The metabolic functions of the liver
Catabolism of haemoglobin, bilirubin
Metabolism of iron
The major metabolic functions of the liver:

– uptake of most nutrients from the gastrointestinal tract,
– intensive intermediary metabolism, conversion of nutrients,
– controlled supply of essential compounds (glucose, VLD lipoproteins, ketone bodies, plasma proteins, etc.),
– ureosynthesis, biotransformation of xenobiotics (detoxification),
– excretion (cholesterol, bilirubin, hydrophobic compounds, some metals).
The hepatocytes (the hepatic parenchymal cells) have an immensely broad range of synthetic and catabolic functions with a substantial reserve metabolic capacity.

Many of them are the **specialized metabolic functions of the liver:**

**Metabolism of saccharides**
- Primary regulation of the **blood glucose concentration**. E.g. in the postprandial state, there is an uptake of about 60% of glucose supplied in portal blood and stored as glycogen, or in hypoglycaemia, glycogenolysis and gluconeogenesis is initiated.
- The liver cells meet their energy requirements preferentially from fatty acids, not from glycolysis. Glucose (also as glycogen store) is altruistically spared for extrahepatic tissues.

**Metabolism of lipids**
- Completion and secretion of VLDL and HDL.
- **Ketogenesis** produces ketone bodies, precious nutrients. They cannot be utilized in the liver, but they are supplied to other tissues.
- Secretion of cholesterol and bile acids into the bile represents the major way of cholesterol elimination from the body.
- **Dehydrogenation of cholesterol to 7-dehydrocholesterol and 25-hydroxylation of calciols** play an essential role in calcium homeostasis.
Metabolism of nitrogenous compounds
– Deamination of amino acids that are in excess of requirements.
– Intensive proteosynthesis of major plasma proteins and blood-clotting factors.
– Uptake of ammonium, ureosynthesis.
– Bilirubin capturing, conjugation, and excretion.

Biotransformation of xenobiotics
– Detoxification of drugs, toxins, excretion of some metals.

Transformation of hormones
– Inactivation of steroid hormones – hydrogenation, conjugation.
– Inactivation of insulin, about 50 % insulin inactivated in its only passage through the liver (GSH:insulin transhydrogenase splits the disulfide bonds, then proteolysis of the two chains).
– Inactivation of catecholamines and iodothyronines, conjugation of the products.

Vitamins
– Hydroxylation of calciols to calcidiols, splitting of β-carotene to retinol.
– The liver represent a store of retinol esters and cobalamin (B₁₂).

Iron and copper metabolism
– Synthesis of transferrin, coeruloplasmin, ferritin stores, excretion of copper.
The liver parenchymal cell (hepatocyte)

Columns (cords) of hepatocytes are surrounded by sinusoids lined by endothelial fenestrated layer (without a basement membrane) and Kupffer cells (mononuclear phagocytes).

Plasmatic membrane directed to the space of Disse – the "blood" pole, the "bile" pole – lateral parts of the membrane with gap junctions and parts forming bile capillaries.
The liver receives venous blood from the intestine. Thus all the products of digestion, in addition to ingested drugs and other xenobiotics, perfuse the liver before entering the systemic circulation.

The mixed portal and arterial blood flows through sinusoids between columns of hepatocytes.

Hepatocytes are differentiated in their functions according to the decreasing $pO_2$. In a simple liver acini, there are **three zones** equipped with different enzymes.
Hexagonal hepatic lobules round terminal hepatic venules are not functional units.

**simple liver ACINUS** – a functional unit

efferent vessels
at least two terminal hepatic venules

arterial blood
terminal branches aa. hepaticae

portal (venous) blood
from intestine, pancreas, and spleen

portal field with afferent vessels

bile ductules
Metabolic areas in the acini

Zone 1 – periportal area

- High $pO_2$
- Cytogenesis, mitosis
- Numerous mitochondria
- Glycogenesis and glycogenolysis
- Proteosynthesis
- Ureosynthesis

Zone 3 – microcirculatory periphery

- Low $pO_2$
- High activity of ER (cyt P450, detoxification)
- Pentose phosphate pathway
- Hydrolytic enzymes
- Glycogen stores, fat and pigment stores
- Glutamine synthesis
Liver of a patient who died in hepatic coma:

Seastar-shaped necrotic lesion around the terminal hepatic venule. This shape is produced by necrosis creeping along zones 3 of the simple acini, intercalating between them and reaching portal spaces.
Liver – production of bile

### Composition of bile

<table>
<thead>
<tr>
<th></th>
<th>Hepatic bile</th>
<th>Gall-bladder bile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inorganic salts</td>
<td>8.4</td>
<td>6.5</td>
</tr>
<tr>
<td><strong>Bile acids</strong></td>
<td><strong>7 – 14</strong></td>
<td><strong>32 – 115</strong></td>
</tr>
<tr>
<td><strong>Cholesterol</strong></td>
<td><strong>0.8 – 2.1</strong></td>
<td><strong>3.1 – 16.2</strong></td>
</tr>
<tr>
<td>Bilirubin glucuronates</td>
<td><strong>0.3 – 0.6</strong></td>
<td><strong>1.4</strong></td>
</tr>
<tr>
<td><strong>Phospholipids</strong></td>
<td><strong>2.6 – 9.2</strong></td>
<td><strong>5.9</strong></td>
</tr>
<tr>
<td>Proteins</td>
<td><strong>1.4 – 2.7</strong></td>
<td><strong>4.5</strong></td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td><strong>7.1 – 7.3</strong></td>
<td><strong>6.9 – 7.7</strong></td>
</tr>
</tbody>
</table>

### Functions

The **bile acids** emulsify lipids and fat-soluble vitamins in the intestine. High concentrations of bile acids and **phospholipids** stabilize micellar dispersion of cholesterol in the bile (crystallization of cholesterol → cholesterol gall-stones).

**Excretion** of cholesterol and bile acids is the major way of removing cholesterol from the body. Bile also removes hydrophobic metabolites, drugs, toxins and metals (e.g. copper, zinc, mercury).

**Neutralization** of the acid chyme in conjunction with $\text{HCO}_3^-$ from pancreatic secretion.
Degradation of haemoglobin to bilirubin– bile pigments

Erythrocytes are taken up by the reticuloendothelial cells (cells of the spleen, bone marrow, and Kupffer cells in the liver) by phagocytosis.

In blood plasma, hydrophobic bilirubin molecules (called unconjugated bilirubin) are transported in the form of complexes bilirubin-albumin.
Bilirubin IXα
configuration 4Z,15Z

Polarity of the two carboxyl groups of unconjugated bilirubin is masked by formation of hydrogen bonds between the carboxyl groups and the electronegative atoms within the opposite halves of bilirubin molecules.
The hepatic uptake, conjugation, and excretion of bilirubin

**UNCONJUGATED bilirubin** (bilirubin-albumin complex) in hepatic sinusoids

- Bilirubin receptor (bilitranslocase)
- Albumin
- Ligandin (protein Y)

**UDP-glucuronate**

- glucosyluronate transferase on ER membranes
- bilirubin monoglucosiduronate
- bilirubin bisglucosiduronate

**CONJUGATED bilirubin**

- is polar, water-soluble – active transport into bile capillaries in the form of micelles depends on the bile acids

The amount that can leak from excretion in the plasma membrane of hepatocytes.
The formation of urobilinoids by the intestinal microflora

Conjugated bilirubin

is secreted into the bile. As far as bilirubin remains in the conjugated form, it cannot be absorbed in the small intestine.

In the large intestine, bacterial reductases and β-glucuronidases catalyze deconjugation and hydrogenation of free bilirubin to mesobilirubin and urobilinogens:

A part of urobilinogens is split to dipyrrromethenes, which can condense to give intensively coloured bilifuscins.

Urobilinogens are partly
– absorbed (mostly removed by the liver), a small part appears in the urine,
– partly excreted in the feces; on the air, they are oxidized to dark brown faecal urobilins.
Healthy individuals:

**Plasma:** unconj. bilirubin-albumin complex < 20 µmol / l

- Uptake of bilirubin, its conjugation and excretion

V. lienalis (the blood from the spleen flows into the portal vein)

- Small amounts of urobinogens not removed by the liver

**Urine:**
- urobinogens < 5 mg / d

**Feces:**
- urobinoids and bilifuscins ~ 200 mg / d

Most of urobinogens are removed in the liver (oxidation?)

Portal urobinogens

Conjugated bilirubin

Urobilinogens and dipyrromethenes
In the **absence of intestinal microflora** (before colonization in newborns or during treatment with broad-spectrum antibiotics):

**Plasma:** normal bilirubin concentration

**Uptake** of bilirubin and its **conjugation**

**Excretion** into the bile

**Conjugated bilirubin**

Intestinal flora is lacking or inefficient (speedy passage in diarrhoea)

**Feces:** **BILIRUBIN** (golden-yellow colour that turns green on the air), urobilins and bilifuscins are absent

**Urine:** urobilinogens are absent
Major types of hyperbilirubinaemias

Hyperbilirubinaemia – serum bilirubin > 20 – 22 µmol / l

Icterus (jaundice) – yellowish colouring of scleras and skin, serum bilirubin usually more than 30 – 35 µmol / l

The causes of hyperbilirubinaemia are conventionally classified as

– **prehepatic** (haemolytic) – increased production of bilirubin,

– **hepatocellular** due to inflammatory disease (infectious hepatitis), hepatotoxictoxic compounds (e.g. ethanol, acetaminophen), or autoimmune disease; chronic hepatitis can result in liver cirrhosis – fibrosis of hepatic lobules,

– **posthepatic** (obstructive) – insufficient drainage of intrahepatic or extrahepatic bile ducts (cholestasis).
Prehepatic (haemolytic) hyperbilirubinaemia
– excessive erythrocyte breakdown

Blood serum:
**unconjugated bilirubin** elevated

**Feces:** polycholic (high amounts of urobilinoids and bilifuscins)

**Urine:** increased urobilinogens (no bilirubinuria)

increased urobininogens are not removed sufficiently

high portal urobininogens

high supply of urobininogens (bilirubin-albumin complexes cannot pass the glomerular filter)

**Blood serum:**
intensive uptake, conjugation, and excretion into the bile

elevated unconjugated bilirubin

increased urobilinogens (bilirubin-albumin complexes cannot pass the glomerular filter)
Hepatocellular hyperbilirubinaemia

The results of biochemical test depend on whether an impairment of hepatic uptake, conjugation, or excretion of bilirubin predominates.

Blood serum: 
- **unconj. bilirubin** is elevated, when its uptake or conjugation is impaired
- **conj. bilirubin** is elevated, when its excretion or drainage is impaired

ALT (and AST) catalytic concentrations increased

Urine: 
- Increased urobilinogens (unless bilirubin excretion is impaired)
- Bilirubinuria (when plasma conj. bilirubin increases)

Feces: normal contents (unless excretion is impaired)

Impairment in uptake, conjugation or excretion of bilirubin.

Portal urobilinogens are not removed efficiently.

Conj. bilirubin (unless its excretion is impaired)

Urobilinogens and conjugated bilirubin pass into the urine (not unconj. bilirubin-albumin complexes)
Obstructive (posthepatic) hyperbilirubinaemia

Blood serum:
- **conjugated bilirubin** elevated
- **bile acids** concentration increased
catalytic concentration of **ALP** increased

leakage of conj. bilirubin from the cells into blood plasma

*uptake* of bilirubin and its *conjugation*

low conj. bilirubin (if obstruction is not complete)

conjugated bilirubin passes into the urine

*Feces:* urobilinoids and bilifuscins **decreased** or **absent** (grey, **acholic** feces)

*Urine:* urobilinogens are lowered or **absent**

**bilirubinuria**
Summary:

<table>
<thead>
<tr>
<th>Type</th>
<th>Bilirubin</th>
<th>Urobilinogens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(derivatives)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Blood serum</td>
<td>Blood</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>Urine</td>
</tr>
<tr>
<td></td>
<td>Feces</td>
<td></td>
</tr>
<tr>
<td>PREHEPATIC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(or haemolytic)</td>
<td>increased (unconjugated)</td>
<td>increased</td>
</tr>
<tr>
<td></td>
<td>absent</td>
<td></td>
</tr>
<tr>
<td>HEPATOCELLULAR</td>
<td>increased (unconjugated)</td>
<td>present</td>
</tr>
<tr>
<td></td>
<td>present (unconjug.)</td>
<td></td>
</tr>
<tr>
<td>OBSTRUCTIVE</td>
<td>increased (conjugated)</td>
<td>present</td>
</tr>
<tr>
<td>(posthepatic)</td>
<td>(unconj.)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>absent or absent</td>
<td></td>
</tr>
</tbody>
</table>

21
Laboratory tests for detecting an impairment of liver functions ("liver tests")

- Plasma markers of hepatocyte membrane integrity

Catalytic concentrations of intracellular enzymes in blood serum increase:
An assay for alanine aminotransferase (ALT) activity is the most sensitive one.
In severe impairments, the activities of aspartate aminotransferase (AST) and glutamate dehydrogenase (GD) also increase.

Increase of catalytic concentrations:

- Moderate injury
- Severe damage

ALT  AST  GD
• **Tests for decrease in liver proteosynthesis**
  Serum concentration of *albumin* (biological half-time about 20 days), *transthyretin* (prealbumin, biological half-time 2 days) and *transferrin*, *blood coagulation factors* (prothrombin time increases), activity of serum non-specific *choline esterase* (ChE).

• **Tests for the excretory function and cholestasis**
  Serum *bilirubin concentration*
  Serum catalytic concentration of *alkaline phosphatase* (ALP) and *γ-glutamyl transpeptidase* (γGT)
  Test for *urobilinogens* and *bilirubin* in urine
  Estimation of the excretion rate of *bromosulphophthalein* (BSP test) is applied to convalescents after acute liver diseases.

• **Tests of major metabolic functions** are not very decisive:
  Saccharide metabolism  low glucose tolerance (in oGT test)
  Lipid metabolism  increase in VLDL (triacylglycerols) and LDL (cholesterol)
  Protein catabolism  decreased urea, ammonium increase
  (in the final stage of liver failure, hepatic coma)

• **Special tests to specific disorders:** serological tests to viral hepatitis, serum α-foetoprotein (liver carcinoma), porphyrins in porphyrias, etc.
Metabolism of iron

The body contains 4 – 4.5 g Fe:
- In the form of haemoglobin: 2.5 – 3.0 g Fe,
- tissue ferritin stores: up to 1.0 g Fe in men (0.3 – 0.5 g in women),
- myoglobin and other haemoproteins: 0.3 g Fe,
- circulating transferrin: 3 – 4 mg Fe.

The daily supply of iron in mixed diet is about 10 – 20 mg. From that amount, not more than only 1 – 2 mg are absorbed. Iron metabolism is regulated by control of uptake, which have to replace the daily loss in iron and prevent an uptake of excess iron.

A healthy adult individual loses on average 1 – 2 mg Fe per day in desquamated cells (intestinal mucosa, epidermis) or blood (small bleeding, so that women are more at risk because of net iron loss in menstruation and pregnancy).

There is no natural mechanism for eliminating excess iron from the body.
Absorption of iron in duodenum and jejunum

Phosphates, oxalate, and phytate (myo-inositol hexakis(dihydrogen phosphate), present in vegetable food) form insoluble Fe\(^{3+}\) complexes and disable absorption. Fe\(^{2+}\) is absorbed much easier than Fe\(^{3+}\). Reductants such as ascorbate or fructose promote absorption, as well as Cu\(^{2+}\).

Gastroferrin, a component of gastric secretion, is a glycoprotein that binds Fe\(^{2+}\) maintaining it soluble and prevents its oxidation to Fe\(^{3+}\), from which insoluble iron salts are formed.

Elimination of insoluble Fe salts in feces
**Transferrin (Trf)**

is a plasma glycoprotein (a major component of $\beta_1$-globulin fraction), $M_r$ 79 600.

Plasma (serum) **transferrin concentration 2.5 – 4 g / l (30 – 50 µmol / l)**

Transferrin molecules have two binding sites for Fe ions,
**total iron binding capacity** (TIBC) for Fe ions is higher than **60 µmol / l**.

**Serum Fe$^{3+}$** (i.e. transferrin-Fe$^{3+}$) concentration is about **10 – 20 µmol / l**,  
14 – 26 µmol / l in men,  
11 – 22 µmol / l in women.

Circadian rhythm exists, the morning concentrations are higher by 10 - 30 % than those at night..

**Saturation of transferrin** with Fe$^{3+}$ equals usually **about 1/3**.

Because the biosynthesis of transferrin is stimulated during iron deficiency (and plasma iron concentration decreases), the decrease in saturation of transferrin is observed.
Iron is taken up by the cells through a specific receptor-mediated endocytosis.

Some receptors are released from the plasmatic membranes. Increase in serum concentration of those **soluble transferrin receptors** is the earliest marker of iron deficiency.
Ferritin

Ferritin occurs in most tissues (especially in the liver, spleen, bone-marrow, and enterocytes).

The protein apoferritin is a ball-shaped homopolymer of 24 subunits that surrounds the core of hydrated iron(III) hydroxide. One molecule can bind few thousands of Fe$^{3+}$ ions, which make up to 23 % of the weight of ferritin.

Minute amounts of ferritin are released into the blood plasma from the extinct cells. Plasma ferritin concentration 25 – 300 µg/l is proportional to the ferritin stored in the cells, unless the liver is impaired (increased ferritin release from the hepatocytes).

If the loading of ferritin is excessive, ferritin aggregate into its degraded form, haemosiderin, in which the mass fraction of Fe$^{3+}$ can reach 35 %.

Ferritin was discovered by V. Laufberger, professor at Masaryk university, Brno, in 1934.
**Hepcidin**

is a polypeptide \( M_r \sim 2000, 25 \) amino acid residues, from which \( 8 \) are Cys), discovered as the liver-expressed antimicrobial peptide, LEAP-1, in 2000. It is produced by the liver (to some extent in myocard and pancreas, too) as a **hormone that limits the accessibility of iron** and also exhibits certain antimicrobial and antifungal activity.

The biosynthesis of hepcidin is stimulated in iron overload and in inflammations (hepcidin belongs to acute phase proteins type 2), and is suppressed during iron deficiency.

Notice the fact that the same two factors stimulating hepcidin synthesis inhibit the biosynthesis of transferrin.

**Effects of hepcidin:** It – reduces \( \text{Fe}^{2+} \) absorption in the duodenum,  
– prevents the release of recyclable \( \text{Fe} \) from macrophages,  
– inhibits \( \text{Fe} \) transport across the placenta,  
– diminishes the accessibility of \( \text{Fe} \) for invading pathogens.

Hepcidin is filtered in renal glomeruli and not reabsorbed in the renal tubules. So the amount of hepcidin excreted into the urine corresponds with the amount synthesized in the body. There is a positive correlation between this amount of hepcidin and the concentration of ferritin in blood plasma.