Interpretation of Urinary Organic Acid Test in Inherited Metabolic Diseases

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Ph.D. of clinical biochemistry

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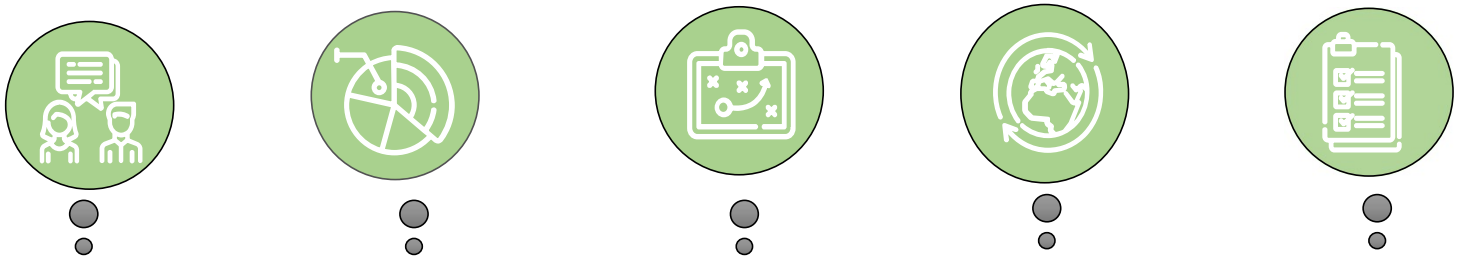
Outlines

- Introduction
- Application
- Confounding factors

Organic acids



Low-molecular weight (mass < 300)

Polar Compounds

Carboxylic groups

Weak acids

Strongly hydrophilic

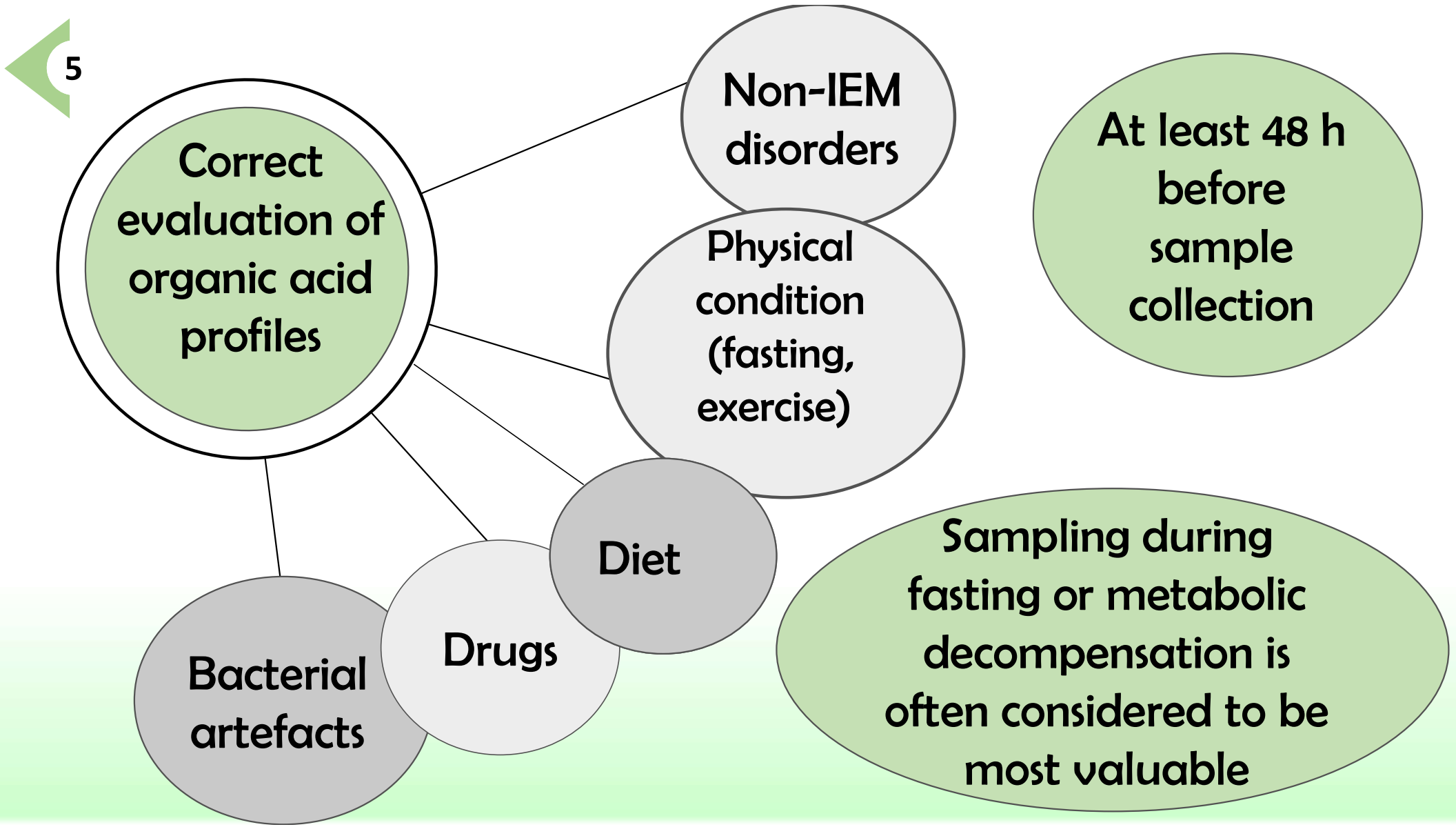
- **Carboxylic Acids:** Methylmalonic acid, succinic acid, Lactic and Pyruvic Acids
- **Dicarboxylic Acids:** Adipic acid and suberic acid
- **Ketones and Hydroxy Acids:** 3-hydroxybutyric acid

Metabolic acidosis

Rapid excretion into the urine

Organic acids

The gold standard method for the analysis of organic acids in a biological matrix is gas-chromatography coupled with a mass spectrometry detector (GC-MS)


Correct evaluation of organic acid profiles
Non-IEM disorders
Physical condition (fasting, exercise)
Diet
Drugs
Bacterial artefacts
At least 48 h before sample collection
Sampling during fasting or metabolic decompensation is often considered to be most valuable

Impact of acylcarnitines in the diagnosis of organic acidurias

Correct evaluation of organic acid profiles

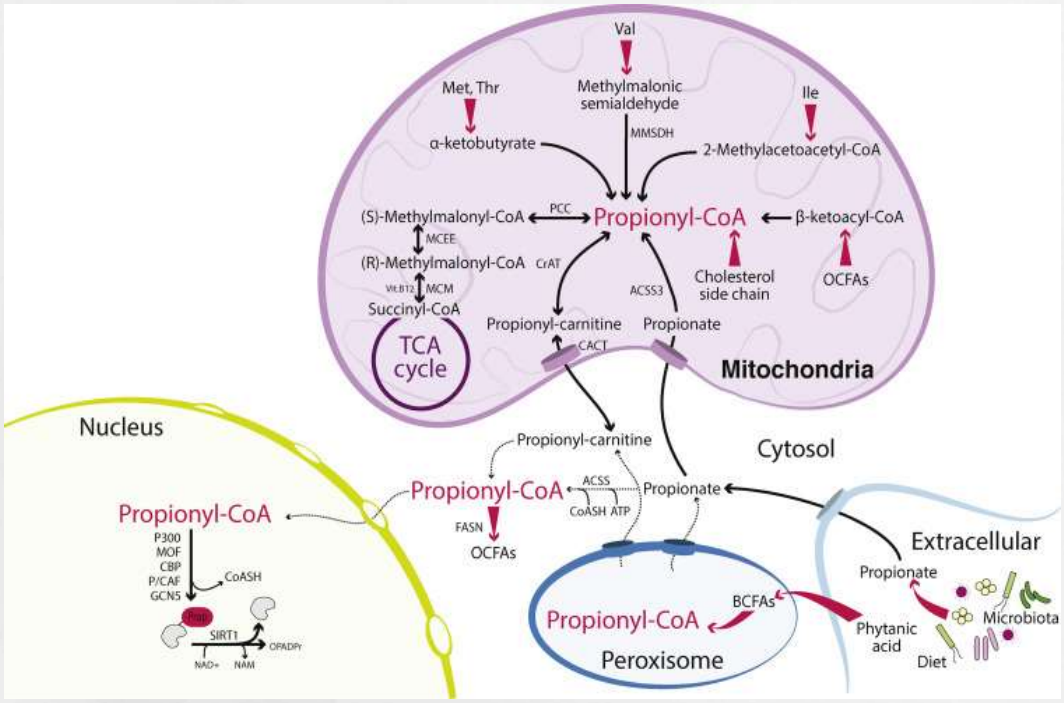
Acylcarnitines

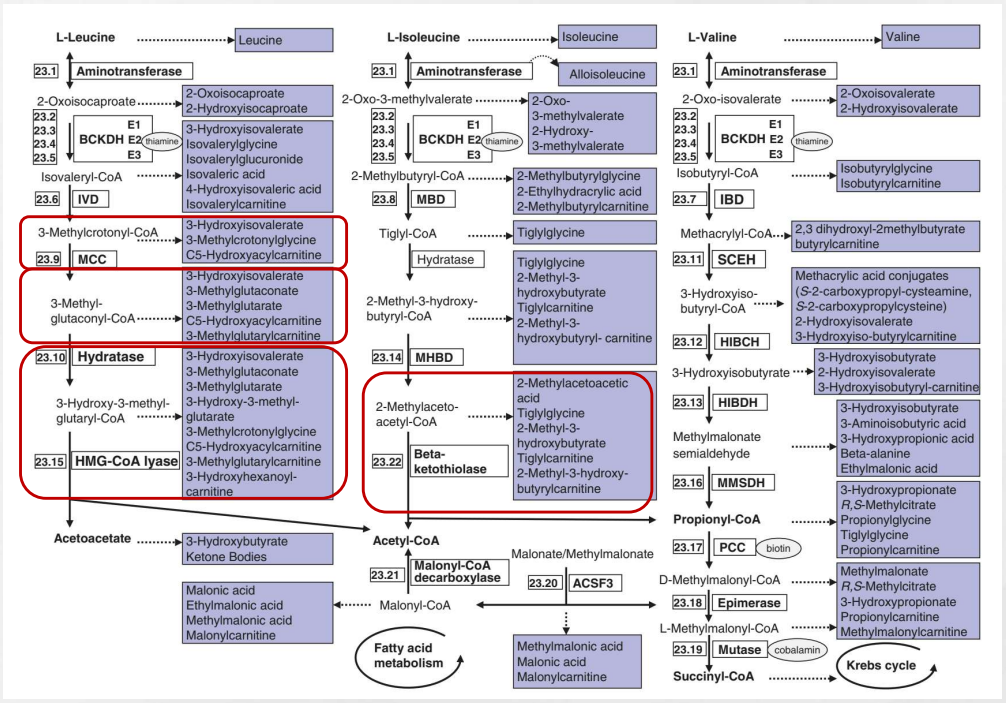
Organic aciduria	Acylcarnitines (P; DBS)
Methylmalonic	C3; C3/C2; C3/C16; C4DC; C16:1OH
Propionic	C3; C3/C2; C3/C16; C16:1OH
Isovaleric	C5; C5/C0; C5/C3; C5/C2
3-Methylcrotonylglycinuria	C5OH; C5OH/C0; C5OH/C8; C5:1
HMG-CoA Lyase deficiency	C5OH; C5OH/C0; C5OH/C8; C6DC
Glutaric type I	C5DC; C5DC/C3DC; C5DC/C5OH; C5DC/C16; C5DC/C8¸ C5DC/C10
Ketothiolase deficiency	C5OH; C5OH/C0; C5OH/C8; C5:1
Biotinidase deficiency/ HCS deficiency	C5OH; C5OH/C0; C5OH/C8; C3; C3/C2; C3/C16
2-Methylbutyrylglycinuria	C5; C5/C0; C5/C3; C5/C2

Abbreviations: *P* plasma, *DBS* dried blood spot, *HMG-CoA* 3-Hydroxy-3-methyl-glutaryl-CoA, *HCS* Holocarboxilase synthetase

Propionyl carnitine (C3)

3-OH-isovaleryl carnitine (C5-OH)





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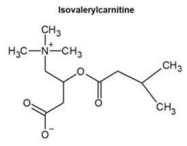
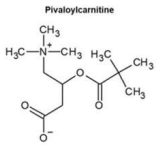
Isovaleryl carnitine (C5)

Butyryl carnitine (C4)

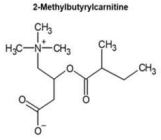
C5 acylcarnitine



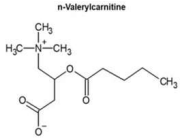
isovalerylcarnitine (isovaleric acidemia)



pivaloylcarnitine (therapy with pivalate-containing medications)

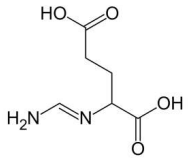


2-methylbutyrylcarnitine (2-methylbutyryl-CoA dehydrogenase deficiency or SBCADD)

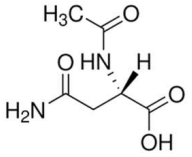


n-valerylcarnitine (metabolic disorders involving fatty acids with odd-numbered carbon chains)

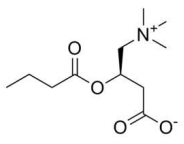
C4 acylcarnitine



Formiminoglutamic acid (glutamate formiminotransferase deficiency)



Isobutyrylcarnitine (isobutyryl-CoA dehydrogenase deficiency)



Butyrylcarnitine (short-chain acyl-CoA dehydrogenase deficiency)

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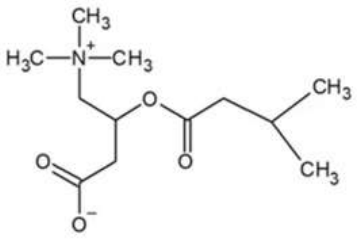
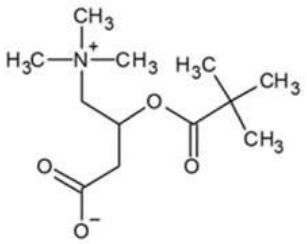
C5 acylcarnitine

Isovalerylcarnitine



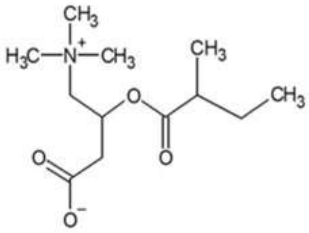
isovalerylcarnitine
(isovaleric acidemia)

Pivaloylcarnitine



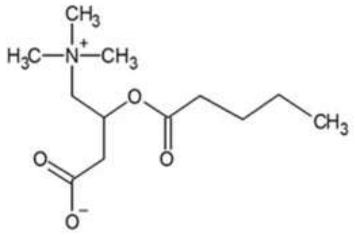
pivaloylcarnitine
(therapy with pivalate-containing medications)

2-Methylbutyrylcarnitine



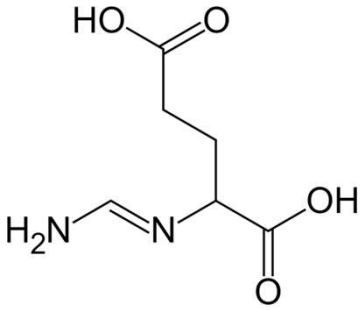
2-methylbutyrylcarnitine
(2-methylbutyryl-CoA dehydrogenase deficiency or SBCADD)

n-Valerylcarnitine

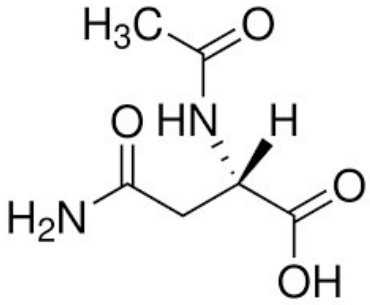


n-valerylcarnitine
(metabolic disorders involving fatty acids with odd-numbered carbon chains)

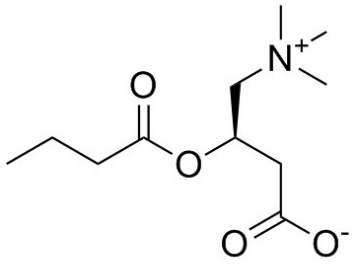
C4 acylcarnitine



Formiminoglutamic acid (glutamate formiminotransferase deficiency)

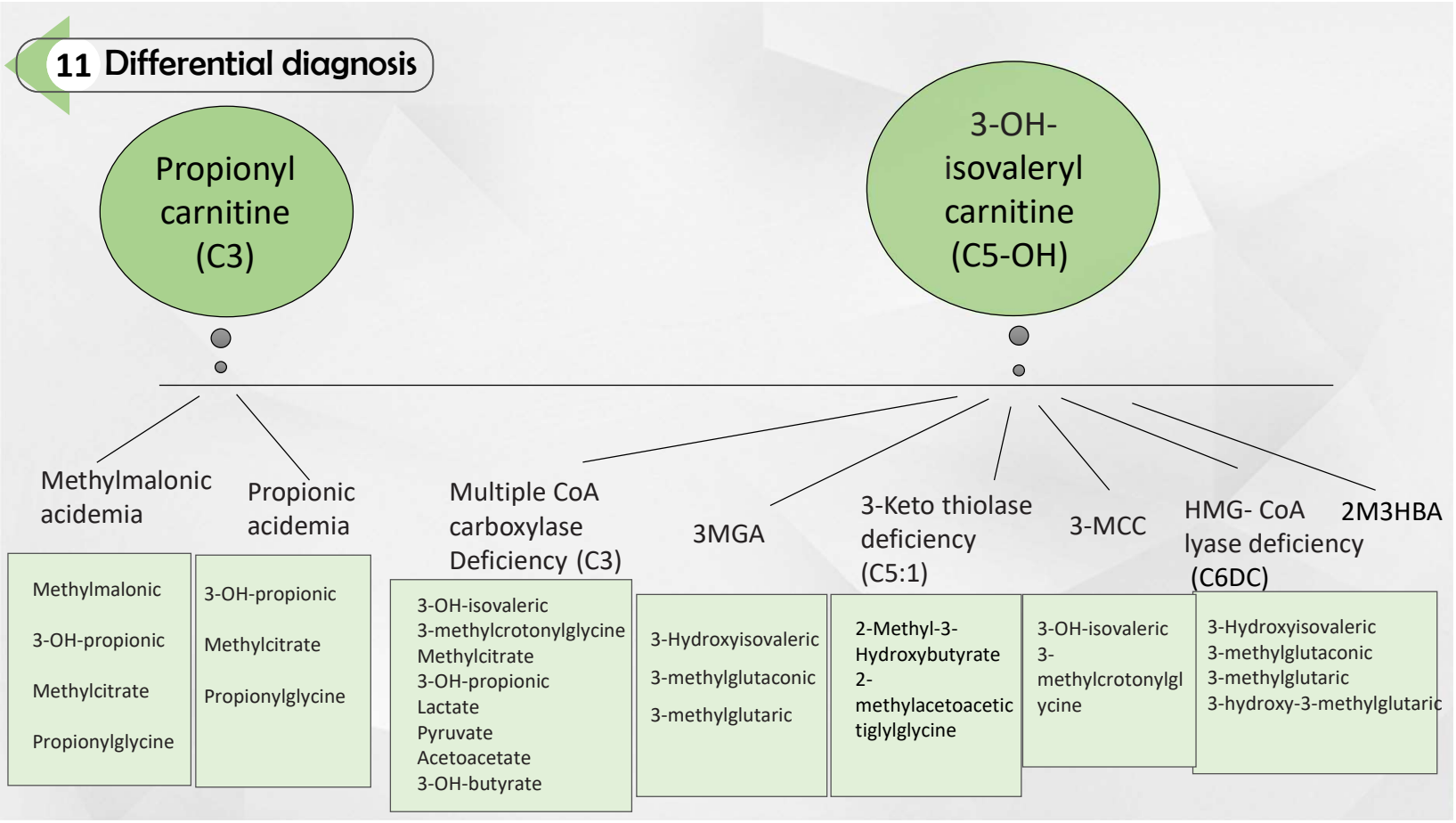


Isobutyrylcarnitine (isobutyryl-CoA dehydrogenase deficiency)

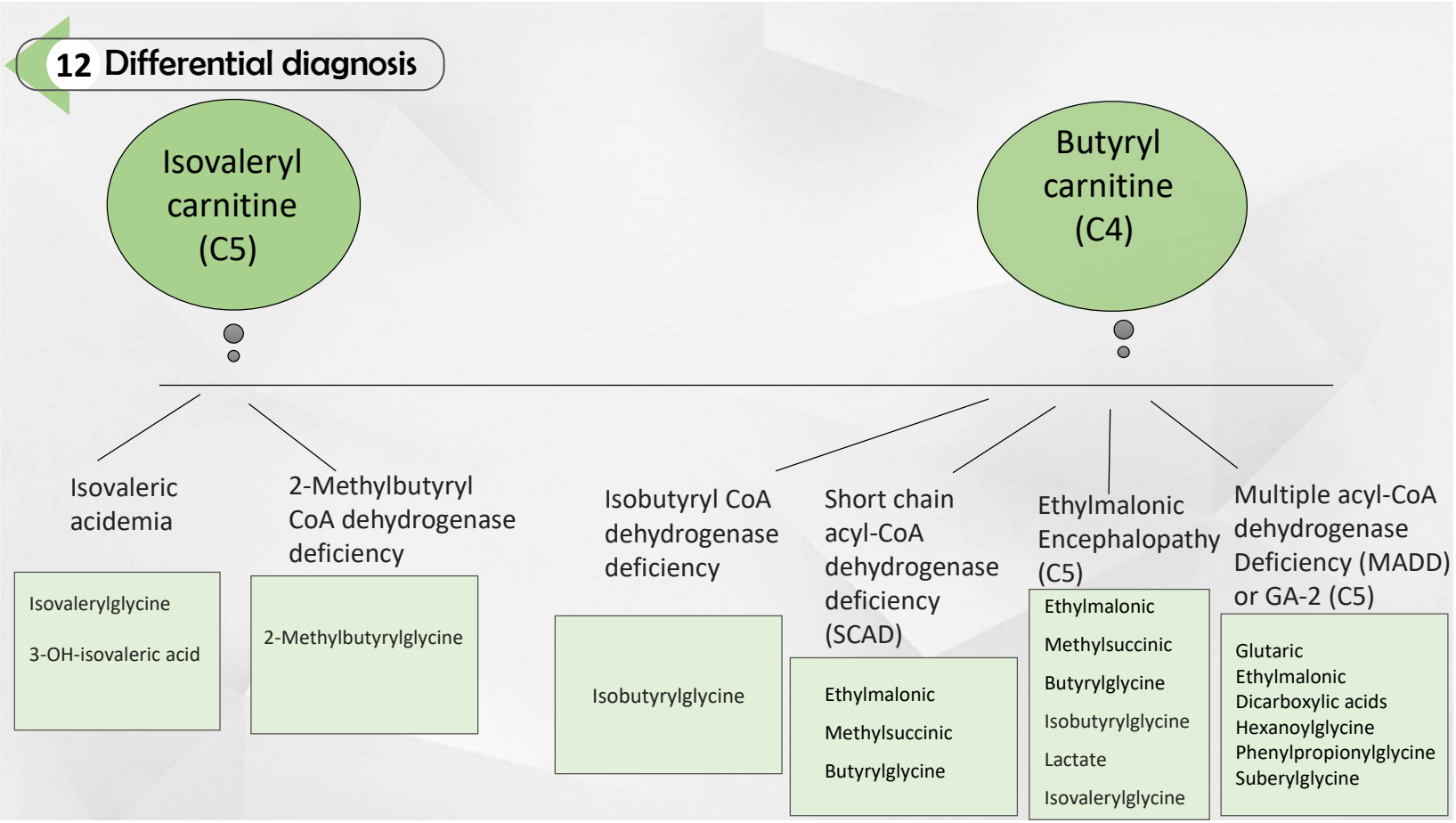


Butyrylcarnitine (short-chain acyl-CoA dehydrogenase deficiency)

11 Differential diagnosis



12 Differential diagnosis

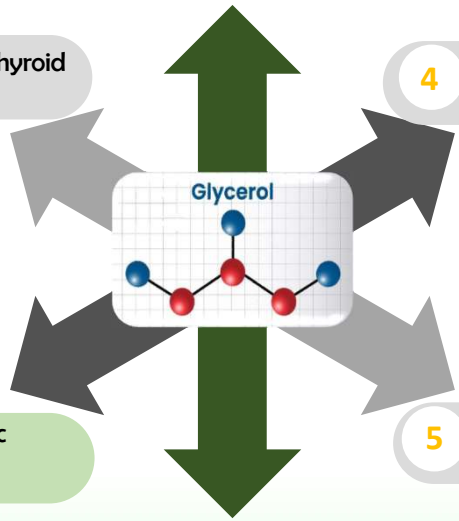


14 Elevated glycerol

1 **Inherited Metabolic Disorders**
Glycerol kinase deficiency (GKD)

2 Hormonal or Enzymatic Disorders (Thyroid Disorders, Pancreatic Insufficiency)

4 Laboratory or Sample Preparation Artifacts



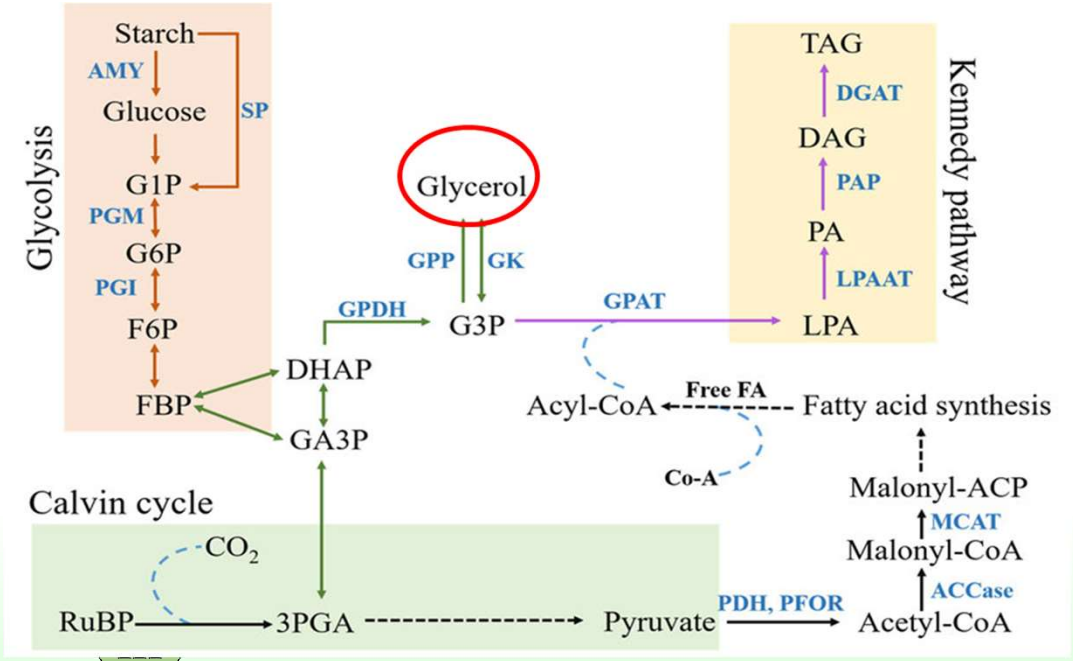
3 Clinical Conditions (Diabetes, Metabolic Syndrome, Liver Diseases)

5 Nutritional or Therapeutic Factors (High Dietary Fat Intake)

Alcohol, Infection or Metabolic Stress

Elevated glycerol

15 **Elevated glycerol** Glycerol kinase deficiency (GKD) or Hyperglycerolemia



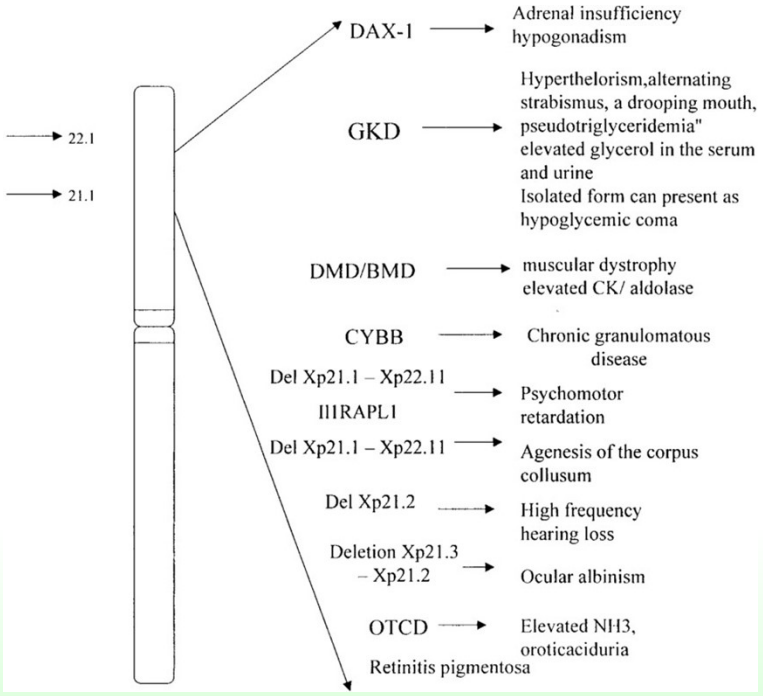
Glycerol metabolism

Elevated glycerol

16 Elevated glycerol

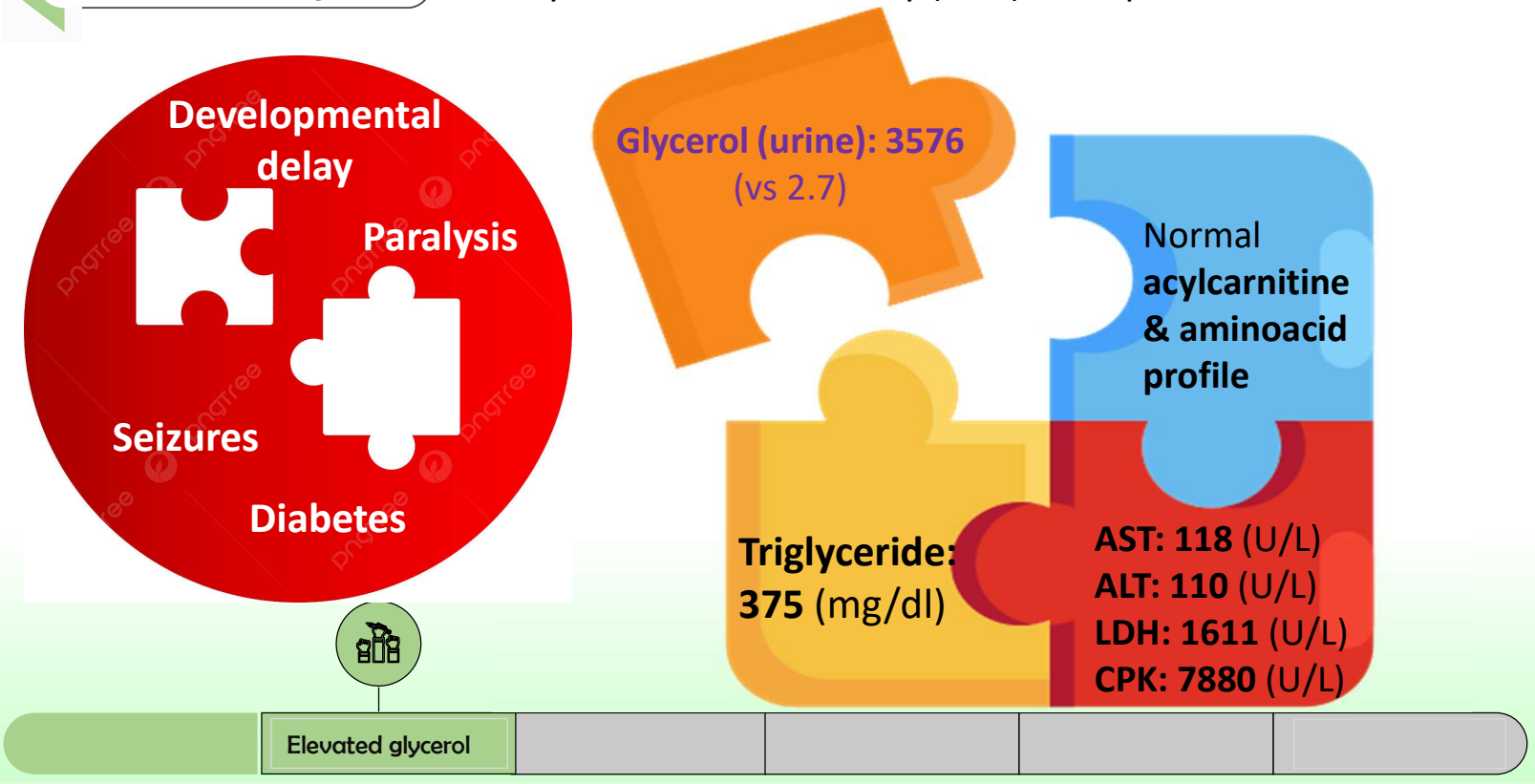
Glycerol kinase deficiency (GKD) or Hyperglycerolemia

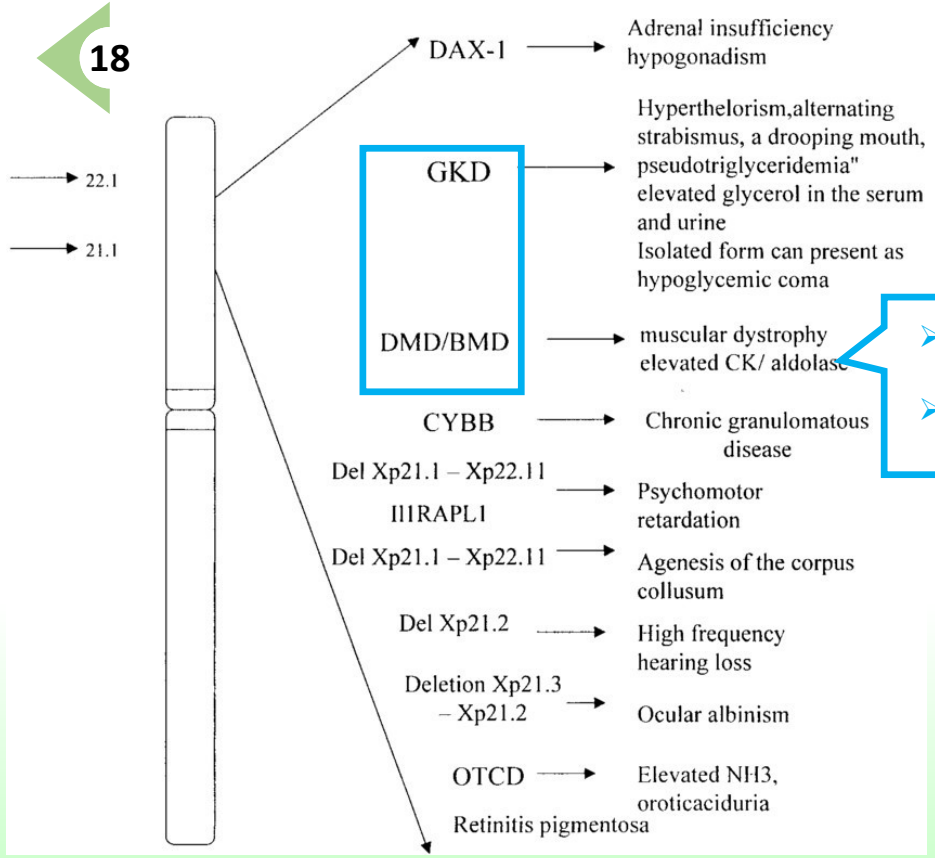
- GKD is due to deletion or mutations within the glycerol kinase (GK) gene on Xp21.2.
- Contiguous gene deletion syndrome.
- These individuals may be at increased risk for insulin resistance and type 2 diabetes mellitus.
- Diagnosis made by:
  - Glycerolemia
  - Glyceroluria
  - Mutations in the GK gene



17 Elevated glycerol

Glycerol kinase deficiency (GKD): Case presentation



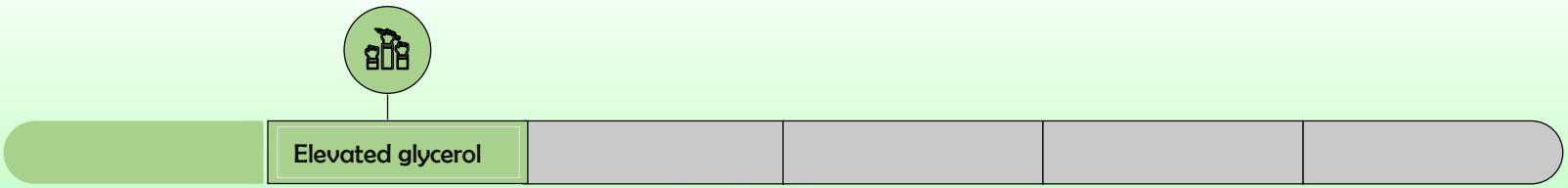


- Severe, progressive muscle weakness as in classical Duchenne muscular dystrophy
- But also has been associated with milder muscle disease

19 Elevated glycerol

Glycerol kinase deficiency (GKD): Case presentation

System	Symptoms and biomarkers	Neonatal (birth–1 month)	Infancy (1–18 months)	Childhood (1.5–11 years)	Adolescence (11–16 years)	Adulthood (>16 years)
Other	No clinical significance	+	+	+	+	+
Metabolic	Hypoglycemia	±	±	±	±	±
	Insulin resistance type II diabetes mellitus					±
Laboratory findings	Glucose (plasma)	↓-n	↓-n	↓-n	↓-n	↓-n
	Glycerol (plasma)	↑	↑	↑	↑	↑
	Glycerol (urine)	↑	↑	↑	↑	↑
	Triglyceride, pseudo (plasma)	↑	↑	↑	↑	↑

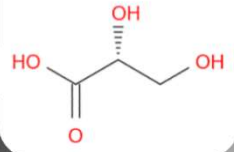


20 Elevated glyceric acid

1 Primary Hyperoxaluria Type II (PH2): glyoxylate reductase/hydroxypyruvate reductase (GRHPR) deficiency

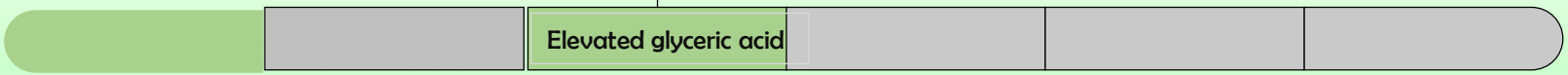
2 Glycerate kinase deficiency

Glyceric acid



3 Defects in Hydroxypyruvate Metabolism

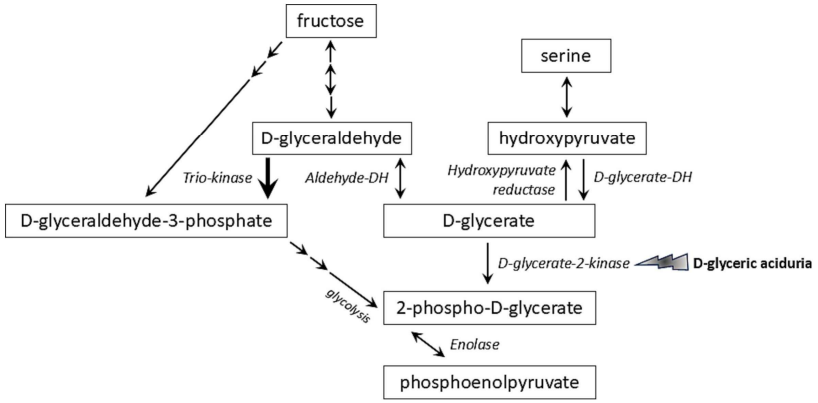
4 Glycerol-Containing Medications or Supplements

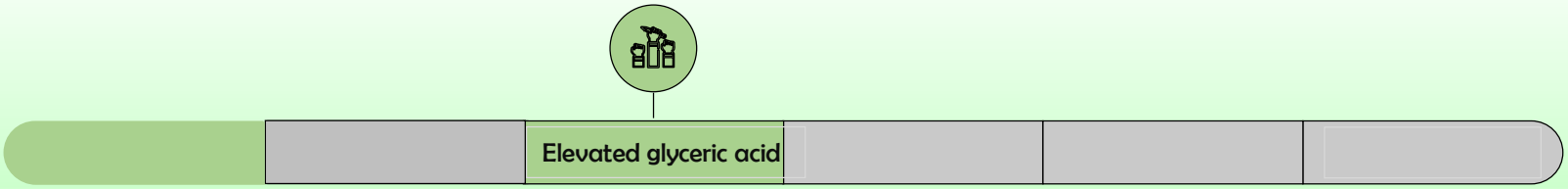


21 Elevated glyceric acid

Glycerate kinase deficiency (GLYCTK-D) or d-glyceric aciduria (DGA)

- Caused by deficiency of d-glycerate kinase (GLYCTK) gene and the glycerate kinase enzyme.
- Inborn error of metabolism of serine and fructose metabolism.
- Only 17 patients were described.
- Diagnosis made by:
  - Elevated d-glyceric acid levels in plasma, urine or CSF
  - Reduced glycerate kinase activity in liver



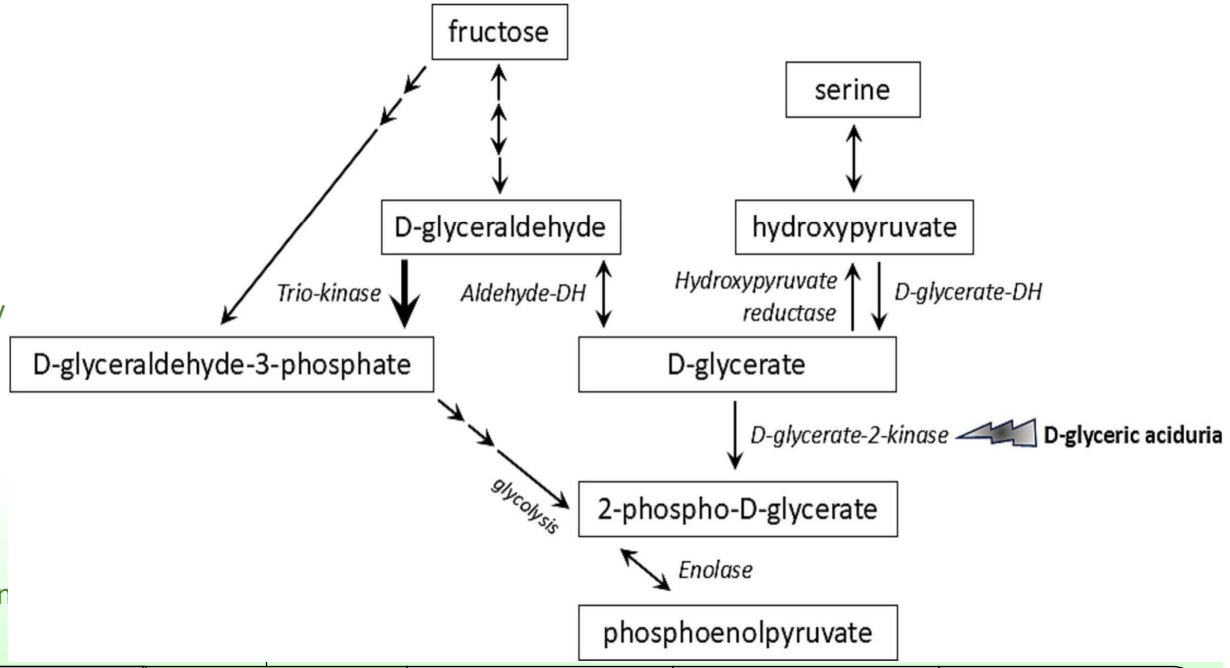


22 Elevated glyceric acid

Glycerate kinase deficiency (GLYCTK-D) or d-glyceric aciduria (DGA)

- Symptoms :
  - Hypotonia
  - Failure to thrive
  - Encephalopathy
  - Seizures
  - Intellectual disability
  - Microcephaly
  - Metabolic acidosis
  - Early death

only mild speech delay
apparently normal developmen



23 Elevated glyceric acid

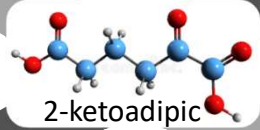
Glycerate kinase deficiency (GLYCTK-D) or d-glyceric aciduria (DGA)

System	Symptoms and biomarkers	Neonatal (birth–1 month)	Infancy (1–18 months)	Childhood (1.5–11 years)	Adolescence (11–16 years)	Adulthood (>16 years)
CNS	**Encephalopathy**	±	±	±		
	Intellectual disability	±	±	±		
	Seizures	±	±	±		
	Speech delay	±	±	±		
Metabolic	Metabolic acidosis	±	±	±	±	
Musculoskeletal	**Hypotonia, muscular-axial**	±	±	±		
	Microcephaly	±	±	±		
Other	**Early death**	±	±	±		
	Failure to thrive	±	±	±		
Laboratory findings	D-glycerate (cerebrospinal fluid)	↑	↑	↑	↑	
	D-glycerate (plasma)	↑	↑	↑	↑	
	D-glycerate (urine)	↑	↑	↑	↑	
	D-glycerate kinase (liver)	↓	↓	↓	↓	

24 2-ketoadipic
2-aminoadipic
2-hydroxyadipic acids

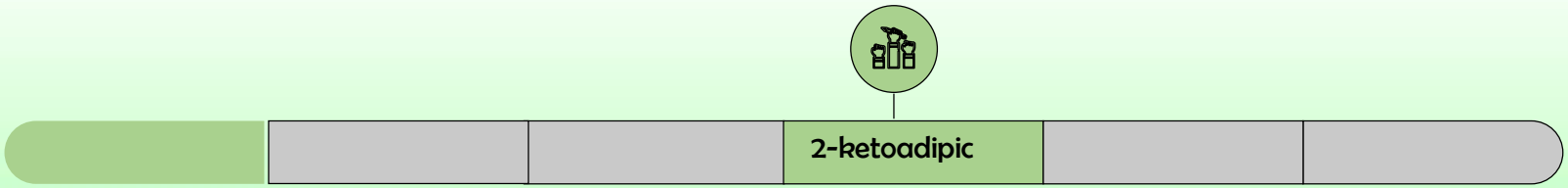
2 **Nutritional and Pharmacological Factors** (Excess Lysine Intake)

1 **Inherited Metabolic Disorders** (2-Aminoadipic and 2-Ketoadipic Aciduria)



3 **Neurological Diseases** (Charcot-Marie-Tooth disease type 2)

4 **Sampling and Storage Issues** (Microbial Contamination of Samples, Sample Degradation)



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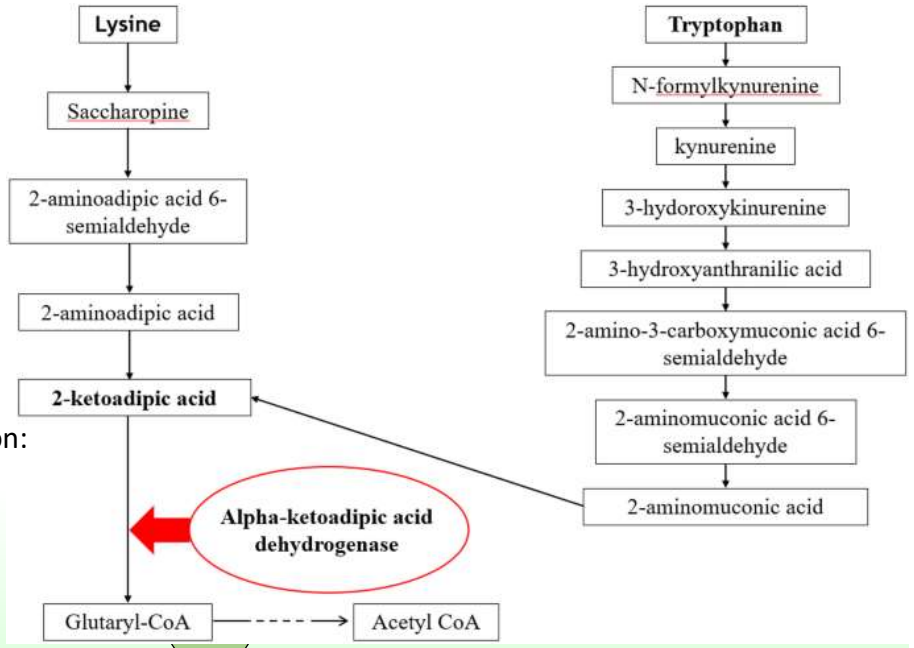
2-ketoadipic 2-aminoadipic 2-hydroxyadipic acids

2-Amino/2-Ketoadipic aciduria

- Caused by mutations in the DHTKD1 gene encoding alpha-ketoadipic acid (KAA) dehydrogenase complex.
- Inborn error of metabolism in the catabolic pathways of lysine, hydroxylysine, and tryptophan.
- Diagnosis made by increased urinary excretion:
  - Alpha-ketoadipic acid (KAA)
  - Alpha-aminoadipic acid (AAA)
  - Alpha-hydroxyadipic acid (HAA)
  - N-acetyl-AAA



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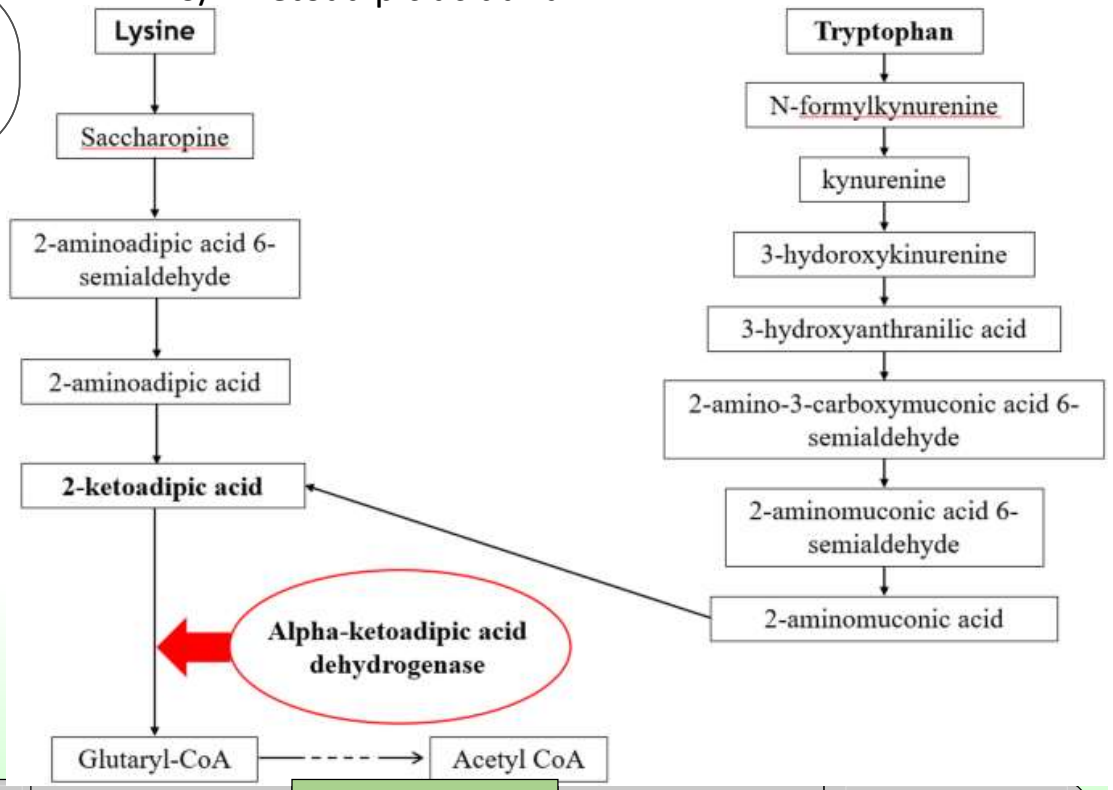
2-ketoadipic
2-aminoadipic
2-hydroxyadipic acids

Symptoms :

- Hypotonia
- Developmental delay
- Mild to severe intellectual disability
- Ataxia
- Epilepsy
- Behavioral disorders (most commonly attention deficit hyperactivity disorder)

Many individuals with the biochemical phenotype are completely asymptomatic

2-Amino/2-Ketoadipic aciduria



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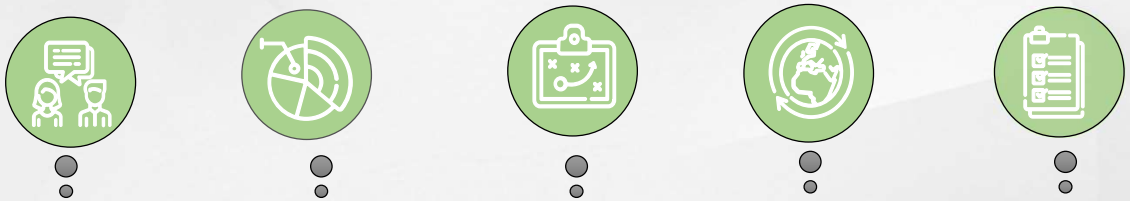
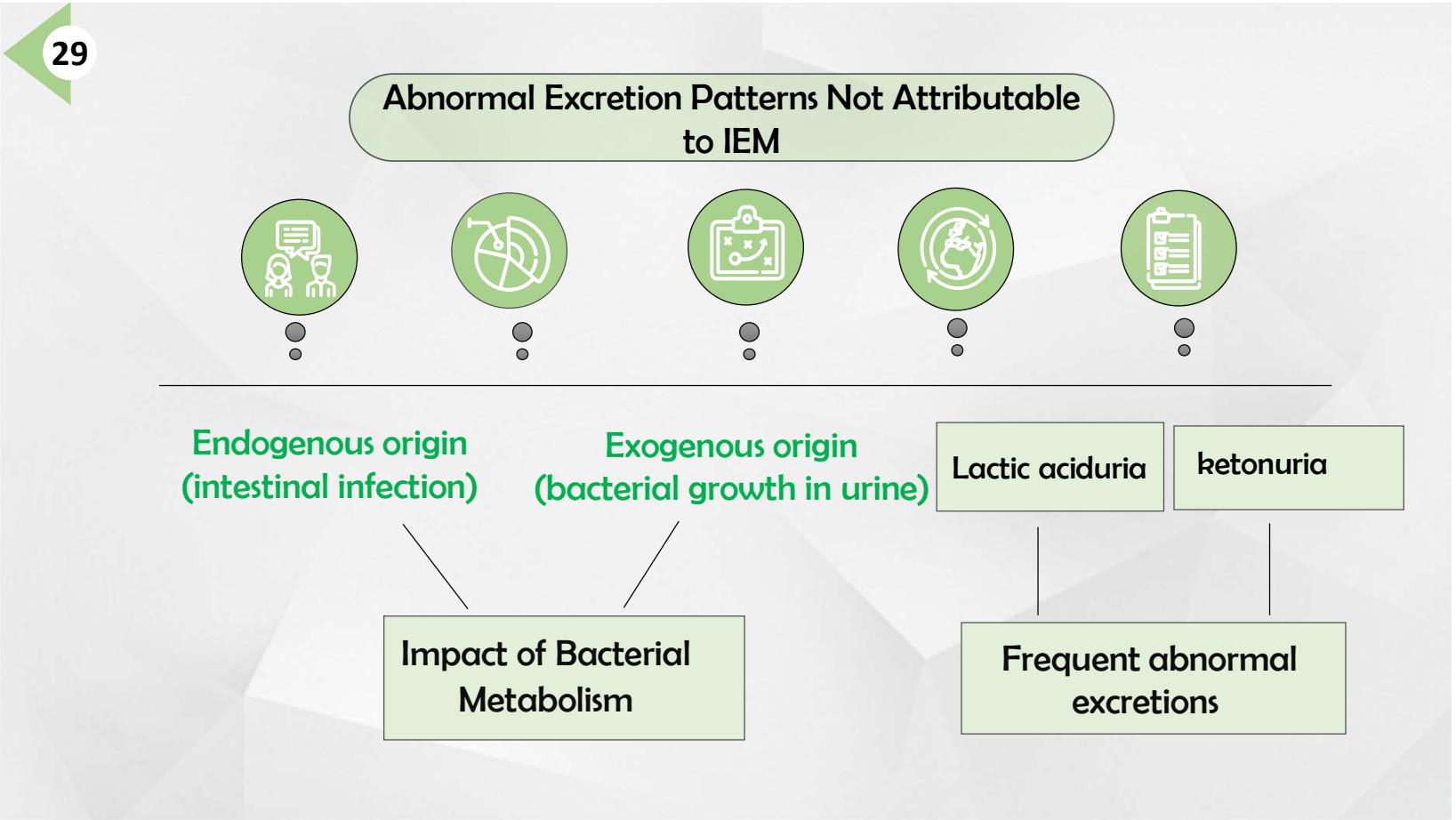
2-ketoadipic
2-aminoadipic
2-hydroxyadipic acids

2-Amino/2-Ketoadipic aciduria

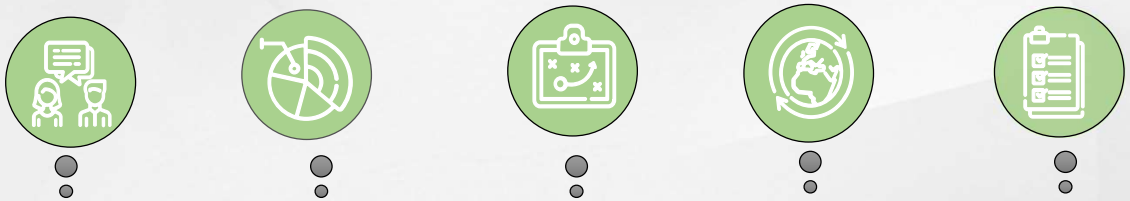
System	Symptoms and biomarkers	Neonatal (birth–1 month)	Infancy (1–18 months)	Childhood (1.5–11 years)	Adolescence (11–16 years)	Adulthood (>16 years)
Other	No clinical significance	±	±	±	±	±
CNS	Developmental delay		±	±		
	Seizures		±	±		
Metabolic	Metabolic acidosis		±	±		
Laboratory findings	2-Aminoadipate (urine)		↑	↑		
	2-Hydroxyadipate (urine)		↑	↑		
	2-Ketoadipate (urine)		↑	↑		
	3-Hydroxyglutaric acid (urine)		↑	↑		
	3-Hydroxyisovaleric acid (urine)		↑	↑		
	3-Methylglutaconic acid (urine)		↑	↑		
	Dicarboxylic acids (urine)		↑	↑		
	Ethylmalonic acid (urine)		n-↑	n-↑		
	Ketones, during hypoglycemia		+	+		



Abnormal Excretion Patterns Not Attributable to IEM


Abnormal Excretion Patterns Not Attributable to IEM
Endogenous origin (intestinal infection)
Exogenous origin (bacterial growth in urine)
Impact of Bacterial Metabolism
Lactic aciduria
ketonuria
Frequent abnormal excretions

Abnormal Excretion Patterns Not Attributable to IEM (Impact of Bacterial Metabolism)



Endogenous origin

Abnormal excretion of d-lactate, methylmalonate, p-hydroxyphenylacetate, p-hydroxyphenyllactate, phenylacetylglutamine, phenylpropionylglycine, glutarate, benzoate, and hippurate

Exogenous origin

d-lactate, 2-ketoglutarate, d-2-hydroxyglutarate, succinate, 3-hydroxypropionate, and phenol derivatives (phenol, p-cresol, hippurate)

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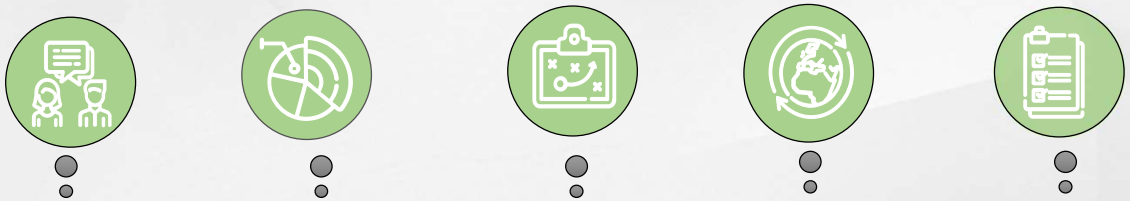

lactic aciduria

- Generally accompanied by Pyruvate, p-hydroxyphenyllactate, 2-hydroxyisovalerate, 2-hydroxybutyrate, branched-chain 2-keto acids
- Differentiation from Dihydrolipoyl dehydrogenase deficiency

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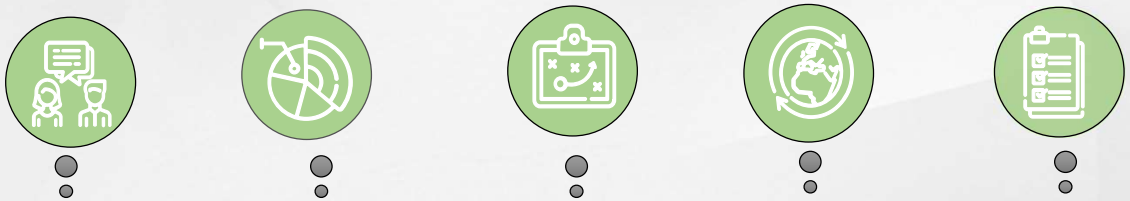
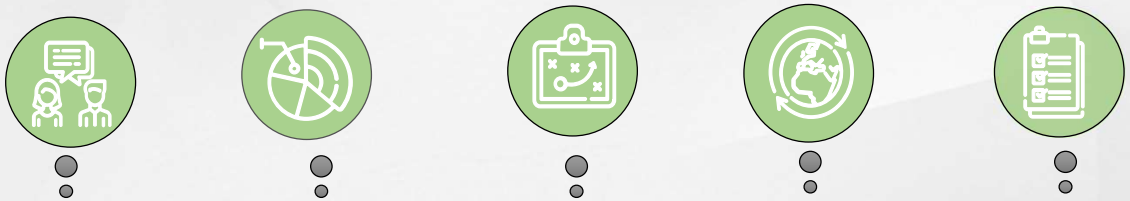


Ketonuria

Generally accompanied by 3-hydroxyisobutyrate, 3-hydroxyisovalerate, 2-hydroxybutyrate, and dicarboxylic acids, particularly their 3-hydroxy derivatives with chain lengths up to C14

Differentiation from long-chain 3-hydroxyacyl-CoA dehydrogenase or trifunctional protein deficiency

Abnormal Excretion Patterns Not Attributable to IEM



Valproic acid — Increased excretion of 3-hydroxyisovalerate, 5-hydroxyhexanoate, 7-hydroxyoctanoate, p hydroxyphenylpyruvate, dicarboxylic acids, and, to a lesser extent, hexanoylglycine, tiglylglycine, and isovalerylglycine

Medium-Chain Triglycerides (MCTs) — Produce a pattern resembling FAOD, with increased saturated even-numbered dicarboxylic acids, particularly sebacate, 5-hydroxyhexanoate and 7-hydroxyoctanoate, the presence of octanoate, and either absence or low excretion of glycine derivatives

Krebs cycle/respiratory chain

	Non-IEM	IEM
2-Ketoglutarate *(38, 72)*	Bacterial contamination; lithium; uremia; increase with younger age	As malate; 2-ketoglutaric DH deficiency; GA I; 2-amino/2-ketoadipate acidemia; dihydrolipoyl DH (E3) deficiency; glycogen storage disorder I; 2-hydroxyglutaric aciduria (D-form); fumarase deficiency
Aconitate		Respiratory chain defects (e.g., complex I); Pearson syndrome
Citrate, isocitrate	High carbohydrate intake; parathyroid extract; saturnism; citrate intake; fruit juice added to urine; hyperparathyroidism; increase with younger age	Dihydrolipoyl DH (E3) deficiency; fumarase deficiency; pyruvate carboxylase deficiency; Pearson syndrome
Fumarate	Lithium; renal tubular reabsorption defect (fumaric aciduria); increase with younger age	As malate; fumarase deficiency
Malate *(73–75)*	Lithium; uremia; increase with younger age	Respiratory chain defects; pyruvate carboxylase deficiency; PDH complex (E1, E3) deficiency; Pearson syndrome
Succinate *(72, 76)*	Bacterial (on storage); 2-ketoglutarate degradation; lithium; ketosis; tissue ischemia; increase with younger age	As malate; malonic aciduria; fumarase deficiency

Pyroglutamate (L- or D-5-oxoproline)

Drug

Acetaminophen; vigabatrin; fludoxacillin, netilmicin

Diet

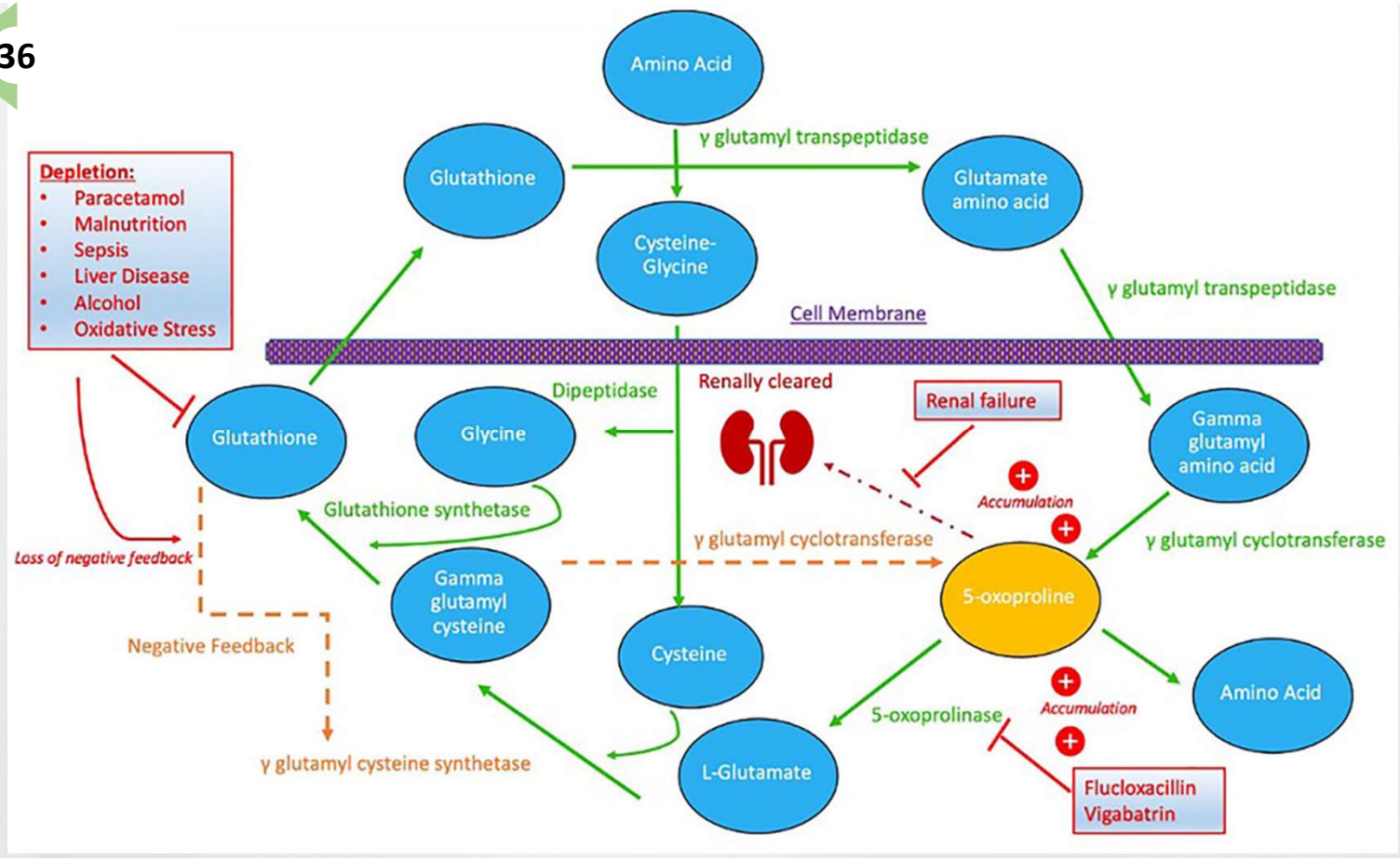
From glutamine of hydrolyzed proteins (infant formula); vegetarian or low-protein diets, undernutrition; glycine deficiency

Disorder

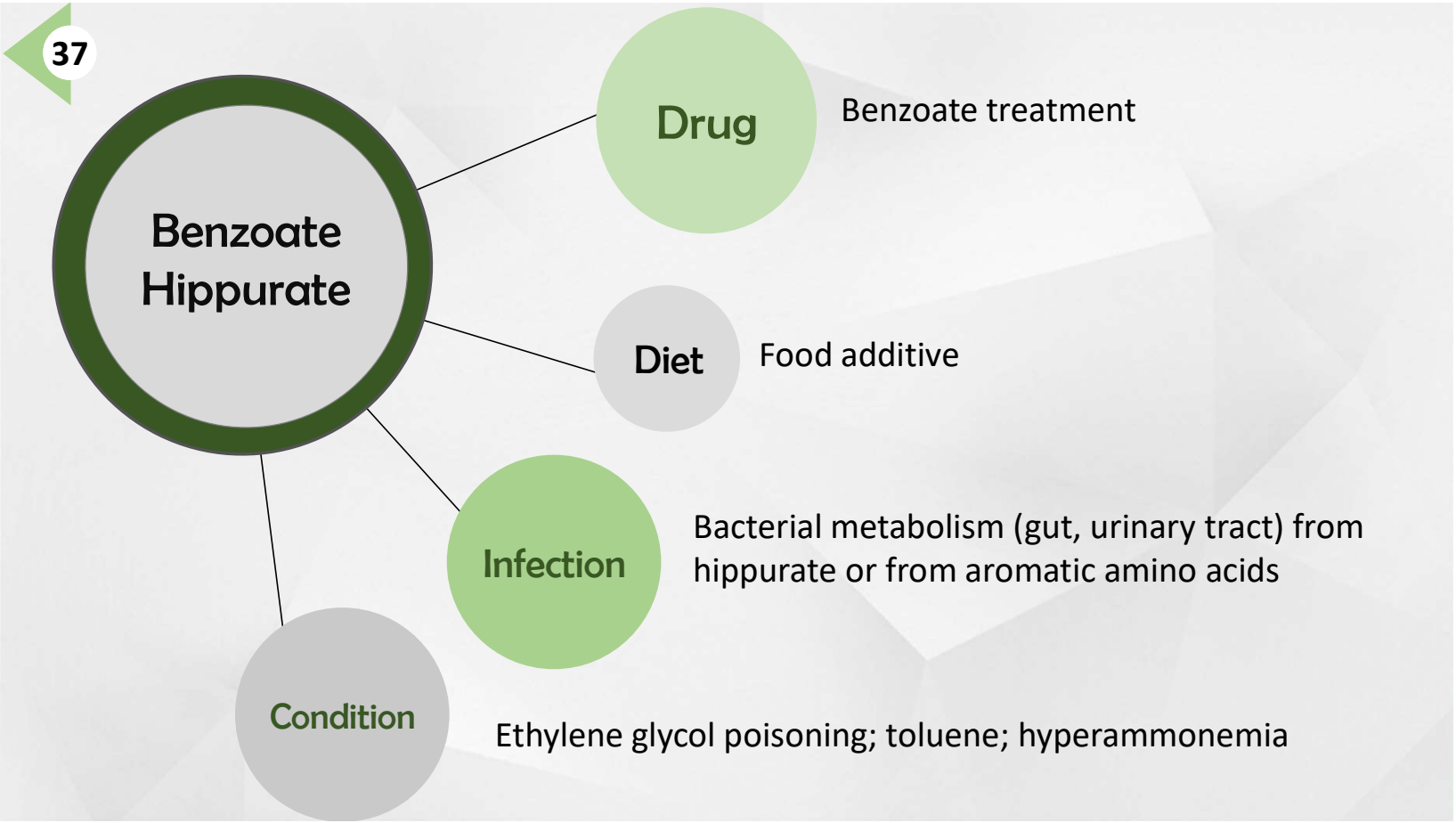
Glutamine degradation (in hyperammonemia, urea cycle defects); Steven–Johnson syndrome; renal insufficiency

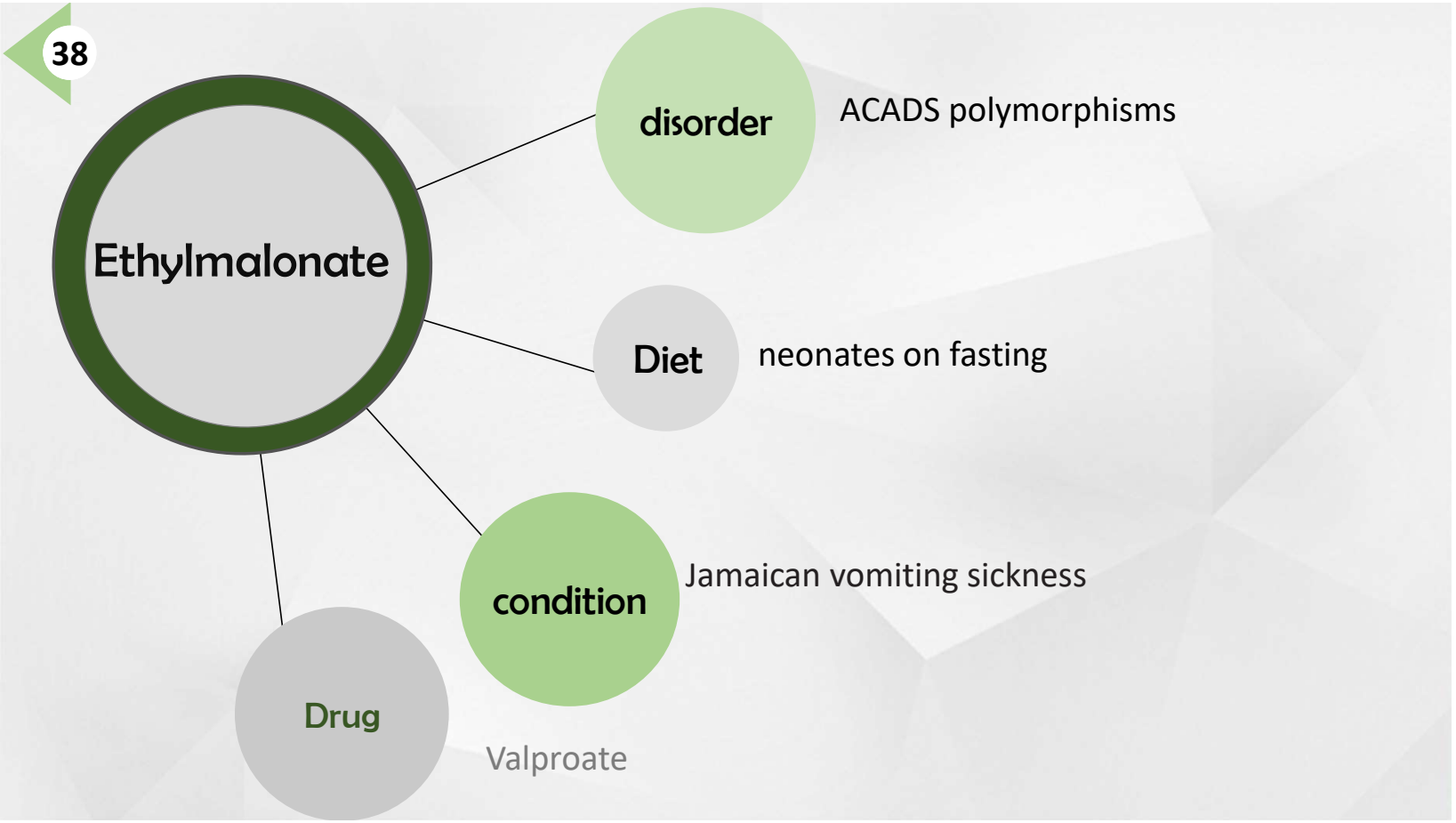
Condition

Burns; premature newborns; pregnancy (increased metabolic demand for glycine) ; increase with younger age


Amino Acid
γ glutamyl transpeptidase
Glutathione
Glutamate amino acid
Cysteine-Glycine
γ glutamyl transpeptidase
Depletion:
Paracetamol
Malnutrition
Sepsis
Liver Disease
Alcohol
Oxidative Stress
Cell Membrane
Renally cleared
Dipeptidase
Renal failure
Glutathione
Glycine
Gamma glutamyl amino acid
Accumulation
Glutathione synthetase
γ glutamyl cyclotransferase
γ glutamyl cyclotransferase
Loss of negative feedback
Gamma glutamyl cysteine
5-oxoproline
Negative Feedback
Cysteine
Amino Acid
5-oxoprolinase
Accumulation
γ glutamyl cysteine synthetase
L-Glutamate
Flucloxacillin
Vigabatrin

37


Benzoate Hippurate
Drug
Benzoate treatment
Diet
Food additive
Infection
Bacterial metabolism (gut, urinary tract) from hippurate or from aromatic amino acids
Condition
Ethylene glycol poisoning; toluene; hyperammonemia


Ethylmalonate
disorder
ACADS polymorphisms
Diet
neonates on fasting
condition
Jamaican vomiting sickness
Drug
Valproate


Thanks