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Iron: The Dual Element

[Demir: İki Yönlü Element]

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ABSTRACT

Iron is one of the most widely spread metals in the earth's crest. In the living body it has a dual nature. The element is essential for almost all living organisms: it participates in important metabolic processes including oxygen transport, DNA synthesis and electron transport, regulation of gene expression. However, excess of iron is highly toxic because of its ability to promote free radical formation via Fenton's reaction. High levels of iron within the body have been associated with increased incidence of some cancers and dysfunction of organs [heart, pancreas, liver] or development of some neurodegenerative diseases. This dual nature has forced the evaluation of systems of transport, store and use of the element in a safe and available manner. The specific binding to proteins provides increased iron solubility and bioavailability, reduces the potential toxicity and exerts control over the metabolism of this essential nutrient.

Recent advances in genetics, molecular biology and biochemistry of iron metabolism provide more detailed understanding of regulatory mechanisms. That knowledge contributes to more effective protection and treatment of disorders, caused by disturbances in iron homeostasis thus making a step ahead in improving health and quality of life.

Key Words: Iron, transport, uptake and storage, regulatory mechanisms, iron absorption

ÖZET

Demir yer kabuğunda en bol bulunan metallerden biridir. Canlı organizmasında ikili bir tabiatı vardır. Hemen hemen bütün canlı organizmalar için alınması zorunlu olan bir elementtir. Oksijen taşıma, DNA sentezi ve electron taşınması, gen ifadesinin düzenlenmesi gibi önemli metabolik işlemlerde rol alır. Öte yandan, demirin fazlası Fenton tepkimesi yoluyla serbest radikal oluşumunu arttırdığı için oldukça toksiktir. Vücuttaki yüksek demir düzeyinin bazı kanserlerle ve organ bozukluğu ile [kalp, pankreas, karaciğer] veya nörodejeneratif hastalıkların gelişmesi ile ilişkili olduğu gösterilmiştir. Bu ikili tabiat, elementin güvenli ve yararlanılabilir şekilde taşınma, depolanma ve kullanım sistemlerinin gelişmesini zorunlu kılmıştır. Proteinlere özgül bağlanma demirin çözünürlüğünü ve biyoyararlanımını arttırıp, potansiyel toksisitesini azaltır ve bu esansiyel besin maddesinin metabolizması üzerinde kontrol sağlar.

Anahtar Kelimeler: Demir, taşınma, alım ve depolama, düzenleyici mekanizmalar, demir emilimi

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Distribution of Iron in Human Body

The average content of iron in the body is approximately 35 and 45 mg/kg body weight in adult women and men respectively [1]. The main part of total body iron [60 – 70 %] is incorporated in the structure of hemoglobin of the developing erythroid precursors and mature erythrocytes. Another 10 % of essential iron is presented in muscle myoglobin and some other tissue enzymes and cytochromes. In healthy individuals, the remaining iron [20-30 %] is stored as ferritin and hemosiderin in hepatocytes and macrophagial systems of liver, spleen and bone marrow.

The iron, bound to plasma transferring is less than 1 % of the total iron store, but its pool is very important with high turnover. Almost 80 % of transferrin-bound plasma iron is transported to the bone marrow for hemoglobin synthesis in the developing erythroid cells [2]. The monocyte-macrophagial system provides the biggest amount of iron, needed for the erythropoesis [3].

Transport And Uptake Of Iron

Within the body, between the sites of absorption, storage and utilization, iron is transported bound to plasma protein transferrin. The protein is also involved in the transport of other metal ions such as Al^{3+}, Mn^{2+} [48, 49], Cu^{2+} and Cd^{2+} [4] although the affinity for binding iron is the highest. Transferrin is mainly synthesized in the liver but is also produced in the brain, testis, lactating mammary glands and in some fetal tissues during the development. In the circulation transferrin molecules exist as a mixture of iron-free [apo], mono-ferric and di-ferric forms. The relative abundance of each form depends on the concentration of iron and transferrin in blood plasma. The complex transferrin-iron enters the cells by a receptor mediated endocytosis with formation of specialised endosomes. Di-ferric transferrin has the highest affinity, followed by monoferric and apotransferrin has the lowest affinity [5].

There are at least two types of transferring-receptors; each has its own distinct cell and tissue-specific expression. The protein structure of the receptor 1 consists of three domains: 1. cytoplasmic region [61 amino residues, amino-terminal]; 2. transmembrane region [28 residues] and 3. extracellular carboxyl-terminus region [671 residues], to which transferring binds [6,7]. Nucleotide sequence analysis of the structure reveals a moderate degree of similarity with prostate-specific membrane antigen [8]. Biological significance of this similarity remains to be elucidated. The form in the circulation lacks the cytoplasmic and transmembrane domains of the intact receptor [6,7]. Serum [soluble] transferrin receptors correlate well with red cell iron turnover and erythropoetic activity.

Transferrin-receptor 1 is mainly expressed in immature erytrocytes, placental tissues, liver and rapidly divided cells [9].

Studies, based on mice models, demonstrate biological significance of this form of the receptor. Mice heterozygous for the disrupted gene develop typical symptoms of iron deficiency [10]. Mice with homozygous mutations of the gene die in utero because of highly defective erythropoesis and heavily disturbed neurological development. The neurological defect raises question weather the defect is caused by the impaired supplying of neurons with iron or the same receptor has some additional yet unidentified function, not related to iron, in these cells.

The second transferring receptor TfR-2 is a transmembrane glycoprotein that shares 66 % similarity with TfR-1 in the extracellular domain. It is expressed in the liver where expression of TfR-1 is relatively low. It could bind iron and play role in cellular iron uptake. Affinity of transferrin is lower for TrR-2 than for TfR-1 [11]. The biological significance of TfR-2 is evident in humans with a homozygous mutation in its gene. These patients develop a non-HFE-linked form of hemochromatosis [12].

Non-transferrin bound iron [NTBI] could be uptaken by pathways, which include production of hydroxyl radicals rather than through increased other transporter synthesis or other type of the cell surface receptors. NBTI uptake also appears to be influenced by other trivalent metals, but how these effects are exerted is unknown [13]. Additional transferrin-independent mechanism by melanotransferrin and lactoferrin exist to transport iron [14, 15].

Iron Storage

Alterations in iron storage capacity are key part for establishing of iron homeostasis. Modulation of iron storage can be performed by changed synthesis or degradation of ferritin molecules.

Tissue ferritin is composed of two type of chains: heavy [H] and light [L]. A third subunit G is found in the serum and is thought to derive from L-subunit by glycosylation [16]. H-ferritin is a ferooxidase and this activity promotes the more rapid incorporation of iron into ferritin structures, rich in this subunit. The H chains predominate in tissues like heart and pancreas. Isoforms, rich of L-chains take up iron more slowly but hold it longer and they are predominant in storage tissues like liver and spleen. A decrease in the amount of H subunits increases compensatory L-subunit synthesis. Therefore, H deficiency can lead to isolated hyperferritinaemia in the absence of iron overload [17].

In the cells, loaded by iron, lysosomal hydrolytic enzymes degrade the aggregates of ferritin to the formation of haemosiderin. This conversation may have a cytoprotective role against iron cytotoxicity..

Regulation of Iron Uptake and Storage

Tight regulation maintains a balance between iron uptake, transport, storage and utilization in response to cellular metabolic needs. In mammalian cells, the expression of many key molecules in iron metabolism is regulated via a feedback regulatory mechanism. In non-

erythroid cells these regulations are occurred at post-transcriptional level and depend of intracellular iron levels [18,19]. They involve interaction between specific sequence – stem-loop structures, termed iron responsive elements [IRE] in mRNA and iron regulatory proteins 1and 2 [IRP]. Depending on their locations in mRNA, IRE-s act as suppressors or enhancers of translation thus resulting in decreased or increased stability of mRNA molecules and therefore in suppressed or stimulated synthesis of the key proteins: ferritin, TfR1, Ireg1, DMT1. In erythroid cells the regulation occurs at transcriptional level during erythroid differentiation without involving IRE and IRP mechanism.

In the patients with hereditary hyperferritinemia-cataract syndrome, an autosomal dominant disorder, a single point mutation in the hexa-nucleotide loop region of the IRE in ferritin light chain has been identified and characterized [20]. Therefore the affinity of IRPs to the mutant IRE is dramatically reduced. The result is overproduction of ferritin irrespectively to cellular iron status.

Disorders of Iron Metabolism

Disorders of iron metabolism are related either to deficiency or to excessive accumulation of iron. To minimize iron-mediated harmful effects and to reduce cost of the treatment, early diagnosis is absolutely necessary.

Iron deficiency

Iron deficiency probably represents the most common nutritional deficiency overall. Once the body stores have been exhausted, a continuing negative iron balance reduces transferrin-iron saturation to less than required to maintain erythropoiesis. Eventually, with continuing iron deficiency, haemoglobin production is impaired and anaemia develops, reducing the availability of oxygen to the tissues. Consequences of iron –deficiency anaemia are well known. Special interest provoke observed non-hematological effects: reduced work performance with lactic acidosis, reduced attention span and learning ability in children, impaired temperature regulation, defects in cell-mediated immunity.

In some periods such as early childhood and adolescence, the iron content in the diet could be not enough to provide proper amounts for the body needs. For the women in active age, the additional iron loss by the monthly cycle increases the daily needs. During pregnancy there is also an increased need of iron. Special attention is concentrated on early diagnosis of iron deficiency in pre-school children because lack of iron in this case may cause deteriorated cognitive function with considerable social implication.

Iron Overload

Hereditary hemochromatosis is inherited autosomal recessive disorder with a high prevalence in Caucasians [0.5-1.20 %] [21]. HFE was discovered as the causative gene for this disease in 1996. Two mutations [C282Y and H63D] account for 90 % of hemochromatosis [1].

The patients with non HFE-hemochromatosis have a homozygous mutation in the gene that codes the structures of the transferrin receptor 2. It seems possible that TfR2 function is associated with regulation of homeostasis rather than directly involved in iron uptake [22].

Excessive absorption may occur in genetically caused African model of iron overload [Bantu siderosis]. Increased deposition of iron is generalised in the macrophages. This fact has a special consequence in Africa where tuberculosis is endemic. The cause of this disturbance is combination between stimulated iron absorption and genetic defect, non-localised into HLA locus [23].

Iron overload is also occasionally seen patients with alcoholic liver disease. The mechanism for stimulation of iron absorption may include the high iron content of some alcoholic beverages, pancreatic exocrine insufficiency or a direct effect of alcohol as a stimulator of iron absorption.

Intestinal iron absorption-physiology and molecular aspects

Iron has no normal pathway for excretion from the body. Therefore the positive iron balance is maintained by regulating predominantly the intestinal absorption of the element.

In man dietary iron intake consists of two components: haem iron [red meat] and non-haem iron [vegetables, cereals]. Non-haem iron exists predominantly in the highly insoluble ferric form Fe[III] and therefore is poorly bioavailable. It is the reason for the high percent of iron deficiency anaemia in the developing countries, where the combination of poor iron supplies and parasitic infections reduces iron stores. Non-haem iron is absorbed early in the duodenum, where the low pH favours the solubility of iron. Low molecular iron complexes and Fe^{2+} are well absorbed. In the jejunum and ileum, the element forms insoluble complexes thus decreasing the bio-availability of iron.

Iron absorption occurs mainly in the proximal intestine with the maximal rate in the duodenum and the lowest in the ileum [24]. The intestinal transit of iron through duodenal enterocytes includes two main components: uptake and transfer [25]. Uptake is defined as a transport of dietary iron across the apical membrane of the enterocyte into the intestinal mucosa. Transfer is the movement of iron from the enterocyte across the basolateral membrane to the circulation.

Mucosal uptake

Apical transporter DMT1/Nramp2

This transporter is a protein that transfers iron across the apical brush-border membrane within the cells. The nature of the transporter has been identified by two independent groups. Gunshin et al. gave the name DMT1, which underlines the capability of the protein to transport divalent ions of some metals [26]. Fleming et al.

gave the name Nramp2 and revealed that the transporter is analogue of protein in the macrophages and it is a part of the defence against intracellular pathogens [27]. DMT1 is not specific only for iron. The ability of DMT1 to transport the ions of some heavy metals such as Pb or Cd provides an explanation for the increased absorption of these toxic metals in iron deficiency state. Zinc and copper have been reported to regulate DMT1 expression, providing another possible mechanism for metal interaction with iron absorption [28].

Duodenal ferri-reductase activity

In mammalians, iron is taken primarily in the the duodenum, where ferrous iron is more efficiently absorbed than ferric iron. DMT1 has been shown to be responsible for the uptake of ferrous iron from the lumen into the mucosa. Most dietary iron is in the form of ferric iron complexes and these must be reduced to ferrous ions before iron can be transported by DMT1 across the apical membrane. A brush-border surface ferric reductase enzyme activity has been demonstrated both in the duodenal mucosa itself and in cultured intestinal cells [24,29]. Reduction and uptake are sequential steps during absorption [24]. The reductase activity is strongly stimulated by hypoxia and iron deficiency, both of which stimulate absorption of dietary non-haeme iron. The reductase activity is highest in the duodenum and lowest in the ileum, compatible with the profile of iron absorption along the gut: highest in the duodenum and lowest in the ileum [24].

McKie et al. identified the gene, responsible for the reducing activity [duodenal cytochrome b -dcytb] by a subtractive cloning strategy [30]. The protein sequence is homologous to cytochrome b561-b-type haem transmembrane reductase, highly expressed in chromaffin granule membranes in the adrenal medulla. Later studies confirm that duodenal ascorbate serves as an electron source for this reductase activity thus providing one mechanism for molecular explanation of interaction between intracellular ascorbate levels and the rate of iron absorption [31,32].

Basolateral transfer

The protein transporter, responsible for the transfer across the basolateral membrane of duodenal enterocytes across the circulation is Ireg 1/Ferroportin. Ireg1 is isolated following the same strategy as for Dcytb [33]. Ireg1 is independently identified in other two labs and has been named ferroportin [34] and MTP1 [35]. Ireg1 likely also plays an important role in iron transport in other organs, notably the placenta, where iron transfers between maternal and fetal circulations. Recycling of iron into circulation from the breakdown of haemoglobin and realising from macrophages to the circulation also probably involves Ireg1. Several groups report mutations in the structure of Ireg1 which cause haemochromatosis [36,37] distinct from HFE mutations. The clinical data show distinct differences for patients with HFE mutations. In patients with Ireg1 mutations, serum ferritin is very high and reticuloendothelial cells are severely loaded in contrast to HFE patients.

Hephaestin - intestinal ferooxidase

Hephaestin is a protein with a structure highly similar to that of ceruloplasmin-multi-copper ferrooxidase. Studies indicate that the protein is required for the release of iron into the circulation and the binding to the transferring. Hephaestin is not a transporter, but it facilitates the iron transport. In normal iron uptake of dietary iron but defective hephaestin microcytic anemia is developed and it is not compensated effectively by oral iron supplementation but by parenteral iron supplying. Hephaestin is highly expressed in small intestine but not predominantly in the duodenum like Ireg1, Dcytb or DMT1. In fact it is expressed in the gut with no obvious gradient. This suggests hephaestin may have additional role in the intestine unrelated to iron absorption [38].

Hepcidin: hormone - key regulator of iron homeostasis

Hepcidin is a small 25-aminoacid disulfide rich peptide, synthesized in the liver and secreted in the plasma [39,40]. The lack of the peptide causes progressive accumulation of iron and the over-expression leads to severe iron deficiency anaemia [41]. Mutations in hepcidin cause early-onset iron overload in man with juvenile hemochromatosis [42]. Refractory iron deficiency anaemia in patients with large liver adenomas is related to markedly increased RNA levels of hepcidin [43]. In vitro data suggest that increased non-transferrin bound iron in thalassemia syndromes and some other hemolytic states, in patients with hereditary hemochromatosis, hypo- and a-transferrinemia could lead to decreased hepcidin secretion: it provides one possible mechanism, explaining increased iron absorption and iron overload in these pathological conditions [44].

The idea that hepcidin could be cytokine-regulating gene both impairing iron recycling from the macrophages and iron absorption is confirmed by the fact that its production is induced by IL-6, but not by IL-1 и TNF-α [45].

Availability of reliable serum and urine hepcidin assay could enlarge the number of studies on hepcidin regulation, performed in man [46].

Regulators of iron absorption

Iron in the diet could modulate the rate of absorption: mechanism referred as “dietary regulator” [1]. The phenomenon “mucosal block” occurs several days after the consumption of dietary iron. Supplementation by high oral iron doses decreases Dcytb and DMT - expression thus suggesting that “mucosal block” operates at the level of protein involving in iron uptake [47].

Iron stores regulator responds rather to total body iron than dietary iron. It probably acts at the level of crypt-cell programming. This regulator facilitates slow accumulation of dietary non-heme iron and does not regulate

hem-iron uptake. It prevents iron overloading once the body iron stores have been repleted [1].

Several other factors could influence the rate of intestinal iron absorption: marrow erythropoetic activity, blood hemoglobin concentration, presence of systematic inflammation [48]. Iron absorption is increased in rapid growing and pregnancy: states with decreased iron in the tissues. During the second pregnancy stage the absorption of iron from the diet is increased as well as absorption of supplemented inorganic iron. The increasing is continuing at about 12 weeks after birth delivery.

Recent knowledge on the mechanism of intestinal non-heme iron absorption confirms earlier hypothesis that hypoxia plays role of independent stimulating regulator [49,50]. Key aspect of iron absorption regulation is separation of absorption mechanism from the presumed sensing mechanism. This separation is supported by recent findings on the crypt-villus localization of proteins concerning absorption [25].

Considerable progress has been made in understanding the mechanisms controlling iron absorption and the whole iron homeostasis. The dual nature of iron in human body determines the essential importance of these regulations. Profound studies of the processes, maintaining normal narrow iron limits contribute to more effective protection and treatment of the main disturbances of iron metabolism [iron deficiency and iron overload] and allow improving health and quality of life.

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References

[1] Andrews NC. [1999] Disorders of iron metabolism. N Engl J Med 341: 1986-95.

[2] Conrad ME, Umbreit JN, Moore EG. [1999] Iron absorption and transport. Am J Med Sci 318: 213-29.

[3] Vogt BA, Alam J, Croatt A et al. [1995] Acquired resistance to acute oxidative stress: possible role of heme oxygenase and ferritin. Lab Invest 72: 474-83.

[4] Moos T, Morgan EH. [2000] Transferrin and transferrin receptor-function in brain barrier systems. Cell Mol Neurobiol 20: 77-95.

[5] Young SP, Bomford A, Williams R. [1984] The effect of the iron saturation of transferrin on its binding and uptake by rabbit reticulocytes. Biochem J 219: 505-10.

[6] Flowers CH, Skikne BS, Covell AM et al. [1989] The clinical measurement of serum transferrin receptor. J Lab Clin Med 114: 368-77.

[7] Huebers HA, Beguin Y, Pootrakul P et al. [1990] Intact transferrin receptors in human plasma and their relation to erythropoiesis. Blood 75: 102-7.

[8] Evans P, Kemp J [1997] Exon/intron structure of the human transferrin receptor gene. Gene 199: 123-131.

[9] Ponka P. [1999] Iron metabolism: physiology and pathophysiology. Trace Elem Res Hum 5: 55-7.

[10] Levy JE, Montross LK, Andrews NC. [2000] Genes that modify the hemochromatosis phenotype in mice. J Clin Invest 105: 1209-16.

[11] Kawabata, H, Germain, RS, Vuong PT et al. [2000] Transferrin receptor 2-alpha supports cell growth both in iron-chelated cultured cells and in vivo. J Biol Chem 275: 16618-25.

[12] Camaschella C, Roetto A, Cali A et al. [2000] The gene TfR2 is mutated in a new type of hemochromatosis mapping to 7q22. Nat genet 25: 14-15

[13] Olakanmi O, Stokes JB, Pathan S. [1997]. Polyvalent cationic metals induce the rate of transferrin-independent iron acquisition by HL-60 cells. J Biol Chem 272: 2599-2606.

[14] Danielsen EM, VanDeurs B. [1995] A transferrin-like GPI-linked iron-binding protein in detergent-isolated noncaveolar microdomains at the apical surface of fetal intestinal epithelial cells. J Cell Biol 131: 939-50.

[15] McAbee DD. [1995] Isolated rat hepatocytes acquire iron from lactoferrin by endocytosis. Biochem J 311: 603-9.

[16] Harrison PM, Arosio P. [1996] The ferritins: molecular properties, iron storage function and cellular regulation. Biochim Biophys Acta 1275: 161-203.

[17] Ferreira C, Santambrogio P, Martin ME et al. [2001] H ferritin knockout mice: a model of hyperferritinemia in the absence of iron overload. Blood 98: 525-32.

[18] Haile DJ [1999] Regulation of genes of iron metabolism by the iron-response proteins. Am J Med Sci 318: 230-40.

[19] Kuhn LC [1996] Control of cellular iron transport and storage at the molecular level. In: Iron Nutrition Health Diseases, Hallberg L [ed], Asp N, London, pp 7-29.

[20] Beaumont C, Leneuve P, Devaux I et al. [1995] Mutation in the iron responsive element of the L ferritin mRNA in a family with dominant hyperferritinemia and catarct. Nat Genet 11: 444-446.

[21] Bell H, Thordal C, Raknerud N et al. [1997] Prevalence of hemochromatosis among first-time and repeat blood donors in Norway. J Hepatol 26: 272-279.

[22] Camaschella C, Zecchina G, Lockitch G et al. [2000] A new mutation G51C in the iron-reponsive element IRE of L-ferritin associated with hyperferritinemia-cataract syndrome decreases the binding affinity of the mutated IRE for iron regulatory proteins. Br J Haematol 108: 480-2.

[23] Kasvosve I, Gangaidzo IT, Gomo ZA et al. [2000] African iron overload. Acta Clin Bel 55: 88-93.

[24] Raja KB, Simpson RJ, Peters TJ. [1992] Investigation of a role for reduction in ferric iron uptake by mouse duodenum. Biochim Biophys Acta 1135: 141-146.

[25] Miret S, Simpson RJ, McKie AT. [2003] Physiology and molecular biology of dietary iron absorption. Annu Rev Nutr 23: 283-301.

[26] Gunshin H, Mackenzie B, Berger UV et al. [1997] Cloning and characterization of a mammalian proton-coupled metal-ion transporter. Nature 388: 482–8.

[27] Fleming MD, Trenor CC, Su MA et al. [1997] Microcytic anaemia mice have a mutation in Nramp2, a candidate iron transporter gene. Nat Genet 16: 383-86.

[28] Tennant J, Stansfield M, Yamaji S et al. [2002] Effects of copper on the expression of metal transporters in human intestinal Caco-2 cells. FEBS Lett 527: 239-44.

[29] Riedel HD, Remus AJ, Fitscher BA et al. [1995] Characterization and partial purification of a ferrireductase from human duodenal microvillus membranes. Biochem J 309: 745-748.

[30] McKie AT, Barrow D, Latunde-Dada GO et al. [2001] An iron-regulated ferric reductase associated with the absorption of dietary iron. Science 291: 1755-59.

[31] Atanasova B, Mudway IS, Laftah A, Latunde-Dada GO, McKie AT, Peters TJ, Tzatchev KN, Simpson RJ. [2004] Duodenal ascorbate levels are changed in mice with altered iron metabolism. J Nutr 134: 501-5.

[32] Atanasova BD, Li AC, Bjarnason I, Tzatchev K, Simpson R. [2005] Duodenal ascorbate and ferric reductase in human iron deficiency. Am J Clin Nutr 81: 130-3.

[33] McKie AT, Marciani P, Rolfs A et al. [2000] A novel duodenal iron-regulated transporter, IREG1, implicated in the basolateral transfer of iron to the circulation. Mol Cell 5: 299-309.

[34] Donovan A, Brownlie A, Zhou Y et al. [2000] Positional cloning of zebrafish ferroportin identifies a conserved vertebrate iron exporter. Nature 403, 776-81.

[35] Abboud S, Haile DJ. [2000] A novel mammalian iron-regulated protein involved in intracellular iron metabolism. J Biol Chem 275: 19906-12.

[36] Montosi G, Donovan A, Totaro A et al. [2001] Autosomal-dominant hemochromatosis is associated with a mutation in the ferroportin [SLC11A3] gene. J Clin Invest 108: 619-23.

[37] Roetto A, Merryweather-Clarke AT, Daraio F et al. [2002] A valine deletion of ferroportin 1: a common mutation in hemochromatosis type 4. Blood 100: 733-34.

[38] Frazer DM, Vulpe CD, McKie AT et al. [2001] Cloning and gastrointestinal expression of rat hephaestin: relationship to other iron transport proteins. Am J Physiol Gastrointest Liver Physiol 281: G931-39.

[39] Park CH, Valore EV, Waring AJ et al. [2001] Hepcidin, a urinary antimicrobial peptide synthesised in the liver. J Biol Chem 276: 7806-10.

[40] Pigeon C, Ilyin G, Courselaud B et al. [2001] A new mouse liver-specific gene, encoding a protein homologous to human antimicrobial peptide hepcidin, is overexpressed during iron overload. J Biol Chem 276: 7811-19.

[41] Nicolas G, Bennoun M, Devaux I et al. [2001] Lack of hepcidin gene expression and severe tissue iron overload in upstream stimulatory factor 2 [USF2] knockout mice. Proc Natl Acad Sci USA 98: 8780-85.

[42] Roetto A, Papanikolau G, Politou M et al. [2003] Mutant antimicrobial peptide hepcidin is associated with severe juvenile hemochromatosis. Nat Genet 33: 21-22.

[43] Suominen P, Punnonen K, Rajamaki A et al. [1998] Serum transferrin receptor and transferrin receptor-ferritin index identify healthy subjects with subclinical iron deficits. Blood 92: 2934-9.

[44] Kulksiz H, Herrmann T, Riedel HD et al. [2003] Expression of hepcidin in hereditary hemochromatosis: evidence for a regulation in response to the serum transferring saturation and to non-transferrin bound iron. Blood 102: 371-6.

[45] Nemeth E, Valore EV, Territo M et al. [2003] Hepcidin, a putative mediator of anemia of inflammation, is a type II acute-phase protein. Blood 101: 2461-3.

[46] Swinkles DW. [2007] Hepcidin: Analysis, regulation and clinical implications. Clin Chem Lab Med, 45, Specila Suppl S43.

[47] Frazer DM, Wilkins J, Becker EM et al. [2003] A rapid decrease in the expression of DMT1 and Dcytb but not Ireg1 or hephaestin explains the mucosal block phenomenon of iron absorption. Gut 52: 340-46.

[48] Fleming RE. [2007] New insights in iron metabolism. Clin Chem Lab Med, 45, Specila Suppl S43.

[49] Mukhopadhyay CK, Mazumder B, Fox PL. [2000] Role of hypoxia inducible factor-1 in transcriptional activation of ceruloplasmin by iron deficiency. J Biol Chem 275: 21048-54.

[50] Raja KB, Pippard MJ, Simpsom RJ et al. [1986] Relationship between erythropoiesis and the enhanced intestinal uptake of ferric iron in hypoxia in the mouse. Br J Haematol 64: 587-93.