SCHOOL OF MEDICINE AND HEALTH SCIENCES
DIVISION OF BASIC MEDICAL SCIENCES
DISCIPLINE OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

PBL SEMINAR: IRON AND IRON BALANCE – An Overview

What are some of the vital functions of Iron in the body?

- Iron has several vital functions:
  - As a component of Hb: It facilitates O_2 transport in blood (OxyHb)
  - As a component of Mb: It facilitates storage of O_2 in muscle,
  - As a component of Cytochromes: It facilitates movement of electrons within specific organelles (e.g., Oxidative Phosphorylation in Mitochondria)
  - As a component of enzymes: Oxidases, Oxygenases, (e.g., Lysosomal enzyme Myelo-Peroxidase, which is required for proper Phagocytosis and Killing of Bacteria by Neutrophils)

How is Iron distributed in the body?

- Iron in the body is distributed or classified as:
  - **Functional Iron** (About 80%):
    - Mainly located in RBC as Hb,
    - Lesser amount in Myoglobin, and Cytochromes
  - **Storage Iron** (About 20%):
    - Stored primarily as **Ferritin**, but some stored as **Hemosiderin**
  - **Transport Iron:**
    - Less than 1.0% of body Iron is in plasma, bound to Transferrin,
      - Each molecule of Transferrin binds $2Fe^{3+}$ (Ferric) ions

- Concentration of Iron in Serum/Plasma vary with Age and Sex
  - Males: 18 – 45umol/L
  - Females: 14 – 32umol/L

- Marked **Circadian Rhythm** (**Diurnal variation**) is observed in Plasma Iron levels in both male and female, with higher values in the morning

What are some of the dietary sources of Iron?

- Normal intake of Iron is about 0.2 – 0.4mmol/day (10 – 20mg/day)
- Good dietary sources of Iron:
  - **Heme Iron is Ferrous Iron (Fe^{2+})**
    - Liver, Fish, Meat, Egg yolk, Oysters,
  - **Non-Heme Iron is Ferric Iron (Fe^{3+})**
    - Green leafy vegetables, Fruits, Dried beans

How is dietary Iron absorbed? (Fig. 1)

- Iron is absorbed mainly in the Duodenum and Upper Jejunum
- Dietary Iron is consumed usually attached to ligand
- In the **Stomach**: **High acidic environment (low pH)**:
  - Non-Heme Iron (Fe^{3+}) is converted to Heme Iron (Fe^{2+}) releasing the Ligand
  - Content of the stomach with Fe^{2+} then enters the Intestine

- In the **Duodenum**: **High alkaline environment (High HCO_3^-)**:
  - Free Fe^{2+} ions are oxidized to Fe^{3+} ions
  - Fe^{3+} ions are taken up by Intestinal Mucosal cells

TAKE NOTE:

- Heme is absorbed directly by Mucosal Cells
- Fe^{2+} then dissociate from Heme

How is absorption of Iron regulated?

- **Apoferritin** (an Iron-binding protein) produced in Intestinal Mucosal cells regulates absorption of Iron

Apoferritin + Fe^{3+} ========➔ Ferritin

- In **Iron-depleted state**
  - Apoferritin level is low, allowing absorbed Fe^{3+} to move freely across the Intestinal Mucosal cell,
  - Fe^{3+} is converted to Fe^{2+} (by Ferric Reductase) and released into plasma,
  - In plasma: Fe^{2+} is convert back to Fe^{3+} (by Ferroxidase) and then bound to and transported by Transferrin

- In **Iron Replete state**: (**Fig. 2**)
  - Increased amount of **Apoferritin** is produced to **trap Iron** as **Ferritin**
    - Apoferritin binds absorbed Fe^{3+} forming Ferritin, which is then retained in the Intestinal Mucosal cells (**Fig. 2**)
  - Iron in Ferritin retained by mucosal cells is lost into the Intestinal lumen when the cells are sloughed
    - Mucosal cell retention is influenced by Iron status of the body:
      - It is reduced in Iron depletion
      - It is Increased in state of Iron overload

What factors influence the capacity of the body to absorb Iron?

- Some factors that can influence Iron absorption from diet:

  State of Iron Stores:
  - Amount of Iron in the body;
    - GIT increases absorption when iron stores are low and decreases absorption when stores are sufficient
    - Percentage of Iron absorbed (i.e., Iron Bioavailability) can vary from less than 1% to greater than 50%

  Rate of Erythropoiesis:
  - Increase rate of RBC production can stimulate Iron uptake several folds

  Chemical State of Iron or kind of Iron in the Diet:
  - Heme Iron (Fe^{2+}) is 2 to 3 times more absorbable than Non-Heme Iron (Fe^{3+}) in Plant-based foods and Iron-fortified foods
  - Gastric Acid enhances uptake of Non-Heme Iron: H^+ ion (low pH,) facilitates conversion of F^{3+} to Fe^{2+} before uptake

- Duodenal Microvilli contain Ferric Reductase that can catalyze conversion of F^{3+} to Fe^{2+} thereby promoting uptake of Fe^{3+}

Content of diet: (presence or absent of Enhancers and Inhibitors):

- Bioavailability of Non-Heme Iron is strongly affected by the kind of other foods Ingested at the same meal
- **Enhancers of Non-Heme Iron absorption:**
  - Vitamin C (Ascorbic Acid and Dehydroascorbate), Reducing agents (e.g., Fructose), Citrate

- **Inhibitors of Iron absorption:**
  - Oxalate, Poly-phenols (in certain vegetables), Tannins (in Tea), Phytates (in Bran), Calcium (in dairy products), Fibers (Vegetarian diets are usually low in Heme Iron), Antacids

TAKE NOTE:

- Iron Bioavailability in a Vegetarian diet can be increased by careful planning of meals to include other sources of Iron and Enhancers of Iron absorption

What are the three mechanisms that regulates uptake of Iron?

- **Stores Regulator**:
  - As body Iron stores fall, the Mucosa is signaled to moderately increase uptake

- **Erythropoietic Regulator**:
  - In response to Anemia, the Erythroid cells will signal the Mucosa to increase Iron uptake more significantly

- **Dietary Regulator**:
  - A short-term increase in dietary Iron is not absorbed as the Mucosal cells have accumulated Iron and "Block" additional uptake

What is the rate of Iron turnover and possible sources of Iron loss?

- **Major turn over of Iron**:
  - Synthesis and Destruction of RBC
  - Adult male: About 95% of Iron for RBC synthesis is recycled from the breakdown of RBC, only 5% comes from dietary sources
  - Infant: Estimated to derive about 70% of Iron for RBC synthesis from the breakdown of RBC and 30% from the diet

- **Some sources of Iron Loss**:
  - Adult male & female: Iron is loss in cells desquamated from the Skin and Intestinal Mucosa
    - Females have additional losses via: Menstruation, Pregnancy, Delivery and postpartum

  - Women of childbearing age require additional Iron to compensate for Menstrual blood loss and for tissue growth during pregnancy

- Pathological GIT Iron loss via GIT bleeding occurs in infants and children sensitive to Cow's milk and in adults who have Peptic Ulcer Disease, Inflammatory Bowel Syndrome, or Bowel Cancer
- Hookworm infections are associated with GIT blood loss and iron depletion

IRON STORES:

How is Iron stored in the body? (Fig. 1)

- Iron not needed for functional purposes is stored mainly as soluble protein complex **Ferritin**
  - Ferritin binds and stores only Ferric (Fe^{3+}) ions
  - Ferritin is present in: Intestinal Mucosal cells, Bone Marrow, Liver, Spleen and Skeletal Muscles

- **When Iron store is adequate**:
  - Large amount of Apoferritin is synthesized to Trap excess Iron within the Mucosal Cell preventing Transfer of Iron to capillary bed

- **When Iron store is low**:
  - Virtually no Apoferritin is synthesized so as not to compete against the transfer of Iron to Plasma

- Excess Iron absorption (e.g., Hemochromatosis: disorder in Iron metabolism):
  - Body stores of Iron are greatly increased with very high deposit of Iron in many organs (e.g., Liver, Pancreas, Skin)
  - Much more Ferritin is present in Liver and Spleen

How significant is Transferrin?

- Transferrin is the major transport protein for Iron in Blood
- Transferrin binds and transport only Fe^{3+} ions ($2Fe^{3+}$ per Transferrin)
  - Most of the Iron-Binding Capacity of blood plasma is due to Transferrin
- Transferrin is usually about one-third (33.0%) saturated with Iron,
- Transferrin saturation below 15%, usually indicates Iron deficiency with some degree of clinical effect expected
- High % saturation of Transferrin is a sensitive marker for Iron overload
- Plasma level of Transferrin may be reduced during:
  - Protein-energy malnutrition, Acute-phase response, Infections, Neo-plastic disease, Chronic Liver disease

What is the role of Ceruloplasmin in Transport of Iron in Plasma? (Fig.3)

- Ceruloplasmin (Ferroxidase) is a copper-containing enzyme involved in Iron transport
- Ferric Reductase converts Fe^{3+} stored by Ferritin to Fe^{2+}, which is then released from Mucosal Cell
- Fe^{2+} crosses the plasma membrane into the blood
- In the blood Ferroxidase (Ceruloplasmin) a copper-containing enzyme catalyzes the conversion of Fe^{2+} to Fe^{3+}
- Transferrin then binds and transports Fe^{3+} in blood

How significant is the Total Iron-Binding Capacity (TIBC)?

- Total Iron Binding Capacity (TIBC) is a measurement of all the proteins available for binding Mobile Iron in blood (**Fig. 4**)
- TIBC is the maximum amount of Iron in blood that can be bound
  - Ferritin is not included in TIBC, because it binds only Stored Iron
- Transferrin represents the largest quantity of Iron-binding proteins
  - TIBC is an indirect, measurement of Transferrin
- During Iron overload, Transferrin levels stay about the same or decrease, whereas the other less common Iron-carrying proteins increase in number
  - Thus, TIBC is less reflective of true Transferrin levels
  - TIBC varies minimally with iron intake
  - TIBC is more a reflection of Liver function (Transferrin is produced in the liver) and Nutrition than of Iron metabolism
- During Iron deficiency or late pregnancy TIBC is increased, but saturation is decreased
- During Iron overload (Hemochromatosis), TIBC is low, but it is saturated
- TIBC can vary depending on the clinical condition (**See Fig. 5**)

Use a properly labeled schematic diagram to give a brief overview of Iron metabolism: SEE FIGURE 6

MANIFESTATIONS OF IRON DEFICIECY:

What is Anaemia?

- Anaemia is indicated when Hb level falls below recommended cutoff points
- Hb cutoff points recommended by WHO are as follows:
  - Pregnant women: **Hb ≥ 110.0g/L** (11.0g/dL)
  - Children 6 to 59 months of age: **Hb ≥ 110.0g/L** (11.0g/dL)
  - Non-pregnant women: **Hb ≥ 120.0g/L** (12.0g/dL)
  - Men: **Hb ≥ 130.0g/L** (13.0g/dL)

<u>TAKE NOTE</u>:

- Hb cutoff values can be affected by Altitude and Race
- Anaemia can be diagnosed by measuring:
  - Hb concentration in blood or
  - Proportion of RBC in Whole blood (Hematocrit)

What Hb cutoff points are used to indicate Anaemia in PNG?

What is Iron deficiency?

- Iron deficiency is a concept that is difficult to define
- During adequate nutrition, Iron is stored in tissues and is used when dietary intake of Iron is inadequate or bioavailability of Iron is low
- Iron stored mainly in the Liver is an Index of Iron Nutritional Status
- **Depletion of the Iron Store in the body constitutes Iron Deficiency**
- Iron deficiency represents a Spectrum ranging from:
  - Iron depletion, which causes no Physiological Impairments, to

- Iron-deficiency Anaemia, which affects the functioning of several organ systems

Iron deficiency usually occurs in Three Sequentially developing stages.

What are the Three Sequential stages of Iron deficiency?

First Stage: Depleted Iron Stores:

- Depletion of Iron Stores: amount of Stored Iron (Serum Ferritin < 12ug/L) is reduced but the amount of Functional Iron may not be affected
  - No stored iron is available to mobilize if the body requires more Iron
  - Hb concentration remains above Cutoff Levels, thus Anaemia is absent
    - Limitations in Diagnosis of the First Stage:
      - Ferritin cannot be used to accurately assess depleted Iron stores in individuals with poor health, **Why?**
      - Because Ferritin is an Acute-phase reactant,
        - Serum Ferritin levels increase during (Subclinical and Clinical) Infectious and Inflammatory diseases

Second Stage: Iron Deficient Erythropoiesis:

- In Iron Deficient Erythropoiesis, Iron stores are depleted and Transport Iron is significantly reduced
- Hb concentration remains above Cutoff Levels, thus Anaemia is absent
- Concentration of Transferrin Receptors are increased
- Shortage of Iron results in increased free Erythrocyte Protoporphyrin concentration in young RBC

Third Stage: Iron Deficiency Anaemia (IDA):

- Iron Deficiency Anaemia is the most severe form of Iron Deficiency,
  - Iron store is depleted
  - There is significant reduction in Iron transport
  - Iron supply is inadequate for Hb synthesis

- Hb concentrations falls below Cutoff Levels, resulting in Anaemia
  - Shortage of Iron leads to underproduction of Iron-containing functional compounds, including Hb
  - In Iron-deficiency Anemia, RBC are Microcytic and Hypochromic

What are some of the consequences of Iron deficiency and IDA?

- In Children, Iron-deficiency Anaemia can cause developmental delays, behavioral disturbances, decreased motor activity and decreased attention to tasks
- Developmental delays may persist past school age (i.e., 5 years) if the Iron deficiency is not fully reversed
- Iron-deficiency Anaemia contributes to Lead poisoning in children by increasing the ability of the GIT to absorb heavy metals, including lead

- Among pregnant women, Iron-deficiency Anaemia during the first two trimesters of pregnancy is associated with a twofold-increased risk for preterm delivery and a threefold increased risk for delivering a low-birth weight baby
- Iron deficiency is associated with decreased Immuno-competence

LABORATORY TEST FOR IRON STAUS:

What are some of the test used to assess Iron status?

- Iron status can be assessed through several laboratory tests, however, no single test is accepted for diagnosing Iron deficiency
- Some Biochemical tests can be used to detect earlier changes in Iron Status

- **Biochemical tests** include the following:
  - Erythrocyte Protoporphyrin Concentration,
  - Serum Ferritin Concentration,
  - Concentration of Transferrin Receptors
  - Transferrin Saturation

- Hematological tests based on characteristics of RBC (Hb concentration, Hematocrit, Mean Cell Volume (or Mean Corpuscular Volume), are generally more available and less expensive than Biochemical tests

What is the significant of the Erythrocyte Protoporphyrin Concentration test?

- Erythrocyte Protoporphyrin is the immediate precursor of Hb
- Concentration of Erythrocyte Protoporphyrin in blood increases when insufficient Iron is available for Hb synthesis
- Iron deficiency, Lead poisoning, Infection and Inflammation cause elevate of Erythrocyte Protoporphyrin concentration
- Assessment of Iron status using Erythrocyte Protoporphyrin has some advantages and disadvantages relative to other laboratory measures
  - Day-to-day variation within persons for Erythrocyte Protoporphyrin concentration is less than that for serum Iron concentration and Transferrin saturation
  - High Erythrocyte Protoporphyrin concentration is an earlier indicator of Iron-deficient Erythropoiesis than is Anaemia,
  - High Erythrocyte Protoporphyrin concentration is not as early an indicator of low Iron stores as is low serum Ferritin concentration

What is the significant of the Serum Ferritin Concentration test?

- Under normal physiological conditions, Serum Ferritin concentration is closely related to body Iron stores
    - About 1.0ug/L of serum Ferritin concentration is equivalent to approximately 10.0mg of stored iron

- Serum Ferritin concentration is an early indicator of the status of Iron stores
- Serum Ferritin is the most specific indicator available of depleted Iron stores, especially when used in conjunction with other tests to assess Iron status
  - For example, for women with low Hb level (Anaemia):

- Serum Ferritin concentration less than or equal to 15ug/L strongly indicates Iron deficiency
- Serum Ferritin concentration greater than 15ug/L suggests that Iron deficiency is not the cause of the Anaemia

- Increased concentration of Serum Ferritin occurs during Iron Overload, irrespective of the cause
- Ferritin is one of the Acute-phase proteins, thus may increase during infection or other acute disorders

- Factors other than the level of stored iron can result in large within-individual variation in serum Ferritin concentration
  - For example, because serum Ferritin is an acute-phase reactant, chronic infection, inflammation, or diseases that cause tissue and organ damage (e.g., hepatitis, cirrhosis, neoplasia, or arthritis) can raise its concentration independent of iron status. This elevation can mask depleted iron stores

Reference:

1. Biochemistry with clinical correlations, Ed. Devlin 4th Edition; Pages 1003 to 1009,1116, 1130.
2. Harper's Biochemistry Ed. R. K. Murray et al, 24th Edition, Pages711 to 715.
3. WWW.telpath2.med.utah.edu/webpath/tutorial/iron/
4. WWW.cdc.gov/epo/mmwr/preview/mmwrhtml/
5. WWW. library.med.utah.edu/NetBiochem/images/intferri.gif

ABSORPTION of IRON (Fig. 1)

In the stomach:

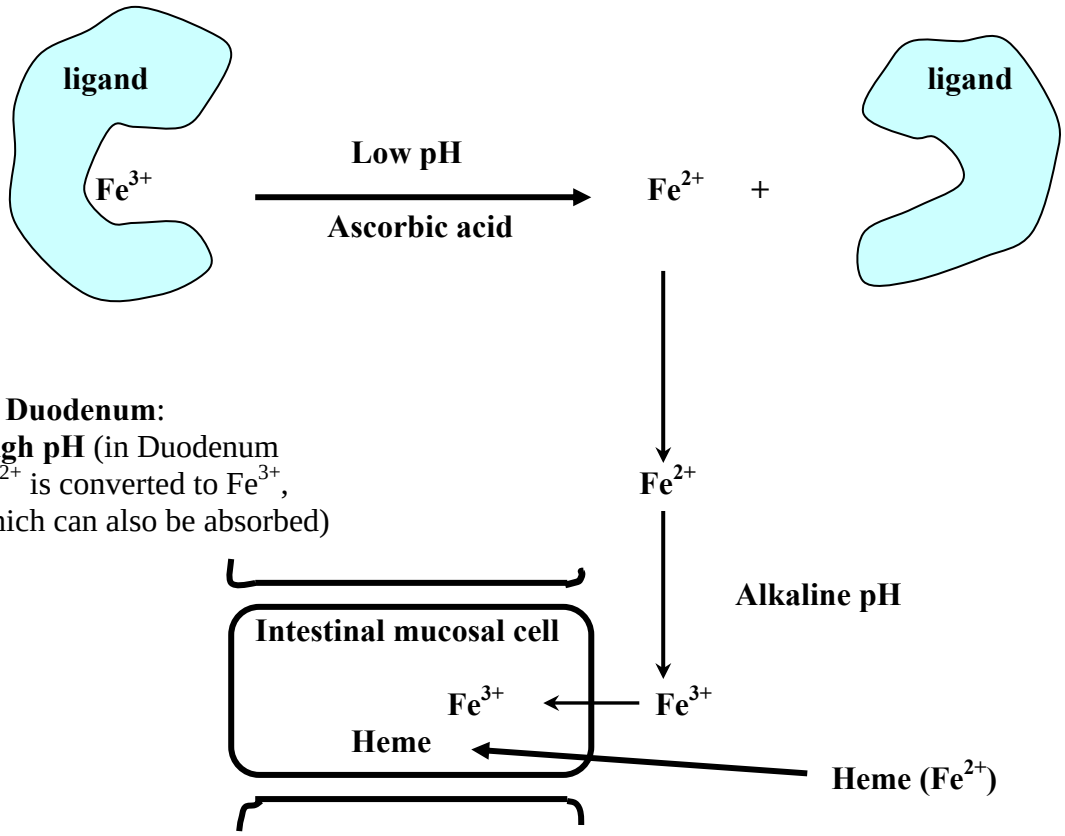

Fig. 1: Simplified Schematic diagram of Absorption of Iron:

Absorption of Fe^{2+} (Heme Iron) is about 2 to 3 times for effective than Fe^{3+} (Non-Heme Iron)

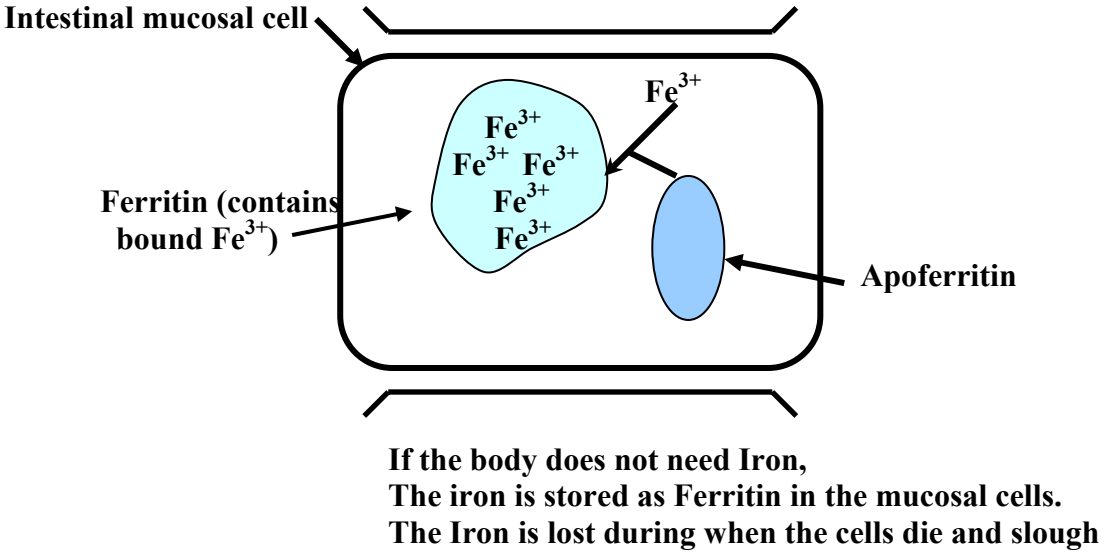

Fig. 2: Storage of Iron as Ferritin in Intestinal Mucosal Cells: Iron Replete state

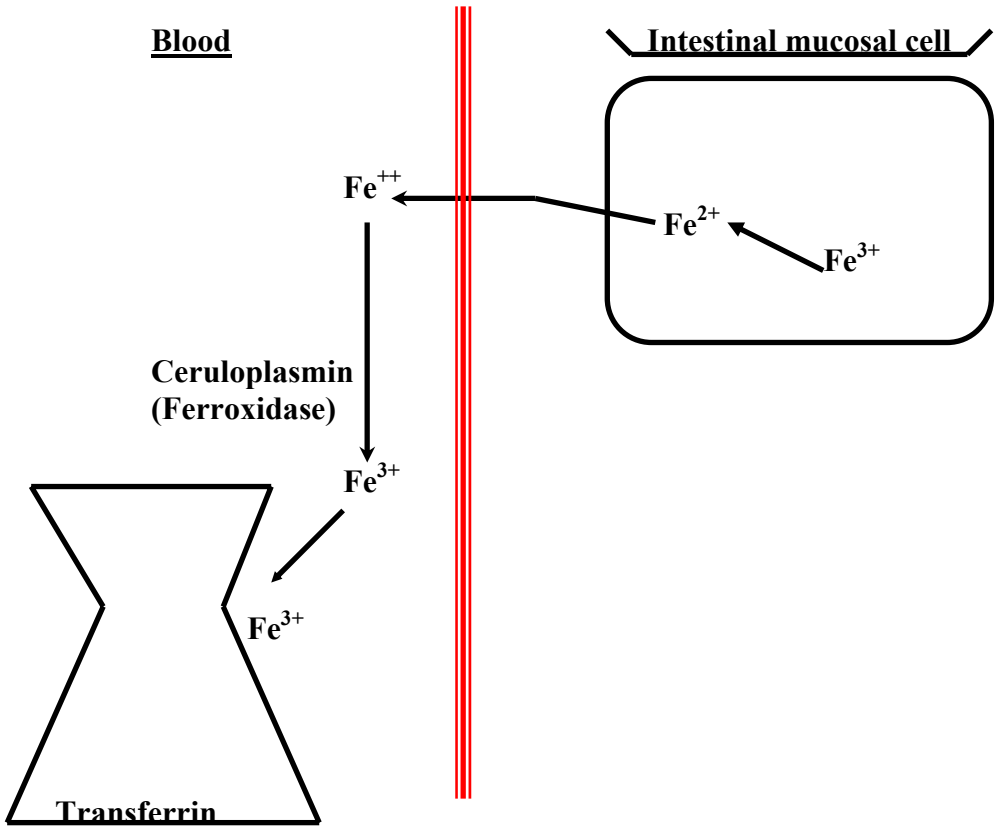

Fig. 3: Role of Ceruloplasmin in transport of Iron by Transferrin in plasma

Ferric Reductase converts Fe^{3+} stored by Ferritin to Fe^{2+}, which is released from Mucosal Cell
Fe^{2+} crosses the plasma membrane into the blood
In the blood Ferroxidase (Ceruloplasmin) a copper-containing enzyme converts Fe^{2+} to Fe^{3+}
Transferrin then binds and transports Fe^{3+} in blood

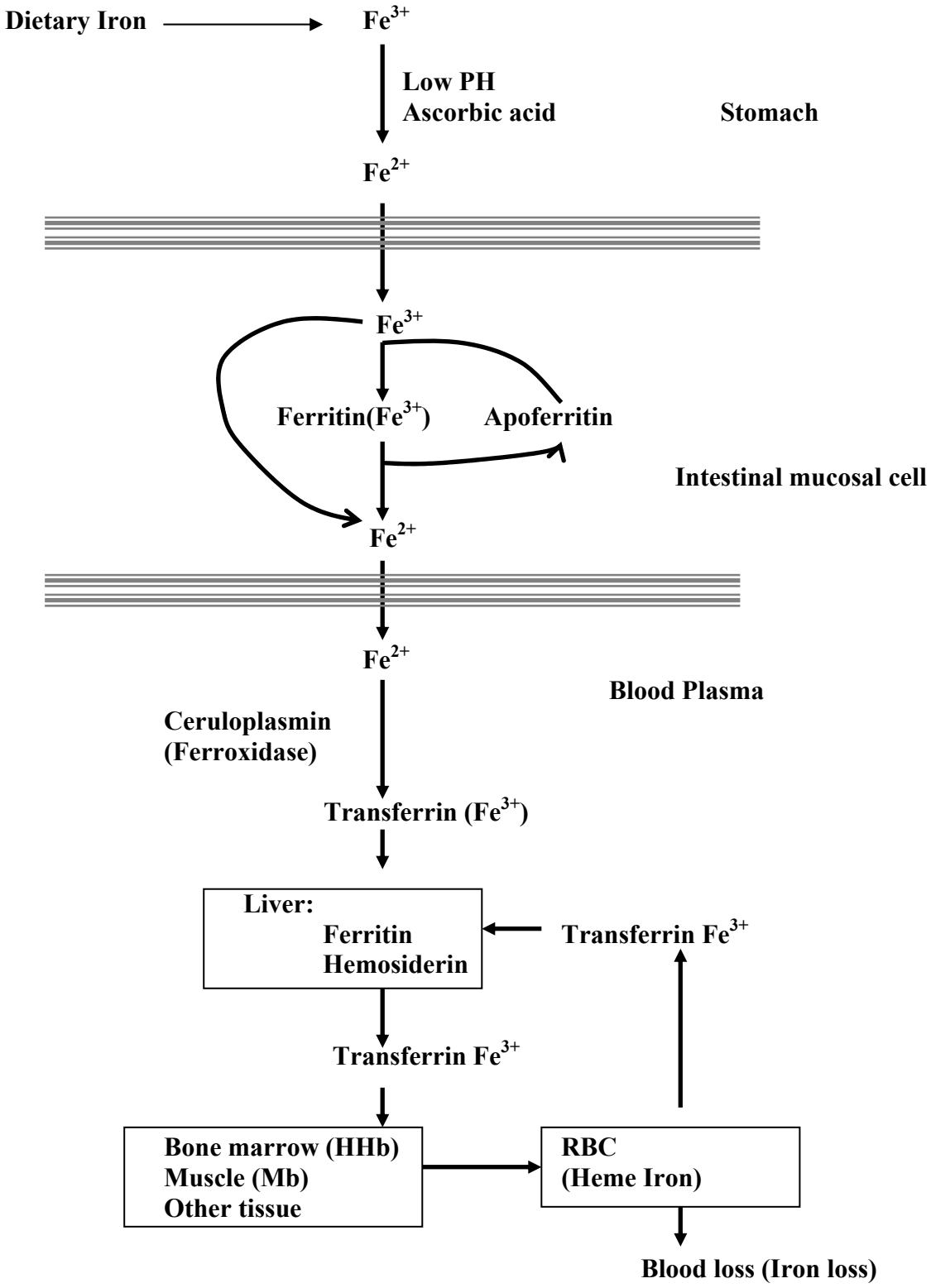

Fig. 5: Schematic representation of an Overview of Iron Metabolism

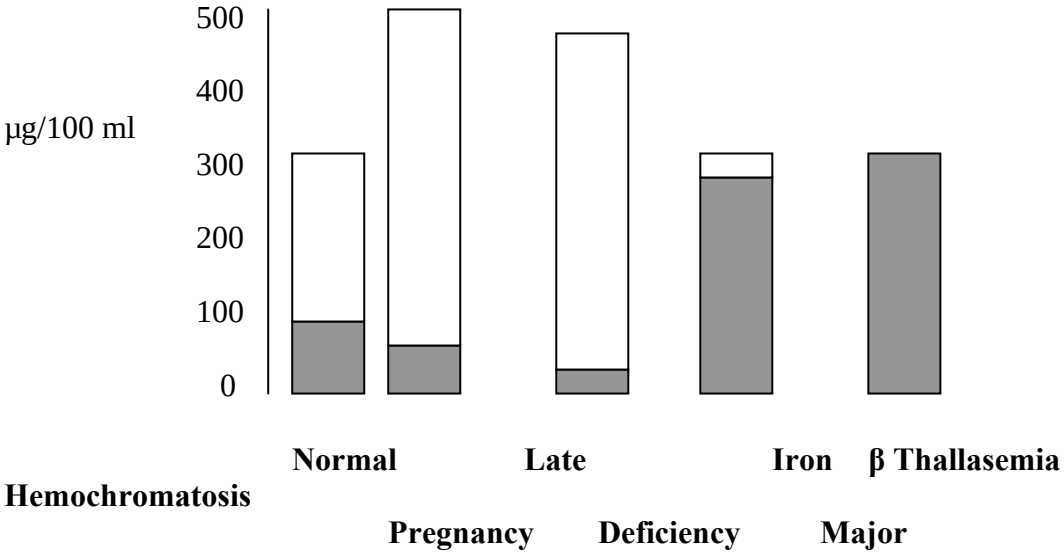

Fig. 6 Serum Iron and Iron Binding Capacities In Various Physiological & Pathological Conditions

Fig. 4: Serum Iron & Iron Binding Capacity