Nitrogen turnover

- Tissue protein, plasma protein, enzymes, hormones, antibodies, hemoglobin
- Dietary Protein
- Glucose, glycogen, Lipids
- Amino Acid Pool
- Non-protein compounds, heme, heterocyclic amines
- Carbon dioxide, water, urea, energy

Nitrogen Balance (NB)

- NB = ingested nitrogen - excreted nitrogen
- Ingested nitrogen – main source – proteins
- Excreted nitrogen – main route – urine
- Reflects the equilibrium of protein metabolism – prevalence of biosynthesis or degradation
Nitrogen Balance (NB)

Types:
1. Positive: Ingested N > excreted N
2. Negative: Ingested N < excreted N
3. Balanced: Ingested N ≈ excreted N

Dietary protein requirements

- Children (until 13 years old) – 13-34 g/24 h
- Adolescents – (13-18 years old) – 34-52 g/24 h
- Healthy adults – 46-56 g/24 h
- Pregnancy – 80 g/24 h

Dietary Proteins - biological value

- Essential and Nonessential Amino Acids in Mammals

Depends on:
1. Amino acid composition – essential vs non-essential amino acids

### Essential and Nonessential Amino Acids in Mammals

<table>
<thead>
<tr>
<th>Essential</th>
<th>Nonessential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>Asparagine</td>
</tr>
<tr>
<td>Histidine</td>
<td>Aspartate</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>Cysteine</td>
</tr>
<tr>
<td>Leucine</td>
<td>Glutamate</td>
</tr>
<tr>
<td>Lysine</td>
<td>Glutamine</td>
</tr>
<tr>
<td>Methionine</td>
<td>Glycine</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>Proline</td>
</tr>
<tr>
<td>Threonine</td>
<td>Serine</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>Tyrosine†</td>
</tr>
</tbody>
</table>

*Arginine and histidine are essential in the diets of juveniles, not adults.
†Tyrosine is classified as nonessential only because it is readily formed from essential phenylalanine.
Dietary Proteins - biological value

Depends on:
2. ability of the organism to assimilate the protein – digest proteins and absorb the amino acids

Dietary Protein Turnover

General pathway of protein digestion
Digestion of dietary proteins

1. Localization: stomach + small intestine;
2. Necessary compounds:
   - proteolytic enzymes;
   - HCl;
   - HCO$_3^-$;
   - transmembrane transporters;
   - local regulations.

Proteolytic enzymes - Proteases

Hydrolases
Proteases or proteinase or peptidases
1. Endopeptidases
2. Exopeptidases:
   a. aminopeptidases
   b. carboxypeptidases
### Proteolytic enzymes

#### Endopeptidases
1. Pepsine
2. Trypsine
3. Chymotrypsine
4. Collagenase
5. Elastase

#### Exopeptidases
1. Aminopeptidase
2. Carboxypeptidase

---

### Specificity of the proteolytic enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Peptide bond formed by:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pepsin</td>
<td>-NH₂ gr. of Phe, Tyr, Trp, Leu, Asp, Glu</td>
</tr>
<tr>
<td>Trypsin</td>
<td>-COOH gr. of Arg and Lys</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>-COOH gr. of Phe, Tyr, Trp</td>
</tr>
<tr>
<td>Carboxypeptidase</td>
<td>Phe, Tyr, Trp, Arg, Lys and hydrophobic C-terminal amino acids</td>
</tr>
<tr>
<td>Aminopeptidase</td>
<td>N-terminal amino acids</td>
</tr>
</tbody>
</table>

### Specificity of the proteolytic enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Produced in:</th>
<th>Produced:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pepsin</td>
<td>Stomach</td>
<td>Non-active</td>
</tr>
<tr>
<td>Trypsin</td>
<td>Pancreas</td>
<td>Non-active</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>Pancreas</td>
<td>Non-active</td>
</tr>
<tr>
<td>Carboxypeptidase</td>
<td>Pancreas</td>
<td>Non-active</td>
</tr>
<tr>
<td>Aminopeptidase</td>
<td>Intestine</td>
<td>Active</td>
</tr>
<tr>
<td>Dipeptidases</td>
<td>Intestine</td>
<td>Active</td>
</tr>
</tbody>
</table>
Activation of the proteolytic enzymes

**Mechanism** – partial proteolysis

**Activators:**
1. Pepsinogen – HCl, pepsin
2. Trypsinogen – enterokinase or enteropeptidase
3. Chymotrypsinogen – trypsin
4. Procarboxypeptidase - trypsin

**Mechanism of partial proteolysis**

- Activation of pepsinogen to pepsin
- Maintenance of optimal pH for pepsin
- Denaturation of dietary proteins
- Contribution to Fe$^{2+}$ absorption
- Antibacterial action

**Role of HCl in the digestion of proteins**
SECRETION OF HYDROCHLORIC ACID

ROLE OF THE CELLS

- **MUCOUS CELLS** - secrete mucus to protect lining from potential destructiveness of acidic gastric juice

- **CHIEF CELLS** - secrete digestive enzymes, mainly pepsinogen

- **PARIETAL CELLS** - secrete HCl and Intrinsic factor

DIGESTION OF PROTEINS IN THE STOMACH

- Entry of protein into the stomach stimulates the secretion of the local hormone gastrin
- Gastrin stimulates secretion of pepsinogen and HCl
- pH – 1,0-2,0
- Denaturation of proteins by HCl
- Activation of pepsinogen to pepsin by HCl and active pepsin
- Pepsin hydrolyzes peptide bonds on the amino-side of Tyr, Phe, and Trp
- Final products of hydrolysis - polypeptides and oligopeptides
Digestion of proteins in small intestine
Role of local hormones

- Secretin stimulates the pancreas to secrete pancreatic juice rich in bicarbonate to neutralize the gastric HCl
- The entry of amino acids into duodenum induce the release of the cholecystokinin, which stimulates secretion of pancreatic juice rich in enzymes

Digestion of proteins in the intestine
Pancreatic Juice

- Is a water solution of enzymes and electrolytes (primarily $\text{HCO}_3^-$)
- Neutralizes acid chyme
  $$\text{HCl} + \text{HCO}_3^- \rightarrow \text{Cl}^- + \text{H}_2\text{CO}_3 \rightarrow \text{H}_2\text{O} + \text{CO}_2 \uparrow$$
- Provides optimal environment for pancreatic enzymes ($\text{pH} \approx 7.0-8.0$)

Digestion of proteins in the intestine
Pancreatic Juice

Enzymes are secreted INACTIVE as ZYMogens or PROenzymes

1. Trypsinogen
2. Chymotrypsinogen
3. Procarboxypeptidase
4. Proelastase
Activation of pancreatic enzymes

[Diagram showing activation of pancreatic enzymes]

Digestion of proteins in the intestine

Intestinal enzymes

The cells of the small intestine secrete active enzymes:

- aminopeptidase - hydrolyze successive amino-terminal residues
- dipeptidases - hydrolase dipeptides

Digestion of proteins in the intestine

Final products

By the sequential action of proteolytic enzymes, ingested proteins are hydrolyzed to yield a mixture of free amino acids.
Acute pancreatitis

In this condition:
- the zymogens of the proteolytic enzymes are converted into their active forms prematurely, inside the pancreatic cells →
- as a result, these proteolytic enzymes attack the pancreatic cell and tissue itself →
- causing a painful and serious destruction of the organ, which can be fatal.

Absorption of amino acids
mechanism I – simport with Na⁺

Absorption of amino acids
mechanism II – γ-glutamyl cycle
Putrefaction of Aminoacids

1. Location – large intestine
2. Mechanism – action of microorganisms
3. Production:
   - Scatol, Indol from Trp
   - Crezol, Phenol from Phe and Tyr
   - Putrescin from ornitine
   - Cadaverine from Lys

Detoxification of putrefaction products

- Location – liver
- Mechanism: I step – oxidation
  II step – conjugation
- Oxidation – microsomal chain of oxidation
- Conjugation – sulfate ion, glucuronic acid, acetyl-, etc.
Detoxification of Indol

Amino acid pool

- 30 g from the total 15 kg of proteins of the organism (if total body mass is about 70 kg)

- Free blood amino acids – 0.35-0.65 g/l.
General pathways of amino acids catabolism

1. Pathways of carboxyl group metabolism – decarboxylation
2. Pathways of amino group metabolism
   - Transamination
   - Deamination
3. Pathways of carbon skeletons catabolism

Pathways of carboxyl group metabolism – decarboxylation

1. α-decarboxylation – formation of biogenic amines
   \[ R\text{-CH-COOH} \rightarrow R\text{-CH}_2\text{-NH}_2 + \text{CO}_2 \]
   \[ \text{NH}_2 \]

2. ω-decarboxylation:
   \[ \text{HOOC-CH}_2\text{-CH-COOH} \rightarrow \text{H}_2\text{C-CH-COOH} + \text{CO}_2 \]
   \[ \text{Asp NH}_2 \quad \text{NH}_2 \quad \text{Ala} \]

Pathways of carboxyl group metabolism – decarboxylation

3. Decarboxylation coupled with transamination
   \[ R\text{-CH-COOH} + R'^{-}\text{C-COOH} \rightarrow R'^{-}\text{C}=\text{O} + R\text{-CH-COOH} + \text{CO}_2 \]
   \[ \text{NH}_2 \quad \text{O} \quad \text{H} \quad \text{NH}_2 \]

4. Decarboxylation coupled with condensation of 2 molecules
   \[ R\text{-CH-COOH} + R'^{-}\text{C-S-CoA} \rightarrow R\text{-CH} \rightarrow C\text{-R}^* + \text{HS-CoA} + \text{CO}_2 \]
   \[ \text{NH}_2 \quad \text{O} \quad \text{NH}_2 \quad \text{O} \]
Amino acids are converted to BIOLOGICAL AMINES by α-decarboxylation.

Histamine synthesis

Substrate – His
Biological action:
1. stimulation of gastric secretion;
2. histaminergic action modulates sleep due to stimulatory effects upon neurons
3. suppressive action that protect against the susceptibility to convulsion, drug sensitization etc.
4. involved in immune system disorders and allergies

GABA synthesis

GABA inhibits synaptic transmission
Antiepileptic remedy
Serotonin synthesis

Serotonin was isolated in 1948 by Page

Catecholamines synthesis

- Tyrosine gives rise to the catecholamines: dopamine, norepinephrine (noradrenaline) and epinephrine (adrenaline)
- Levels of catecholamines are correlated with blood pressure
- The neurological disorder Parkinson’s disease is associated with an underproduction of dopamine

Serotonin role

- neurotransmitter, allowing numerous functions in the human body including the control of appetite, sleep, memory and learning, temperature regulation, mood, behaviour, cardiovascular function, muscle contraction, endocrine regulation and depression.
- a powerful vasoconstrictor in blood
Biological Amines

- overproduction of dopamine in the brain is associated with psychological disorders such as schizophrenia
- low serotonin levels are associated with obsessive-compulsive tendencies or seasonal depression
- histamine excess can be manifest as asthma, vasomotor rhinitis, allergic skin disorders with pruritis, excess stomach acid production, saliva, tears, thin nasal and bronchial secretions, and certain types of vascular headaches

Inactivation of biological amines

\[ \text{H}_2\text{O} + \text{FAD} \xrightarrow{\text{MAO}} \text{FADH}_2 + \text{NH}_3 \]
Common metabolic pathways of the amino-group

1. Transamination
2. Deamination:
   a. direct
   b. indirect

Transamination reaction

- Removal of the α-amino groups from the α-L-amino acids, by →
- Transfer to the α-keto carbon atom of an α-keto acid, mainly α-keto glutarate
- Enzymes – aminotransferases, class transferases
- Goal of transamination – collection of the amino groups from different amino acids in only one – L-glutamate
**Vitamine B6**

Pyridoxine  Pyridoxal  Pyridoxamine

**Vitamine B6**

Pyridoxal phosphate (PALP)  Pyridoxamine phosphate (PAMP)

**PALP and PAMP (deriv. Vit B₆)**

- covalently bound to the E through ε-amino group of Lys
- intermediate carrier of amino groups during transamination by →
- reversible transformations between pyridoxal phosphate (PALP), which accept the amino group, and pyridoxamine phosphate (PAMP), which donates its amino group to the α-keto acid
Transamination of alanine

\[
\text{COO}^- + \text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2^+ \xrightarrow{\text{AT}} \text{CH}_3\text{CH}_2\text{CH}_2\text{COO}^- + \text{COO}^- + \text{H}^+
\]

\[
\text{Alanine} \quad \alpha\text{-ketoglutarate} \quad \text{pyruvate} \quad \text{glutamate}
\]

Transamination of aspartic acid

\[
\text{COO}^- + \text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2^+ \xrightarrow{\text{AsAT}} \text{CH}_3\text{CH}_2\text{CH}_2\text{COO}^- + \text{COO}^- + \text{H}^+
\]

\[
\text{Aspartate} \quad \alpha\text{-ketoglutarate} \quad \text{oxaloacetate} \quad \text{glutamate}
\]
Diagnostical value of aminotransferases

Myocardial infarction
- aminotransferases, among other enzymes, leak from the injured heart cells into the bloodstream
- increased concentration in the blood serum of AsAT
- increased concentration of other heart enzymes creatine kinase (CK), lactate dehydrogenase (LDH) and proteins (myoglobin, troponins etc.)
- can provide information about the severity and the stage of the heart damage

Diagnostical value of aminotransferases in liver diseases

Acute viral hepatitis: ALT – 25-50 times ↑
AST – 15-30 times ↑
maximal values - 2 weeks of the jaundice period
Type A – high values from the beginning and rapidly decrease
Type B – moderate progressive increase during 1-2 months

Deamination

Removal of -NH₂ group from the amino acid in form of ammonia

Types:
1. Direct
2. Indirect
Direct deamination

- oxidativ
  \[
  R-\text{CH-COOH} + \frac{1}{2} \text{O}_2 \rightarrow R-\text{C-COOH} + \text{NH}_3 + \text{NH}_2\text{O}
  \]
- intramolecular
  \[
  R-\text{CH}_2\text{CH-COOH} \rightarrow R-\text{CH}=\text{CH-COOH} + \text{NH}_3 + \text{NH}_2\text{O}
  \]

Direct oxidative deamination of amino acids

Non-oxidative direct deamination of Serine

Serine Dehydratase
Non-oxidative direct deamination of treonine

Histidine non-oxidative direct deamination

Direct deamination of glutamic acid
Direct deamination of glutamic acid

- L-glutamate dehydrogenase (M, 330,000).
- Located in mitochondria
- Co-enzymes - NAD⁺ or NADP⁺
- Final products: ammonia – detoxification
  - NADH.H⁺ – oxidation
  - α-keto glutarate – oxidation

Regulation of Glu deamination

- glutamate dehydrogenase is an allosteric enzyme
- positive modulator – ADP
- negative modulator – GTP

Indirect deamination of aminoacids

2 steps:
1 step – transamination – goal: collection of the -NH₂ groups in the structure of GLU
2 step – direct deamination of Glu – goal: removal of -NH₂ group in form of ammonia
Sources of blood ammonia

- Amino acids deamination
- Biogenic amine detoxification
- Catabolism of nitrogen bases
- Absorption from large intestine (putrefaction of amino acids)
Ammonia toxicity

- Liver diseases →
- Disorders of the urea cycle →
- Increased concentration of ammonia in the blood (hyperammoniemia)

Ammonia toxicity

Detoxification of the excess ammonia disturbs the energetic metabolism due to:

- reductive amination of α-keto glutarate to Glu – depletes cellular NADH and α-keto glutarate, required for ATP production
- conversion of Glu to glutamine by glutamine synthetase – depletes ATP itself

Brain ammonia toxicity

- ammonia passes brain blood barrier
- disorders or absence of aerobic oxidative phosphorylation and TCA cycle activity damage neural cell
Brain ammonia toxicity

- Increased ammonia leads to glutamine formation from Glu
- This depletes glutamate stores which are needed in neural tissue for neurotransmitters synthesis (glutamate itself and GABA)
- Therefore, reductions in brain glutamate disturb energy production as well as neurotransmission

Mechanisms of ammonia detoxification

- Temporary detoxification – biosynthesis of Asn and Gln
- Final detoxification:
  a) formation and renal excretion of ammonia salts
  b) synthesis of urea

Mechanism of Asn and Gln biosynthesis (1)
Mechanism of Asn and Gln biosynthesis (2)

Biological role of Asn and Gln

- Transport of NH$_3$ from different tissues to the site of excretion (kidneys) and the site of final detoxification (liver)
- Asn and Gln are nontoxic, neutral compound that pass through cell membranes, whereas aspartate and glutamate, which bears a net negative charge, cannot.

Ammonia release from Asn and Gln

- Asparagine is converted to aspartate by asparaginase, releasing NH$_3$ into the circulation.
Ammonia release from Asn and Gin

- NH₂ release from Asn and Gin

Urea cycle
Krebs-Henseleit cycle

- Role – final and complete detoxification of ammonia
- Final product – urea
- Location – hepatocytes – partial mitochondria, partial cytosol
- Energy consumption – 3 mol of ATP or 4 high energetic bonds
- Sources of NH₂ groups: NH₃ and Asp
Carbamoyl phosphate synthetase reaction of urea synthesis

E - Carbamoyl phosphate synthetase I (CPS I)
mitochondrial matrix enzyme
is positively allosterically regulated by \( \text{N-acetylglutamate} \)

1st reaction of the urea cycle

E – carbamoylphosphate ornitine transferase
Citrouline transported to cytoplasm by specific transporter; mechanism – antiport with ornithine

2nd reaction of the urea cycle

E – argininosuccinate synthetase
Consumed – 1 ATP=2 high energetic bonds
3rd reaction of the urea cycle

E – argininosuccinate lyase

4th reaction of the urea cycle

Regulation of the activity of the urea cycle

- **Long term** – gene level – biosynthesis of the enzymes; regulating factor – ammonia concentration in the blood
- **Short term** – allosteric regulation – carbamoylphosphate synthetase; positive modulator – N-acetylglutamate
Metabolism of the carbon skeletons of the amino acids

Carbon skeletons – the α-keto acids produced during the deamination of the amino acids

Amino acids are degraded to:
- pyruvate,
- α-ketoglutarate,
- succinyl-CoA,
- fumarate,
- oxaloacetate,
- acetyl-CoA,
- acetoacetate.

Utilisation of the carbon skeletons:
1. enter into the Krebs cycle and are oxidased
Metabolism of the carbon skeletons of the amino acids

Utilisation of the carbon skeletons:

2. are transformed into glucose – glucogenic amino acids

3. are transformed into ketone bodies – ketogenic amino acids
Glucogenic amino acids

Carbon skeletons of glucogenic amino acids are degraded to:
- pyruvate, or
- intermediates of Krebs Cycle:
  - 4-C (succinil, succinate, fumarate or OA)
  - 5-C (α-ketoglutarate).

Glucogenic amino acids

1. are the major carbon source for gluconeogenesis when glucose levels are low.
2. can be converted to glycogen or or fatty acids for energy storage.
3. can be catabolized for energy

Ketogenic amino acids

Carbon skeletons of ketogenic amino acids are degraded to:
- acetyl-CoA, or
- acetoacetate.
Can be converted to:
1. ketone bodies
2. fatty acids
Can be catabolized for energy in Krebs Cycle
Metabolism of individual amino acids

Tetrahydrofolic acid
Folic acid (or vit. B₉ or vit. B₃)

Functions of the Tetrahydrofollic Acid

- Carry and transfer one carbon units from catabolic reaction to biosynthetic one
- The one carbon units are:
  - methyl (-CH₃)
  - methylene (-CH₂-)
  - methenyl (=CH-)
  - formyl (-COH)
  - formimino (-CO=NH)
  - etc.

One carbon units attached to the active center of FH₄
Folic acid - RDA

- RDA (recommended daily allowance) for adults – 400 μg
- RDA for pregnant women – 600 μg

- the best sources for folic acid:
  - beef liver;
  - baked beans,
  - raw spinach,
  - green peas,
  - broccoli,
  - avocado,
  - peanuts,
  - wheat germ,
  - tomato juice,
  - orange juice.

Functions of the Tetrahydrofolic Acid

- Nucleic Acid Synthesis
- Methylion Reactions

Metabolism of Gly and Ser
Metabolism of Gly and Ser

1. Non-essential amino acids
   - Gly is produced from Ser and Tre
   - Ser is produced from Gly and 3-phosphoglycerate
2. Glucogenic amino acids

Metabolism of Gly and Ser

Interconversion of Gly and Ser

Biosynthesis of serine
Metabolism of Asp

Lys METABOLISM

Lys – essential amino acid

Lysine catabolism
Pro metabolism
Pro biosynthesis

Pro hydroxylation

Metabolism of cromoproteins
Cromoproteins

- Hempoproteins
- Flavoproteins

Hempoproteins

- Hemoglobin
- Myoglobin
- Cytocromes
- Catalase
- Peroxidase
- Etc.
Hemoglobin

Heme synthesis

1. Globin synthesis – ribosomes
2. Hem synthesis:
   a. heme is synthesized in all tissues
   b. the principal sites of synthesis are:
      – erythroid cells (≈85%)
      – hepatocytes (accounting for nearly all the rest of heme synthesis)

Heme synthesis

– 1st and the last 2 reactions – in the mitochondria
– the rest – in the cytosol
Heme synthesis – regulation

1. **transcription of ALA synthase** (1 reaction) –
   controlled by $\text{Fe}^{2+}$-binding elements →
   prevents accumulation of porphyrin intermediates in the absence of $\text{Fe}^{2+}$

2. **ALA synthase** is inhibited by heme and hemin

Disorders of Heme synthesis - porphyrias

<table>
<thead>
<tr>
<th>Intermediates</th>
<th>Enzymes</th>
<th>Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine + Glutamic Acid</td>
<td>Aminolevulinic Acid Synthase</td>
<td>Acute Intermittent Porphyria</td>
</tr>
<tr>
<td>δ-Aminolevulinic Acid</td>
<td>δ-ALA Dehydratase</td>
<td>Porphyria Cutanea Tarda</td>
</tr>
<tr>
<td>Porphobilinogen</td>
<td>Uroporphyrinogen III Cosynthase</td>
<td>Hereditary Coproporphyria</td>
</tr>
<tr>
<td>Coproporphyrinogen</td>
<td>Uroporphyrinogen Decarboxylase</td>
<td>Erythropoietic Protoporphyria</td>
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<tr>
<td>Protoporphyrinogen</td>
<td>Coproporphyrinogen Oxidase</td>
<td>Vanillic Acid Porphyria</td>
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<tr>
<td>Coproporphyrinogen</td>
<td>Ferrochelatase</td>
<td></td>
</tr>
</tbody>
</table>
Hemoglobin catabolism

Conjugated bilirubin
### Differential diagnosis of jaundice

<table>
<thead>
<tr>
<th>Type of Jaundice</th>
<th>Hyperbilirubinemia</th>
<th>Urine Bilirubin</th>
<th>Urine Urobilinogen</th>
<th>Other Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-hepatic (hemolytic)</td>
<td>Unconjugated</td>
<td>Absent</td>
<td>Increased</td>
<td>Degree of urine urobilinogen increases directly related to increased hemoglobin catabolism</td>
</tr>
<tr>
<td>Intrahepatic (hepatocellular)</td>
<td>Conjugated and unconjugated</td>
<td>Increased</td>
<td>Normal to decreased</td>
<td>Increased liver enzymes: alanine transaminase (ALT), aspartate transaminase (AST)</td>
</tr>
<tr>
<td>Post-hepatic (obstructive)</td>
<td>Conjugated</td>
<td>Increased</td>
<td>Decreased</td>
<td>Increased alkaline phosphatase and cholesterol; may have increased ALT and AST</td>
</tr>
</tbody>
</table>