

Hemoglobin and its derivatives (LF MU)

Hemoglobin is a red blood pigment that ensures the transport of oxygen from the lungs to the tissues and the transport of CO₂ and protons from the peripheral tissues to the respiratory organs. The concentration of hemoglobin in a healthy adult man is approximately 150 g/l, in an adult woman about 140 g/l. One gram of hemoglobin can bind up to 1.34 ml of oxygen. [1]

The structure of hemoglobin

It is a **tetrameric protein** made up of four subunits. Always two and two subunits are identical. In physiologically occurring hemoglobins, there are four types of polypeptide chains α , β , γ , and δ , which differ in the number and sequence of amino acids. The tetramer is made up of two α chains and two other types of chains that give the character of the entire hemoglobin molecule. In adults, hemoglobin A predominates, the structure of which includes, in addition to two α chains (141 amino acids), two β chains (146 amino acids).

Each subunit includes a polypeptide chain to which one heme is covalently bound. The basis of the heme molecule is protoporphyrin, formed by 4 pyrrole nuclei connected by methenyl bridges with centrally bound iron. Heme iron is six-bonded overall – it is connected to the nitrogen atoms of the pyrrole nuclei by four coordination bonds. Another coordination valency binds iron to the imidazole group of the amino acid histidine in the globin chain. The sixth valency of Fe is assigned to the oxygen molecule (O₂).



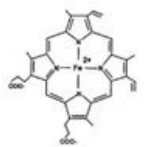
Difference between venous and arterial blood

Hemoglobin in the blood

Determination of hemoglobin in the blood is one of the most basic laboratory tests. The concentration of hemoglobin in the blood is the main criterion for assessing whether it is anemia. The term anemia is used when hemoglobin or erythrocytes fall below the lower limit of physiological values. Anemia is one of the most common clinical findings. It is a condition that leads to a decrease in the binding capacity for oxygen and a subsequent disorder of tissue respiration.



Hemoglobin



Heme

Causes of anemia

Anemia occurs when erythropoiesis is unable to meet the requirements for the production of new red blood cells. It develops as a result of blood loss or increased destruction of red blood cells or insufficient production of red blood cells. The following overview lists some specific causes of anemia:

1. *Anemia from increased blood loss* :
 - Acute blood loss.
 - Chronic blood loss.
2. *Anemia from increased breakdown of erythrocytes* (hemolytic states).
 - Autoimmune hemolytic anemia (presence of antibodies against one's own erythrocytes).
 - Erythrocyte membrane disorder (deviation in the composition of the erythrocyte membrane).
 - Hereditary enzyme defects of erythrocytes (pyruvate kinase, glucose-6-phosphate dehydrogenase).
 - Unstable hemoglobins – hemoglobinopathy (e.g. hemoglobin S in sickle cell anemia).
3. *Anemia from reduced erythrocyte production* .
 - Lack of substances needed for erythropoiesis (lack of iron, lack of vitamin B12, lack of folic acid, lack of erythropoietin - chronic renal diseases, lack of other substances, e.g. vitamins B1, B6).
 - Anemia due to chemical, physical and radiation damage.
 - Anemia in chronic inflammatory, infectious and tumor diseases.

An increase in hemoglobin values can be a manifestation of dehydration of the organism or chronically reduced pulmonary ventilation. Rarely, it can be caused by some myeloproliferative conditions, eg polycythemia vera.

Principle of determination of hemoglobin in blood

Oxidation of hemoglobin to methemoglobin:

HbFe II	+	[Fe III (CN) 6] 3–	→	HbFe III	+	[Fe II (CN) 6] 4–
Hemoglobin				Methemoglobin		

Conversion of methemoglobin to cyanomethemoglobin:

HbFe III	+	CN –	→	HbFe III CN
Methemoglobin				Cyanmethemoglobin

The photometric determination is based on the oxidation of divalent iron in hemoglobin by potassium hexacyanoferrate to trivalent iron. In further reaction with potassium cyanide, the resulting methemoglobin is converted into very stable cyanomethemoglobin with a single broad absorption maximum in the visible region at 540 nm.

Evaluation: The reference range of hemoglobin concentration in the blood (hemoglobin B) for an adult man is 130-180 g/l and for a woman 120-160 g/l.

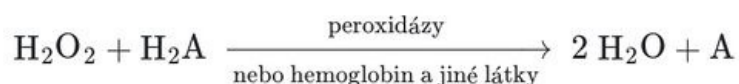
Task: Determination of hemoglobin in blood (https://portal.med.muni.cz/index.php?f=chyba&redirect_pg=download&redirect_id=658&error=2¤t_priv=2) (pdf)

Hemoglobin in urine

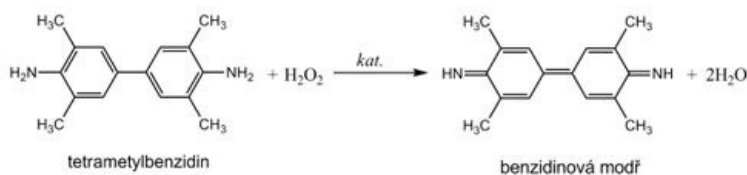
Up to a million erythrocytes per day are excreted in the urine of completely healthy people. This very small quantity cannot be demonstrated by ordinary chemical tests. The occurrence of a large number of erythrocytes (**hematuria** , erythrocyturia) or penetration of free hemoglobin, or of muscle myoglobin into definitive urine (**hemoglobinuria** , or myoglobinuria) is almost always a pathological finding. **Macroscopic hematuria** is observed with the naked eye; urine has a pinkish color (comparable to water from washed meat) and hemoglobin can be detected in it spectroscopically. Urine contains at least 1 g of hemoglobin per liter. In massive hemoglobinuria, the urine can even have a dark beer color (degradation of hemoglobin to hematin). **Microscopic hematuria** is demonstrable only biochemically.

Determination of hemoglobin in urine

Hemoglobin catalyzes, similarly to peroxidase , the oxidation (dehydrogenation) of some substrates (e.g. benzidine derivatives) by hydrogen peroxide:



However, it is not an enzyme activity (catalysis is conditioned by heme iron), and therefore it is not lost even after thermal denaturation. We are talking about **pseudoperoxidase activity** , which is used for sensitive but non-specific evidence of hemoglobin or trace amounts of blood. It is advantageous to use a chromogenic substrate to monitor the reaction, i.e. a substance that gives a distinctly colored product by dehydrogenation (often benzidine or its non-carcinogenic derivatives, aminophenazone, etc.).



The reagent zone of diagnostic strips contains a chromogen (e.g. tetramethylbenzidine) with stabilized hydrogen peroxide (e.g. cumene hydroperoxide). In the presence of free hemoglobin (hemoglobinuria), the indicator zone becomes uniformly blue. If erythrocytes are present in the urine (**erythrocyturia**), intensely greenish-blue colored dots or spots are formed.

Hemoglobinuria can be seen in intravascular hemolysis . Damage to the glomerular membrane (glomerular hematuria) and bleeding from any part of the urinary tract lead to more frequent erythrocyturia. It is often found in urinary tract infections , urolithiasis and tumors of the urogenital tract .

In addition to hemoglobin, myoglobin also provides a pseudoperoxidase reaction , which can be excreted in the urine during the breakdown of skeletal muscle (rhabdomyolysis , crush syndrome). The positivity of the test can also be caused by peroxidases of leukocytes or some bacteria, yeasts or fungi, which can occur in urine, especially during urinary tract infections . If we want to exclude the possibility of a false positive reaction due to the effect of cellular peroxidases, the reaction must be performed with boiled urine.

Contamination of the collection vessel with strong oxidizing agents also causes a false positive reaction. On the other hand, the presence of strongly reducing substances (e.g. ascorbic acid) can slow down or stop the pseudoperoxidase reaction and thus be the cause of false negative results.

Assignment: Detection of blood and blood dye in urine (https://portal.med.muni.cz/index.php?f=chyba&redirect_pg=download&redirect_id=652&error=2¤t_priv=2) (pdf)

Hemoglobin in the stool - occult bleeding

Evidence of occult (hidden) bleeding is used to detect the early stages of colorectal cancer, when radical and effective treatment is possible. The examination consists of capturing traces of blood in the stool, various methodological procedures are used:

- The methods use **the pseudoperoxidase activities** of hemoglobin. The patient must follow a diet for 3 days before the examination, exclude uncooked meat, salami, bananas, tomatoes from the diet, must not take medicines containing ascorbic acid or acetylsalicylic acid. After that, the patient himself takes samples from three consecutive stools and applies them to the test cards. The evaluation is carried out in the laboratory, the principle is similar to that of *hemoPHAN diagnostic strips*
- Other methods are based on **the immunochemical detection** of hemoglobin using an antibody against human hemoglobin. Immunochemical methods are more sensitive and specific, there is no need to keep a diet before the examination. Positive results must be verified by other diagnostic methods.

Task: Test for occult bleeding in the digestive tract (https://portal.med.muni.cz/index.php?f=chyba&redirect_pg=download&redirect_id=660&error=2¤t_priv=2) (pdf)

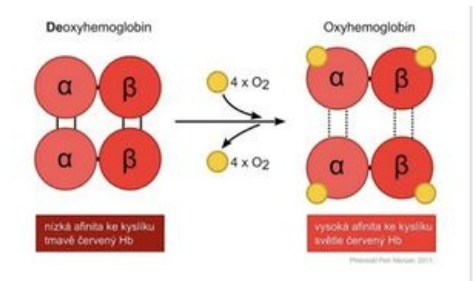
Hemoglobin derivatives

Hemoglobin derivatives include the following types:

Oxyhemoglobin and deoxyhemoglobin

Oxyhemoglobin and deoxyhemoglobin

Hemoglobin carrying oxygen is referred to as **oxyhemoglobin (oxyHb)**. Each Hb molecule can bind 4 oxygen molecules. After the release of oxygen, we speak of **deoxyhemoglobin (deoxyHb)**. In both forms, iron is divalent, as only hemoglobin containing Fe II+ can reversibly bind and transport an oxygen molecule. Oxygenation of the hemoglobin molecule changes the electron state of the Fe II+ -heme complex, which is manifested by a change in the color of the dark red shade typical of venous blood to the bright red color of arterial blood. In the human body, about 98.5%^[2] of oxygen is bound to hemoglobin.



Oxyhemoglobin and deoxyhemoglobin

Carbaminohemoglobin

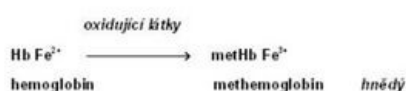
Carbaminohemoglobin is hemoglobin to which CO₂ is bound. Carbon dioxide binds to the globin chain of hemoglobin. The binding of CO₂ to hemoglobin reduces the affinity of hemoglobin for oxygen.

Methemoglobin

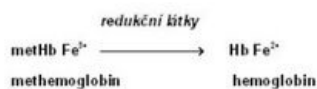
Methemoglobin (metHb; also *hemiglobin* or *ferrihemoglobin*) is characterized by the presence of **trivalent** iron, which is formed by the oxidation of divalent iron in hemoglobin^[3]. Methemoglobin loses the ability to reversibly bind oxygen. In its place, Fe III+ binds a water molecule with a sixth coordination bond. The color of methemoglobin is chocolate brown.

Methemoglobin is present in erythrocytes in small amounts even physiologically (about 1–3% of the total hemoglobin concentration^[4]). This is mainly due to the effect of nitrites, which arise from nitrates contained in food. The reverse reduction of methemoglobin to hemoglobin is primarily ensured by the NADH-dependent cytochrome-b 5 reductase (also methemoglobin reductase). A minor role is played by NADPH-dependent methemoglobin reductase, which is dependent on the supply of NADPH from the pentose cycle and on the presence of another electron carrier (e.g. flavin)^[5]. Non-enzymatic mechanisms include the action of glutathione and ascorbic acid.

1. vznik methemoglobinu



2. redukce methemoglobinu na hemoglobin



Methemoglobin- origin and therapy

An increased concentration of methemoglobin in the blood is referred to as **methemoglobinemia**. The causes are various:

- **Hereditary methemoglobinemia** is usually caused by a congenital defect in NADH-dependent methemoglobin reductase or the presence of abnormal hemoglobin M.
- **Acquired methemoglobinemia** is the most common form of methemoglobinemia. It can be caused by the action of oxidizing substances:^[6]
 - poisoning by certain substances (nitrobenzene , aniline and its derivatives - e.g. some dyes),
 - under the influence of certain drugs (local anesthetics – benzocaine , phenacetin , sulfonamides),
 - increased content of nitrates and nitrites in water and food.

Newborns are especially sensitive to the increased content of these substances due to the immaturity of the reduction systems and the increased proportion of fetal hemoglobin, which is more easily oxidized. Methemoglobinemia is manifested by cyanosis with a characteristic gray-brown shade and hypoxia.

Symptoms of methemoglobinemia

Methemoglobin values	Symptoms
0-2%	normal value
< 10%	cyanosis
< 35%	cyanosis and other symptoms (headache, shortness of breath)
70%	lethal concentration

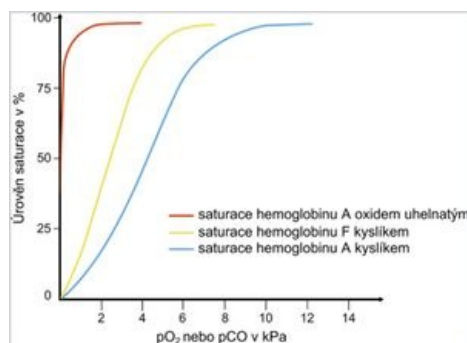
Part of the therapy of acquired methemoglobinemia is the administration of some reducing agents - methylene blue or ascorbic acid.

Carbonylhemoglobin

Carbonylhemoglobin (COHb, *carboxyhemoglobin*) is formed **by the binding** of carbon monoxide to hemoglobin . The bond formed is 250–300 times stronger than that of oxygen . Carbonylhemoglobin cannot transport oxygen, and cellular hypoxia develops as a result of the blood's reduced ability to carry oxygen . In an excess of oxygen, the binding of carbon monoxide to hemoglobin is reversible. That is why inhalation of O₂ is most important in carbon monoxide poisoning .

Small amounts **of COHb** can also occur in healthy individuals. Values of around 2% are found in urban dwellers, and COHb can rise up to 10% of total hemoglobin in heavy smokers . A few minutes' stay in an environment containing 0.1% CO can increase the concentration of carbonyl hemoglobin to 50%.

Carbon monoxide is produced during incomplete combustion of fuels, it is also contained in exhaust gases and in smoke during fires in closed rooms. Hemoglobin saturation curve



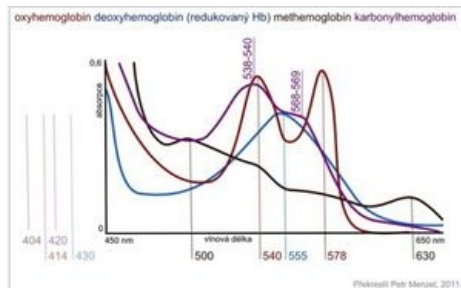
Hemoglobin saturation curve

Symptoms of carbon monoxide poisoning

COHb values in %	Symptoms
10	shortness of breath with greater exertion
20-40	headaches , shortness of breath, fatigue, vomiting
40-60	hyperventilation, tachycardia, syncope, convulsions
60-80	coma, death

Carbonylhemoglobin is characterized by a crimson red coloration; also people with severe carbon monoxide poisoning have a "healthy" pink skin color. Compared to hemoglobin, carbonylhemoglobin is more resistant to chemical influences, it changes more slowly under the influence of various agents.

Spectrophotometry of hemoglobin derivatives



Absorption spectra of hemoglobin and its derivatives

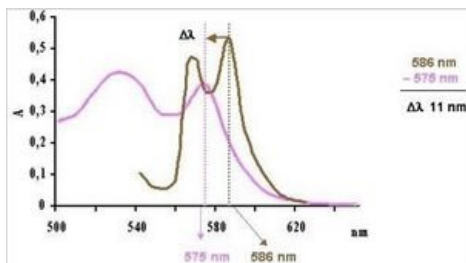
Absorption spectra of hemoglobin and its derivatives Hemoglobin and its derivatives have characteristic absorption spectra in the visible light region, which are used for their analysis and rapid identification. Significant absorption maxima in the 400–430 nm region, the so-called Soret band, are typical for all hemoproteins. Other absorption peaks are considerably lower. Oxyhemoglobin is characterized by two incompletely separated maxima in the region of 540 and 578 nm. Deoxyhemoglobin has a single absorption maximum at 555 nm. The main absorption maximum of methemoglobin is at 630 nm and a second faint peak at 500 nm is pH dependent. When methemoglobin reacts with potassium cyanide, the maximum at 630 nm disappears, as cyanmethemoglobin is formed. The decrease in absorbance at 630 nm is proportional to the methemoglobin concentration.

Cyanmethemoglobin shows a broad absorption maximum at 540 nm, which is used in determining the concentration of hemoglobin in the blood. The spectrum of carbonylhemoglobin resembles that of oxyhemoglobin, but the position of the peaks is shifted towards lower wavelengths.

Absorption maxima of hemoglobin and its derivatives

Hemoglobin derivative	Absorption maxima [nm]
Hemoglobin reduced	431, 555
Oxyhemoglobin	414, 540, 578
Methemoglobin	404, 500, 630
Carbonylhemoglobin	420, 538–540, 568–569
Cyanmethemoglobin	421, 540

Determination of carbonyl hemoglobin:



Determination of carbonylhemoglobin-spectrophotometry

The determination of carbonylhemoglobin in the blood is one of the basic toxicological examinations. It is an objective criterion in the assessment of acute and chronic carbon monoxide poisoning.

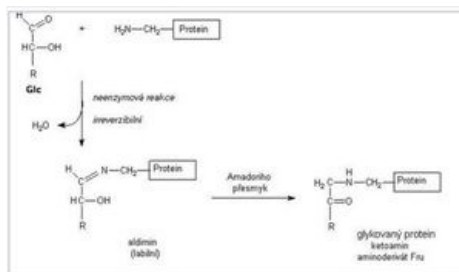
- *Spectrophotometric evaluation* . Carbonylhemoglobin can be determined quickly spectrophotometrically based on reading the shift of the absorption maximum of diluted blood from 586 nm^[7]. The shift of the maximum in the spectrum is dependent on the ratio of COHb and O₂ Hb in the sample.
- *Reaction with tannin* . As a guide, carbonylhemoglobin can be determined by reaction with tannin or Ajatin (from about 10% COHb). Tannin forms a strawberry-red precipitate in the presence of carbonylhemoglobin. In the absence of carbonyl hemoglobin, the color of the precipitate is brownish gray.
- *Acid-base balance analyzers* . The analysis of the toxicologically most important derivatives of hemoglobin COHb and metHb is also made possible by modern acid-base balance analyzers that have a built-in photometric system for their measurement.

Task: Spectrophotometric examination of hemoglobin and its derivatives (https://portal.med.muni.cz/index.php?f=chyba&redirect_pg=download&redirect_id=655&error=2¤t_priv=2) (pdf)

Task: Indicative determination of carbonylhemoglobin (https://portal.med.muni.cz/index.php?f=chyba&redirect_pg=download&redirect_id=650&error=2¤t_priv=2) (pdf)

Glycated hemoglobin HBA₁

Glycated hemoglobin is produced by a non-enzymatic reaction between hemoglobin and blood glucose . His work is irreversible.



The principle of non-enzymatic glycation of proteins

The level of glycated hemoglobin therefore reflects the concentration of glucose in the blood throughout the life of the erythrocyte , i.e. about **120 days** , and is used to assess **the success of the treatment/compensation of diabetes in the period 4-8 weeks** before the examination. The form of the stable fraction HbA 1c is most often determined .

Terminology

- *Glycated hemoglobin* – the sum of carbohydrate adducts at the N-terminal end or ε amino groups of lysine in hemoglobin.
- *HbA 1* - the sum of various minor fractions of hemoglobin (glycated), including HbA 1c , HbA 1a1/a2 , HbA 1b1/b2/b3 , HbA 1d1/d2/d3 and HbA 1e .
- *HbA 1c* - valine glucose adduct at the N-terminal end of β-globin; corresponds to the so-called stable ketoamine (N-[1-deoxyfructosyl]hemoglobin).

Glycated hemoglobin can be determined using ion exchange chromatography followed by spectrophotometry .

Evaluation

The amount of glycated hemoglobin is expressed in % of total hemoglobin or now in **mmol/mol** according to the IFCC (International Federation of Clinical Chemistry).

Reference limits

- in healthy adults up to **39 mmol/mol**^[8], (2.8–4.0%)
- in diabetics, HbA 1c concentrations up to 45 mmol/mol (4.5%) indicate excellent diabetes compensation, up to 60 mmol/mol (6.0%) acceptable, and higher values indicate unsatisfactory diabetes compensation^[9]

Task: Determination of glycated hemoglobin (https://portal.med.muni.cz/index.php?f=chyba&redirect_pg=download&redirect_id=657&error=2¤t_priv=2) (pdf)

Iron

Iron is one of the most important elements in the human body. The body of an adult contains more than 70 mmol (4.0-4.5 g) of iron. In women, this amount is lower than in men, which is attributed to blood loss during menses .

Distribution of iron in the organism

Form	Function	Protein	Quantity according to
Active iron	oxygen transport	hemoglobin	2.5-3.0
		myoglobin	0.3
	electron transfer	cytochromes , cytochrome oxidase	0.2
	decomposition of hydrogen peroxide	catalase , peroxidase	
Stock iron		ferritin , hemosiderin	0.8-1.0
Transport iron		transferrin	0.003

Iron metabolism

The presence of iron is essential for cell function. As a component of heme, it participates in oxygen transport and as a component of cytochromes, it conditions the transfer of electrons in the respiratory chain. An undesirable effect of iron as a transient and very reactive element is participation in radical reactions, during which the so-called reactive forms of oxygen are formed . These can damage cell membranes, proteins and DNA.

Iron is absorbed as Fe 2+ by active transport in the duodenum and upper jejunum in two ways:

1. porphyrin-bound Fe in the form of a stable lipophilic complex;
2. Fe II+ – chelates soluble in water.

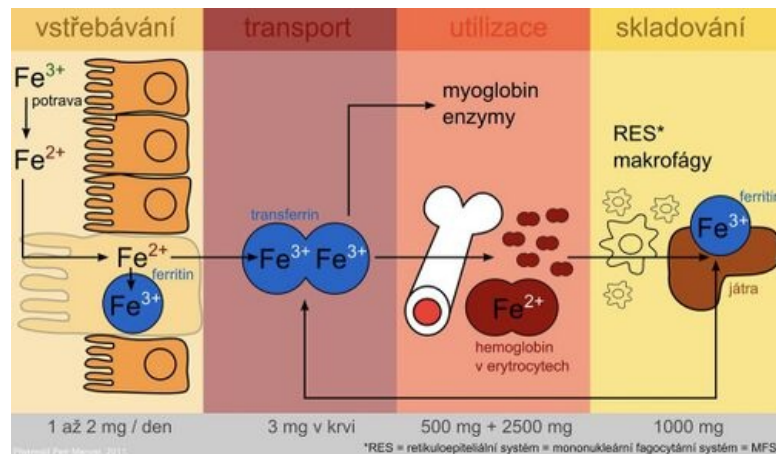
Only a small part is absorbed in ionized form.

There is an average of 10-50 mg of iron per day in the diet, but only 10-15% is absorbed. In heme compounds (meat) it is absorbed better, non-heme Fe in plant food much worse. In addition, plants contain oxalates, phytates, tannins and other phenolic compounds that form insoluble or chelate complexes with Fe that are difficult to absorb. Ascorbic acid, on the other hand, improves iron absorption.

After being absorbed by the intestinal mucosa, part of the iron is incorporated into the storage form - ferritin in the intestinal cells. Part of the absorbed iron passes into the plasma, where it is transported bound to transferrin. **The protein ferroportin** plays an important role in the transfer of iron across the basolateral membrane of enterocytes (it is also found in the membrane of macrophages and hepatocytes). It is the main place of regulation of iron homeostasis in the organism. A key regulatory factor is the protein **hepcidin**, which is synthesized in the liver. By binding to ferroportin, it inhibits the transport of iron from cells and thus contributes to its sequestration in them. Hepcidin levels increase during inflammation. Hepcidin is also partly responsible for **the anemia of chronic diseases**. Mutations in the hepcidin gene lead to **juvenile hemochromatosis type 2B**.

Plasma iron is taken up by cells of target tissues via the transferrin receptor and is either incorporated into heme or stored in the form of ferritin. The use of the specific transport protein transferrin and the storage protein ferritin for iron storage represent protective mechanisms to prevent the toxic effects of redox-active iron.

During desquamation of dead mucosal cells, unused iron leaves the stool together with unabsorbed iron.



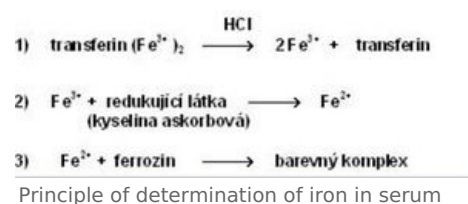
Investigation of iron metabolism

In practice, we commonly encounter diseases associated with changes in metabolism and utilization of iron. Laboratory examination of iron metabolism includes the following examinations:

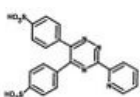
- serum iron
- serum transferrin and iron binding capacity
- serum ferritin
- transferrin receptor

The mentioned parameters are important diagnostic indicators for demonstrating a decrease or increase in iron reserves even in stages that are not accompanied by significant clinical manifestations.

Serum iron determination



Colorimetric methods, atomic absorption spectrophotometry and other special techniques are used to determine iron in serum. The most widely used are photometric methods, based on the reaction of iron with a complexing substance. All procedures include the following steps:



Ferrozine

1. Release of Fe^{3+} from binding to transferrin using acids or surfactants (e.g. HCl).
2. Reduction of Fe^{3+} to Fe^{2+} , which is necessary for the reaction with the complexing agent. E.g. ascorbic acid is used for reduction.
3. Reaction of Fe^{2+} with a complexing agent containing reactive groups $-\text{N}=\text{C}-\text{C}=\text{N}-$ to form a colored complex. Metal ions form chelates with two nitrogen atoms. Currently, two complexing substances are mainly used - **bathofenthroline** and **ferrozine** (3-(2-pyridyl)-5,6-bis(4-sulfophenyl)-1,2,4-triazine - PST, trademarked name

FerroZine®), which has higher absorption coefficient and is more soluble in water.

Evaluation

Serum iron concentrations are subject to a circadian rhythm and are influenced by other factors as well. This limits the diagnostic value of this parameter. It is a poor indicator of tissue iron stores and must always be assessed in combination with serum transferrin and iron binding capacity. Reduced concentrations accompany iron deficiency, caused, for example, by large or repeated blood losses, insufficient iron intake through food or impaired absorption. The finding is not specific, as reduced levels are also encountered in acute infections or chronic inflammatory diseases (transfer of iron to tissues). High levels of iron occur in hemochromatosis (see below), iron overdose or intoxication, increased breakdown of erythrocytes, and some liver diseases.

Reference values

men: 9–29 $\mu\text{mol/l}$

women: 7–28 $\mu\text{mol/l}$

Serum transferrin and iron binding capacity

Iron is transported through the blood bound to a specific protein with β 1 -electrophoretic mobility – transferrin , which is synthesized in the liver. The rate of its formation is inversely proportional to the iron reserves in the body; it increases with iron deficiency and decreases with excess. The biological function of transferrin consists in the ability to easily form non-toxic complexes with iron and transfer Fe absorbed by the mucosa of the small intestine to the bone marrow or to storage forms (ferritin or hemosiderin). Each transferrin molecule binds two Fe $3+$ atoms (1 g of transferrin binds 25.2 μmol of iron). Transferrin can be determined directly using immunochemical methods or indirectly as the ability of transferrin to bind iron - the so-called iron binding capacity. **Total iron binding capacity** (TIBC) is the amount of iron that transferrin is able to bind if all binding sites are occupied. Usually, only 1/3 of transferrin is saturated with iron - **the bound capacity** . Free transferrin without bound iron represents **the free binding capacity** (2/3 of transferrin) available for iron transport under increased demands.

Conversion between transferrin concentration and total binding capacity:

$$\text{Total binding capacity } [\mu\text{mol/l}] = \text{transferrin } [\text{g/l}] \cdot 25.2 .$$

The reference range for serum transferrin concentration (S-transferrin) is 2.0–3.6 g/l for a total binding capacity of 50–70 $\mu\text{mol/l}$.

Transferrin saturation

From the values of iron and transferrin concentration we can calculate **transferrin saturation** (TfS) , which is defined as the ratio of serum iron concentration to the total binding capacity of transferrin for iron. It is a sensitive parameter for detecting latent iron deficiency.

Extra open brace or missing close brace

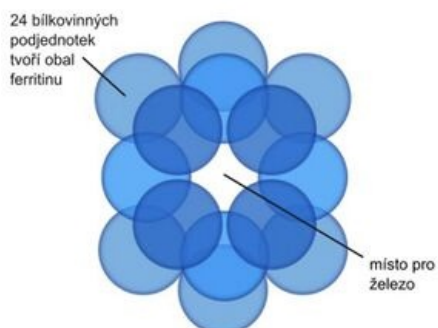
Assessment of transferrin saturation

physiological values: 25-50%

reduction of saturation in iron deficiency: < 15%

increase in saturation with excess iron: > 50%

Ferritin and hemosiderin



(Překlad Petr Menšík, 2011)

Schematic structure of ferritin

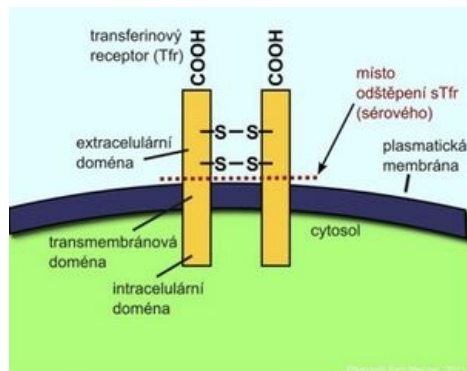
Ferritin is the most important storage protein for iron. The ferritin molecule is adapted to bind a large amount of Fe $3+$ in a soluble and non-toxic form for the organism. Ferritin is formed by an outer protein shell of 24 subunits - apoferritin (Mr 440,000), delimiting a cavity in which up to 4,500 iron atoms can be concentrated in the form of iron oxyhydroxide (FeO·OH) in microcrystalline form with phosphates (FeO·OPO 3H_2). The entry and exit of iron atoms allow the pores between the individual subunits of the shell of the ferritin molecule. Normally, its capacity is used at about 20%. It is stored in cells in the liver, spleen and intestinal mucosa.

In the blood serum, ferritin is found in a very low concentration. Serum ferritin concentrations are **a measure of iron stores** in the body. Low concentrations indicate depletion of the body's total iron reserve and serve to detect iron deficiency anemia early in its prelatent phase. Increased ferritin concentrations are a concomitant phenomenon of high tissue iron stores. We also encounter them in many patients with liver disease, some malignant tumors (tumor marker) or inflammatory diseases (positive acute phase reactant).

The reference range for serum ferritin concentration (S-ferritin) is 30–300 µg/l for men and 20–120 µg/l for women.

Hemosiderin is another storage protein for iron. It is formed by the aggregation of denatured ferritin with other components. It produces particles 1 to 2 µm in size that are visible under a light microscope when iron staining is used. Hemosiderin contains more iron than ferritin, but due to its poor water solubility, it is difficult to obtain. It is formed when the amount of iron in the body exceeds the storage capacity of ferritin.

Transferrin receptor



Transferrin receptor

Iron transported in the blood by transferrin is taken up by cells through a specific transferrin receptor (TfR). At a certain stage of development, it is found on the surface of all cells, but it is most expressed on the surface of precursor cells of the red line in the bone marrow. TfR is a transmembrane protein that consists of two identical subunits joined by a disulfide bond. **By separating the extracellular domains of the receptor, the so-called soluble fraction of the transferrin receptor (sTfR)**, which can be in the form of a dimer or a monomer, is released into the circulation. Cells respond to reduced iron stores by synthesizing increased amounts of transferrin receptors.

An increase in sTfR is a reliable **indicator of iron deficiency for hematopoiesis**. Elevated levels of sTfR are found in *iron-deficiency anemias* or *hemolytic anemias*. Determination of sTfR is valuable in anemic patients in whom ferritin is elevated due to the acute phase reaction. Determining the concentration of sTfR can also be used in patients with a bone marrow transplant to monitor the progress of erythropoiesis.

Immunochemical methods are used for determination.

Disorders of iron metabolism

Iron deficiency (sideropenia)

Lack of iron in the body is usually caused by insufficient absorption from the intestine or chronic blood loss. It can result in sideropenic anemia (**hypochromic microcytic anemia**), which is one of **the most common hematological diseases**. However, anemia is usually a late symptom of gradually developing sideropenia. It will show up in the blood count only after the iron has almost completely disappeared. Therefore, it is necessary to detect iron deficiency at an early stage, which is not yet accompanied by anemia.

Based on the determination of the basic parameters of iron metabolism, we distinguish three degrees of deficiency:

- **Prelatent iron deficiency** is the name for a condition where there is a gradual decrease in stores, but the supply of iron to the erythroblasts of the bone marrow is not yet affected. About half of patients have serum ferritin levels below 12 µg/l.
- With **latent iron deficiency**, its reserves are essentially exhausted. Ferritin is reduced below the lower limit of the norm and is already accompanied at this stage by a decrease in the level of iron in the serum and a reduced supply to the erythroblasts of the bone marrow. The binding capacity for iron increases. A sensitive indicator of latent iron deficiency is a drop in transferrin saturation below 15%. However, anemia does not yet develop.
- With **a manifest iron deficiency**, anemia develops with a drop in hemoglobin values below the lower limit of the norm. Iron deficiency anemia is characterized by low serum iron and ferritin, and an increased concentration of transferrin (binding capacity for iron). In hemolytic anemias or iron overload, on the other hand, serum iron is increased, while the total binding capacity for iron is reduced.

Prelatent iron deficiency	Latent iron deficiency	Manifest iron deficiency
reduction of stored iron – decrease of ferritin	lack of stored iron – decrease in ferritin	lack of stored iron – decrease in ferritin
	decrease in serum iron	decrease in serum iron
	drop in transferrin saturation below 15%	transferrin drop below 10%
	increasing the total binding capacity for iron	increasing the total binding capacity for iron
	increase in sTfR	increase in sTfR
		decrease in hemoglobin concentration - anemia

Excess iron

The organism is not equipped with an excretory pathway for iron, and therefore, under certain circumstances, excess iron can accumulate in the tissues. Early diagnosis can prevent tissue damage from excess iron. Iron overload usually develops very slowly. We distinguish 3 stages:

- In the stage **of prelatent iron excess**, its content in the organs increases, but without exceeding their storage capacity.
- During **the latent stage of iron overload**, the storage capacity of the cells is exceeded, but the function of the organs is not yet damaged, the level of ferritin and the level of iron in the serum increase, and the saturation of transferrin rises above 55%.
- In the stage **of manifest iron excess**, some organs are already damaged.

Laboratory finding in excess of iron

Prelatent iron overload	Latent iron overload	Manifest iron excess
increasing iron stores - increasing ferritin	increase in iron reserves – increase in ferritin above 300 µg/l	increase in iron stores - increase in ferritin (with severe impairment above 2000 µg/l)
	increase in serum iron	significant increase in serum iron
	increase in transferrin saturation above 55%	increase in transferrin saturation (can exceed 90% in severe cases)

Hemochromatosis

Accumulation of iron in tissues is related to a disease we refer to as hemochromatosis .

- **Primary hemochromatosis** is a hereditary disease caused by increased absorption of iron from the intestine. Excess iron is stored in parenchymatous organs such as the liver, heart, pancreas, and adrenal glands. It has a toxic effect on affected organs and disrupts their function by catalyzing chronic reactions leading to the formation of free radicals. The main clinical manifestations are skin hyperpigmentation, hepatosplenomegaly and diabetes mellitus .
- **Secondary hemochromatosis** can develop as a result of, for example, repeated transfusions, excessive intake of iron-containing preparations or hemolytic anemia. In the biochemical picture, we find increasing levels of ferritin and iron in the serum, the saturation of transferrin increases while it simultaneously decreases.

Iron poisoning

Children are at risk of accidental ingestion of large quantities of preparations (lentil-like tablets). The lethal dose for a child is 600 mg. For an adult, an iron intake of 40 mg/kg is toxicologically serious, an intake of 60 mg/kg is fatal .

Symptoms include nausea, vomiting (even vomiting blood), abdominal pain, diarrhea (sometimes bloody). Large fluid losses cause shock, kidney failure, and death. If the patient survives this stage of poisoning, he may fall into unconsciousness, convulsions and liver failure after 12 hours. If he survives even this second phase, the poisoning can leave permanent consequences (intestinal damage).

Treatment of acute poisoning

- Gastric lavage.
- Administer the chelating agent deferoxamine (5–10 g in 50–100 ml of water) through a nasogastric tube.
- Consider intravenous desferoxanin to flush out absorbed iron. A pink-red complex of deferoxamine with iron appears in the urine. The treatment should be repeated until the urine color returns to normal^[10] .

Task: Determination of Fe in serum by colorimetric method (https://portal.med.muni.cz/index.php?f=chyba&redirect_pg=download&redirect_id=656&error=2¤t_priv=2) (pdf)

Links

related articles

- Carbonylhemoglobin
- carbaminohemoglobin
- Methemoglobin
- Examination of hemoglobin and it's metabolism
- Heme

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