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Iron Metabolism: A Comprehensive Review

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Despite its abundance in the earth's crust, iron deficiency is a serious health issue in many parts of the world. Although fundamental observations about iron metabolism and the significance of iron nutriture were first noted some time ago, the molecular mechanisms involved in iron metabolism are just now being defined.

Historical Perspective

Among all of the micronutrients, iron has the longest and best described history. Iron is the fourth most abundant terrestrial element, comprising approximately 4.7% of the earth's crust in the form of the minerals hematite, magnetite, and siderite. Primordial iron compounds were probably responsible for the catalytic generation of some of the atmospheric oxygen upon which most modern life forms depend.[1] Iron is an essential nutrient for all living organisms with the exception of certain members of the bacterial genera *Lactobacillus* and *Bacillus*. In these organisms, the functions of iron are replaced by other transition metals, especially manganese and cobalt, which reside next to iron in the periodic table. In all other life forms, iron is an essential component of, or cofactor for, hundreds of proteins and enzymes.

Based on extrapolations made from modern aboriginal societies, prehistoric humans had an adequate intake of iron.[2] The ancient Arabs, Chinese, Egyptians, Greeks, and Romans, although ignorant about the nutritional importance of iron, attributed therapeutic properties to iron.[3] The ancient Greeks administered iron to their injured soldiers to improve muscle weakness, which probably derived from hemorrhagic anemia.[4] Alchemists and physicians of the 16th century prescribed iron for medicinal use.[5,6] Iron salts were given to young women to treat what was then described as chlorosis, an arcane term for anemia usually due to iron or protein deficiency.[4] Various physicians during this time also prescribed iron pills for anemia and were unceremoniously ridiculed by their successors in the medical profession.[7,8]

Iron was identified as a constituent of animal liver and blood in the early 18th century.[3,9,10] In 1825, hemoglobin (Hb) iron content was determined to be 0.35%,[4] a value extremely close to the Hb iron content of 0.347%[11] calculated by modern methods. Between 1832 and 1843 chlorosis was defined by low levels of iron in the blood and reduced number of red cells.[3,9,10] Boussingault first described the nutrition essentiality of iron in 1872.[12] In 1895, Bunge accurately described the anemia of chlorosis in terms of nutritional iron deficiency.[13]

Key observations about iron nutrition were made during the first half of this century.[4,9,10,14] Moore discovered the enhancing effect of ascorbic acid on iron absorption.[15] Granick later proposed the "mucosal block" theory for control of body iron.[16] Although these fundamental observations on the metabolism of iron and its nutritional significance were made some time ago, the molecular mechanisms involved in iron metabolism are just now being described.

Whole Animal Metabolism

Food Sources

Despite its abundance in the earth's crust, iron deficiency is a serious health issue in many parts of the world. The iron nutrition status of an individual and of populations is largely a function of the amount of dietary iron, the bioavailability of that iron, and the extent of iron losses. Many foods that are potentially good sources of iron are limited by the bioavailability of that iron.[17] The bioavailability

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of iron is a function of its chemical form and the presence of food items that promote or inhibit absorption.[15,18–24] Basal obligatory iron losses in humans are approximately 1 mg/day and must be replaced by an equivalent amount of iron derived from the diet. The typical Western diet provides an average of 6 mg of heme and nonheme iron per 1000 kcal of energy intake.[26] Heme iron is an important dietary source of iron because it is more effectively absorbed than nonheme iron. From 5% to 35% of heme iron is absorbed from a single meal, whereas nonheme iron absorption from a single meal can range from 2% to 20%, depending on the iron status of the individual and the ratio of enhancers and inhibitors in the diet. Thus, although it constitutes about 10% of the iron found in the diet, heme iron may provide up to a third of total absorbed dietary iron.[27]

Nonheme iron, which constitutes 90% of the remaining dietary iron, accounts for 60% of the iron from animal sources and about 100% of the iron found in vegetable material. Intentional iron fortification or iron contamination of food during preparation may account for as much as 10–15% of dietary nonheme iron.[28] The primary overall effect of food on nonheme iron absorption is inhibitory. The rat, which has been the standard model of iron absorption for humans, appears to be less sensitive to these factors compared with humans. Therefore, the actual influence of these factors in humans may be underestimated.[29] Conversely, the long-term contributions of these enhancers and promoters to body iron stores may be more limited than first thought.[30–32]

For the most part, attempts to alleviate iron deficiency through food fortification and diet supplementation have been successful in the United States. Many different forms of iron have been employed for this purpose. In the United States, fortification of food with iron started in the 1940s. The Food and Drug Administration has since upwardly revised standards for iron enrichment of cereal products and has issued guidelines for fortification of infant formulas. These actions have prompted much debate about potential iron overloading in people with adequate iron status.[33–37]

Supplementation, although more able to target specific populations at risk for iron deficiency, is limited by dosage requirements and potential side effects, both of which lead to noncompliance. Side effects of high-dose (>200 mg) iron supplementation include gastrointestinal distress and constipation. The additions of bovine Hb to foods[38] and bovine lactoferrin to infant formulas[39] appear to be effective methods of iron supplementation that have minimal side effects.

Iron Absorption

General

The vast amount of work that has contributed to our knowledge of iron absorption will not be extensively rereviewed here. We refer the readers to other recent and more comprehensive documents.[40,41] We would, however, like to direct our attention to some of the mechanistic aspects of iron absorption that have been suggested in recent years.

The process of iron absorption can be divided into three stages: (1) iron uptake, (2) intraenterocyte transport, and (3) storage and extraenterocyte transfer (Figure 1). During the intestinal phase of digestion, iron binds to specific mucosal membrane sites, is internalized, and then either is retained by the mucosal cell or is transported to the basolateral membrane where it is bound to transferrin (Tf) in the plasma pool. The process of iron absorption is controlled by intraluminal, mucosal, and somatic factors. A multitude of intraluminal factors affect the amount of iron available for absorption as either inhibitors or promoters. Mucosal factors include the amount of mucosal surface and intestinal motility. Somatic factors that influence iron absorption include erythropoiesis and hypoxia.

Luminal Phase

No absorption of iron occurs in the mouth, esophagus, or stomach. However, the stomach does contribute hydrochloric acid. This not only helps to remove protein-bound iron by protein denaturation but also helps solubilize iron by reducing it from the ferric to the ferrous state. Reduction of ferric iron is necessary because the majority of iron in the diet is found in the relatively insoluble ferric form ($K_{sp} = 10^{-17}$ M) and is poorly absorbed.[42,43] Decreased stomach acidity due to over consumption of antacids, ingestion of alkaline clay, or pathologic conditions such as achlorhydria or partial gastrectomy may lead to impaired iron absorption.[44,45] The combined actions of gastric acid and pepsin account for slightly less than half of the release of conjugated dietary iron and the reduction of a third of total dietary ferric iron.

Other gastrointestinal components also have roles in iron absorption. Pancreatic ductal cells secrete bicarbonate, which, by increasing the pH of the lumen, potentially decreases iron absorption. This effect is counterbalanced by pancreatic proteases that liberate nonheme iron from digestiva. Alterations in this balance, as seen in patients with pancreatic insufficiency or cystic fibrosis, may have potential adverse consequences.[46] It has been suggested that consumption of pancreatic enzymes by these patients may predispose them to iron overload.[47]

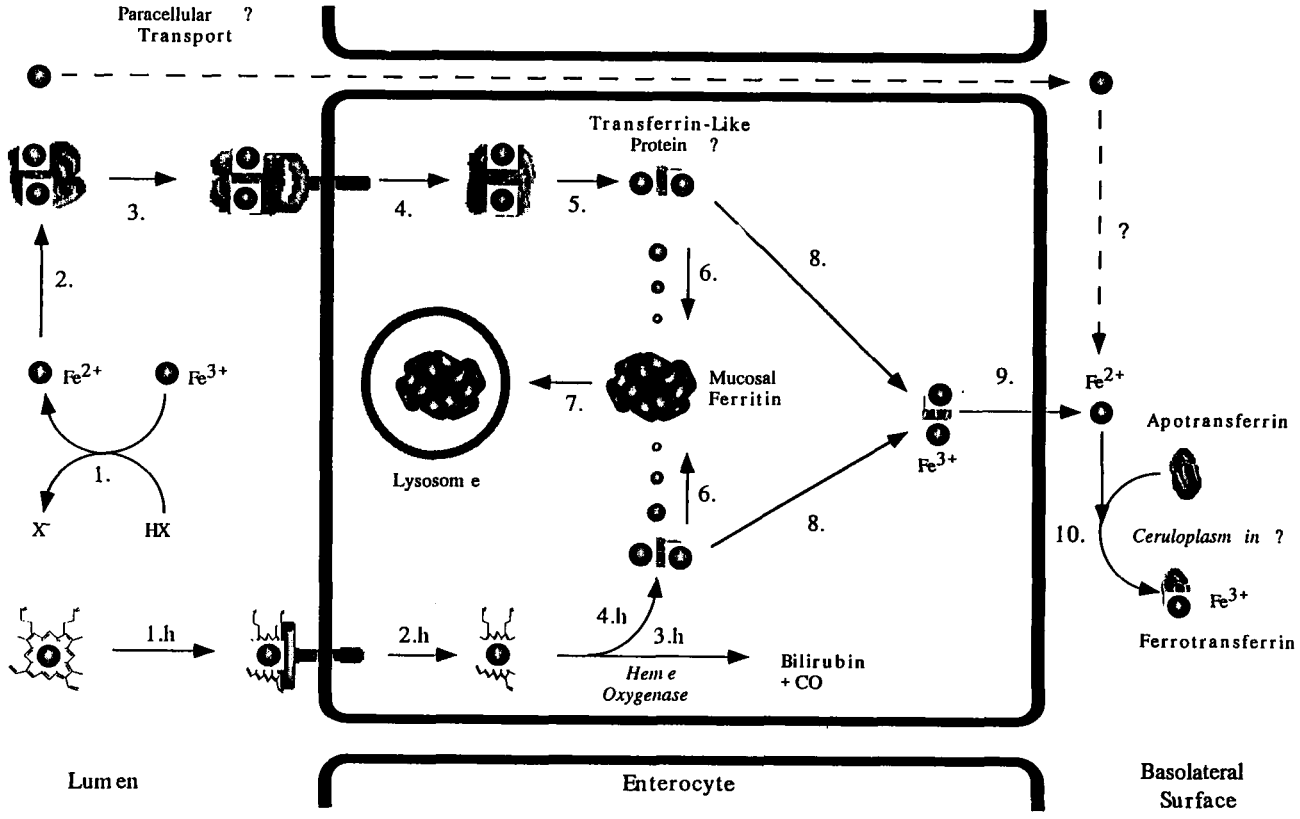

Figure 1. Enterocyte uptake and transfer of iron. Nonheme iron: A reductant such as ascorbate reduces nonheme ferric iron to ferrous iron (**1**). Chelators sequester and solubilize nonheme iron. Nonheme iron is then transferred to a binding protein(s) within the lumen (**2**). The iron-binding protein binds to a specific transporter on the luminal surface of the enterocyte (**3**). Nonheme iron is transported to the enterocyte interior (**4**). This iron is either transferred to low molecular weight chelates or to a transferrin-like protein (**5**). The transferrin-like protein delivers iron to either mucosal cell ferritin (**6**) or to the basolateral surface of the enterocyte (**8**). Absorbed iron that is not sequestered by ferritin is delivered to the basolateral surface of enterocytes (**9**) and oxidized for binding to transferrin (**10**). Heme iron: Heme binds to its receptor (**1h**) and is internalized (**2h**). After entering the cell, heme is degraded to iron, carbon monoxide, and bilirubin IXa by the enzyme heme oxygenase (**3h**). This iron enters the common intracellular (enterocyte) pool of iron (**4h**) (**5**) and is processed like nonheme iron (**6–10**).

The majority of iron absorption takes place in the duodenum and upper jejunum.[48,49] Factors that increase transit through these areas decrease iron absorption.[50] A multitude of dietary factors affect iron absorption during this phase. Heme iron appears to be affected only by animal proteins, which facilitate its absorption,[51,52] and calcium, which inhibits its absorption.[53] In contrast to heme iron, many factors affect nonheme iron absorption.[20,24,41,54–56] The extrinsic intraluminal factors that decrease iron absorption include bran,[57] hemicellulose, cellulose, pectin,[58] phytic acid found in wheat and soy products,[59,60] and polyphenolic compounds.[21] The absorption of iron is also affected by interactions with other metal ions or minerals. Generally, very high amounts of divalent cations in the diet inhibit iron absorption. The absorption of metal ions or minerals is affected in the same way by iron. Some of the nutritionally significant known metals/minerals interactions are summarized elsewhere.[61–71]

Mechanisms of Intestinal Cell Uptake

The complete pathway of iron absorption is largely unknown and is currently a matter of controversy. Current knowledge is summarized in Figure 1. At physiologic levels iron uptake is mediated by a series of receptors and binding proteins. At higher levels, iron seems to be absorbed passively via a paracellular pathway. During the intestinal phase of digestion, iron is present in the lumen as either heme iron or nonheme iron chelates. Heme iron is taken up directly by the enterocyte and, after enzymatic action, is processed in a manner analogous to nonheme iron. Nonheme iron is transferred to binding proteins within the lumen. Specific transporters exist for nonheme iron binding proteins on the luminal surface of enterocytes.[72] Nonheme iron is transported to the enterocyte interior, where it is bound to an iron-binding protein(s). This iron is transferred either to ferritin or to the basolateral side

of the enterocyte. Given the observation of increased iron absorption with low iron stores and decreased absorption with high iron stores, it is tempting to speculate that there is genetic regulation of both receptors and binding proteins. This regulation appears to be exerted across the basolateral membrane in a manner that is correlated to whole-body iron stores. Iron is then either lost when the cell is sloughed or bound to Tf in the circulation. The tools of molecular biology are now being used to determine what receptors, binding proteins, and cells contribute to iron absorption. At this time, unfortunately, the description of the process of iron absorption is incomplete.

Heme Iron

Heme is soluble in an alkaline environment. Hence, no binding proteins are necessary for its luminal absorption. Although specific transporters exist for heme on the surface of rat enterocytes,[73,74] rats do not absorb heme iron as efficiently as do humans.[75] A specific receptor/transporter for heme has not yet been described in humans. After binding to its receptor, the heme molecule is then internalized. After entering the cell, heme is degraded to iron, carbon monoxide, and bilirubin IXa by the enzyme heme oxygenase.[27,76] This enzyme is not induced by oral administration of Hb (a source of heme) but is induced by iron deficiency.[76] Its distribution in the intestine is identical to the areas of maximal heme iron absorption.[77] It is thought that the iron that is liberated from heme by heme oxygenase enters the common intracellular (enterocyte) pool of iron.

Luminal Nonheme Iron-Binding Proteins

Ferrous iron that has been liberated by gastric and pancreatic proteases is readily oxidized to the ferric form in an alkaline environment. It would be rendered insoluble and biologically unavailable except for the presence of intraluminal iron-binding molecules. Various attempts have been made to identify these molecules. The interpretation of these and other studies seeking to identify physiologic iron-binding molecules is difficult because of the large amount of nonspecific binding by iron. It was originally proposed that the enterocyte or some other gastrointestinal entity such as the stomach or liver synthesized Tf and that Tf was extruded into the lumen to sequester iron.[78] This Tf, replete with dietary iron, was then absorbed by the enterocyte. Transferrin protein has been detected within duodenal mucosal cells[79,80]; however, most investigators have failed to detect Tf mRNA in these cells.[81,82] Although one study has claimed to detect Tf receptors (TfR) on the luminal surface of enterocytes,[83] most others have failed to do so.[84,85] Furthermore, even though Tf-bound iron is efficiently absorbed by the rat,[78] when administered to patients with achlorhydria, it is not effectively absorbed.[86] In addition, humans and mice with hypotransferrinemia become iron overloaded rather than iron deficient.[87] Although the intestinal absorption of Tf-bound iron is an attractive hypothesis, most of the available evidence argues against this scenario and suggests a very limited role for Tf in the direct absorption of iron.

Several investigators have reported the presence of luminal iron-binding proteins that are distinct from Tf. One of these proteins is mucin, which binds (K_d = 1.1 × 10^{-4} M) and solubilizes ferric iron in an acidic milieu.[88] Mucin also binds zinc but with lower affinity.[88] Iron chelates of histidine, ascorbate, and fructose, which enhance iron absorption in vivo, donate iron to mucin at neutral pH and may represent true in vivo complexes. More stable chelates of anions that inhibit iron absorption in vivo, such carbonate and oxalate, do not seem to donate iron to mucin in vitro.

At least two groups have described a 160-kDa glycoprotein from human microvillous membrane vesicles that may participate in facilitated transport of iron.[72,89] This protein is composed of three identical 54-kDa monomers.[89] Other iron-binding proteins of 35, 95, and 120 kDa have been isolated from cultured rat intestinal epithelial cells.[90,91]

Intraenterocyte Transport and Storage

Transport of absorbed iron through the enterocyte may involve a Tf-like protein.[92] One candidate for this Tf-like protein might be mobilferrin, a 56-kDa cytosolic protein isolated from rat and human duodenal mucosa that can bind iron (K_d = 9 × 10^{-5} M).[92] It is a homologue of calreticulin and can also bind calcium, copper, and zinc.[93] The multiple metal ion-binding properties of mobilferrin have been suggested as explanations of the interactions between the absorption of these elements. Mobilferrin coprecipitates with the α and β subunits of integrin during its purification, prompting Conrad to suggest that mobilferrin is involved in cytosolic acceptance of iron from membrane-bound integrin.[91]

In the conceptual model of feedback regulation of iron absorption, an adequate iron status increases the amount of iron retained by the enterocyte. In this regard, ferritin has been proposed to act as an "iron sink" for intestinal mucosal cells. Iron that is not transferred to the plasma is stored in mucosal cell ferritin and is lost when the enterocyte dies and is subsequently shed.[94,95] It is unlikely that any mucosal cell ferritin reaches the circulation before the enterocyte is shed. The lower duodenal levels of ferritin mRNA found in iron-deficient subjects and

higher duodenal levels of ferritin mRNA found in secondary iron overload support the role of mucosal ferritin as a major regulator of iron absorption.[82] If ferritin is the mucosal "iron sink," dysregulation of mucosal ferritin mRNA expression could lead to iron deficiency or iron overload. Consistent with this hypothesis is the fact that the concentrations of mucosal cell ferritin mRNA and ferritin protein in patients with familial hemochromatosis are lower than those of patients with secondary iron overload.[80,82,96]

Extraenterocyte Transfer

Absorbed iron is delivered and bound to Tf at the basolateral surface of enterocytes. It has been proposed that ceruloplasmin is the protein responsible for oxidation of iron, which is necessary for its binding to Tf at the basolateral membrane.[97,98] The evidence for involvement of ceruloplasmin in this process is largely circumstantial. Copper deficiency results in accumulation of iron in the mucosa and liver, reduced iron transport to peripheral tissues, and anemia.[99] The classic explanation for this type of anemia has been attributed to lack of basolateral mucosal cell ferroxidase I (ceruloplasmin) action. This explanation is not fully adequate, as brindled mice and patients with Menkes' or Wilson's disease all have low serum ceruloplasmin levels but do not exhibit iron deficiency anemia.[99]

Regulation of Iron Absorption

Mucosal Factors

The majority of iron absorption takes place in the duodenum and upper jejunum. These areas also adapt during iron deficiency to promote iron absorption.[49] In these areas the amount of functional absorptive mucosal surface is important for iron absorption. Consequently, surgical removal of any part of the duodenum and upper jejunum or the presence of factors that increase enterocyte turnover decreases iron absorption.[50] Clinical disorders that affect iron absorption at this level include malabsorption syndromes such as steatorrhea and tropical sprue.[50]

Somatic Factors

The regulation of iron absorption involves somatic factors that notify the enterocyte of the need for iron absorption. It is clear that a person's iron status correlates inversely with the amount of iron absorbed.[100] More recent investigations have shown that iron deficiency is the most potent somatic inducer of both heme and nonheme iron absorption. The mechanism(s) for this induction is largely unknown. One possible contributing factor is intestinal heme oxygenase, which is activated by somatic iron deficiency.[76]

Hemoglobin and serum ferritin apparently have limited roles in signaling the enterocyte about the need for iron absorption.[82,101,102] It has been suggested that internalized plasma ferro-Tf may allow the enterocyte to monitor body iron status and regulate iron absorption. Exposure to low intracellular amounts of plasma ferro-Tf would signal the enterocyte to up-regulate iron entry into the body. Transferrin receptors are only found at the basolateral surface of enterocytes.[103,104] The amount of enterocyte TfR mRNA and protein increases during iron deficiency and decreases during secondary iron loading.[82,103,105] Acute hemolysis, which stimulates iron absorption, does not influence enterocyte basolateral TfR number.[105] Of interest is that the levels of TfR and TfR mRNA in mucosal cells of people with hemochromatosis are higher than suitably matched controls.[82,104]

Active erythropoiesis, induced either by bleeding[100] or by acute hemolysis,[106] increases the absorption of iron. It has therefore been proposed that erythropoietin is an endogenous signal for iron absorption; there is limited evidence for this hypothesis. In fact, recombinant human erythropoietin does not increase intestinal iron absorption when given to iron-overloaded rats.[107] Moreover, exchange transfusion of reticulocytes with a large number of TfR into rats stimulates iron absorption in iron-replete animals independently of erythropoietin production or active erythropoiesis.[108]

Hypoxia increases iron absorption[109] independently of erythropoiesis.[110,111] Increased plasma iron turnover, which occurs not only in erythropoiesis but also in disorders of ineffective erythropoiesis such as thalassemia, hemolytic anemias, and sideroblastic anemias, is associated with increased iron absorption.[112] Other clinical disorders such as hemochromatosis, congenital ferrochelatase deficiency, and porphyria cutanea tarda[50] result in an increased iron absorption by mechanisms that are yet unexplained. Finally, inflammatory processes may decrease iron absorption,[113] probably by eliciting the production of cytokines that have a direct effect on the mucosal cell.

Transport of Iron

In addition to being oxidized to the insoluble ferric state in an oxygen-rich environment such as that found under physiologic conditions, free iron is an extremely toxic substance capable of catalyzing many deleterious reactions. Quantitatively, the most significant iron transport molecule in vertebrates is Tf. Not only is it responsible for delivery of iron

from the basolateral surface of enterocytes to peripheral tissues, but it is also responsible for redistribution of iron to various body compartments and protection of iron from glomerular filtration. A number of other systems may make small but important contributions in transporting iron to the tissues including heme-hemopexin (HPX), ferritin, lactoferrin, and the as yet uncharacterized low-molecular weight pool of iron.

Transferrin is a single-chain, 80-kDa protein composed of two iron-binding half-site motifs. Each site binds ($K_d = 10^{-22}$ M) ferric iron in a ternary complex of protein ligands, bicarbonate, and water. Under physiologic conditions, Tf can also bind manganese and aluminum.[114] Some investigators have suggested that because the two half-sites of Tf release iron at different pHs, they may be functionally distinct.[115] However, recent evidence suggests that they are physiologically indistinct.[116] In vivo Tf is normally 25–50% saturated with iron.[117] Thus, under normal physiologic circumstances, the iron-binding capacity of plasma is always in excess of iron concentration.

Transferrin belongs to a family of proteins that includes ovotransferrin, lactoferrin, melanotransferrin (p97 antigen), and a newly described protein, hemiferrin.[118] In rats and humans, the primary site of synthesis is the liver. However, other sites also synthesize Tf, including brain, kidney, testes, and fetal muscle. The human Tf gene has been localized to the 3q21–25 region of chromosome 3. The gene contains 17 exons and 16 introns. The coding region is 2.3 kb, which is lengthened to 3.5 kb by elongation of the intron regions. The 5′ sequence of the human Tf gene contains elements that allow transcriptional regulation by heavy metals, glucocorticoids, and the acute-phase reaction signal.[119] The Tf gene is also transcriptionally regulated by insulin-like growth factor, epidermal growth factor, platelet-derived growth factor,[120] and retinoic acid.[121] Iron regulates Tf gene expression in liver[122] but not in other tissues.[81] Using the chloramphenicol acetyl transferase (CAT) reporter gene, it has been determined that Tf synthesis is regulated by iron post-transcriptionally.[123]

Free heme is detected in serum only in hemolytic conditions. Normally serum heme is bound to either albumin ($K_d = 1 \times 10^{-8}$ M) or HPX ($K_d < 1 \times 10^{-13}$ M).[107] Hemopexin is a 60-kDa glycoprotein.[124] The gene for human HPX has been localized to chromosome 11.[124] Hemopexin is synthesized by the liver and circulates in the plasma. Lactoferrin is an 80-kDa iron-binding protein found in milk, plasma, and mucous secretions such as tears. It is secreted from some glandular epithelial tissues as well as from activated neutrophils. Human lactoferrin is composed of 703 amino acids and exhibits 59% sequence homology with Tf.[125] Unlike Tf, however, iron does not appear to affect the transcription of the lactoferrin gene. Specific uptake of lactoferrin by hepatocytes has been described.[126] Although its role in iron transport remains unclear, lactoferrin is considered to be part of the acute-phase response and to be bacteriostatic and fungistatic by virtue of its ability to sequester iron.[127]

In addition to its role as an iron storage protein, ferritin can act as a cellular iron delivery agent. Kupffer cells release some of the iron that has been salvaged from senescent erythrocytes in the form of H-ferritin. This ferritin is ultimately scavenged by hepatocytes. Halliday et al. have described ferritin receptors of rat ($K_d = 1.0 \times 10^{-8}$ M),[128] pig ($K_d = 2.9 \times 10^{-9}$ M),[129] and human($K_d = 6.0 \times 10^{-8}$ M)[130] hepatocytes. Separate, specific binding sites on Molt-4 cells have also been described for H-ferritin ($K_d = 6.5 \times 10^{-7}$ M).[131]

Storage and Mobilization of Iron

The molecular distribution of iron in the human body is summarized elsewhere.[132] The concentration of iron in the human body is approximately 30–40 mg/kg body weight. However, that concentration varies as a function of age and gender and the specific tissues and organs examined. About 85–90% of nonstorage iron is found in the erythroid mass. The storage iron concentration in the body varies from 0 to 15 mg/kg body weight depending on gender and iron status. The distribution of this stored iron is not uniform, as the liver contains approximately 60% of the ferritin in the body. The remaining 40% is found in muscle tissues and cells of the reticuloendothelial system.[117] Normally, 95% of the stored iron in liver tissue is found in hepatocytes as ferritin. Hemosiderin constitutes the remaining 5% and is found predominately in Kupffer cell lysosomal remnants. However, during iron overload, the mass of hemosiderin in the liver accumulates at 10 times the rate of ferritin.[133]

Ferritin

The overall structure of ferritin is conserved among higher eukaryotes. In humans it comprises 24 polypeptide subunits. At least two distinct isoforms of the polypeptide subunits exist, and combinations of these subunits allow for considerable heterogeneity in the structure of the full protein. The isoform designated H ferritin is a 22-kDa protein composed of 182 amino acids. The L isoform is a 20-kDa protein containing 174 amino acids. The subunit composition of ferritin seems to be tissue specific. For example, the H form predominates in heart, whereas the L form predominates in liver.[134] Although nu-

merous pseudogenes exist for ferritin on multiple chromosomes, the actively transcribed gene for the H subunit is found on chromosome 11[135] and for the L form on chromosome 19.[136,137] The genes are 3 kb each, with four exons that are processed into 1-kb transcripts. The synthesis of both subunits of ferritin is stimulated by iron.[138,139] The H subunit appears to be regulated only at the level of translation.[140] The L subunit is apparently regulated at the level of transcription[140,141] and translation.[138] By coupling these two mechanisms a 25–50-fold change in the level of ferritin mRNA can be achieved.[142] Theil[143] has proposed that differential ferritin gene expression plays a role in the "housekeeping" of cellular iron, and that the proportion of H-to L-chain ferritin is related to the developmental demands of the cells.

Theoretically, up to 4500 ferric iron atoms can be stored in ferritin.[144] Even though ferritin with 1200–1400 molecules of iron appears to be the most efficient in the acquisition or release of iron, in vivo ferritin is normally 20% saturated (800 out of 4500 iron sites occupied).[145] The structure and composition of the mineralized core is analogous to a polymer of ferrhydrite ($5Fe_2O_3 \cdot 9H_2O$) with a variable amount of phosphate.[146]

Ferritin iron core formation was recently reviewed by Crichton and Ward.[146] First, ferrous iron enters the protein through specific channels. Then iron is oxidized either at various sites within the protein or on the core surface. The H chain possesses a distinct ferroxidase site(s), and the homogeneous polymer of H-chain ferritin is capable of self-loading.[147] The L chain of ferritin lacks this site, but the homogeneous polymer of L-chain ferritin is evidently also capable of some self iron loading at physiologic pH.[148] The L chain is also more efficient than the H chain in the formation of a mineralization nuclei. Therefore it has been suggested that there is cooperativity between the H and L subunits in the process of iron loading.[149] Alternatively, some investigators have proposed a model of ferritin iron loading by which ceruloplasmin is responsible for iron oxidation and subsequent incorporation into ferritin.[150]

Iron is rapidly released from ferritin by reduction of the iron core. It has been suggested that ascorbic acid or reduced flavin mononucleotide is the endogenous reductant in this process in vivo. At the present moment however, the identity of the reductant is unknown. In vitro studies of iron oxidation sometimes use ascorbic acid to mobilize iron from iron-loaded ferritin; some authors have suggested that excessive ascorbic acid intakes could lead to increased mobilization of storage iron, which could promote oxidative tissue damage.[151] There is little in vivo evidence to suggest that this occurs in people with normal iron status or normal iron-handling capabilities. However, in iron-loaded people with thalassemia who are treated with deferoxamine, supplemental ascorbic acid may be toxic.[152]

The rate of iron release from ferritin is influenced by several factors. For example, the last iron atoms entering the mineralized core of ferritin are more easily liberated than those loaded first.[153] The H-chain ferritin also releases iron more rapidly than does the L-chain.[149] In addition, heme binds to ferritin,[154] which increases the rate of iron release.[155]

It has been proposed that ceruloplasmin is necessary for the oxidation of ferritin-derived iron and for its subsequent attachment to Tf. Copper-deficient rats accumulate liver iron in the form of ferritin.[156] Liver perfusion of these animals with blood containing ceruloplasmin causes immediate transfer of ferritin-bound iron to Tf.[157]

Hemosiderin

When the average tissue iron stores of ferritin approach about 4000 atoms of iron per ferritin molecule, ferritin is degraded by lysosomal proteases to form hemosiderin, an insoluble iron storage protein.[158] In this process the protein shell of ferritin is partially degraded so that up to 40% of the mass of hemosiderin consists of iron. The description of the type of iron stored in hemosiderin depends on the sources and conditions under which it was obtained. These forms of iron include amorphous ferric oxide, ferrhydrite, and goethite.[146] These forms of iron are less chemically reactive than those found in ferritin and may be less available for mobilization.

Iron Turnover and Redistribution

The absorption and loss of iron are balanced in individuals with normal iron status. However, disruptions of this balance are commonly seen during menstruation, pregnancy, and gastrointestinal bleeding. In order to meet tissue needs, iron must either be mobilized from storage or be recycled. Iron turnover is a significant means of recycling iron in the body. For example, in a 70-kg individual with a normal iron status, about 35 mg/day of iron are turned over in the plasma[159] (Figure 2). Iron turnover is primarily mediated by destruction of senescent erythrocytes by the reticuloendothelial system.[159] Erythrocytes, which contain about 80% of the body's functional iron, have a mean functional lifetime of 120 days in humans. At the end of their functional lifetime, they are recognized as senescent by changes in the structure of their membranes and are catabolized at extravascular sites by Kupffer cells and spleen macrophages. After phagocytosis, the globin chains of Hb are denatured, which re-

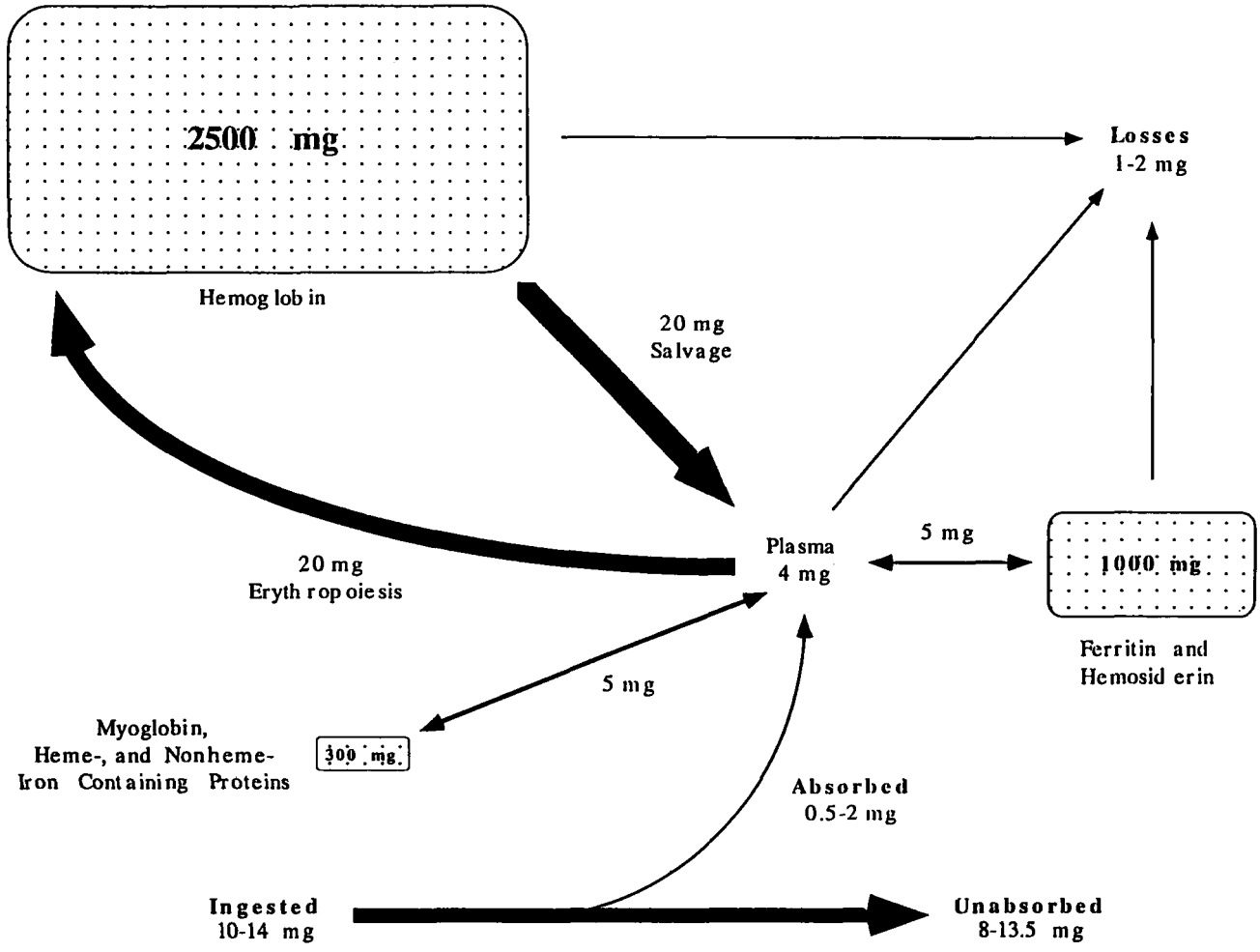

Figure 2. Iron distribution and exchange pools.

leases bound heme. Intracellular unbound heme is ultimately degraded by heme oxygenase, which liberates iron. About 85% of the iron derived from Hb degradation is rereleased to the body in the form of iron bound to Tf or ferritin. Each day 0.66% of the body's total iron content is recycled in this manner.[159] Smaller contributions are made to plasma iron turnover by the degradation of myoglobin and iron-containing enzymes.

Iron Losses

The low solubility of iron precludes excretion as a major mechanism for maintaining iron homeostasis. In contrast to most other trace minerals whose homeostasis in maintained by excretion, the primary mechanism of maintaining whole-body iron homeostasis is to regulate the amount of iron absorbed so that it approximates iron losses. Iron losses can vary considerably with gender. In human males, total iron losses from the body have been calculated to be 1 mg/day. For premenopausal female humans, this loss is slightly higher. The predominant route of loss is from the gastrointestinal tract, with losses amounting to 0.6 mg/day in adult males.[160] Fecal iron losses derive from shed enterocytes, extravasated red blood cells, and biliary heme breakdown products that are poorly absorbed. Urogenital and integumental iron losses have been estimated at >0.1 mg/day and 0.3 mg/day, respectively, in adult males.[161] Menstrual iron loss, estimated from an average blood loss of 33 mL/month, equals 1.5 mg/day, but may range as high as 2.1 mg/day.[160] Oral contraceptives reduce this loss[160,162] and intrauterine devices increase it.[160,163,164] Pregnancy is associated with losses approximating 1 g over the pregnancy course, consisting of basal losses of 230 mg iron; increased maternal red cell mass of 450 mg iron; fetal needs of 270–300 mg iron; and placenta, decidua, and amniotic fluid iron content of 50–90 mg.

A number of clinical and pathological conditions are accompanied by variable amounts of blood loss. These include hemorrhage, hookworm infestation, peptic gastric or anastomotic ulceration, ulcerative colitis, colonic neoplasia, cow's milk feeding to in-

fants, aspirin, nonsteroidal anti-inflammatory drugs or corticosteroid administration, and hereditary hemorrhagic telangiectasia.[165–170] In addition to these conditions, a significant amount of iron (210–240 mg/unit) can be lost with regular blood donation.[171]

Intracellular Metabolism of Iron

Acquisition of Iron via the Transferrin Receptor

Because most cellular iron acquisition occurs via Tf uptake, we will focus on the role of the TfR in maintaining intracellular iron homeostasis. The TfR is a 180-kDa glycoprotein composed of two identical 95-kDa subunits that are linked by two disulfide bridges (Cys-89 and Cys-98).[172] The human TfR gene has been localized to chromosome 3, region q26.2 qter.[173,174] The promoter region of this gene contains several metal response elements and appears to be transcriptionally (two- to threefold decrease)[175] and translationally[176] regulated by iron. Gene transcription is also negatively regulated by retinoic acid[177] and variably regulated by 1,25-dihydroxyvitamin D_3.

Each subunit of the TfR is composed of 760 amino acids.[178] Specific sequences in the intracellular domain composed of Tyr-Thr-Arg-Phe (YTRF) appear to be necessary for aggregation in clathrin-coated pits.[179] Serine 24 on the cytoplasmic domain of the TfR is phosphorylated by protein kinase C.[180] The functional consequences of this phosphorylation are unknown, but it is not required for internalization.[181]

The transmembrane region of the TfR consists of a single hydrophobic domain (24–28 amino acids) above position 65. The transmembrane segment of the human TfR functions as a signal peptide and is necessary for translocation to the cell surface.[182] This hydrophobic domain is also acylated in positions Cys-62 and possibly Cys-67.[172] This acylation is apparently not required for transport to the cell surface. The receptor is (N-linked) glycosylated on Asn-251, Asn-317, and Asn-727.[178] Glycosylation facilitates Tf–TfR binding through its effects on the tertiary and quaternary structure of the TfR. Each TfR subunit binds one Tf molecule with high affinity.[183] The affinity of the TfR for Tf increases with Tf iron-binding site occupancy. The affinity is highest for diferric Tf ($K_d = 1.1 \times 10^{-8}$ M) and lowest for apo-Tf ($K_d = 4.6 \times 10^{-6}$ M).[184] Because the concentration of Tf in plasma is $30–40 \times 10^{-6}$ M, cell surface TfRs are usually saturated with Tf. Therefore, the regulation of cellular iron uptake is mediated by altering the number of TfRs present on the cell surface. At any one time about a third of the cellular mass of TfRs is found on the cell surface. This number can be increased either by immediate translocation of cytoplasmic receptors to the surface or by de novo synthesis. The number of receptors present on the cell surface is a function of intracellular iron status, cell proliferative status, and metabolic need such as production of hemoglobin and myoglobin. Consequently, erythroblasts (1×10^5) and reticulocytes (8×10^5) have the highest number of TfRs per cell, as their iron needs are very high. As these cells mature into erythrocytes, they lose functioning TfR on their cell surface.[185]

Ferro-transferrin is taken up by TfR-mediated endocytosis. Some researchers have demonstrated that internalization of the TfR can occur with apo-Tf attached,[186] although others have shown that only ferro-Tf is internalized.[187] After internalization, the endosomal compartment containing the Tf–TfR complex sheds its clathrin coat. An endosomal proton pump (H^+-ATPase) then lowers the pH in the endosome to pH 5–6. This acidic milieu lowers the affinity of Tf for iron. Binding of chloride to an anion-binding site of TfR-bound Tf facilitates the removal of iron from Tf.[188] Additionally, part of the TfR also participates in this process.[189] Some investigators have described an endosomal enzyme that uses NADH to reduce Tf-derived ferric iron to the ferrous state.[190,191] Other investigators have suggested that ascorbate may nonenzymatically assist in this action.[192]

After iron is removed from Tf, the iron-containing portion of the endosome separates from the compartment containing the Tf–TfR complex. Iron in the endosomal compartment is transported across the membrane to the cytosol where it appears either to enter a pool of low molecular weight iron chelates[193] or attach to an intracellular iron-binding protein. Recent evidence suggests that the endosomal proton pump (H^+-ATPase) may participate in the transport of iron from the endosome to the cytosol.[194] This iron is then channeled into one of three pathways: iron-regulatory protein(s), iron-utilizing proteins, or storage iron.

The endosomal portion containing the TfR–apo-Tf complex travels to the Golgi apparatus, where it is packaged, along with newly synthesized receptors, and is translocated to the cell surface. The affinity of the TfR for apo-Tf at pH 7.4 is much lower than that at pH 5.5. Consequently, apo-Tf is released when the TfR–apo-Tf complex returns to the cell surface. The complete cycling of TfR–Tf occurs in about 10 minutes and can occur repeatedly about 100 times before either TfR or Tf is degraded. In sheep and rat reticulocytes the TfR receptor can be actively shed from the cell surface.[195,196] A truncated form of the TfR that lacks cytoplasmic and transmembrane regions is found in human plasma bound to Tf.[197,198] It is not known whether the human TfR fragment arises from alternate splicing of

the TfR gene or posttranslational cleavage. The fragment of TfR that circulates in plasma can be detected by enzyme-linked immunosorbent assay and is the basis of a new method for determining an individual's iron status.

Intracellular Low Molecular Weight Species

Several investigators have reported the presence of an intracellular pool of low molecular weight iron-containing species. The nature of this pool is largely speculative and suggestions of its composition range from citrate, nucleotide, pyrophosphate, amino acid, and/or protein chelates or complexes of iron.[199–201] The intracellular concentration of this pool is constant throughout conditions ranging from iron deficiency to iron overload.[200]

Intracellular Iron Homeostasis

Iron Response Elements

Intracellular iron homeostasis requires the coordinated regulation of the synthesis and action of proteins involved in iron acquisition, utilization, and storage. When intracellular iron is scarce the cell needs to increase its acquisition of iron either by mobilization of storage iron or acquisition of plasma iron. The cell also needs to prioritize its iron utilization so that life-sustaining, iron-containing proteins preferentially receive iron. In animals, much of this process is regulated at the genetic level by iron.[123,175,202–205] In addition to the ill-defined transcriptional regulation by iron of Tf, TfRs, and ferritin, iron participates directly in its own homeostasis. It binds to a *trans*-acting element(s) known as the iron responsive element binding protein(s) (IRE-BP[s]). During intracellular iron deficiency, an IRE-BP binds to *cis*-acting iron regulatory or responsive elements (IREs) located in either the 3′ or 5′ untranslated region of some mRNAs. These IREs are a family of stem loop structures,[206] which are highly conserved among species.[207] Their secondary and tertiary structures are important for high-affinity binding of the IRE-BP. Five IREs have been identified in the 3′ untranslated region of TfR mRNA.[176,208–210] A single IRE has been located in the 5′ untranslated region of the mRNAs of ferritin–erythroid D-aminolevulinate synthase (e-D-ALAS)[202] and a mitochondrial isoform of aconitase (m-aconitase),[211] which repress translation of these genes when an IRE-BP is bound.[212–214] An IRE has also been located in the amyloid precursor mRNA.[215,216] In contrast to e-D-ALAS, which contains a functional IRE, examination of the mRNA for housekeeping D-aminolevulinate synthase (h-D-ALAS) did not reveal an IRE.[202] Finally, although Tf synthesis may be regulated at the level of translation by iron,[123] no IRE has yet been identified in the mRNA for Tf. Thus, translational control of iron-containing proteins by the IRE–IRE-BP system is widespread in animals but may not be universal.

Iron Response Element-Binding Protein(s)

The IRE-BPs, also known as the ferritin repressor protein,[217] the iron regulatory factor,[218] or P-90,[219] show broad tissue distribution.[220] The gene for one IRE-BP has been localized to chromosome 9[221] and has been cloned from a number of species.[222] This IRE-BP has a molecular mass of 98 kDa, shows 95% homology between four different species, and shows considerable homology to m-aconitase (30%) and isopropylmalate isomerase.[223] This IRE-BP has been putatively identified as the cytosolic form of aconitase (c-aconitase).[224] Iron-replete c-aconitase is an enzyme that converts citrate to isocitrate. The regulation of translation of mRNA by this IRE-BP does not involve changes in the level of IRE-BP.[225] Disassembly of its iron–sulfur cluster (4Fe–4S) to (3Fe–4S) results in loss of aconitase activity and promotes high-affinity binding to mRNA.[226–228] However, simple reduction and removal of the iron bound in the fourth coordination site of c-aconitase is insufficient to produce the high-affinity RNA binding properties found in the native IRE-BP. It appears that endogenously produced nitric oxide serves to promote disassembly of the iron–sulfur cluster of IRE-BP and enhances the high-affinity binding of IRE-BP to mRNA.[229,230] The IRE-BP is also phosphorylated by protein kinase C, which enhances the high affinity binding of IRE-BP to mRNA.[231] Although the hypothesis of 3Fe/4Fe switching is attractive with regard to signaling IRE-BP binding to mRNA, other mechanisms also seem likely; further study of the binding region of IRE with IRE-BP is needed.

A second IRE-BP has recently been identified that differs from the above IRE-BP in size (105 kDa) and tissue distribution (primarily brain and intestine).[232] The location of the IREs and their binding proteins in tissues and cellular organelles will help clarify the regulatory system within and between organ systems.

Chemical Properties and Biochemical Functions of Iron

Introduction

Iron is a *d*-block transition element, which can exist in oxidation states ranging from −2 to +6. In biologic systems, these oxidation states are primarily limited to the ferrous (+2), ferric (+3), and ferryl (+4) states. The interconversion of iron oxidation states is not only a mechanism whereby iron partic-

ipates in electron transfer but also a mechanism whereby iron can reversibly bind ligands. Iron can bind to many ligands by virtue of its unoccupied *d* orbitals. The preferred biologic ligands for iron are oxygen, nitrogen, or sulfur atoms. The electronic spin state and biologic redox potential (from +1000 mV for some heme proteins to −550 mV for some bacterial ferredoxins) of iron can change according to the ligand to which it is bound. By exploiting the oxidation state, redox potential, and electron spin state of iron, nature can precisely adjust the chemical reactivity of iron. Thus, iron is particularly suited to participate in a large number of useful biochemical reactions.[233,234] The activity of many of the enzymes involved in these biochemical reactions decreases during tissue iron deficiency. Only rarely, however, have direct connections between biochemical events and clinical manifestations been firmly established.

Four major classes of iron-containing proteins carry out these reactions in the mammalian system: iron-containing proteins (Hb and myoglobin), iron–sulfur enzymes, heme proteins, and iron-containing enzymes (noniron sulfur and nonheme enzymes). In iron–sulfur enzymes, iron can be bound to sulfur in four possible arrangements (FeS, 2Fe–2S, 4Fe–4S, 3Fe–4S). In humans only three of these occur. In heme proteins, iron is bound to various forms of heme that differ not only in the composition of their side chains but also in the methods whereby they are attached to proteins. In humans, however, the predominant form of heme is protoporphyrin IX (PP-IX).

Oxygen Transport and Storage

The movement of oxygen from the environment to terminal oxidases is one of the key functions of iron. Dioxygen is bound to porphyrin ring iron containing molecules either as part of the prosthetic group of Hb within red blood cells or as the facilitator of oxygen diffusion in tissue—myoglobin. Hemoglobin is a tetrameric protein with two pairs of identical subunits (α2, β2, MW 64 kDa) with either 141 or 142 amino acids in the a chain and 146 in the b chain. Each subunit has one prosthetic group, Fe–PP-IX, whose ferrous iron reversibly binds dioxygen. The four subunits are not covalently attached to each other but do react cooperatively with dioxygen with specific modulation by pH, pCO_2, organic phosphates, and temperature. These modulators determine the efficiency of oxygen transport from the alveoli capillary interface in the lung to the red cell → capillary → tissue interface in peripheral tissues. The allosteric effect of decreasing pH—the Bohr effect—decreases binding affinity of heme–Fe for dioxygen via protonation of His-146 on β chains and Val-1 on α chains in the presence of Cl^- and CO_2. CO_2 forms a Schiff base with the terminal amino acids of each chain and decreases dioxygen affinity. This favors the unloading of oxygen in tissues where the pH is lower and pCO_2 is higher than in arterial blood. 2,3-Diphosphoglycerate is a product of a side pathway within erythrocytes and binds to a specific region of the β chain to decrease Hb–O_2-binding affinity. This right shift of the dissociation curve is evident in times of greater need for oxygen delivery such as in anemia, when the blood content of Hb is significantly reduced and increased cardiac output is only partially compensatory.

Myoglobin is the single chain hemoprotein of 17 kDa MW in cytoplasm. Its role is to increase the diffusion rate of dioxygen from capillary red blood cells to cytoplasm and mitochondria. The concentration of muscle myoglobin is drastically reduced in tissue iron deficiency, thus limiting the rate of diffusion of dioxygen from erythrocytes to mitochondria.[235]

Electron Transport

The cytochromes contain heme as the active site with the Fe-porphyrin ring functioning to reduce ferric iron to ferrous iron with the acceptance of electrons. The iron–sulfur proteins also act as electron carriers via the action of iron bound to either two or four sulfur atoms and cysteine side chains. The 40 different proteins comprising the respiratory chain contain 6 different heme proteins, 6 iron–sulfur centers, 2 copper centers, as well as ubiquinone to connect NADH to oxygen. Several hundred enzyme activities have also been ascribed to the cytochrome P450 family of enzymes. Some demonstrate limited substrate specificity, but most exhibit broad substrate specificity. They have been termed the mixed function oxidases. At least 39 rat and 28 human cytochrome P450 genes have been identified so far and more are likely to be discovered.[236] The microsomal P450 enzyme system participates in the biosynthesis of steroid hormones such as pregnenolone, corticosterone, aldosterone, and 1,25-OH-vitamin D_3. The microsomal P450 enzyme system participates in the metabolism of xenobiotics such as drugs and aromatic hydrocarbons. This enzyme system includes cholesterol 7α-monooxygenase, the rate-limiting step in bile acid synthesis. This enzyme system is also responsible for the formation of prostacyclin, thromboxane, and leukotrienes.

With the exception of the glutathione peroxidases, all mammalian peroxidases contain iron. Catalase degrades hydrogen peroxide formed as a byproduct of some oxidase reactions. Myeloperoxidase forms hypochlorite anion, which is an impor-

tant cytotoxic molecule produced by neutrophils. Thyroperoxidase is responsible not only for the organification of iodide but also for the conjugation of iodinated tyrosine residues on thyroglobulin. Nitric oxide ($NO^{\bullet}$) is a potent biologic effector molecule. Its synthase is a cytochrome P450-like protein that occurs in at least four isoforms. At least two of these forms, NO synthase I and II, contain iron in the form of PP-IX.[237,238] They may also contain a catalytically active nonheme iron.[239]

Physiologic Functions of Iron and Signs of Iron Deficiency

The overt physical manifestations of iron deficiency are glossitis, angular stomatitis, koilonychia (spoon nails), blue sclera, esophageal webbing (Plummer–Vinson syndrome), and anemia. Behavioral disturbances such as pica (characterized by abnormal consumption of nonfood items such as dirt [geophagia] and ice [pagophagia]) are often present in iron deficiency. The physiologic manifestations of iron deficiency have also been noted in immune function, cognitive performance and behavior, thermoregulatory performance, energy metabolism, and exercise or work performance.[235,240] Many of these manifestations of iron deficiency are not mutually exclusive events and do not occur independently of each other. Furthermore, many of these manifestations occur only during certain stages of iron deficiency.

The progression of iron deficiency occurs in two steps related to depletion of iron stores prior to depletion of functional iron: (1) bone marrow, spleen, and liver stores depletion; and (2) diminished erythropoiesis due to a negative iron balance leading to anemia and decreased activity of iron-dependent enzymes. Clinical iron deficiency is frequently diagnosed by virtue of anemia secondary to long-term diminished erythropoiesis. With a few exceptions, depletion of the storage iron pool is generally without influence on physiologic function.[241,242] In those studies, correlations were noted between electroencephalogram asymmetry (a central nervous system abnormality) and plasma ferritin within the iron-adequate range. Nonetheless, nearly all functional consequences are more strongly related to "anemia" rather than to tissue iron deficits. The challenge of separating O_2 transport events from tissue iron deficits still looms large. Good examples are the decreases in muscle myoglobin content, cytochrome oxidase activity, and electron transport.

Several well-known consequences of iron deficiency occur after the depletion of iron stores—decline in Hb concentration, decrease in mean corpuscular Hb concentration, a decrease in the size and volume of the new red cells, reduced myoglobin, and reduced amounts of both Fe–S and heme iron-containing cytochromes within cells. Diffusion of dioxygen from Hb into tissue becomes limited in this situation due to fewer erythrocytes packed close together in capillaries, increased membrane diffusivity, and a decreased tissue myoglobin concentration. The heterogeneity of distribution of mitochondria around and adjacent to capillary walls is well known but not well studied in the iron-deficient human or animal model. The delivery of red cells to tissue is under a complex regulation by both systemic and local regulatory features. The reader is urged to consider that the matching of oxygen delivery to tissue needs for oxygen is the ultimate goal of these regulators. In severe anemia, oxygen transport is clearly limiting to tissue oxidative function at anything but the resting condition.[235] This is despite a significant right shifting of the Hb–O_2 dissociation curve (decreased affinity) and an increase in cardiac output in an attempt to increase TaO_2. Tissue extraction of oxygen is increased by this compensation and mixed venous PO_2 is significantly lower in anemic individuals. While Hb–O_2 affinity compensation is reasonable at sea level, the opposite direction of compensation occurs in anemic people at high altitudes (4000 m). The Hb–O_2 dissociation curve is "left-shifted" in these hypobaric hypoxic conditions to increase O_2 loading in the lung at the expense of tissue delivery.[243] The very significant decrease in myoglobin and other iron-containing proteins in skeletal muscle seen in iron-deficiency anemia contributes significantly to the decline in muscle aerobic capacity.[235]

A recent study[244] used ^{31}P nuclear magnetic resonance spectroscopy to examine the functional state of bioenergetics in iron-deficient and iron-replete rat gastrocnemius muscle at rest and during 10 seconds of contraction at 2 Hz. Iron-deficient animals had a clear increase in phosphocreatine (PCr) breakdown and a decrease in pH compared to controls. They also had a slower recovery of PCr and inorganic phosphate concentrations after exercise. Repletion for 2–7 days with iron dextran showed no substantial improvement in these indicators of muscle mitochondrial energetics. These authors concluded that "tissue factors" such as decreased mitochondrial enzyme activity, decreased number of mitochondria, and altered morphology of the mitochondria might be responsible for these observations. It is not uncommon for phosphate : oxygen ratios to be normal in iron deficiency despite very significant alterations in activity of iron-containing respiratory chain enzymes.

A more typical repair curve for muscle iron-containing and oxidative enzymes during iron repletion experiments has been described.[245] Pyruvate and malate oxidase were decreased to 35% of normal in iron-deficient muscle and improved to 85%

Table 1. Diagnostic Criteria for Iron Deficiency (Adapted from Herbert[246] and Ferguson et al.[247])

	Iron Overload	Normal	Iron Depletion	Iron-Deficient Erythropoiesis	Iron-Deficiency Anemia
Erythrocyte morphology	Normal	Normal	Normal	Normal	Microcytic/ hypochromic
Hemoglobin (g/L^{-1})					<120 (female) <135 (male)
Hematocrit					<0.35 (female) <0.40 (male)
Plasma iron ($\mu g/dL^{-1}$)	>175	115 ± 50	115	<60	<40
RBC protoporphyrin ($\mu g/dL^{-1}$)	30	30	30	100	200
RE marrow Fe	4+	2–3+	0–1+	0	0
Sideroblasts (%)	40–60	40–60	40–60	<10	<10
TIBC ($\mu g/dL^{-1}$)	<300	300 ± 30	360	390	410
Transferrin saturation (%)	>60	35 ± 15	30	<15	<15
Plasma ferritin ($\mu g/dL^{-1}$)	>300	100 ± 60	20	10	<10
TfR relative amount		1	1.5	3	3–4
TfR ($mg\ dL^{-1}$)[a]		5.36 ± 0.82			13.9 ± 4.6

[a] This will vary depending on the antibody used.

of normal in 10 days of treatment. 2-Oxoglutarate oxidase was decreased to 47% of normal and improved to 90%; in contrast, succinate oxidase was only 10% of normal in iron deficiency and improved to only 42% of normal after 10 days. Cytoplasmic enzymes hexokinase and lactate dehydrogenase were unaffected by iron status. The 50–90% decrease in both the Fe–S enzymes and in the heme-containing mitochondrial cytochromes is consistent with many other observations over the last two decades.[235] What seems to determine the amount of decline in activity with iron deprivation is the rate of turnover of that particular iron-containing protein in the time of cellular deprivation of iron.

The last stage of iron deficiency occurs when stores are depleted and there is no longer sufficient iron to meet daily requirements. This stage of iron-deficient erythropoiesis leads to a significant compromise in cellular function in many organs.[235] The rate at which individual tissues and cellular organelles within those tissues develop a true iron "deficit" is dependent on the rate of turnover of iron-containing proteins and rate of cell growth, as well as the intracellular mechanisms for recycling iron.[235]

The reader is encouraged to look at recent reviews that describe a number of functional consequences of iron deficiency.[235,240]

Assessment of Iron Status

The iron status of humans can range from iron overload to iron-deficiency anemia. Historically, many different methods have been used to assess the iron status of an individual including dietary intake, hematocrit, Hb, mean cellular Hb, mean cell volume, erythrocyte mean index, free erythrocyte protoporphyrin, bone marrow iron stain, serum iron, total iron-binding capacity, serum Tf, Tf saturation, serum ferritin, and serum TfR. These methods vary considerably in their sensitivity and selectivity. The more commonly accepted diagnostic tests and their associated values are summarized in Table 1.[246,247]

Hemoglobin/Hematocrit

Several reviews of iron metabolism in the last decade[235,248,249] have noted that the term "iron deficiency" has no universal interpretation. Clinical sequelae are most frequently recognized at the end stages of the iron depletion process when body iron stores have been depleted. For clinicians, the prevalence of iron deficiency is equated with the prevalence of iron-deficiency anemia. The use of anemia as a clinical indicator may be due either to the ease of assessment in measuring Hb concentration[248] or to the assumption that iron deficiency exerts its deleterious effects only if anemia is present.[249] The use of Hb and hematocrit as indices of iron status must be used carefully, as significant false-positive indications occur.[250] Although a single replicate has been determined to accurately predict iron status, adjustments must be made for significant variations due to age, gender, and race in clinical assessment of iron status of individuals or populations. Hemoglobin concentrations are also altered in/by polycythemia, dehydration, cigarette smoking, chronic inflammation, chronic infection, hemorrhage, protein-energy malnutrition, vitamin B_{12} deficiency, folic acid deficiency, hemoglobinopathies, and pregnancy.[251] Thus, considerable information about nu-

trition and health status is needed in addition to Hb determination if one is to use Hb to assess iron status.

Ferritin

A long-term negative iron balance eventually leads to the depletion of the storage iron pool, leading to dramatic declines in plasma ferritin concentrations. The most realistic tool to date in a nonclinical setting for assessment of the size of the storage pool is the measurement of serum or plasma ferritin concentrations. The concentration of serum ferritin reflects the size of the storage iron compartment if the subject is not also in an inflammatory state.[252] Serum ferritin values usually fall in the range of 20–300 mg/L, with each mg/L representing 10 mg of storage iron.[145] Plasma ferritin concentration can increase dramatically with both acute and chronic inflammations, vitamin B_{12} deficiency, folic acid deficiency, liver disease, leukemia, Hodgkin's Disease, alcohol intake, and hyperthyroidism.[117,253–255] In addition, it is now known that there is a large within-subject day-to-day coefficient of variation (25–40%) in plasma ferritin concentrations.[250]

Transferrin Saturation

Once the storage iron pool is depleted due to a prolonged or acute negative iron balance, there is a decline in the Tf saturation. Consequently, less than adequate iron is available for essential body iron proteins.[256,257] People in this stage of iron depletion have a Tf saturation below 15–16% and an inadequate supply of iron for bone marrow to support normal erythropoiesis.[257–259] The measure of erythropoiesis is clearly an important aspect in this iron delivery scheme, as decreased erythropoiesis can lower iron transport requirements by 50–80%.

Transferrin Receptor

The measurement of TfR concentration in the plasma has diagnostic value for the assessment of iron deficiency anemia and ineffective erythropoiesis.[260–262] The amount of TfR in circulation has been shown to vary with individual iron status. Plasma TfR concentrations increase even in mild iron deficiency of recent onset.[263,264] The plasma concentration of TfR is increased in β-thalassemia, autoimmune hemolytic anemia, sickle cell anemia, hereditary spherosis, Hb H disease, polycythemia vera, secondary polycythemia, myelofibrosis, and chronic lymphocytic leukemia.[260–262] The plasma TfR concentration is decreased in hemochromatosis, aplastic anemia–bone marrow ablation, posttransplantation anemia, and chronic renal failure.[247,260–262] Unlike ferritin, levels of plasma TfR are not significantly affected by inflammation[264,265] or by liver disease.[247] Unlike most other methods of assessment, TfR concentration can be used to distinguish between iron-deficiency anemia and other anemias, including the anemia of chronic disease.[247] Hence, the TfR is particularly useful in assessing iron status.

Populations at Risk for Iron Deficiency

Despite the effectiveness of intervention therapies, iron deficiency is the primary nutrition deficiency in the United States.[266] One to six percent of the U.S. population has impaired iron status, including 3.5–12.1% of males 11–14 years of age and 2.5–14.2% of females 15–44 years of age.[267] Approximately 6–11% of reproductive age females, 14% of 15–19-year-old females, and 25% of pregnant women were iron deficient in the United States and Canada in the 1980s.[267,268] Fortification of the food supply in combination with additional intakes of iron from supplements, as well as changes in dietary patterns have effectively eradicated iron deficiency from nearly all segments of the U.S. population. The exceptions are pregnant women and a small proportion of young children, adolescents, and reproductive age women. However, special clinical populations, some elderly, and perhaps athletes may still require targeted intervention.

International statistics demonstrate the immensity of the problem,[269,270] with 15% of the world's population having iron-deficiency anemia. The World Health Organization estimates that 1.3 billion people are anemic, and that nearly half (500–600 million) have iron deficiency as the causal agent. For populations at risk the prevalence of iron deficiency can approach 50%. As noted in the various sections of this review, iron requirements are determined by growth and maintenance requirements.[271] There are additional requirements associated with clinical and pathological conditions leading to increased blood loss. Iron requirements in infancy, childhood, adolescence, and during pregnancy are covered in detail elsewhere.[272,273] The increased dietary requirements during these periods of accelerated growth amount to 1 mg/kg/day in infants, 10 mg/day in children, up to 15 mg/day in female adolescents, and an additional 5–6 mg/day above the Recommended Dietary Allowance during the second and third trimester of pregnancy. The rapid expansion of the maternal blood volume, placental growth, and fetal growth place tremendous demands on maternal stores of iron. It is not uncommon for >70% of women to emerge from pregnancy with iron-deficiency anemia. Menstrual blood losses during reproductive years elevate iron requirements in female on average 5 mg/day above that of males.

The requirements for adult male and postmenopausal women is 10 mg/day.

Public health nutritionists are more interested in how much of the population is at risk of iron depletion and are thus more concerned with iron status adequacy and prevalence of depleted iron stores. Iron deficiency can be defined as that moment in time when body stores of iron—ferritin and hemosiderin—become depleted of iron and a restriction of supply of iron to various tissues becomes apparent.[117] Conceptually, the process of depletion of iron stores can occur rapidly or very slowly and is dependent on the balance between iron intake and iron requirements.

Clearly, iron intake depends on the quantity of iron in the diet in addition to the presence of a number of inhibitors and a smaller number of enhancers of iron absorption in food.[249] Iron requirements depend on body needs for tissue growth and tissue maintenance, which vary with the life cycle and certain environmental factors. The systematically greater prevalence of iron deficiency in women than men is likely due to a shift in the iron balance equation and not due to "femaleness or maleness." The gradual increase in iron status in females after menopause regardless of estrogen replacement supports this concept. Requirements for iron in pregnancy are increased dramatically to approximately 4 mg/day above normal and perhaps even higher if the pregnancy occurs in adolescents.[273,274] The requirements for iron are of course, higher in the growing child and give rise to the concept of "critical periods" during both prenatal and postnatal growth.[275] That is, there exists a particular period of time during early development when the brain and other specific organs are especially susceptible to a nutrient deficiency state. The restoration of normal dietary iron intake at later times may not restore normal function or tissue content of these organs and "catch-up growth" may not occur.

A number of clinical and pathologic conditions associated with blood loss can cause iron deficiency. In addition, treatments that actively increase iron requirements, such as erythropoietin therapy, can lead to iron deficiency.[276,277]

Conclusions and Future Research Perspectives

This review is not a comprehensive examination of all aspects of iron nutrition in health and disease. Rather, our intent is to review the current knowledge in key aspects of the field of iron nutrition and biology and to, hopefully, stimulate new intellectual efforts by scholars to better understand the role of iron in human biology. Scientific investigations spanning several decades have increased our understanding of the role of this mineral in many aspects of metabolism, but it is clear that many questions remain. For example, what are all of the components of the iron absorption pathway? What is the somatic regulator of iron absorption? What is the nature of the low molecular weight intra-and extracellular pools of iron? What are the full consequences of iron deficiency, particularly as related to cognitive function, and are they reversible? How does hemochromatosis arise? Finally, what role does iron, as found in the body under normal nutrition states, play in oxidative stress? Hopefully, scientists in the next decade will unravel some of these mysteries.

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