Iron is an integral part of haemoglobin molecule. Its deficiency leads to the commonest type of anaemia in all geographic regions and racial groups. Its overload, if associated with presence of unbound iron in tissues, is characterized by tissue injury which may be severe enough to cause significant morbidity as a result of damage to heart, liver, pancreas, endocrines and almost any tissue. During the last decade, many poorly understood areas of iron homeostasis have been unraveled, especially pertaining to the influx of iron in the upper part of small intestine, its trafficking in the plasma, metabolism in the macrophage, red cell precursors & hepatocytes, and role of genes in iron metabolism.

Distribution of Iron in the Body

Iron is the commonest element (after oxygen, silicon and aluminum) and the second most abundant metal in the crust of earth. And still, its deficiency is the commonest cause of anaemia (1). In a healthy adult male total body iron content is 3-5 grams, of which nearly 60% is present in haemoglobin. Every two ml. of blood contains one mg of elemental iron; therefore, a continual blood loss, e.g., from gastro-intestinal tract, can lead to a negative iron balance.

Nearly one third of total body iron (1-1.5 grams) is stored in the liver, spleen and bone marrow macrophages in the form of ferritin and haemosiderin; in these two storage forms, iron is nearly equally distributed. Small quantities of iron are distributed in myoglobin (in the muscles) and in certain enzymes (cytochrome oxidase, xanthine oxidase, ribonucleotide reductase, etc). This is an essential form of iron, and once incorporated in the molecules, is not available for metabolism elsewhere. In very severe iron deficiency, particularly in children, a deficiency in aerobic enzymes can lead to irreversible tissue injuries, manifested by mental retardation, confusion state, etc.

A very small quantity of iron (about 3 mg) is present in the plasma, and is bound to an iron carrying protein, transferrin. This pool is of immense physiological importance as it is responsible for a daily trafficking of about 20 mg of iron between the sites of absorption, storage and haemoglobin synthesis (2). Ferritin (Mol Wt 480 kDA) is composed of a protein, apoferritin and about 4,500 iron atoms. Apoferritin comprises of 24 subunits, which assemble peripherally in a spherical form, enclosing a central hollow space where iron atoms are attached chemically. Iron atoms can move in and out through six channels (3). Ferritin safeguards iron entry into the body and maintains iron in a safe and readily accessible form.

Trace amount of ferritin present in the plasma (μg/l) is an index of ferritin in the stores. Thus, in iron deficiency serum ferritin level is <12 μg/l, whereas in conditions characterized by iron overload (β-thalassaemia major, underlying disorders, haemochromatosis, etc), the levels may be >1,000 μg/l. If iron chelation is not given, these values may be markedly raised (even up to or >5,000μg/l). The effect of iron chelation by desferrioxamine or oral chelators is best monitored by regular serum ferritin levels.

Erythron itself requires about 20 mg elemental iron daily for haemoglobin synthesis, whereas the daily iron absorption ranges between 1 and 2 mg. The remaining, major bulk of iron required for haemoglobin synthesis is provided from iron stores (ferritin) and from the iron released from breakdown of senescent red cells.

Haemosiderin: It is a water soluble, but more stable form of storage iron. Iron content of haemosiderin is much greater than ferritin. It may be stained by Prussian blue reaction. The main sites of storage of ferritin and haemosiderin are macrophages in the...
Liver, spleen and bone marrow. (5)

**Iron in Diet**

Iron is present in meat (muscle, liver, kidney, etc) as an integral part of haem molecule. The haem molecule is at least three times more readily absorbable as compared to elemental iron and iron salts (6). The latter form of iron is present in leafy vegetables where it is bound to organic compounds, phytates and phosphates; in these forms, iron is less readily absorbable. Milk is a poor source of iron; therefore, a delayed weaning is a common cause of iron deficiency anaemia in infants and young children.

The diet of a normal adult contains 10-20 mg of elemental iron, of which only 10% is absorbed; the remaining iron is lost in the stool unabsorbed. Most of the dietary iron is present in oxidized form (Fe³⁺), which must be reduced to ferrous state (Fe²⁺) before absorption. This reduction is accomplished in the acidic pH of stomach by reducing agents, particularly ascorbic acid, which is effective only if taken concomitantly with iron. It can keep the iron ions in Fe²⁺ state even in alkaline pH.

Iron is solubilized in acidic pH of stomach, and gets insoluble again and thus unabsorbable in alkaline pH of small intestine. The factors facilitating and blocking iron absorption are depicted in table 2.

**Excretion**

There is no specific excretory mechanism of iron. Nearly 1 mg of iron is shed off with the sloughing epithelial cells in the urine, sweat, and GIT every day. In females, an additional 20 mg is lost in menses every month, making the females more vulnerable to iron deficiency anemia during reproductive life.

**Intestinal Iron Transport**

Iron is absorbed, in ferrous form, across the enterocytes lining the absorptive villi in duodenum and proximal part of jejunum. This process is facilitated by two trans-membrane transporter proteins, i.e., Divalent Metal Transporter-1 (DMT-1) and Ferroportin and redox enzymes. (7-9) (1,29,47 Nancy)

A cytochrome b like protein (Dcyt b), present on the apical surface of enterocytes reduces dietary ferric (Fe³⁺) into ferrous (Fe²⁺) iron.

DMT-1 located on the same surface facilitates entry of these Fe²⁺ molecules into the cells (10) (Nancy 42), where mobilferrin facilitates the across the cytosol.

Ferroportin, present on the basolateral surface of enterocytes, serves to help iron egress from the cell into circulation. On release from the cell, Fe²⁺ is instantly oxidized to Fe³⁺, probably with the help of a ferroxidase “hephaestin” (11)(64 Nancy) which chemically has a 50% identity with ceruloplasmin. It is noteworthy that both DMT-1 and Ferroportin mRNAs contain Iron Responsive Element (IRE) in the untranslated portions (in the 3- and 5- ends respectively (7,812-14) (1,19,28,29,47 Nancy). This (IRE in DMT-1) stabilizes the molecule in iron deficiency resulting in increased DMT-1 expression (15) (2 of Chaim Hersch)

**Intestinal Regulation**

The undifferentiated precursor cells in the intestinal crypts appear to sense the body iron needs and become programmed by that information, as they differentiate into mature absorptive enterocytes. This programming establishes a “Ferrostat”, which subsequently dictates the rate of apical iron absorption, the fate of iron within the cell and the rate of baso-lateral iron egress. (16)(Nancy). However, how the signals from erythroid and store regulators are transduced is not fully understood. The Ferrostat referred to above is influenced by:

a). “Mucosal Block”: A recent exposure to an iron bolus in the diet makes the absorptive enterocytes refractory to acquiring additional iron for several days probably due to accumulation of ferritin. The latter makes the enterocytes perceive that its “set-point” has been reached, thereby blocking further entry of iron into the cell.

b). “Stores Regulators”: The Ferrostat is modulated by total body iron, so that the iron flux is upregulated two to three folds in iron deficiency versus iron replete states (17)(17: Nancy). This modulation is probably accomplished through sensing variation in the iron occupancy of plasma transfusion by the “programmed” enterocytes.

c). “Erythropoietic Regulator”: The absorptive enterocytes sense the proliferative activity of erythron through some mysterious signals. It has been observed that increased iron absorption is associated with erythroid hyperplasia in iron deficiency and some other conditions (like thalassaemia, congenital dyserythropoietic anemia, sideroblastic anemia, etc.) in which erythropoiesis is ineffective. It is possible that from the destroyed precursors, some biochemical
signal is sent to affect and modulate the enterocytes (18)(22: Nancy)

The researchers have probed into the possibility of some soluble or cellular signals, which would integrate the role of liver, intestine and bone marrow in iron homeostasis. A possible such regulator is **Hepcidin**. The latter is a small peptide of 20-25 amino acids, which is cleaved from a larger precursor. It is present in the plasma, and is synthesized in the liver and excreted through the kidney. Hepcidin has been hypothesized as a component of both erythroid and stores regulator. It is proposed that secondary to a low erythroid or store Ferrostat, a decrease in Hepcidin expression signals for an increased cellular iron release from stores and increased iron absorption from the intestines. On the contrary, with a high erythroid or store Ferrostat, Hepcidin expression is accentuated. It results in a decrease in the cellular iron release from hepatocytes and macrophages, and a decreased iron absorption from the intestine (50:p 234) Fig 232

**Hepatocyte Regulator (p230)**

**Transport of Iron in the Blood:** After the absorption across the enterocyte, Fe$^{++}$ is instantly oxidized to Fe$^{+++}$ by hephaestin. This protein forms a complex with another iron transporter called IREG (Ferroportin; MTP1) thereby shuttling the Fe$^{+++}$ across the cell membrane into the plasma. (20)(8:Sysmax)

In the plasma, about 4 mg of iron is present in a bound state with a specific transport protein, Transferrin (TF). This is a dynamically important compartment of iron, as it is vitally important for conservation and recycling of iron, and is responsible for the supply of about 30 mg of iron for the daily needs for hemoglobin synthesis.

TF (Mol. Wt. 79.5 kDa) is a single chain glycoprotein, synthesized by the parenchymal cells of the liver. Its synthesis increases inversely proportionately to the amount of iron in the stores, and thus in iron deficiency anemia, transferrin levels are high. Each molecule of transferrin can bind a maximum of two atoms of iron. In a normal individual, only one third of transferrin iron binding sites are saturated, so that the serum transferrin saturation is 30-33% of total iron binding capacity.

Transferrin serves at least three purposes: it solubilizes the Fe$^{+++}$, attenuates its reactivity and facilitates its delivery to cells. (16)(Nancy C Andrews). Plasma TF can bind a maximum of 12 mg of iron in a normal adult. This TIBC is rarely utilized, and most individuals have about 3 mg of circulating iron bound to TF. Consequently, there is little, if any, free iron in the plasma. (16)(Nancy) Transferrin acts as a transporter of iron in the plasma, from site of absorption to body stores, and is also responsible for trafficking of iron between sites of store and sites of haemoglobin synthesis (hemoglobin synthesizing erythroblasts)

**Role of Macrophage in Iron Regulation**

The macrophages especially in the liver, spleen and bone marrow, play an important role at least at two levels:

**a). Heme Oxygenase Activity:** The macrophages recognize and remove senescent and damaged red cells from the circulation. The erythrocytes are decomposed within the macrophages, and iron is removed from hemoglobin in a step catalyzed by the enzyme Heme Oxygenase-1. This enzyme is subject to regulation, and its activity can be induced by a variety of cell stresses including inflammatory cytokines. 21-23)(15,38,61:Nancy)

**b). Iron Egress from the Macrophage:** Ferroportin is highly expressed in macrophages, and is probably implicated in egress of iron from the cell (7,12)(1,19:Nancy) It has been shown that mutations in Ferroportin lead to iron overload, characterized by macrophage iron retention. (24,25)(49,52:Nancy) Ceruloplasmin is also implicated in iron egress from the macrophage 26-30)(32,33,53,62,65)

It is interesting to observe that in HFE-hemochromatosis, iron egress appears to be modulated such that macrophages are iron-depleted. On the contrary, iron modulation is characterized by its retention within the macrophages in case of anemia of chronic disorder (31,32)(9,42:Nancy)

**Transferrin Receptors (TfR)**

On the surface of erythroblasts, transferrin gets specifically attached to TfR (CD71); these are of two types:

**1). TfR-1:** It is a transmembrane glycoprotein having two identical 85 KDa polypeptide chains, joined by disulfide bonds in their extracellular (ecto-) domain. Each ecto-domain can bind one transferrin molecule. (33)(Seligman 1979)

TfR-1 has (O- and N-) glycosylation sites.
Receptors without O-linked glycosylation are cleaved inside the cell, with release of extracellular domains into the plasma as serum TfR.

Proliferating cells have a high expression of TfR as compared to non-proliferating cells (34). The gene for human TfR is located on chromosome 3, and is 32 kb in length. In the same chromosome and region, gene for TF and hypoxia responsive elements is also represented. (35)

2. TfR-2, highly expressed in the liver (36), has its genes on chromosome 7q22. TfR-2 plays some role in iron uptake by erythroid cells; however, it is not regulated by cellular iron status, and has no IRE in the region of the gene (37). It has a lower affinity for TF(Fe)2 (38,39)(35, 36 Nancy). Its role in cellular iron uptake has not yet been defined, but human patients with mutations in the gene encoding TfR-2 develop iron overload. (40)(13 of Nancy)

Serum TfR is elevated in iron deficiency, but may also increase in conditions in which there is increased erythropoietic activity, including hemolytic anemias, thalassemias, polycythemia vera and other myeloproliferative disorders. (41,42)(Beginn 1992, Hueber 1990) In fact sTfR concentration mainly reflects the iron demand of erythropoietic tissue, i.e., both increased erythropoiesis and severe iron deficiency will produce an increase in sTfR (43)(Sysmax J—Rolf Hunzmaan)

Contrary to ferritin concentration during the course of development of iron deficiency, the sTfR concentration remains stable initially. Only when the amount of iron available is so low that erythropoiesis is affected, will the sTfR concentration rise. So, sTfR is the only available serum marker reflecting iron deficient erythropoiesis (44)(14 of Sysmax J). It is also a practically useful observation that the sTfR concentration remains unchanged during acute phase reaction and pregnancy. In this regard it is a superior marker of iron deficiency during infections and other underlying disorder, as compared to serum ferritin level. Furthermore, the diagnostic sensitivity of sTfR measurement is greater than that of the transferrin saturation. (43)(Sysmax J)

It has also been reported that an elevated sTfR/log ferritin ratio (sTfR-F index) reflects functional iron deficiency better than any of the above mentioned parameters. It may be mentioned that the term “functional iron deficiency” is based on the assumption that neither the demand for iron nor the state of iron stores is important alone, but the relationship of the two is (45,46)(15,16 of Sysmax). Serum TfR-F index is influenced by the rise of ferritin during acute phase response. It is suggested that different cut-off values are required for patients with normal CRP (≤5mg/l) and elevated CRP (>5 mg/l)

The specific TfR is located on the surface of many cells, but is most highly represented on those requiring large amounts of iron. The Tf-(Fe)2-TfR interaction allows iron to enter cells through receptor mediated endocytosis (Transferrin cycle) Fig. This mechanism is efficiently displayed in erythroid precursors (47)(43 of Nancy—Levy JE))

The Genes Encoding Ferritin and TfR Synthesis appear to be closely co-ordinated in their expression (48)(Casey:Proven). It has been observed that when iron is abundant, ferritin levels rise with a concomitant fall in sTfR concentration. Conversely, when iron is in short supply, ferritin levels fall and sTfR levels are upregulated to facilitate iron uptake (49)(Proven). Both these processes are regulated simultaneously by an elegant reciprocal and coordinated translational control involving cytoplasmic messenger RNA by the Iron Responsive Element (IRE) and Iron Responsive Protein (IRP) mechanism. The former is a stem loop structure present on ferritin messenger RNA 5'-unsaturated regions, and the latter is an 85 KDa soluble polypeptide that recognizes the IRE. (49)(Proven) IRP-1 has homology to citric acid cycle aconitase (50)(Ronault 1990:Proven)

When cytoplasmic iron is low, IRP-1 binds to the IRE of both TfR and ferritin mRNA. This binding stabilizes TfR mRNA and results in increased TfR translation. At the same time, IRP-1 binds to IRE of ferritin mRNA repressing its translation and thus inhibiting the formation of new apoferritin.

Conversely, when cytoplasmic iron is high, IRP-1 dissociates from IRE on both ferritin and TfR mRNA, resulting in de-repression of apoferritin synthesis and destabilization and accelerated degradation of TfR mRNA. A reciprocal response is exhibited to iron deficiency by increasing TfR production and decreasing iron storage in ferritin, whereas in iron overload, the same mechanism results in decreased TfR production and increased iron accumulation in newly formed ferritin (51,52)(Chain Herscho; Kaptain:Proven)

Therefore, IRP-1 is believed to display as a dual function protein whose activity is controlled through cytoplasmic iron level via the 4Fe-4S clusters, as shown in FIGURE. (Proven)

TfR gene is located at 3q26.2q ter, close to the gene encoding transferrin. The 3'UTR of TfR gene contains five potential stem loop structures that are very similar to ferritin IRE (53,54)(Muller &
Uptake of Iron by Normoblast and Transferrin Cycle (Fig Nancy p.1987)

The circulating iron-transferrin complex (Fe2-Tf) specifically binds to transferrin receptors (TfRs) on the surface of erythroblasts. The clathrin coated pits, where these complexes are formed, invaginate to form specialized endosomes. Since the cytoplasmic membrane also contains transmembrane DMT-1 molecules, the latter are present in the membrane coating the endosomes. The pH within the endosomes decreases significantly due to an active proton pump. This results in the release of iron from transferrin. The released iron moves out of the endosome into the cytosol through transporter DMT-1. The endosome with attached transferrin and TfR re-attaches to the cell membrane, and becomes an integral part of cell membrane again, so that it takes part again in entry of iron into the cell cytoplasm. Figure

In the cytosol, iron moves either to get incorporated and stored in ferritin and haemosiderin, or to enter into the mitochondria. The mitochondria play a unique role in iron metabolism: the terminal step in heme biosynthesis, the incorporation of iron into protoporphyrin IX by the enzyme ferro-chelatase, which occurs in mitochondrial matrix (55,)(Mark D Fleming:p 270, Sem. Hematol 2002).

Mitochondria also play a key role in biogenesis of iron-sulfur (Fe-S) clusters, which are versatile enzymatic co-factors and regulating or structural components of many mitochondrial and several cytoplasmic proteins. Among these, an important example is of the iron sensing activity of IRP-1, which is dependant on the assembly and disassembly of Fe-S clusters (56)(9 of Fleming MD. Semin Hematol p271)

Iron Uptake in Mitochondria: In the cytoplasm of erythroid precursors, iron probably travels in iron3+ form, because Fe2+ is extremely unstable in the presence of oxygen. However, iron must be converted to Fe2+ form in order to cross the inner mitochondrial membrane (57)(13 of Hematology2002). The conversion of Fe3+ to Fe2+ probably occurs due to provision of electrons necessary for the reaction by mitochondrial respiratory chain (58,59)(14,15). The mitochondrial iron uptake is energy dependant, as it requires a membrane potential over the inner membrane surface (57)(13). This membrane potential is created by the respiratory chain which pumps protons out of the mitochondrial matrix and thereby creates a strong proton gradient (Fig5A, p516).

Maintenance of Iron in Fe2+ State: Within the mitochondria, iron must be maintained in Fe2+ form, as iron in Fe3+ state is unavailable for the action of ferro-chelatase in incorporating iron in heme molecule) (60,61)(17; 36 of Fleming MD. Hematology 2002). The prevention of re-oxidation is probably prevented by the Uncoupling Protein-2 (Unp-2), which allows protons to slip back into mitochondrial matrix, thereby diminishing the proton gradient (62)(16). This stimulates the respiratory chain to run faster to restore the gradient. In this way greater heat is generated and more oxygen is consumed.

Mitochondrial iron accumulation resulting in the formation of ring sideroblasts in refractory anemia probably occurs because iron ion the mitochondria is not in the right form (i.e., Fe2+). It has been shown that iron accumulates in sideroblasts in Fe3+ form, mainly as ferric phosphate (63)(10). Since ferro-chelatase accepts only Fe2+ for heme synthesis, it cannot be utilized in Fe3+ form, and thus it accumulates within the mitochondrial matrix (64)(11). It has now been clearly shown that in Refractory Anemia (RA), there is no deficiency of ferro-chelatase (64)(11).

<table>
<thead>
<tr>
<th>Amount of Iron in Average Adult</th>
<th>Male (g)</th>
<th>Female (g)</th>
<th>Percentage of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>2.4</td>
<td>1.7</td>
<td>65</td>
</tr>
<tr>
<td>Ferritin and</td>
<td>1.0</td>
<td>0.3</td>
<td>30</td>
</tr>
<tr>
<td>haemosiderin</td>
<td>(0.3-1.5)</td>
<td>(0-1.0)</td>
<td></td>
</tr>
<tr>
<td>Myoglobin</td>
<td>0.15</td>
<td>0.12</td>
<td>3.5</td>
</tr>
<tr>
<td>Haem enzymes (e.g. cytochromes, catalase)</td>
<td>0.02</td>
<td>0.015</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Table 3.1: The Distribution of Body Iron
peroxidases, flavoproteins

Transferin-bound iron 0.004 0.003 0.1

References

6. Suominen P. Serum transferrin receptor and transferrin receptor-feritin index identify subjects with subclinical iron deficits. Blood. 1990;89:2934-2938


