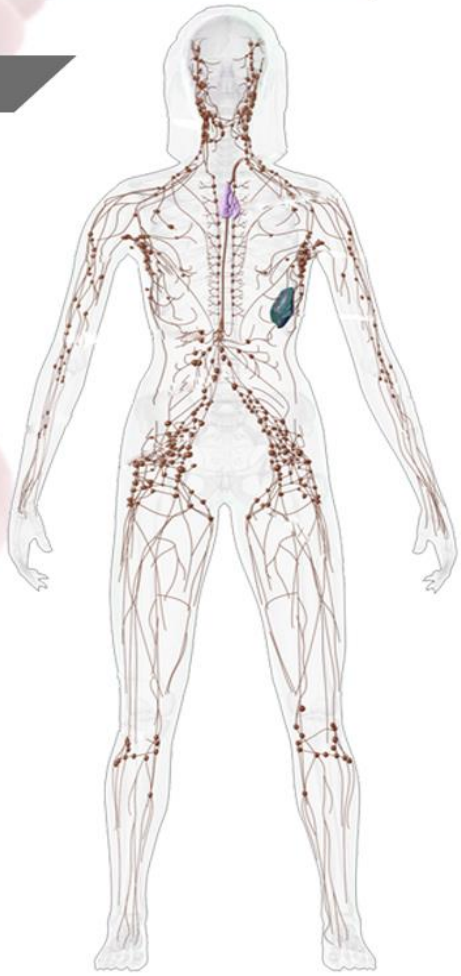
Sheet 5

Hematology and Lymphatic system

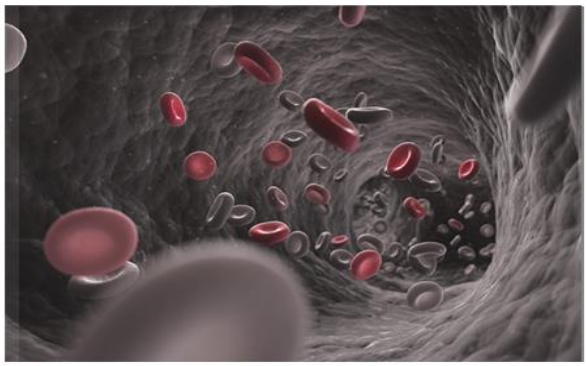
Subject | Biochemistry



Done by | Abdallah & Razi

Corrected by | Bann

Doctor | Dr. Mamoun



The doctor started the lecture by correcting a mistake regarding pyruvate kinase and the G6PD enzyme from the previous lecture, I will mention what he said here (Check Video 4 / timestamp 04:40).

Pyruvate Kinase (PK)

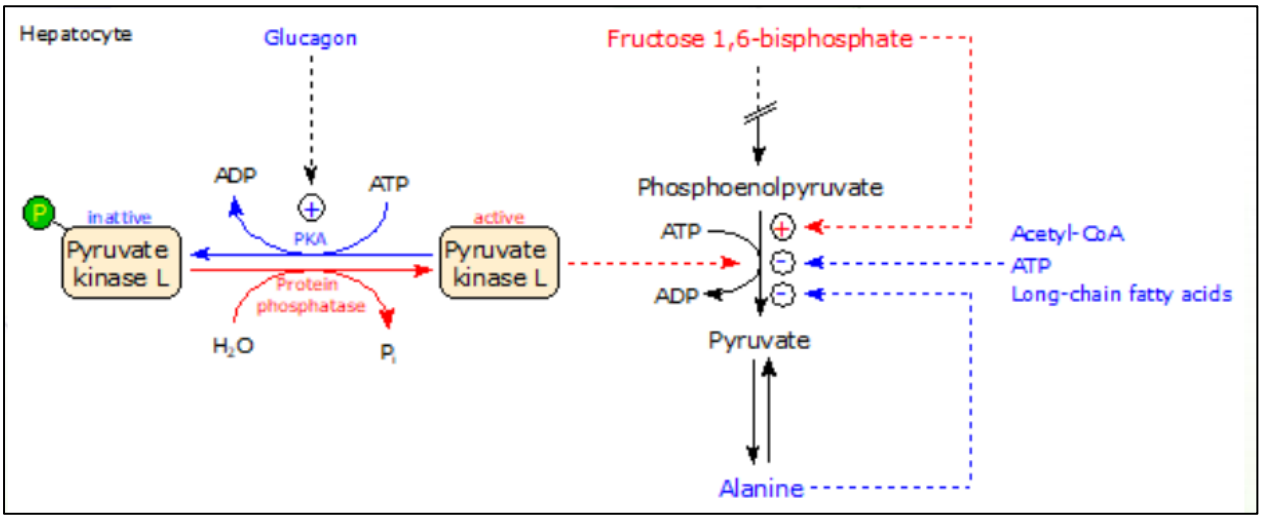
- There are **four** isozymes of PK: **PKL** *(liver)* and **PKR** *(erythrocytes)*, which are produced from PKLR gene. **PKM1** *(muscle and brain)* and **PKM2** *(fetal and most tissues)* produced from PKM gene (both isozymes from PKM ARE NOT allosteric).

The PKLR isozymes are allosterically regulated:

Remember: Allosteric regulation is the regulation of an enzyme by binding an effector molecule at a site other than the enzyme's active site. The site to which the effector binds is termed the allosteric site or regulatory

- **Inhibited** by:
  - -Phosphorylation by protein kinase A,
  - -ATP,
  - -acetyl-CoA,
  - -Alanine,
  - -long-chain fatty acids.
- **Activated** by: Fructose 1,6-Bisphosphate

The liver enzyme is also controlled at the level of synthesis. Increased carbohydrate ingestion induces the synthesis of PK.

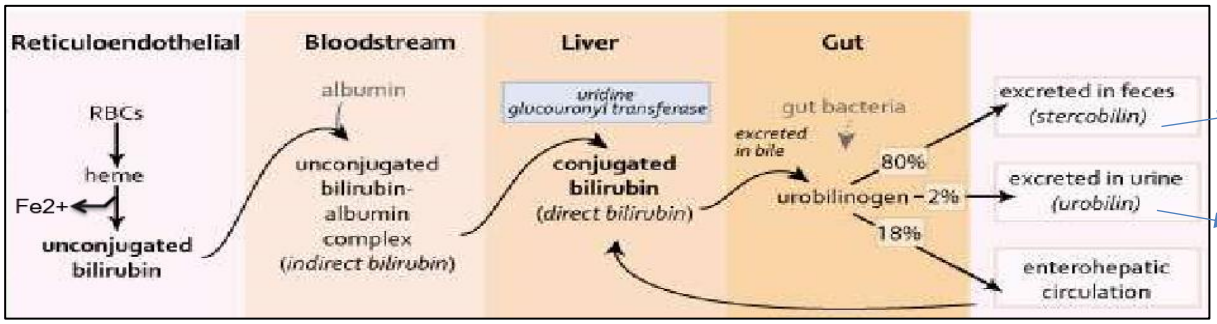


Fetal PK

In erythrocytes, the fetal PK isozyme has a much greater activity than the adult isozymes, thus, fetal erythrocytes have lower concentrations of glycolytic intermediates including 1,3BPG, (and 2,3BPG).

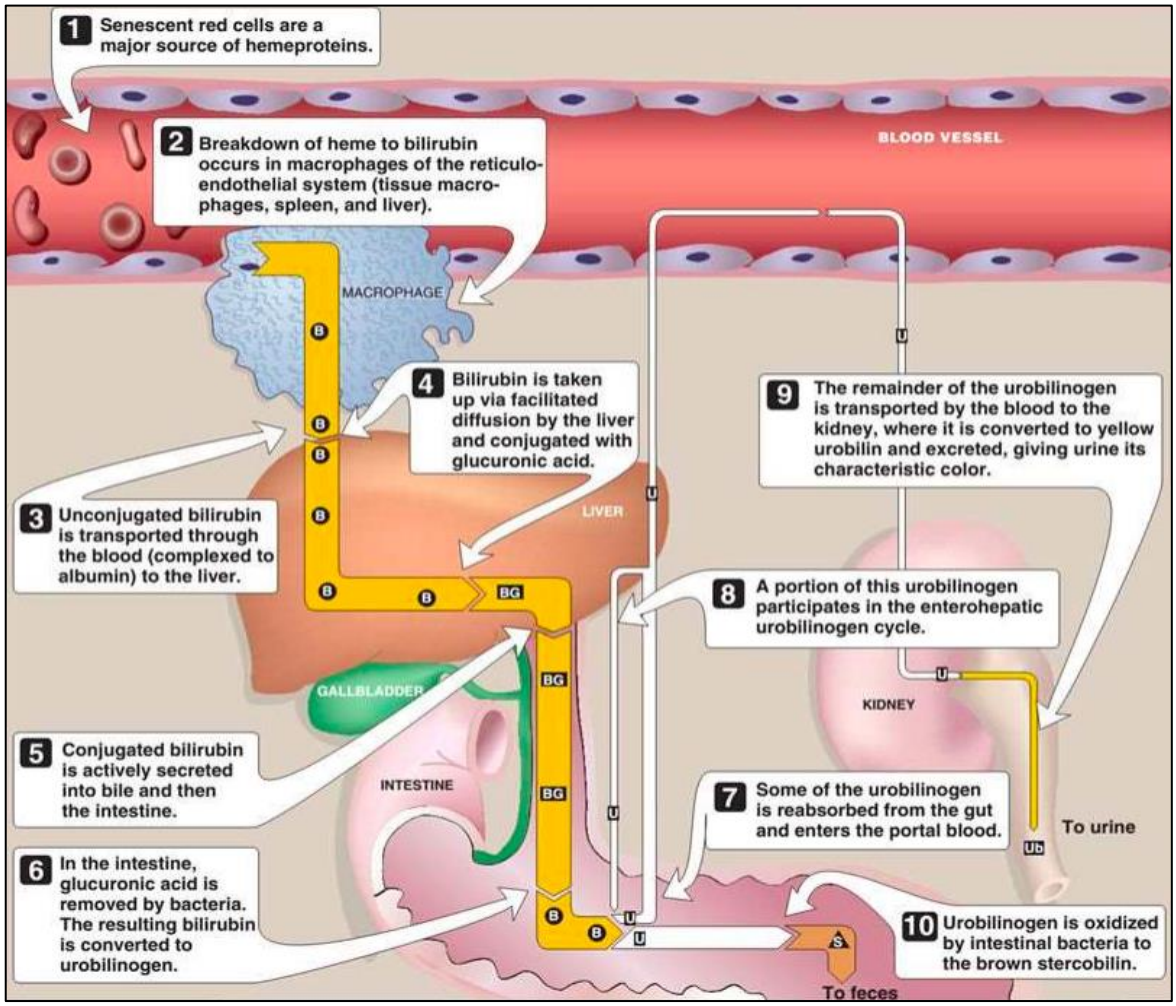
The second point the doctor mentioned was about **G6PD**, and that its activity **decreases** with aging RBCs and **not the age of the person**. At the age of 120 days of an erythrocyte, there is hardly any G6PD activity and that goes well in line with the age of an RBC (120 days); so, it is G6PD that determines the age of an RBC.

Catabolism of Heme



-The iron from heme degradation must be preserved because it is essential to our body. (heme degradation and synthesis will be discussed in more detail in a separate lecture)

- **Please pay attention to the picture**



Jaundice

Jaundice is the accumulation of bilirubin in the plasma and tissues.

In jaundice, tissues such as skin, nails, and sclerae (whites of eyes) have a yellow color.

Types of jaundice:

- Hemolytic jaundice: Massive lysis of RBC causes higher levels of unconjugated bilirubin.
- Hepatocellular jaundice: Liver damage, decreased conjugation efficiency, defective secretion of conjugated bilirubin into bile increase bilirubin in blood. Levels of urobilinogen increase in urine (dark) and pale stool.
- Jaundice in newborns: inefficient conjugating enzyme.
- Biliary obstruction: subnormal amounts of conjugated bilirubin reach the intestine for conversion to urobilinogen and conjugated bilirubin is excreted in urine (dark).

(This part was not mentioned by the doctor in the video, so I copied what was in the slides)

Now we will start the lecture which is about Iron metabolism in our bodies.

Iron

Iron is very precious to our body.

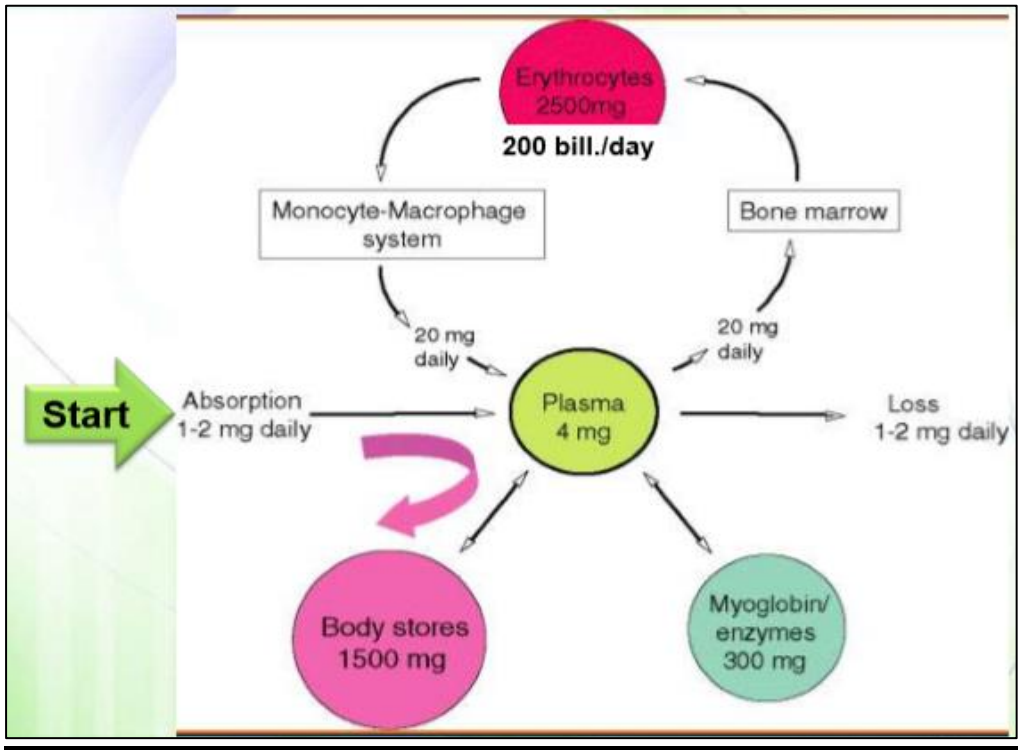
Within the body, iron exist in two oxidation states: ferrous (Fe^{2+}) or ferric (Fe^{3+}).

Iron is important for oxygen metabolism and transport. It is also the prosthetic group for a number of enzymes such as: **redox cytochromes**, and the **P450 class** of detoxifying cytochromes.

Iron is very precious to our body, yet, iron can be potentially toxic due its ability to form free radicals, for example, the Fenton reaction.

In Solution: iron is not free.

The life cycle of Iron in our bodies



From the previous picture, the doctor focused on a couple of points:

1- The RBC's posses almost 2500 mg of iron (the largest proportion).

2-Absorbtion is almost equal to excretion (there is no excretion pathway for iron in our bodies)

3- 3-4 mg circulates through the plasma. Small amounts are lost (about 1–2 mg/day) and are replaced by dietary absorption. 1 mg a day for men and 1.5–2 mg a day for women with regular menstrual period

4-Little amounts of Iron are absorbed from food but most is preserved, foods that contain high iron are lentils and meat

5- Well-nourished people have 3-4 grams of iron in their bodies.

6- Iron is mainly used for hemoglobin synthesis (70% of all iron). The iron for hemoglobin synthesis is recovered from dead erythrocytes. The recycled iron binds to transferrin in blood circulation and is distributed to iron-consuming cells, mainly erythrocytes. Additional iron (0.3–0.4 g) is channeled to other cellular proteins (myoglobin and cytochromes).

Sources of Iron

Most of the iron in the body is recycled from destroyed red blood cells. The released iron is scavenged by macrophages in the reticuloendothelial system. A significant portion (the majority of iron) is stored in the liver, both in the hepatocytes, and in the Kupffer cells (reticuloendothelial cells)

Iron Absorption

In Neutral or alkaline pH → iron is in Ferric (+3) form → Duodenum for example

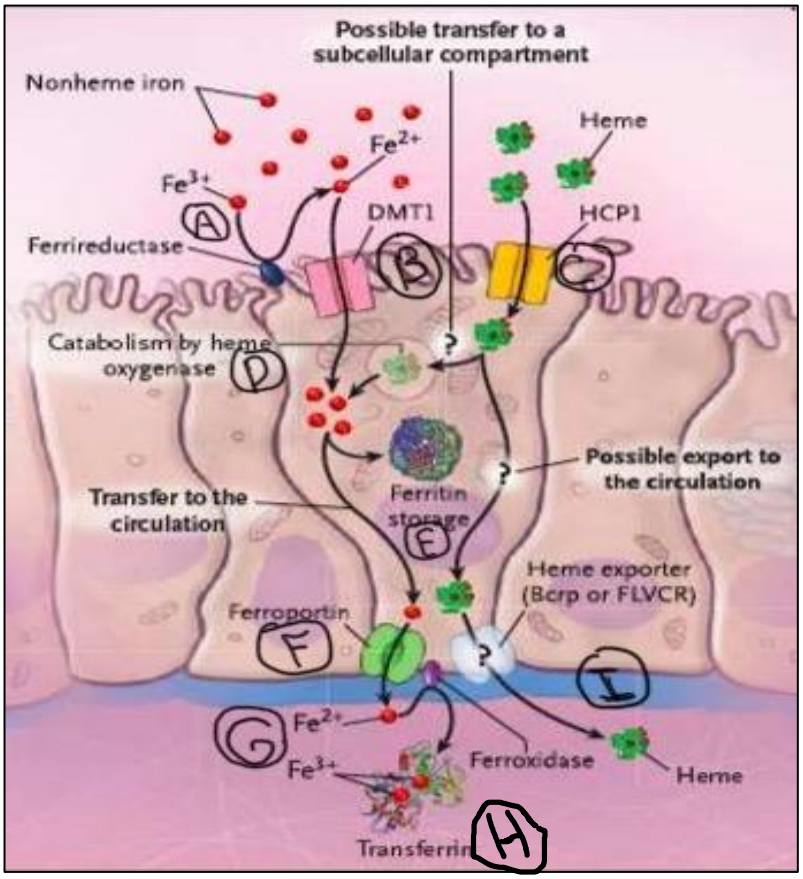
In Acidic pH → iron is In the Ferrous (+2) form → Stomach for example

However, to be absorbed, dietary iron must be in its ferrous Fe+2 form.

And for Iron to be stored, it must be in the ferric Fe+3 form.

The main sight of Iron Absorption is the enterocytes of the duodenum.

Before reading the text, take a good look at the picture, at each step in the picture I added a letter, check the corresponding letter throughout the text for the explanation of that part. Each corresponding letter in the text is CAPITAL, **bold** and underlined. From **A** to **I**



Non-heme Iron:

A- A ferric reductase enzyme (ferrireductase, which is membrane bound) on the enterocytes' brush border, Dcytb (duodenal cytochrome B), reduces ferric Fe3+ to Fe2+; so that it can be absorbed.

B- Divalent metal transporter 1 (DMT1) transports iron into the cell. (DMT-1 can also transport other metal ions such as zinc, copper, cobalt, manganese, cadmium or lead)

Just to refresh your memory:

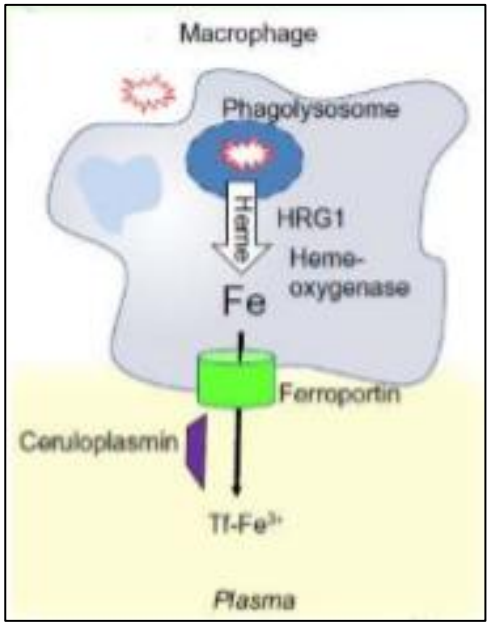
HEME IRON	NONHEME IRON
Iron that comes from the animal sources	Iron that comes from plant sources
Occurs in oysters, red meat, poultry, beef liver, and fish like sardines	Occurs in beans, nuts, lentils, greeny-leaves such as spinach, and pumpkin seeds
Consists of a heme protein attached to the iron	Does not contain a heme protein attached to the iron
Absorption rate is high	Absorption rate is comparatively low
Excess heme iron can cause health risks	Does not cause health risks

Heme Iron:

C-Iron can also be obtained from ingested heme; meat is a good source of heme. Heme is absorbed by a receptor called **heme-carrier protein (HCP)** and

D- iron (fe+2) is released from heme by **heme oxygenase1 (HO-1).**

In other cells such as macrophages, which destroy RBCs, heme oxygenase is also found and is useful in extracting iron from heme



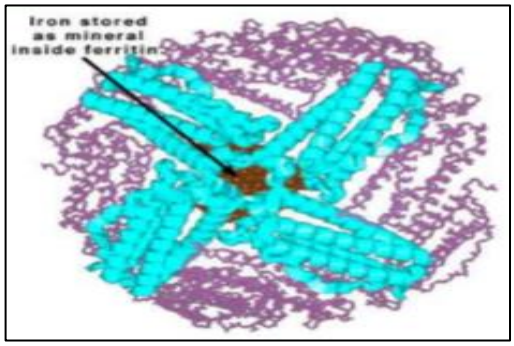
Up until now, we have iron stored in the enterocytes, now it has 2 different fates;

1- **E**-stored in ferritin.

2- **F**-transported out of these cells.

Storage in ferritin

- Cells can then store iron as ferritin. Each Ferritin complex can store about *4500* iron (Fe3+) ions.



But if ferritin stays in the cells and then these cells are sloughed off from the tip of the villus into feces (mucosal renewal of the GIT), iron will be eliminated from the body (lost), so iron must be transported out of the enterocytes, so fate 2 is:

Transport

Iron is transported out via a basolateral transporter known as *ferroportin* which transports iron outside of cells, which is distributed throughout the body on all cells.

G- Once iron leaves intestinal cells, an iron oxidase, known as hephaestin or ferroxidase (which is bound to the membrane), converts iron from the ferrous state to the ferric state.

- *Note*: Macrophages have ferroportin too, but the enzyme that catalyzes the oxidation of iron once it is out of the macrophage is ceruloplasmin rather than the ferroxidase.
- Note: Nonintestinal cells use the plasma protein ceruloplasmin to oxidize iron, just like in the macrophage picture page 4

There is a condition related to iron deficiency known as Aceruloplasminemia, (deficient ceruloplasmin).

H-Iron is rapidly bound to transferrin, a **large** iron-binding **protein** of the blood/plasma that delivers iron to liver cells and from liver cells to other tissues via receptor-mediated endocytosis

Clarification: Ferroportin is the major iron export protein located on the cell surface of enterocytes, macrophages and hepatocytes, the main cells capable of releasing iron into plasma for transport by transferrin.

I-Note: heme can be transported out as it is, but there is a question mark about that as in it is still unclear, nonetheless it gets degraded quickly in blood (since is hydrophobic and doesn't dissolve well in blood)

Properties of Transferrin

Apotransferrin (transferrin without its prosthetic group) can bind several metals, and ferric, not ferrous, iron has highest affinity forming ferrotransferrin. Transferrin contains two sites that bind ferric iron

1/9 of the transferrin molecules have iron bound at both sites

4/9 of them have iron bound at one site

4/9 have no iron bound

This means that iron-binding sites of transferrin are normally only about 33% (1/3) - 45% saturated with iron and when iron exceeds normal levels, nontransferrin bound iron (NTBI) appears, which targets parenchymal cells, especially the hepatocytes.

Note: nontransferrin bound iron (NTBI): This is iron that is bound to proteins other than transferrin (and is not free iron). It can make up to 75% of iron in the system if iron levels exceeds normal levels and this is considered a pathological condition.

Receptor mediated endocytosis

- Transferrin transports iron throughout the body in the blood and binds to a receptor known as Transferrin receptor 1, there is another receptor known as Transferrin receptor 2.

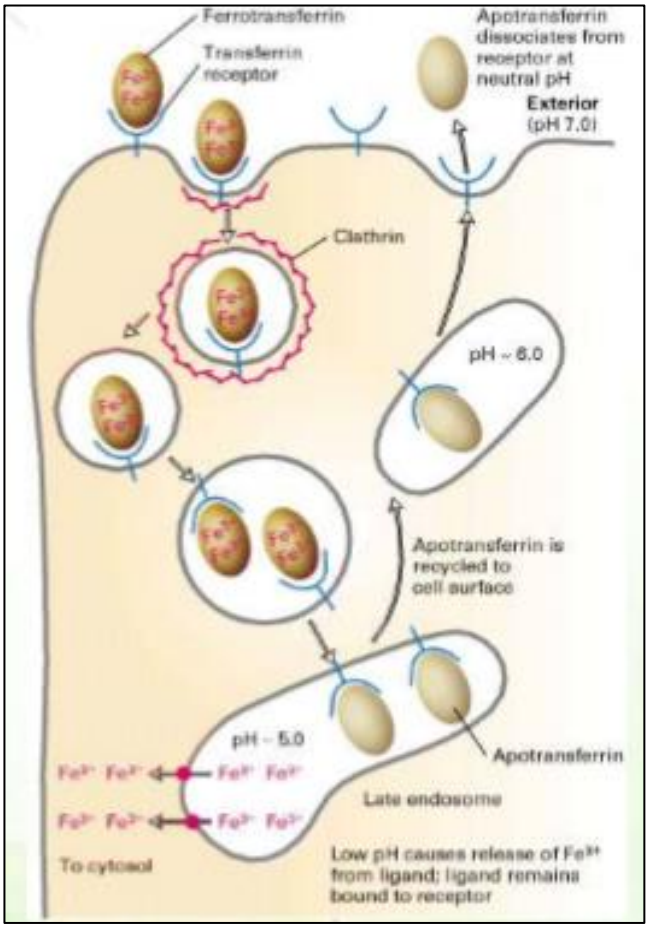
The mechanism:

1-Ferrotransferrin binds to a transferrin receptor triggering endocytosis into early endosomes (pH of 6.0).

2-Early endosomes are transformed into late endosomes (pH of 5.0) where Fe3+ atoms dissociate from transferrin and are transported into the cytosol.

3-The apotransferrin-transferrin receptor complex is recycled back to the surface membrane, apotransferrin dissociates from the receptor, and the receptor binds another ferrotransferrin.

Iron can then be used in the cell, for example it can be stored in the liver in the ferritin form, or be used as a prosthetic group of an enzyme such as cytochrome or myoglobin …



Quick recap of the enzymes and transporters:

1-ferrireductase: on the apical border of the enterocytes (but other isoforms are present in the body), reduces ferric to ferrous

2-DMT-1: transport of Fe+2 into enterocytes

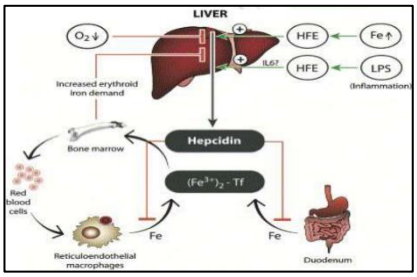
3-HCP-1: transports heme iron into enterocytes

4-Heme oxygenase: releases iron from heme

5-Ferroportin: on the basolateral membrane of the enterocyte (also on a lot of other cells throughout the body), transports Fe+2 out of the cell

6-Ferroxidase: membrane bound enzyme, oxidizes the Fe+2 that just came out of the enterocyte to Fe+3 so that it can bind transferrin.

Regulation of protein function

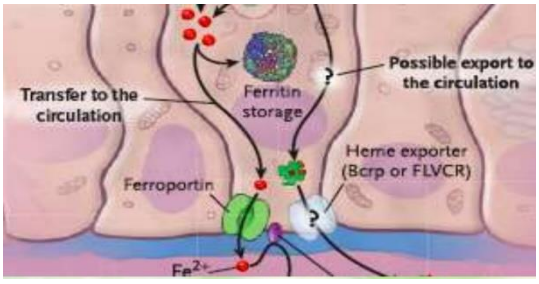


Hepcidin, is a short peptide hormone, composed of 25 amino acids which is mainly released from the liver, it's an inhibitor of iron absorption and transport (inducing internalization and degradation of ferroportin.)

Its production is increased by iron overload and inflammation and is suppressed by iron deficiency.

It acts on a number of tissues including intestinal cells, and it can also act on macrophages.

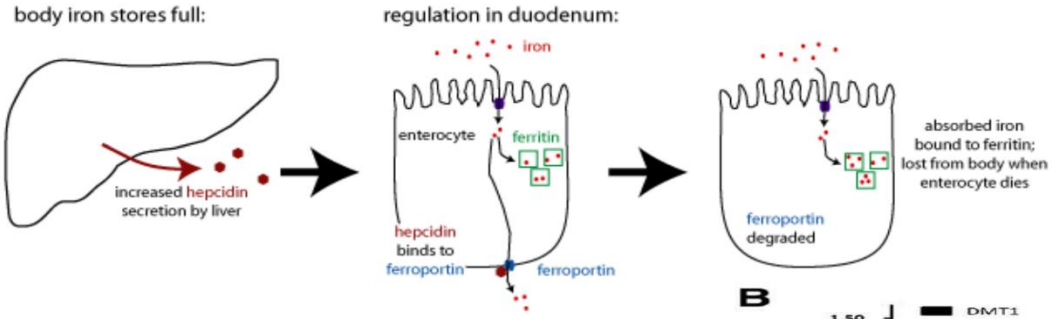
Mechanism of action:

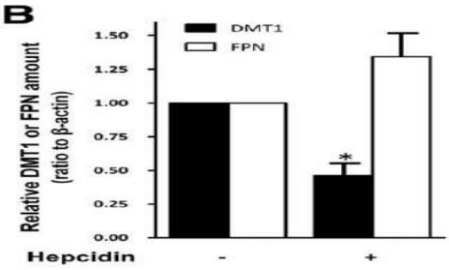


It acts on ferroportin, so that iron is not released from intestinal cells into the circulation. When these intestinal cells die, the iron stored in them is also lost at their death.

Hepcidin can also act **DMT1**, reducing its expression so less iron would be absorbed.

The iron release from macrophages is also reduced by inhibiting their ferroportin.



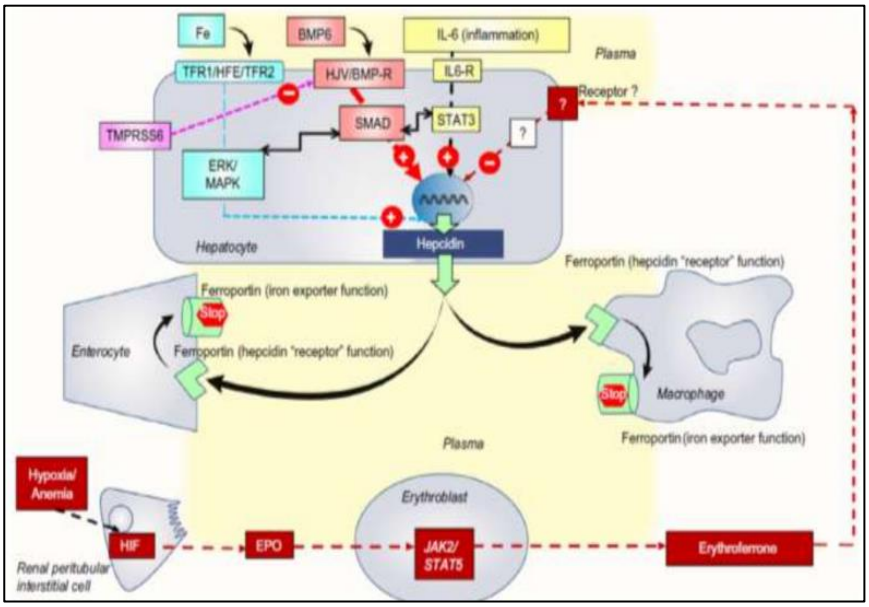


Another research article suggests an alternate mechanism of action for hepcidin, which is by acting on and inhibiting DMT1 resulting in a **significant** decrease of its activity and simultaneously increasing the activity of ferroportin. The star shown in the diagram shows a significant difference in the activity of DMT1 caused by hepcidin. The absence of the star shows an insignificant difference in the activity of FPN caused by hepcidin.

Regulation of hepcidin

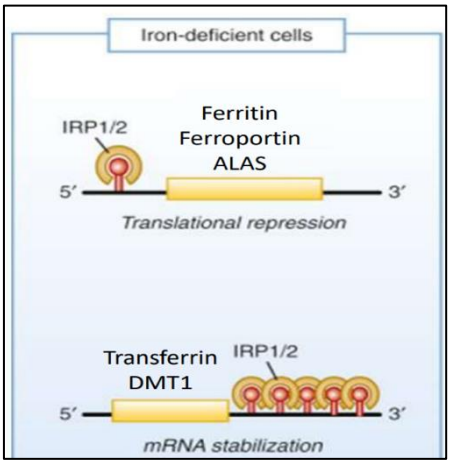
Anemia: decreased levels of iron in our body induces the release of **erythropoietin** by the kidneys, which induces the release of a protein hormone known as **erythroferrone** from the erythroblasts, which acts on the liver cells to reduce the production of hepcidin.

On the contrary, inflammatory cytokines and, iron load increases the production of hepcidin.



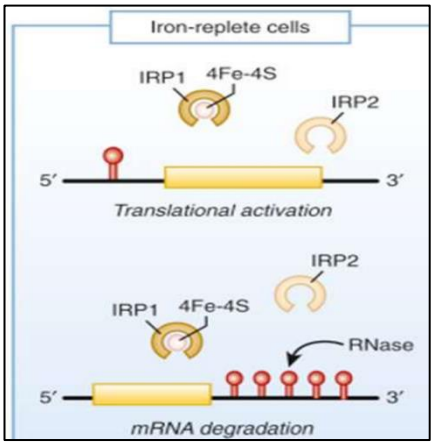
Post-transcriptional regulation of expression

We have 2 groups of iron response elements at the mRNA, one at the 5' UTR for the regulation of **ferritin**, **ferroportin (in macrophages)** and **ALAS (ALA synthase)**. The other one is located at the 3' UTR for the regulation of **transferrin receptor** and **DMT1**.



- **Low iron levels**:
  1) iron response element binding proteins (**IRE-BP**) bind to the **IRE** on the **5'**, **repressing** the translation and production of ferritin, ferroprotin, and ALAS**.**
  2) **IRE-BP** bind the **IRE** on the **3'** to stabilize the mRNA and **increase** the **production** of transferrin and DMT1.

- **High iron levels:**
  1) Iron prevents the **IRE-BP** from binding to the **IRE** on the **5'**, which leads to the **production** of ferritin, ferroportin, and ALAS.
  2) Iron prevents the **IRE-BP** from binding to the **IRE** on the **3'**, which leads to the **degradation** of the mRNA, therefore transferrin and DMT1 are **not expressed.**



Iron-related diseases

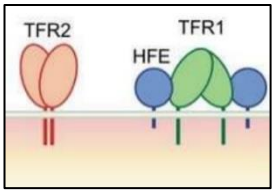
1) Hemochromatosis (HC)

is an autosomal recessive disorder in iron metabolism that is characterized by excess iron absorption, saturation of iron-binding proteins and deposition of hemosiderin in the tissues, which is **toxic** to tissues.

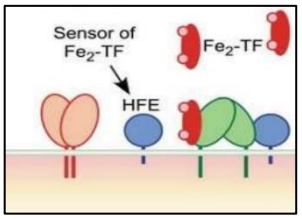
- it's more common in males than females, because females **lose iron** from the blood loss of menstruation and childbirth.

- The primary cause of hemochromatosis is the inheritance of an autosomal recessive allele designated as **HFE** (human homeostatic iron regulator protein), but four other genes that regulate the hepcidin–ferroportin axis can also be involved.
- Tissues that are affected by the accumulation of iron:
  - Liver (hepatic fibrosis)
  - Pancreas (diabetes mellitus)
  - Joints (arthropathy)
  - Skin (pigmentation)
  - Heart (cardiomyopathy)
  - Gonadotrophin-secreting cells (hypogonadotrophic hypogonadism)
- Classes of hemochromatosis:
  - Type 1 (hemochromatosis protein, HFE dependent), which is most common
  - Type 2A [HFE2 (HJV) dependent]
  - Type 2B (hepcidin, HAMP dependent)
  - Type 3 (TfR2, TfR2 dependent)
  - Type 4 (ferroportin dependent).

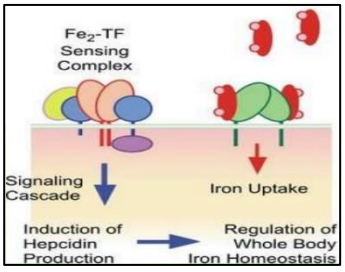
Normally:



At low serum iron conditions, **TFR1** is bound to and inhibited by **HFE [a major histocompatibility complex (MHC) class-1 gene]**, so there's no iron transport. **β2-microglobulin** is required for this interaction.

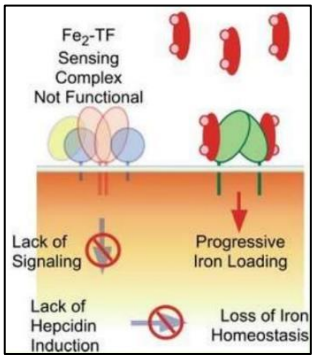


When iron levels increase, it binds to transferrin and **Fe^{2+}-TF** complex starts competing with **HFE** to bind to **TFR1**, which results in the dissociation of **HFE** and the uptake of iron through endocytosis.



After **HFE** dissociates from **TFR1**, it binds to **TFR2** inducing its signal and therefore increasing the expression of hepcidin, which maintains the balance of iron uptake

Abnormally:

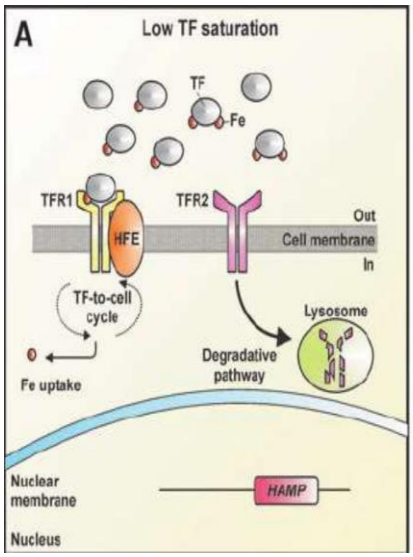


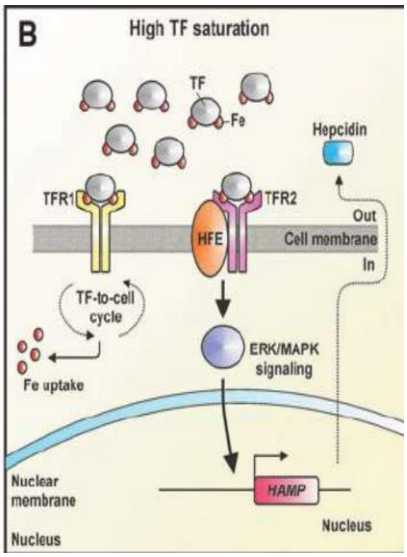
Mutation or absence of **HFE** (eg. C282Y) or **TFR2** prevents formation of a functional iron sensor and signal transduction effector complex leading to dysregulation of systemic iron homeostasis, therefore iron uptake is not inhibited, iron accumulates into the cells leading to hemochromatosis.

Also, the lack of **HFE-TFR2** interaction means no hepcidin is produced

Usually the mutation is due to a cystine replaced by a tyrosine. Tyrosine can't form a disulfide bond with **β2-microglobulin**, therfore there's no interaction between **HFE** and **TFR1**.

- Mutations in **β2-microglobulin** might also cause hemochromatosis

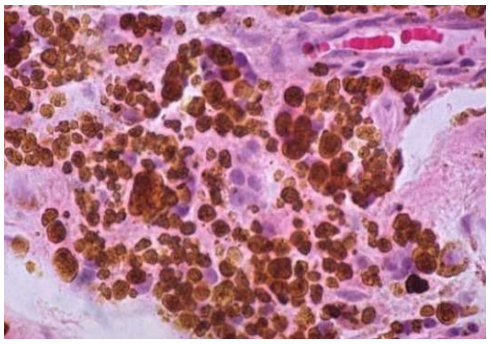




Juvenile hemochromatosis (also known as: HFE2 (HJV)-dependent hereditary hemochromatosis) ~ Type *2A* Hereditary Hemochromatosis

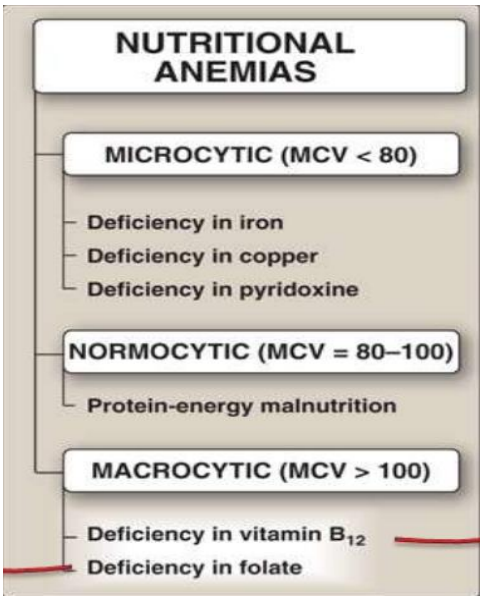
- It's a very rare, severe juvenile form of hemochromatosis is due to a homozygous deletion of the gene for hepcidin.
- Mutations in HJV gene, which encodes the protein "hemojuvelin", account for the majority of JH.
- HJV upregulates expression of hepcidin.
- *Type 2B* is also juvenile hemochromatosis, but is caused by mutations in hepcidin gene.

- The normal total body iron stores may range from 2 to 6 gm, but people with hemochromatosis have much greater stores.
- When iron accumlates inside cells (might reach 50 gm), a toxic clusters of iron form, known as **hemosiderin**, it causes cellular dysfunction and damage .



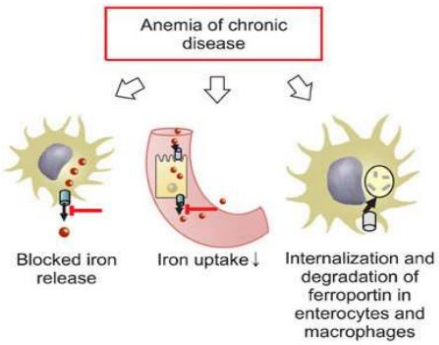
2) Anemia

- Anemias are characterized by a deficiency in the number of mature erythrocytes in the circulation, lowering the oxygen-carrying capacity of the blood, causing tissue hypoxia, and clinical symptoms such as fatigue, weakness, increased cardiac output, as well as increased morbidity and mortality.

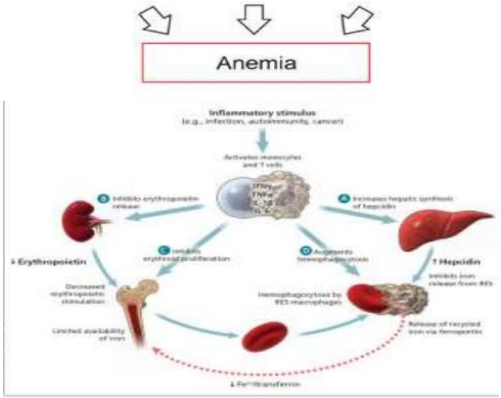


It has many causes and can be diagnsoed by examining the erythrocyte size (check out this figure on the right)

- Folic acid is important for DNA replication and its deficiency means cells cannot replicate so they become larger
- Vitamin B_{12} is necessary for folate's regeneration



Anemia of **chronic** disease is mainly prevalent in rich countries due to infections. Inflammatory cytokines (IL-6) present in infections promote the synthesis of hepcidin, which downregulates the expression of ferroportin leading to decreased enteric iron absorption and increased iron retention within splenic macrophages and hepatocytes.



GOOD LUCKkkkkkkkkkkkkkk!!!!!