Iron Metabolism in Man

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Abstract

Iron metabolism in man is a highly regulated process designed to provide iron for erythropoiesis, mitochondrial energy production, electron transport, and cell proliferation. The mechanisms of iron handling also protect cells from the deleterious effects of free iron, which can produce oxidative damage of membranes, proteins, and lipids. Over the past decade, several important molecules involved in iron homeostasis have been discovered, and their function has expanded our understanding of iron trafficking under normal and pathological conditions. Physiologic iron metabolism is strongly influenced by inflammation, which clinically leads to anemia. Although hepcidin, a small circulating peptide produced by the liver, has been found to be the key regulator of iron trafficking, molecular pathways of iron sensing that control iron metabolism and hepcidin production are still incompletely understood. With this review, we provide an overview of the current understanding of iron metabolism, the recently discovered regulators of iron trafficking, and a focus on the effects of inflammation on the process. (JPEN J Parenter Enteral Nutr. 2013;37:599-606)

Keywords

iron absorption; iron uptake; hepcidin; inflammation; hepcidin gene regulation

Introduction

Iron is the most abundant element on earth, comprising approximately 32% of the earth’s mass. Iron is a critical element, participating in many cellular functions in mammals. For nutritionists, perhaps the best known role for iron is as a major constituent of hemoglobin (Hb) since Hb is the critical protein involved in oxygen delivery to tissues. But iron also plays an important role in other cellular functions, including DNA synthesis through the reduction of ribonucleotides to their corresponding deoxyribonucleotides, the cytochrome system involved in mitochondrial electron transport, and muscle function as part of the major muscle protein, myoglobin, in addition to myocyte mitochondrial function.

However, although iron is vital for so many metabolic reactions, free iron is toxic to tissue, damaging cellular membranes, proteins, and DNA through the Fenton reaction. Free iron will interact with hydrogen peroxide (H$_2$O$_2$) to form the highly reactive hydroxyl free radical (•OH). Moreover, catalytic amounts of iron facilitate the production of molecular oxygen, hydroxyl radical, and hydroxyl anion from superoxide radicals and hydrogen peroxide (Haber-Weiss reaction). These reactions are summarized below.

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\begin{align*}
(1.1) \quad Fe^{2+} + H_2O_2 &\rightarrow Fe^{3+} + •OH + OH^- \\
(1.2) \quad Fe^{3+} + O_2^- &\rightarrow Fe^{2+} + O_2 \\
(1.3) \quad O_2^- + H_2O_2 &\rightarrow O_2 + •OH + OH^- 
\end{align*}
\]

Because of the highly reactive and potentially damaging properties of free iron, the body has developed elaborate mechanisms to control both the intra- and extracellular trafficking of iron, which is bound to proteins such that only an extremely small amount of iron is found free within cells or in the extracellular fluid. In iron-loading conditions, including the primary iron-loading disorders (such as hereditary hemochromatosis), the iron-loading anemias (such as thalassemia major), and transfusional iron overload, increased intracellular free iron can eventually lead to parenchymal organ damage.

This review discusses normal iron homeostasis in man and how normal homeostasis is disturbed in one of the most common problems encountered clinically—inflammation. Particular attention is given to hepcidin, a relatively recently discovered
small molecule that appears to be a key regulator of iron trafficking.6

Normal Iron Balance

There is no regulated mechanism of iron excretion in mammals.7 Consequently, iron balance is maintained almost exclusively at the point of iron absorption. The daily iron needs of an adult approximate 1 mg in men and 1.5–2.0 mg in women who are in their childbearing years.8 In steady state, this amount of iron replaces iron lost from the body through the desquamation of epithelial cells from the gut, skin, and genitourinary tract.7

The amount of iron in a normal adult is 3–4 g and distributed in several major pools, as shown in Figure 1. These iron pools include the plasma transferrin pool (3 mg), the tissue (predominantly the liver and, to a lesser extent, the reticuloendothelial [RE]) storage pool (approximately 200–300 mg in adult women and 1 g in adult men), and the myoglobin pool (300 mg).8 The pools that are actively turning over include the transferrin-bound iron pool and the RE storage pool. Transferrin-bound iron is essentially the sole supplier of iron to developing erythroblasts in the bone marrow.

Red blood cells have a life span of 120 days; thus, the body must replace 0.8%–1.0% of its red cells each day. In an average-size adult, the red cell mass is between 1500 and 2000 mL. This translates into a need to produce 15–20 mL of packed red cells each day. Since each milliliter of packed red cells contains 1 mg of elemental iron, 15–20 mg of iron needs to be delivered to the erythroid marrow to support normal red cell production; even more iron is required under conditions of erythropoietin stimulation, such as blood loss or increased red cell destruction (hemolysis).7 This means that the transferrin-bound iron pool turns over 5–7 times per day, delivering the iron needed to support new red cell formation. As the body only absorbs 1–2 mg of iron each day under normal circumstances, most of the iron needed for new red cell production comes from iron stores, and most of the iron used for new red cell production comes from iron that is efficiently reused from the breakdown of senescent red cells by resident macrophages. Because the transferrin iron pool turns over 5–7 times every 24 hours, anything that interferes with the release of iron from RE stores or iron absorption from the gut leads to a rapid fall in circulating iron levels (see below).

Mechanisms of Iron Absorption

Iron is complexed in the food we eat, and the nature of the foodstuff determines iron bioavailability. In most cases, the iron in food has to be dissociated and made soluble in order for the iron to be absorbed. The normally low pH of the stomach serves this function, releasing iron and maintaining it in the ferric (Fe³⁺) state.7,8 Once released, the free iron comes into contact with absorptive cells of the proximal small intestine. It is here that most iron absorption takes place. A number of common clinical conditions or medical interventions are known to interfere with acid production or with iron absorption. Examples are gastric atrophy, chronic antacid use, proton pump inhibitors, age, gastric bypass surgery, and Helicobacter pylori infection.

At the absorptive surface, the ferric iron encounters a reductase, at least one of which is DcytB (duodenal cytochrome B), which converts ferric iron to the ferrous (Fe²⁺) form (Figure 2). It is ferrous iron that is then taken into the cell via DMT-1 (divalent metal-ion transporter 1, also known as Nramp2 [natural resistance-associated macrophage protein family 2] or SLC11A2 [solute carrier family 11, member 2]).9,10 In contrast, heme iron is absorbed via a different pathway, which has not yet been elucidated. This is notable since heme iron is much more efficiently absorbed (by a factor of 5–10) than elemental iron.8

How iron is handled once it is inside the cell is not completely understood, but in general, there are two paths iron can follow. One path is to be complexed with apoferitin and stored within the cell as ferritin. The second path is to be transported to the basolateral membrane of the absorptive cell and be exported from the cell via ferroportin, the only known iron export protein in mammalian cells (Figure 2). One reason that the proximal jejunum is the major site of iron absorption is that ferroportin-rich cells are found in greatest abundance in the
duodenum and proximal jejunum, falling off with increasing distance from the pylorus.\(^{11}\)

Important new findings implicate the absorptive cell in the regulation of iron uptake, independent of long-range regulators, such as hepcidin\(^ {12,13}\) (see below). Levels of key proteins in the absorptive enterocyte are influenced by oxygen tension in the cell, which, in turn, affects the transcription factor hypoxia-inducible factor 2α (HIF-2α).\(^ {14}\) This leads to subsequent changes in transcription of DMT1 and ferroportin. In addition, the content of iron within the enterocyte regulates iron absorption through its effects on iron regulatory proteins (IRP) types 1 and 2 and their subsequent effect on messenger (m) RNAs encoding DMT1, ferroportin, ferritin, and HIF-2α.\(^ {15}\) The IRPs bind to sequences (iron-responsive elements [IREs]) that influence mRNA translation (with respect to ferroportin, ferritin, and HIF-2α) or stability (with respect to transferrin receptor 1 [TfR1] and DMT1).\(^ {16}\) Consequently, in the face of hypoxia or cellular iron deficiency, DMT1 and ferroportin are upregulated in a manner that promotes iron absorption from the diet.

Iron being transferred from the absorptive cell to the circulation must undergo another change in valence state. This is affected by hephaestin (ferroxidase), a multicopper oxidase homologous to ceruloplasmin. Hephaestin converts Fe\(^ {2+}\) to Fe\(^ {3+}\), the form required for binding to transferrin.\(^ {7}\)

**Iron Transport and Cellular Iron Uptake**

Transferrin is the major iron transport protein in the body. Transferrin-bound iron circulates to the bone marrow, where virtually all of it is taken up by developing erythroid cells via TfR1. The iron-TfR1 complex enters the erythroid cell via clathrin-coated pits and is taken up into endosomes\(^ {7}\) (Figure 3). Although all cells require iron at some time in their development, erythroid cells have the highest concentration of TfR1, with several hundred thousand receptors per cell, depending on the stage of maturation of the cell.

The uptake of transferrin-bound iron by the TfR1 depends on the iron content of transferrin. Transferrin is a bilobed protein, capable of binding 2 iron atoms. Fully loaded transferrin is referred to as holo- or diferric transferrin. Transferrin without iron is apotransferrin. The affinity of diferric transferrin for TfR1 is several-fold greater than that for monoferric transferrin and a thousand-fold greater that of apotransferrin.\(^ {17}\)

For iron to be released within the immature erythroid cell, an active proton pump is required to lower the pH of the endosome. The iron is then exported from the endosome via DMT1 into the cytosol after another change in valence from the ferric to the ferrous form facilitated by Steap3, an endosomal ferrireductase.\(^ {10}\) Once in the cytosol, virtually all of the iron is transported to the mitochondria, where it is incorporated into heme and, eventually, hemoglobin. Following release from the marrow, newly formed red cells remain for 120 days in the circulation before being recognized as senescent and removed by the reticuloendothelial system (RES).

Transferrin and TfR1 protein are transported back to the cell surface and effectively recycled to function again (Figure 3). Part of the TfR1 protein is proteolytically cleaved or released and circulates in the plasma as soluble TfR1 protein. The concentration of TfR1 protein in circulation reflects the iron status of the patient as well as total erythropoietic activity.\(^ {18,19}\)

**Regulation of TfR1 Gene Expression**

TfR gene expression is affected by iron status.\(^ {20}\) The TfR1 gene has IREs in the 3′-untranslated region that regulate gene expression. In the presence of adequate iron, IRPs interact with iron and are not available to bind to the IREs. This renders the poly-A tail of the mRNA susceptible to proteolytic cleavage. In the absence of iron, the IRPs can interact with the IREs to stabilize the poly-A tail, which prolongs the half-life (and therefore the function) of the mRNA. This leads to
increased expression of TfR1 on the surface of erythroid cells, and that, in turn, results in elevated circulating levels of TfR1. Thus, soluble TfR1 protein serves as a specific marker for iron deficiency. However, in the iron-replete state, the level of soluble TfR1 is also useful to estimate the size of the erythroid marrow.

**Iron Recycling**

When the red cell reaches the end of its life span, it is recognized as senescent by resident macrophages through poorly understood signals. The senescent red cell is taken up mostly by macrophages in the spleen and liver. Within the macrophage, the globin and heme are broken down and their components returned to the amino acid pool, while the iron is rapidly transported to the cell membrane, where it is released via ferroportin to be bound to transferrin. This process of “iron recycling” is extremely efficient, and under normal conditions, 80%–90% of the iron is reused for Hb synthesis. The remaining 10%–20% of the iron recycled from senescent red cells is stored in the macrophage as ferritin.

Because of the large amount of iron required to support the normal daily turnover of red cells, anything that interferes with iron availability can rapidly result in a decrease in the serum iron (see below).

**Hepcidin—Key Regulator of Iron Trafficking**

Although hepcidin had been studied originally as an antimicrobial peptide, the profound effect of hepcidin on iron metabolism was not appreciated until 2000. At that time, quite by accident, investigators studying gluconeogenesis silenced the gene for hepcidin in the mouse. The result was an unexpected phenotype of parenchymal (but not reticuloendothelial) iron overload. Rather than iron accumulating in the spleen (macrophages) of the mouse as it aged, iron loading occurred in the pancreas and liver—the same organs affected in patients with hereditary hemochromatosis. It was quickly recognized that hepcidin was necessary to control iron uptake from the diet as well as iron release from storage sites. In the absence of hepcidin, iron continued to be absorbed and released from stores, mimicking the human condition of hereditary hemochromatosis. Mice genetically engineered to overexpress hepcidin in utero were born with severe iron deficiency, implicating hepcidin in the transport of iron across the placenta. Subsequent work has clarified, at the molecular level, the role of hepcidin in controlling iron movement.
The mechanism by which hepcidin controls iron uptake and release was elucidated by Nemeth et al. Hepcidin regulates the cell surface expression of ferroportin, which is expressed on the surface of cells involved in iron handling—most specifically, iron-absorbing enterocytes and cells of the RES. Hepcidin binds to ferroportin on the cell surface, and the complex is internalized and degraded. Thus, ferroportin serves as the receptor for hepcidin. When hepcidin levels are elevated, ferroportin expression on the cell surface is reduced and iron is retained within the absorptive and RE cells. This interaction effectively shuts down iron absorption from the gut and also results in the trapping of iron in cells of the RES. The net effect is a fall in the serum iron, which can be rapid and profound. In animal models, the administration of a single dose of hepcidin causes a very rapid drop in the serum iron, which is maintained for up to 2–3 days (Figure 4). If this condition persists, the patient or animal will develop anemia due to the restrictions placed on Hb synthesis (“iron-restricted erythropoiesis”).

Hepcidin is produced primarily in the liver but also by resident macrophages. Hepcidin is a small molecule—25 amino acids—and tightly folded with 4 internal disulfide bonds. Regulation of hepcidin gene expression is complex, involving inflammatory signals, signals related to iron balance in the body, signals related to erythroid activity, and the state of tissue oxygen availability.

Iron Balance Signals
Iron availability has been recognized for decades to affect iron absorption and trafficking. With iron deficiency, iron absorption increases, as do circulating levels of transferrin and soluble TfR1 protein. Some of the iron-dependent alterations in iron absorption are now known to be regulated at the level of the enterocyte itself (and see above), and hepcidin is largely, but not solely, responsible for these changes.

Although a number of molecules are involved in regulating hepcidin gene expression, how these molecules interact and the cross-talk between receptor-associated pathways has only recently come into sharper focus (Figure 5). Key is signaling through the bone morphogenetic protein receptor (BMPR) pathway. Known or potential contributors to BMPR signaling include TfR1 and -2, the hemochromatosis protein (HFE), hemojuvelin (HJV), neogenin, and matriptase-2 (also known as transmembrane protease, serine 6 [TMPRSS-6]). The interaction between these molecules orchestrates hepcidin gene expression.

HFE has been suggested to act as a bimodal switch between the 2 sensors of the concentration of diferric transferrin (Tf-Fe3⁺), TfR1 and TfR2, on the surface of hepatocytes. This model is supported by the following findings: HFE binds the ubiquitously expressed TfR1 at a site that overlaps the transferrin binding domain, and Tf-Fe3⁺ thus competes with HFE binding to TfR1. By contrast, TfR2 can bind both Hfe and Tf-Fe⁺ simultaneously. Mice bearing an engineered TfR1 mutation with increased HFE binding show low hepcidin expression and systemic iron overload similar to HFE-deficient mice, suggesting that TfR1 sequesters HFE to prevent its participation in hepcidin activation. Conversely, mutations that abolish the HFE-TfR1 interaction or mice with increased HFE levels display elevated hepcidin expression and succumb to iron deficiency. Full hepcidin activation by diferric transferrin requires both HFE and TfR2. These observations support a model in which high concentrations of Tf-Fe3⁺ displace HFE from TfR1 to promote its interaction with TfR2, which is further stabilized by increased Tf-Fe⁺ binding to the lower affinity TfR2. The HFE-TfR2 complex then activates hepcidin gene transcription.

Although HFE and TfR2 clearly contribute to hepcidin activation, the BMP signaling pathway is quantitatively the most critical. BMP signaling integrates signals from the “Tf-Fe3⁺ sensing complex” and hepatocyte iron stores. Central to the latter is bone morphogenetic protein 6 (BMP6), which is positively regulated by iron. How BMP6 mRNA expression is activated by increased iron levels and repressed by iron deficiency is unknown. BMP6 knockout mice show hepcidin deficiency and tissue iron overload, although BMP2 and BMP4 can also bind to HJV. HJV is a BMP coreceptor that adapts BMP receptors for iron regulation. As might be expected, mutations in a variety of the genes encoding proteins critical to hepcidin gene regulation or iron trafficking can lead to clinical phenotypes of iron overload or iron deficiency.

Inflammatory Signals
Many of the inflammatory signals that regulate hepcidin gene expression are well known. Interleukin (IL)–6, known to be elevated in inflammation, has been shown to be a key positive
regulator of hepcidin levels. In elegant experiments, Nemeth et al.33 showed that IL-6 injection into human volunteers resulted in a rapid increase of hepcidin in the urine. In a parallel series of experiments in mice, inflammation was created by injecting turpentine into the foot pad. This led to increased hepcidin gene expression in the liver. Similarly, when normal mice that had been maintained on a low iron diet were switched to a diet high in iron, hepcidin gene expression was increased. When these same experiments were carried out in IL-6 knockout mice, the induction of inflammation failed to increase hepcidin gene expression, whereas the hepcidin response to dietary iron loading remained intact. These findings convincingly demonstrated that hepcidin gene expression was regulated by at least 2 distinct pathways—an inflammatory pathway and an iron-sensing pathway—and that IL-6 was necessary for the inflammatory response pathway but was not part of the iron-sensing pathway. It is now recognized that at least 4 identifiable pathways regulate, either alone or in concert, hepcidin gene expression: inflammation, iron balance, hypoxia, and erythropoiesis.25

However, IL-6 may not be the sole inflammatory mediator of hepcidin gene expression. Recently, it was reported that inflammation caused by either Brucella abortus or Mycobacterium plus Freund’s complete adjuvant in hepcidin knockout mice also resulted in hypoferremia34 (T. Ganz, personal communication, 2012). What mediates iron trafficking under these conditions is unknown, but if confirmed, there must be additional mediators of inflammation that are capable of inducing hepcidin levels.

Clinical Consequences of Inflammation

In man, chronic inflammation can lead to anemia. The anemia of inflammation (AI; also referred to as the anemic of chronic disease [ACD]) is among the most common forms of anemia encountered clinically. A broad spectrum of inflammatory conditions such as malignancy, autoimmune diseases, tissue injury, and infection are accompanied by an increase in inflammatory cytokines.

One of the most dramatic effects of inflammation, as noted above, is the effect on iron trafficking. As discussed, the best understood inflammatory cytokine in this context is IL-6. With the upregulation of hepcidin, iron absorption from the gut and release of iron into circulation from storage cells are blocked by the downregulation of the cellular iron exporter, ferroportin.23,33 Under these conditions, “relative” or “functional” iron deficiency ensues. Despite normal, or even increased, iron stores, circulating iron levels fall and evidence of iron-restricted erythropoiesis is seen. If the so-called inflammatory blockade of iron release is sustained over a long period of time, the red cells can become microcytic.

Several other mechanisms contribute to AI. In the presence of inflammatory cytokines, erythropoietin (Epo) production by the kidneys is impaired. This has been shown both in vitro35,36 and in vivo,35 and the degree of suppression is dependent on the concentration of the cytokines. Cytokines implicated include IL-1, tumor necrosis factor, and the interferons. Interestingly, IL-6, in vitro, had no suppressive effect on Epo
production by hypoxia-sensitive human hepatoma cell lines.\textsuperscript{36} In addition to impaired Epo production, AI is characterized by a blunted response of Epo-responsive cells in vitro.\textsuperscript{37}

The cellular target for inflammatory cytokines was worked out many years ago either by administering the cytokine of interest to experimental animals or by overexpressing the cytokine by genetic engineering. When normal mice were injected with IL-1\textalpha, the number of erythroid colony-forming cells (CFU-E) in the spleen fell by 60\%, whereas there was no effect on the numbers of the more primitive progenitor, the erythroid burst-forming cell (BFU-E).\textsuperscript{38} In mice engineered to overexpress tumor necrosis factor (TNF), only the CFU-E were negatively affected, whereas more primitive progenitors of all lineages were either unaffected or increased in number.\textsuperscript{39} Consequently, inflammation contributes to anemia via a number of mechanisms.

### Summary

Iron is a critical element for many physiological processes as they relate to red cell and energy production. Iron balance is regulated at the level of absorption since the body lacks mechanisms for iron excretion. Iron absorption is controlled both locally, by the absorptive enterocyte, and from a distance by the liver-derived peptide, hepcidin. The discovery and hormonal function of hepcidin has provided enormous insights into normal iron metabolism as well as iron metabolism in inflammatory states. Despite all of the advances, the molecular pathways regulating iron metabolism under physiological and pathophysiological conditions remain incompletely understood and are subject to intense research.

### References


