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Review

Current Knowledge of Iron Metabolism

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ABSTRACT

Iron plays many roles in human physiology. In this article, we summarize the basic and current knowledge of this essential micronutrient on human metabolism.

Index Entries: Iron, metabolism; absorption; anemia; deficiency.

HISTORICAL BACKGROUND

Iron is the most abundant metal in the universe and the fourth most abundant element in the Earth's crust. It is naturally found in the soil as a constituent of different minerals, in the water, and in many foods *(1,2)*.

Iron coexisted with humans from the beginning of history and was utilized by them in many different ways. One of mankind's milestones was iron forging; its medical use is known since the oldest prescriptions as Eber's Papyrus (Egypt), 1500 yr B.C., in which ferric oxide was used as an ointment for the treatment of baldness. In Greece, 1200 yr B.C., the same substance was mixed with wine to treat masculine impotence. Susruta, an Indian physician contemporary to Buddha, 500 yr B.C., mentions beneficial effects on human health of different iron preparations *(3)*.

In the Middle Ages and the Renaissance, iron was used for the treatment of different diseases but without much knowledge of its cause–effect relationship. Only in the 16th century was iron deficiency related to green-

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sickness or chlorosis, a name given at that time to ferropenic anemia, which affected mostly adolescent women with symptoms such as weakness, fatigue, and paleness. The first to use iron for the specific treatment of chlorosis was Sydenham, who eliminated bleedings and cathartics that were used commonly at that time *(3,4).*

Lemery and Geoffry demonstrated for the first time in 1713 that iron is present in blood ashes, relating this tissue with this metal and establishing, in this way, the scientific basis of the therapy of its deficiency. In 1832, the French physician Pierre Blaud began the treatment of chlorosis with the oral administration of iron using a pill composed by ferrous sulfate and potassium carbonate, which was called "Blaud's pill." Afterward and for many years, as late as the last decade of the 19th century, chlorosis was treated following the principles established by Sydenham and Blaud. However, Bunge, one of the first scientists able to quantify iron in the organism and in many foods, undervalued Blaud's pill, which at that time was used almost exclusively, because he found iron in the feces of persons who consumed those pills, concluding, therefore, that the iron in the pills was not absorbed. Moreover, taking into account the vitalist theories prevailing at that time, Bunge believed that no inorganic form of iron could be a precursor of blood. Even though Blaud's theory was attacked by many scientists before the end of the century, in 1920 it acquired a standing again when Whipple et al. demonstrated that cooked liver was more efficient than ferrous carbonate for blood regeneration. However, in 1932, Castle et al. demonstrated the effectiveness of inorganic iron in the regeneration of hemoglobin when it was administered parenterally to patients with hypochromic anemia *(3,4).*

In 1937, McCance and Widdowson began the first work on iron balance, which suggested a limited absorption and elimination of this metal. In the same year, Heilmeyer and Plotner determined iron plasma concentrations and postulated its transport mechanism. Laurell completed these studies in 1947, giving the actual name of transferrin to the plasma protein that carries iron in plasma. As late as 1943, using radioisotopes for the study of human metabolism, Hahn et al. were able to quantify iron absorption and the regulatory capacity of the intestinal mucous membrane in this process. In 1950, Huff et al. completed these studies, determining the distribution, the metabolism, and the balance of iron in the human body; these concepts are still accepted today *(3,4).*

IRON SOURCES

In order to understand iron metabolism, it is necessary to first know how this metal is found in foods, because these are the primary and natural sources of this mineral. The form in which iron is found is a primary factor of the metabolism of this vital element *(5).*

Iron is found in foods in two different groups: one of hemic iron and the other one of nonhemic iron *(2).* The heme-type iron is a part of hemo-

globin, myoglobin, cytochromes, and many other heme proteins, which are present principally in animal foods. The heme group, which is present in all these proteins, is formed by a complex organic ring, called protoporphyrin, to which a divalent iron atom is bound, which is able to form six coordinated bounds—four of them with the protoporphyrin, one with a nitrogen atom of the protein fraction, and the last remaining free as a binding site for an oxygen molecule *(6).*

The nonheme iron type corresponds to iron that is not bound to an heme group; it includes basically inorganic salts of this metal and they are found principally in vegetal foods as well as in the principal pharmaceutical preparations utilized for the therapy against iron deficiency *(2,4).*

IRON ABSORPTION

Iron absorption takes place at the duodenum and the upper jejunum of the gastrointestinal system. Even if there is no absorption at the stomach, this organ contributes to the process by means of the secretion of hydrochloric acid and enzymes, which helps not only to set free the iron from the food matrix but also to make it soluble, as hydrochloric acid favors the reduction of this cation to the ferrous form *(7–9).*

The process of iron absorption may be divided sequentially in the following steps.

Uptake

The ingested iron may be found in the intestinal lumen in the hemic or the nonhemic forms; as a consequence of these alternatives, the iron will be transferred from the intestinal lumen into the enterocyte in a different way *(10).*

In a first stage, the nonhemic iron should be soluble in order to be absorbed, because insoluble forms cannot be absorbed and are excreted together with the feces. Ferrous iron is much more soluble than the ferric forms, because these precipitate rapidly in the intestinal alkaline medium. For this reason, the iron that is set free through the action of gastric and pancreatic proteases is bound to intraluminal ligands, the function of which is to stabilize the ferrous form, keeping the iron soluble and, consequently, biologically available for its uptake and transfer into the enterocyte *(5,11).*

Even though there are some controversies with regard to the identification of this specific binding agent, many authors agree that it may be a glucoprotein called mucine. Synergic with mucine function, there are other low-molecular-weight iron-binding agents such as histidine, ascorbate, or fructose that enhance the enterocytic iron uptake *(12–14).*

Subsequently, this fixing protein bound to iron may be bound to a specific carrier at the enterocyte lumen surface called integrin, to which

iron may also be transferred. In this way, iron is introduced into the cell interior, in which it is transferred to low-molecular-weight ligands or to a protein similar to transferrin, called mobilferrin by some authors *(10,11,15–18)*

Hemic iron is soluble in an alkaline medium and, therefore, intraluminal ligands are not necessary. Its uptake mechanism is still somewhat controversial with regard to the existence of a specific carrier or receptor for this type of iron. However, once this iron is in the enterocyte interior, the heme is degraded to iron, carbon monoxide, and bilirubin IXa through the action of the enzyme heme-oxygenase. The iron released through this mechanism is bound to low-molecular-weight ligands or to a protein similar to transferrin, integrating in this way, together with the nonheme iron, a portion of the enterocyte intracellular iron pool *(5,19,20).*

Intraenterocyte Transport and Storage

Once iron is in the enterocyte interior, it is not free, but it is bound to different ligands; one of them, eventually the most relevant, is a protein that is capable of binding two iron atoms with a high-affinity constant, having characteristics similar to transferrin. This protein has been called mobilferrin and it is homologous to calreticulin, which can bind not only iron but also other cations such as calcium, copper, and zinc. The iron bound to this protein is carried to the basal pole of the enterocyte and subsequently transferred to transferrin. Mobilferrin has been assigned to having a potential effect to modulate the regulation of iron absorption, playing a role in one of the first steps of the homeostasis of the metabolism of this metal *(17,21,22).*

The iron not transferred to transferrin integrates the intraenterocyte storages as ferritin; this iron will most probably be lost with feces once the enterocyte dies and, consequently, undergo desquamation. It has been observed that individuals with iron deficiency have a lower concentration of mRNA for ferritin; in those individuals who underwent an overdose of this metal, these values are increased. In this way, the ferritin in the enterocyte interior has an important primary regulatory function of iron absorption *(5,23–25).*

Transfer to Plasma

The iron in the enterocyte interior, not stored as ferritin, is transferred to transferrin, which will distribute it to the different tissues of the organism. The process of transfer takes place at the basal pole of the enterocyte, where, prior to the binding to transferrin, iron has to be oxidized to its ferric form. In this oxidative process, a copper-depending enzyme with ferroxidase I activity is involved. According to some authors, ceruloplasmin plays a role in this process, even though there are some contradictions with regard to this point *(26–31).*

FACTORS MODIFYING IRON ABSORPTION

Iron absorption may be affected by a combination of different factors, such as the type of ingested iron, the nutritional status of the individual for this element, and the presence of absorption activators or inhibitors existing in the intestinal lumen together with iron *(32–37).*

Because nonhemic iron is found in a higher proportion in the diet, its absorption will be significantly modified by the nutritional status of the individual for this element. Thus, if the iron stores are depleted, iron absorption will increase, and if stores are replete, iron absorption will be decreased. There are different physiological states that produce a substantial increase of the absorption of this metal, such as growth and pregnancy, as a consequence of an increase of the synthesis of new biomolecules that have iron in their structure *(38–40).*

Those factors that have an increasing influence on nonhemic iron absorption at the level of intestine lumen are called activators, whereas those that decrease are called inhibitors *(41).*

Among absorption activators, there are substances such as ascorbic acid that not only produces the reduction of iron to its ferrous form but also is able of chelating it, maintaining in this way the iron in a soluble form and, consequently, biologically available for absorption. There are also other organic acids that produce absorption increase of this type of iron, such as citric, malic, and tartaric acids *(36,42–45).*

Meat also increases iron absorption, but the mechanism by which this occurs has not been clearly established. However, there is experimental evidence that suggests that the composition of amino acids of the meat proteins would be a determinant factor, assigning to cysteine and other sulfur-containing amino acids, as well as to the peptides with these amino acids, the role of promoting iron absorption *(46–51).*

Recently, several studies demonstrated that vitamin A as well as β-carotenes increase the solubility of the iron contained in the food, decreasing also the inhibitory effect of phytates and polyphenols present in the diet. Even if the mechanism through which these compounds produce this effect has not been explained, it may be supposed that it takes place through the formation of complexes that would maintain the iron in a soluble form in the intestinal lumen, precluding in this way the inhibitory effects of tannins and polyphenols on iron absorption *(52–54).*

Among iron absorption inhibitors, fundamentally phytates and tannins are found; both are present in foods of vegetal origin *(32,55–59).* In foods of animal origin, the animal proteins with the most significant inhibitory effect are casein, milk serum proteins, and bovine serum albumin and egg yolk proteins. Among vegetal proteins, the most important is a fraction derived from soy protein called 7S congicine, which demonstrated an inhibitory effect on the nonhemic iron absorption, similar to that of phytates *(47,60–63).*

Phosphates and calcium are present in many foods and are also potential inhibitors of iron absorption. Phosphates produce insoluble

compounds, principally with ferric ions, consequently inhibiting its absorption *(59,64–66).*

In the case of calcium, there are some contradictions with regard to the degree of iron absorption inhibition as well as to the mechanism through which this effect takes place. Minotti et al. studied the effect of different calcium sources on iron absorption and demonstrated that the chemical form in which calcium is found as well as the physiological iron status are factors that determine the inhibitory effect of calcium on iron absorption *(67–72).*

Other metals close to iron in the periodic table could have a potential effect on iron absorption. Among these, the most significant is zinc, because it is frequently used together with iron as supplements in certain physiological conditions such as during pregnancy and in infant formulas. It has been demonstrated that zinc interferes with iron absorption only if its molar concentration is much higher than that of iron and if both minerals are given with no food. However, if both compounds are administered with foods in doses within the daily requirements, no reciprocal interaction between the absorption of either metal was found *(73–79).*

Even though the proportion of hemic iron in food is low compared to that of nonhemic iron, its high absorption determines that the fraction of the absorbed iron corresponding to hemic iron becomes significant. The absorption of hemic iron is slightly variable with regard to the nutritional status for this mineral in the individual and the inhibitors of nonhemic iron absorption have a low or even no effect on the bioavailability of this type of iron, with the exception of calcium, which produces a statistically significant absorption decrease *(41,80).*

PLASMATIC IRON TRANSPORT

Free ionic iron is highly toxic, because in an oxygen-rich aqueous medium it may catalyze different chemical reactions, the products of which are noxious for different cell structures. For this reason, the iron in the organism is bound to different ligands *(3,81–84).*

The principal plasmatic iron transport protein is transferrin, having a molecular weight of 80 kDa and two ferric iron-binding sites; in normal physiological conditions, it is saturated up to 30% *(3,85,86).*

The function of this protein is to carry iron from the enterocyte basal pole to the different tissues of the organism, after the absorption of iron takes place. It also redistributes the iron in the organism, principally from storage to the tissues with a higher iron demand *(87–90).*

The synthesis of this protein takes place fundamentally in the liver even though other tissues, such as the kidneys, the brain, the testicles, and the fetal muscle also synthesize this protein. Even if the plasma iron concentrations regulate the transferrin liver biosynthesis, this is not the case of the transferrin synthesized in other tissues *(91–93).*

There are other ligands such as hemopexin, ferritin, lactoferrin, and not yet fully characterized low-molecular-weight ligands that are present in a small proportion; even so, they may contribute in a small but significant way to the iron transport between tissues *(5,94)*.

IRON DISTRIBUTION IN THE ORGANISM

The total iron amount in the organism is about 30–40 mg per kilogram body weight. This value varies according to different factors such as individual age, sex, and the type of nurture; because iron is not distributed homogeneously in the organism, the studied tissue or organ should also be considered *(2,4,5)*.

From the functional point of view, iron in the organism may be included in two large groups: the essential iron compounds in which fundamentally hemoglobin, myoglobin, cytochromes, and different iron-containing enzymes are found and that of the storage iron compounds such as ferritin and hemosiderin *(2,3,5)*.

Essential Iron Compounds

They include hemoglobin, quantitatively the most preponderant protein because it contains more than 65% of the total iron of the organism. It is contained within the erythrocytes and its principal function is to carry oxygen from the lungs to the remaining tissues. This protein is a tetramer formed by four globin chains and a heme group that contains one iron atom per globin molecule *(2,5,6,95)*.

Myoglobin also contains iron and is found in muscle. One globin molecule and one heme group form it. Its function is to transport and to store the oxygen to be utilized during the process of muscle contraction. From the quantitative point of view, myoglobin contains approx 10% of the total iron in the organism *(2,6)*.

Basically, one globin molecule and one heme group form the cytochromes, another group of molecules with important metabolic functions. They are found principally in the mitochondrias and in other cell organelles. Its basic function is to intervene in the electron transport processes, as in the oxidative energy production in mitochondrias or in the case of the P450 cytochrome, which regulates the oxidative degradation processes of endogenous compounds or different drugs *(2,4,6,97–100)*.

Many enzymes also include iron in their structures, which may be found in the heme form, as in the case of catalases and peroxidases, or in the nonheme form, as in the dehydrogenase of the reduced adenine dinucleotide nicotinamide. Even if the total iron content in the enzymes represents only 3%, its presence is physiologically essential, because these enzymes would be metabolically inactive in the absence of iron *(2,5,6,101–106)*.

Iron Storage Compounds

The iron not momentarily used in the different metabolic processes is stored. Its amount ranges between 0 and 15 mg per kilogram body weight, depending on several physiological and nutritional factors *(4,5).*

The principal storage tissue of this metal is the liver, which contains 60% of the stored iron, whereas the reticuloendothelial cells and the muscle contain the remaining 40%. The stored iron is bound to specific proteins; ferritin contains 95% of the hepatic iron, whereas its degraded form, hemosiderin, contains the remaining 5% *(2,5,104).*

The function of ferritin is to store iron within the cell. It is formed by 24 subunits of polypeptides, having a molecular weight of 20–22 kDa. There are two isoforms: one called L, having 20 kDa, which is found principally in the liver, and the other one, called H, with 22 kDa, is predominant in the heart. The biosynthesis of both isoforms is regulated by the intracellular iron concentrations and the oxidative stress, among other factors *(105–110).*

Ferritin has the capacity to contain up to 4500 iron atoms per molecule, even though under normal conditions it is saturated to a 20%. The iron within this protein is stored mainly as a hydrated polymolecular phosphate and ferric oxide, among other complex forms of inorganic iron compounds *(111–114).*

Iron in its ferrous form enters into the ferritin molecule through specific pores; once in the molecule interior, iron is oxidized to its ferric form through a process in which the stoichiometric ratio of Fe(II) to O_2 is close to 3.8 in the presence of ceruloplasmin. Finally, Fe(III) is included within the crystallization nucleus in the ferritin interior. Each ferritin chain (H and L) has a cooperative function during this iron incorporation process; the L chain has a more important capacity to promote crystallization nuclei than the H chain, whereas the H chain has a higher capacity than the L chain to induce the oxidation of Fe(II) mediated by the presence of O_2/ceruloplasmin. If it is necessary to liberate iron from its stores, the iron is reduced in a first step to its ferrous form in the interior of the ferritin molecule, in order to be liberated subsequently through the pores of ferritin. In this process of reduction, there is evidence showing that ascorbic acid and the reduced flavin mononucleotide are involved as mediators *(109,114–120).*

Once the ferritin molecule iron content increases up to 4000 atoms per molecule, lysosomal enzymes degrade ferritin to hemosiderin. This protein is insoluble, having an iron content of 40% of its weight, with a composition corresponding to chemical forms of iron less reactive than those present in ferritin *(82,109,112,121,122).*

BIOLOGICAL CYCLE OF IRON

Under normal conditions, the iron daily intake is approx 10–14 mg. In the duodenum and the upper portion of the small intestine, about 0.5–2

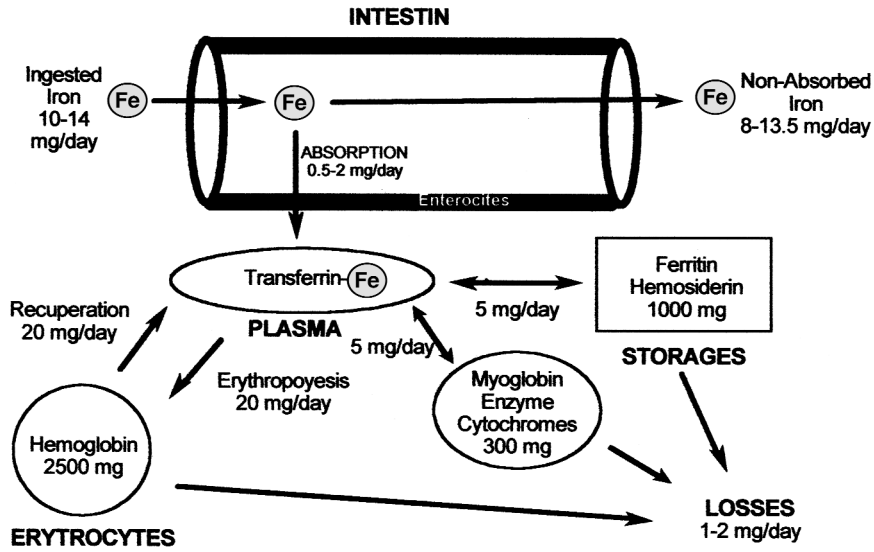

Fig. 1. The biological cycle of iron. Distribution and interchange between the different compartments. (Adapted from ref. *123*.)

mg of iron are absorbed, depending on several factors. For example, adult men absorb approx 1 mg per day, whereas females in reproductive age need 2 mg per day, because their requirements are higher owing to menstrual bleedings *(4,5)*.

Once iron is absorbed by the enterocytes from the intestine mucous membrane, it is passed to plasma, where it is transported by transferrin to the different organs and tissues. As is shown in Fig. 1, the highest internal iron recirculation takes place among the plasma, the reticuloendothelial cells, and the erythroid medulla, which synthesizes erythrocytes that are afterward liberated into the circulation *(123,124)*.

In the human being, the red blood cells life cycle is about of 120 d. In this situation, they are recognized by the reticuloendothelial cells as old erythrocytes and are destroyed. In this process, the protein fraction of hemoglobin is degraded into its constitutive amino acids and the heme group is degraded through the action of hemoxygenase, liberating the iron. The major proportion of this iron is rapidly liberated into the plasma, in which transferrin carries it to the erythroid medulla to be reutilized for the biosynthesis of new hemoglobin molecules, which are subsequently incorporated into new erythrocytes *(2,5,125)*.

Transferrin also carries iron to other tissues that require it for several metabolic processes, because many of their biomolecules, such as myoglobin, cytochromes, and some enzymes, require iron in their structure to be metabolically active. In this case, the turnover rate between the iron of

these molecules and the plasma is highly variable and the iron mean life depends principally on the turnover rate of the subcellular structures to which the iron is associated *(2,126).*

With the purpose of maintaining plasma iron concentration within a constant range, there is a permanent interchange of iron between transferrin and the iron storages (ferritin and hemosiderin). Thus, after an abundant iron intake, transferrin will transport a significant iron amount to the storage organs. On the contrary, if there is a demand for this metal by some tissue, transferrin will take iron from the storages in order to carry it to this tissue *(126,127).*

In the particular case of iron, which differs in this sense from all of the remaining trace minerals, its homeostasis in the organism is regulated through its absorption and not by its excretion or elimination. Even so, there are losses of this metal as a result the desquamation of enterocytes, erythrocyte extravasations, bile products resulting from heme degradation, and so forth. It can be estimated that these losses are approx 1 mg per day for adult men and postmenopausal women; for females at the reproductive age, these values range between mean daily values of 1.5 and 2 mg of iron, owing to, menstrual bleeding. The amount depends also on the contraceptive method used, as it is known that intrauterine devices increase bleedings and, consequently, iron losses, whereas oral contraceptives decrease these losses *(3,128–131).*

On the other hand, pregnancy is associated to a loss of approximately 1 g of iron, which is significant for the organism, especially in the case of repeated pregnancies. There are other particular cases in which iron losses occur, such as hemorrhages, infections resulting from heme phagocytizing parasites, utilization of nonsteroid anti-inflammatory drugs, blood donations, and so forth *(4,5).*

IRON CELL METABOLISM

Iron cell uptake is carried out by a transferrin receptor (TfR), which is a glucoprotein with a molecular weight of 180 kDa; it is formed by two equal subunits of 95 kDa bound by disulfur bridges, each of which has 760 amino acids *(133,134).*

Each subunit has the capacity to bind one transferrin molecule. The affinity of TfR is substantially higher for diferric transferrin than for apotransferrin, the respective dissociation constants (K_d) of which are 1.1×10^{-8} *M* and 4.6×10^{-6} *M.* However, transferrin plasma concentration is about $(30–40)\times10^{-6}$ *M,* which implies that the TfRs at the cell surface are saturated. Therefore, the number of TfRs present on the cell surface, which depends on the intracellular status for iron, regulates iron cell uptake. Thus, for example, in those metabolically active tissues with increased intracellular iron requirements, a higher number of TfRs will be found, a value that will

be increased through the synthesis of new TfRs or by increasing the translocation rate of this receptor. Through this mechanism, approx one-third of the total TfR mass is present at the cell surface *(135–138).*

Once the transferrin with iron (FeTf) is bound to the TfR at the cell surface, the TfR–FeTf complex undergoes uptake through endocytosis. In this process, the cytoplasm fraction of the receptor plays an essential role in the process of TfR–FeTf internalization, a process that is regulated by the activation of the protein quinase C. Within the endosome, a pH change takes place to values close to 5.5, mediated through an ATP-dependent proton pump, which produces a decrease of the affinity of transferrin for iron. The binding of Cl^- to an anion fixation site of the complex facilitates the separation of iron and a reduction of ferric to ferrous iron, a process that decreases even more the affinity of transferrin for this metal. This last process may be mediated by ascorbic acid or, enzymatically, by an endosome enzyme NADH dependent. It has recently been demonstrated that phosphate and pyrophosphate groups also facilitate the liberation of iron bound to transferrin. This effect has been observed not only at acid pH but also at pH 7.4, thus supplying evidence of a secondary mechanism of iron liberation from the TfR–FeTf complex. On the other hand, it has been observed that the liberation of the first iron atom from diferric transferrin produces a stability change of the TfR–FeTf complex as a consequence of the interaction of transferrin with the receptor, which destabilizes the binding of the remaining iron atom, facilitating, in this way, its liberation *(88,139–152).*

Afterward, the iron-containing endosome fraction is separated; the iron of its interior is transferred to the cell cytoplasm, a process that may be apparently mediated through an ATP-dependent proton pump. Once the iron is in the cytoplasm, it is bound to iron-fixing proteins or to low-molecular-weight ligands. This iron may be bound afterward to iron-regulating proteins or be integrated into protein structures having iron, or it may be included in the cell storages of this metal *(5,153–156).*

The other part of the endosome that contains the apoTf–TfR complex is taken by the Golgi apparatus in order to be packed together with freshly synthesized transferrin receptors. These vesicles head toward the cell membrane, merging with it and putting the apoTf–TfR complexes into contact with the extra cell space. At the pH of the extra cell space (7.4), the affinity of TfR for apoTf decreases substantially. Thus, apoTf is liberated in order to engage in its functions again. This cycle takes approx 10 min and it can be repeated about 100 times until transferrin or its receptor is degraded *(5).*

BIOCHEMICAL AND PHYSIOLOGICAL FUNCTIONS OF IRON

The principal biological functions of iron are based on its oxidation and reduction properties. Its oxidation state varies from –2 to +6. The inter-

conversion among them confers to this element particular properties that allow it to participate in electron transfer mechanisms or to be reversibly bound to different ligands such as oxygen, nitrogen, and sulfur. As a consequence, this element will also have special biological properties, by which it participates in many biochemical processes, generally through its association with several biomolecules, especially proteins, many of them with enzyme activity *(1,2,5).*

Among the proteins associated with this element, we find those containing iron in its structure, such as hemoglobin and myoglobin, enzymes containing iron bound to sulfur, enzymes containing hemic iron, and enzymes containing iron but not in the heme form nor bound to sulfur *(3,5,6).*

These particular characteristics of iron as well as the great variety and diversity of biological structures to which it is associated determine that this element plays a role in multiple and vital biochemical and physiological processes such as the transport and storage of oxygen by hemoglobin; in the muscle metabolism, in which the iron contained in myoglobin allows the passage of oxygen from erythrocytes to the muscle mitochondria; as heme, it is included in the active site of cytochromes, which play an active role in multiple and varied metabolic routes such as those related to the energy metabolism; with the microsome enzyme P-450 system that participates in the synthesis of various steroids as aldosterone, corticosterone, pregnenolone, vitamin D_3, and so forth. This system also participates in the degradation of different metabolites, drugs, pharmaceuticals, and several toxic substances. Finally, it should be mentioned that iron is also included in most of the mammalian oxidases; thus, its involvement in a very wide variety of physiological and metabolic processes is evident *(3–5,98,157,158).*

BIOCHEMICAL PARAMETERS RELATED TO IRON STATUS

There are several parameters related to iron metabolism that reflect its status in the organism. Among those of greatest relevance we may find the following.

Hemoglobin

Hemoglobin is the red pigment of erythrocytes; its principal function is related to the transport of oxygen. A protein fraction (globin) and four heme molecules form hemoglobin. Because iron is an essential component of this molecule, its iron content undergoes variations according to the status for this element. Thus, a low hemoglobin concentration will produce hypochromia, a condition related to anemia provoked by iron deficiency. The use of hemoglobin as a tracer for iron status has some limitations, as in dehydration, chronic inflammatory states, polycytemia, smoking habits,

chronic infection, hemorrhages, vitamin B_{12} and folic acid deficiencies, protein-energetic malnutrition, pregnancy, and hemoglobin pathologies. Considering normal hemoglobin values, it is necessary to take into account that the existing variations depend on age, sex, and race of the person under consideration, as these values have slight but significant variations in each particular case *(159,160).*

Hematocrit

Hemocrit is determined with total blood with heparinized capillary tubes after centrifugation, until a constant cell pellet volume is obtained. The hematocrit value, which is given as the percentage of red cells, is obtained comparing the height of the red cells with regard to the height of the cells and plasma column. The normal hematocrit values are given in tables and depend on the age, sex, and race of the individual *(159).* The use of this procedure to obtain the iron status has some disadvantages as a consequence of its low sensitivity and specificity, because, as in the case of hemoglobin determination, the hematocrit is affected by different factors. Another disadvantage is its lack of precision, especially if capillary blood is taken as a sample. In spite of these limitations, the hematocrit has the advantage of being a simple, economic, and rapid method *(159,160).*

Red Cell Indices

These indexes are as follows: the mean cell volume (MCV), the mean cell hemoglobin (MCH), and the mean cell hemoglobin concentration (MCHC). These parameters are useful for measuring the size of red cells, as well as its hemoglobin content and concentration. They may be easily calculated from the hemoglobin concentration, hematocrit, and number of red blood cells. The MCV is the mean red blood cell volume and it is the ratio between the hematocrit and the number of red blood cells. MCH is the mean hemoglobin content in the erythrocytes and it is given by the ratio between hemoglobin concentration and number of red blood cells. The MCHC is the mean hemoglobin concentration in a given red blood cell volume; it is calculated as the ratio between hemoglobin concentration and hematocrit. The normal values for these parameters are also given in tables and they show variations according to the individual's age and sex. The abnormal values of these parameters are especially useful for the characterization of the different morphological types of anemia *(159,160).*

Serum Iron, Total Iron-Binding Capacity, and Transferrin Saturation Percentage

Serum iron (SI) and total iron-binding capacity (TIBC) are parameters related to the iron interchange between the reticuloendothelial system and the bone medulla. Transferrin is the principal protein related to iron blood

transport. As a consequence, the serum iron content is related to the number of iron atoms bound to transferrin. Each of its molecules is able to bind up to two iron atoms, a reason for which TIBC is related to the fraction of free sites of transferrin able to transport iron. Consequently, transferrin saturation percentage (TSP) is calculated as the percentage ratio between SI and TIBC. These three parameters are particularly useful to differentiate an iron-deficient status resulting from nutritional causes from that resulting from to different pathologies associated with chronic infectious or inflammatory processes. Normal values for these parameters are given in tables and they depend fundamentally on individual's age and sex. It should, however, be taken into account that several factors as circadian variations, the use of oral contraceptives, chronic diseases, as well as other factors may modify their values *(159,160).*

Serum Ferritin

The serum ferritin (SF) is in equilibrium with its intracellular form and is proportional to the iron storage content. The relationship between these parameters is that a SF value of 1 µg/L is equivalent to approx 8–10 mg of storage iron. Factors such as acute or chronic infections, vitamin B_{12} and folic acid deficiencies, excessive alcohol consumption, leukemia, hepatic diseases, and so forth produce a significant SF increase. On the contrary, SF values lower than 12 µg/L are related to an evident iron storage deficiency. Falsely reduced SF values resulting from other causes have not been detected. Normal SF values are given in tables and depend fundamentally on the age and sex of each person. However, it should be emphasized that there is a significant intraindividual variation coefficient of approx 15% for the SF values *(159,160).*

Erythrocyte Protoporphyrin Concentration

The physiological basis for the utilization of erythrocyte protoporphyrin concentration (EPC) to evaluate iron metabolism is the fact that protoporphyrin IX is the heme precursor. Under normal conditions, EPC in red blood cells is low, but as the available iron quantity for hemoglobin synthesis decreases, the EPC value increases proportionally to the metal availability decrease. The EPC determination is carried out fluorometrically on total blood and is given as micrograms per deciliter or micromoles per liter of red blood cells. Normal values depend on several factors such as the individual's age, sex, and race. Even if an EPC increase is related to an iron-deficient status, there are other factors such as chronic diseases, infection, inflammation, and cancer, which are also associated with high EPC levels. In the case of lead intoxication, EPC increases are also produced, as a consequence of the interference of this metal with heme synthesis. Taking these considerations into account, the EPC determination is considered as a simple, economic, and reliable method to evaluate iron

metabolism if the obtained values are normal. In the case of increased EPC values, more data should be obtained to draw conclusions on the individual's iron metabolism *(159,160).*

Transferrin Receptor Plasma Concentration

Transferrin receptor plasma concentration (TfRPC) changes with the iron nutritional status of the person, being increased with a slight deficiency of this metal. TfRPC is also increased in certain pathologies as β-thalassemia, autoimmune hemolytic anemia, chronic lymphocytic anemia, and so forth. However, TfRPC decreases in hemachromatosis, aplastic anemia, and chronic renal insufficiency. In contrast to the other parameters used for the iron status evaluation, inflammation, infection, or hepatic diseases do not significantly affect TfRPC. Therefore, the clinical usefulness of TfRPC determination is the result of the fact that it allows a differentiation of an anemia caused by iron deficiency with regard to other types of anemia, principally in those regions in which the prevalence of infections is high. It has recently been found that this biochemical parameter for determining iron status is the best estimator to detect iron deficiency during pregnancy *(5,161–163).*

VARIATION OF THE BIOCHEMICAL PARAMETERS ASSOCIATED TO THE IRON STATUS

Table 1 shows the variations of the biochemical parameters associated with iron metabolism during the progressive development of iron deficiency until anemia is produced.

In the first stage, a decrease of the organic iron stores content is observed, which is related to a reduction of serum or plasma ferritin concentration. In the second stage of iron deficiency, a decrease of plasma iron concentration, lower than 60 μg/dL, is shown together with an increase of total iron-binding capacity and, consequently, a decrease of TSP to less than 15%. At the same time and as a consequence of the insufficient iron supply for heme synthesis, an increase of free erythrocyte protoporphyrin concentration higher than 100 μg/dL of red blood cells is produced. However, at this stage, a significant modification of hemoglobin concentration is not yet observed, because its value remains within the normal range according to sex and age. Finally, at the third and last stage, anemia resulting from iron deficiency is produced, which is determined by a clear decrease of the hemoglobin concentration and the hematocrite, reflected at the erythrocyte level such as hypochromia with microcytosis and a decrease of total iron-binding capacity. This stage is also characterized by a decrease of plasma iron concentration (lower than 40 μg/dL) and of ferritin (lower than 10 μg/dL) and a substantial increase of free erythrocyte protoporphyrin (more than 200 μg/dL of red blood

Table 1
Sequential Stages of the Progressive Development of Iron Deficiency

PARAMETER	NORMAL	IRON DEPLETION	IRON DEFICIENT ERYTHROPOIESIS	IRON DEFICIENCY ANEMIA
Bone marrow iron RE	2-3 +	0-1 +	0	0
TIBC (μg/dl)	330±30	360	390	410
Plasma ferritin (μg/l)	100±60	20	10	< 10
Iron absorption (%)	5-10	10-15	10-20	10-20
Plasma iron (μg/dl)	115±50	115	< 60	< 40
Trasferrin saturation (%)	35±15	30	< 15	< 15
Free erytrocyte protoporphyrin (μg/dl,CR)	30	30	100	200
Erytrocytes (morfology)	Normal	Normal	Normal	Microcytic Hypochromic

Note: RE = reticuloendotelial; TIBC = total iron-binding capacity, RC = red cell.
Source: Data from refs. *4, 159,* and *164.*

cells). At this stage, a large increase of the total iron-binding capacity is also produced, with values higher than 410 μg/dL *(159).*

Table 1 shows that a lack of adequate intake of bioavailable iron, according to physiological and/or metabolic requirements of the organism, may provoke an initial stage of iron deficiency that, if it is not corrected, may reach a stage of anemia resulting from iron deficiency. The knowledge of the physiological and biochemical conceptual basis through which this pathological situation takes place, as well as the economic and social consequences of the involvement of a substantial portion of the population of a country or region in such a disabling disease, is a fundamental requirement to be able to take adequate measures to avoid this situation.

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