inborn errors of metabolism - biochemical basis
What is an inborn error of metabolism (IEM)?

Genetically determined biochemical disorders, that affect an individual’s ability to convert nutrients or to use them for energy production.
• Majority of IEMs are due to defect in a single gene that code for an:
  - **ENZYME**
  - **COFACTOR**
  - **TRANSPORTER**

• *Most of them are inherited in an autosomal recessive pattern*
Fundamental design of the normal metabolic pathways and effects of metabolic aberrations

METABOLIC BLOCK

Substrate accumulation

Product deficiency

Accumulation of byproducts

GARROD’S HYPOTHESIS
IEMs affecting mother and fetus

Fig. 2 Maternal - fetal impact in IEM
diagnosis

- Prenatal diagnosis
- Newborn screening
- Evaluation of symptomatic patients
- Postmortem screening

Analysis of
1) Metabolites 2) Enzymatic activity 3) Molecular structure
classification

- Three broad categories:
- Based on the effects of their metabolic derangement.

1. Intoxication type

2. Energy deficiency type

3. Disorders of complex molecules (storage type)
Disorders of amino acid metabolism
Disorders of amino acid metabolism

Defects in aminoacid catabolism

Urea cycle defects
- Nonketotic hyper glycemia, Primary hyper oxaluria

Glycine

S containing aminoacids
- cystinuria
- homo cystinuria
- cystathioninuria

Branched chain a.a
- MSUD

Aromatic a.a
- PKU, alkaptonuris, hyper tyrosinemia

Defects in aminoacid membrane transport
- Cystinuria
- Hartnup’s disease
Aromatic amino acids

Phenylalanine → Phenylpyruvic acid

Phenylketonuria block

Phenylalanine hydroxylase

Tyrosine block

Tyrosinemia II

Tyrosine transaminase

Tyrosinemia III

Tyrosinemia I

Phenylalanine

Albinism block

3,4-Dihydroxyphenylalanine (DOPA)

Melanin pigments

CO₂ + H₂O

Citric acid cycle

Fumaric acid

Acetoacetic acid

Fumarylacetoacetatic acid

Fumarylacetoacetase

Tyrosinaemia III

p-Hydroxyphenylpyruvic acid

Tyrosinase

Homogentisic acid (2,5-dihydroxyphenylacetic acid)

Homogentisic acid oxidase

Alkaptonuria block

Maleylacetoacetatic acid
Classic Phenylketonuria
And
Other Hyperphenylalaninemias

Phe

COO \(^{-}\)

+ O\(_2\)

\[\text{Phe-hydroxylase}\]

H\(_4\) Biopterine

\[\text{DHP reductase}\]

H\(_2\) Biopterine

NADP\(^{+}\)

NADPH + H\(^{+}\)

Tyr

COO \(^{-}\)
phenylketonuria

- Blood phenylalanine (30-80mmol/L)
- Urine – phenyllactic, phenylacetic
- Urine pterin profile - Neopterin and biopterin, primapterin
- Activity of dihydropteridine reductase in blood cells
- 5-HIAA and HVA in CSF
**Guthrie’s test**

*Bacillus subtilis* needs Phenylalanine to grow
- Normal urine + B. Subtilis = no growth
- PKU urine + B. Subtilis = growth of bacteria

---

**Ferric chloride test**

- 5 ml fresh urine
- 3-4 drops of 10 gm% FeCl₃
- Blue-green colour indicates phenylpyruvate

---

**Dinitrophenyl hydrazine test (DNPH)**

- 2 ml fresh urine
- 2 ml DNPH
- Mixed
  - Examined immediately and after 10 minutes
- Yellow ppt after 30 mins indicate Keto acids
**tyrosinaemias**

**TYPE I** - Hepatorenal tyrosinaemia

- Elevated conc. of tyrosine
- Urine - succinylacetone
- ALA and succinylacetone in blood
- Alpha-fetoprotein

Fumaryl acetoacetate → Succinyl acetone

- Fumaryl acetoacetate hydrolase
- Fumarate
- Acetoacetic acid

\[
\text{Fumaryl acetoacetate} \xrightarrow{\text{hydrolase}} \text{fumarate} \xrightarrow{\text{Fumaryl acetoacetate hydrolase}} \text{acetoacetic acid}
\]

\[
\text{ALA} \xrightarrow{\text{ALA dehydratase}} \text{PBG}
\]
**Tyrosine transaminase**

Tyrosine $\rightarrow$ P-hydroxy phenyl pyruvate

- Oculocutaneous tyrosinaemia
- painful corneal erosions and plaques, inflammation and
- keratosis of palmar surface
alkaptonuria

Homogentisate

Maleyl acetoacetate

benzoquinone acetate (urine)

black alkapton bodies

Ferric chloride test - purple black
Benedict's test - brown colour
Tyrosine → Tyrosinase → DOPA → Tyrosinase → DOPA quinone → Dopachrome → Melanine

ALBINISM
Disorders of branched chain amino acid metabolism

- High plasma conc of branched chain aa’s
- BC ketoacids, oxoacids and hydroxy acids – urine
- Odor of maple syrup - smelling ear wax
- alloisoleucine
- Positive DNPH test
- FeCl test- greenish grey color
Disorders of sulfur containing aa’s
Biosynthesis of cysteine

\[
\text{Methionine} \quad \rightarrow \quad \text{Homocysteine} \quad \rightarrow \quad \text{Cystathionine} \quad \rightarrow \quad \text{Cysteine}
\]

\[
\text{Methionine} \quad \rightarrow \quad \text{Homocysteine} \quad \rightarrow \quad \text{Cystathionine} \quad \rightarrow \quad \text{Cysteine}
\]

\[
\text{Methionine} \quad \rightarrow \quad \text{Homocysteine} \quad \rightarrow \quad \text{Cystathionine} \quad \rightarrow \quad \text{Cysteine}
\]

\[
\text{Methionine} \quad \rightarrow \quad \text{Homocysteine} \quad \rightarrow \quad \text{Cystathionine} \quad \rightarrow \quad \text{Cysteine}
\]

\[
\text{Methionine} \quad \rightarrow \quad \text{Homocysteine} \quad \rightarrow \quad \text{Cystathionine} \quad \rightarrow \quad \text{Cysteine}
\]

\[
\text{Methionine} \quad \rightarrow \quad \text{Homocysteine} \quad \rightarrow \quad \text{Cystathionine} \quad \rightarrow \quad \text{Cysteine}
\]

\[
\text{Methionine} \quad \rightarrow \quad \text{Homocysteine} \quad \rightarrow \quad \text{Cystathionine} \quad \rightarrow \quad \text{Cysteine}
\]

\[
\text{Methionine} \quad \rightarrow \quad \text{Homocysteine} \quad \rightarrow \quad \text{Cystathionine} \quad \rightarrow \quad \text{Cysteine}
\]

\[
\alpha\text{-Ketobutyrate} \quad \rightarrow \quad \alpha\text{-KB dehydrogenase} \quad \rightarrow \quad \text{Propionyl Co A}
\]

\[
\text{Propionyl Co A}
\]

\[
\text{Homocystinuria}
\]
Accumulation of homocysteine,

Deposited in various tissues

Binds copper and interferes with maturation of collagen and elastin

Skeletal deformities, posterior dislocation of lens, mental retardation, increased susceptibility to thrombosis.
• Disulfide homocystine levels

• Total and free homocysteine levels (immunoassay, HPLC, tandem mass spectrometry)

• Methionine levels (B.U)
Hyperammonemia without metabolic acidosis - defect of the UREA CYCLE
Acetyl-CoA + glutamate

N-acetyl-glutamate

CO₂ + NH₃ + 2 ATP

Carbamoylphosphate

Mitochondrion

Ornithine

Orc1

Aspartate + Citrulline

Arginosuccinic acid

Aspartate synthase

Aspartate lyase

Arginase

Urea

CPS-I

Fumarate + Arginine

Hyperammonaemias

Hyperammonaemia, abnormal liver functions, elevated transaminases, PT/PTT ratio, plasma amino acids (glutamine and alanine), urine amino acids

Low conc of citrulline with elevated glutamine - NAGS, CPS-1 and OTC deficiency

Elevated citrulline - citrullinaemia type 1 (ASA deficiency) - type 2 (citrin deficiency)

Urine orotic acid - OTC deficiency

Orotic acid

UTP, CTP

Carbamoylphosphate

Orc1 (HHH)

Cytoplasm

Citrulline Synthase

Citrulline

Orc1 deficiency - HHH syndrome - hyperammonaemia, hyperornithinaemia, homocitrullinuria
Disorders of amino acid transport
Hartnups disease

Results from defects in intestinal & renal transport of neutral aas like tryptophan (neutral AA transporter).

- Degradation of tryptophan - indoleyl acetic acid and indolyl acetyl glutamine - neurotoxic action - neurological symptom
- Pellagra like symptoms
  - Blood: plasma levels of tryptophan ↓
  - Urine shows ↑ amounts of IAA (Obermeyer test)
CYSTINURIA:
- renal and GIT transport of cystine and dibasic aa’s - defective
- Cystine - insoluble → Precipitates in the renal tubules
- Cystine calculi - nephrolithiasis.
- U- cystine, lysine, ornithine, arginine

Lysinuric protein intolerance
- Cationic amino acid transporter
- Failure to thrive, alveolar proteinosis, hepatosplenomegaly, pancreatitis, diarrhea, osteoporesis, hypotonia, postprandial hyperamonemia
- Lysine, arginine, ornithine - U
- Orotic acid - U
Organic acidaemias

Hyperammonaemia with metabolic acidosis and ketosis - ORGANIC ACIDAEMIAS
Accumulation of intermediates in the catabolic pathways of amino acids

- intermediates - water soluble (carboxyl groups)

- Physiologic metabolites present in excessive amounts/ metabolites not normally present

Metabolic acidosis - 6.85 to 7.3
Low bicarbonate - <5-15 mmol/L
Significant anion gap
Propionic aciduria
Methyl malonic aciduria

- Valine
- Isoleucine
- Methionine
- Cholesterol
- Odd chain fatty acids

\[ \text{Propionyl-CoA} \]

\[ \text{D-Methylmalonyl-CoA} \]

\[ \text{L-Methylmalonyl-CoA} \]

- Propionyl carnitine
- Methyl citric acid
- MMA

- Propionyl CoA carboxylase
- Methyl malonyl CoA mutase
- Vitamin B12 lysosomal release
Biochemical effects in propionic acidaemia

- Increased organic acids
  - Ketosis and metabolic acidosis with secondary Hyperammonaemia and Hyperglycinemia

- Increased glucose utilization
  - Hypoglycemia and Ketosis
Severe metabolic acidosis
Hyperammonaemia
Characteristic odor of sweaty feet
Urine organic acid analysis -
3-OH glutaric acid - Urine
Glutaryl carnitine - plasma
Carbohydrate metabolism
GALACTOSE METABOLISM

GALACTOKINASE DEFICIENCY
- Rare autosomal recessive disorder
- Causes elevation of galactose in blood (galactosemia) and urine (galactosuria)
- Causes galactitol accumulation if galactose is present in the diet.

ALDOSE REDUCTASE
- The enzyme is present in liver, kidney, retina, lens, nerve tissue, seminal vesicles, and ovaries.
- It is physiologically unimportant in galactose metabolism unless galactose levels are high (as in galactosemia).
- Elevated galactitol can cause cataracts.

CLASSIC GALACTOSEMIA
- Uridylytransferase deficiency.
- Autosomal recessive disorder (1:30,000 births).
- Causes galactosemia and galactosuria, vomiting, diarrhea, and jaundice.
- Accumulation of galactose 1-phosphate and galactitol in nerve, lens, liver, and kidney tissue causes liver damage, severe mental retardation, and cataracts.
- Antenatal diagnosis is possible by chorionic villus sampling. Newborn screening is available.
- Therapy: Rapid diagnosis and removal of galactose (and therefore, lactose) from the diet.
- Despite adequate treatment, at risk for developmental delays and, in females, premature ovarian failure.
Galactose-1-phosphate uridyl transferase
Galactitol accumulates in lens

Excess gal-1-p inhibits glycogen phosphorylase and phosphoglucomutase - impede glycogenolysis - accumulation of glycogen in the liver-jaundice; Feeding difficulties and vomitings - poor weight gain

Renal failure- albuminuria and aminoaciduria

Galactosaemia - persistent stimulus for insulin secretion by pancreas - hypoglycaemia, Abnormal lethargy or irritability
Effect on erythrocytes:
Galactose $\rightarrow$

Inhibits glucose-6-phosphate dehydrogenase $\rightarrow$

HMP - Shunt $\rightarrow$

Reduces the supply of NADPH $\rightarrow$

haemolysis
FRUCTOSE METABOLISM

GLUCOSE
- Hexokinase
- Glucose 6-phosphatase

SUCROSE
- Sucrase

FRUCTOSE
- ATP
- Fructokinase
- ADP
- Fructose 1-P
- Aldolase B

ESSENTIAL FRUCTOSURIA
- Lack of fructokinase.
- Autosomal recessive (1:130,000 births)
- Benign condition.
- Fructose accumulates in the urine.

HEREDITARY FRUCTOSE INTOLERANCE (FRUCTOSE POISONING)
- Autosomal recessive (1:20,000 births)
- Absence of aldolase B leads to intracellular trapping of fructose 1-P.
- Causes severe hypoglycemia, vomiting, jaundice, hemorrhage, hepatomegaly, lactic acidemia, and hyperuricemia.
- Can cause hepatic failure and death.
- Therapy: Rapid detection and removal of fructose and sucrose from the diet.

GLYCOGEN
- Phosphogluco-isomerase
- Fructose 6-P
- Fructose 1,6-bisphosphatase

GLUCONEOGENESIS
- Fructose 1,6-bis-P
- Fructose 1,6-bis-P

GLYCOLYSIS
- Dihydroxyacetone P
- Triose P isomerase
- Triose P kinase
- Glycerol 3-P

PHOSPHOGLYCERIDES
- Glycerol kinase
- ADP
- Glycerol-P

TRIACYLGLYCEROLS
- Glycerol dehydrogenase
- Glycerol-P
- ADP

PYRUVATE
Disorders of the carnitine cycle and fatty acid oxidation

*Hypoketotic hypoglycaemia with/without hyperammonaemia - FATTY ACID OXIDATION IMPAIRMENT*
Fasting or high energy demand - fatty acids - main substrates for energy production in liver, cardiac and skeletal muscle.

- Fasting or high energy demands
- Fatty acids are released from adipose tissue
- Impaired oxidation of fatty acids
- Accumulate in liver, heart, skeletal muscle
- Cardiomyopathy, myopathy, fatal arrhythmias
Lack of acetyl CoA
Dec. Gluconeogenesis
Excessive utilization of glucose
Hypoglycaemia
Decreased availability of ketones
Decrease in energy supply to the brain
LOC, seizures and coma
CARNITINE CYCLE

Carnitine – essential role in the transport of LCFA inside mitochondria for \( \beta \)-oxidation

- Medium and short chain fatty acid - independent
  - OCTN2 – carnitine transporter
  - CPT-1 – carnitine palmitoyl transferase 1
  - CACT - carnitine-acyl carnitine translocase

- Hypoglycaemia
- No ketone bodies
- Hyperammonaemia
- Elevated liver transaminase
- Occasionally - Creatine kinase activity

Carnitine uptake defect
- Urinary carnitine wasting
- Low serum free, total and acylated carnitine conc (25-50µmol/L)

CPT-I deficiency:
- Elevated FFA levels
- Low long-chain carnitine
- Elevated free carnitine/(C16+C18) ratio

Carnitine-acyl carnitine translocase deficiency
- Elevated conc of long chain acyl-carnitine levels
- Low free carnitine levels

CPT 2 deficiency
- Elevated long chain acyl carnitine levels with no carnitine deficiency

Medium and short chain fatty acid metabolism
**β-Oxidation defects**

- **VLC acyl-CoA dehydrogenase deficiency**
- **Trifunctional protein deficiency**
- Hypoketotic hypoglycaemia
- Elevated CK and transaminase
- Elevated acyl carnitine
- Specific enzyme assay

- **Medium chain acyl-CoA dehydrogenase deficiency** → urine acyl glycine, dicarboxylic acids
- **Short chain acyl Co-A dehydrogenase deficiency** → butyryl CoA – ethyl malonic acid, butyryl glycine and butyryl carnitine levels
# Glycogen storage disorders

<table>
<thead>
<tr>
<th>Type</th>
<th>Defective enzyme</th>
<th>Organ affected</th>
<th>Glycogen in the affected organ</th>
<th>Clinical features</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-Von Gierke</td>
<td>Glucose 6-phosphatase or transport system</td>
<td>Liver and kidney</td>
<td>Increased amount; normal structure.</td>
<td>Massive enlargement of the liver. Failure to thrive. Severe hypoglycemia, ketosis, hyperuricemia, hyperlipemia.</td>
</tr>
<tr>
<td>II-Pompe</td>
<td>α-1,4-Glucosidase (lysosomal)</td>
<td>All organs</td>
<td>Massive increase in amount; normal structure.</td>
<td>Cardiorespiratory failure causes death, usually before age 2.</td>
</tr>
<tr>
<td>III-Cori</td>
<td>Amylo-1,6-glucosidase (debranching enzyme)</td>
<td>Muscle and liver</td>
<td>Increased amount; short outer branches.</td>
<td>Like type I, but milder course.</td>
</tr>
<tr>
<td>IV-Andersen</td>
<td>Branching enzyme (α-1,4 → α-1,6)</td>
<td>Liver and spleen</td>
<td>Normal amount; very long outer branches.</td>
<td>Progressive cirrhosis of the liver. Liver failure causes death, usually before age 2.</td>
</tr>
<tr>
<td>V-McArdle</td>
<td>Phosphorylase</td>
<td>Muscle</td>
<td>Moderately increased amount; normal structure.</td>
<td>Limited ability to perform strenuous exercise because of painful muscle cramps. Otherwise patient is normal and well developed.</td>
</tr>
<tr>
<td>VI-Hers</td>
<td>Phosphorylase</td>
<td>Liver</td>
<td>Increased amount.</td>
<td>Like type I, but milder course.</td>
</tr>
<tr>
<td>VII</td>
<td>Phosphofructokinase</td>
<td>Muscle</td>
<td>Increased amount; normal structure.</td>
<td>Like type V.</td>
</tr>
<tr>
<td>VIII</td>
<td>Phosphorylase kinase</td>
<td>Liver</td>
<td>Increased amount; normal structure.</td>
<td>Mild liver enlargement. Mild hypoglycemia.</td>
</tr>
</tbody>
</table>

Note: Types I through VII are inherited as autosomal recessives. Type VIII is sex linked.
We are currently in the classroom of the lysosomes. They break down larger good molecules into smaller molecules. Notice how small and round they are.

We are now in the classroom of the peroxisomes. They break down very long of fatty acids! Any questions?

No, let’s move on!
### Lipid storage disorders

<table>
<thead>
<tr>
<th>Disease</th>
<th>Enzyme Deficiency</th>
<th>Lipid Accumulating&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Clinical Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tay-Sachs disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fabry’s disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metachromatic leukodystrophy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Krabbe’s disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gaucher’s disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Niemann-Pick disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farber’s disease</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>NeuAc, N-acetylneuraminic acid; Cer, ceramide; Glc, glucose; Gal, galactose. ↓↑, site of deficient enzyme reaction.
Peroxisomes

**Alpha Oxidation**
- AMACR
- Pristanoyl-CoA
- Acyl-CoA oxidase etc.

**Beta Oxidation**
- C11-carnitine

**Peroxisomal disorders**
- X linked adrenoleukodystrophy
- Zellweger syndrome
- Refsum disease
Thank you
references

- Teitz textbook of clinical chemistry and molecular diagnostics - fifth edition

- Clinical biochemistry- metabolic and clinical aspects - churchill livingstone

- Indian journal of Practical pediatrics. Vol.12 No.2 APR.-JUN.2010