Iron and blood donation: A Review

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Abstract

Iron is essential mineral necessary for the transport of oxygen. Humans can get iron from oral iron supplement or IV injections. It can also be obtained from blood transfusion. Iron content of the diet depends on the type of food eaten and the total daily caloric intake. Absorption of iron is a very complex process that takes place in the duodenum and upper jejunum. This process involves number of proteins that transport iron across the apical membrane, proteins that transport iron through the basolateral membrane of enterocyte , proteins that change redox state of iron and thus assist in its transport. The paper reviewed iron and blood donation.

Keywords: Iron, Iron absorption, Iron metabolism, Blood donation

Introduction

Iron is essential mineral necessary for the transport of oxygen. Disorders of iron homeostasis are among the most common human disorders. Although it is the fourth most abundant element in Earth´s crust, iron bioavailability is very low and despite a low daily requirements iron deficiency is the most common nutritional disorder in the world.

According to Andrew (2002), humans can get iron from oral iron supplement or IV (intravenous) injections.It can also be obtained from blood transfusion.Bishop *et al.* (1996) noted that most of the iron is obtained from food. Iron content of the diet depends on the type of food eaten and the total daily caloric intake.

Good sources of dietary iron include red meat, fish, poultry, lentils, beans, leaf vegetables, chickpeas, black-eyed peas, and fortified bread.Dietary iron is available in two forms called haem and non-haem. Haem iron is present in fish, meat and chicken, while non-haem iron is available in vegetables, grains and cereals (Sembulingam *et al.,* 2006).Iron is present in food as ferric hydroxides, ferric-protein complexes and haem protein complexes (Hoffbrand *et al.*, 2004). The recommended daily allowance (RDA) represents a daily nutrient intake goal for healthy individuals that should prevent deficiency disease in 97% of the healthy population. Tolerable upper intake level (UL) is the largest amount of a nutrient that healthy individuals can take each day

without being placed at increased risk of adverse health effects or any kind of adverse reaction (negative effect).The UL for iron is 45gm per day. The RDA for iron and all other nutrients is established by the food and nutrition board of the National Academies.

The daily requirement for iron varies according to age, sex, weight, and state of health (Bishop *et al.*, 1996).A well-balanced diet contains sufficient iron to meet up to body requirements. In adult males, the daily requirement is approximately 10 mg/day, which is easily obtained by a well-balanced diet. Table 2.1 shows RDA for iron that is age and sex specific.

Table 1 Minimal Daily Iron Requirements

Age Group	Amount that must be absorbed daily for haemoglobin synthesis (mg)	Minimal amount that should be ingested daily (mg) (RDA)
Infants	1	10
Children	0.5	5
Young nonpregnant women	2	20
Pregnant women	3	30
Men and postmenopausal women	1	10

Lichtman et al*., 2006.*

Iron Metabolism

Iron metabolism is a set of chemical reactions maintaining homeostasis of iron. Bishop *et al.,* (1996) asserts that iron homeostasis is maintained under tight control to ensure that appropriate requirements for normal physiological and biochemical functions are maintained. Any alteration due to deficit or an excess of iron in the body often produces significant biochemical alterations. Iron is involved in a broad range of biologically important reactions. It is a component of innumerable haemoproteins including oxygen transport proteins,haeme containing enzymes and many essential non-haeme iron proteins that catalyse a wide range of reactions and have a central role in mechanism for oxygen sensing (Papanikolaou *et al.,* 2005; Andrew *et al.,* 2002).Iron is a transition metal and it exists in two readily reversible redox states: reduced ferrous (Fe(II)) form and oxidized ferric (Fe(III)) form. At physiological oxygen concentrations the stable state of iron in most of its biological complexes is Fe(III) (Aisen *et al.*, 2001).Reduction reactions play a crucial role in the iron metabolism, because only reduced iron ion can be a substrate for transmembrane transport of iron, loading and releasing of iron from ferritin and for haeme synthesis (Aisen *et al.*, 2001; Watt, 2010).Although biological function of iron is largely attributable to its chemical properties as a

transition metal, exactly these properties render it potentially toxic. Iron excess is believed to generate oxidative stress due to its ability to generate ROS (Puntarulo, 2005).Fe(II) catalysed reduction of one electron in O_2molecule results in the formation of superoxide anion which further leads to the sequence of well-known Haber-Weis-Fenton´s reactions that generate ROS which can possibly damage macromolecules such as proteins, lipids and nucleic acids (Aisen *et al.*, 2001).

Iron Absorption

The body has no effective means of excreting iron and thus the regulation of absorption of dietary iron from the duodenum plays a critical role in iron homeostasis in the body.

A. Intestinal iron absorption

Absorption of iron is a very complex process that takes place in the duodenum and upper jejunum.This process involves number of proteins that transport iron across the apical membrane (importers), proteins that transport iron through the basolateral membrane of enterocyte (exporters), proteins that change redox state of iron and thus assist in its transport.There is no known regulated pathway of iron excretion so body iron content is regulated by precisely controlled intestinal absorption.

The intestinal mucosa responds to changes in body iron stores, tissue hypoxia, and demand for iron, and it alters absorption accordingly (Leida *et al.*, 2012).Absorption is increased in iron deficiency while is reduced in the iron overload. Dietary iron is present in food as one of two forms - as inorganic or haeme iron.Inorganic form is dominant in the standard diet, and makes up about 90% of the total amount present in food. Haeme accounts for only 10% of the dietary iron (Munoz *et al.*, 2011).Despite the relatively low participation in diet, haeme is a highly bioavailable source of iron whose absorption is significantly more efficient than the absorption of inorganic form because it is not affected by the dietary constituents that adversely affect the absorption of inorganic form (Conra *et al.*, 2002)

B. Absorption of inorganic iron

The most dietary inorganic iron is in ferric form and it must be first reduced by brush border ferrireductase, duodenal cytochrome B (DcytB) (Mckie *et al.*, 2001).Recently, there are some data showing that members of six-transmembrane epithelial antigen of the prostate (STEAP) family are also expressed in intestine (Atanasova *et al.*, 2005).The exact role of both types reductases especially in human iron absorption remains uncertain (Ohagami *et al.*, 2006). Ferrous iron is then transported across the membrane into the cytoplasm via divalent metal transporter 1 (DMT1) expressed on apical membrane of the duodenal enterocytes (Canonne *et al.*, 1999). DMT1 is not specific for iron transport but also mediates transport of other divalent metal cations including zinc, manganese and copper although it seems that its primary physiological role is iron transport (Aisen *et al.*, 2001; Canonne *et al.*, 1999). This transport protein is also expressed on the membrane of endosomes where mediates iron transport from endosomes into the cytoplasm during transferrin cycle (Andrew, 2002). DMT-1 seems to have a role in transport of non transferrin bounded iron (NTBI), especially in conditions of iron excess (Garric *et al.*, 2006).

Some researchers have shown that duodenal ferric iron uptake proceeds through a separate, although less understood pathway. While ferrous iron uses DMT-1, ferric uses integrin-mobilferrin pathway (IMT) that solely transports ferric iron, not other metals of nutritional importance (Conrad *et al.*, 2002; Conrad *et al.,* 2000).This pathway involves several proteins like mobilferrin, beta-3-integrin and flavin-monooxygenase. Flavin-monooxygenase has a role of ferrireductase. In the cell cytosol these proteins are integrated into a large protein complex called paraferritin (Umbreit *et al.*, 2002). Western Blott analysis of paraferritin revealed that it also contains beta-2-microglobulin and DMT-1.The presence of considerable fraction of DMT-1, mobilferrin and hephaestin in the cytoplasm of cells indicates a possible intracellular role of these proteins (Ranganathan *et al.*, 2012; Simovich *et al.*, 2002).

C. Absorption of haem iron

Transport of haem across the membrane is not required only for heme iron absorption in gut but also for cellular haeme turnover. Synthesis of haeme partially takes place in the mitochondria and after that haeme must be transported to the endoplasmic reticulum to be included in haemoproteines.Haeme is involved in transcriptional regulation of some genes therefore also needs to be transported in the cell nucleus (Hou *et al.*, 2006). Studies have shown that intact haeme moiety is absorbed by intestinal enterocytes via haeme-carrier protein 1 (HCP1), transport protein expressed at high levels in duodenum (Lantunde *et al.*, 2006; Shayeghi *et al.*, 2005).Some recent investigation indicated that described haeme intestinal transporter might be folate transporter. Within the cell iron is released from protoporphyrin ring by heme oxygenase 1 (HO-1) and converges with the cytosolic iron pool of enterocyte.Afterwards, two forms of iron share the same pathway - enter the inorganic iron pool of the enterocytes.It seems that step catalysed by HO-1 is limiting factor in the absorption of haeme.

Iron Transport

A. Intracellular iron transport

Once iron enters the intestinal epithelial cells through the apical membrane, it could be sequestrated as ferritin or transported into circulation across the basolateral membrane.

Absorptive enterocytes perform their function for two days and then are being shed into intestinal lumen. Iron that is not exported from enterocytes into the plasma is lost by exfoliation of intestinal epithelium. Therefore, transport of iron by ferroportin across the basolateral membrane determines whether iron is delivered into the circulation or removed from the body with shed enterocytes(Leida *et al.*, 2012). Exfoliation of epithelial cells of intestinal mucosa may represent pathway of regulated iron excretion because these cells at the basolateral membrane express transferrin receptors type 1 (TfR1) and iron from the plasma can enter the cell by receptor mediated endocytosis, but the capacity of this mechanism of excretion in response to the accumulation of iron is very limited (Gantz *et al.*, 2006). To be transported through the basolateral membrane iron must first be transported through the cell cytoplasm. Transport of iron across the enterocyte cytoplasm is the least understood step in iron absorption. There are two possible mechanisms of transport that do not exclude each other: transport of iron associated with some proteins (chaperones) or transcytosis (Ma *et al.,* 2006).Although the molecular details have yet to be explored, cytosolic monothiol glutaredoxins and Poly(rC)-binding proteins could act as iron chaperones and thus may represent the basic cellular machanism for intracellular iron delivery (Philpott *et al.*, 2012; Shi *et al.*, 2008).

B. Iron transport in plasma

The plasma transferrin compartment functions as transit compartment through which flows about 20 mg of iron each day (Gkouvatsos *et al*., 2012). Principles of iron transport are partially dictated by its chemical properties. Binding of iron to transferrin, a major iron transporter in the blood, provide solubility, reduce reactivity and thus provides a safe and controlled delivery of iron to all cells in the body.Transferrin is serum glycoprotein with molecular weight about 75–80 kDa. Molecule is folded into two globular domains, each containing a specific binding site for single Fe(III). Affinity of transferrin molecule for iron at physiological pH is extremely high so almost all non-heme iron in circulation is bounded to transferrin (Baker *et al.*, 2003). Under circumstances of iron overload NTBI appears in plasma. NTBI refers to all forms of iron in the plasma that binds to ligands other than transferrin. Iron is bounded to these ligands with substantially less affinity than to transferrin. This form of iron is very reactive and could enter Fenton reaction. NTBI is likely to be a major contributor to iron loading in hepatocytes under conditions of elevated transferrin saturation, capable to freely enter the cell so it is considered to be a marker of iron toxicity (Duk-Hee *et al.*, 2004; Brissot *et al.*, 2012).

C. Iron uptake in the tissues

Transferrin is taken up into the cell by transferrin mediated endocytosis in so called transferrin cycle. Under physiological conditions this cycle enables controlled access of iron to cells because individual cells can efficiently regulate the entry of iron by regulating the expression of TfR1 at the surface, according to their iron needs (Leida *et al.*, 2012).

(i)   Transferrin receptors

Two types of functionally different transferrin receptors are described, TfR1 and transferrin receptor 2 (TfR2). TfR1 is expressed by all iron-requiring cells but level of expression varies greatly (Aisen *et al.*, 2004). It is highly expressed on immature erythroid cells, rapidly dividing cells (normal and malignant), placental tissue. TfR1 is a transmembrane glycoprotein comprised of two identical disulfide bounded subunits with a molecular mass of approximately 90 kDa. Each subunit possesses one binding site for the transferrin. Diferric transferrin has a higher affinity for TfR1 than monoferric form or iron-free apotransferrin (Leida *et al.*, 2012). Besides the membrane-associated TfR1, a soluble form of this receptor exists in human serum which represents a soluble fragment of the extracellular receptor domain. Soluble transferrin receptor (sTfR) is released by proteolytic cleavage of the protein C-terminal end. It is proposed that release of sTfR is regulated by binding of its ligand transferrin (Dassler *et al.*, 2006). The level of sTfR reflects the availability of functional iron.

TfR2 is predominantly expressed in liver, hematopoetic cells, duodenal crypt cells, and it overlaps with hereditary hemochromatosis protein (HFE). TfR2 binds to HFE and transferrin, but interacting domains of HFE with TfR2 are different from those of TfR1. It is assumed that TfR2/HFE complex is required for transcriptional regulation of hepcidin production by diferric transferrin (Gao *et al.*, 2009; Rapisarda *et al.*, 2010; Falzacappa *et al.*, 2005; Goswami *et al.*, 2006; Wallace *et al.*, 2007).

(ii)   Transferrin cycle

Binding of diferric transferrin to TfR1 at the surface of the cell triggers clathrin-mediated endosome formation and initiate transferrin cycle. Action of proton pump on endosome membrane acidifies endosome content and leads to a conformational changes of transferrin as well as transferrin receptor, resulting in iron release (Ponka *et al.*, 1999). Fe(III) is then reduced by ferrireductase STEAP3 and iron is transported across the membrane of endosome into the cytoplasm via DMT1 (Ohgami *et al.,* 2005). Apotransferrin is then returned to the cell surface completing the transferrin cycle and is released to be recharged with iron. TfR1 is presented for a new uptake cycle. During its lifetime transferrin makes around 100-200 cycles of iron transport.

After cellular iron uptake iron enters poor characterized "labile iron pool" (LIP). LIP is defined as pool of iron complexed with low affinity ligands (citrate, ATP, amino acids, ascorbic acid or by unidentified chaperones). Recent study identified iron(II)glutathione as the dominant component of this pool (Hider *et al.,* 2011). LIP represents < 5% of the total cellular iron (Andrew 2005). It is dynamic compartment that supplies iron to the mitochondrion for haeme and iron sulfur cluster synthesis or could be used for synthesis of iron-containing proteins in cytosole thereby controlling numerous metabolic reactions. Iron in the LIP that exceeds requirement for the synthesis of haeme and non-haeme iron containing proteins is stored within ferritin to minimize free iron because LIP is catalytically active and capable of initiating free radical reactions (Schneider *et al.*, 2000). Quantification of LIP is possible with novel non-disruptive technique that use fluorescent metalosensors and may be clinically significant in states of iron overload (Kakhlon *et al.*, 2002).

Under normal circumstances entry of transferrin bounded iron is the main route of iron entry into cells but the pathological accumulation of iron leads to transferrin saturation and appearing of NTBI which can enter into cells via transferrin-independent pathway (Brissot *et al.*, 2012)

D   Liver Iron Transport

The liver is the main storage organ for iron. In iron overload, free radical formation and generation of lipid peroxidation products may result in progressive tissue injury and eventually cirrhosis or hepatocellular carcinoma. Iron is sequestrated in hepatocytes predominantly in the form of ferritin or haemosiderin. The uptake of transferrin-bound iron (TBI) by the liver from plasma is mediated by two transferrin receptors - transferrin receptor 1 (TfR1) and TfR2. In iron overload, TfR1 is down-regulated in hepatocytes and in untreated HH subjects there is a complete absence of TfR1 expression on hepatocytes (Trinder *et al.*, 1990). The haemochromatosis protein (HFE*)* is also expressed by hepatocytes and is likely to regulate TfR1- mediated uptake of TBI.

TfR2 is highly expressed in human liver and is likely to play an important role in liver iron loading in iron overload states (Kawabata *et al.*, 1999). Unlike TfR1, TfR2 lacks an iron response element and thus is not reciprocally regulated in response to the level of plasma iron. Instead, TfR2 protein expression is regulated by transferrin saturation. TfR2 protein is up-regulated in iron overload and in the Hfe knockout mouse model of HH and may contribute to increased TBI uptake by the liver in iron overload (Johnson *et al.*, 2004). It has a 30-fold lower affinity than TfR1 for TBI but has a higher capacity to transport TBI into the hepatocyte. In normal and iron loaded conditions, expression of TfR2 exceeds that of TfR1 suggesting that TfR2 plays an important role in hepatic iron loading in HH.

As transferrin becomes saturated in iron overload states, excess iron is also found as non transferrin-bound iron (NTBI) and is likely to play an important role in hepatocyte iron loading in HH and other iron overload conditions. NTBI is extremely toxic and is cleared rapidly from plasma by the liver. It has been shown that humans and mice lacking transferrin develop massive iron overload in non-haematopoietic tissues such as the liver and pancreas (Trenor *et al.*, 2000). In Hfe knockout mice plasma NTBI is increased and hepatocyte NTBI uptake is increased 2.5 fold. NTBI is reduced and transported across the hepatocyte membrane via a carrier-mediated process consistent with DMT1. Fpn1 is likely to mediate the transport of iron out of hepatocytes, which is then oxidised by caeruloplasmin and bound to transferrin (Pietrangelo *et al* .,2004, Abboud *et al.*, 2000).

(i)   TfR1 and HFE play key roles in enterocyte iron absorption.

TfR1 (Transferrin- Receptor 1) is ubiquitously expressed and transferrin mediated iron uptake is thought to occur in most cell types. HFE (Haemochromatosis Protein) however is highly expressed in crypt cells. HFE is a MHC class 1-like molecule which interacts with 2-microglobulin and forms a complex with TfR1. Its role in the regulation of TfR1 mediated transferrin-bound iron (TBI) uptake still remains unclear (Waheed *et al.,* 1999). It has been shown that HFE competitively inhibits the binding of TBI to TfR1, reduces the cycling time of the HFE/TfR1- TBI complex through the cell and reduces the rate of iron release from transferrin inside the cell. Conversely, when HFE and 2-microglobulin are over-expressed in Chinese Hamster Ovary cells, uptake of TBI was enhanced due to increased recycling of TfR1 through the cell and produces the opposite effect of higher intracellular iron concentrations (Waheed *et al.,* 2002). In human human haemochromatosis (HH), and in the Hfe knockout mouse model, the lack of HFE results in decreased TBI uptake from plasma into the enterocyte suggesting that HFE usually functions by enhancing TBI uptake from the plasma into the crypt cell via the TfR1 and may also inhibit the release of iron from the cell via

ferroportin 1 (Trinder *et al,* 2002; Townsend *et al.*, 2002).

TfR2 is restricted to hepatocytes, duodenal crypt cells and erythroid cells suggesting a more specialised role in iron metabolism. It is expressed at lower levels in the duodenum and does not interact with HFE in vitro. The role of TfR2 in the genesis of iron overload in the liver may be more important than its role in absorption of iron in the duodenum. The amount of iron absorbed by the enterocyte in the villus region is determined by regulation of the expression of the iron transporters DMT1 and Fpn1 in response to iron and other signalling peptides such as hepcidin. The intracellular iron concentration controls the interaction of cytosolic iron regulatory proteins (IRPs) 1 and 2 with iron regulatory elements (IREs) in the 3' and 5' regions of mRNA (Klauser *et al.,* 1993). IRP1 contains an iron-sulfur cluster and in the presence of iron acts as an aconitase (interconverting citrate and isocitrate). In the absence of iron, IRP1 binds to IREs. IRP2 undergoes iron-dependent degradation in iron-replete cells and is not available to bind to IREs. IRP2 is sensitive to degradation in the presence of nitrous oxide (NO), whereas IRP1 is activated by NO (Beutler, 2004). IRP1 operates as an iron sensor only in high oxygen environments whereas IRP2 is the major sensor of iron in mammalian cells at physiological oxygen tensions (Meyron *et al.,* 2004). When IRP1 and IRP2 bind to the IRE in the 3'-untranslated region of TfR1 or DMT1 mRNA, the transcript is stabilised, translation proceeds, and the proteins are synthesised. Thus, a high IRP binding activity reflects low body iron stores and results in up-regulation of DMT1 and TfR1 levels in the duodenum and increased dietary iron absorption. When IRPs bind to the 5'-untranslated region of ferritin mRNA, translation of the transcript is blocked and synthesis is halted. Thus, ferritin levels are reciprocally regulated – being increased in iron replete states and decreased in iron deplete states. Fpn1 contains IRE and is regulated by intracellular iron levels as well as post-translational by hepcidin. HFE and TfR2 do not contain IRE and their expression is not iron regulated.

Regulation of systemic iron homeostasis

Although iron is very abundant element in environment its bioavailability is very low so body uses iron very rationally. In the same time iron is potentially toxic and there is no pathway of regulated excretion so absorption in gut must be strictly controlled. Those facts dictate the principles of systemic iron homeostasis. The bone marrow is the main consumer of circulating iron and the most of the daily iron need is used for hemoglobin synthesis in 200 billion new erythrocytes. In balance, macrophages recycle 10–20 times more iron than the intestine absorbs providing most of daily iron supply. RES macrophages in the spleen and elsewhere phagocytize and lyse aged or damaged red blood cells. Haeme is degraded by HO-1 and iron is liberated from protoporphyrin ring and released *via* ferroportin back to plasma transferrin (Knutson *et al.*, 2005). Changes in iron flux through macrophages affect the maintenance of iron homeostasis more rapidly than changes in iron absorption in enterocytes. The body loss 1-2 mg of iron per day and about the same amount is absorbed in gut in order to provide enough but not too much iron to keep stores replete. Therefore, systemic iron homeostasis regulates intestinal iron absorption, its entry and mobilization from the stores in order to meet erythropoietic needs. It also assures a stable milieu where each cell regulates iron uptake according to its own requirements.

Since its discovery many studies confirmed role of liver hormone hepcidin as key regulator of iron homeostasis and placed liver as the central organ of system iron homeostasis. This organ synthesizes hepcidin, main iron transport protein transferrin and stores the most of iron (Detivaud *et al.*, 2005; Weinstein *et al.*, 2002).

There is some evidence of kidney involvement in iron homeostasis at least when iron demand is high. Recently, several iron transport pathways have been identified in the kidney but the role of kidneys in iron metabolism should be explained by future studies (Veuthey *et al.*, 2008; Kulaksiz *et al.*, 2005; Smith *et al.*, 2009). The crypt programming model proposes that the crypt cells sense body iron levels, which in turn regulate the absorption of dietary iron via the mature villus enterocytes. The second model proposes that liver hepcidin, which is regulated by a number of factors such as liver iron levels, inflammation, hypoxia and anaemia, is secreted into the blood and interacts with villus enterocytes to regulate the rate of iron absorption.

A. Hepcidin

Hepcidin is negative regulator of iron metabolism. On the molecular level it binds to its functional receptor ferroportin promoting internalization, and finally lysosomal degradation of this iron exporter (De Domenico *et al,* 2007). Loss of ferroportin from cell membrane causes cellular iron retention and represses iron efflux from sites of main iron flow (macrophages, hepatocytes and enterocytes) into the blood decreasing thus transferrin saturation and reducing iron availability (Figure 3) (Nemeth *et al.*, 2004).
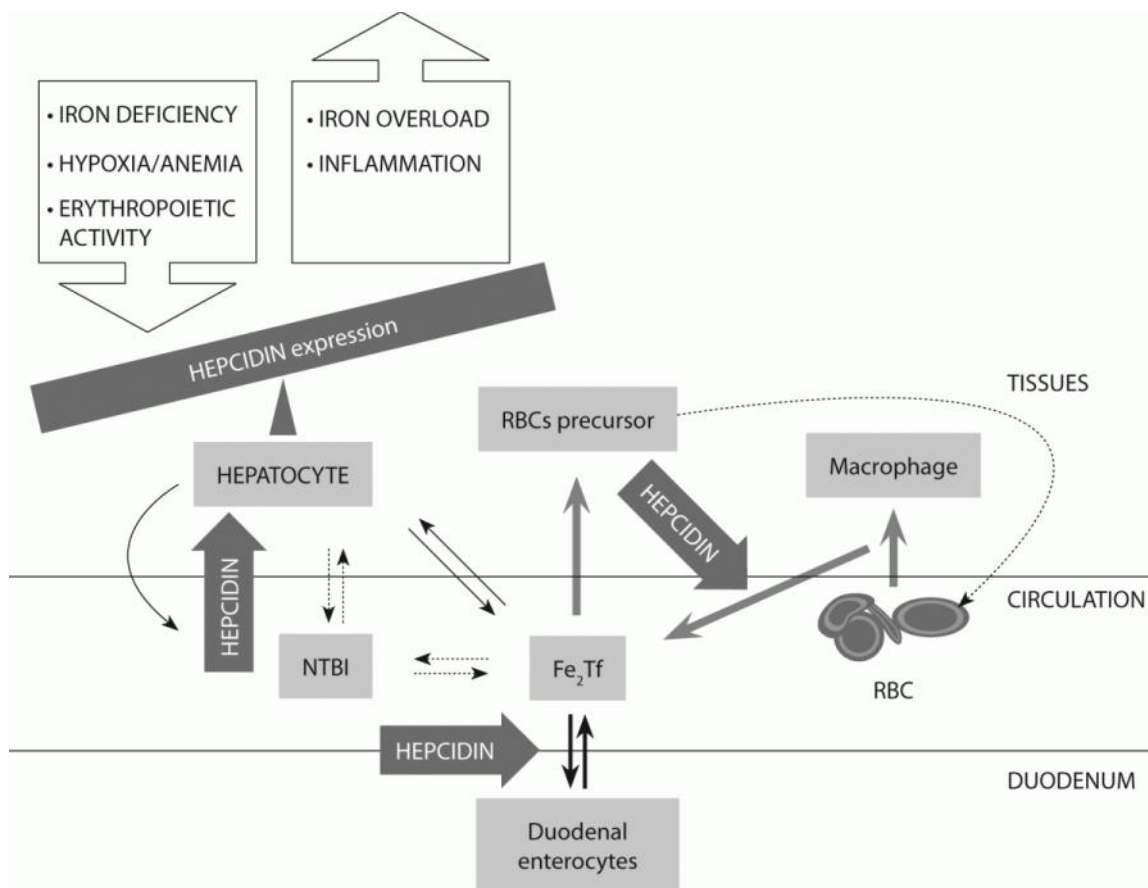
Leida *et al.,* 2012

Figure 1. Maintenance of systemic iron homeostasis by action of hepatic hormone hepcidin.

Liver cells receive multiple signals related to iron balance and respond by transcriptional regulation of iron regulatory hormone hepcidin. Hepcidin is negative regulator of iron metabolism that represses iron efflux from sites of main iron flow: macrophages, hepatocytes and enterocytes decreasing thus transferrin saturation and reducing iron availability. Iron deficiency, hypoxia/anemia and increased erythropoietic activity decrease hepcidin expression while iron overload (except HH) and inflammation increase it.

Dysregulation of hepcidin production, whether genetic or acquired, causes iron disorder. In healthy individual, an increase of body iron would lead to increased hepcidin expression and therefore to decreased iron absorption. In patients affected by HH, because of inadequate or ineffective hepcidin-mediated down-regulation of ferroportin, iron absorption continues despite high body iron load (Sangwaiya *et al.*, 2011; Anderson

et al., 2006).Oppositely, overexpression of hepcidin gene is associated with a hypoferremic, microcytic, iron refractory anemia (Chung *et al.*, 2006; Nicholas *et al.*, 2002).

Hepcidin was isolated from human blood in the year 2000, during the searching for cysteine rich antimicrobial peptides. It is named LEAP-1 (liver expressed antimicrobial peptide) (Krause *et al.*, 2000). Almost at the same time, a peptide was isolated from urine and named hepcidin after its hepatic origin and antimicrobial effect *in vitro* (Park *et al.*, 2001). Studies demonstrated that hepcidin is not liver specific but is also expressed in other tissues: the kidney, heart, lungs (Kulaksiz *et al.*, 2004). Hepcidin is synthetized as an 84-amino acid (aa) prepropeptide, and is subsequently processed into 60–64-aa prohepcidin. Mature and biologically active 25-aa hepcidin is produced by the removal of the proregion with prohormon convertase furin (Valore *et al.*, 2008). Hepcidin forms simple

hairpin structure stabilized by four disulfide bonds. It also circulates in plasma as 22-aa peptide and as prohormone pro-hepcidin that lacks biological activity (Kulaksiz *et al*, 2004; Gagliardo *et al.*, 2009). Recent study revealed that proregion of hepcidin may have bacteriostatic effects, and as such may contribute to the innate immune response (Barthe *et al.*, 2011).

Hepcidin is transcriptionally regulated and there is no evidence of other control types. Numerous molecules are involved in regulation of its expression. There are at least four major, separate pathways in hepcidin regulation: regulation by iron status, dietary iron and iron stores; regulation by inflammation; regulation by hypoxia/anemia; and regulation by erythroid factors (Zhang *et al.*, 2009; Kemna *et al.*, 2008).

(i)   Hepcidin regulation by iron status

Molecular mechanism by which iron stores regulate hepcidin synthesis is not completely described. HH caused by homozygous disruption of HFE, TfR2 and haemojuvelin (HJV) is characterized by low level of hepcidin inspite of iron overload indicating that these molecules act as direct or indirect regulators of hepcidin synthesis (100,101). At the subcellular level HJV and TfR2 are localized on the same basolateral membrane domain indicating that interaction of these proteins is possible. Localization enables direct contact with blood and sensing of signals influenced by iron metabolism (Merle *et al*, 2007; Chen *et al,* 2012).

HJV gene is identified in the year 2004 and its mutation is identified as a cause of type 2 hereditary juvenile hemochromatosis (HJH) (Papanikolaou *et al*, 2004). Patients with HJH mutations have very low urinary level of hepcidin and unlike other form of HH, early age of symptoms onset. HJV-mutant mouse exhibit severe iron overload phenotype and complete lack of hepcidin expression. HJV is expressed predominantly in skeletal and cardiac muscle and liver. This protein can be expressed as a membrane bound and soluble form (sHJV) detected in human plasma. It has been proposed that HJV act as co-receptor that binds to bone morphogenetic protein (BMP) ligands and BMP

type I and type II receptors on the cell surface. This complex (HJV-BMP ligand-BMP receptors) consequently induces an intracellular BMP signalling pathway which in turn activates the SMAD4 signalling pathway. SMAD complex translocate to nucleus and directly increases hepcidin gene transcription (Wang *et al.*, 2005; Babitt *et al.*, 2006). BMP/SMAD signaling cascade of HJV is important for basal regulation of hepcidin transcription. Recently, liver transmembrane serine protease, matriptase-2 (type II transmembrane serine proteinase; TMPRSS6) emerged as an essential component of a pathway that detects iron deficiency. It cleaves membrane bound HJV increasing sHJV that competitively impairs BMP signaling and blocks transcription of hepcidin gene permitting enhanced dietary iron absorption. Recent study presented data that do not confirm cleavage of membrane HJV by matriptase-2 in vivo suggesting that its role in hepcidin gene regulation could be more complex (Krijt *et al.*, 2011).

(ii)   Hepcidin regulation by inflammation

Hepcidin is considered to be the mediator of ACD. Hepcidin synthesis is markedly induced by infection and inflammation. Interleukin 6 (IL-6) is apparently the key inducer of hepcidin synthesis during inflammation (Kemna *et al.*, 2005). It regulates hepcidin expression through signal transducer and activators of transcription (STAT3) signaling pathway (Wrighting *et al.*, 2006). IL-6 acts on hepatocytes and stimulate hepcidin production resulting in cellular iron retention and hypoferremia, thus limiting iron availability to pathogens (Kemna *et al.*, 2005). Restricted iron availability is limiting factor in hemoglobin synthesis and results in development of anemia.

Almost all known bacterial pathogens require iron to multiply. Iron-withholding strategy is important component of innate immunity. Numerous studies confirm increased susceptibility to infection in patients with hemochromatosis due to increased iron availability (Khan *et al.*, 2007). Decreased serum iron is believed to contribute to host defense against invading pathogens and cancer cells (Ong *et al.*, 2006). In this sense hepcidin emerged as

link between immunity and iron metabolism and the key mediator of anemia of chronic disease (Theur *et al.*, 2011).

(iii) Hepcidin regulation by hypoxia/anemia and erythroid factors

When anemia/hypoxia occurs, erythropoietyn expression increases leading to stimulation of the erythropoiesis. In parallel, hepcidin gene expression decreases, allowing rapid mobilization of iron from reticuloendotelial cells and more iron is absorbed from the duodenal enterocytes to supply sufficient iron to erythrocyte precursor cells (Nicholas *et al.*, 2005). Several studies indicated that suppression of hepcidin is not directly mediated by anemia or but requires tissue hypoxia increased erythropoiesis (Pak *et al,* 2006; Vokurka *et al.,* 2006). Signals which regulate hepcidin expression are hierarchically arranged. In diseases characterized by ineffective erythropoesis, like talassemias, dominance of the stimulus of erythropoietic demand over the inhibition by iron stores can cause iron overload (Kattamis *et al.*, 2006). Studies have shown that regulation of hepcidin by erythropoiesis is probably mediated by bone marrow-derived signal molecules: growth differentiation factor 15 (GDF15), twisted gastrulation protein homologe 1 (TWSG1) and hormone erytropoetin (Pinto *et al.*, 2007). Suppression of hepcidin in hypoxia is mediated by hypoxia inducible factors (HIF) (Lakhal *et al.*, 2011; Peyssonnaux *et al.*, 2007).

Regulation of Cell Iron Homeostasis

Since both cellular iron deficiency and iron overload are detrimental for cell, iron uptake, storage, export and cellular distribution must be tightly controlled. Tight regulation of iron assimilation prevents an excess of free intracellular element that could lead to oxidative stress and damage of cellular structures like DNA, proteins and lipid membranes by ROS. At the same time it provides enough iron in order to meet the metabolic needs (Leida *et al.*, 2012).

Cellular iron uptake and storage are coordinatively regulated at the posttranscriptional level by well-known IRE/IRP system. Cytoplasmic proteins known as IRP1 and IRP2

have the ability to ˝sense˝ level of iron in transit pool. This proteins bind specifically to RNA stem-loops known as IRE and posttranscriptionally modify the expression of proteins involved in iron metabolism.

IREs are stem-loop RNA motifs present on 3´ or 5´-untranslated mRNA regions (3´-UTR ur 5´-UTR) that can interact with IRP. Thus iron itself modulates the synthesis of variety of proteins involved in iron metabolism, heme synthesis, tricarboxylic acid cycle:

· IRE at 5´-UTR mRNA ferritin, ferroportin, cALAS, HIF-2-alpha;
· IRE at 3´-UTR mRNA TfR1, DMT1.

Recently, 35 novel mRNAs that bind IRP1 and IRP2 were identified as well as cellular mRNAs with exclusive specificity for IRP1 or IRP2. Spontaneous mutations in IRE have been described in humans. Some genetic defects in L-ferritin IRE result in hyperferritinemia-cataract syndrome with prominent ocular findings and elevated serum ferritin but with no evidence of disturbed iron homeostasis. Mutations in H-ferritin IRE have been observed in cases with familiar iron overload disorder (Kato *et al.*, 2001).

IRP1 is ubiquitously expressed cytosol iron-sulfur protein. When cellular iron concentration is sufficient IRP1 acts as an aconitase, cytosol iron-sulfur protein, and it lacks RNA binding activity (Clarke *et al.*, 2006). IRP2 functions only as an RNA binding protein which is degraded in the presence of iron but in the absence of iron it binds to IRE. Alternatively, genetic ablation of IRP1 and IRP2 revealed that IRP2 dominates iron homeostasis (Meyron *et al.*, 2004).

IRE-IRP binding has two different effects depending on the IRE location relative to coding region. Binding of IRP to IREs in 3´-UTR region stabilize the transcript and prevents mRNA degradation thus increasing mRNA translation and protein synthesis. IRP binding to IREs at 5´-UTRs transcript results in translational repression by precluding ribosome binding and interrupting protein synthesis.
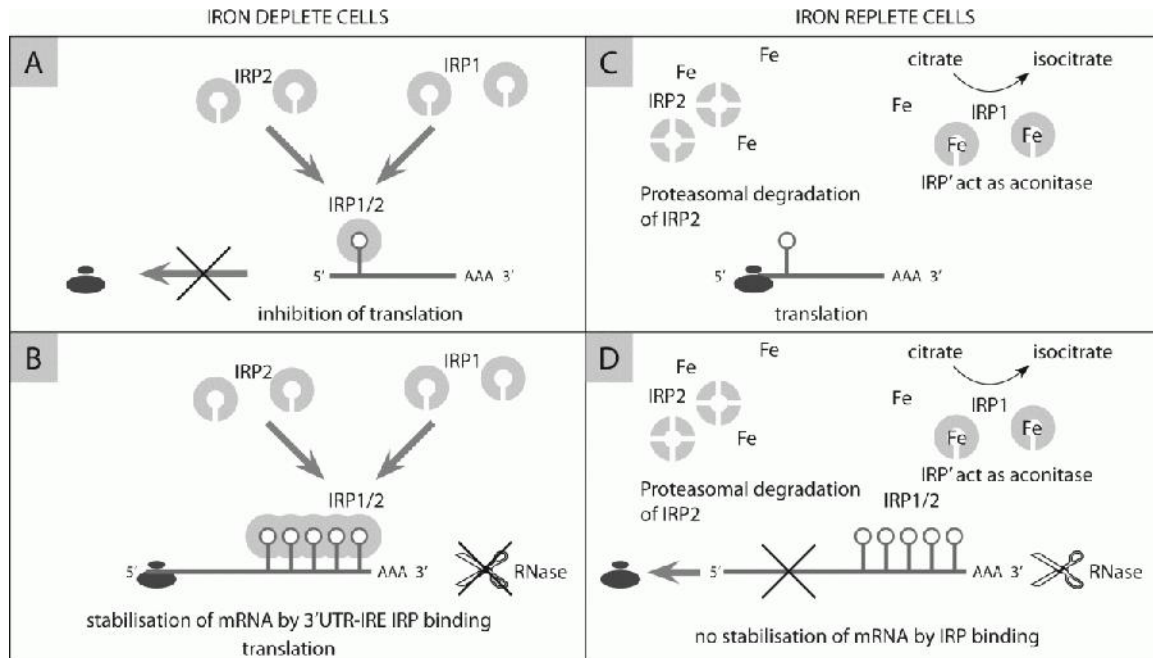
Leida et al., 2012

Figure 2 Maintenance of cellular iron homeostasis by IRE/IRP system.

In iron deplete cells binding of IRP1/2 at 5´UTR IRE of ferritin and ferroportin mRNA block translation hampering its initiation (A); Binding of IRP1/2 at 3´UTR IRE of TfR mRNA stabilize its transcript (B). In iron replete cells IRP1 act as aconitase and IRP2 is degradated by proteases so translation of 5´UTR IRE mRNA of ferritin and ferroportin is carried out undisturbed (C). In presence of sufficient iron there is no binding of IRP1/2 and stabilization of 3´UTR IRE transcript of TfR (D).

When cells are iron-sufficient, IRP1/2 lose their affinity for RNA binding, consequently ferritin synthesis is activated while TfR1 mRNA is degraded. Opposite, when intracellular iron concentration is low IRPs bind to IREs of ferritin mRNA at its 5´-UTR and block translation, whereas stabilize TfR mRNA by binding at 3´-UTR and thus increasing iron uptake by cell. Iron is neither the only modulator of IRP-1 activity nor level of IRP2. Besides iron, this regulatory system is influenced by nitric oxide, phosphorilation by protein kinase C, oxidative stress and hypoxia/reoxygenation and this provides a molecular basis by which agents other than iron can selectively modulate iron metabolism in cells and tissues (Eisensten *et al.*, 1998).

Body Iron Distribution

Lichtman *et al.* (2006) noted that once iron enters the body, it is distributed in various body compartments either as storage iron or circulating iron. Body iron content of an adult is 3-5 g (~ 45 mg / kg woman, ~ 55 mg / kg for men). Between diet and transportation of iron to body system, iron is found in the following body compartment.
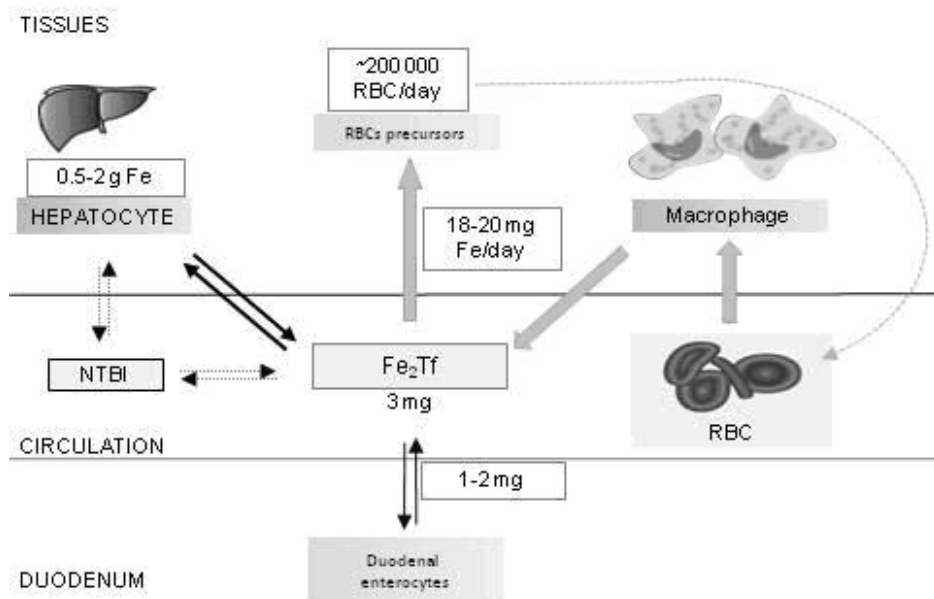
Figure 3 Body iron distribution.

Most of the body iron is incorporated in hemoglobin of circulating erythrocytes (60-70%). Approximately 20-30% of iron in the body is in the form of ferritin and haemosiderin in hepatocytes and RES macrophages as a spare iron. The amount of iron bounded to transferrin is about 3 mg but plasma transferrin compartment functions as transit compartment through which flows about 20 mg of iron each day. Under circumstances of iron overload NTBI can appear in plasma. The bone marrow is the main consumer of circulating iron. 18-20 mg of iron, mostly recycled, is used for hemoglobin synthesis in 200 billion new erythrocytes every day. Healthy people absorb 1-2 mg of iron per day which compensates for iron loss.
NTBI - non-transferin bound iron; RBCs - red blood cells; Tf - transferrin.

A   Iron in Haemoglobin

Haemoglobin is the iron containing oxygen transport metalloprotein in the red blood cell (Hopkins *et al.*, 2000). It transports oxygen from the lungs to body's cells where it releases the oxygen for cell use. 80-90% of iron transported by transferrin in the plasma is delivered to the erythroid marrow for the synthesis of haemoglobin. According to Fleming *et al.* (2002), once within the developing erythroblast, iron must be transported to mitochondria to be incorporated into haem. Within the vesicle, another protein (DMT-1) affects the release of fe^{3+} into cytosol, where it is taken up by mitochondria for haem synthesis. In mitochondria, iron is inserted into protoporphyrin IX by haem synthetase (ferrochelatase) which combines with globin to form haemoglobin.

Protoporphyrin IX+ Fe $\xrightarrow{\text{Glutamate haem synthase}}$ Haem   Globin
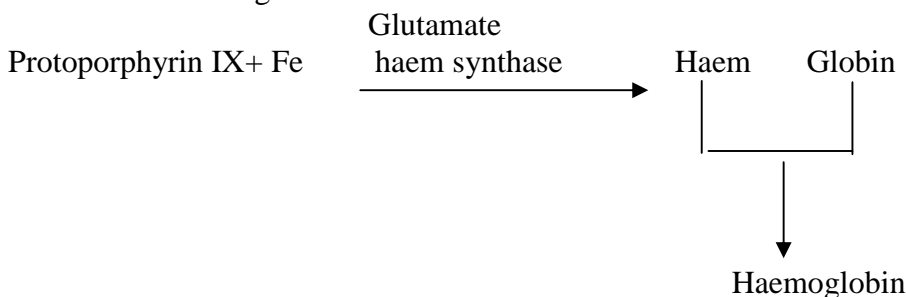
Haemoglobin

Haemoglobin, which is 0.34 percent iron by weight, contains approximately 2g of body iron in men and 1.5g in women. One milliliter of packed erythrocytes contains approximately 1mg of iron.

Hillman *et al*. (2006) noted that the process of new red cell production is not perfect, and 10-20% of precursor red blood cells are destroyed by marrow reticuloendothelial cells prior to release. In addition, about 1% of the red blood cells in circulation are destroyed each day as they reach the end of their lifespan. Together, these two processes result in the recovery of 25-30mg of iron each day by the reticuloendothelial cells of the marrow and spleen. This iron can then be transported back to marrow by transferrin for new cell production.

Role of Iron in Haemoglobin

According to Perutz *et al.* (1990), haemoglobin contains four subunit; each subunit of haemoglobin is a globular protein with an embedded haem group. Each haem group contains one iron atom that can bind one oxygen molecule through ion-induced dipole forces. Hopkins *et al.* (2000) stated that a haem group consists of an iron (Fe) ion (charge atom) held in a heterocyclic ring, known as porphyrin. A porphyrin ring

consists of four pyrole molecule cyclically linked together with the iron ion bound in the centre. The iron ion, which is the site of oxygen binding (i.e. transporting the oxygen and CO_2 in the blood), coordinates with the four nitrogen in the centre of the ring, which all lie in one plane (Perutz *et al.*, 1990). The iron is responsible for the red color of blood (Hopkins *et al*) and is bound strongly to the globular protein via the imidazole ring of the F8 histidine residue below the porphyrin ring. The iron ion may either be in the Fe^{2+} or Fe^{3+} state but ferri haemoglobin (methaemoglobin) Fe^{3+} cannot bind oxygen (Perutz *et al.,* 1990). In binding, oxygen temporarily oxidizes Fe^{2+} to Fe^{3+}, so iron must exists in the +2 oxidation state to bind oxygen. The enzyme methaemoglobin reductases reactivates haemoglobin found in the inactive Fe^{3+} state by reducing the iron centre.

Harrison *et al.* (1996) opines that iron is necessary for oxygen transport in the blood. It is the central atom of the haem group, a metal complex that binds molecular oxygen (O_2) in the lungs and carries it to all cells in the body (e.g., the muscles) that need oxygen to perform their activities. Andrew (2002) noted that without iron in the haem group, there would be no site for oxygen to bind, and thus no oxygen would be delivered to the cells.
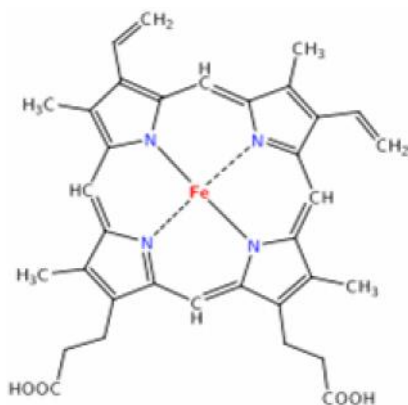

Figure 4 Structure of haem

B. Storage Iron

Harrison *et al.* (1996) asserts that all cells require iron for the synthesis of proteins but also have the ability to store excess iron. About 100-1000mg iron is stored in the reticuloendothelial cells and liver hepatocytes as ferritin and haemosiderin, the amount varying widely according to overall body iron status (Sembulingam *et al.,* 2006, Hoffbrand

et al., 2004, and Dacie *et al.,* 2006). When cellular iron exceeds requirements the excessive iron is stored in bioavailable form as ferrritin which protects cells from potentially toxic reactions catalyzed by iron (Kurz *et al.*, 2011). Ferritin thus has got dual function of iron

detoxification and reserve. It is a water-soluble protein-iron complex of molecular weight 465 000. It is made of an outer protein shell, apoferritin, consisting of 22 subunits and an iron-phosphate-hydroxide core. Walter *et al.* (1996) noted that different cell types may have different forms of ferritin, which are called isoferrtins. It has been suggested that there are at least two different subunits of ferritin (Walter *et al.*, 1996). These 24 subunits have been designated Heavy (H) and Light (L) chain type : H (heavy or hart Mr~ 21 kDa) and L (light or liver Mr ~19 kDa). The H type is the more acidic human isoferritin, such as those found in the heart and kidney cells, and the L subunit is the more basic isoferritin such as in liver and spleen isoferritin. It contains up to 20% of its weight as iron and is not visible by light microscopy (Hoffbrand *et al.*, 2006). Each molecule of apoferritin may bind up to 4000-5000 atoms of iron. The ratio of H and L subunits within the assembled ferritin protein varies depending on tissue type. Incorporation of iron into ferritin requires ferroxidaze activity that is attributed to H subunit while L subunit has a role in mineralization (Chasteen *et al.*, 1999). Mature ferritin has got a molecular weight of about 450 kDa and is capable to accumulate up to 4,500 iron atoms (Aisen *et al.*, 2001). Ferritin stored iron will be utilized when cell become iron deficient but mechanism underlying the ferritin iron release have yet to be completely elucidate (Watt, 2010, De Domenico *et al.*, 2009, Asano *et al.*, 2011).

According to Lichtman *et al*. (2005), ferritin is found in all cells and in the highest concentration in liver, spleen and bone marrow. Although the most of mature ferritin is located in the cytoplasm of cells, a small fraction was also found in nucleus of some cells. In the nucleus, ferritin could serve for delivering iron to iron-dependent enzymes or transcription factor activities but could also have a role of free iron "scavenger" that might otherwise catalyze DNA oxidative damage (Surguladze *et al.*, 2004; Alkhateeb *et al.*, 2010).

Although most ferritin is used to store iron within cells, very small amount enters into circulation. The source and detailed secretory pathway of ferritin are not completely understood but its biophysical characteristics imply active secretion through the lysosomal secretory pathway (Choen, 2010; Wang, 2010). Plasma ferritin is almost non-ferous, and its exact biologic purpose is still unknown. Some authors hypothesize that it may have a role as iron scavenger and modulator of inflammation (De Domenico *et al.*, 2011). Studies have shown that extracellular ferritin can function as an iron carrier to provide iron to cells (Wang *et al*, 2005; Fisherr *et al.*, 2007). Ferritin receptors are presented on lymphocytes and on some other cell types, but their physiological functions have not been fully defined. The plasma ferritin concentration is used as useful indicator of iron stores (Cavill, 2002). It has been estimated that plasma ferritin concentration of 1 µg/L corresponds to 8-10 mg tissue iron stores (Finch, 1994). In blood, plasma ferritin is present in minute concentration.

The plasma (serum) ferritin concentration usually correlates roughly with total body iron stores, making measurement of serum ferritin levels important in the diagnosis of disorders of iron metabolism (Hoffbrand *et al* .,2004, Hillman *et al.,2005*, Lichtman *et al* .,2006;Dacie *et al*., 2006). The size of the storage compartment is quite variable. Normally in adult men it amounts to 800 to 1000mg; in adult women it is a few hundred milligrams. The mobilization of storage iron involves the reduction of Fe^{3+} to Fe^{2+}, its release from the core crystal, and its diffusion out of the apoferritin shell. As it passes from cytosol to plasma, it must be reoxidized, either by hephaestin in the cell membrane or by caeruloplasmin in plasma, before it binds to transferrin (Townsend *et al*., 2002).

Another form of stored iron in the cell is hemosiderin, insoluble degradation product of incomplete lysosomal degradation of ferritin. In iron overloading conditions hemosiderin becomes the predominant iron storage protein. Under physiological conditions hemosiderin is not an effective iron donor but plays a protective role. Subject to conditions such as inflammation and hypoxia it could become an iron donor promoting free radical production and tissue damage in iron overloaded cells (Ozaki *et al*, 1988).

Haemosiderin is an insoluble protein-iron complex of varying composition containing about 37% of iron by weight. It is derived from partial lysosomal digestion of aggregates of ferritin molecules and is readily visualized with the aid of the light microscope as areas of Prussian-blue postivity after staining of tissue with potassium ferricyanide in acid.

C   Myoglobin

Myoglobin is a red protein containing haem, which carries and stores oxygen in muscle cells. It is structurally similar to haemoglobin, but it is monomeric: each myoglobin molecule consists of a haem group nearly surrounded by loops of a long polypeptide chain containing approximately 150 amino acid residues. Myoglobin contains 3-5 percent of the total body iron. Iron remains in the ferrous state when oxygen combines with the haem of myoglobin which at a lower partial pressure has greater affinity for oxygen than haemoglobin.

D   Tissue Iron Compartment

Tissue iron normally amount to 6 to 8 mg, this includes cytochromes and iron-containing enzymes (catalase and peroxidases). According to Fleming *et al.*(2002), although a small compartment, it is an extremely vital one that is sensitive to iron deficiency. Cytochromes are conjugated proteins which carry iron porphyrin prosthetic groups. The first of the cytochromes series function by reduction of the iron from the ferrous to the ferric state thereby carrying electrons from other enzymes systems such as flavoproteins. Catalase and peroxidase are iron porphyrin proteins in which iron appears to remain in the ferric form. Both systems catalyze the reduction of substrate hydrogen peroxidase and related peroxides by means of donated atoms (Haper, 2004).

E   Labile Iron Pool

The existence of a labile pool was postulated from studies of the rate of clearance of injected ^{59}Fe from plasma (Lichtman *et al.*, 2006). Iron leaves the plasma and enters the interstitial and intracellular fluid compartments for brief time before it is incorporated into haem or storage compounds. Some of the iron re-enters plasma, causing a biphasic curve of ^{59}Fe clearance 1 to 2 days after injection. Beutler *et al.* (1993) opined that the change in slope defines the use of the labile pool, normally 80 to 90mg of iron. It is now sometimes considered to be equivalent to the chelatable iron pool

Iron Excretion

The body conserves iron with remarkable efficiency. Most iron loss occurs by way of desquamated intestinal cells in the feces and it normally amounts to about 1 mg per day, less than one thousandth of total body iron (Hillman *et al.,* 2005). Exfoliation of skin and dermal appendages and perspiration result in much smaller losses. Even in tropical climates, the loss of iron in sweat is minimal. Very small amounts of iron are lost in the urine. Lactation may cause excretion of about 1 mg iron daily, thus doubling the overall rate of iron loss. Although total daily iron loss is normally about 1 mg for males, it averages about 2 mg for menstruating women (Lichtman *et al.*, 2005). Persons with marked iron overload, as in haemochromatosis, may lose as much as 4 mg of iron daily, probably because of the shedding of iron-laden cells, principally macrophages.
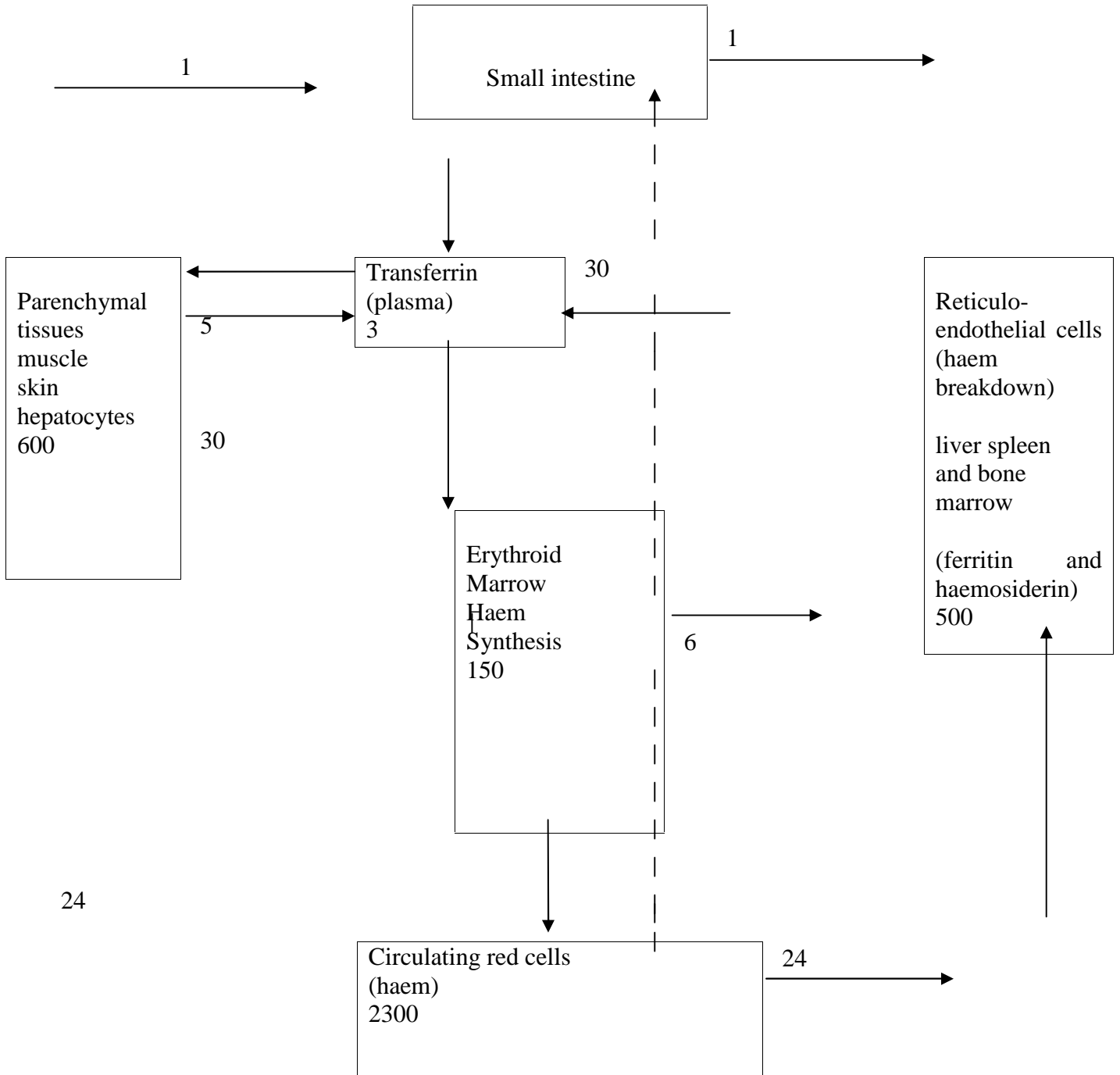
Dacie et al. (2006)

Figure 5 Iron exchange within the body**.** Numbers in the box refers to amount of iron (mg) in the various compartments and the numbers alongside arrows indicate transfer in mg/d. The dotted line indicates the small loss of iron (9.5mg) into the gut from red cells.

Table 2 Distribution of Iron in the Body (70-kg man)

Protein	Location	Iron content (mg)
Haemoglobin	Red blood cells	2000
Myoglobin	Muscle	400
Cytochromes and iron sulphur proteins	All tissues	50
Transferrin	Plasma and extravascular Fluid	5
Ferritin and haemosiderin	Liver, spleen, and bone marrow	100-1000

Dacie et al. (2006)

Disorders of Iron Metabolism

Iron Deficiency

Lichtman *et al.* (2006) defines iron deficiency as a state in which the content of iron in the body is less than normal. Iron deficiency is a leading cause of microcytic anaemia in children and adults and occurs in a varying degree of severity that merges imperceptibly into one another. When iron supply to erythroid marrow is deficient, red blood cell production is impaired and new cells released into circulation are poorly haemoglobinized (Hillman *et al.*, 2005). The severity of the anaemia and the degree of microcytosis and hypochromia generally reflect the severity and chronicity of the iron deficiency state.

A   Etiology of Iron Deficiency

Iron deficiency may occur as a result of chronic blood loss, frequent blood donation, inadequate dietary iron intake, malabsorption of iron, pregnancy and lactation, intravascular haemolysis, or a combination of these factors (Hillman *et al*., 2005). Lichtman *et al.* (2006) asserts that in men and postmenopausal women the occurrence of iron deficiency anaemia in the absence of any haemorrhage is tantamount to a diagnosis of occult gastrointestinal bleeding. In adult the most common cause of gastrointestinal bleeding are peptic ulcer, hiatal hernia, gastritis, haemorrhoids, vascular anomalies, and neoplasm. Haemostatic defeats, particularly those related to abnormal platelet function or number, may also lead to gastrointestinal bleeding.

Mcphee *et al.* (2007) noted that menstrual bleeding is a very common cause of iron deficiency in pre-menopausal women. The amount of blood lost with menstruation varies markedly from one woman to another and is often difficult to evaluate by questioning the patient. Gross menorrhagia is seldom overlooked, but moderate increase in menstrual blood loss, sufficient to create a negative iron balance, may escape notice. The average menstrual flow in normal, healthy women is about 40 ± 20 ml and is relatively constant from one period to the next (Brittenham, 1991). Most women who lose 80 ml or more of blood each period become iron deficient.

In pregnancy, the average iron resulting from diversion to the fetus, blood loss at delivery, and lactation approximates 900 mg, this is equivalent to the loss of over 2 liters of blood. Iron depletion has been reported in 85 to 100 percent of pregnant women. The incidence is low in women who take oral iron supplementation (Mcphee *et al.*, 2007).

According to Andrew (2002), iron deficiency in infants is mostly as a result of the use of unsupplemented milk diets, which contains inadequate amount of iron. During the first year of life, the full-term infant requires approximately 160 mg and the premature infant about 240 mg of iron to meet the needs of an expanding red cell mass. He went further to state that about 50 mg of this need is met by the destruction of erythrocytes, which occurs physiologically during the first week of life. The rest must come from the diet. Milk product are very poor sources of iron, and prolonged breast-feeding or bottle feeding of infants frequently leads to iron-deficiency anaemia unless there is iron supplementation. This is especially true of premature infants.

In older children, an iron-poor diet may also contribute to the development of iron-deficiency anaemia, particularly during rapid growth periods. According to Lichtman *et al.* (2006) the scanty iron supply in diet places young women and children to risk of negative iron balance. Among men in the 18-to-20 year age, iron deficiency may be found without bleeding, presumably because of the iron demands found by the recent growth spurt. Intestinal malabsorption of iron is quite an uncommon cause of iron deficiency expect after gastrointestinal surgery and in malabsorption syndromes.Feder (2003) stated that from 10 to 34 percent of patients who have undergone subtotal gastric resection develop iron- deficiency anaemia years later. In malabsorption syndrome, absorption of iron may be so limited that iron-deficiency anaemia develops over a period of years (Perutz *et al.*, 1990).

B   Epidemiology of Iron Deficiency Anaemia (IDA)

Few studies have reported the prevalence of iron deficiency or anaemia in the Nigeria population. Among infants, studies in Ibadan suggest IDA prevalence is high (Akinkugbe *et al.*, 1999). Other studies show the prevalence of anaemia (all causes) among non-pregnant young women to be 15.7%, (Okafor, 2013) and 20.0% in pregnant women. United States data show that 11% of men and 10% of women older than 65 years have anaemia, with iron deficiency accounting for 20%. Other high-risk groups include blood donors, where the prevalence of iron deficiency among females exceeds 20%.

C   Pathogenesis of Iron Deficiency

Iron deficiency develops in stages (Mcphee *et al.*, 2007). As it develops, different compartment are depleted in iron in a sequential, overlapping fashion. In iron deficiency, plasma iron clearance is rapid and is closely inversely correlated with the serum iron concentration. The plasma iron transport rate may be normal or increased. The percentage of iron utilization in haemoglobin synthesis is normal or increased. There is usually little or no evidence of ineffective erythropoiesis.
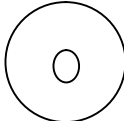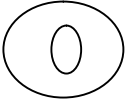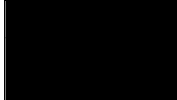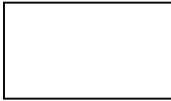
There exist three overlapping phases in which iron loss can manifest:

1.   The first phase is the occurrence of iron depletion which Hoffbrand *et al.* (2004) describes as pre-latent iron deficiency.Haemosiderin and ferritin virtually disappear from marrow and other storage sites in this phase.
2.   In the second phase, iron loss or iron requirement increases iron absorption from food. The normal haemoglobin concentration is maintained in this phase. The transferrin saturation percentage then diminishes to below 15% a situation which Hoffbrand *et al.* (2004) describes as latent iron deficiency.
3.   In the third phase of iron deficiency, iron loss or iron requirement can become so great that despite maximum absorption from food, the iron supply is not sufficient to maintain a normal haemoglobin concentration.

In general, clinical state of iron deficiency can be classified as iron-store depletion, iron-deficient erythropoiesis, or iron-deficiency anaemia (Hillman *et al.*, 2005). Iron depletion is the earliest stage of iron deficiency, in which storage iron is decreased or absent but serum iron concentration, transferrin saturation and blood haemoglobin levels are normal. Iron depletion is generally not associated with any of abnormalities associated with anaemia (such as altered red cell indices or morphology).Iron deficiency sufficient to affect erythropoiesis is preceded by latent phase during which symptomless biochemical aberrations are measurable. These aberrations

include reduction in serum iron, increase in free erythrocyte protoporphyrin, and decreases in tissue haeme enzymes (Andrew, 1999). According World Health Organisation, anaemia is present in adult if the haematocrit is less than 41% (haemoglobin< 13.5 g/dL) in males or less than 37% (haemoglobin < 12 g/dL in females. Iron deficiency anaemia is the most advanced stage of iron deficiency. It is characterized by decrease or absent iron stores, low serum concentration, low transferrin saturation, and low blood haemoglobin concentration.

	Normal	Latent Iron Deficiency	Iron deficiency anaemia
Red cell iron (peripheral film and indices)	Normal	Normal	Hypochromic microcytic MCV, MCH,MCHC↓
Iron stores (bone marrow macrophages iron)	++	0	0

Hoffbrand et al. (2004).

Figure 6 The development of iron deficiency anaemia. Reticuloendothelial (macrophage) stores are lost completely before anaemia develops. MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume.

D   Clinical   Manifestation   of   Iron Deficiency
i   Anaemia

The anaemia in iron-deficient patients can be very severe, with blood haemoglobin levels less than 4 g/dl being encountered in some patients (Hillman *et al.*, 2004). Severe iron-deficiency anaemia is associated with all of the various symptoms of anaemia, resulting from hypoxia and the body's response to hypoxia. Thus, tachycardia with palpitation and pounding in the ears, headache, light-headaches, and even angina pectoris may all occur in patients who are severely anaemic (Lichtman *et al.*, 2006).

ii   Fatigue and Cardiovascular Symptoms

Most patients with gradually progressive anemia do not sense fatigue or easy fatigability until haemoglobin levels fall below 7 to 8 g/ dl. The cardiovascular sign of chronic anemia generally develop at about the same time beginning with tachycardia, palpitation, pounding in ears, dizziness, and something breathlessness during intense exertion (Lichtman *et al*, 2006). Anemia per se is primarily responsible for the early symptom of intolerance to intense exercise. The biochemical effects of iron deficiency on skeletal muscle are disputed, but it appears that muscular endurance during prolonged work slowly becomes compromised by tissue iron deficiency (Fleming, 2002)

iii   Behavioral Effect of Iron Deficiency

In children the effect of iron deficiency upon growth and development can be measured. Test of sensory development, motor function, and language skills in iron deficient children show mental and behavioral impairment that can be gradually improve by administration of iron (Andrew, 1999). Iron deficient infant and children commonly display depressed spontaneous activity, a reversal in the diurnal pattern of such activity, and impaired cognitive function. Numerous underlying noncognitive disturbances have hampered accurate measure of the effect of iron deficiency in early life on 1Q score and development test performance; among these are short attention span, fearfulness, withdrawal, increased body tension, and a characteristic silent solemnity (Bishop *et al.,* 1996).

iv   Oral and nasopharyngeal Symptoms

The most prevalent and visible sign of chronic iron deficiency is absence or flattening of the papillae of the tongue (Brittenham, 1991). This is sometimes accompanied by soreness and burning, and because of epithelial thinning, the tongue may be dark red, contrasting strongly with the facial pallor. During severe, protracted iron deficiency the tongue becomes smooth, glistering, and slightly shrunken. Painful, reddened fissures and ulcerations may appear at the corner of the mouth as the epithelial dysplasia worsens (Andrew, 2002).

v   Nails: koilonychias

According to Hoffbrand *et al.* (2004), "during chronic iron deficiency the fingernails commonly become brittle, breakable, and coarsely ridged". Normally nails are extruded by the committed epithelial stem cells of the nail bed at a rate of about 0.1 mm/day. This growth rate slows, and newly formed nails are abnormally thin, fragile, and easily split at the ends. With loss of structural resilience, the nails become splayed and flattened from use.Eventually the terminal halves of the fingernails become concave rather than convex (Brittenham, 1991).

vi.   Skeletal changes

Most iron deficient children suffer some degree of general malnutrition and show retarded skeletal growth development. Fleming (2002) noted that infants and young children having severe iron deficiency anaemia display skull changes similar to those of congenital haemolytic anaemias. As the result of ineffective erythropoiesis, the marrow cavity is forced to expand by the massive accumulation of arrested, unhaemoglobinized erythroblasts.

E   Laboratory Studies of Iron Deficiency

Accurate diagnosis of an iron-deficiency state requires several laboratory tests (Table 4). Measurement of the red cell indices and blood film, serum iron, transferrin iron-binding capacity, serum transferrin receptor and the serum ferritin level are of greatest importance.Other measurements used include direct inspection of marrow reticuloendothelial iron stores using a marrow aspirate and measurement of the red cell protoporphyrin level.

i   Red cell indices and blood film

According to Hoffbrand *et al.,* (2004), even before anaemia occurs, the red cell indices (MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume) fall and they fall progressively as the anaemia becomes more severe. The blood film shows hypochromic, microcytic cells with occasional target cells and pencil-shaped poikilocytes. The reticulocyte count is low in relation to the degree of anaemia. In a significant anaemia, the red cell count may be within normal limits and PCV only moderately lowered because many poorly filled cells are present (Dacie *et al.*, 2006). The bone marrow shows normoblasts hyperplasia with decreased cellular haemoglobin in the majority of the normoblasts.
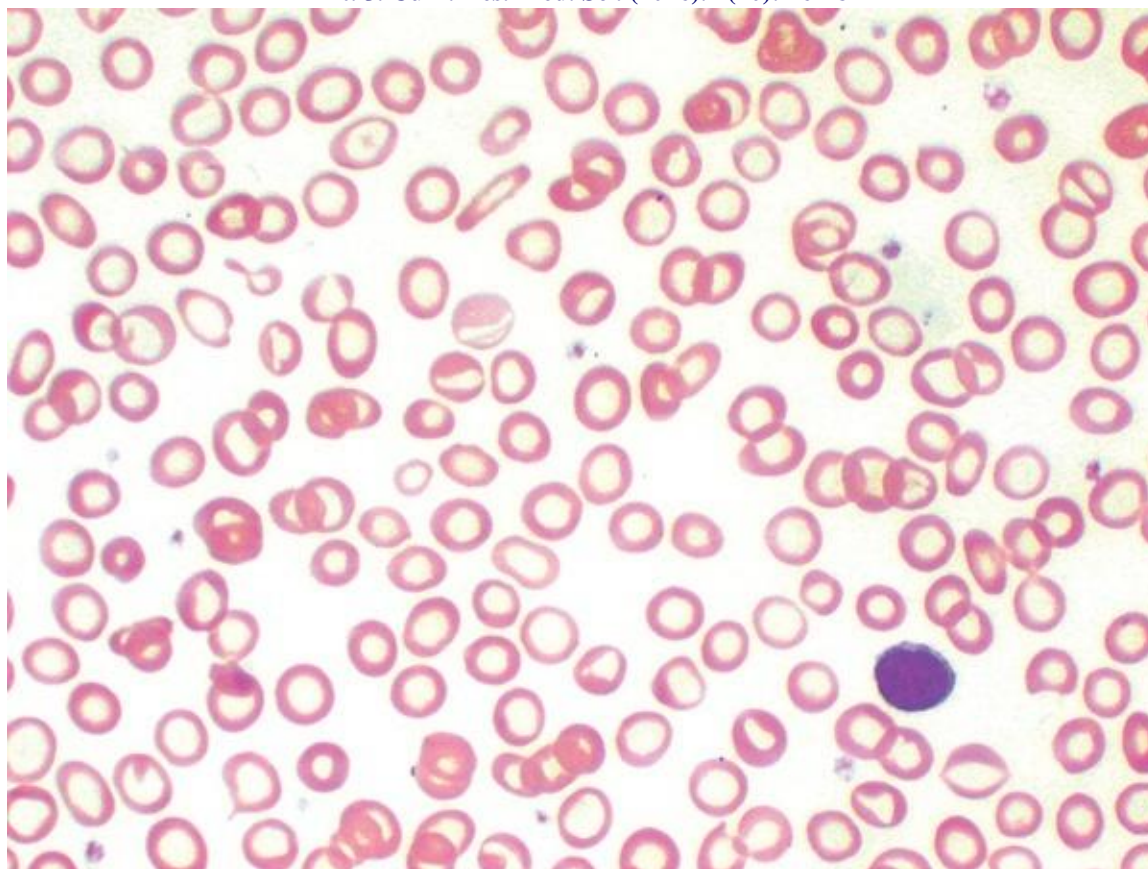
Figure 7 The peripheral blood film in severe iron deficiency anaemia. The cells are microcytic and hypochromic with occasional target cells.

ii   Serum Iron and Total Iron-Binding Capacity

The serum iron (SI) is a direct measure of the amount of iron bound to transferrin (Hillman *et al.*, 2005). A normal individual has an SI of 50-150 µg/dL. The serum iron concentration is usually low in untreated iron-deficiency anaemia; however it may be normal (Lichtman *et al.*, 2006). The total iron-binding capacity (TIBC) is a measure of the amount of iron that can be bound by transferrin and, therefore, is a surrogate measure of the serum transferrin level. The normal TIBC is 300-360 µg/dL. In iron-deficiency anaemia TIBC are often increased. Together, the SI and TIBC (transferrin level) are used to calculate the percent saturation of transferrin with iron (SI/TIBC = percent saturation). In state of normal iron balance, the percent saturation is between 20% and 50%. When it falls below 20%, the erythroid marrow has difficulty obtaining sufficient iron to support increased levels of erythropoiesis.

iii   Serum ferritin

A small fraction of body ferritin circulates in the serum, the concentration correlates with total-body iron stores. With the recognition that the small quantity of ferritin in human serum reflects body iron stores, measurement of serum ferritin has been widely adopted as a test for iron deficiency and iron overload (Dacie *et al.*, 2006). Each nanogram per milliliter of serum ferritin correlates to 8-10mg of iron stores (Hillman *et al.*, 2005). In children and pre-menopausal women, typically have ferritin levels of 7-187 ng/ml (200-500mg of iron stores), whereas adult men and postmenopausal women have levels of 21-385 ng/ml (500-2000mg of iron stores), depending on their age and level of dietary iron.

iv   Serum Transferrin-Receptor

Serum transferrin receptor (sTfR) can be used in conjunction with the serum ferritin measurement in diagnosing iron store depletion and defects in iron delivery to the marrow. Levels of sTfR (normal = 5-9 µg/L) increase rapidly once iron stores are exhausted (serum ferritin < 40 µg/L). Iron deficiency is accompanied, therefore, by an elevation of sTfR proportional to the severity of the anaemia. Hoffbrand *et al.* (2004) asserts that the level of sTfR is also raised if the overall level of erythropoiesis is increase.

Table 3 Laboratory Studies in Iron Deficiency

	Iron-Store Depletion	Iron-Deficient Erythropoiesis	Iron-Deficiency Anaemia
Haemoglobin	Normal	Slight decrease	Marked decrease (microcytic hypochromic)
Iron-stores	<100mg (0-1+)	0	0
Serum iron (µg/dL)	Normal	<60	<40
TIBC (µg/dL)	360-390	>390	>410
Percent saturation	20-30	<15	<10
Ferritin (µg/L)	>40	<20	<12

Hillman et al. (2004)

v   Bone Marrow Iron

Bone marrow examination is not essential to asses iron stores except in complicated cases. In iron deficiency anaemia there is a complete absence of iron from stores (macrophages) and from developing erythroblasts. The amount of iron in the reticuloendothelial cells can be estimated by inspection of a Prussian blue stain of marrow aspirate particles. A simple scale of 0 to 4+ iron store is generally used in reports. This grading system correlates fairly well with the amount of iron available for erythropoiesis.

Table 4   Measurement of Iron Stores

Iron Stores	Serum Ferritin (ng/mL)	Marrow Iron stain (0-4+)
0	<12	0
1-300 mg	12-200	1+
300-800 mg	20-50	2+
800-1000mg	50-150	3+
>1-2g	150-300	4+
Iron overload	>500	

Hillman et al. (2004).

Iron Overload

Levy *et al.* (2000) states that iron overload is a condition in which an abnormality in the intestinal epithelium leads to an uncontrolled passage of iron from the diet into the lumen. Here, absorption of iron is greatly more than the body's need for iron. Since excretion is limited, the intestinal defect gradually leads to excessive accumulation of iron within the body.

A   Causes of Iron Overload

Because body iron content is maintained by regulation of absorption, excess body iron can accumulate only when absorption is dysregulated or when iron is injected into the body, either in the form of medicinal iron or as transfused erythrocytes.

i   Deregulation of Iron Absorption

A variety of mutation cause iron absorption in experimental animals and in man. The precise biochemical abnormality associated with this disorder is not understood but iron continues to be actively absorbed even in the face of high transferrin saturations (Fleming, 2002).

ii   Transfusion or Iron Therapy

Iron overload can be iatrogenic in origin. Erythrocytes contain 1 mg of iron per milliliter, transfusion of 450 ml of whole blood or 200 ml of red cells add 200 mg of total iron to the body, iron that will not be excreted. Thus, a patient who required 2 U of blood per month accumulates 4.8 g of iron per year. If the need for transfusion is necessitated by a disorder in which ineffective erythropoiesis plays a prominent role, the accumulation of iron is even greater.

The homeostatic mechanisms of the body are such that the inappropriate administration of iron by the oral route is highly unlikely to produce clinically significant iron overload. Documented iron overload after iron injection is even less common and was not accompanied by demonstrable tissue damage (Ludlam, 1992). Industrial exposure to iron dust (Welder siderosis) results in deposition of iron in the lungs, and increased ferritin levels have been documented (Feder *et al.*, 1996).

B   Pathology of Iron Overload

Affected tissue and organs exhibit a deep brown color. Histologic examination reveals prominent haemosiderin deposition in many tissues and organs. The liver often is enlarged. After cirrhosis develops, the liver becomes granular or coarsely nodular. The cirrhosis of haemochromatosis usually has a micronodular appearance. In the original description of African iron overload, the liver pathology was deemed to be indistinguishable from that of classical haemochromatosis, (Ludlam, 1992) but in more recent studies (Fleming, 2002) it seems that only some of the affected patients manifest iron storage primarily in kupffer cells

In patients with transfusional iron overload, macrophages are heavily laden with iron, presumably derived from transfused red cells. The myocardium is thickened and the heart is often enlarged, testes are often atrophic.

C   Clinical Features of Iron Overload

The clinical features of the most common form of hereditary haemochromatosis are cirrhosis of the liver, darkening of the skin, cardiomyopathies, and diabetes (Lichtman *et al.*, 2006). Many symptoms have been attributed to hereditary haemochromatosis. These include abdominal pain, weakness, lethargy, fatigue, loss of libido, impotence, and arthropathies.The arthralgia of patients with haemochromatosis is claimed to have characteristic features (Feder *et al.*, 1996). It is said to tend to begin at the small joints in the hands, especially the second and third metacarpal joints, and that in some cases episodes of acute synovitis.

D   Laboratory Features of Iron Overload

The main laboratory features of hereditary haemochromatosis are increased transferrin saturation and increased serum ferritin level. Serum iron concentration greater than 300µmol/g is considered strong evidence for haemochromatosis when factors such as transfusions are eliminated as the cause (Lichtman *et al.*, 2006).

Blood Donation

Blood for transfusion is obtained by bleeding healthy male and female donors into suitable blood bag with the right anticoagulant (Ukaejiofor, 2004). A blood donation is when a healthy person voluntarily has blood drawn. The blood is used for transfusion or made into

medications by a process called fractionation or apheresis. Blood is the most donated tissue in medical practice and it is a veritable tool in many life-saving situations if used appropriately and judiciously. It is a life-saving procedure and health services are challenged to maintain an adequate supply of safe blood, and to ensure that it is used appropriately. Blood plays an important role in the transport of oxygen from the lungs to various tissues of the body and transports carbon dioxide from tissue to the lungs. Red blood cells, white blood cells, platelets, plasma etc are components of blood. Nutrients are transported in the blood to the tissues after absorption from the intestinal tract or after release from the storage depots such as the liver.

Donated bloods are used for blood transfusion in cases of blood loss due to accident, trauma, complications of pregnancy and childbirth and can also be used to replace blood lost during surgery. Many donors donate as an act of charity, but some are paid for their own future use. Today there are mainly three types of donors namely: voluntary, relative, and commercial donors. Ideally blood donation should be voluntary i.e. with no financial reward because the desire for money may make the donor not reveal all their medical history or life style or frequency of donation (Ukaejiofor, 2002). In tropical Africa, for example Nigeria, these non remunerated donors are in a very short supply, sometimes because of certain superstitions beliefs relating to blood. Relative donors are relations of the patient requiring transfusion e.g. husband whose wife requires blood transfusion in obstetric cases etc. Because the two above types are in short supply commercial donors have emerged. These are individuals who are remunerated for every pint of blood donated.

A   Types of Donation

Blood donations are divided into groups based on who will receive the collected blood (Brecher, 2005).An allogenic (also called homologous) donation is when a donor gives blood for storage at a blood bank for transfusion to an unknown recipient. A directed donation is when a person, often a family member, donates blood for transfusion to a specific individual (Wales, 2001).

A replacement donor donation is a hybrid of the two and is common in developing countries. In this case, a friend or family member of the recipient donates blood to replace the stored blood used in a transfusion, ensuring a consistent supply. When a person has blood stored that will be transfused back to the donor at a later date, usually after surgery, that is called an autologous donation (AABB, 2008).

B   Blood Donor Eligibility

Donor eligibility rules are intended to protect the health and safety of the donor as well as the patient who receive the transfusion. The criteria listed below are provided as guidelines to assist in determining whether one may be eligible to be a good donor. Making donations for one's own use during surgery (Autologous blood transfusion) is considered a medical procedure and the rule for eligibility are less strict than for regular volunteer donation (ARC, 2007).

Criteria for selection of whole blood and apheresis donors are divided into two groups, which are: nature of donation and informed consent. Nature of donation involves the donation of blood only on a voluntary basis and donor shall not be remunerated for the donation. Financial reward for the donation of blood or blood components shall be prohibited. Donor appreciation by the giving of tokens, certificates, badges and the refund of direct transport expenses are acceptable (NBTS, 2007).

Informed consent however, involves the prospective donor, whether of whole blood or apheresis, been given adequate verbal and / or written information about the procedure. And every blood donor shall give informed consent prior to each blood donation (NBTS, 2007). The criteria are been listed as follows:

1.   Age
2.   All forms of creutzfeldt-Jacob Disease
3.   Bleeding condition
4.   Donation interval
5.   Full blood count
6.   General health
7.   Haemoglobin, haematocrit
8.   Hepatitis

9.   HIV/AIDS
10.   Identification
11.   Malaria
12.   Pulse and blood pressure (high and low).
13.   Pregnancy, nursing
14.   Potential exposure to blood and blood fluid
15.   Sickle cell
16.   Syphilis/gonorrhea

(American Red Cross, Bethsda, 2007; Cheesbrough, 2004; Dutta, 2006; NBTS, 2006 NBTS, 2007)

i.   Age

Blood donors shall be healthy person between the ages of 16 and 65 years. Persons under the age of 18 years shall require parental consent to donate blood (American Red Cross 2007; NBTS, 2007).

ii   Haemoglobin and Haematocrit

Each prospective donor shall be screened for haemoglobin concentration or haematocrit prior to donation. The prospective blood donors is accepted if his or her haemoglobin is at or above 12.5 g/dl; alternatively his / her haematocrit value at or above 38% (ARC, 2007; NBTS, 2007).

iii.   Hepatitis

Hepatitis A is seldom transmitted via blood transfusion. Potential donors who admit a recent bout of hepatitis A or contact with a case are regarded as unsuitable for 6 months from last contacts (Baker *et al*, 2001; Newman *et al*, 2001). Individuals with history of jaundice or hepatitis shall be deferred for 12 months after recovery. At this stage, test for hepatitis B (HbsAg) and hepatitis C (HCV) antibodies and/or antigens shall be negative before donation is allowed (NBTS, 2007). Also prospective donors who have been in close contacts with an individual with hepatitis shall be deferred for a 12 months period. (American Red Cross 2007; NBTS, 2007)

iv   HIV (Human Immunodeficiency Virus 1 and 2)

The risk of developing HIV disease/AIDS after being transfused with HIV infected blood is high (greater than 95%). All blood donors must be screened for antibody to HIV-1 and HIV-2 using a sensitive test. When an antibody-screening test is negative, blood may still contain HIV. This can happen when blood is collected during the window period, i.e. soon after a donor becomes infected with HIV when antibody to the virus is not yet detectable in the serum (Cheesbrough, 2004).

Donors are considered to be high risk for HIV if they have ever used intravenous drugs (other than prescribed medications), have been in certain African countries where HIV is extremely common, engaged in high-risk sexual behaviour, or had sex with anyone who is in any of those risk groups (Bethesda, 2007; ARC, 2007).

v.   Malaria

Red cells of donors who have recently visited or who have lived in a country where malaria is present are also discarded, although their plasma may be used. Malaria parasite resists storage at 4 ^{0}C and may therefore be transmitted to the recipient of infected red cells (Baker *et al*, 2001).

vi.   Pulse and Blood Pressure (High And Low)

The donor's pulse rate shall not exhibit any irregularity and shall be between 50 and 100 beats per minute, except in highly trained athletes where a lower pulse rate may be accepted (NBTS, 2007). The donor's blood pressure (high) is accepted as long as his systolic blood pressure does not disqualify the donor from donating (Alfred *et a.l*, 2007).

Blood pressure (low) however, will be acceptable as long as the donor feels well when he/she comes to donate. If his/her blood pressure normally runs low, it may be more difficult for his/ her body to adjust to the volume loss following donations, especially if he/she is dehydrated. Drinking extra water before and after donation is important (Alfred *et al*, 2007; Dutta, 2006).

vi.   Donation Interval

The interval between consecutive whole blood donations shall not be less than 90 days unless otherwise authorized by a medical officer (NBTS, 2007).

C   New Strategies in Recruitment of Donors

Many technique of blood donor recruitment have been advocated. The best methods are understandably based on personal contact between motivator and prospective donor or at least contacts between motivator and group. Person to person contact may be difficult but person to group contact is pragmatic. The Red Cross conducts its recruitment activities within the community, encouraging the public to come forward to give the 'river of life'. A team of volunteer Red Cross blood donation campaigners conducts talk at tertiary institutions, community organisations and companies, informing the public on the importance of blood donation and the need for regular blood donors (ARC, 2007). Besides public education activities, motivators also recruit companies and organisations to become catalysts in encouraging their employees and members to be regular blood donors. These companies and organisations become corporate blood donors and do their part in meeting the national blood requirement by pledging to collect 200 to 300 units of blood a year (WHO, 2007).

It should be noted that recruitment and retention are about people and community, about understanding them, capturing their interest and influencing their behaviour. One key secret of successful blood donor recruitment is to take the beds to the donors as close as possible on their convenient date and time rather than expecting the donor to come to the blood bank (NBTS, 2006; NBTS, 2007). Other requirement strategies involves launching a campaign for voluntary blood donation on a particular fixed day via posters, folder, stickers, the media (print and electronic), banners, rallies etc.

D   Complications in Blood Donation

Donors are screened for health problems that would put them at risk for serious complications from donating. First-time donors, teenagers and women are at a higher risk of a reaction (Eder, 2008). One study showed that 2% of donors had an adverse reaction to donation (ARC, 2008). Most of these are minor. Newman *et al*. (2001) reported that in a study of 194, 000 donations, only one donor were found with long-term complication. In Denmark the national Blood Donor Organisation (the Blood Donors in Denmark (BID) have for many years registered all severe complication related to blood donation, because they were administering the economical compensation in the severe cases. Analysis of data from Denmark on incidence and outcome of blood donation has shown that the vasovagal reaction is the most common complication (58% of all), and followed by needle injury (48%). However, the incidence of severe outcome is 5 time higher in the needle injury group, making it the most signification group regarding donor security. Bruising of the arm from the needle is the most common concern. One study found that less than 1% of donors have this problem (Harrison *et al.*, 2000).

Bolan, (2001) reported that donors sometimes have adverse reaction to the sodium citrate used in apheresis collection procedure to keep the blood from clotting. Since the anticoagulant is returned to the donor along with blood components that are not being collected, it can bind calcium in the donor's blood and cause hypocalcaemia. Hypovolemic reaction can occur in blood donation, because of a rapid change in blood pressure. Fainting is generally the worst problem encountered. The process has similar risks to other forms of phlebotomy. In the United State, a blood bank is required to report any death that might possibly be linked to a blood donation.

Iron Status of Blood Donors.

Regular blood donors have being observed to undergo a progressive decline in iron reserves, while some develop frank iron-deficient erythropoiesis. The prevalence of iron depletion is significantly higher in menstruating women and

increases progressively as the rate of donation increases. In a study conducted by Ositadinma *et al*, 2015 in Enugu state Nigeria, iron depletion was seen in 1.3% in group 2 (1-3 times) and also in 13.3% of group 4 (7-9 times), iron deficiency was present in 4.4% of group 3 (4-6 times) and in 20% of group 6 (13-15 times) and iron deficiency anaemia was discovered in 4.4% of group 3 (4-6 times). Blood donors with more than seven times instances of blood donation (*P<0.05*) showed a significant relationship between iron depletion and iron deficiency. In a similar study on the Iron stores of regular blood donors in Lagos, Nigeria, Adeniran *et al* a total of 52 regular (study) and 30 first-time (control) volunteer blood donors were studied prospectively. Mean hemoglobin and packed cell volume in the study group (13.47 ± 2.36 g/dL and 42.00 ± 7.10, respectively, P = 0.303) were not significantly higher than in the control group (12.98 ± 1.30 g/dL and 39.76 ± 4.41, respectively, P = 0.119). Mean serum ferritin was 102.46 ± 80.26 ng/mL in the control group and 41.46 ± 40.33 ng/mL in the study group (P = 0.001). Mean serum ferritin for women in the study group (28.02 ± 25.00 ng/mL) was significantly lower than for women in the control group (56.35 ± 34.03 ng/mL, P = 0.014). Similarly, men in the study group had a lower mean serum ferritin (48.57 ± 45.17 ng/mL) than men in the control group (145.49 ± 87.74 ng/mL, P = 0.00). The mean serum transferrin receptor value was higher in the study group (1.56 ± 0.88 µg/mL) than in the control group (1.19 ± 0.38 µg/mL, P = 0.033).

The findings suggest that hemoglobin concentration, packed cell volume, and serum iron levels are not significantly affected by regular blood donation and that regular blood donors appear to have reduced iron stores compared with controls.

Abdullah et al, studied the effect of repeated blood donations on the iron status of male Saudi blood donors, the result shows the mean serum iron was significantly higher among subjects with no previous history of blood donation (group I) than among regular donors who had donated twice or more. The difference in serum ferritin concentration was statistically significant (p<0.05) when comparing regular donors in group III (72.4 µg/L), group IV (67.4 µg/L) and group V (26.2 µg/L) with first-time blood donors (131.4 µg/L). In contrast, the difference in the concentration of serum ferritin between subjects in group II (98.9 µg/L), who had donated once in the last 3 years, and in first-time blood donors (131.4 µg/L) was not statistically significant (p<0.131). None of the group I donors suffered from iron deficiency, whereas 2.8% of the donors who had donated between two to five times had iron deficiency. The prevalence of erythropoiesis with iron deficiency in regular blood donors was 4.3%

The results of this study show that an increase in the number of donations results in an increase in the frequency of depleted iron stores and subsequently in erythropoiesis with iron deficiency, although the level of haemoglobin remained acceptable for blood donation. This result may indicate the need to review the guidelines on acceptance of donors.

Conclusion

Iron is essential mineral necessary for the transport of oxygen. Humans can get iron from oral iron supplement or IV injections.It can also be obtained from blood transfusion. Iron content of the diet depends on the type of food eaten and the total daily caloric intake. Absorption of iron is a very complex process that takes place in the duodenum and upper jejunum.This process involves number of proteins that transport iron across the apical membrane, proteins that transport iron through the basolateral membrane of enterocyte , proteins that change redox state of iron and thus assist in its transport.

Blood for transfusion is obtained by bleeding healthy male and female donors into suitable blood bag with the right anticoagulant. The blood is used for transfusion or made into medications by a process called fractionation or apheresis. Blood is the most donated tissue in medical practice and it is a veritable tool in many life-saving situations if used appropriately and judiciously. It is a life-saving procedure and health services are challenged to maintain an adequate supply of safe blood, and to ensure that it is used appropriately. Donors are screened for

health problems that would put them at risk for serious complications from donating.

Regular blood donors have being observed to undergo a progressive decline in iron reserves, while some develop frank iron-deficient erythropoiesis. The prevalence of iron depletion is significantly higher in menstruating women and increases progressively as the rate of donation increases.

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