

# Iron Metabolism: From Health to Disease

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## Abstract

### Background

Iron is vital for almost all living organisms by participating in a wide range of metabolic processes. However, iron concentration in body tissues must be tightly regulated since excessive iron may lead to microbial infections or cause tissue damage. Disorders of iron metabolism are among the most common human diseases and cover several conditions with varied clinical manifestations.

### Methods

An extensive literature review on the basic aspects of iron metabolism was performed, and the most recent findings on this field were highlighted as well.

### Results

New insights on iron metabolism have shed light into its real complexity, and its role in both healthy and pathological states has been recognized. Important discoveries about the iron regulatory machine and imbalances in its regulation have been made, which may lead in a near future to the development of new therapeutic strategies against iron disorders. Besides, the toxicity of free iron and its association with several pathologies has been addressed, although it requires further investigations.

### Conclusion

This review will provide students in the fields of biochemistry and health sciences a brief and clear overview of iron physiology and toxicity, as well as imbalances in the iron homeostasis and associated pathological conditions.

## CHEMICAL PROPERTIES AND BIOLOGICAL FUNCTIONS OF IRON

Iron is a part of the subfamily of transition elements and is one of the most abundant metals on Earth, as well as an essential nutrient. It is a component of several metalloproteins and plays a vital function in essential biochemical activities, such as oxygen sensing and transport, electron transfer, and catalysis. This unique ability of iron to serve as an electron donor and acceptor renders this metal irreplaceable and indispensable for life. Iron exists in two steady oxidative states: ferrous ( $\text{Fe}^{2+}$ ) and ferric ( $\text{Fe}^{3+}$ ). In aqueous

media,  $\text{Fe}^{2+}$  is spontaneously oxidized by molecular oxygen to  $\text{Fe}^{3+}$  to form ferric hydroxide ( $\text{Fe}(\text{OH})_3$ ). Therefore, the maximal solubility of iron in an oxidative environment like extracellular fluids is limited by the product solubility constant of  $\text{Fe}(\text{OH})_3$ . At pH 7.0, the maximal solubility of  $\text{Fe}^{3+}$  is very low, whereas  $\text{Fe}^{2+}$  solubility is much higher. On the other hand, when the absorbed iron is not bound to proteins, it is capable of catalyzing reactions that produce harmful free radicals (refer to *Toxicity of iron*). As a result of the toxicity of free iron and its low solubility in the presence of oxygen and neutral pH conditions, organisms have been forced to develop proteins (e.g., transferrin) that are able to bind  $\text{Fe}^{3+}$  and maintain its stable form but, simultaneously, make it available for biological processes. Moreover, the poor solubility of iron is an important chemical property since it renders iron difficult to access by pathogenic microorganisms, thus avoiding their proliferation.

The majority of iron is intracellular, sequestered within the iron storage proteins (ferritin and hemosiderin) or associated with proteins in the form of heme, which is a common prosthetic group composed of protoporphyrin IX and a  $\text{Fe}^{2+}$  ion. The insertion of  $\text{Fe}^{2+}$  into protoporphyrin IX, catalyzed by ferrochelatase in the mitochondria, determines the final step of the heme biosynthetic pathway. Subsequently, heme is exported to the cytosol for incorporation into hemoproteins. The microsomal heme oxygenases 1 (HO-1), 2 (HO-2), and 3 (HO-3) catalyze heme degradation. The released  $\text{Fe}^{2+}$  is reutilized. This reaction also generates carbon monoxide (CO), which is thought to be involved in signaling pathways, and biliverdin, which is further enzymatically converted to the antioxidant bilirubin.

The most abundant mammalian hemeproteins, hemoglobin and myoglobin, serve as oxygen carriers in the erythroid tissue and in the muscle, respectively. Oxygen binding is mediated by the heme moieties. Another important class of hemeproteins are cytochromes, which play an important function in redox reactions and electron transport.

Iron–sulfur clusters (e.g., 2Fe-2S, 3Fe-4S, or 4Fe-4S) are the most prevalent forms of nonheme iron in metalloproteins. They play a wide range of functional roles, such as electron transfer (e.g., the Rieske proteins in complex III of the respiratory chain), structural stabilization (bacterial endonuclease III), transcriptional regulation (the bacterial SoxR and FNR transcription factors), and catalysis (e.g., aconitase, an enzyme of the citric acid cycle). Other forms of protein-associated iron may include iron-oxo clusters (e.g., in ribonucleotide reductase, an enzyme required for DNA synthesis) or mononuclear iron centers (e.g., in cyclooxygenase and lipoxygenase, enzymes involved in inflammatory responses).

It is also known that nonheme iron plays an important role in a mechanism for oxygen sensing, via the hypoxia-inducible factor (HIF). Under normoxic conditions, HIF undergoes hydroxylation that makes it rapidly degraded via the ubiquitin-proteasome pathway, in a process catalyzed by iron-dependent enzymes. On the other hand, under hypoxic conditions, HIF is stabilized and allows the activation of genes involved in angiogenesis, glycolysis, cell proliferation and survival, and erythropoiesis. This process is of extreme importance to cellular adaptation to low oxygen conditions.

## BODY IRON HOMEOSTASIS

### Iron Distribution in the Human Body

The adult human body contains approximately 3–5 g of iron (45–55 mg/kg of body weight in adult women and men, respectively), with more than two-thirds (~2 g) incorporated in the hemoglobin of developing erythroid precursors and mature red blood cells. The remaining body iron is mostly found in a transit pool in reticuloendothelial macrophages (~600 mg) or stored in hepatocytes (~1000 mg) within ferritin, an iron storage protein. A smaller fraction is found in muscles within myoglobin (~300 mg), while only a minuscule amount (~8 mg) is constituent of other cellular iron containing proteins and enzymes. A healthy individual absorbs daily 1–2 mg of iron from the diet, which compensates nonspecific iron losses by cell desquamation in the skin and the intestine (Fig. 1). Furthermore, menstruating women physiologically lose iron from the blood. Recycling of iron via reticuloendothelial macrophages provides the amount of iron required for erythropoiesis (30 mg/day). Iron bound to plasma transferrin corresponds to less than 0.1% of total body iron, but represents, in kinetic terms, the most active pool.

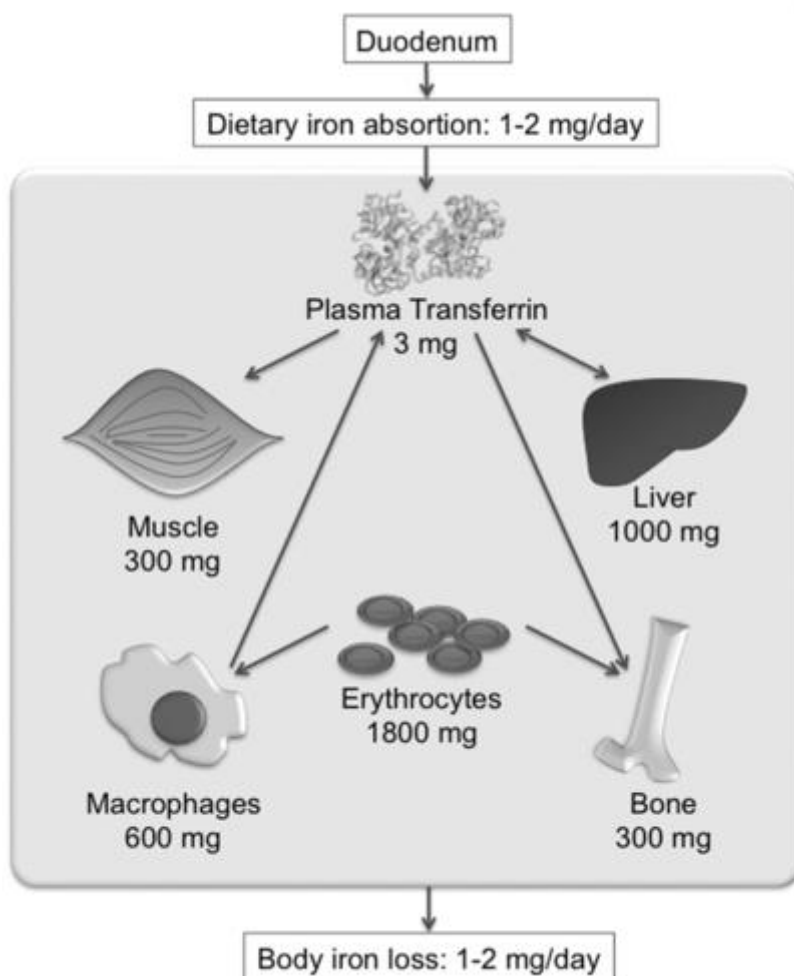


Figure 1. Iron distribution within the body.

## Iron Absorption

Dietary iron absorption occurs mostly at the duodenum and the upper portions of the jejunum. The body has no effective means of excreting iron and thus the regulation of absorption of dietary iron plays a critical role in iron homeostasis. Multiple steps are involved in iron absorption, including the reduction of iron to a ferrous state, apical uptake, intracellular storage or transcellular trafficking, and basolateral release. Dietary iron is found in heme (10%) and nonheme (90%) forms and their absorption occurs under different mechanisms.

Absorption of dietary nonheme iron involves the release of elemental iron from digested food and its maintenance in a soluble form, which is accomplished in part by gastric acid. The low pH of the gastric effluent dissolves ingested inorganic iron and facilitates its enzymatic reduction ( $\text{Fe}^{3+}$  is reduced to  $\text{Fe}^{2+}$  by ferric reductases present on the apical surface of duodenal enterocytes). Then, iron is transported across the intestinal epithelium by a transporter called divalent metal transporter 1 (DMT-1), which also traffics other metal ions such as copper, zinc, and cobalt by a proton-coupled mechanism.

Heme iron is absorbed into the enterocytes by a putative, not completely identified, heme carrier protein 1, a membrane protein found in the proximal intestine, where heme absorption is greater. Once internalized in the enterocytes, it is likely that most dietary heme iron is metabolized by heme oxygenase to release  $\text{Fe}^{2+}$ , which enters a common pathway with dietary nonheme iron before it leaves the enterocytes. Nevertheless, it remains uncertain whether some intact heme iron crosses the cell, leaving the enterocyte through the action of the recently characterized heme exporters. These exporters are also expressed in liver, kidney, and erythroblast, suggesting that they may act at those sites.

Once inside the intestinal epithelial cell, iron is exported across the basolateral membrane of the enterocyte into the circulation (absorbed iron), where it binds to transferrin, a potent chelator, able of binding iron tightly but reversibly, and is transported to sites of use and storage. Transferrin-bound iron enters target cells (erythroid, immune, and hepatic cells) through a process of receptor-mediated endocytosis. Senescent erythrocytes undergo phagocytosis by reticuloendothelial system (RES) macrophages, heme iron is metabolized by heme-oxygenase, and iron is stored as ferritin (the major iron storage protein). Later, iron is released from macrophages and bound by transferrin, which transports iron to the bone marrow. This internal turnover of iron is essential to meet the requirements for erythropoiesis.

Basolateral iron transport is mediated by ferroportin 1 and then iron is oxidized by a multicopper oxidase protein called hephaestin (an enzymatic protein similar to plasma ceruloplasmin) before being bound by plasma transferrin. The absorption of iron is dependent on the body iron stores, hypoxia, and rate of erythropoiesis.

## **MAINTENANCE OF IRON HOMEOSTASIS**

Since iron is required for a number of diverse cellular functions, a constant balance between iron uptake, transport, storage, and utilization is required to maintain iron homeostasis.

Mammals regulated systemic iron stores at the level of intestinal absorption, although iron elimination from the body is not regulated and is entirely dependent upon physiological and nonphysiological blood and epithelial cell loss. It is believed that three regulatory signals contribute to the maintenance of homeostasis. First signal is called “dietary regulator”—after the ingestion of a dietary iron bolus, absorptive enterocytes are resistant in acquiring additional iron for several days. This probably results from the accumulation of intracellular iron because high intracellular iron may suppress the expression of DMT1. The second signal, called “stores regulator,” controls iron uptake in response to body iron stores. In iron-deficient conditions, iron absorption is significantly stimulated by two- to threefold. When iron stores are replenished, iron absorption returns to basal levels. Third signal is called “erythropoietic regulator” and modulates iron absorption in response to erythropoiesis. This signal has a dominant role in the control of iron homeostasis and has a great capacity to increase iron absorption because most of the body iron is utilized by the bone marrow for hemoglobinization of red blood cells.

More recently, two models have been proposed to explain how the absorption of iron is regulated: the crypt programming model and the hepcidin model. The first one proposes that enterocytes in the crypts of the duodenum take up iron from the plasma. The intracellular iron level of the crypt cells corresponds to the body iron stores, which in turn determines the amount of iron absorbed from the gut lumen. The crypt cells express both transferrin receptor 1 (TfR1) and TfR2, which mediate the cellular uptake of transferrin-bound iron from plasma. The hepcidin model suggests that hepcidin is secreted into the blood and interacts with villous enterocytes to regulate the rate of iron absorption. Hepcidin is a circulating regulatory hormone peptide produced by hepatocytes that functions as the master regulator of cellular iron export by controlling the amount of ferroportin (the iron exporter present on the basolateral surface of intestinal enterocytes and macrophages). The binding of hepcidin to ferroportin induces ferroportin internalization and degradation, resulting in cellular iron retention and decreased iron export. It is hypothesized that when hepcidin levels are reduced, as in iron deficiency (ID), ferroportin 1 expression and iron release from intestinal cells, liver, and cells of RES are increased. On the other hand, when hepcidin levels are increased, as in iron overload (by the uptake of transferrin-bound iron) or inflammation, iron release from intestinal crypt cells, liver, and macrophages is reduced.

Currently, there are evidences to support both models and it is possible that both control mechanisms involved in the regulation of iron homeostasis.

## **IRON AND INFECTION**

Iron is an indispensable nutrient in the life of several organisms, including bacteria. Bacteria require iron and other transition metals so that they can replicate, and eventually cause disease. Therefore, the withholding of iron is an effective strategy of the host in the prevention of infection, a process commonly referred as nutritional immunity. As previously stated, the poor solubility of iron is an important chemical property in this context. Nevertheless, there are some iron-binding proteins that maintain low levels of free, circulating iron, as well as hinder the uptake of iron by bacteria, including transferrin, lactoferrin, and siderocalin.

Both transferrin and lactoferrin have great affinity for iron. Moreover, most of the iron-binding sites of these proteins are normally unoccupied, thus making the amount of free iron very small to support bacterial growth. Therefore, the antimicrobial properties of these two proteins were solely attributed to their ability to sequester iron. However, and in the case of lactoferrin, new insights emerged regarding to its antimicrobial properties. It is now well established that lactoferrin possesses bactericidal activity against a wide range of microorganisms. Nevertheless, these bactericidal properties appear to be iron independent and are probably a result of a direct interaction of lactoferrin with bacterial surface. Receptors for the N-terminal region of lactoferrin have been discovered on the surface of some microorganisms. It is thought that lactoferrin binds to these receptors by inducing cell death in Gram-negative bacteria. On the other hand, the bactericidal activity of lactoferrin in Gram-positive bacteria seems to be mediated by electrostatic interactions between the negatively charged lipid layer and the positively charged lactoferrin surface that cause changes in the permeability of the bacterial membrane.

Siderocalin (also known as neutrophil gelatinase-associated lipocalin (NGAL) or lipocalin 2) is a member of the lipocalin family of binding proteins. During infection, bacteria secrete iron chelating compounds called siderophores, which allow the uptake of iron from the host. Siderocalin is released by neutrophils at sites of infection and inflammation, and blocks bacterial proliferation through binding to bacterial siderophores, which prevents iron uptake by bacteria.

### **IMBALANCE OF IRON HOMEOSTASIS**

The imbalance of iron homeostasis is associated with the development of several diseases. For instance, iron can be toxic and damaging when it is in excess and accumulates in different human organs. This excess iron in the body is usually associated with some iron-overloading disorders, such as hereditary hemochromatosis (HH) and thalassemias. On the other hand, low levels of iron in the right place could lead, for example, to the development of ID anemia (IDA). Therefore, a proper balance of iron concentration must be achieved.

### **Toxicity of Iron**

Although iron is indispensable for life, its excess can be toxic to tissues. Iron has the ability to produce oxygen free radicals under aerobic conditions, which turns it into a potential harmful component.

Free radicals are generated within the cell as part of normal cellular mechanisms. However, the overproduction of reactive oxygen species (ROS), such as superoxide ( $\bullet\text{O}^{2-}$ ) and hydroxyl ( $\bullet\text{OH}$ ) radicals may lead to cellular damage. When iron exceeds the metabolic needs of the cell, it can generate oxidative stress, characterized by an increase in the basal concentration of ROS. The reactions between iron and ROS are shown in Figure 2.

<b>Reduction</b>
$\bullet\text{O}^{2-} + \text{Fe}^{3+} \rightarrow \text{O}_2 + \text{Fe}^{2+}$
<b>Fenton reaction</b>
$\text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \bullet\text{OH} + \text{OH}^- + \text{Fe}^{3+}$
<b>Harber-Weiss reaction</b>
$\bullet\text{O}^{2-} + \text{H}_2\text{O}_2 \rightarrow \bullet\text{OH} + \text{OH}^- + \text{O}_2$

Figure 2. Reactions between iron and reactive oxygen species.

Generally, the main sources of OH radicals are Fenton and Harber–Weiss reactions. In relation with  $\bullet\text{O}^{2-}$ , this radical has an extremely short half-life and rapidly undergoes dismutation, yielding hydrogen peroxide ( $\text{H}_2\text{O}_2$ ). In biological systems living under aerobic conditions, OH radicals are formed from oxygen as a result of both normal cellular metabolism and oxidative stress associated with several conditions (e.g., inflammation, platelet aggregation, etc.). These radicals are the most reactive free radical species known and have the capacity to react with a wide range of cellular components, such as DNA, proteins, and cell membranes. The resulting effects are impaired synthesis of proteins, membrane lipids, and carbohydrates; induction of proteases; and altered cell proliferation.

In the last few years, several studies have shown a relationship between free radicals and the development of certain conditions, such as cancer, diabetes, cardiovascular, neurodegenerative and ophthalmologic diseases, and aging. In diseases of iron overload (e.g., HH), the generation of free radicals leads to tissue damage and organ failure. Moreover, excess of free iron has been considered carcinogenic, once the generation of free radicals by this metal can promote DNA strand breaks, oncogenes activation, and tumor suppressor genes inhibition. More recently, the role of iron in neurodegenerative disorders such as Parkinson's and Alzheimer's diseases has been the target of intensive study, since it has been suggested that iron overload increases brain oxidative stress status.

## ID Diseases

ID is defined as the reduction of the total iron content of the body, and results when iron losses or requirements exceed its absorption. ID is the most common nutritional deficiency and the leading cause of anemia worldwide, remaining as one of the most important public health issues.

ID can result from several causes, which are normally classified into two categories: increased iron needs and decreased iron intake or absorption (Table 1).

**Table 1. Causes for Iron Deficiency**

Increased iron needs	Decreased iron intake or absorption
Increased demand	Impaired absorption
Rapid growth rate in adolescents	Inadequate dietary intake
Pregnancy and lactation	Intestinal malabsorption
Increased blood loss	
Heavy menstruation	
Gastrointestinal bleeding	
Blood donation	

## ID anemia

ID is usually seen as a continuous process, comprising three elementary steps: iron depletion, iron-deficient erythropoiesis (IDE), and IDA. Basically, IDA occurs when ID is sufficiently severe to the point of reducing erythropoiesis. IDA is the most frequent type of chronic anemia. Iron is an essential mineral for several metabolic reactions in our body. IDA is associated with several conditions, such as preterm delivery, defects in cognitive and psychomotor development, impaired work capacity, diminished growth, alterations in bone mineralization, and diminished immune response. Furthermore, increased oxidative stress in patients with IDA has been reported. Iron is an essential cofactor for enzymes involved in several cellular processes, namely in antioxidant metabolism. Therefore, the increased oxidative stress, and consequent DNA damage, seems to play a critical role in the pathogenesis of IDA.

## Iron Overload Diseases

Iron overload could be defined as an excess of iron in the body, regardless of the presence or absence of tissue damage. However, progressive accumulation of iron in vital organs increases the risk of hepatic, cardiovascular, and pancreatic dysfunctions. Moreover, and in line with previously discussed, individuals with iron overload diseases have an increased susceptibility to infectious diseases.

Iron overload is commonly classified into two categories: primary (or hereditary) and secondary (or acquired) (Table 2). Primary iron overload is the result of an inherited defect in the regulation of iron



balance, whereas secondary iron overload is almost always the consequence of other genetic or acquired disorders.

**Table 2. Classification of Iron Overload Diseases**

<b>Primary iron overload/hereditary hemochromatosis</b>
<i>HFE</i> related
C282Y/ C282Y
C282Y/ H63D
Non- <i>HFE</i> related
Hemojuvelin
Transferrin receptor-2
Ferroportin
Hepcidin
Secondary iron overload
Iron-loading anemias
Thalassemia
Sideroblastic anemia
Chronic haemolytic anemia
Parenteral iron overload
Red blood cell transfusions
Long-term hemodialysis

### **Primary iron overload**

The classical example of primary iron overload is HH. HH is a common genetic disorder among Caucasian population, with an autosomal recessive inheritance, and is characterized by an excessive absorption of dietary iron. Excess iron progressively accumulates in different organs, particularly in liver, heart, and pancreas. The common complications are cirrhosis and carcinoma of the liver, cardiomyopathy, diabetes mellitus, and if not treated, death.

HH occurs in patients with mutations in specific genes involved in iron metabolism (e.g., HFE gene), and has been classified into four subtypes (types 1, 2, 3, and 4). The majority of patients (approximately 85–90%) have type 1, which is associated with mutations in HFE gene (OMIM 235200). This gene is located on the short arm of chromosome 6 (6p21.3) and encodes for a transmembrane protein. This protein associates with  $\beta$ -2-microglobulin and regulates the interaction between transferrin and its receptor (Fig. 3). Two *missense* mutations in this gene were identified: C282Y (C, cysteine replaced by Y, tyrosine) and H63D (H, histamine replaced by D, aspartic acid). Although the whole mechanism leading to iron overload remains to be elucidated, there are evidences that it could also arise from hepcidin dysregulation.

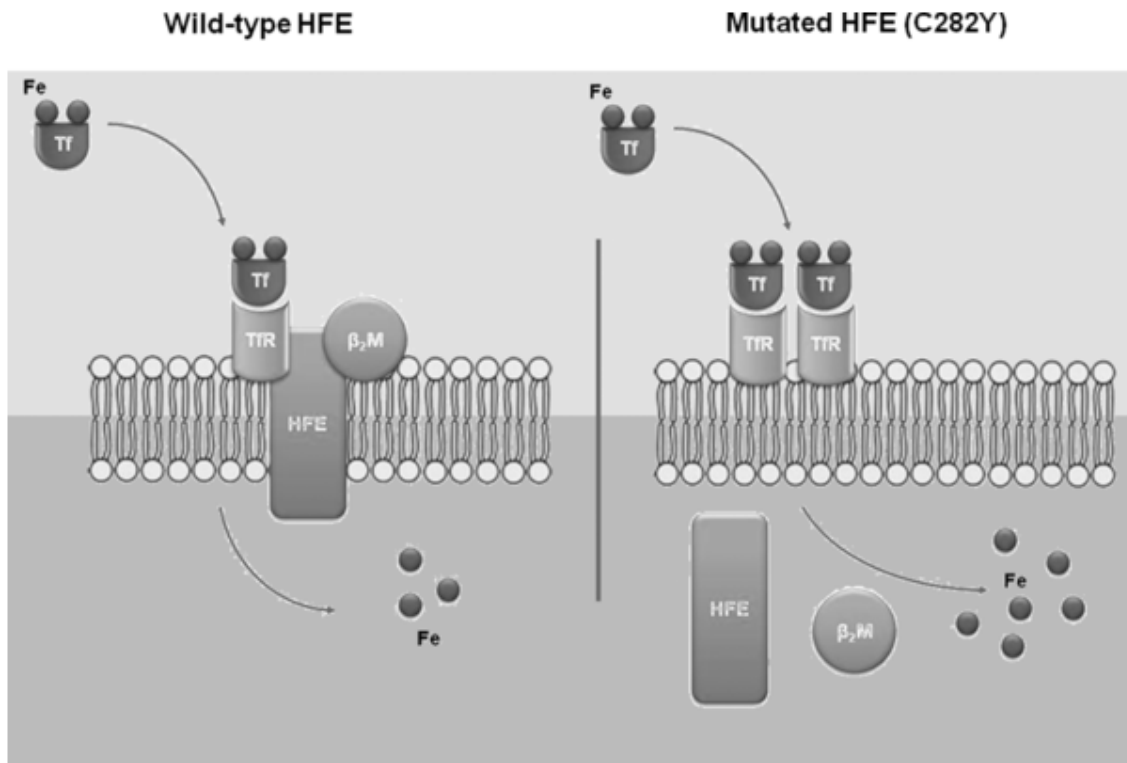


Figure 3. Interactions between wild-type/ mutated HFE protein and transferrin receptor: in normal conditions, wild-type HFE protein binds to  $\beta$ -2-microglobulin ( $\beta_2M$ ), thus reducing the affinity of transferrin receptor (TfR) for transferrin (Tf) associated iron (Fe), and preventing excessive iron uptake. On the other hand, mutated HFE proteins does not bind to  $\beta_2M$  and the affinity of transferrin receptor (TfR) for transferrin-associated iron is not affected, leading to an excessive iron accumulation in the intracellular space.

On the other hand, mutations of other genes coding for iron regulatory proteins (ferroportin, hemojuvelin, hepcidin, and TfR 2) have been implicated in primary iron overload diseases, accounting for most of the non-HFE forms of HH, such as juvenile hemochromatosis or ferroportin disease.

### Secondary iron overload

Secondary iron overload typically occurs as a result of a wide range of conditions, namely ineffective erythropoiesis, chronic liver diseases, parenteral administration, or ingestion of excessive amounts of iron.

One of the best studied examples of iron overload secondary to ineffective erythropoiesis and blood transfusions is thalassemia. Thalassemia involves a group of recessively inherited defects in the synthesis of globin chains of hemoglobin. The two major forms of the disease are  $\alpha$ -thalassemia (defect in the production of  $\alpha$ -globin chain) and  $\beta$ -thalassemia (defect in the production of  $\beta$ -globin chain). Thalassemia is characterized by chronic anemia as a result of ineffective erythropoiesis, and a variety of secondary complications such as iron overload. This complication is particularly severe in  $\beta$ -thalassemia, and has become a leading cause of morbidity and mortality among thalassemic patients. Moreover, the standard

treatment is chronic blood transfusion to increase the hemoglobin levels. Initially, it was thought that iron overload in these patients was exclusively caused by this therapeutic procedure. However, it is known that thalassemic patients have increased gastrointestinal iron absorption (three to four times greater than normal), as a consequence of ineffective erythropoiesis. This process is mediated by downregulation of hepcidin and upregulation of ferroportin, which together with chronic transfusion therapy seriously aggravate the clinical setting of the patients.

The treatment of some diseases, such as thalassemia (previously described) or myelodysplastic syndromes is based on repeated blood transfusions. Although this procedure allows the suppression of endogenous erythropoiesis and the correction of the anemia, chronic transfusion therapy remains one of the most important cause of secondary iron overload. Our body is not capable to remove excess iron, thus repeated transfusion can rapidly result in iron overload. Each unit of packed red blood cells contains about 200–250 mg of iron. For instance, a treatment program with 100 units involves loading with 20 g of iron, an amount five to six times greater than the normal content in the organism (3–5 g). In order to overcome this problem, in the last few years, iron-chelating therapy has been used for patients with transfusional iron overload.

## **CONCLUSIONS**

In the last few decades, several studies concerning iron metabolism have been carried out. Although it seems to be a simple process, new insights on iron metabolism have allowed the perception of its real complexity. The extreme importance of iron in almost all living organisms, including humans, and its role in healthy and pathological conditions has now been recognized.

Although iron plays an important function in several biochemical processes, its concentration in body tissues needs to be tightly and constantly regulated. Important discoveries about this complex regulatory machinery have been made in the past few years. On the other hand, and taking into account that disorders of iron metabolism are among the most common human diseases, imbalances in this regulatory mechanism and associated complications have also been the target of intensive investigations.

However, there is a wide range of aspects in this field that needs further elucidation. For instance, a detailed understanding of the molecular mechanisms involved in iron absorption and metabolism might be the key to the development of novel therapeutic strategies in iron overload and ID diseases. Moreover, the toxicity of free iron and its association with the development of some pathological conditions, especially neurodegenerative and malignant diseases, require further investigations.

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