Iron Metabolism and Disease

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Iron

- Element (Fe)
- Molecular weight 56
- Abundance
- May be 2+ or 3+
  - Ferrous (2+) “reduced” - gained an electron
  - Ferric (3+) “oxidised” - lost an electron

$$Fe^{+++} + e^{-} \leftrightarrow Fe^{++}$$

- Redox states allows activity passing electrons around body
- Redox change required for iron metabolism

Iron functions

- Oxygen carriers
  - haemoglobin
- Oxygen storage
  - Myoglobin
- Energy Production
  - Cytochromes (oxidative phosphorylation)
  - Krebs cycle enzymes
- Other
  - Liver detoxification (cytochrome p450)

Iron Toxicity

- Iron can damage tissues
- Catalyzes the conversion of hydrogen peroxide to free-radical ions
- Free-radicals can attack:
  - cellular membranes
  - Proteins
  - DNA
- Iron excess possibly related to cancers, cardiac toxicity and other factors

Iron Distribution

- 35 – 45 mg / kg iron in adult male body
- Total approx 4 g
  - Red cell mass as haemoglobin - 50%
  - Muscles as myoglobin – 7%
  - Storage as ferritin - 30%
    - Bone marrow (7%)
    - Reticulo-endothelial cells (7%)
    - Liver (25%)
  - Other Haem proteins - 5%
    - Cytochromes, myoglobin, others
  - In Serum - 0.1%

Iron Transport in Blood

- Red cells
  - As haemoglobin
  - Cannot be exchanged
- Plasma
  - Bound to Transferrin
  - Carries iron between body locations
  - eg between gut, liver, bone marrow, macrophages
  - Iron taken up into cells by transferrin receptors

Iron status

DIAGNOSTIC TESTS

Transferrin

- Protein MW 77,000
- Synthesised in the liver.
- Two diantennary carbohydrate chains.
- Each molecule binds can bind two Fe^{3+} molecules (oxidised) (transferrin binds to iron at 18mcmol/L. It is capable of binding up to 54mcm/L and therefore is unsaturated in normal state i.e. 33% saturated)
- Contains 95% of serum Fe.
- Usually about 30% saturated with Fe.
- Production decreased in iron overload.
- Production increased in iron deficiency.
- Measured in blood as a marker of iron status.

Total Iron Binding Capacity (TIBC)

- *High levels*:
  - Low body iron stores.
- *Low levels:*
  - High body iron stores.
- *Other conditions*
  - Decrease TIBC: high oestrogen states (pregnancy, OCP)
  - Increase TIBC: malnutrition, chronic liver disease, chronic disease (eg malignancy), protein-losing states, congenital deficiency, neonates, acute phase (negative reactant).

Transferrin Receptors

- Collects iron from transferrin for uptake into cells
  - Recognises and binds transferrin
  - Receptor + transferrin endocytosed
  - Iron released into cell via Iron transporter (DMT1)
  - Receptor + transferrin return to cell surface
  - Transferrin released

Soluble Transferrin Receptors

- Truncated form of cell surface receptors
- Found in the circulation
- High levels with iron deficiency
- Low levels with iron overload
- Possible role in diagnosis of iron deficiency compared in setting of inflammation

Serum Iron

- The serum contains about 0.1% of body iron
- Over 95% of iron in serum bound to transferrin
- Measures all serum iron (not in red cells)
- Of limited use on its own
- Useful for interpretation of iron status only if grossly abnormal – eg iron poisoning
- Commonly combined with serum transferrin to express transferrin saturation

Serum Iron Measurement

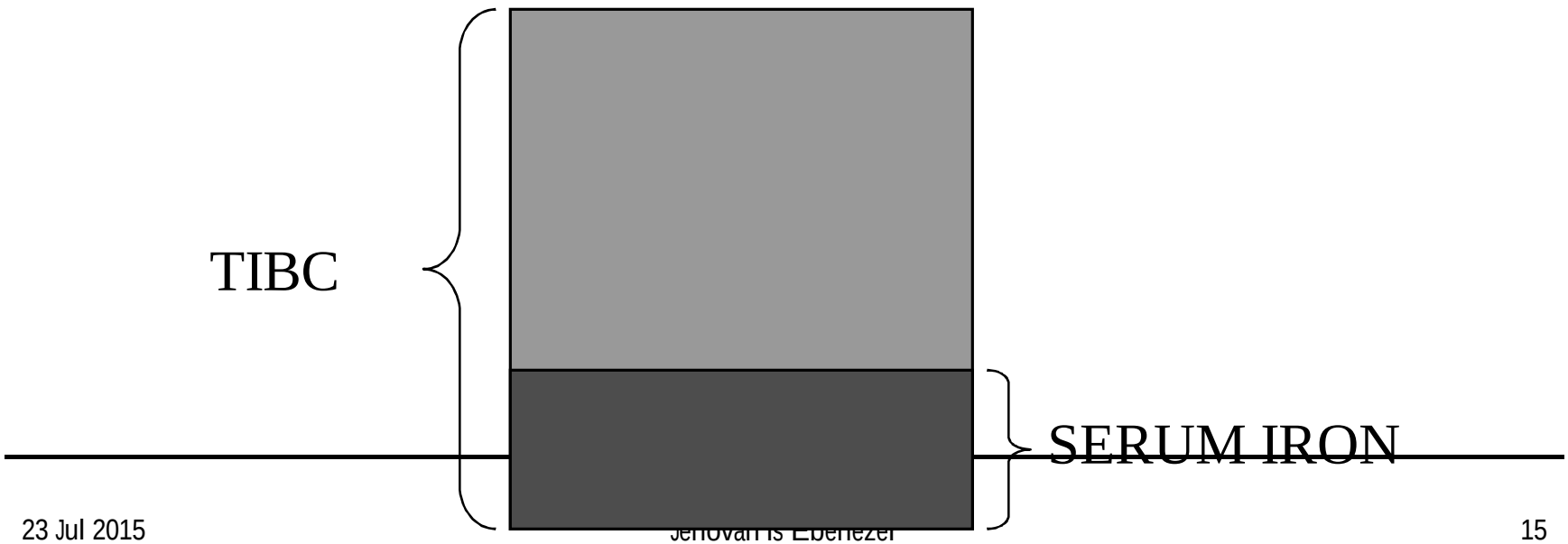
- Investigation of haemochromatosis and in diagnosis and management of iron poisoning
- Low levels:
  - Iron deficiency
  - Other: Random variation; acute or chronic inflammation; pre-menstrual.
  - Associated with infection, trauma, rheumatoid arthritis, neoplasia
- High levels:
  - Iron Overload: hepatitis
  - Other: Random variation, OCP, pregnancy, recent iron ingestion

Transferrin Saturation

- Percent of transferrin (TIBC) iron-binding sites which are filled with iron
- Combines two factors to improve sensitivity
- Iron overload
  - High iron plus low transferrin
  - High saturation (50 – 100%); normal 33%
- Best serum marker of increased body iron
- Used as a screen for iron overload

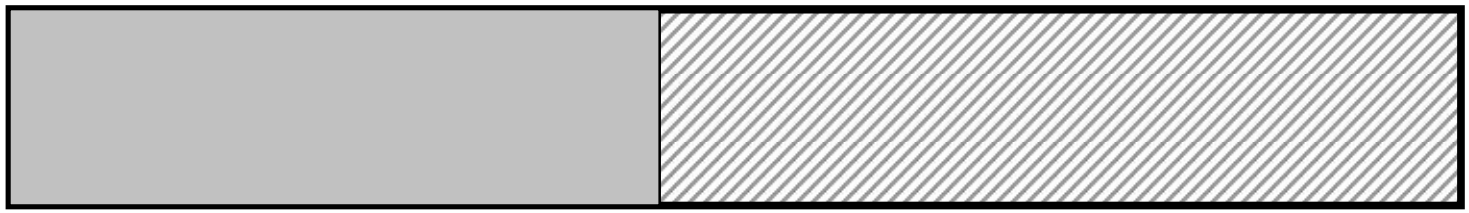
PERCENT SATURATION

$$\text{PERCENT SATURATION} = \frac{\text{SERUM IRON}}{\text{TIBC}} \times 100$$



Transferrin Saturation

NORMAL IRON STATUS



Normal iron Normal transferrin Saturation 40%

IRON OVERLOAD

High iron Low transferrin Saturation 80%

Iron Storage - Ferritin

(When there is insufficient apoferritin, iron is usually in the plasma as COLLOIDAL IRON OXIDE)

- Iron store in the liver and nearly all other cells.
- MW 460,000.
- Outer shell: apoferritin, consists of 22 protein subunits
- Iron-phosphate-hydroxide core.
- 20% iron by weight, binding up 4,500 atoms of iron per molecule.
- Small fraction found in circulation (contains less than 1% of serum iron).
- Stores iron and releases it in a controlled

Ferritin - Measurement

- A routine blood test – reflects iron stores
- Low serum levels
  - Indicate Iron deficiency (high specificity)
- High serum levels
  - Iron overload e.g haemachromatosis
- Other - Ferritin may be increased in serum by:
  - Tissue release (hepatitis, leukaemia, lymphoma)
  - Acute phase response (tissue damage, infection, cancer)
- Interpretation
  - Low levels always indicate Fe deficiency.

Iron Loss

- Physiological
  - Cell loss: gut, desquamation
  - Menstruation (1mg/day)
  - Pregnancy, lactation
- Pathological
  - Bleeding
  - Gut, menorrhagia, surgery, gross haematuria

Iron re-use

- Old cells broken down in macrophages in spleen and other organs
- Iron transported to liver and other storage sites

- Red cell iron recovered from old red cells
- Very little iron lost in routine metabolism

Iron Scavenging

- Intravascular haemolysis
- Breakdown of red cells in the circulation
  - Free haemoglobin binds haptoglobins -> taken up by liver
  - Free haem binds haemopexin -> taken up by liver
  - Haem passing through kidney resorbed
  - Three mechanisms to conserve iron in pathological situations

Iron Loss

- An unregulated process
- No mechanisms to up- or down-regulate iron loss from the body
- Over-intake cannot be matched by increased loss
- Under intake cannot be matched by decreased loss
- Thus iron homeostasis is regulated by adjusting iron intake

Iron Absorption

- 1 – 2 mg iron are absorbed each day
- (in iron balance 1 – 2 mg iron leaves the body each day)
- Occurs in the duodenum
- Taken up as ionic iron or haem iron
- Only 10% of dietary iron absorbed
- Dietary iron usually in excess
  - either not absorbed, or kept in enterocytes and shed into the gut

Haem iron absorption

- Haem split from globin in intestine
- Absorbed into enterocyte as haem
- Iron freed into enterocyte pool or absorbed intact

Iron absorption regulation

- Increased
  - Low dietary iron
  - Low body iron stores
  - Increased red cell production
  - Low haemoglobin
  - Low blood oxygen content
- Decreased
  - Systemic inflammation

IRON ABSORPTION AND REGULATION

- HEPICIDIN is the key regulator of iron in our body.

HEPICIDIN

- Is a peptide hormone which was first identified in human urine and plasma in 2000.
- Its molecular weight is 25 Kda.
- Highly folded structure.
- Present in inactive form;prohepcidin(60aa) and its active form is hepicidin(25aa).

Hepicidin, Primary regulator

- Increased expression of hepicidin leads to Decrease iron absorption and release.
- Mutation :Hemochromatosis
- Increased expression:Iron deficiency
- Hepicidin mRna expression is reduced by erythropoetin,hypoxia & inflammation.
- Also binds to ferroportin.

Ferroportin

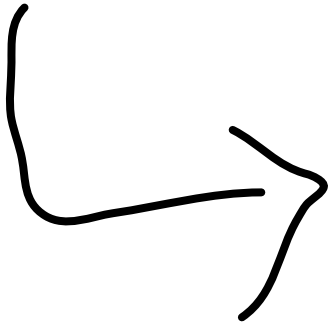
- The only cellular iron exporter in vertebrates.
- Present in macrophages,placenta and the hepatocytes.

Mechanism of action of hepicidin

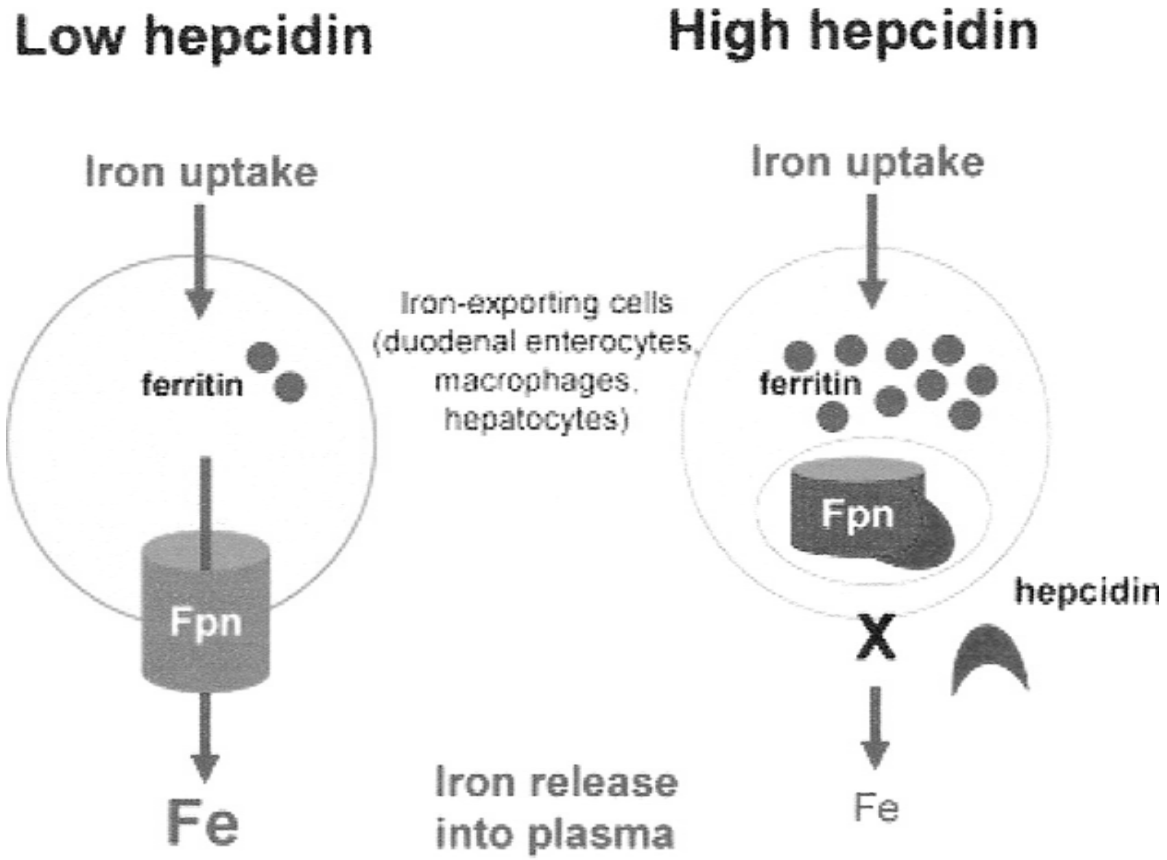
- The major mechanism of hepicidin is THE REGULATION OF TRANSMEMBRANE IRON TRANSPORT.
- It binds to FERROPORTIN ,forms hepicidin-ferroportin complex ,which is degraded in the lysosomes and iron is locked inside the cells(mainly enterocytes,hepatocytes and macrophages).

THEREFORE,

- Hepcidin lowers iron absorption in the intestine ,lowers iron releasing from hepatocytes and macrophages



Serum iron is decreased.



Hepicidin Regulation

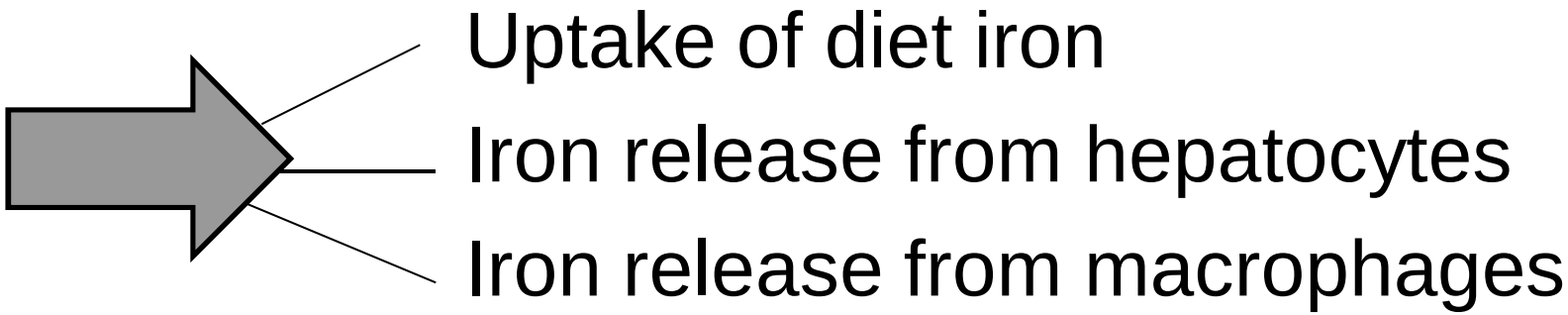
So when hepicidin levels are low ,iron exporting cells have abundant ferroportin and thus releases iron into plasma.When hepicidin concentration increases it binds to ferroportin and thus iron is retained in the cells.

Regulation of Hepicidin

- Hypoxia/Anemia
- Inflammation

Regulation of Hepcidin synthesis by anemia and hypoxia

- Oxygen ↓ ⇒ Hepcidin ↓



Regulation of Hepcidin synthesis by inflammation

- Interleukin-6 ↑ ⇒ Hepcidin ↑

⇒ iron ↓ ⇒ anemia of chronic disease.

- Generally when iron level ↑ ,ROS(Reactive oxygen species) that leads to ↑ in thiobarbutyric acid ↑ ⇒ activation of NF Kappa proteins activates IL-6 , hepicidin synthesis ↑

Disease States

- Hepcidin deficiency, physiological = Haemochromatosis
- Hepcidin excess – anaemia of chronic disease

Increased Iron Uptake

- Low dietary iron
- Leads to increased activity of:
  - DCytB and DMT1
- Caused by local factors in gut

Increased Iron Uptake

- Signal from body to gut in response to increased needs
- Hepcidin
  - Increased levels decrease iron absorption
  - Low levels increase iron absorption

Iron absorption regulation

- Increased
  - Low dietary iron
  - Low body iron stores *****
  - Increased red cell production *****
  - Low haemoglobin *****
  - Low blood oxygen content *****

  ***** lead to decreased hepcidin production
- Decreased
  - Systemic inflammation
  - leads to increased hepcidin production

The liver and iron metabolism

- Hepcidin production by the liver controls gut iron absorption and therefore body iron stores
- HFE and haemojuvelin involved in hepcidin regulation

Iron Release from cells

- Ferroportin present on cell surface to release iron
- Found on gut cells, liver cells and macrophages
- Requires cofactor to oxidise iron to allow for binding to transferrin
  - Hephaestin in gut
  - Caeruloplasmin in other cells
- Hepcidin blocks iron release from all cells
- A possible mechanism for anaemia of chronic disease

Iron Deficiency

- Extremely common
- Due to reduced intake, increased loss or increased demands
- Stores reduced before deficiency seen
- **Iron deficiency is not a diagnosis**
  - A cause needs to be identified!
  - Eg obstetric causes, low intake, malabsorption, bowel cancer, haemorrhoids, inflammatory bowel disease

Iron Deficiency

- Laboratory changes:
  - Low iron (poor specificity)
  - Low ferritin (excellent specificity)
  - Elevated Transferrin (TIBC)
  - Low transferrin saturation
  - Hypochromia, microcytosis
  - Anaemia
- Stages
  - Reduced iron stores
  - Iron deficient erythropoiesis
  - Iron deficient anaemia

Anaemia of chronic disease

- Infection, inflammation, malignancy
- Low iron absorption
- Low serum iron
- Stainable iron stores in RE cells
- Hepcidin is an acute phase protein
- Increased hepcidin
  - blocks iron in gut cells
  - Traps iron in macrophages and liver cells
- Produces a functional iron deficiency
  - Not responsive to iron therapy

Anaemia of chronic disease

- Hard to separate from iron deficiency anaemia
- May co-exist
- **Ferritin:** low with pure iron deficiency but increased with acute phase response
- **Iron:** low in both conditions
- **Transferrin**: high in pure iron deficiency but decreased with acute phase response

Genetic haemochromatosis

- Iron overload disease
- Caused by increased iron absorption
- May affect liver, pancreas, skin, heart, joints, endocrine organs (bronze diabetes)
- Gradual accumulation of iron over the life of the person (positive iron balance)
  - Iron overload detectable in teens and 20s
  - Organ overload in 30s
  - Organ damage in 40s and 50s
- Cirrhosis and liver disease main cause of increased mortality

Genetic Haemochromatosis

- >95% defect in HFE gene (C282Y)
- Associated with low hepcidin
- Leads to overactivity of ferroportin
  - Increased gut absorption of iron
- Also other mechanisms
  - Increased DMT1 and DcytB activity
  - Not related to hepcidin
- Limited penetrance (1 – 50%)
  - May require other genes to be involved

Other tests related to iron status

- Haemoglobin
  - Low with iron deficiency, anaemia of chronic disease
- Mean Cell Volume
  - Low with iron deficiency, thallassaemia
- Liver iron
  - High with iron overload
  - Better marker for GH when corrected for age
  - (Hepatic iron index)
- Bone marrow iron
  - Low with iron deficiency

CASE

- A 45 year old man presented with weight loss, lassitude and weakness. His skin was noticeably bronzed. On examination, he was found to have hepatosplenomegaly, rather sparse body hair and small testes. On further questioning, he admitted he had lost his libido and become impotent.
- Lab findings:
  - Urine positive for glucose
  - Blood glucose (fasting) 10 mmol/L (2.8-6.0)

Case

hemochromatosis!

Serum:	Iron	70umol/L (9-29)
	iron-binding capacity	67 umol/L
	ferritin	5000 ug/L (20-300)
	testosterone	9nmol/L (9-30)
	luteinizing hormone	2U/L (2-10)

- Comment on the results
- Give the management and prognosis

Conclusions

- Iron related diseases are common and clinically important
- Recent advances have changed our understanding
- Groups of tests “Iron studies” are the best first line investigation
- New tests and therapies will follow the new understandings.

Reading

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