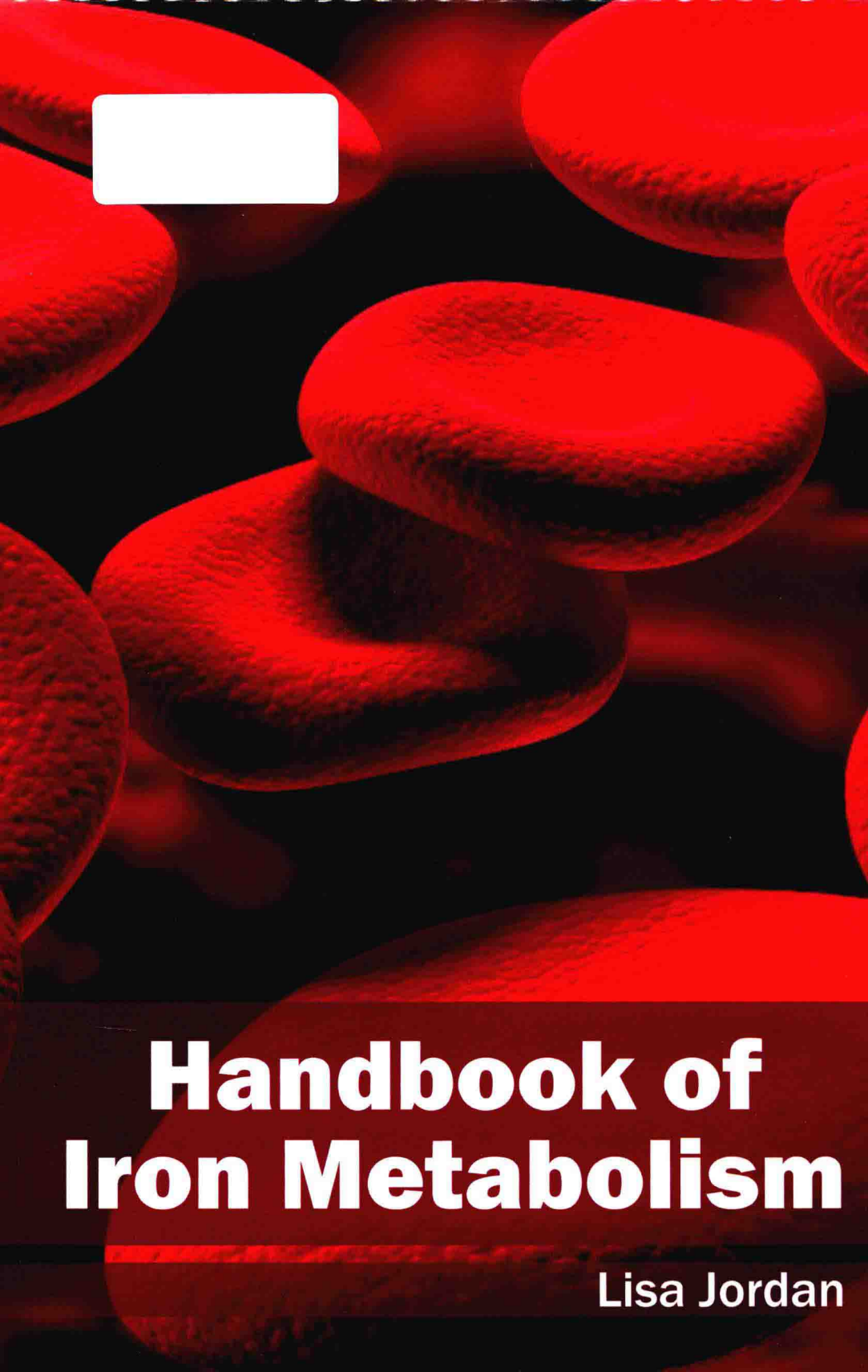

Handbook of Iron Metabolism

Lisa Jordan

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Edited by **Lisa Jordan**

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Preface

This book has been an outcome of determined endeavour from a group of educationists in the field. The primary objective was to involve a broad spectrum of professionals from diverse cultural background involved in the field for developing new researches. The book not only targets students but also scholars pursuing higher research for further enhancement of the theoretical and practical applications of the subject.

Iron is an essential of human life. This book deals with various aspects related to iron metabolism. Iron has a variety of functions in the body, inclusive of the metabolism of oxygen in a range of biochemical activities. Iron, as heme or in its nonheme form, plays a significant role in key reactions of DNA fusion and energy creation. Low solubility of iron in body fluids and the capability of iron to form toxic fluids in contact with oxygen make iron consumption, use and storage a serious issue. The detection of new metal transporters, receptors and peptides, and detection of innovative cross-interactions between known proteins are now leading to a development in the comprehension of systemic iron metabolism.

It was an honour to edit such a profound book and also a challenging task to compile and examine all the relevant data for accuracy and originality. I wish to acknowledge the efforts of the contributors for submitting such brilliant and diverse chapters in the field and for endlessly working for the completion of the book. Last, but not the least; I thank my family for being a constant source of support in all my research endeavours.

Editor

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Systemic Iron Metabolism in Physiological States

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Iron Metabolism in Humans: An Overview

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1. Introduction

Iron is the most abundant element on earth, yet only trace elements are present in living cells. The four major reasons leading to limited availability of iron in living cells despite environmental abundance would be:

1. When iron was available some 10 billion years ago, it was available as Fe (II), but Fe (II) is not a very strong Lewis acid. Thus, it does not bind strongly to most small molecules or activate them strongly toward reaction.
2. Today iron is not readily available from sea or water solutions due to oxidation and hydrolysis.
3. Iron in ferrous state is not easily retained by proteins since it does not bind very strongly to them.
4. Free Fe (II) is mutagenic, especially in the presence of dioxygen.

To overcome, the above problems with availability of iron, specific ligands have evolved for its transport and storage because of its limited solubility at near neutral pH under aerobic conditions [1].

Iron is involved in many enzymatic reactions of a cell; hence it is believed that the presence of iron was obligatory for the evolution of aerobic life on earth. Furthermore, the propensity of iron to catalyze the oxygen radicals in aerobic and facultative anaerobic species indicates that the intracellular concentration and chemical form of the element must be kept under tight control.

2. Overview of iron metabolism

2.1 Oxidation states

The common oxidation states are either ferrous (Fe^{2+}) or ferric (Fe^{3+}); higher oxidation levels occur as short-lived intermediates in certain redox processes. Iron has affinity for electronegative atoms such as oxygen, nitrogen and sulfur, which provide the electrons that form the bond with iron, hence these atoms are found at the heart of the iron-binding centers of macromolecules. When favorably oriented on the macromolecules, these anions can bind iron with high affinity. During formation of complexes, no bonding electrons are

derived from iron. The non bonding electrons in the outer shell of iron (the incompletely filled *3d* orbitals) can exist in two states. When bonding interactions with iron are weak, the outer non-bonding electrons will avoid pairing and distribute throughout the *3d* orbitals. When bonding electrons interact strongly with iron, there will be pairing of the outer non-bonding electrons, favoring lower energy *3d* orbitals. These two different distributions for each oxidation state of iron can be determined by electron spin resonance measurements. Dispersion of *3d* electrons to all orbitals leads to the high-spin state, whereas restriction of *3d* electrons to lower energy orbitals, because of electron pairing, leads to a low-spin state.

3. Distribution and function

The total body iron in an adult male is 3000 to 4000 mg. In contrast, the average adult woman has only 2000-3000 mg of iron in her body. This difference may be attributed to much smaller iron reserves in women, lower concentration of hemoglobin and a smaller vascular volume than men.

Iron is distributed in six compartments in the body.

i. Hemoglobin

Iron is a key functional component of this oxygen transporting molecule. About 65% to 70% total body iron is found in heme group of hemoglobin. A heme group consists of iron (Fe^{2+}) ion held in a heterocyclic ring, known as aporphyrin. This porphyrin ring consists of four pyrrole molecules cyclically linked together (by methene bridges) with the iron ion bound in the center [Figure 1] [2]. The nitrogen atoms of the pyrrole molecules form coordinate covalent bonds with four of the iron's six available positions which all lie in one plane. The iron is bound strongly (covalently) to the globular protein via the imidazole ring of the F8 histidine residue (also known as the proximal histidine) below the porphyrin ring. A sixth position can reversibly bind oxygen by a coordinate covalent bond, completing the

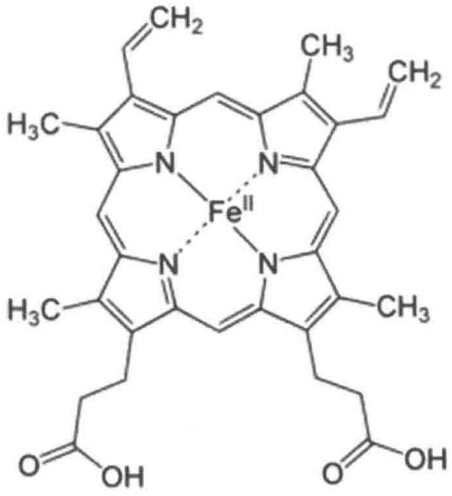

Fig. 1. Structure of heme showing the four coordinate bonds between ferrous ion and four nitrogen bases of the porphyrin rings.

octahedral group of six ligands [Figure 2]. This site is empty in the nonoxygenated forms of hemoglobin and myoglobin. Oxygen binds in an "end-on bent" geometry where one oxygen atom binds Fe and the other protrudes at an angle. When oxygen is not bound, a very weakly bonded water molecule fills the site, forming a distorted octahedron.

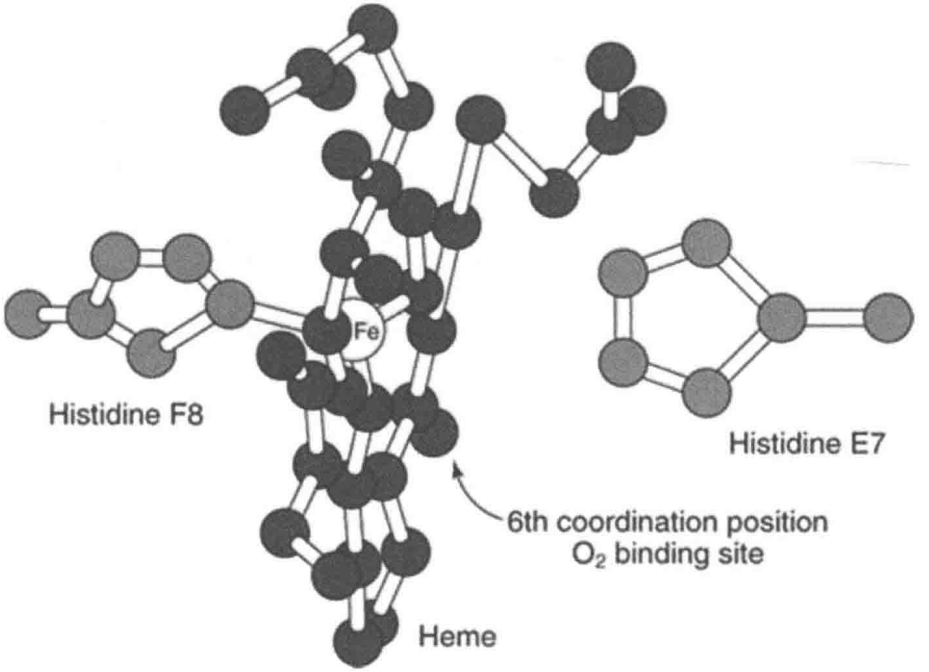

Fig. 2. Structure of heme showing the square planar tetrapyrrole along with the proximal and the distal histidine.

Even though carbon dioxide is also carried by hemoglobin, it does not compete with oxygen for the iron-binding positions, but is actually bound to the protein chains of the structure. The iron ion may be either in the Fe^{2+} or in the Fe^{3+} state, but ferrihemoglobin also called methemoglobin (Fe^{3+}) cannot bind oxygen [3]. In binding, oxygen temporarily and reversibly oxidizes (Fe^{2+}) to (Fe^{3+}) while oxygen temporarily turns into superoxide, thus iron must exist in the +2 oxidation state to bind oxygen. If superoxide ion associated to Fe^{3+} is protonated the hemoglobin iron will remain oxidized and incapable to bind oxygen. In such cases, the enzyme methemoglobin reductase will be able to eventually reactivate methemoglobin by reducing the iron center.

ii. Storage Iron- Ferritin and Hemosiderin

Ferritin is the major protein involved in the storage of iron. The protein consists of an outer polypeptide shell (also termed apoferritin) composed of 24 symmetrically placed protein chains (subunits), the average outside diameter is approximately 12.0 nm in hydrated state. The inner core (approximately 6.0 nm) contains an electron-dense and chemically inert inorganic ferric "iron-core" made of ferric oxyhydroxyhydroxide phosphate [$(FeOOH)_8(FeO\text{-}OPO_3H_2)$]. [Figure 3]. The ferritins are extremely large proteins (450kDa)

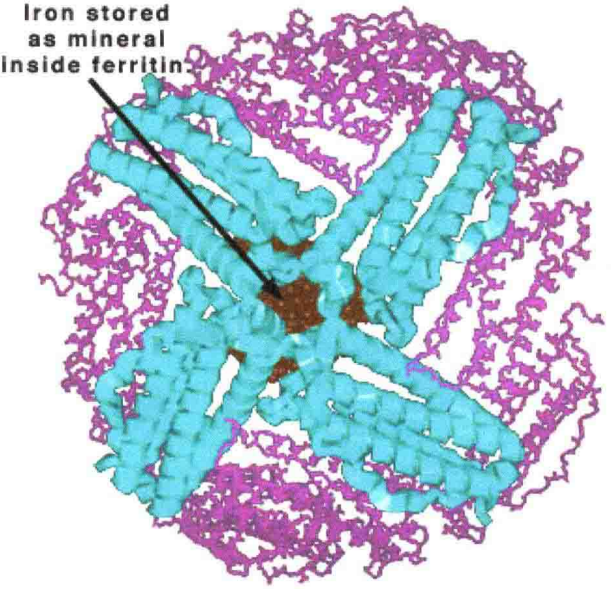

Fig. 3. Structure of ferritin showing the outer polypeptide shell with inner iron-core containing iron stored as mineral -ferric oxyhydroxyhydroxide phosphate [$(FeOOH)_8$ ($FeO\text{-}OPO_3H_2$)].

which can store upto 4500 iron atoms as hydrous ferric oxide. The ratio of iron to polypeptide is not constant, since the protein has the ability to gain and release iron according to physiological needs. Channels from the surface permit the accumulation and release of iron. All iron-containing organisms including bacteria, plants, vertebrates and invertebrates have ferritin [4,5].

Ferritin from humans, horses, pigs and rats and mice consists of two different types of subunits- H subunit *(heavy; 178 amino acids)* and L *(Light, 171 amino acids)* that provide various isoprotein forms. H subunits predominate in nucleated blood cells and heart. L - subunits in liver and spleen. H-rich ferritins take up iron faster than L-rich *in -vitro* and may function more in iron detoxification than in storage [6]. Synthesis of the subunits is regulated mainly by the concentration of free intracellular iron. The bulk of the iron storage occurs in hepatocytes, reticuloendothelial cells and skeletal muscle. When iron is in excess, the storage capacity of newly synthesized apoferritin may be exceeded. This leads to iron deposition adjacent to ferritin spheres. This amorphous deposition of iron is called **hemosiderin** and the clinical condition is termed as hemosiderosis.

Multiple genes encode the ferritin proteins, at least in animals, which are expressed in a cell-specific manner. All cells synthesize ferritin at some point in the cell cycle, though the amount may vary depending on the role of the cell in iron storage, i.e housekeeping for intracellular use or specialized for use by other cells.

Expression of ferroportin (FPN) results in export of cytosolic iron and ferritin degradation. FPN-mediated iron loss from ferritin occurs in the cytosol and precedes ferritin degradation

by the proteasome. Depletion of ferritin iron induces the monoubiquitination of ferritin subunits. Ubiquitination is not required for iron release but is required for disassembly of ferritin nanocages, which is followed by degradation of ferritin by the proteasome [7].

iii. Myoglobin

Myoglobin is an iron- and oxygen-binding protein found in the muscle tissue of vertebrates in general and in almost all mammals. It is a single-chain globular protein of 153 or 154 amino acids [8,9], containing a heme prosthetic group in the center around which the remaining apoprotein folds. It has eight alpha helices and a hydrophobic core. It has a molecular weight of 17,699 daltons (with heme), and is the primary oxygen-carrying pigment of muscle tissues [9]. Unlike the blood-borne hemoglobin, to which it is structurally related [10], this protein does not exhibit cooperative binding of oxygen, since positive cooperativity is a property of multimeric /oligomeric proteins only. Instead, the binding of oxygen by myoglobin is unaffected by the oxygen pressure in the surrounding tissue. Myoglobin is often cited as having an "instant binding tenacity" to oxygen given its hyperbolic oxygen dissociation curve [Figure 4].

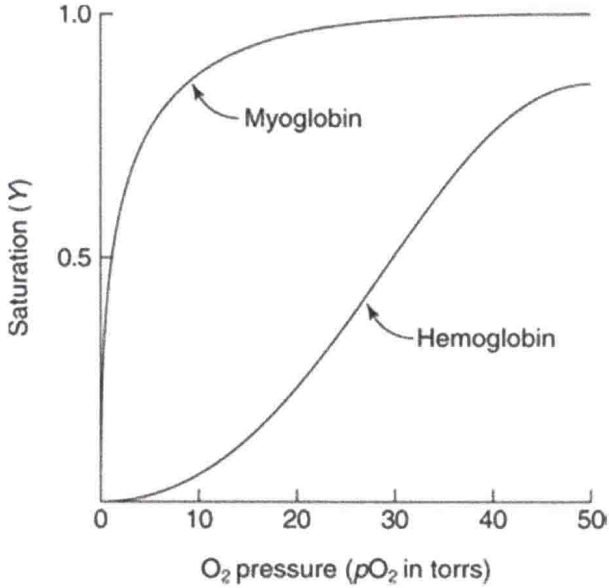

Fig. 4. Iron dissociation curve of hemoglobin and myoglobin.

iv. Transport Iron- Transferrin

Transferrin is a protein involved in the transport of iron. The transferrins are glycoproteins with molecular weight of approximately 80, 000 Da, consisting of a single polypeptide chain of 680 to 700 amino acids and no subunits. The transferrins consist of two non cooperative iron- binding lobes of approximately equal size. Each lobe is an ellipsoid of approximate dimensions 55 x35 x 35A° and contains a metal binding site buried below the surface of the protein in a hydrophilic environment [Figure 5]. The two binding sites are separated by 42 A° [11]. There is approximately 40% identity in the amino acid sequence between the two

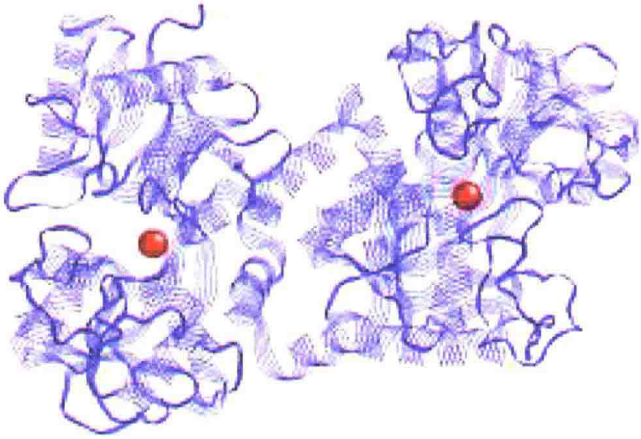

Fig. 5. Bilobar structure of Human transferrin

lobes [12, 13]. The protein is a product of gene duplication derived from a putative ancestral gene coding for a protein binding only one atom of iron.

The transferrins are highly cross-linked proteins, the number of disulfide bridges varying between proteins and between domains within each protein. There are six disulfide bonds conserved in each of the two-domains of all the transferrins, plus additional ones for the individual proteins. Human serum transferrin is the most cross- linked, having 8 and 11 disulfide bridges in the N- and C- terminal metal-binding lobes. The transferrins, with the exception of lactoferrin, are acidic proteins, having an isoelectric point (pI) value around 5.6 to 5.8.

Several metals bind to transferrin; the highest affinity is for Fe^{3+}; Fe^{2+} ion is not bound. Various spectroscopic and chemical modification studies have implicated histidine, tyrosine, water (or hydroxide) and (bi) carbonate as ligands to the Fe^{3+} in the metal-protein complex.

The transferrins are unique among proteins in their requirement of coordinate binding of an anion (bicarbonate) for iron binding [14,15]. Several studies suggest that the bicarbonate is directly coordinated to the iron, presumably forming a bridge between the metal and a cationic group on the protein. In the normal physiological state, approximately one-ninth of all the transferrin molecules are saturated with iron at both sides; four-ninths of transferrin molecules have iron at either site; and four-ninth of transferrin molecules are free of iron.

Transferrin delivers iron to cells by binding to specific cell surface receptors (TfR) that mediate the internalization of the protein. The TfR is a transmembrane protein consisting of two subunits of 90,000 Da each, joined by a disulfide bond. Each subunit contains one transmembrane segment and about 670 residues that are extracellular and bind a transferrin molecule, favoring the diferric form. Internalization of the receptor- transferrin complex is dependent on receptor phosphorylation by a Ca^{2+}- Calmodulin- protein kinase C complex. Release of the iron atoms occurs within the acidic milieu of the lysososme after which the receptor- apotransferrin complex returns to the cell surface where the apotransferrin is released to be reutilized in the plasma [Figure 6]. Inside the cell, iron is used for heme synthesis within the mitochondria, or is stored as ferritin.

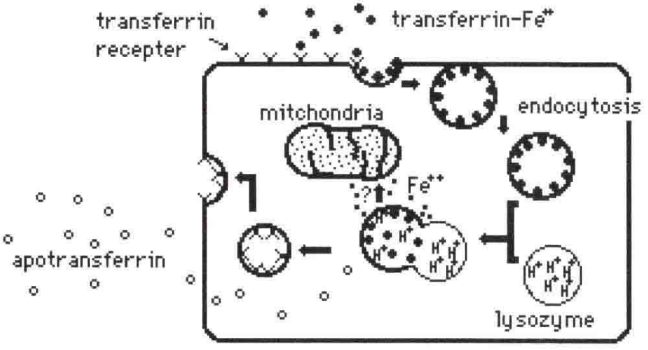

Fig. 6. Cellular uptake of iron by transferrin receptor

v. Labile iron Pool

The uptake and storage of iron is carried out by different proteins, hence a pool of accessible iron ions, called labile iron pool (LIP) exists, that constitutes crossroads of the metabolic pathways of iron containing compounds [16]. The LIP is localized primarily but not exclusively, within the cytoplasm of the cells. It is bound to low-affinity ligands [17] and is accessible to permeant chelators and contains the cells' metabolically and catalytically reactive iron. LIP is maintained by a balanced movement of iron from extra- and intracellular sources [18].

The trace amounts of "free" iron can catalyse production of a highly toxic hydroxyl radical via Fenton/Haber-Weiss reaction cycle. The critical factor appears to be the availability and abundance of cellular labile iron pool (LIP) that constitutes a crossroad of metabolic pathways of iron-containing compounds and is midway between the cellular need of iron, its uptake and storage. To avoid an excess of harmful "free" iron, the LIP is kept at the lowest sufficient level by transcriptional and posttranscriptional control of the expression of principal proteins involved in iron homeostasis [19].

vi. Other heme proteins and flavoproteins

Certain enzymes also contain heme as part of their prosthetic group (e.g. catalase, peroxidases, tryptophan pyrrolase, guanylate cyclase, Nitric oxide synthase and mitochondrial cytochromes).

Iron readily forms clusters linked to the polypeptide chain by thiol groups of cysteine residues or to non-proteins by inorganic sulphide and cysteine thiols leading to generation of iron- sulphur clusters. Examples of iron-sulphur proteins are the ferredoxins, hydrogenases, nitrogenases, NADH dehydrogenases and aconitases. Structure of most of these proteins dictates their function.

4. Physiological turnover of iron in the body

Daily requirements for iron vary depending on the person's age, sex and physiological status. Although iron is not excreted in the conventional sense, about 1 mg is lost daily

through the normal shedding of skin epithelial cells and cells that line the gastrointestinal and urinary tracts. Small numbers of erythrocytes are lost in urine and feces as well. Humans and other vertebrates strictly conserve iron by recycling it from senescent erythrocytes and from other sources. The loss of iron in a typical adult male is so small that it can be met by absorbing approximately 1 mg of iron per day [20] [Figure 7]. In comparison, the daily iron requirement for erythropoiesis is about 20 mg. Such conservation of iron is essential because many human diets contain just enough iron to replace the small losses. However, the blood lost in each menstrual cycle drains 20 to 40 mg of iron, so women in their reproductive years need to absorb approximately 2 mg of iron per day. However, when dietary iron is more abundant, absorption is appropriately attenuated.

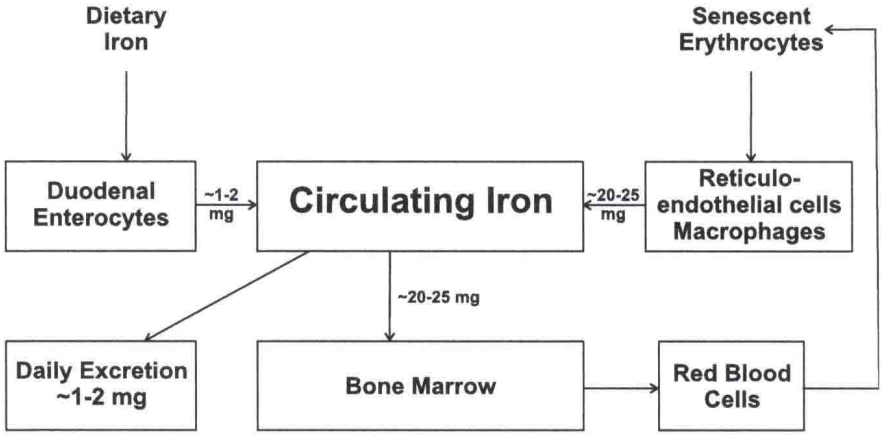

Fig. 7. Diagram showing the physiological turnover of iron in the body

5. Mechanisms regulating Iron absorption

The iron stores in the body are regulated by intestinal absorption. Intestinal absorption of iron is itself a regulated process and the efficacy of absorption increases or decreases depending on the body requirements of iron.

The dietary iron, which exists mostly in the ferric form, is converted to the more soluble ferrous form, which is readily absorbed. The ferric form is reduced to ferrous by the action of acids in stomach, reducing agents such as ascorbic acid, cysteine and -SH groups of proteins. Entry of Fe^{3+} into the mucosal cells may be aided by an enzyme on the brush-border of the enterocyte (the enzyme possesses ferric reductase activity also). The ferrous ion is then transported in the cell by a divalent metal transporter (DMT1) [Figure 8].

In the intestinal cell, the iron may be (a) stored by incorporation into ferritin in those individuals who have adequate plasma iron concentration. A ferroxidase converts the absorbed ferrous iron to the ferric form, which then combines with apoferritin to form ferritin, or (b) transported to a transport protein at the basolateral cell membrane and released into the circulation. However, the basolateral-transport protein has not yet been identified, It is believed to work in combination with hephaestin, a copper-containing protein, which oxidizes Fe^{2+} back to Fe^{3+}.