

Review

The Molecular Biology of Human Iron Metabolism

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Lab Med Spring 2014;45:92-102

DOI: 10.1309/LMF28S2GIMXNWHMM

ABSTRACT

Iron is one of the most important nonorganic substances that make life possible. Iron plays major roles in oxygen transport (eg, hemoglobin; ~67% of total body iron [TBI]), short-term oxygen storage (eg, myoglobin; ~3.5% of TBI), and energy generation (eg, cytochromes; ~3% of TBI).¹ Iron also serves vital roles in various nonheme-containing enzymes (~2% of TBI). **Figure 1** lists heme-containing and nonheme iron-containing proteins. TBI is controlled by the rate of iron absorption; there are no physiologic mechanisms to excrete excess iron.

Iron deficiency has many adverse consequences, including anemia, and in children, behavioral and learning disorders.²⁻⁴ Iron excess is toxic to the body, harming the heart, liver, skin, pancreatic islet beta cells, bones, joints, and pituitary gland. Maintaining proper iron balance is essential for maintaining homeostasis and health.

TBI in adults normally ranges between 3.5 and 5.0 g.⁵ A total of 75% of TBI is functional, and 25% is stored within cells as ferritin or hemosiderin. Ferritin contains 24 subunits of light chains (L chains; 19.7 kDa) and heavy chains (H chains; 21.1 kDa). The L chains are encoded on chromosome 19q13.33 and are 175 amino acids long. The H chains are encoded on chromosome 11q1 and are 183 amino acids long. Each ferritin molecule can contain as many as approximately 4500 ferric ions. Because the major role of iron is in hemoglobin synthesis, this review will focus on iron, iron transport, and hematopoiesis.

Keywords: bone morphogenetic protein, ceruloplasmin, divalent metal ion transporter-1, ferritin, ferroportin, hemochromatosis, hepcidin, hephaestin, HFE, iron, transferrin, transferrin receptor, total body iron

Abbreviations

BMP, bone morphogenetic protein; CCL2, chemokine (C-C motif) ligand 2; CXCL8, C-X-C motif chemokine 8; DAMP, danger (or damage)-associated molecular pattern; GDF-15, growth differentiation factor-15; HAMP, hepcidin (hepatic) anti-microbial peptide; HCP-1, heme carrier protein-1; HFE, hemochromatosis; HIF, hypoxia-inducible factor; HIF-2A, hypoxia-inducible factor-2 alpha; HJV, hemojuvelin; HRG1, solute carrier family 48 (heme transporter), member 1; IL-6, Interleukin-6; IL-6R, interleukin-6 receptor; IRE, iron-responsive element; IRP, iron regulatory protein; JAK, Janus kinase; MAPK, mitogen-activated protein kinase; PAMP, pathogen-associated molecular pattern; PRR, pattern recognition receptor; RBC, red blood cell; SLC11A2, solute carrier family 11 (proton-coupled divalent metal ion transporter), member 2; SLC40A1, solute carrier family 40 (iron-regulated transporter), member 1; SLC46A1, solute carrier family 46 (folate transporter), member 1; SMAD, a “mothers against decapentaplegic” homolog; STAT, signal transducer and activator of transcription; STEAP-3, six-transmembrane epithelial antigen of the prostate-3; TBI, total body iron; Tfr1, transferrin receptor 1; Tfr2, transferrin receptor 2; TWSG1, twisted gastrulation protein homolog 1

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The Iron Circuit

The normal iron circuit includes the uptake of iron from transferrin by developing erythroblasts, the incorporation of iron into heme, red blood cell (RBC) production, RBC survival, and RBC senescence in the spleen with return of iron to the bone marrow via transferrin (**Figure 2**).⁶ Uptake of iron from the diet is necessary to replace the small amount of iron normally lost daily via the sloughing off of intestinal mucosal cells and menstrual blood loss in women of reproductive age.

The presence of phytates, oxalates, phosphates, carbonates, and tannates decreases nonheme iron absorption, whereas nonheme iron absorption is increased by ascorbic acid, protein in the diet, and a low gastric pH that fosters more efficient digestion of food. Hypo- or achlorhydria, which results in higher gastric pH, reduces iron release from food. Causes of hypo- or achlorhydria include atrophic gastritis, *Helicobacter pylori* infection, gastrectomy, and vagotomy.⁷⁻⁹ Iron malabsorption can result from celiac disease or small-bowel resection. Dietary iron can be bound to starch or clay, inhibiting absorption.

Heme-containing proteins

Oxygen transport and binding
 Hemoglobin
 Myoglobin

Peroxidases

Lactoperoxidase
 Eosinophil peroxidase
 Thyroid peroxidase
 Myeloperoxidase

Cytochrome: redox cytochromes, P450 class of detoxifying cytochromes

Cytochrome c oxidase heme a
 Cytochrome b/b6
 Cytochrome b5
 Cytochromes c (Classes III and IV)
 Cytochrome c554
 Cytochrome cd1 nitrite reductase (c-domain)
 Hydroxylamine oxidoreductase
 Soluble cytochrome b562
 Cytochrome c
 Cytochrome f
 Cytochrome cd1 nitrite reductase
 Bacterioferritin
 Cytochrome c oxidase heme a3
 Cytochrome c
 Hydroxylamine oxidoreductase

Other enzymes

Nitric oxide synthase (NOS)
 Prostaglandin synthase (cyclooxygenase)
 Catalase

Nonheme proteins

Iron storage or transport
 Transferrin
 Ferritin
 Haptoglobin (with hemoglobin)
 Hemopexin (with heme)
 Lactoferrin (in breast milk)

Nonheme enzymes

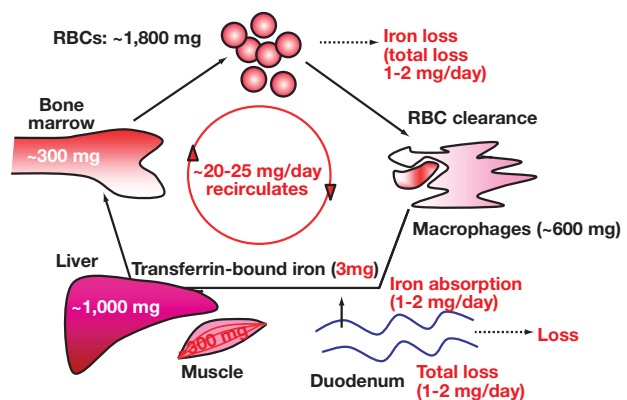
Ribonucleotide reductase
 Iron-sulfur proteins
 Aconitase
 Ferredoxin
 Iron-sulfur cluster scaffold homolog (*Escherichia coli*)
 Nucleotide binding protein-like

Figure 1

Heme and nonheme containing proteins.

Figure 2

Normal iron biology. This process involves the transit of iron from the bone marrow as red blood cells (RBCs) to the spleen for removal by macrophages with iron recycled to the bone marrow via transferrin. The intestine absorbs iron to balance the iron that is lost daily.

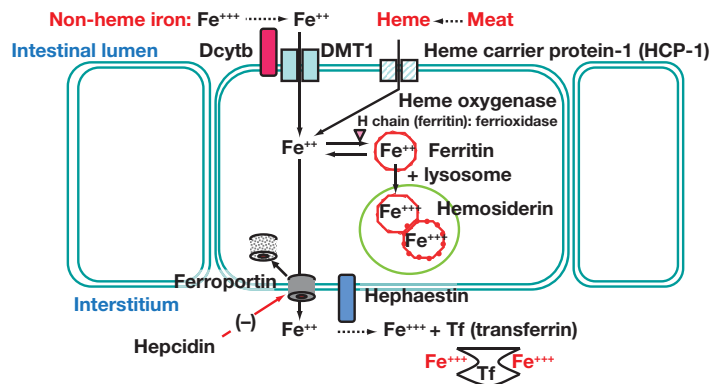


In healthy adults, 20 to 25 mg of iron cycles daily. Absorption of 1 to 2 mg of iron daily from the diet balances the loss of iron from the sloughing off of intestinal mucosal cells. The usual adult diet contains 10 to 15 mg of elemental iron; thus, 7% (1 of 15 mg) to 20% (2 of 10 mg) of dietary iron may be absorbed daily. Menstruating women may absorb as much as 3 mg of iron daily because of their

increased iron loss compared with men. Transferrin-bound iron totals approximately 3 mg. Therefore, the circulating iron pool turns over approximately 7 to 8 times per 24 hours (ie, 3 mg in transferrin in the circulation versus the recycling pool of 20-25 mg). Also, from these numbers, one can observe that only 4% (1 of 25 mg) to 10% (2 of 20 mg) of cycling iron comes from dietary sources. Most cycling

Figure 3

Depiction of the clearance of ferroportin from the cell membrane by hepcidin, the export of ferrous iron by ferroportin, the oxidation of ferrous iron to ferric iron by hephaestin, and the subsequent loading of ferric iron onto transferrin. Enterocytes regulate total body iron through the absorption of iron. Heme iron from meat is better absorbed than elemental iron. Hepcidin is a major regulator of this process.



iron comes from the spleen, where RBCs are destroyed by splenic macrophages; the iron is recycled to the bone marrow via transferrin. The distribution of iron throughout the body is as follows: bone marrow, 300 mg; circulating RBCs, 1800 mg; splenic macrophages, 600 mg; muscle, 300 mg; and liver, 1000 mg.

Cells of the Iron Circuit

The 4 key cell types involved in iron cycling are intestinal enterocytes, erythroblasts, splenic macrophages, and hepatocytes.¹⁰ The enterocytes are the site of iron absorption and regulate TBI (excluding iron acquired through transfusions or parenteral iron injections or infusions). Erythroblasts use iron, giving rise ultimately to anucleated RBCs. Splenic macrophages degrade aged RBCs and release iron back to the bone marrow via transferrin. The liver plays a key role in monitoring transferrin saturation, monitoring hepatocellular (tissue) iron content, regulating iron absorption from the gut, and regulating iron release from the spleen. A hepatic hormone, hepcidin, regulates iron absorption from the gut and iron release from the spleen.¹¹⁻¹³ We will now review the function of these 4 key cell types.

Enterocytes

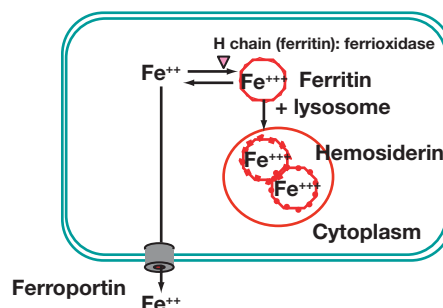
Via their apical membrane, enterocytes absorb ferrous iron or heme that contains ferrous iron (**Figure 3**). Ferric iron in the diet is reduced to ferrous iron via duodenal cytochrome b, which is expressed on the luminal (apical) surface of the enterocyte. Duodenal cytochrome b is

also known as cytochrome b reductase 1 (gene location: chromosome 2q31; 286 amino acids, 31.6 kDa). Ferrous iron then enters into the cytoplasm of the enterocyte via the divalent metal ion transporter-1 (DMT1). The formal name for DMT1 is solute carrier family 11 (proton-coupled divalent metal ion transporter), member 2, or SLC11A2. DMT1 (568 amino acids; 62.3 kDa) also transports cobalt, cadmium, lead, manganese, nickel, and vanadium. The *DMT1* gene is located on chromosome 12q13. Heme iron is more readily absorbed than elemental iron. Heme uptake occurs via the heme carrier protein-1 (HCP-1). The formal name for HCP-1 (459 amino acids, 49.8 kDa) is SLC46A1: solute carrier family 46 (folate transporter), member 1. The *HCP-1* gene is located on chromosome 17q11.2.

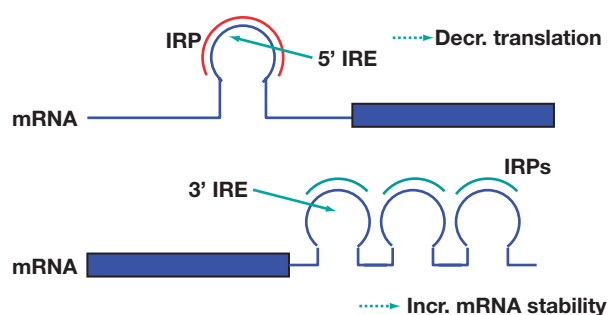
Within the enterocyte, heme oxygenase (288 amino acids, 32.8 kDa; gene location: chromosome 22q1) releases ferrous iron while converting protoporphyrin IX to biliverdin. Within the enterocyte, ferrous iron can be exported from the cell into the interstitium via ferroportin.¹⁴ Alternatively, the ferrioxidase activity of the H chain of ferritin oxidizes ferrous iron to ferric iron to be incorporated into ferritin. The uptake of aggregated ferritin molecules into lysosomes produces hemosiderin. Hemosiderin is detected histologically with a tissue iron stain (ie, to detect siderosis). For example, hemosiderin stains blue with the Perls Prussian Blue stain. Within hemosiderin-laden lysosomes, ferritin is partially degraded. Hemosiderin can be removed with chelation therapy. *Hemosiderosis* is defined as the buildup of stainable iron (hemosiderin) in tissues, especially in the liver and spleen. Hemosiderosis is a descriptive histological term, not a clinical diagnosis.

Figure 4

Depiction of the within-cell process of ferrous iron oxidized to ferric iron to be incorporated into ferritin, or ferrous iron exported outside the cell by ferroportin.

**Figure 5**

Iron regulatory proteins (IRPs) that bind to the 5' iron-responsive elements (IREs) within messenger RNA (mRNA) impairs the translation of such chains. IRPs that bind to the 3' iron-responsive elements (IREs) within mRNA stabilizes the mRNA, leading to the increased translation.



Ferritin is released into the bloodstream; plasma (or serum) ferritin concentrations typically reflect iron stores. However, ferritin can be elevated in conditions other than iron overload, including liver disease (with release of ferritin from hepatocytes), the metabolic syndrome, inflammatory states, chronic illness, and the hemophagocytic syndrome. Plasma ferritin is lower in premenopausal women compared with men. Enterocyte absorption of iron is regulated by 3 factors, namely, tissue oxygen tension, intracellular iron stores, and the systemic iron needs that are mediated by hepcidin.

Mechanism 1: A decrease in enterocytic oxygen tension enables the transcription factor hypoxia-inducible factor-2 alpha (HIF-2A; aka, endothelial p-aminosalicylic acid [PAS] domain protein 1; 870 amino acids; 96.5 kDa; chromosome 2p21) to increase DMT1 and ferroportin messenger RNA (mRNA) transcription. In the enterocyte, the role of ferroportin is to transport ferrous iron across the basal surface of the cell into the interstitium (**Figure 4**).¹⁵

Mechanism 2: Low intracellular iron modulates the iron regulatory proteins (IRPs) types 1 and 2.¹⁶ When cellular iron is reduced, IRPs do not bind to the 5' iron-responsive

elements (IREs) of mRNAs, allowing an increased rate of ferroportin and HIF-2 alpha mRNA translation. However, the mRNA for DMT1 is stabilized by IRP bound to the 3' IRE of the DMT1 mRNA, leading to increased DMT1 production via increased mRNA translation. High iron levels suppress these changes. Note that much of the regulation of iron metabolism occurs via the regulation of translation and not transcription (**Figure 5**). This allows for rapid and precise changes in the absorption of iron from the diet. Transferrin is also transcriptionally regulated.

Mechanism 3: The liver senses low transferrin saturation and responds by reducing hepcidin secretion, allowing increased ferroportin activity to promote transport of ferrous iron out of the enterocytes and into the interstitium.¹⁷ Ferroportin is a member of the solute carrier family of proteins (solute carrier family 40 [iron-regulated transporter], member 1; 571 amino acids; 62.5 kDa; chromosome location, 2q32). Ferroportin is expressed in enterocytes, fixed phagocytes (eg, Kupffer cells and splenic macrophages), and hepatocytes. Hepcidin binds to ferroportin, causing it to be internalized and degraded, leading to the reduced export of iron. When cellular iron is low, IRP binds to the 5' IRE of the ferroportin mRNA and

Figure 6

Depiction of the transferrin glycoprotein. Transferrin is composed of 2 lobes, namely, the C-lobe and the N-lobe. Each lobe binds 1 ferric atom. There is a hinge between the C1 and C2 domains of the C-lobe and also a hinge between the N1 and N2 domains of the N-lobe.

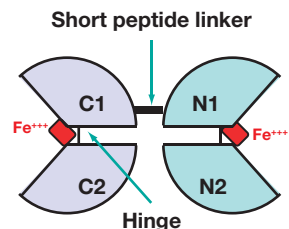
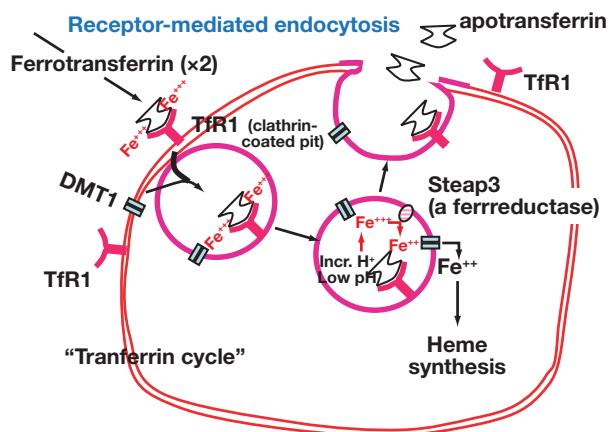


Figure 7

Depiction of the erythroblasts that take up transferrin by the binding of transferrin to the transferrin receptor 1 (TfR1). For simplicity, the drawing shows 1 transferrin molecule binding to TfR1. However, according to the correct stoichiometric information, TfR1 can bind 2 transferrin molecules. The divalent metal ion transporter 1 (DMT1) is internalized with the internalization of the transferrin-TfR1 complex. With acidification of this vesicle, transferrin releases ferric iron. By reduction to ferrous iron via the 6-transmembrane epithelial antigen of the prostate-3 (STEAP-3), iron enters the cytoplasm of the cell via DMT1. Transferrin is then released from the cell as the TfR1 recycles to the erythroblast plasma membrane. This process is termed the *transferrin cycle*.



increases translation, leading to the release of ferrous iron by cells that express ferroportin. This is logical because more iron will be absorbed, adding iron to the TBI pool, while also releasing more ferrous iron from splenic macrophages. In this scenario, more iron is available (via transferrin transport) for hematopoiesis. In contrast to translational regulation, hepcidin regulates ferroportin post-translationally.

For iron to be carried on transferrin, the ferrous iron must be oxidized to ferric iron. Oxidation occurs on the basal membrane of the enterocyte by the enzyme hephaestin (1158 amino acids; 130.4 kDa; chromosome location, Xq1). Each transferrin molecule (698 amino acids; 77.0 kDa; chromosome location, 3q2) can bind 2 ferric ions.¹⁸ Transferrin is a beta globulin that has very high affinity for iron (Kd ~10⁻²² M). Transferrin without bound iron is called apotransferrin; when iron is bound to transferrin, the complex may be termed ferrotransferrin, diferrotransferrin, or holotransferrin. Transferrin consists of 2 homologous lobes (N- and C-lobes) that are connected by a short peptide (Figure 6). C1 and C2 are domains within the C-lobe, and N1 and N2 are domains within the N-lobe. The lobes are 48%

homologous. In the clinical laboratory, transferrin is usually measured via turbidimetry or nephelometry.

Erythroblasts

Bone-marrow erythroblasts take up transferrin-bound iron to synthesize hemoglobin (Figure 7). The earliest blast that is dedicated to erythropoiesis is the proerythroblast, which can be recognized by the presence of nucleoli where ribosomal RNA synthesis is ongoing (Figure 8). The next stage in erythroid development is the development of basophilic erythroblasts that usually lack nucleoli; their basophilic cytoplasm indicates active protein synthesis. The polychromatophilic erythroblast follows, leading to the orthochromic erythroblast. By this stage in erythropoiesis, the nucleus has become dense due to chromatin condensation, and the nucleus is located eccentrically. When the nucleus is lost by extrusion or karyolysis, a reticulocyte is formed. Reticulocytes are larger than mature RBCs. Reticulocytes and RBCs have a pink cytoplasm. With supravital staining, the reticulocytes display a reticular pattern that indicates the continued presence of polyribosomes. When the polyribosomes are no longer present, the cell is a mature RBC.

Figure 8

Outline of the stages of red blood cell (RBC) development. Hb refers to hemoglobin; nRBC, nucleated red blood cell.

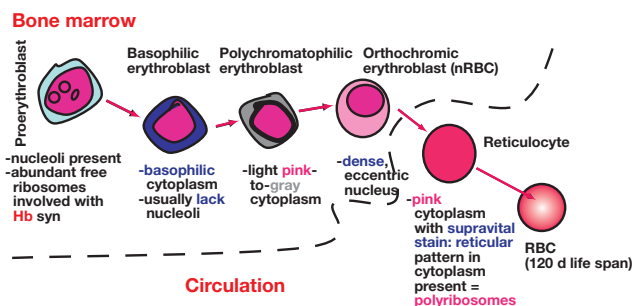
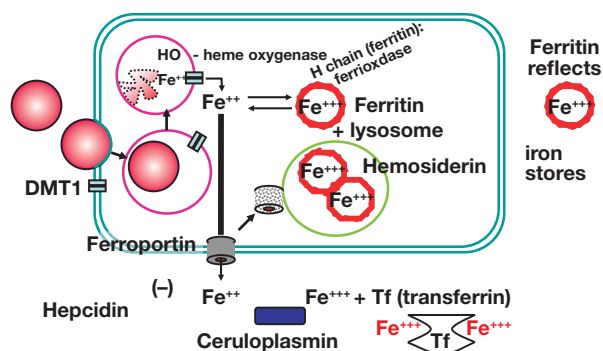
**Figure 9**

Illustration of splenic macrophages phagocytizing damaged red blood cells (RBCs). Similar to erythrocytes, divalent metal ion transporter-1 (DMT1) is internalized concurrent with this process. With digestion of the RBC, heme is released. Heme oxygenase (HO) then removes ferrous iron from heme that enters the cytoplasm via DMT1. Ferrous iron that exits the cell via ferroportin is oxidized to ferric iron for loading onto transferrin via the action of ceruloplasmin.



It is important to note that unbound (free) iron does not normally exist in the circulation, other than the fleeting moment when iron is handed off from ferroportin to transferrin.^{20,21} Erythroblasts express the transferrin receptor 1 (TfR1) that each binds 2 transferrin proteins. TfR1 is a homodimer with each chain composed of 760 amino acids (84.9 kDa; gene location, chromosome 3q29). When transferrin binds to TfR1, the ligand-receptor complex is internalized. Ferric iron is released from transferrin at the low pH within the vesicle. STEAP-3 (6-transmembrane epithelial antigen of the prostate-3) is a ferrireductase that reduces ferric iron to ferrous iron, which subsequently enters the cytoplasm of the RBC via DMT1.²² DMT1 is expressed on the surface of erythroblasts and is internalized with the ligand-receptor complex. The low pH in the vesicle does not destroy transferrin or TfR1; both are recycled to the erythroblast surface. TfR1 remains on the surface (as a transmembrane receptor) whereas apotransferrin (ie, transferrin that lacks ferric iron) enters the extracellular space. This process is referred to as the *transferrin cycle*.

Within the erythroblast, iron enters the mitochondrion, where ferrous iron is added to protoporphyrin IX via

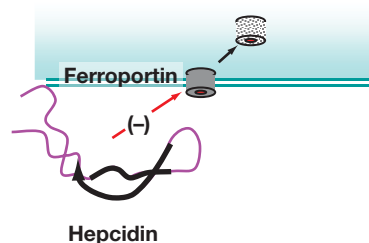
ferrochelatase (423 amino acids; 47.9 kDa; gene location, chromosome 18q21.3), forming heme.²³ Heme leaves the mitochondrion and is added to the globin chains, forming hemoglobin. Iron availability within erythroblasts regulates TfR1 expression via a 3' IRE (low iron causes IRP to bind to the 3' IRE of the TfR1 mRNA, stabilizing the TfR1 mRNA and leading to increased TfR1 expression) and erythroid-specific 5-aminolevulinic synthase (regulated via mRNA translation). Increased iron promotes translation of 5-aminolevulinic synthase mRNA. Erythroid-specific 5-aminolevulinic synthase is the first enzyme in the metabolic pathway for protoporphyrin IX synthesis.

Splenic Macrophages

RBCs that lose their flexibility, presumably due to cumulative membrane damage, are trapped within the spleen and phagocytized by fixed macrophages (**Figure 9**).²⁴ During phagocytosis, DMT1 is also internalized by the macrophages. When senescent RBCs are digested, heme is released from globulin, and the globin chains are digested to amino acids. As in enterocytes, macrophage heme oxygenase removes ferrous iron from heme,

Figure 10

Line drawing of the currently understood tertiary structure of hepcidin. Hepcidin binds to ferroportin to initiate the internalization and metabolism of ferroportin. Consequently, cellular release of iron is reduced.



and the ferrous iron enters into the cytoplasm from the phagolysosome via DMT1. HRG1 (solute carrier family 48 [heme transporter], member 1; 146 amino acids; 16.4 kDa; gene location, chromosome 12q13.11) appears to be able to transport heme directly from the macrophage phagolysosome into the cell cytoplasm. HRG1 may also be functional in enterocytes.

Just as in enterocytes, ferrous iron can be stored as ferric iron within ferritin or hemosiderin, or ferrous iron can be exported outside the macrophage by ferroportin. Also, similar to enterocytes, hepcidin regulates ferroportin expression in macrophages; increased hepcidin leads to increased internalization and degradation of ferroportin with reduced iron export from the macrophage to transferrin.

One difference between iron transport in enterocytes and macrophages is that in the spleen the oxidation of ferrous iron to ferric iron to load iron from macrophages onto transferrin is accomplished by the plasma protein ceruloplasmin, which is a ferrioxidase. Ceruloplasmin (1065 amino acids; 122.2 kDa; gene location, chromosome 3q2) is an alpha-2 globulin that includes 6 to 7 copper atoms. Approximately 90% of circulating copper is bound to ceruloplasmin; however, ceruloplasmin is not a copper-transport protein. Copper is transported in the plasma by albumin and transcuprein.²⁵ Transcuprein is an alpha-1 globulin with a molecular weight of 270 kDa and consists of 2 subunits (~60 kDa and ~200 kDa). Overall, transferrin-bound ferric iron from the splenic macrophages is delivered back to the bone marrow for erythropoiesis.

Hepatocytes

Hepatic cells monitor several aspects of TBI. Hepatocytes regulate iron absorption and recycling via their production and secretion of hepcidin.²⁶ Hepcidin is a product of the

HAMP [hepcidin (hepatic) antimicrobial peptide] gene that is located on chromosome 19q13.1. When hepcidin binds to ferroportin, iron release from cells expressing ferroportin as the iron transport protein (eg, duodenal enterocytes [the major site of iron absorption] and splenic macrophages) is impaired as ferroportin is internalized and degraded (**Figure 10**). The nascent 84 amino acid (9.4 kDa) protein includes a potential signal peptide (amino acids 1-24), a propeptide region (amino acids 25-54), a 25-amino-acid region spanning residues 60 to 84, and a 20-amino-acid region spanning residues 65 to 84. In the 20- and 25-amino-acid sequences, there are 4 disulfide bonds (66-82; 69-72; 70-78; and 73-81). A 22-amino-acid sequence has also been reported.²⁷ Thus, hepcidin may be 20, 22, or 25 amino acids in length. Only the 25-amino-acid form is bioactive; it is present in picomolar concentrations in the plasma. Measurement of hepcidin has been challenging; clinically robust assays are not yet available.²⁷

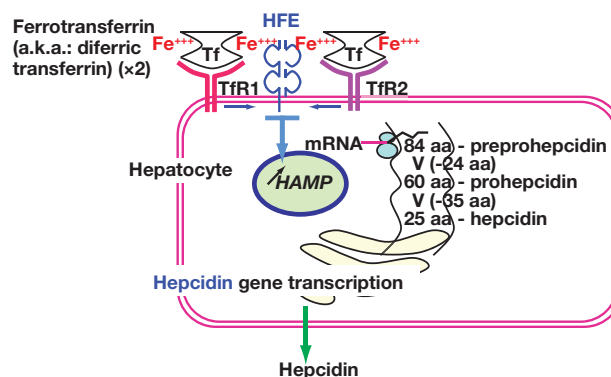
There are 4 major mechanisms regulating hepcidin secretion: inflammation, the iron content of hepatocytes and transferrin saturation, erythropoietic activity, and the oxygen tension within hepatocytes.

Inflammation and Hepcidin

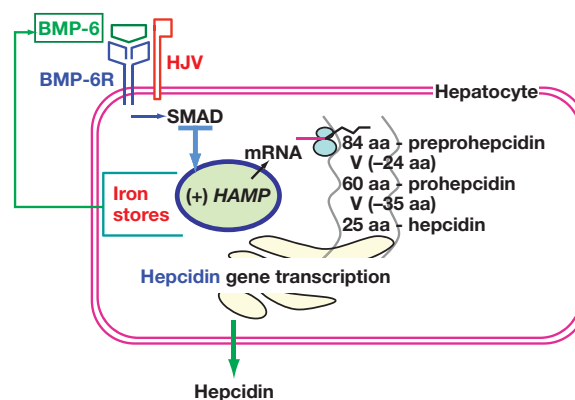
With sterile or nonsterile injury, macrophages act as sentinel cells and detect pathogen-associated molecular patterns (PAMPs) and danger- (or damage-) associated molecular patterns (DAMPs) via pattern-recognition receptors (PRRs) such as toll-like receptors (TLRs), chemokine receptors, integrins, the inflammasome, and various other receptors. In turn, activation of the transcription factor nuclear factor kappa beta (NF- κ B) elicits the production and secretion of a huge number of cytokines, such as tumor necrosis factor- α , interleukin-1 β (IL-1 β), CXC chemokine ligand 8 (CXCL8), CC chemokine ligand 2 (CCL2), and interleukin 6 (IL-6).

Figure 11

Depiction of how hepatocytes monitor transferrin saturation via transferrin receptor 1 (TfR1) and transferrin receptor 2 (TfR2). HFE is involved in this process. mRNA refers to messenger RNA; HAMP, hepcidin (hepatic) antimicrobial peptide.

**Figure 12**

Depiction of hepatocyte monitoring of tissue iron content. This process is afforded by the secretion of bone morphogenetic protein 6 (BMP-6). BMP-6 binds to its receptor, BMP-6R. Hemojuvelin (HJV) is involved in this signaling process.



IL-6 binding to the hepatic IL-6 receptor (IL-6R) increases hepcidin release and lowers the concentration of plasma ferric iron that is normally transported by transferrin.²⁸ This results from decreased ferroportin expression and decreased transport of ferrous iron to the outside of enterocytes and splenic macrophages as ferroportin is internalized and degraded. Signaling distal to the IL-6R involves the Janus kinase–signal transducer and activator of transcription (JAK–STAT) pathway. Hepcidin's reduction of plasma iron levels may have an evolutionary basis by providing a survival advantage because certain bacteria (eg, *Clostridium* species) have high iron requirements.

Iron Content of Hepatocytes and Transferrin Saturation

Hepatic uptake of iron and iron sensing are afforded by TfR1 and transferrin receptor 2 (TfR2; **Figure 11**).²⁹ In this process, transferrin saturation is sensed by hepatocytes. If there is a higher level of transferrin saturation by ferric iron, this is detected by TfR1 and HFE, which correspondingly increase hepcidin concentrations; this reduces iron

absorption from the gut and iron recycling from splenic macrophages.

HFE is a class I major histocompatibility complex (MHC)–like protein that is necessary for the iron-sensing signal of TfR1 that regulates plasma hepcidin concentrations via expression of the HAMP gene.³⁰ The *HFE* gene (348 amino acids; 40.1 kDa) is encoded on chromosome 6p2. Mutations in *HFE* cause classic type 1 hemochromatosis; a lack of perception of transferrin saturation causes hepcidin deficiency and excess absorption of dietary iron, leading to TBI overload.³¹ TfR2 binding to transferrin also regulates hepcidin. Mutations in TfR2 (type 3 hemochromatosis) produce iron overload clinically similar to type 1 hemochromatosis.

As cellular iron increases, bone morphogenetic protein 6 (BMP-6) is secreted (**Figure 12**).³² Just as TfR1 uses HFE in iron sensing and signal transduction to control the HAMP gene and hepcidin, the hepatocyte BMP-6 receptor requires hemojuvelin (HJV) for signaling to control hepcidin secretion.

Table 1. Regulation of Hepcidin Level

Factor Regulating Hepcidin Level	Mechanism/Direction of Change	Effect on Hepcidin Level	Effect on Iron Cycling
Inflammation	Increased IL-6	Increased	Decreased
Iron			
Hepatic iron	Increased	Increased	Decreased
Tf saturation	Increased	Increased	Decreased
Erythropoietic activity	Increased	Decreased	Increased
O ₂ tension	Low cellular pO ₂	Decreased	Increased

IL-6, interleukin 6; Tf, transferrin.

HJV is a 426–amino acid protein (45.1 kDa) encoded by its gene on chromosome 1q21.

The BMP-6/BMP-6 receptor/hemojuvelin circuit operates on an autocrine basis. The BMP-6 receptor/hemojuvelin puts out signals via SMAD. (SMAD is a homolog protein made up of the *Caenorhabditis elegans* protein SMA and the *Drosophila* protein colloquially known as “mothers against decapentaplegic” because a mutation in the gene *MAD* in the mother represses the gene *decapentaplegic* in the embryo.) The BMP-6/BMP-6 receptor/hemojuvelin circuit may also use MAPK (mitogen-activated protein kinase). Similar signaling is involved for TfR1/HFE. Mutations in hemojuvelin and hepcidin cause hemochromatosis in children (eg, type 2 [juvenile] hemochromatosis).³³

Erythropoietic Activity of the Bone Marrow

The erythropoietic activity of the bone marrow influences the liver and regulates hepcidin release. This process begins with peritubular cells in the kidney where hypoxia and/or anemia, detected by a hypoxia inducible factor (HIF), release(s) erythropoietin.³⁴ The increased erythropoietic drive stimulated by elevated erythropoietin levels appears to cause the release of subsequent signals from the bone marrow that reduce hepcidin secretion.³⁵ This promotes increased dietary iron absorption and increased iron release from splenic macrophages. Ultimately, more transferrin-bound ferric iron is made available to erythroblasts.

Signals that may suppress hepatic hepcidin release include growth differentiation factor–15 (GDF-15) and twisted gastrulation protein homolog 1 (TWG1), both of which are secreted by the bone marrow with increased erythropoiesis. This mechanism explains cases of acquired iron overload in various types of anemia in the absence of chronic transfusion therapy. Examples of nontransfusion iron overload anemias include thalassemia, X-linked sideroblastic

anemia, sickle-cell anemia, pyruvate kinase deficiency, hereditary spherocytosis, and congenital dyserythropoietic anemia. Bone-marrow erythropoietic signals appear to dominate (aka “trump”) elevated hepatic iron stores, resulting in suppressed hepcidin levels in the plasma.

The Oxygen Tension Within Hepatocytes

Just as enterocytes and renal peritubular cells monitor their cellular oxygen tension, hepatocytes also monitor intracellular hepatocyte oxygen tension. Hypoxia allows HIFs to increase the expression of a membrane protease (namely, matriptase-2) that cleaves hemojuvelin, reducing BMP-6R signaling.³⁶ In turn, *HAMP* gene expression is reduced, which decreases hepcidin production. HIFs also increase furin, a proprotein convertase that cleaves hemojuvelin, releasing a hemojuvelin decoy receptor for BMP-6.³⁷ This reduces BMP-6 signaling and coordinates with the previously described mechanism to inhibit hepcidin release.

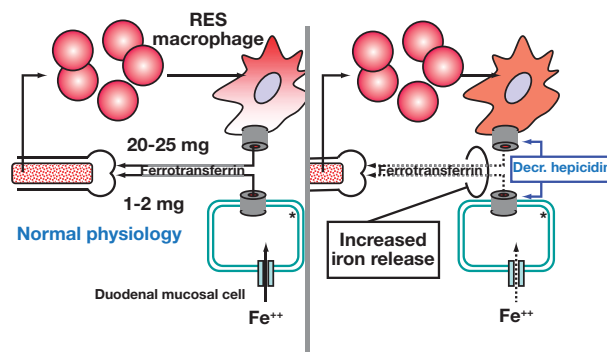
Matriptase-2 (a transmembrane protease, serine 6) is encoded on chromosome 22q1, consists of 811 amino acids, and has a molecular weight of 90 kDa. In the secretory pathway within cells, the proteolytic maturation of proprotein substrates is catalyzed by furin.³⁸ Furin (aka paired basic amino acid–cleaving enzyme) is encoded on chromosome 15q26.1, consists of 794 amino acids, and has a molecular weight of 86.7 kDa.

Discussion

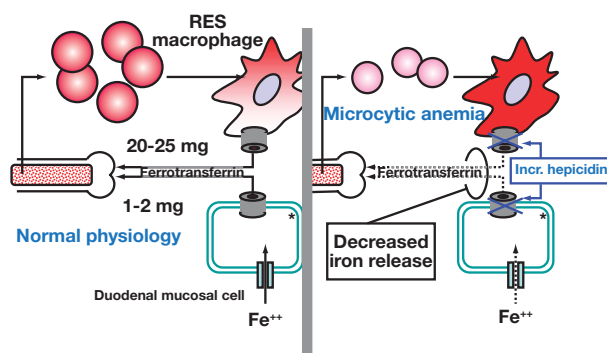
Low transferrin saturation, hepatocyte oxygen tension, and hepatocyte tissue iron content, along with increased erythropoietic activity, reduce hepcidin concentrations in the plasma, permitting increased iron absorption from the gut and increased iron recycling from splenic

Figure 13

Depiction of iron cycling and hepcidin deficiency with decreased plasma hepcidin concentrations. **A**, The normal state of iron cycling. **B**, hepcidin deficiency. Decreased plasma hepcidin concentrations permit hyperabsorption of iron and iron overload.

**Figure 14**

Depiction of iron cycling and hepcidin deficiency with increased plasma hepcidin concentrations. **A**, The normal state of iron cycling. **B**, Hepcidin excess. Increased plasma hepcidin concentrations impair iron availability to the bone marrow, causing iron-restricted anemia.



macrophages (**Table 1, Figure 13**). In non-transfusion induced iron overload, a deficiency of hepcidin causes pathologic iron overload from hyperabsorption of iron. The opposite events (namely, increased transferrin saturation, increased hepatocyte oxygen tension, increased hepatocyte tissue iron content, and decreased erythropoietic activity) increase hepcidin levels, leading to decreased iron absorption from the gut and decreased iron recycling from splenic macrophages (**Table 1, Figure 14**). A pathologic excess of hepcidin causes deficient iron absorption from the gut, impaired release of iron from splenic macrophages, and iron-restricted anemia. Hepcidin deficiency allows excess iron absorption; therefore, nature has selected iron excess over iron deficiency, most likely because iron is not abundant in the human diet. Nutritional iron deficiency is common worldwide.

Although the pathophysiologic nature of iron overload is beyond the scope of this review,^{39,40} iron homeostasis is important to understand the range of disorders that involve iron deficiency or iron excess. Factors that reduce hepcidin, including essentially any form of chronic liver disease, can lead to the hyperabsorption of iron and to

iron overload.⁴¹⁻⁴³ Conversely, excess hepcidin, as in states of chronic disease or chronic inflammation, impairs iron recycling, causing iron-restricted anemia. **LM**

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