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REVIEW

Molecular and Cellular Bases of Iron Metabolism in Humans

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Abstract—Iron is a microelement with the most completely studied biological functions. Its wide dissemination in nature and involvement in key metabolic pathways determine the great importance of this metal for uni- and multicellular organisms. The biological role of iron is characterized by its indispensability in cell respiration and various biochemical processes providing normal functioning of cells and organs of the human body. Iron also plays an important role in the generation of free radicals, which under different conditions can be useful or damaging to biomolecules and cells. In the literature, there are many reviews devoted to iron metabolism and its regulation in pro- and eukaryotes. Significant progress has been achieved recently in understanding molecular bases of iron metabolism. The purpose of this review is to systematize available data on mechanisms of iron assimilation, distribution, and elimination from the human body, as well as on its biological importance and on the major iron-containing proteins. The review summarizes recent ideas about iron metabolism. Special attention is paid to mechanisms of iron absorption in the small intestine and to interrelationships of cellular and extracellular pools of this metal in the human body.

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BIOLOGICAL ROLE OF IRON

Iron is an essential microelement that is a component of iron-containing organic compounds and is necessary for functioning of the human body. Important chemical features of iron are its ability for reversible oxidation/reduction ($\varphi_{Fe^{2+}/Fe^{3+}} = +772$ mV) and for generating compounds involved in such important processes as catalysis, electron transport, neurotransmission, free radical reactions, etc. [1-3].

Iron plays a key role in the formation of iron- and heme-containing proteins involved in energy metabolism, oxygen transport, production and release of neurotransmitters, synthesis of collagen, DNA, and steroid hormones, detoxification of xenobiotics, provision of nonspecific resistance of the body, etc. [3-7].

The involvement of iron in the generation of free radicals in cells is usually considered a process leading to damage of biomolecules (proteins, lipids, nucleic acids) and to development of oxidative stress [8]. However, free radicals concurrently are regulators of many vitally important processes, such as myocardium contractility and vascular tension, the immune response, control of cell division and differentiation, destruction of damaged cells, etc. [9]. Moreover, not all modifications of biomolecules caused by free radicals are deleterious: carbonylation, S-nitrosylation, and nitration of proteins are important for their degradation and necessary for normal functioning of cells [8].

In the human body, iron occurs as inorganic compounds (oxides, salts) and as organic ones (iron-containing proteins, low-molecular-weight organic complexes) [10]. Ionized iron is present in the body in oxidized and reduced forms, or respectively, as trivalent and divalent irons. At the physiological concentration of oxygen, trivalent iron is more stable and forms complexes with proteins acting as a transport (transferrin) and reserve (ferritin) forms of the metal in the human body. Reduced iron plays an important role in metabolism because only divalent iron is a substrate for transmembrane carriers, participates in heme synthesis, and interacts with ferritin [10-12].

In the human body, iron is present inside and outside cells. Extracellular iron is present in various biological fluids such as blood, lymph, liquor, interstitial fluid, etc.

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Iron acts as a cofactor or a component of prosthetic groups of enzymes and performs the following functions: first, iron ions have catalytic properties and are a part of the catalytic site of the active center of enzymes; second, they promote the interaction or provide the formation of a complex between substrate and binding sites of the active centers of enzymes (stabilization of the substrate molecule or of tertiary/quaternary protein structure); third, iron ions can act as acceptors/donors during electron transport, oxidation/reduction, and free radical processes [10, 13].

Moreover, iron regulates expression of numerous genes, many of which are involved in its metabolism on the transcriptional and posttranscriptional levels; iron also participates in the regulation of enzyme activities and enzymatic degradation of proteins in cells [14].

EXTERNAL METABOLISM OF IRON

In the adult human body, there is 3-5 g of iron: 45 mg/kg body weight in women and 55 mg/kg body weight in men. In the blood plasma, there is only 3-5 mg [10, 15, 16]. The iron content in blood serum is 0.1-0.5% of its total amount in the body [17]. Hemoglobin contains 65-80%, ferritin – 20-30%, myoglobin – 5-15%, and transferrin contains 0.1-0.5% of the total amount of iron in the body [6, 15, 18].

The human body receives iron from food (exogenous iron) and from the recirculation of iron released upon the degradation of iron-containing proteins (endogenous iron). The daily human requirement for iron is 20-25 mg, and it is mainly satisfied due to return into the circulation of iron released upon the degradation of iron-containing proteins, mainly hemoglobin [6, 18-20].

Absorption of iron from food depends on the gastric secretion, chime pH, intestinal motility, and the morphologic and functional state of the gastrointestinal tract (GIT). Normally only a small part of the iron in food is absorbed, and the absorption level is quite variable [21, 22]. Iron assimilation in the GIT also depends on the ratio in the food of products of animal and plant origin. The lower uptake of iron from plant food than from animal food sources is an explanation of the higher frequency of iron-deficient states in vegetarians. On the average entrance with food of 10-20 mg iron per day in the GIT of a healthy person, no more than 1-2 mg is absorbed [22]. The iron concentration in the cell is significantly higher than the solubility of free trivalent iron in water (10^{-18} M) and is explained by its binding with different proteins and non-protein chelators. High intracellular iron concentrations result in an electrochemical gradient (~10^{14}) of this element between the cytosol (10^{-4} M) and the extracellular medium (10^{-18} M) [23, 24].

There are two forms of iron in food: heme and nonheme iron [21, 25, 26].

Heme iron is iron bound with porphyrin, and in this form, it is present in the prosthetic group of hemoglobin and myoglobin. Heme iron has high bioavailability, and although it is only a small part of the iron content in food, it provides a significant part of the assimilated exogenous iron [16, 27]. Up to 20-30% of heme iron is absorbed from food, and its uptake is not influenced by other food components. Due to high solubility of heme iron at the alkaline pH of the small intestine, it is absorbed more efficiently than nonheme iron [28, 29].

Nonheme iron in food is presented as free iron and as iron bound with proteins and low-molecular-weight chelators. Different quantities of nonheme iron are found in virtually all products and form the major part of food iron (>90%) [21, 27, 30]. The bioavailability of food iron is influenced by phytates, oxalates, tannins, phosphates, and some drugs, which suppress its uptake, as well as by some amino acids and ascorbic acid, which increase its uptake. As a rule, ~5% of nonheme iron of food is absorbed [21, 22].

Nonheme and heme iron entering with food, i.e. exogenous iron, is more intensively absorbed in the proximal parts of the small intestine: in the duodenum (90%) and the jejunum. The stomach does not play a significant role in the assimilation of iron – here it is absorbed no more than 1-2% of the total iron entering the GIT [31]. The half-elimination period of iron from the human body is ~1800 days [15].

UPTAKE OF IRON IN THE GASTROINTESTINAL TRACT

To penetrate from cavities of the GIT organs into the interstitial connective tissue of the *lamina propria* of the small intestine mucosa and to enter the blood plasma, iron has to pass across the apical and basolateral parts of the enterocyte plasmalemma [25, 31]. The paracellular transport of iron across the epithelium of the GIT organs is negligibly small and of no importance for its assimilation [5]. The transmembrane transfer of iron ions occurs due to combined activities of special proteins: a transporter with the conjugated enzyme able to reduce/oxidize iron.

UPTAKE OF HEME IRON IN THE GASTROINTESTINAL TRACT

Heme iron is transported from the small intestine cavity into enterocytes by the transmembrane transporter HCP1/PCFT (Heme Carrier Protein 1/Proton-Coupled Folate Transporter). This protein is localized in the apical part of the enterocyte plasmalemma and is a component of the membrane of their endosomes. Heme binds with HCP1 and induces receptor-mediated endocytosis [31, 32]. It is thought that at least a part of the heme iron is reabsorbed during the endocytosis and not because of

heme translocation across the HCP1 plasmalemma [32, 33]. Then heme is degraded under the influence of the enzyme heme oxygenase (EC 1.14.99.3) with release of reduced iron. Heme oxygenase catalyzes the oxidative degradation of heme *b* into biliverdin IXα. This reaction can occur in the cavity of endosome/lysosome and in the cytosol, because heme oxygenases are present in the plasmalemma and in the endoplasmic reticulum (ER) membrane [34]. HCP1 found in the ER membrane is required for transporting heme into its lumen and inclusion into heme-containing proteins [35]. HCP1 has been found in the plasma membrane of astrocytes [36].

Moreover, heme iron can be transported across the plasmalemma after formation of a complex with hemopexin – a 63-kDa glycoprotein of blood plasma that has a high affinity for heme [37, 38]. Hemopexin binds one molecule of heme [39]. The complex is recognized by hemopexin receptors localized on the cell membrane (CD 91, the receptor of low-density lipoproteins) and is absorbed by receptor-mediated endocytosis. After the complex has been dissociated in the acidic medium of endosomes, hemopexin and its receptor come back onto the plasmalemma and can participate in transporting other heme molecules [40]. Then heme is degraded by heme oxygenase (HO1 or HO2) inside endosomes, and the released iron is transferred across their membranes into the cytosol (and participates in regulatory processes), stored in ferritin, included into iron-containing proteins, or exported [18]. Thus, the binding of heme by hemopexin protects cells against its toxic action [41, 42].

Heme iron can penetrate into monocytes and macrophages within the hemoglobin/haptoglobin complex during endocytosis mediated through the scavenger receptor CD 163 present on their plasmalemma. Free hemoglobin is produced during intravascular hemolysis of erythrocytes and bound by the plasma glycoprotein haptoglobin (85 kDa) [18, 43].

UPTAKE OF NONHEME IRON IN THE GASTROINTESTINAL TRACT

Nonheme iron of food enters the small intestine lumen mainly in its oxidized state [6] because divalent iron is oxidized by gastric juice components [3, 5, 44, 45]. Because divalent iron is more soluble than trivalent iron (at physiological pH their solubilities are, respectively, 10^{-1} and 10^{-18} M) [46, 47], oxidized iron in the stomach cavity interacts with mucin (mainly MUC2), ascorbic acid, amino acids, monosaccharides, amines, or amides for increasing the solubility on passage into the alkaline medium of the small intestine [5]. Oxidized iron is precipitated in solution at pH > 3, and therefore it has to be bound with chelators at the lower pH in the stomach where it has good solubility [22]. When the gastric content enters the intestine, the pH of the food lump increases and, if there is no binding with chelators, oxidized iron produces insoluble salts. The solubility of trivalent iron salt can also be retained due to its reduction under the influence of ferrireductase of the brush border of enterocytes [48].

Before delivery into enterocytes, all trivalent iron has to be reduced. Trivalent iron of chyme is reduced by duodenal cytochrome *b* (DCYTB, intestinal ferrireductase), which is a glycoprotein localized in the apical part of the enterocyte brush border plasmalemma. DCYTB is a heme-containing protein of the cytochrome b_{561} family present on the membrane of erythroblasts and enterocytes and using ascorbic acid to reduce iron [49-51]. On the apical part of the enterocyte plasmalemma, there is also the protein STEAP3 (Six-Transmembrane Epithelial Antigen of the Prostate 3) that can reduce the iron of chyme [52].

Upon reduction, divalent iron is transferred across the apical membrane of enterocytes by the divalent metal transporter (DMT1, DCT1, NRAMP2). DMT1 (Divalent Metal Transporter 1) is a transmembrane one-chain 61.5-kDa hydrophobic glycoprotein that acts a symporter of protons and divalent cations (Fe^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+}, Co^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+}) at the ratio 1 Me^{2+} : 1 H^+ [11]. The same protein is present on the membrane of erythroid series cells (except erythrocytes) and of nephrocytes of the proximal convoluted tubules of the nephron [53-56]. However, it does not transfer iron ions across the plasmalemma of hepatocytes, macrophages, neurons, and symplastotrophoblasts [22, 57]. DMT1 is present in the membrane of endosomes and mediates the passage of divalent iron from their lumen into the cytosol on the transferrin-mediated uptake of iron by erythroid series cells and nephrocytes [58].

Some authors think that DCYTB is not the only iron-reducing component of the enterocyte plasmalemma and that iron can also be reduced by other reductases and through nonenzymatic mechanisms [59-62]. Alternative concepts on the uptake of divalent iron in the GIT are based on the existence of membrane reductase (not DCYTB) that reduces iron using NAD^+/NADH or oxidized/reduced ascorbate with subsequent transfer of divalent iron by DMT1 [18].

It has been shown that iron in complex with citrate can be reduced in the brush border without enzymes by ascorbate, which is oxidized into dehydroascorbate during the reduction of the iron. Reduced iron is transferred across the enterocyte plasmalemma by DMT1. The extracellular reduced ascorbate crosses the membrane under the influence of glucose transporters (GLUT1) and regenerates in the cytosol due to the conjugated oxidation of GSH/GSSG or NADPH/$NADP^+$ with subsequent transfer of oxidized ascorbate outside across the plasmalemma by an unidentified transporter (possibly an anionic channel). This mechanism seems possible for rodents, because they are able to synthesize ascorbic acid, which allows them to excrete it into the intestinal lumen to provide the transport of iron [18].

Conrad and coworkers postulated the possibility of transport into enterocytes of nonheme trivalent iron [63]. According to their concepts, in the acidic medium of the stomach oxidized iron binds with low-molecular-weight organic compounds (ascorbate, fructose, histidine, etc.), which deliver it at neutral pH onto mucin-2 (MUC-2). The glycoprotein MUC-2 is the major component of mucus of salivary glands, small intestine, and colon. The trivalent iron complex with mucin prevents production of poorly soluble salts and makes the metal available for uptake in the alkaline medium of the duodenum [63]. Each molecule of mucin binds with several iron atoms ($K_d = 10^{-5}$). The complex of oxidized iron with mucin interacts with the heterodimeric transmembrane protein ITGB3 (b_3-integrin, CD 61, 230 kDa) localized on the apical part of the enterocyte plasmalemma, then iron is passed onto integrin, whereas mucin-2 is released into the intestinal lumen. ITGB3 is a receptor for fibronectin, laminin, vitronectin, matrix metalloproteinase 2, thrombospondin, and von Willebrand factor. On the plasmalemma inner surface, the cytosolic protein mobilferrin binds with the α-chain of b_3-integrin [64]. Mobilferrin is a cytosolic iron-binding monomeric glycoprotein (56 kDa, isoelectric point 4.7) of hepatocytes, enterocytes, and tissue macrophages that interacts only with reduced iron. Mobilferrin is an analog of calreticulin, and each molecule binds one atom of reduced iron ($K_d = 10^{-6}$), but with lower efficiency it can bind other divalent cations (Ca^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}) [16, 65].

In the cytosol, the integrin–oxidized iron–mobilferrin complex is adjoined with some other proteins that results in formation of paraferritin (520 kDa). Paraferritin is a multiprotein complex similar to ferritin in molecular weight and hydrodynamic volume but not related to it. Paraferritin consists of several glycoproteins: b_3-integrin (250 kDa), mobilferrin (56 kDa), NADPH-dependent flavin(FAD)monooxygenase (FMO, 60 kDa), β-2-microglobulin, and DMT1 [6, 57, 64-66]. Iron is reduced by paraferritin, which has a ferrireductase activity due to FMO regenerating its coenzyme (FAD) due to the conjugated reaction with NADPH/$NADP^+$ [65]. Because b_3-integrin and FMO are associated with the plasmalemma, paraferritin is also formed on the cytosolic surface of the plasma membrane [16, 67, 68]. A similar mechanism of trivalent iron transport exists in hepatocytes and macrophages [15]. We could not find in the literature data on the endocytosis-mediated uptake of trivalent iron bound with b_3-integrin.

It seems that all pathways of nonheme iron uptake in the intestine function in parallel [22, 57].

In enterocytes, divalent iron is used either for synthesis of iron-containing proteins or is transported across the basolateral membrane into the intercellular space (transient iron) [69, 70], or interacts with ferritin of these cells (stored iron) [19, 71].

IRON TRANSPORT ACROSS THE BASOLATERAL PART OF ENTEROCYTE PLASMALEMMA

Iron ions can be transported further across the basolateral part of the plasmalemma only in the case of passage across the enterocyte cytosol. Divalent iron is transported in this way either in a complex with mobilferrin or on association with other proteins: monothiolglutoredoxins (thioltransferases using glutathione and NADPH as coenzymes) and RNA-binding proteins: PCBP 1 and 2 (poly(rC)-binding proteins 1 and 2) [10, 14]. These cytosolic proteins act as chaperones and underlie mechanisms of the intracellular transport of iron. Recent studies have shown that the divalent iron complex with glutathione is a dominant intracellular compound of the labile iron pool in the cell [72]. Free iron ions in the cytosol are virtually absent [14, 73]. The labile iron pool is no more than 5% of its total content in the cell, and this pool is used by mitochondria for synthesis of heme and iron–sulfur proteins and for synthesis of iron-containing proteins of the cytosol [74]. The remaining iron of the cell binds with ferritin, which limits its reactivity [75].

Divalent iron is transported across the basolateral membrane of enterocytes by the transmembrane glycoprotein ferroportin (Ireg 1, MTP1), which interacts only with divalent iron. Ferroportin is a membrane glycoprotein consisting of 571 amino acid residues and localized on the basolateral surface of enterocytes [76]. This protein has also been detected in the plasmalemma of tissue macrophages, adipocytes, symplastotrophoblasts, and hepatocytes and is responsible for iron release from these cells [77-81].

Ferroportin has been described on the membrane of erythroblasts, which can increase its expression under conditions of iron deficiency and seem to partially compensate the deficiency of food iron for other cells [82]. Similarly to DMT1, ferroportin is responsible for the transmembrane transfer of various divalent cations besides iron [11].

Divalent iron passed across the basolateral membrane of enterocytes is oxidized by hephaestin. Hephaestin is a glycoprotein associated with the outer surface of the basal part of the enterocyte plasmalemma and is a homolog of the plasma protein ceruloplasmin [15, 83-85]. Hepatocytes and macrophages use hephaestin and ceruloplasmin for oxidizing the exported iron [55].

IRON OXIDATION AND TRANSPORT IN BLOOD PLASMA

Ceruloplasmin (ferroxidase) is a copper-containing enzyme of blood plasma α2-globulins. Ceruloplasmin oxidizes divalent iron ions and is a transporter protein for

copper ions. One ceruloplasmin molecule carries six to eight copper atoms. This glycoprotein is synthesized mainly by hepatocytes. Ceruloplasmin does not penetrate across the blood–brain barrier and is synthesized in the human brain by astrocytes [86] and in the eye retina by cells of the inner nuclear layer [87]. Patients with hereditary aceruloplasminemia have a low concentration of iron in blood plasma and high content of iron in the liver, pancreas, brain, and kidneys [88].

In blood plasma, iron binds with the protein apotransferrin, which interacts only with trivalent iron. Upon binding one or two atoms of oxidized iron, apotransferrin is converted into mono- or diferric transferrin, respectively. Transferrin transfers nearly all iron of blood plasma, whereas a very small number of iron ions are transported as complexes with albumins and low-molecular-weight organic compounds producing the pool of non-transferrin-bound iron [6, 18].

PENETRATION OF IRON INTO CELLS

The transfer of trivalent iron into cells is regulated by expression of type-1 and type-2 transferrin receptors. The number of such receptors is maximal on the plasmalemma of erythroid series cells and decreases with maturation of these cells [6, 89]. These receptors are also present on the basolateral part of the enterocyte plasmalemma, where they are responsible for the regulation of iron uptake from food. Thus, the entrance of iron into enterocytes from blood plasma decreases the uptake of food iron [90]. The transferrin receptor binds two molecules of diferric transferrin [11]. The receptors have an extracellular *C*-end, cytoplasmic *N*-end, and the transmembrane domain that contains 62 a.a. and is covalently bound with a residue of palmitic acid [91]. The association constant of diferric transferrin with its receptors is 10^{-7}-10^{-9}, which is 30 and 500 times higher than the constants of monoferric transferrin and apotransferrin, respectively [92]. There are no receptors for transferrin in the membrane of erythrocytes [93].

The type-1 transferrin receptor (TfRI) is a transmembrane glycoprotein with molecular weight of 180 kDa consisting of two identical 90-kDa polypeptides bound by disulfide bonds. The type-1 transferrin receptors (CD 71) have high affinity for diferric transferrin and are expressed on virtually all cells (erythroid cells, symplastotrophoblasts, etc.) [84]. Blood serum contains a soluble form of the type-1 transferrin receptor (sTfR, 95 kDa), which is produced by shedding of the receptor membranous form during its hydrolysis and is its extracellular fragment [10]. The concentration of the soluble transferrin receptor in human blood plasma is 5.5 mg/liter [7].

The type-2 transferrin receptor (TfRIIα and TfRIIβ) is present on enterocytes, hepatocytes, and erythroblasts and regulates the expression of hepsidin through interaction with transferrin and the protein of hereditary hemochromatosis (HFE). The TfRII–HFE complex is necessary for transcriptional regulation of hepsidin production [10, 84, 94, 95].

Hepsidin, the major down-regulator of iron metabolism in the human body, is a peptide consisting of 25 a.a.; its secondary structure is characterized by a hairpin united by two disulfide bonds. Hepsidin is produced from a precursor (84 a.a.) mainly by hepatocytes and by macrophages, adipocytes, and cardiomyocytes; it circulates in the blood plasma, is filtrated by kidneys, and is excreted with urine [44, 71, 96, 97]. Hepsidin interacts with ferroportin of enterocytes, macrophages, symplastotrophoblasts, etc. and causes its internalization and lysosomal degradation. The loss of ferroportin by the plasmalemma of these cells results in a decrease in iron release into blood plasma, its retention in these cells, and decreases transferrin saturation [98, 99]. An increase in the amount of iron in depots of the body leads to stimulation of synthesis of hepsidin, which decreases the iron uptake by enterocytes and reabsorption by nephrocytes that lowers the iron concentration in blood plasma. On the contrary, a decrease in iron concentration in the cellular depots leads to suppression of hepsidin synthesis by hepatocytes and to recovery of iron transport [44].

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) found in the plasmalemma of macrophages functions as a receptor of transferrin. The GAPDH–transferrin complex is absorbed by endocytosis. Exhaustion of intracellular iron stores stimulates GAPDH expression on the plasmalemma of macrophages [100].

Transferrin bound with trivalent iron interacts with its receptors on erythroid, lymphoid, muscle, and nervous cells and on hepatocytes and macrophages [10]. This interaction initiates the interaction of clathrin pits and clathrin vesicles. Proton pumps (H-ATPase) of early endosomes acidify their contents [89, 101].

In the acidic medium (pH 5.4) of early endosomes, iron is released from the complex [trivalent iron–transferrin–transferrin receptor] and after that [apotransferrin–transferrin receptor] returns on the plasmalemma inside the recycling vesicle [6, 51, 101, 102]. Each transferrin molecule can realize 100-200 cycles associated with the transport of iron [10]. At neutral pH in the intercellular fluid, apotransferrin changes its conformation, separates from the receptor, and becomes ready to participate again in the transport of oxidized iron ions. Trivalent iron in the cavity of late endosomes or lysosomes has to be reduced for being transported into the cytosol by DMT1 (NRAMP2-Natural Resistance-Associated Macrophage Protein 2). This function in the membrane of these organelles is performed by the protein STEAP3 (Six-Transmembrane Epithelial Antigen of the Prostate family member 3), which is a metalloreductase [10, 50, 103-106].

Hepatocytes are able to absorb and accumulate iron in ways other than the transferrin-dependent pathway. Non-transferrin-bound iron begins to enter hepatocytes when the iron concentration in blood plasma becomes higher than the ability of transferrin to bind it. This transport does not depend on DMT1; protein candidates transporting non-transferrin bound iron are L-type calcium channels and transporters of other metal ions, e.g. the zinc transporter ZIP14 (Zrt-Irt-like Protein 14) [107-110]. Potential-dependent L-type calcium channels deliver reduced iron into cardiomyocytes on an increase in the concentration of non-transferrin-bound iron in blood plasma [93, 101, 111].

The iron uptake results in formation in the cytosol of the labile iron pool, which either is spent to synthesize iron-containing proteins or is stored in ferritin. The labile iron pool consists of highly reactive bi- and trivalent iron ions bound with low-molecular-weight chelators (citrate, organic phosphates, ATP, ascorbic acid, amino acids, etc.) or proteins (e.g. poly(rC)-binding protein 1). This protein transfers the iron delivered to ferritin [14]. It should be noted that some authors describe also the nuclear and mitochondrial pools of labile iron [112]. After the uptake (in non-erythroid cells), 70-80% of labile iron ions are directed to ferritin [18].

MACROPHAGE-MEDIATED RECIRCULATION OF HEMOGLOBIN IRON IN HUMANS

It has been mentioned that ~70% of iron in the body is a component of hemoglobin localized in erythrocytes. For erythropoiesis, production of $20 \cdot 10^6$ new erythrocytes, a human body needs daily ~25 mg of iron [113]. Only 1-2 mg of iron, or 4-8% of the daily necessity, a human receives with food, whereas the remaining part is covered due to recirculation of iron released during erythrophagocytosis. Iron for hemopoiesis is delivered mainly due to degradation of hemoglobin of old and damaged erythrocytes. The main role in the utilization of erythrocytes belongs to macrophages of the liver and of the spleen red pulp [6, 113, 114]. Erythrocytes taken up during erythrophagocytosis are degraded with intravesicular release of hemoglobin. Proteolysis of hemoglobin by lysosomal enzymes results in release of heme, which under the influence of the membrane enzyme heme oxygenase 1 is divided into divalent iron and protoporphyrin IX. Similarly to iron released because of autophagosomal destruction of ferritin, upon entrance into the lysosome cavity iron is delivered into the cytosol by the transmembrane carrier NRAMP1. NRAMP1 is homologous to DMT1 and is exclusively expressed in macrophages and neutrophilic granulocytes [115, 116]. In the cytosol of phagocytes, this iron can either be stored by apoferritin or be exported into blood plasma under the influence of ferroportin, bind with apotransferrin, and thus be delivered to erythropoietic cells of the red bone marrow. If the stored iron of macrophages is required, iron released from ferritin also enters the blood plasma, binds with apotransferrin, and is delivered into the red bone marrow [51, 117-119].

IRON METABOLISM IN MITOCHONDRIA

Mitochondria play an important role in iron metabolism because they provide the synthesis of heme, which is an important structural component of many vitally important proteins. Heme biosynthesis begins under the influence of 5-aminolevulinate synthase (EC 2.3.1.37) and terminates under the influence of ferrochelatase (EC 4.99.1.1) in the mitochondrial matrix, whereas the intermediate stages of this metabolic pathway occur in the cytosol [113, 120-123]. Iron easily penetrates from the cytosol into the intermembrane space through pores of the outer mitochondrial membrane in complexes with low-molecular-weight or protein chelators and due to combining of the iron-containing endosome membrane with the outer membrane of mitochondria (the "kiss-and-run" mechanism). The latter mechanism is widely distributed in hemoglobin-synthesizing cells of red bone marrow (e.g. in reticulocytes) [124].

Divalent iron is transported across the inner mitochondrial membrane under the influence of the integral glycoprotein mitoferrin. In the case of genetic deficiency of mitoferrin, the absorbed iron does not penetrate from the cytosol into mitochondria, which prevents production of protoporphyrin IX [6, 18, 125, 126]. Mitoferrin binds with Abcb10 (ATP-binding cassette transporter 10), an ATP-binding transporter, which is intensively expressed in mitochondria of erythroid cells and is an integral glycoprotein of the inner mitochondrial membrane. The interaction of mitoferrin with Abcb10 increases its stability and the efficiency of the iron transport because it interacts with ferrochelatase [123, 127, 128].

In the inner mitochondrial membrane of yeast cells, the iron transporter Mrs3/4p has been identified, and its homolog has been described in humans [129].

The transport of reduced iron into the cytosol across the inner mitochondrial membrane is also mediated by a hydrophilic protein of the matrix, frataxin (210 a.a.), which is encoded by the gene associated with Friedreich's ataxia. A GAA repeat in the first intron of the frataxin gene leads to low and sometimes undetectable level of this protein in nearly all patients with Friedreich's ataxia [130]. The deletion of a frataxin homolog (*yfh1p*) in yeasts results in disorders of iron metabolism in the mitochondria and is accompanied by its excessive accumulation in the matrix and intensification of free radical oxidation [131, 132]. It seems that Friedreich's ataxia in humans is also underlain by disorders in the iron metabolism in mitochondria. Frataxin is incapable of binding iron [133]. Its role in the iron metabolism is not quite clear, but it is

known to interact with ABC7 (ATP-binding cassette transporter 7). ABC7 is a protein of the inner mitochondrial membrane that is responsible for the divalent iron export from the matrix and for its regulation. Defects of the frataxin gene lead to sideroblastic anemia combined with Friedreich's ataxia [6, 134-136].

Iron is accumulated in the mitochondrial matrix binding with mitochondrial ferritin or is used by ferrochelatase, which associates divalent iron with protoporphyrin IX [18]. Mitochondrial ferritin is homologous to the H-subunit of cytosolic ferritin, is encoded in the nucleus, and has on the *N*-end a sequence with 60 a.a. that allows it to penetrate into mitochondria, where it forms a homopolymer in the matrix [137, 138]. The highest expression of mitochondrial ferritin is observed in the sex cells of testes, whereas it is absent in liver and spleen macrophages. Similarly to the H-subunit of cytosolic ferritin, mitochondrial ferritin has ferroxidase activity and stores trivalent iron [137]. Because mitochondria contain large amounts of iron-containing proteins, free iron, and free radicals, mitochondrial ferritin plays a protective role [123, 127].

Heme is transported from mitochondria into the cytosol under the influence of protein FLVCR (Feline Leukemia Virus subgroup C Receptor-related protein) [139].

Heme iron is transferred across the inner mitochondrial membrane by an unknown transporter [140]. The protein ABC6 (ATP-binding cassette transporter 6) of the outer mitochondrial membrane is responsible for transfer of heme iron from the intermembrane space into the cytosol [141, 142].

IRON TRANSPORT ACROSS THE BLOOD–BRAIN BARRIER

In the brain, capillary endothelium type-1 transferrin receptors are expressed, which provide uptake of diferric transferrin and release of iron ions into the cytosol. On the adlumenal surface of endotheliocytes, ions of reduced iron are exported from the cytosol into the extracellular space under the influence of ferroportin. Astrocytes and neurons take up this iron under the influence of DMT1 [143, 144]. Under physiological conditions, the pH of the intercellular fluid in brain parenchyma is 7.2, whereas inside astrocytes the pH is ~7.4. This feature determines the entrance of iron into astrocytes, because DMT1 is a symporter of protons and needs them for transporting iron [145-147]. Moreover, neurons are shown to take up trivalent iron complexed with transferrin. Oxidized iron of the intercellular fluid binds with transferrin, which is produced by oligodendrogliocytes, and then this complex interacts with type-1 transferrin receptors on the plasmalemma of neurons, initiating endocytosis [148].

INTRACELLULAR IRON

Hepatocytes and mononuclear phagocytes contain the greatest amount of intracellular iron [149]. Intracellular iron compounds with different functions and characteristic activities and biological roles can be subdivided into four groups [15, 18]. The *first group* consists of hemoproteins, i.e. proteins containing heme as a prosthetic group: hemoglobin, myoglobin, neuroglobin, cytoglobin, cytochromes, cyclooxygenase, NO-synthase, cytochrome *c*-oxidase, catalase, guanylate cyclase, and some peroxidases such as thyroperoxidase, myeloperoxidase, lactoperoxidase, and eosinophilic peroxidase. The *second group* includes nonheme iron-containing enzymes: flavoproteins with iron–sulfur centers, such as succinate dehydrogenase, NADH-dehydrogenase, xanthine oxidase, aconitase, and ferrochelatase; or enzymes with iron as a cofactor: lipoxygenase, ribonucleotide reductase, superoxide dismutase, and tyrosine, tryptophan, and phenylalanine hydroxylases, as well as prolyl hydroxylase [10]. The *third group* comprises the iron-binding cytosolic proteins ferritin and hemosiderin, which are responsible for accumulation of trivalent iron ions and prevention of their toxic action in cells [71]. High levels of plasma iron stimulate the synthesis of ferritin in cells, in particular in hepatocytes. It is known that hepatocytes and stellate macrophages are involved in creation of the stored pool of iron in the body, and the largest part of this iron (~1/3 of the body iron) is found in the liver in hepatocytes as the ferritin component. Stores of ferritin iron can be mobilized for the body's needs. Overloading hepatocytes with iron is hepatotoxic and causes damage to these cells that is accompanied by inflammation [71]. Inflammation in the liver and necrosis of hepatocytes cause an increase in ferritin concentration in the blood plasma [17, 149].

There are data indicating that serum ferritin (isoferritin) is a product of hepatocyte secretion and not a result of their degradation. Serum ferritin contains a small amount of iron and is likely to bind free iron of blood plasma. It seems also that serum ferritin provides some cells with iron – the presence of ferritin receptors on neurons, lymphocytes, and other cells has been described [10, 150, 151]. Serum ferritin can reflect tissue stores of iron. Thus, the ferritin concentration 1 μg/liter corresponds to 8-10 mg iron bound with ferritin in the tissues [152]. The ferritin concentration in blood serum in men is higher than in women (81-600 and 23-350 pM, respectively) [153].

Ferritin is a multimeric water-soluble glycoprotein complex consisting of 24 subunits (apoferritin): heavy or heart subunits (H) with weight of 21 kDa and light or liver subunits (L) with weight of 19 kDa and of different number of trivalent iron atoms. The ratio of H and L protomers in apoferritin determines its tissue isoforms. The subunits form a spherical ensemble with external diame-

ter of 12 nm and internal cavity with 8-nm diameter. The envelope of this ensemble has six channels 0.3-0.4-nm in diameter, and iron is delivered and released through these channels. The molecular weight of apoferritin is 450 kDa [7, 120, 154-157]. The heavy chains of apoferritin have a ferroxidase center and oxidize divalent iron, and afterwards the light chains complex trivalent iron. One ferritin complex can bind up to 4500 atoms of oxidized iron, but usually their number is no more than 3000 atoms [10]. Iron is deposited in the ferritin cavity in salts – hydroxyphosphates – and does not interact with the protein moiety of ferritin [158].

Ferritin performs a double role: first, it stores iron for the body's needs, and second, it protects the cell against free radical reactions with participation of free iron [10, 159]. Ferritin is mainly localized in the cytosol, but it has also been found in the nuclear matrix [160, 161]. It seems that in the nuclear matrix ferritin provides enzymes and/or transcriptional factors with iron or binds free iron to prevent DNA damage by free radicals [162, 163]. Mitochondrial ferritin has also been described, which provides for the biosynthesis of heme-containing and iron–sulfur mitochondrial proteins and protects mitochondria against the action of free iron [164].

Iron is mobilized from ferritin due to its proteolytic degradation. Hemosiderin is a product of incomplete intralysosomal degradation of ferritin [101, 165-167].

The conversion of ferritin into hemosiderin begins from the oversaturation of the ferritin molecules with iron ions (on excess of intracellular iron, it interacts with the protein moiety of ferritin). Then an autophagosome is produced, and ferritin overloaded with iron ions undergoes lysosomal degradation. Hemosiderin is a water-insoluble iron-binding protein that stores a significantly greater number of iron ions than ferritin does (more than 4500 atoms per hemosiderin complex). The iron content in hemosiderin can reach 40% of the weight of the complex. As discriminated from ferritin, hemosiderin under physiological conditions is not a donor of iron ions for its pool in the body but plays only a protective role [10, 168, 169]. Hemosiderin contains iron but is unable to release it [170]. About 5% of the stored pool of iron in the human body is in hemosiderin, which is mainly localized in the liver stellate macrophages [6]. The *fourth group* includes low-molecular-weight inorganic and nonprotein organic iron compounds consisting of complexes with citrate, ATP, cysteine, etc. [19, 171]. Nitric oxide (II) and carbon monoxide (II) generated under the influence of NO-synthase and heme oxygenase, respectively, have high affinities for reduced iron. Iron complexes with NO and CO influence the expression of iron regulatory proteins (IRP), which control the synthesis of ferritin, transferrin receptors, and iron transporters (ferroportin and the divalent metal transporter). Carbon monoxide produced in macrophages due to degradation of hemoglobin under the influence of heme oxygenase binds reduced iron, acting as an antioxidant. Divalent iron preparations are well known as antidotes to intoxication by carbon monoxide [172].

EXTRACELLULAR IRON

Iron is necessary not only for various metabolic processes, but it is also an important participant of free radical oxidation of biomolecules inside and outside the cell [18]. Free reduced iron initiates the formation of reactive oxygen species and triggers peroxidase-type damage in cells [158]. Free iron has high toxicity because as a metal with variable valence it is able to trigger free radical chain reactions leading to generation of reactive oxygen species, which can damage organelles, membranes, the genetic material, as well as biomolecules (proteins, nucleic acids, lipids) and cause oxidative stress [3, 6, 71].

In biological fluids of the human body, iron is mainly in the bound state as iron–protein complexes. Iron concentration in blood plasma widely varies from 9 to 32 μM (18 μM, on average) with rather large daily fluctuations [152]. Extracellular iron-binding proteins in humans include transferrin, lactoferrin, and siderocalin [6].

Transferrin (siderophillin) is a plasma single-chain glycoprotein with molecular weight of 75-80 kDa synthesized by hepatocytes (apotransferrin). Transferrin contains two domains, each of which is able to bind an atom of trivalent iron [120, 154]. Small amounts of transferrin are synthesized by circulating lymphocytes, macrophages, Sertoli cells, etc. [91, 173]. Carbohydrate residues with sialic acid in the end constitute ~6% of transferrin. Apotransferrin binds trivalent iron through oxygen atoms of the phenol rings of two tyrosine residues and through the imidazole nitrogen of a histidine residue and through the oxygen of the γ-carboxylic group of an asparagine acid residue [174]. These ligands occupy four of six coordination bonds of the iron atom and form an octahedron, whereas the remaining two bonds bind bicarbonate ions [119]. One molecule of apotransferrin binds two iron atoms as bicarbonate, and 1 g of transferrin corresponds to ~1.14-1.25 mg iron of blood plasma [6, 7, 10]. Transferrin is the major carrier protein of blood plasma that provides the internalization of iron into cells and prevents body tissues against its toxic action. Transferrin provides the solubility of trivalent iron ions in blood plasma and is responsible for the controlled and targeted delivery of iron into cells [154, 175]. The half-life of transferrin is from 8 to 12 days [7].

Under physiological conditions the transferrin concentration in human blood plasma is relatively constant at 2-4 g/liter (50 μM), with 10% as diferric transferrin. Normally, transferrin has 20-30% saturation with iron, whereas in rodents the transferrin saturation is 60-80%

[15, 71, 92, 176]. Only one third of the ability of transferrin for binding iron is used in human; therefore, in the blood flow it is present in the state of apo-, mono-, or diferric transferrin [21]. In humans, the saturation of transferrin with iron *in vivo* is characterized by the free iron-binding ability of blood plasma. The free (unsaturated) iron-binding ability of blood plasma is determined as the amount of iron that can be additionally bound by transferrin. The free iron-binding ability of blood plasma together with iron bound by transferrin *in situ* (plasma iron) is called the total iron-binding ability of blood plasma [177]. The total iron-binding ability of human blood plasma varies from 44.7 to 71.6 μM (56 μM on average), and the free iron-binding ability, or the storage capacity of transferrin, is 28.8-50.4 μM [119, 153].

Due to the high affinity of transferrin for oxidized iron, virtually all iron of blood serum/plasma is bound with transferrin, i.e. is inactive in redox reactions. The binding constant of iron with transferrin is 10^{24} (K_d = 10^{-23}); therefore, in blood plasma there is less than one free ion of trivalent iron per liter [3, 119].

All iron not bound with transferrin is called non-transferrin-bound iron (NTBI – Non-Tf-Bound Iron). It is iron bound with low-molecular-weight chelators such as citrate and ATP and proteins of blood plasma (serum ferritin, albumin) or of intercellular fluid. Albumin has a weak affinity for iron ions and begins to bind with them at iron concentration above 0.5 mM [178, 179]. This situation is observed in diseases and states associated with excess entrance and accumulation of iron (e.g. hemochromatosis, hypotransferrinemia, hemolytic anemia). At transferrin saturation above 60% in the blood of a healthy person, iron can be detected unbound with transferrin [21, 180, 181]. Normally, the concentration of non-transferrin-bound iron is <1 μM, but it can increase to 10-20 μM upon an increase in the entrance of iron. Many cells, including hepatocytes, erythroid cells, and erythrocytes, are able to take up non-transferrin-bound iron [182-184].

A direct correlation has been established between transferrin saturation, the level of non-transferrin-bound iron, and the entrance of iron into tissues [181]. Non-transferrin-bound iron is captured by liver cells by a positive feedback mechanism, and this can lead to damage of hepatocytes and stellate macrophages [94].

Bone marrow, liver, and small intestine are the main tissue and organs of iron metabolism, and each has a system of tissue receptors specific for transferrin. Reticulocytes of the bone marrow, similarly to cells of the intestinal mucosa, have increased ability to capture iron from saturated (diferric) forms of transferrin. The main sources of plasma iron are mononuclear phagocytes of the internal organs (liver, spleen, red bone marrow) where hemoglobin is subjected to degradation. A small amount of iron enters blood plasma from the stores and on uptake from food in the gastrointestinal tract [6, 71].

The iron-binding protein apolactoferrin has been found in various biological fluids – milk, tears, bile, saliva, synovial fluid, gastric and pancreatic juice, the small intestine secrete, and bronchial mucosa [15, 185, 186]. Moreover, apolactoferrin is present in the secondary (specific) granules of neutrophils produced in myeloid series cells beginning from the promyelocyte stage. There are insignificant amounts of apolactoferrin in blood plasma released into it from neutrophils. Similarly to apotransferrin, apolactoferrin binds two atoms of trivalent iron. Lactoferrin consists of one polypeptide chain of 80 kDa, and under physiological conditions, it is 20% saturated with iron. Iron-free lactoferrin, apolactoferrin, has bacteriostatic features, which disappear upon its saturation with iron [7]. The iron–apolactoferrin complex penetrates into the cell by endocytosis mediated through receptors to lactoferrin. Receptors to lactoferrin responsible for lactoferrin uptake from the intestine have been described on erythrocytes of fetuses and newborns [187, 188].

Siderocalin (lipocalin 2, NGAL-Neutrophil Gelatinase-Associated Lipocalin) is a protein of the acute phase that limits the concentration of free iron in intercellular fluid [189]. Siderocalin, which was initially identified as a component of specific granules of neutrophils, is a 25-kDa glycoprotein bound with gelatinase of neutrophilic granulocytes [190]. Siderocalin is synthesized by immune cells, hepatocytes, adipocytes, glandular cells of the prostate, cells of kidney tubules, etc. Siderocalin exists as a monomer, homodimer, and heterodimer; it binds bacterial siderophores, limits iron consumption by bacteria, and regulates its consumption by body cells. A siderocalin receptor, megalin, has been identified as a member of the family of low-density lipoprotein receptors [191].

EXCRETION OF IRON FROM THE HUMAN BODY

Iron losses of an adult human are about 1 mg/day and are determined by exfoliation of epitheliocytes, slight loss of blood (about 1 ml/day), and also by releasing iron ions with sweat, urine, and feces [15, 44, 101]. The losses of iron mainly occur through the GIT – due to desquamation of epithelial cells of the intestine and with bile. About 250 g of exfoliated epitheliocytes of the small intestine mucosa are delivered into the intestinal lumen during a day. About 10% of the enterocyte mass consists of proteins that are degraded during digestion, and most of their degradation products are reabsorbed [192]. Iron is also lost at the desquamation of epithelial cells of the skin, sweating, and to the lesser degree with urine and during menstruation and labor in women of childbearing age. In mammals, there is a system of iron recirculation; therefore, the daily need for exogenous iron is, on average, 1.5-2 mg. In the absence of hemorrhages and hemoglobin-

uria, maximal daily iron losses are no more than 4 mg [19, 193, 194].

There are no mechanisms of iron excretion, it is eliminated passively [185, 195, 196]. However, it is known that the daily loss of iron can decrease to 0.5 mg/day in patients with iron deficiency anemia and increase in states associated with an excess entrance and accumulation of iron [152]. On excess entrance of iron, enterocytes can accumulate it and exfoliate into the intestinal lumen, executing a protective function [5].

Being released on degradation of erythrocytes, iron is reutilized, and this process is accompanied by a partial release of 5-25 mg iron with bile into the small intestine and then by its reabsorption with involvement of enterocytes into the blood and re-inclusion into the total pool of body iron [22, 44, 185, 186]. An adult man loses 1 mg iron per day, whereas a childbearing age woman loses 1.5 mg. During menstruation, pregnancy, and breastfeeding, iron losses increase to 2 mg per day [10]. To cover the natural losses, the consumption of elementary iron has to be 1 mg/day for men and 1.4 mg/day for women during menstruation. To compensate the losses, it is necessary to assimilate 1-2 mg exogenous iron per day, which approximately corresponds to 8-10 mg iron in food per day [197].

Iron metabolism is a complex multi-stage process with involvement of cellular (membrane and cytosolic) and extracellular (plasma) proteins and low-molecular-weight organic compounds (ascorbate, etc.), which promote its penetration into the cells, oxidation/reduction, storage, and export. The indispensability of iron for all organisms is determined by its importance for cell life, and its complex metabolism explains difficulties of correcting clinical disorders associated with its deficiency or excess. During recent years, significant progress has been achieved in the understanding of iron metabolism in humans and details of its uptake, transport, and accumulation.

On one hand, iron is an indispensable microelement, but on the other hand, it is a metal with high toxicity because it is able to catalyze the formation of free radicals. Differently directed biological effects of iron determine the multi-stage and complex character of its metabolism in the body.

Although the prevalence of iron in the Earth's crust is the fourth among the chemical elements, pathological conditions associated with its insufficient entrance into the body because of its low bioavailability and the complex assimilation mechanism characterize common nutritional disorders (~2 billion people suffer from iron-deficiency anemia) [7, 10, 51] leading to negative systemic effects. Considering the iron role in the normal vital activity of the human organism, it is obvious that disorders in its intra- and extracellular metabolism can underlie various diseases, many of which are now difficult to treat. Thus, disorders in iron metabolism in nervous tissue are associated with pathogenesis of such diseases as Parkinsonism, Alzheimer's disease, and Friedreich's ataxia. Excess accumulation of iron in the human body, exceeding 20 g in different variants of hemochromatosis, is accompanied by development of myocardiopathies, arthropathies, hypogonadism, diabetes mellitus, decrease in resistance to infections, and acceleration of aging [198-200]. It should be noted that for effective treatment of diseases associated with iron deficiency or excess, it is necessary to understand in detail mechanisms of its transformation in the body and to study further molecular bases of the metabolism of this microelement.

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