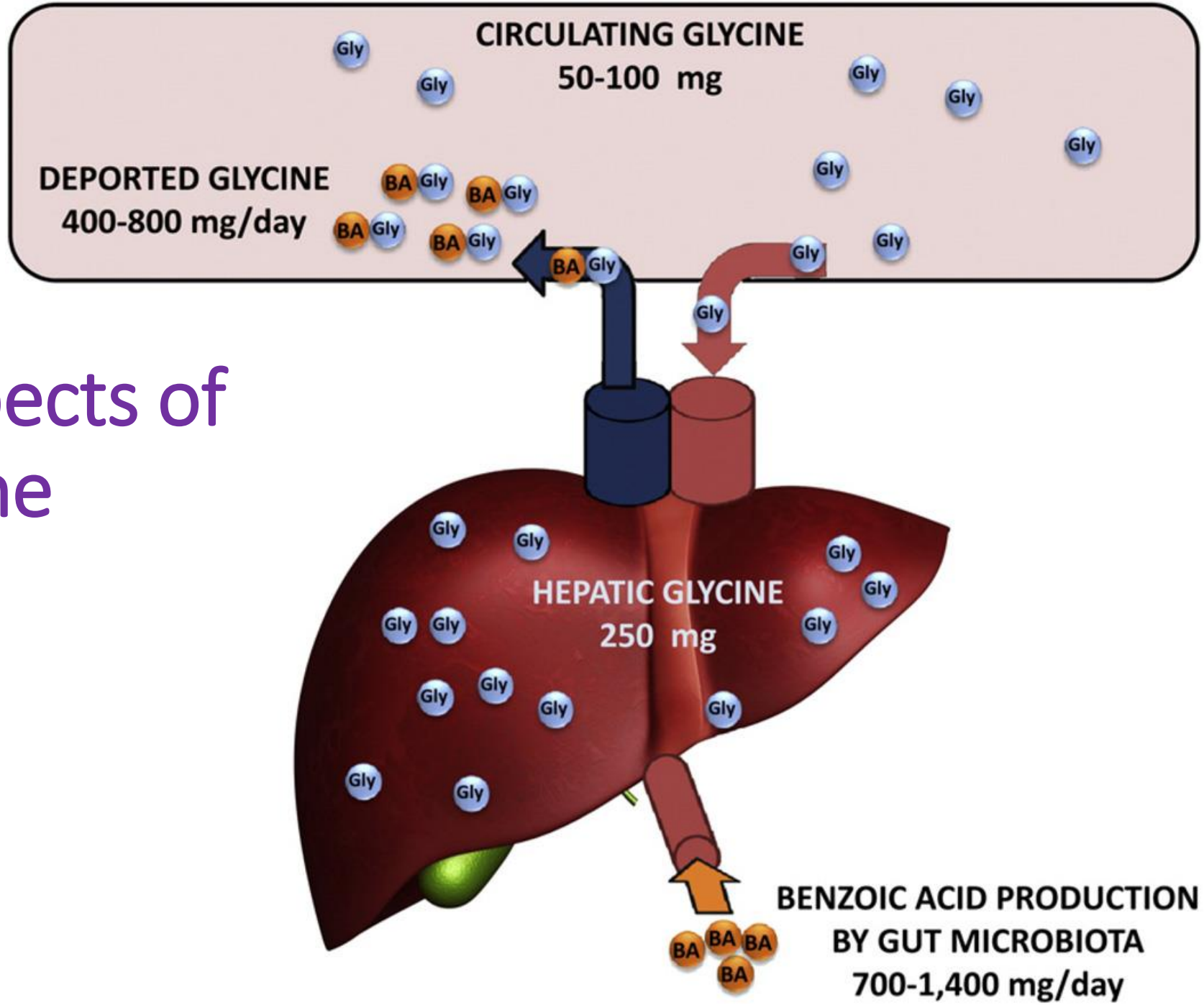


The metabolic role of glycine conjugation

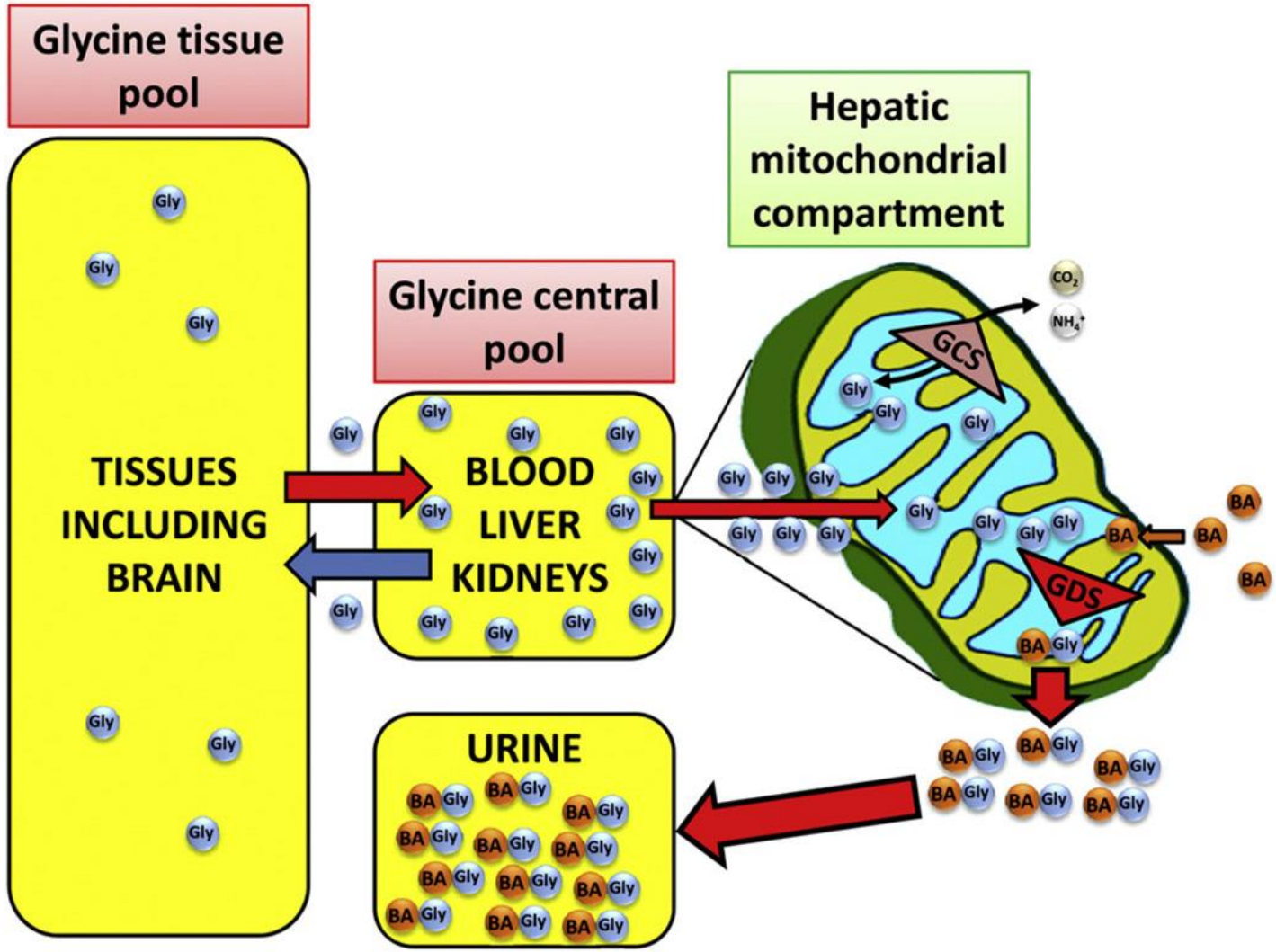
- Facilitating the excretion of xenobiotics
- Restoration of CoASH levels
- Homeostasis of glycine


BIOSYNTHESIS
GLYCEROL 3-PHOSPHATE
$CO_2 + NH_4^+$
5,10-MeTHF
THF
GLYCINE CLEAVAGE SYSTEM
NADH
NAD+
SER
SHMT
THF
H_2O
5,10-MeTHF
GLY
DAAO
NAD+
GLYOXYLATE
NADH
LDH
OXALATE
NADH
NAD+
GLYCINE CLEAVAGE SYSTEM
THF
5,10-MeTHF
$CO_2 + NH_4^+$
5,10-MeTHF
SHMT
H_2O
THF
SER
PYRUVATE
DEGRADATION
DIET/MICROBIOTA
BENZOATE
ATP
CoASH
AMP + PPi
BENZOYL-CoA
H_2O
GLYCINE DEPORTATION SYSTEM

Quantitative aspects of the fate of glycine



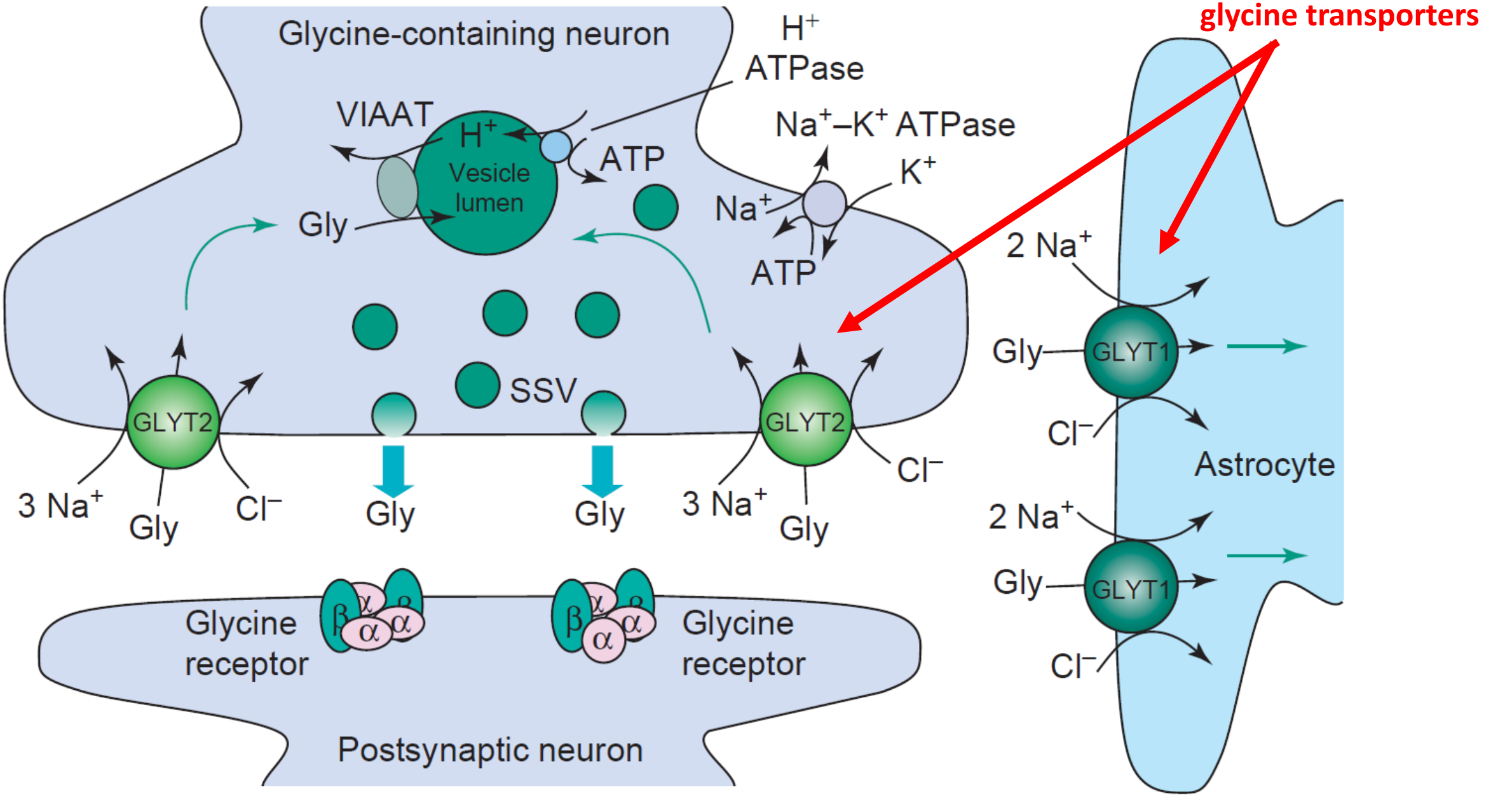
Glycine (GLY) molecules in tissues such as brain and muscle form part of a large volume compartment which is in equilibrium with a smaller central compartment that comprises the blood, liver, and kidneys where GLY is both synthesized and removed, both by metabolism and deportation into urine.



Glycine

- Glycine is the major inhibitory neurotransmitter in posterior areas of the vertebrate CNS.

- Glycine acts as an essential co-agonist of glutamate at NMDA receptors

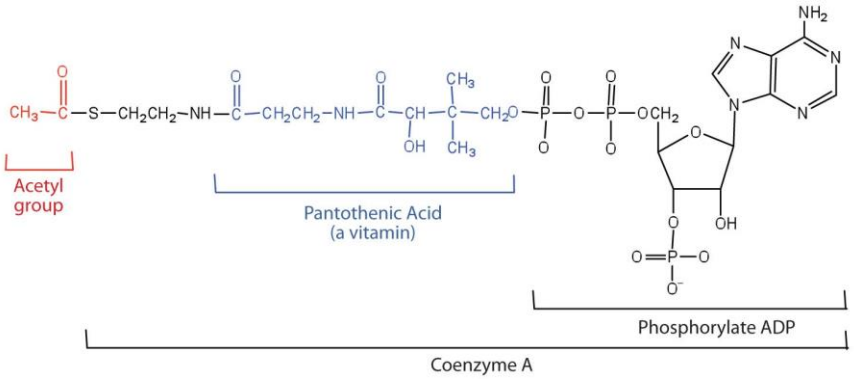

Plasma membrane
glycine transporters
Glycine-containing neuron
H+
ATPase
VIAAT
H+
Vesicle
lumen
ATP
Na+–K+ ATPase
K+
Na+
ATP
Gly
SSV
GLYT2
3 Na+
Gly
Cl−
Gly
Gly
GLYT2
3 Na+
Gly
Cl−
2 Na+
Gly
GLYT1
Cl−
Astrocyte
2 Na+
Gly
GLYT1
Cl−
Glycine
receptor
Glycine
receptor
Postsynaptic neuron

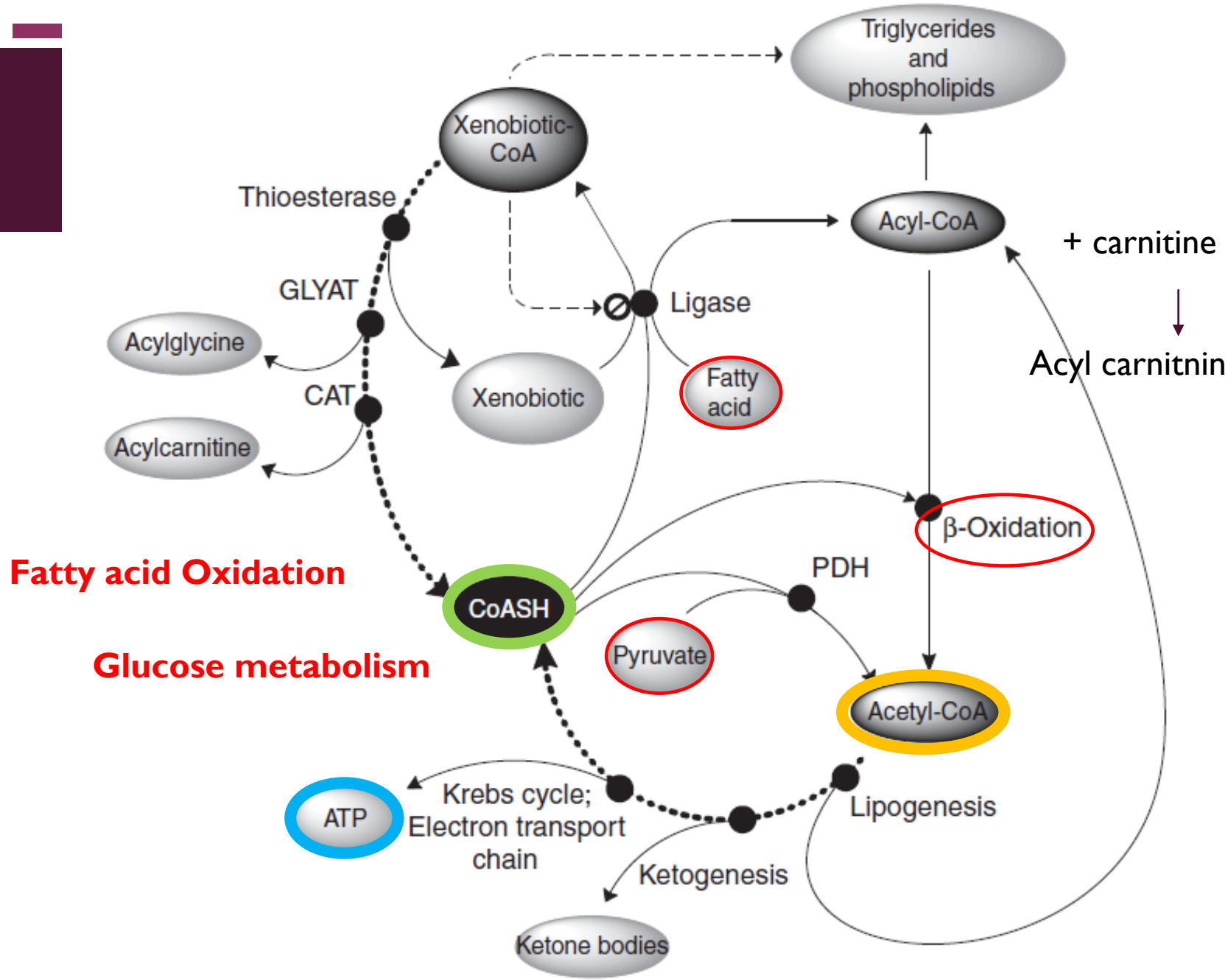
Mechanisms of acyl-CoA toxicity and pathogenesis

1. **Depletion of CoASH**
2. **Toxic effects of accumulating acyl-CoAs**

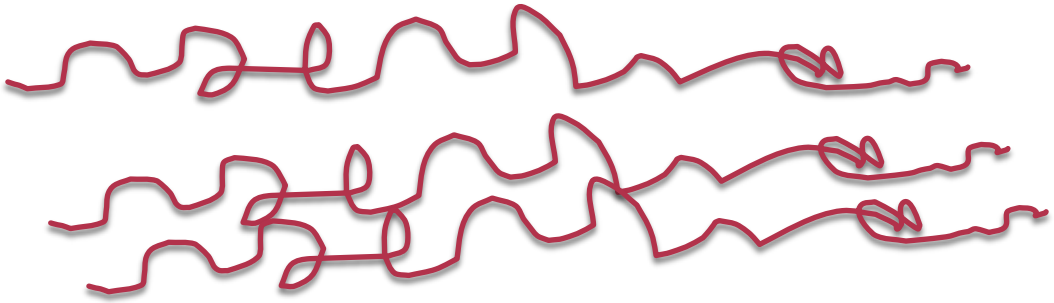
Depletion of CoASH

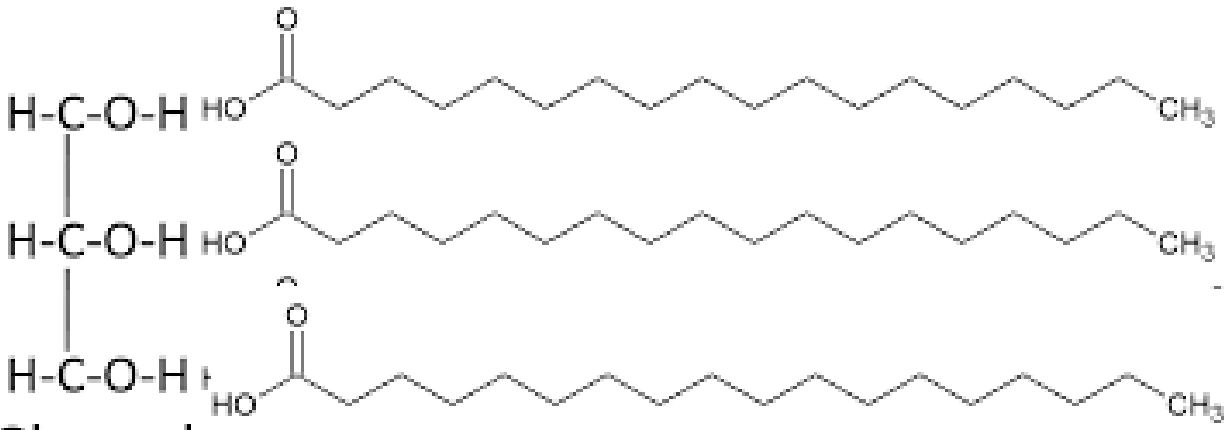
- Role of Coenzyme A in metabolism



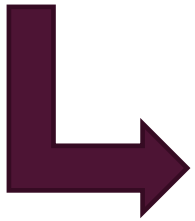


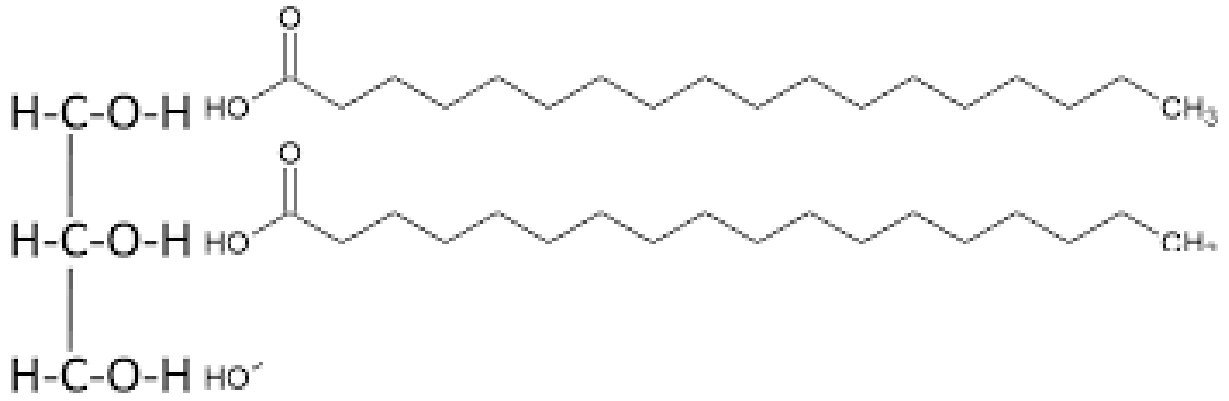
Xenobiotic CoA





Glycerol 3 Fatty Acids





Glycerol 3 Fatty Acids

Accumulating acyl-CoAs

1. **Depletion of carnitine**
   - When an acyl-CoA accumulates to high enough amounts, it may become a substrate for carnitine acyltransferases, resulting in the formation of an acyl-carnitine that can be excreted in the urine
2. **Substitution for acetyl-CoA in lipogenesis**
   - Resulting in odd-chain, branched-chain, aromatic, and other unnatural fatty acids, which cannot be properly catabolized and may be incorporated into cell membranes
   - It has also been shown that 2-arylpropionyl-coa esters, metabolites of NSAIDS, can be incorporated into adipocyte triglycerides
3. **Inhibition of enzymes by acyl-CoAs (competitively or allosterically)**
   - Protein kinase C activity (important in signal transduction) is perturbed by ciprofibroyl-coa, a metabolite of the hypolipidaemic drug ciprofibrate
   - Propionyl-coa, at high concentrations, inhibits formation of n-acetylglutamate by n-acetylglutamate synthetase, resulting in urea cycle dysfunction and hyperammonemia
4. **Function of acyl-CoAs as bioactive lipids**

Conclusions

- GLYAT is the enzyme responsible for glycine conjugation of the Acyl-CoA esters of several xenobiotic organic acids.
- GLYAT activity affects
  - Toxicity of various organic acids.
  - Mitochondrial ATP production
  - Glycine availability and homeostasis
  - CoASH availability

Plasma Amino Acid HPLC Analys

Amino Acid	Res.	Refere.	Amino Acid	Res.	Refere.
Aspartic acid	5	0-24	Alanine	184	210-661
Glutamic acid	138	14-192	Tyrosine	34	22-87
Aspargine	32	30-69	Tryptophane	11	25-191
Serine	119	65-193	Methionine	16	6-40
Histidine	32	32-107	Valine	241	141-317
Glutamine	609	369-711	Phenylalanine	25	48-109
Arginine	64	21-138	Isoleucine	59	37-98
Citruline	42	10-45	Leucine	85	75-175
Glycine	507	120-554	Ornithine	37	28-110
Threonine	85	79-193	Lysine	162	83-238

Notice: Reference value reported in the paper is the related to the adult and interpretation in different ages refer

Outcome of Treatment

Improvement in

- Amino acid profile
- Lipid profile
- Height velocity
- Cognition and behavior and developmental millstones

ACKNOWLEDGMENT

- Maryam Razzaghy Azar
- Mitra Nourbakhsh
- Ali Tale
- Seyed mohammad miryounesi
- Saeid talebi
- Parvane karimzadeh
- Rozzita Hosseini
- Nakisa hooman

Thank you for your attention