Biochemistry - HLS

Done By

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Corrected By

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Metabolism of iron

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Hematopoietic-lymphatic system

Resources

- This lecture
- Yiannikourides and Latunde-Dada. A Short Review of Iron Metabolism and Pathophysiology of Iron Disorders. Medicines 2019, 6, 85. https://www.mdpi.com/2305-6320/6/3/85
- Lippincott's Biochemistry, 7th edition

1JD for whoever reads this name ☺

- The Medical Biochemistry page, Iron and Copper Metabolism https://themedicalbiochemistrypage.org/iron-and-copper-homeostasis/
- Fleming and Ponka, Iron Overload in Human Disease, N Engl J Med 2012;366:348-59, https://www.nejm.org/doi/full/10.1056/nejmra1004967
- Brissot and Loréal, Iron metabolism and related genetic diseases: A cleared land, keeping mysteries, Journal of Hepatology 2016 vol. 64 j 505–515, https://www.sciencedirect.com/science/article/pii/S0168827815007424?via%3Dihub

Importance of iron

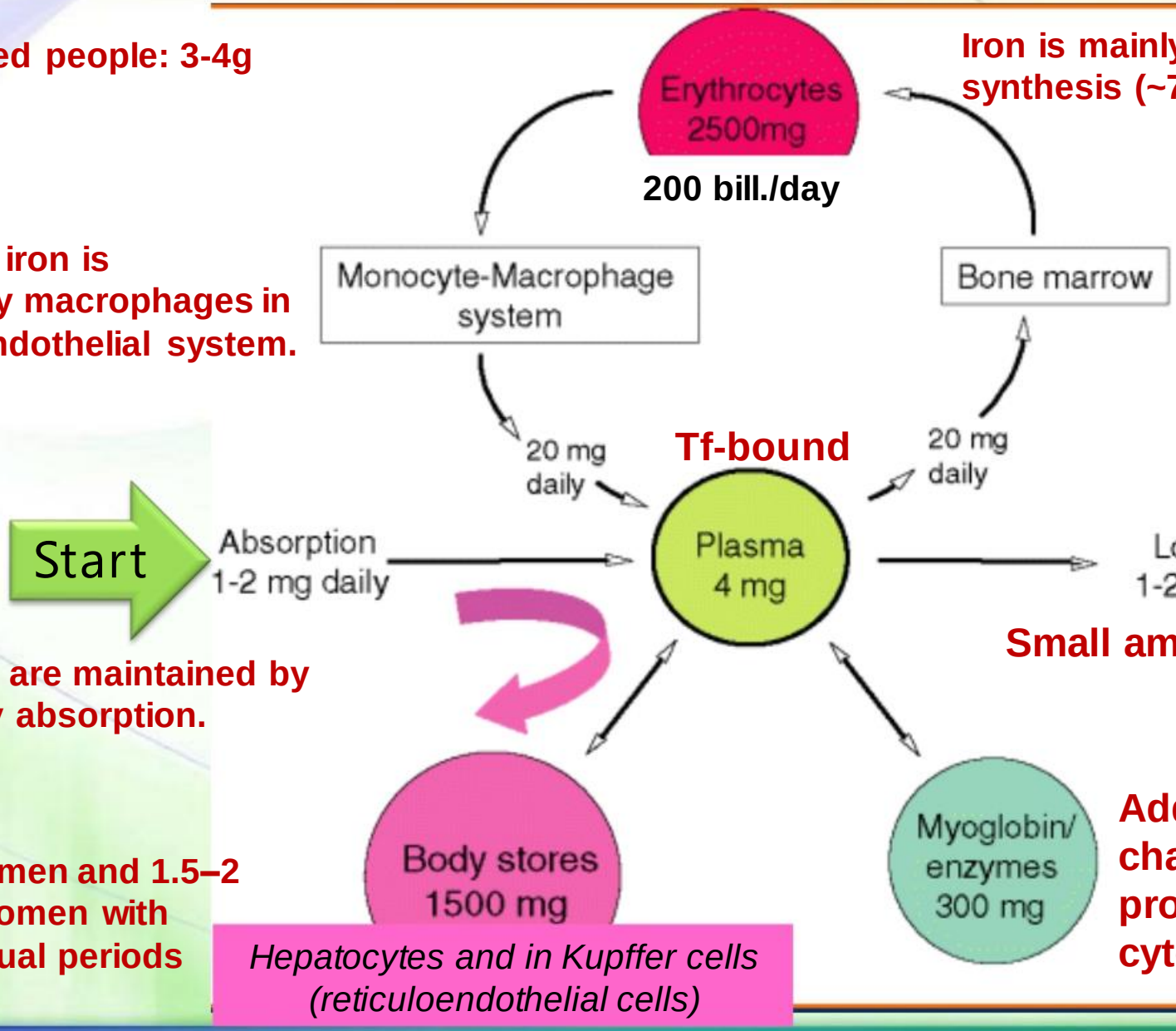
- Within the body, iron exists in two oxidation states: ferrous (Fe^{2+}) or, the highly insoluble, ferric (Fe^{3+}), this is important when it comes to iron absorption and transport.
- It is also the prosthetic group of a number of enzymes such as redox cytochromes and the P450 class of detoxifying cytochromes. It binds covalently to the enzyme and it is important for the enzyme function.
- Iron is important for metabolism and oxygen transport, in hemoglobin.
- Yet…
- Iron can be potentially toxic due its ability to form free radicals, and cause tissue damage. If it is present in high concentrations in the body.

Solution: iron is not free. It is found mainly bound to other proteins.

What is life cycle of iron in the body?

Well-nourished people: 3-4g

The released iron is scavenged by macrophages in the reticuloendothelial system.



Iron is mainly used for hemoglobin synthesis (~70% of all iron).

Small amounts are lost

Levels are maintained by dietary absorption.

1 mg a day for men and 1.5–2 mg a day for women with regular menstrual periods

Additional iron (300 mg) is channeled to other cellular proteins (myoglobin and cytochromes).

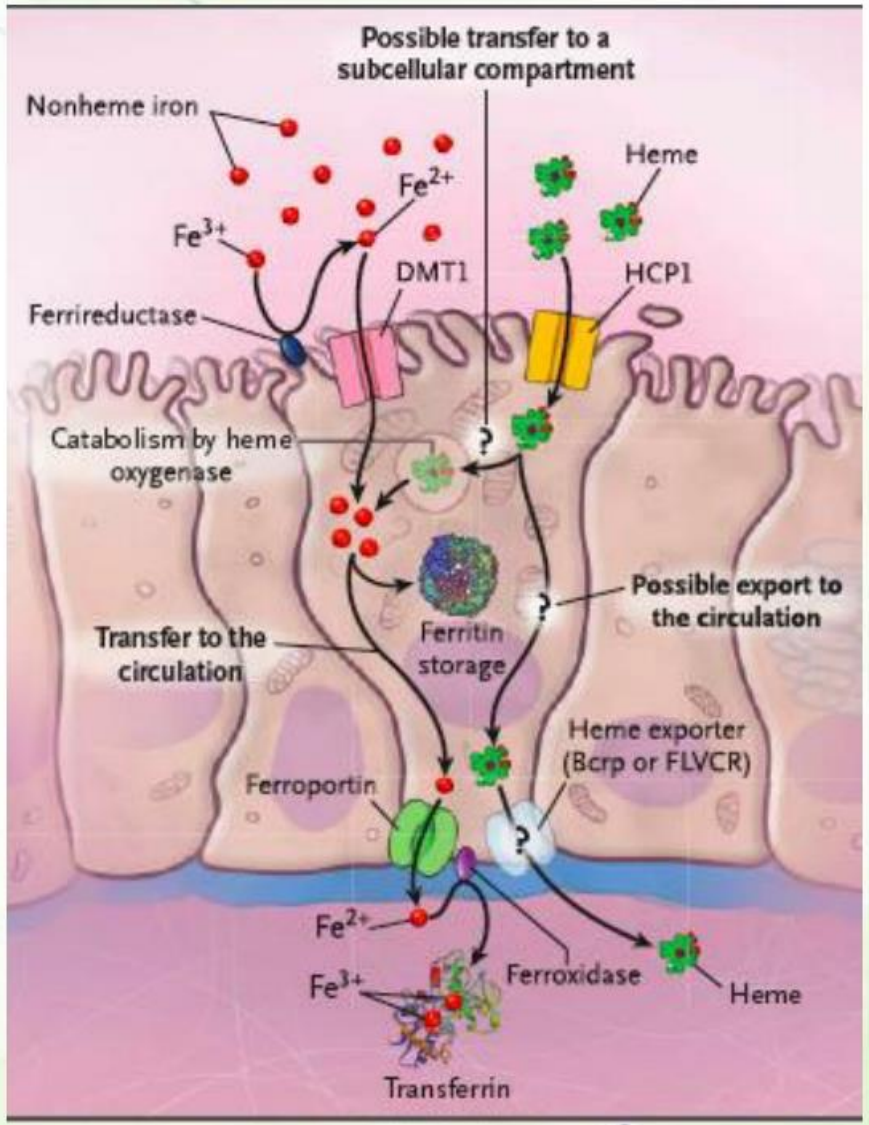
What is life cycle of iron in the body?

- Iron is precious in our system. Normal individuals have about 3-4g of iron. The amount of iron that is absorbed daily is about 1-2 mg daily and that is very little compared to the amount of the iron in our body.
- The amount of iron lost is also very little 1-2 mg daily.
- Women would have more iron loss than men, due to the menstrual cycle.
- Most of the iron in our system exists in our erythrocytes, about 2500mg of iron are in RBCs.
- We have cell renewal, a lot of RBCs die daily, and they release heme and release iron. This iron is sequestered very efficiently by the cells of the reticuloendothelial system, specifically the macrophages.
- Some of the released iron would be immediately bound to transferrin in the plasma, so we have about 4mg of iron bound to the transferrin protein.
- Released iron can be recycled by bone marrow cells, to produce more erythrocytes and more hemoglobin.
- Some iron can be stored in hepatocytes as well as Kupffer cells, about 1,500mg would be stored this way.
- About 300mg of iron would be used to produce myoglobin as well as enzymes.

Iron absorption

State of iron

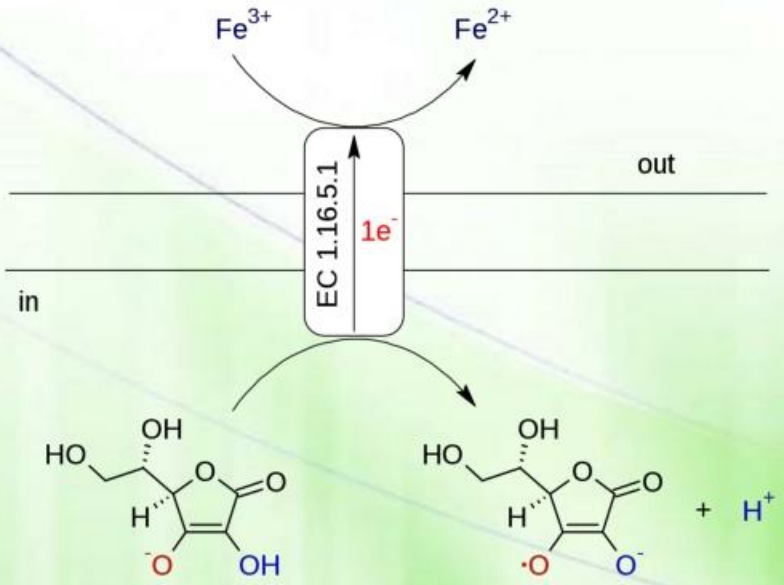
- Under conditions of neutral or alkaline pH, iron is found in the **Fe^{3+} state** and in the **Fe^{2+} state** at acidic pH.
  - In the stomach, iron will be in the ferrous state.
  - In the duodenum, iron is in the ferric state.
- However, to be absorbed, dietary iron must be in its ferrous Fe^{2+} form.

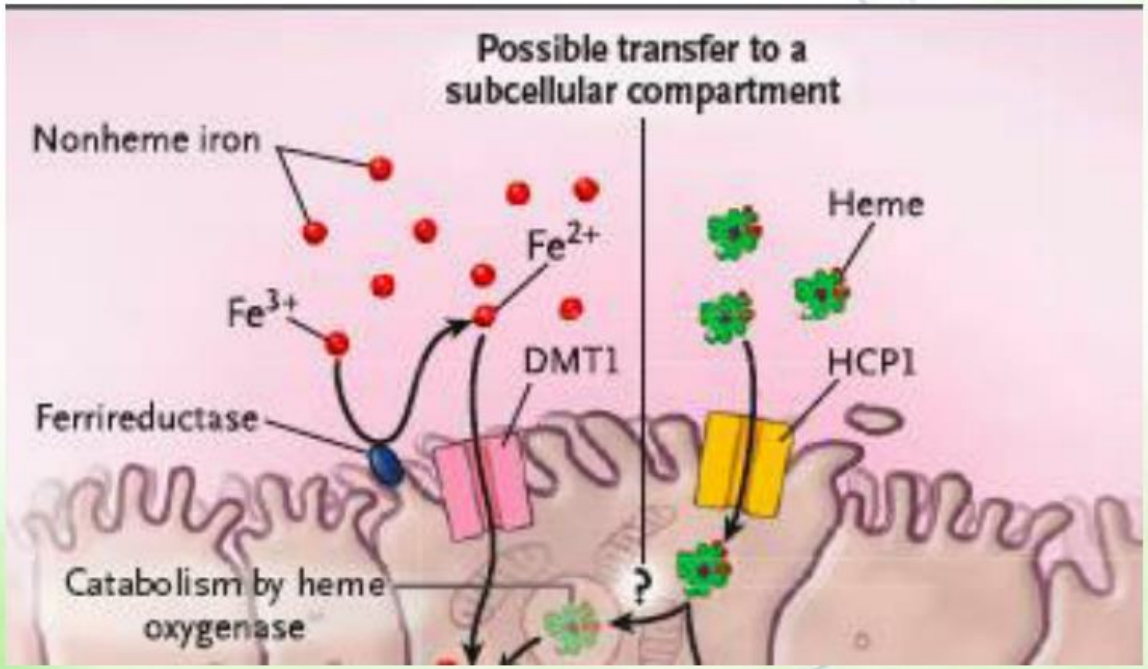


- Once it reaches the small intestine it gets converted from the ferrous state to the ferric state, and this ferric iron must me converted to ferrous again in order to be absorbed, this is done by a membrane-bound enzyme called ferrireductase.

Site of absorption

- A ferric reductase enzyme on the enterocytes' brush border, Dcytb (duodenal cytochrome b), reduces Fe^{3+} to Fe^{2+} .
- Divalent metal transporter 1 (DMT1) transports iron into the cell.
  - DMT-1 can transport other metal ions such as zinc, copper, cobalt, manganese, cadmium, and lead.



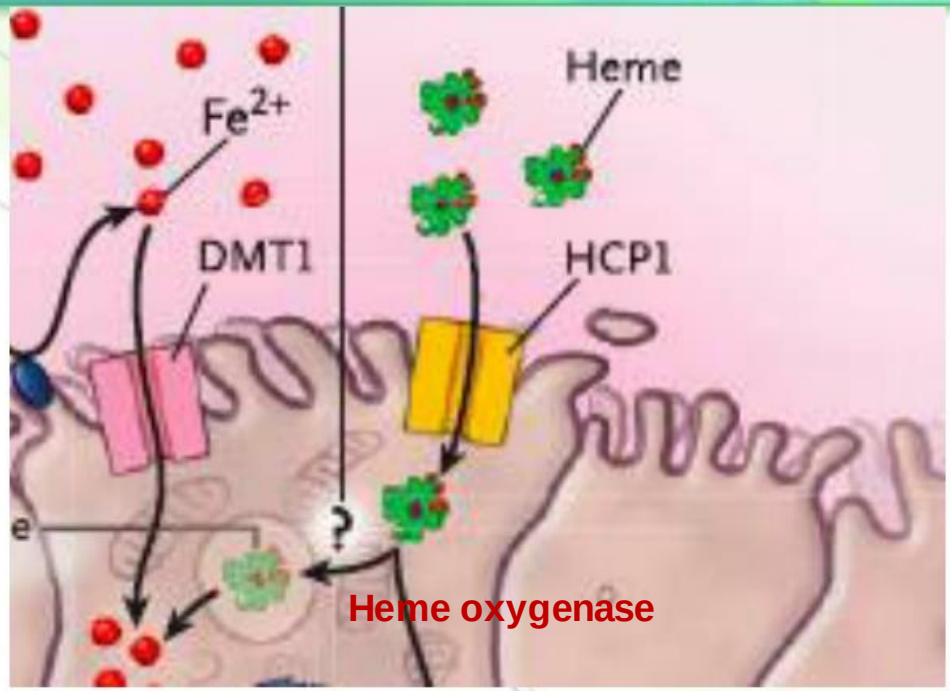


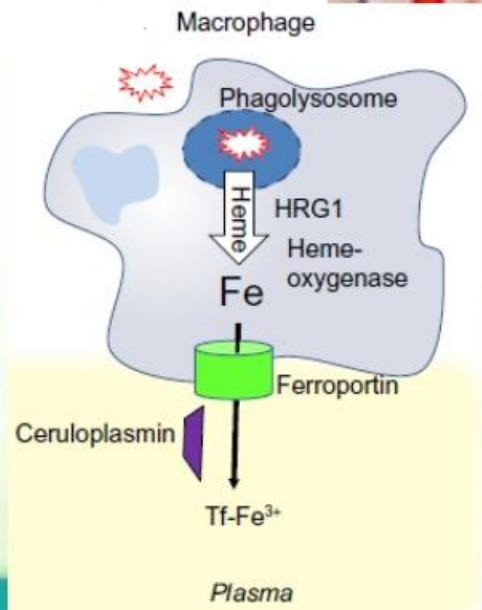
Site of absorption

- Dcytb exists on the cell surface, and it is associated with the transporter known as the DMT1. this is an important transporter that exists not only on the surface of enterocytes but other cells as well.
- Where does the ferric reductase get the electron from to donate to iron. The answer is from Vitamin C. the reductase extracts an electron from vitamin C in the cytosol, this electron can then move to the outward portion of the enzyme and iron can then be reduced.
- This is why vitamin C is considered as an important supplement for iron metabolism and absorption.

Heme as a source of iron

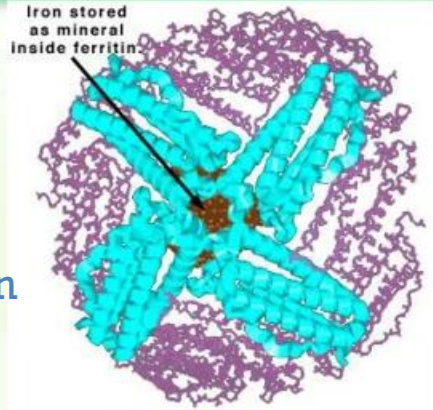
- Iron can also be obtained from ingested heme.
- Heme is absorbed by a receptor called heme-carrier protein 1 (HCP-1) and iron is released by heme oxygenase-1 (HO-1), in enterocytes.
- In other cells such as macrophages, heme oxygenase also extracts iron from heme.





- Another important source of iron is heme itself, and heme comes mainly from eating meat.
- HCP1 takes up the whole heme molecule and then iron is extracted by an enzyme called heme oxygenase 1.

Fates of iron

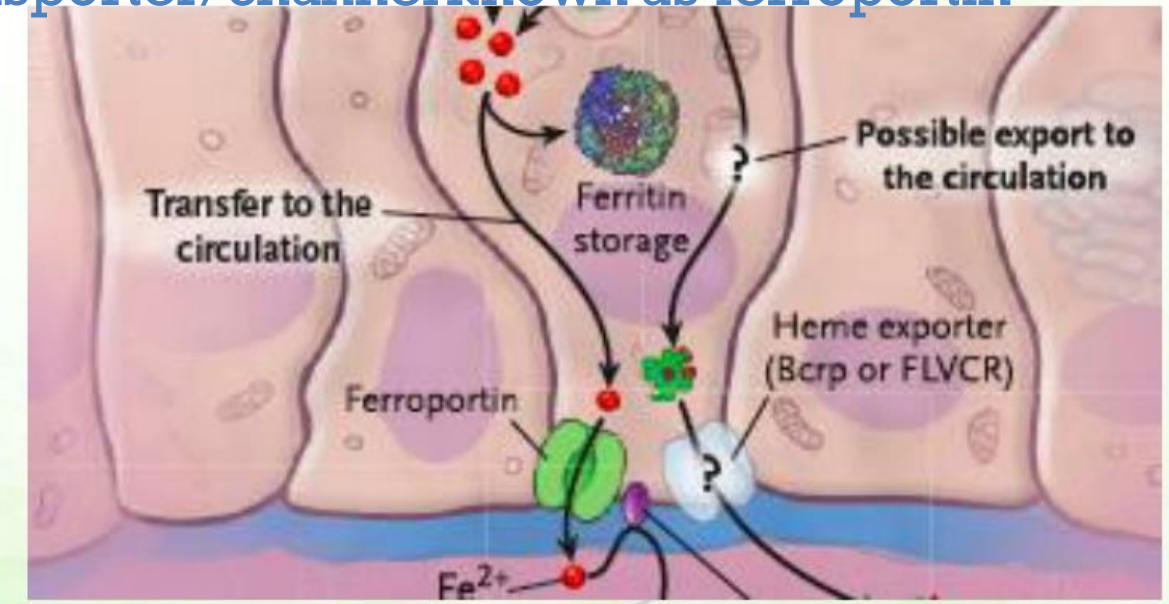


Fate 1: storage

- Cells can then store iron as **ferritin**. Iron binds to a protein called ferritin
  - Each Ferritin complex can store about 4500 iron (Fe^{3+}) ions.
- But, if cells (intestinal cells) are sloughed off from the tip of the villus into feces, iron is eliminated from the body. So, to take advantage of stored iron, it can be transported outside these cells via a transporter/channel known as ferroportin

Fate 2: Transport

- Iron is transported out via a basolateral transporter known as ferroportin, which is distributed throughout the body on all cells (not specific for intestinal cells, can be found in cells like mcrophages)

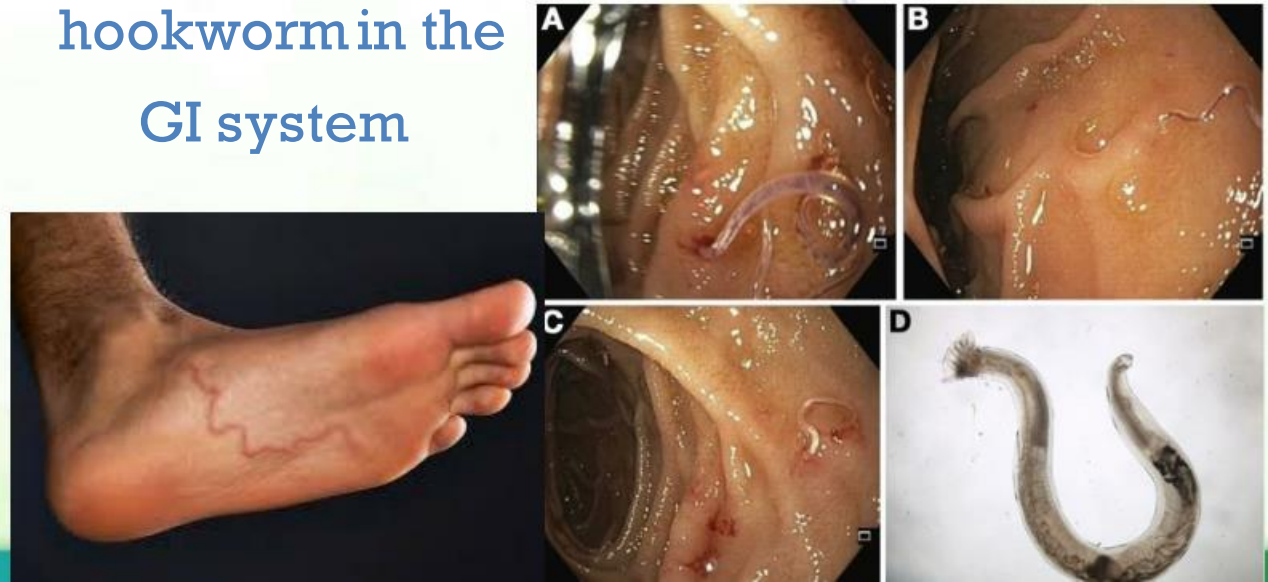


Intestine-related iron metabolism disorders

In both cases, the patient suffers a low level of iron in the body

- Iron malabsorption (problem in absorbing iron)
  - Gastrectomy (total or partial)
  - Celiac disease (villous atrophy) death of enterocytes
  - Crohn's disease
  - Helicobacter pylori (it causes ulcers, and it has a different mechanism to cause malabsorption)
- Intestinal hemorrhage (gastrointestinal-mediated iron loss)
  - Gastric cancer
  - Ulcers (stomach or duodenal)
  - Inflammatory bowl disease
  - Hookworm infection (it's a parasite)

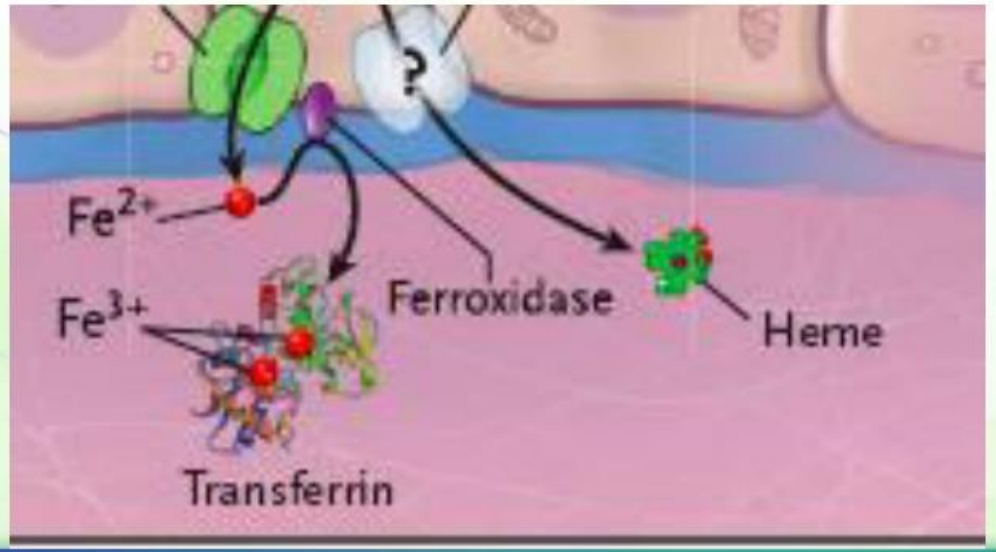
hookworm in the GI system

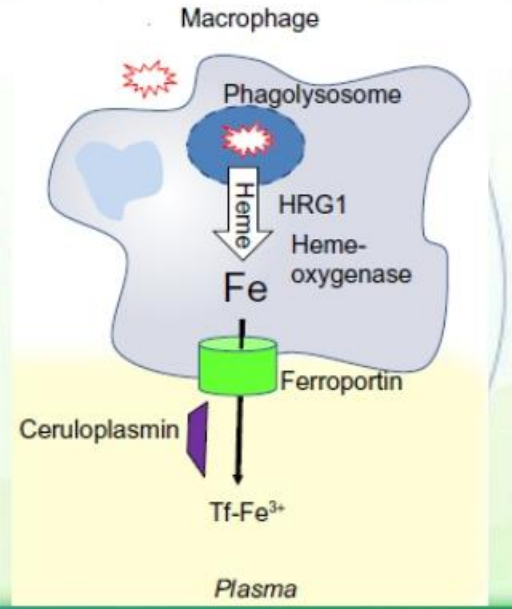


Intestinal hemorrhage is abnormal bleeding that leads to inability of cells to absorb iron and it causes excessive loss of iron

Ferroxidase and transferrin

- Once iron leaves intestinal cells, an iron oxidase, known as hephaestin or **ferroxidase**, converts iron from the ferrous state to the ferric state.
  - Nonintestinal cells use the plasma protein ceruloplasmin to oxidize iron.
- Once it is in the ferric state Iron is rapidly bound to transferrin, an iron-binding protein of the blood that delivers iron to liver cells and from liver cells to other tissues via receptor-mediated endocytosis.

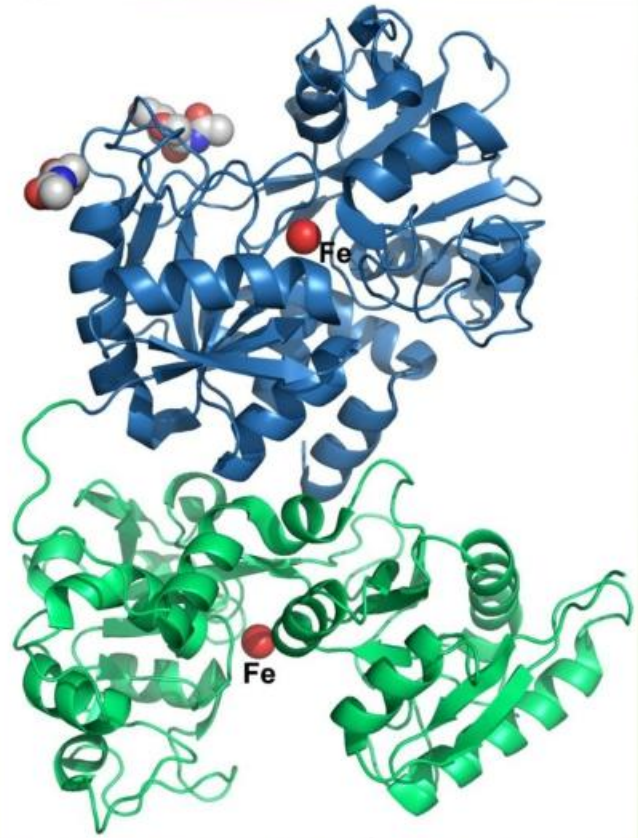




Properties of transferrin it's an iron binding protein

Transferrin binds iron covalently , transferring the apoprotein (apotransferrin) into a holoprotein (transferrin)

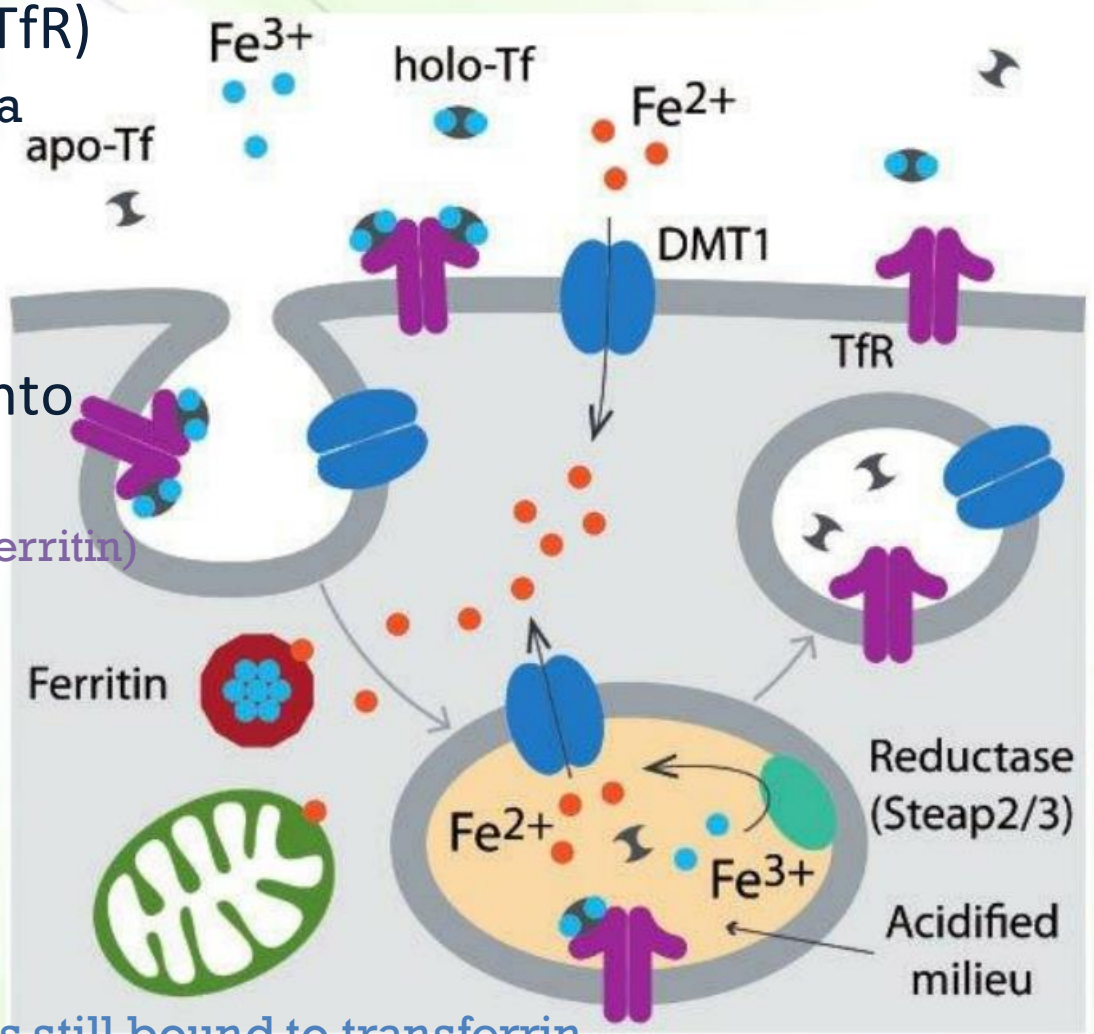
- Apotransferrin can bind several metals, but ferric, not ferrous, iron has highest affinity forming ferrotransferrin.
- Transferrin contains two sites that bind ferric iron:
  - 1/9 of the transferrin molecules have iron bound at both sites
  - 4/9 of them have iron bound at one site
  - 4/9 have no iron bound
    - This means that iron-binding sites of transferrin are normally only about 1/3 saturated with iron
- When iron exceeds normal levels all iron binding sites will be saturated , non-transferrin- bound iron (NTBI) appears.



Receptor-mediated endocytosis

Cells in need of iron display a receptor on their cell surface known as transferrin receptor 1. TfR has the ability to bind to 2 transferrin molecules (di-ferric transferrin)

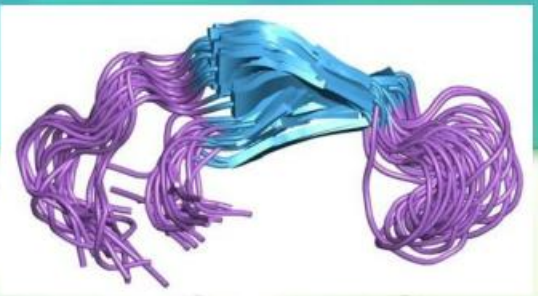
- Ferrotransferrin binds to a transferrin receptor (TfR) triggering endocytosis (invagination, formation of a vesicle) into early endosomes (pH of 6.0).
- Early endosomes are transformed into late endosomes (pH of 5.0) where Fe3+ atoms dissociate (due to acidic environment), get reduced into Fe2+ by the ferrireductase **STEAP3**, and are transported into the cytosol via **DMT1** (stored as ferritin)
- The apotransferrin-transferrin receptor complex is recycled back to the surface, apotransferrin dissociates, and the receptor binds another transferrin.
- Affinity of TfR to iron: diferric Tf (Fe2Tf) >monoferric Tf (Fe1Tf) >apo-Tf



(note that transferrin is still bound to transferrin receptor even though iron was released)

Regulation of protein function

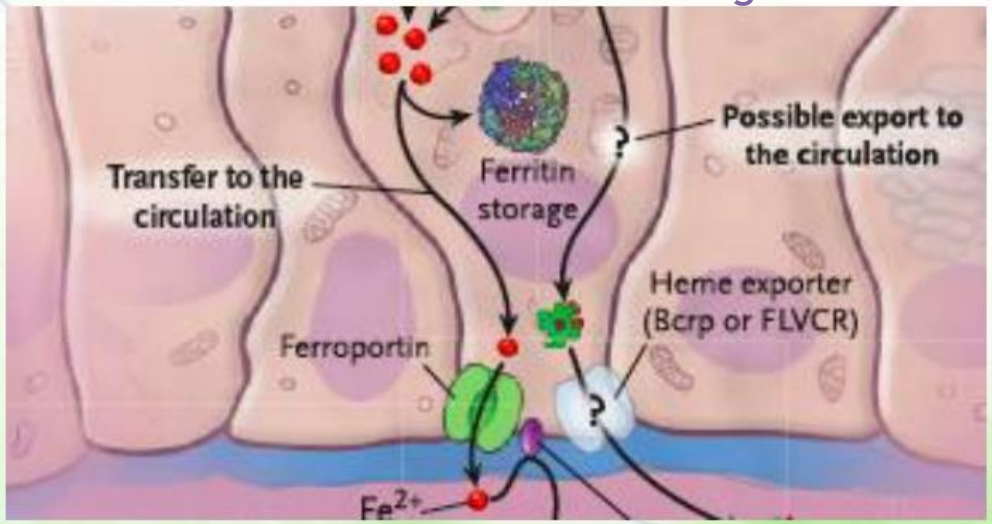
Hepcidin (iron sensor)



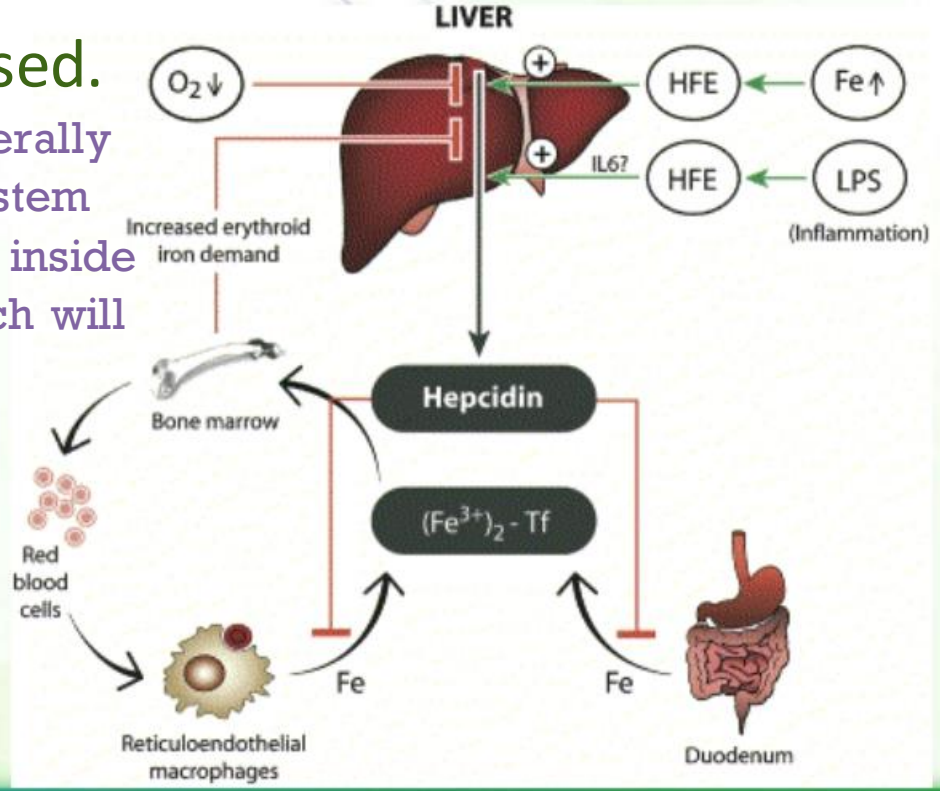
Main iron regulator and sensor in our system

- Hepcidin is a peptide hormone (25 amino acids) secreted by the liver and regulates iron level
  - When iron level increases and in cases of inflammation, hepcidin secretion increases → reducing iron absorption .
  - When iron levels are low, its release is suppressed.

Hepcidin reduces the amount of ferroprotein existing basolaterally in intestinal cells thus reducing the release of iron into our system

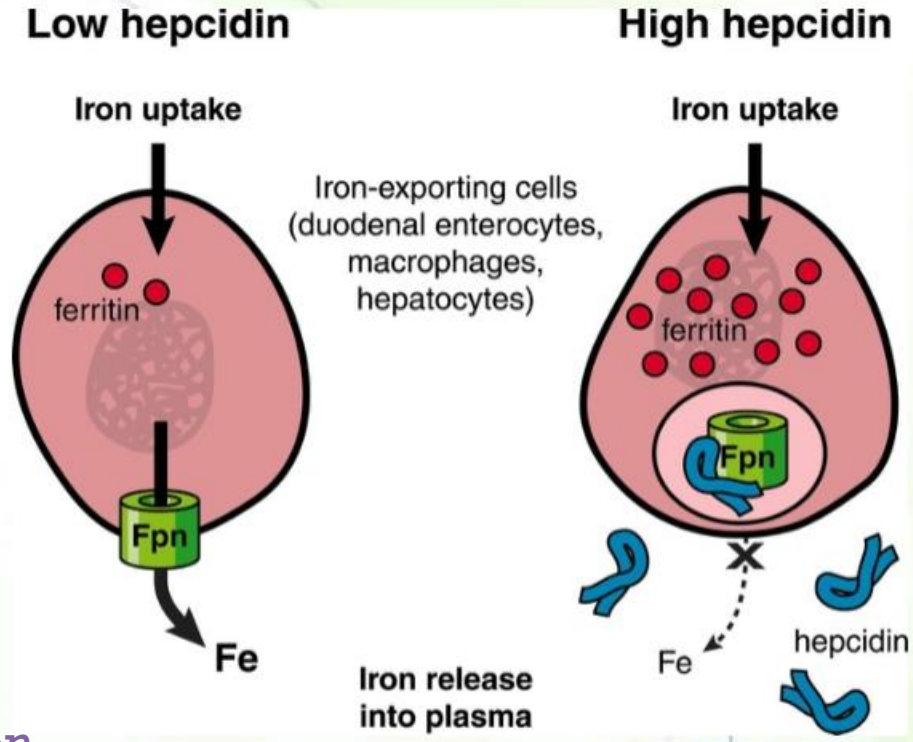


So, iron would stay inside intestinal cells which will be sloughed off & consequently, iron will be lost



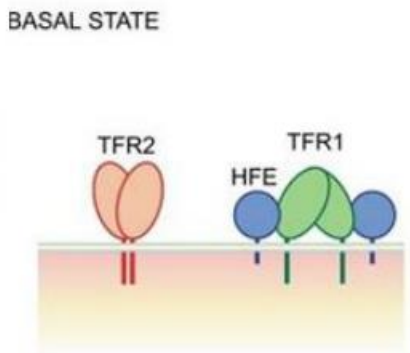
How does hepcidin reduce iron levels in the body?

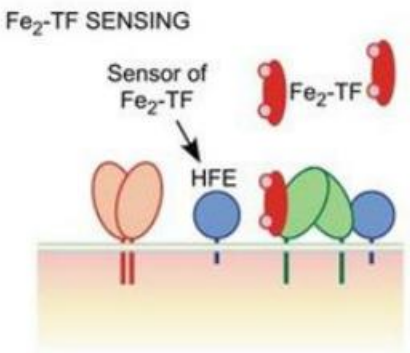
- Hepcidin binds to the basolateral iron transporter ferroportin inducing ferroportin *internalization* and degradation (within lysosomes)
  - This results in higher iron storage.
- Hepcidin also inhibits the presentation of the iron transporters (e.g. DMT1) in intestinal membranes decreasing iron absorption (DMT1 exists on the apical portion of intestinal cells and it's responsible for absorbing iron into intestinal cells)

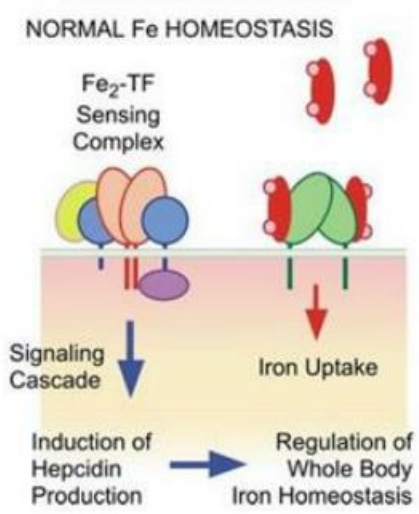


High levels of iron increase the release of hepcidin.
Low levels of iron decrease the release of hepcidin.

Regulation of hepcidin it has to be highly regulated







TFR1 complexes with HFE during low or basal serum iron conditions. It prepares TfR1 to bind ferrotransferrin

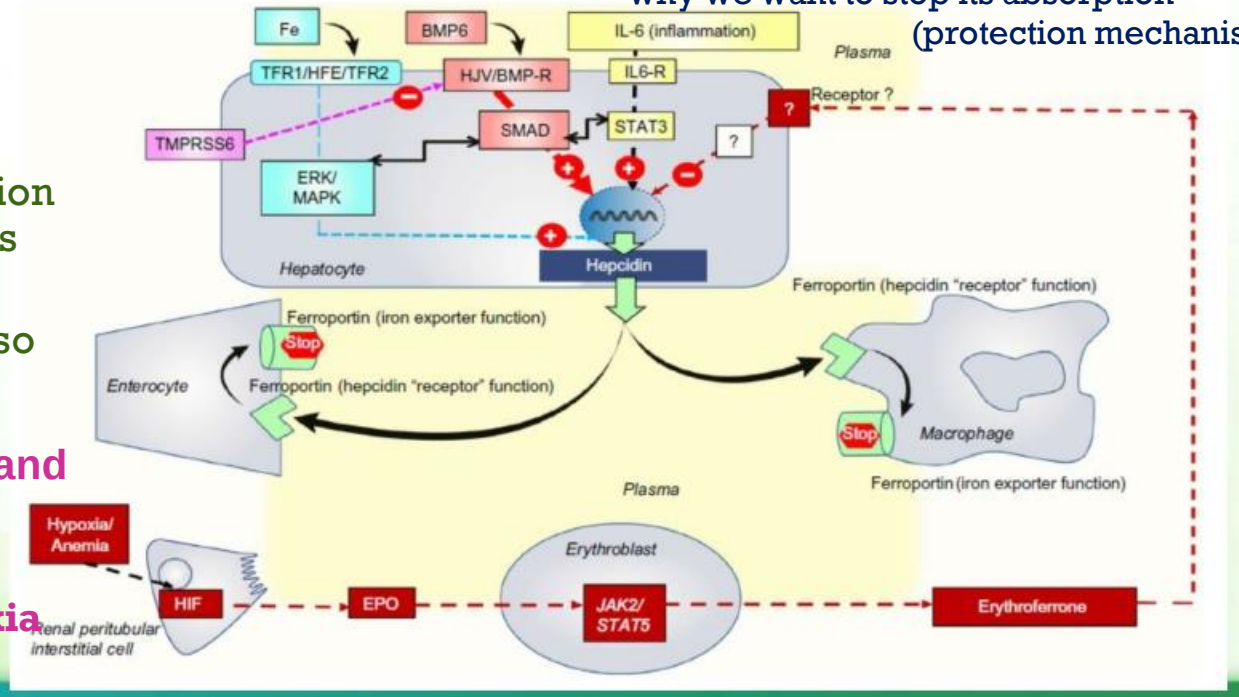
Fe2-Tf binds TFR1 releasing HFE. It dissociates and it goes to bind TfR2

It is regulated using 4 mechanisms (4 different colors)

HFE binds TFR2 and induces an intracellular signaling that stimulates hepcidin production saying we have enough iron in the body → It balances how many iron should be in the blood and inside cells

Inflammatory cytokine, IL6 binds its receptor inducing another transcriptional factor known as STAT3 **which induces expression of hepcidin.** Inflammation indicates infections and pathogens like bacteria which might flourish on iron and that's why we want to stop its absorption (protection mechanism)

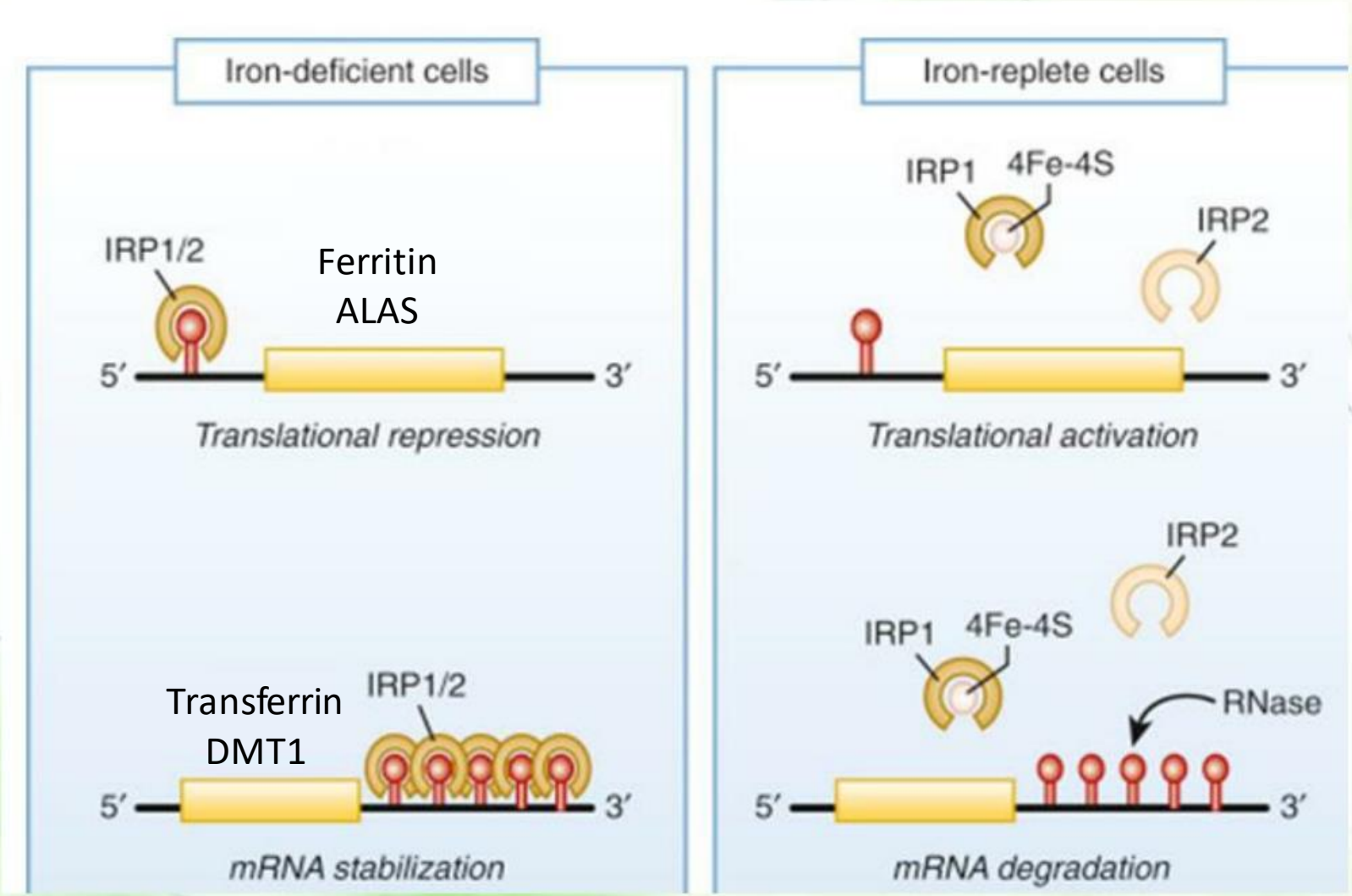
Bone morphogenic protein 6 (BMP6) binds to its receptor (BMPR) and activates expression of hepcidin (signal transduction pathway mediated by a transcriptional regulatory protein known as SMAD that induces hepcidin expression). **BMPR is bound to hemojuvelin (HJV) protein and is degraded by TMPRSS6** (it also activates the spike proteins of Covid-19)



The expression of hepcidin is negatively regulated by anemia and hypoxia through erythroferrone, which is produced by the erythroblasts in response to EPO (erythropoietin) synthesis by the kidney. Erythropoietin is induced by the hypoxia induced factor. (Hormonal regulation)

Post-transcriptionl regulation of expression

Iron-response element and its binding protein



Iron-response element and its binding protein

- Iron regulatory proteins have something in common and that is they all have iron response elements in their mRNA molecules.
- These elements are the sites whereby iron regulatory protein can bind to. Note that the position of iron response elements is different among different mRNA molecules.
- For the ferritin mRNA, the ion response element exists at the 5' untranslated region, on the other hand for the transferrin receptor. the iron response element exists at the 3' untranslated region.
- Why is that important ?
  - Because when iron regulatory protein binds to the iron response element at the 5' untranslated region, it prevents translation from this mRNA.
  - If it binds to the iron response element at the 3' untranslated region, mRNA becomes stable.
- So, if there are low levels of iron in the system, iron regulatory proteins would bind to the iron response elements, and there will be low production of ferritin (no iron then there's no need to make ferritin to store iron) so translation of the first mRNA would be blocked.
- At the same time, we want to increase the production of transferrin and DMT1 by stabilizing the mRNA, because there is a low level of iron
- If there is a high level of iron in the system, iron would bind to the iron regulator protein releasing it from the elements at the 5' & 3' untranslated regions, thus translation resumes increasing the level of ferritin protein (and storing excess iron), and reducing the level of transferrin & DMT1 (destabilization of their mRNA leading to degradation of the mRNA) because there is no need to absorb and transport these proteins.

Iron-related diseases

- Hereditary hemochromatosis (HH) a group of disorders
- Iron-deficiency anemia

Hereditary hemochromatosis

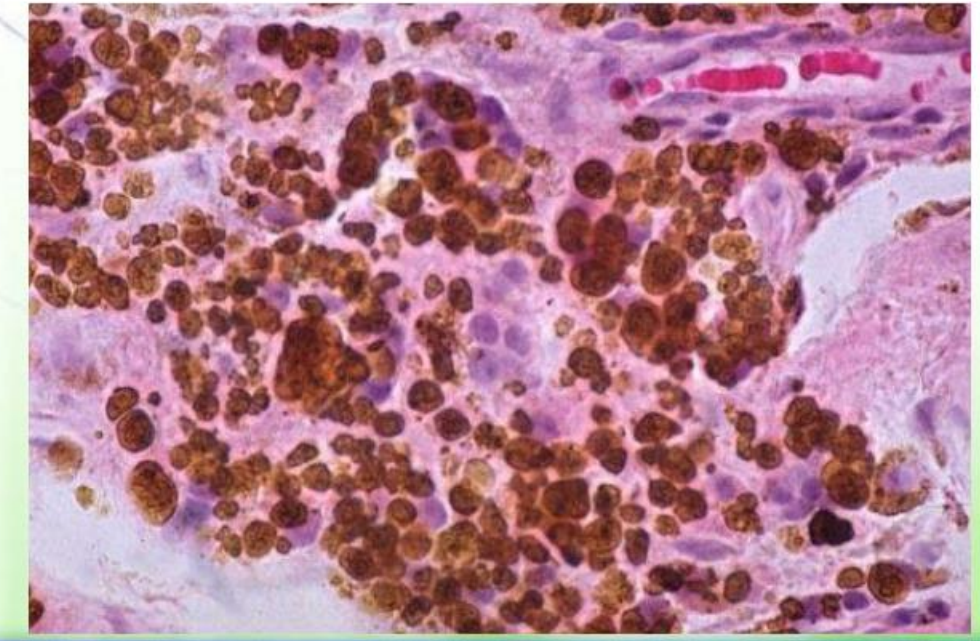
- It is a group of disorders in iron metabolism that is characterized by excess iron absorption, saturation of iron-binding proteins and deposition of hemosiderin in the tissues (Hemosiderin is toxic to tissues)
  - more commonly in males than in females (why?) Due to menstruation
- There are different classes of HH, the primary cause of hemochromatosis is the inheritance of an autosomal recessive allele designated as HFE (type I or primary HH) , but four other genes that regulate the hepcidin–ferroportin axis can also be involved.

Groups/classes of hereditary hemochromatosis

- Type 1 (hemochromatosis protein, HFE-dependent)
  - Most common
- Type 2A [HFE2 (HJV) dependent] HJV is associated with BMP6R
- Type 2B (hepcidin, HAMP-dependent)
- Type 3 (TfR2-, TfR2-dependent)
- Type 4 (ferroportin dependent)
  - Autosomal dominant disorder (all others are autosomal recessive)

Hemosiderin

- The normal total body iron stores may range from 2 to 6 gm, but persons with hemochromatosis have much greater stores.
- The total iron stores of affected persons may exceed **50 gm**.
- If the capacity for storage of iron in ferritin is exceeded, iron is stored as water-insoluble deposits known as hemosiderin, mainly in macrophages.
- Excess hemosiderin leads to cellular dysfunction and damage.



Hemosiderin is seen in this brown color

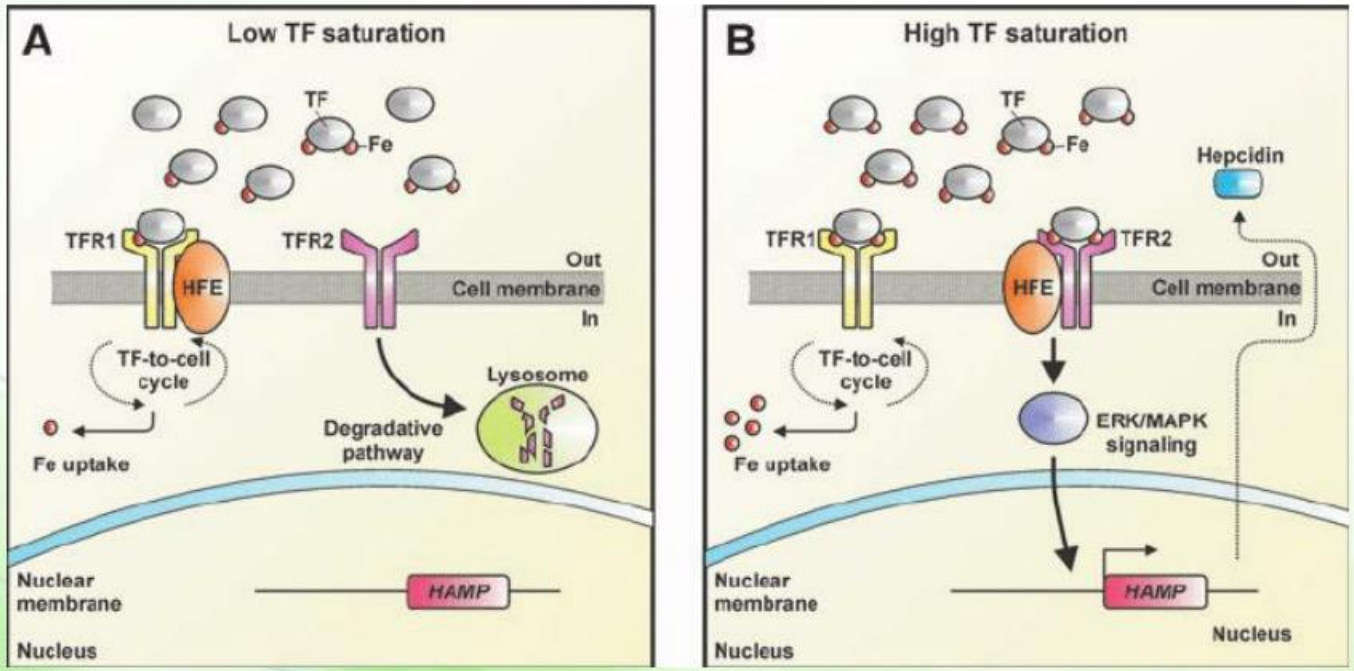
Affected organs and conditions

- Liver (hepatic fibrosis)
- Pancreas (diabetes mellitus)
- Joints (arthropathy)
- Skin (pigmentation)
- Heart (cardiomyopathy)
- Gonadotrophin-secreting cells (hypogonadotrophic hypogonadism)

Regulation of transferrin receptor

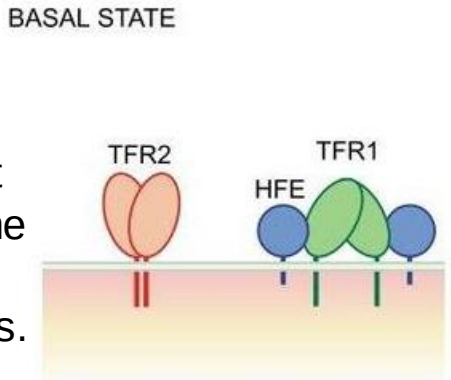
Hemochromatosis disorders all have something in common which is high level of iron in the system

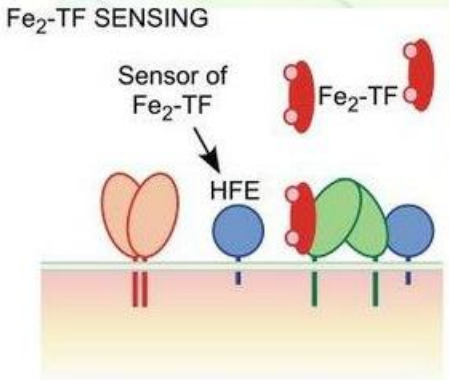
- HFE is a major histocompatibility complex (MHC) class-1 gene.
- Normal HFE complexes with TfR1 reducing iron transfer into cells.
- Mutated HFE (e.g. C282Y) has reduced presence on membrane and/or lack of interaction with Tfr1, loss of inhibition of transferrin receptor, and, therefore increased iron uptake and storage (continuous entry of iron into the system with little production of hepcidin → increasing intracellular iron)



Mechanism of action

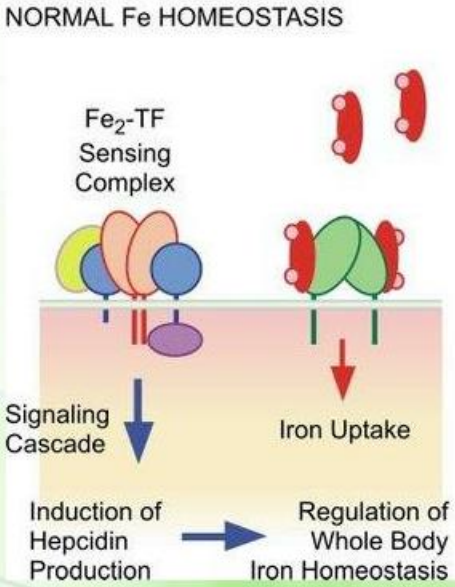
TFR1 exists as a complex with HFE at the plasma membrane during low or basal serum iron conditions.

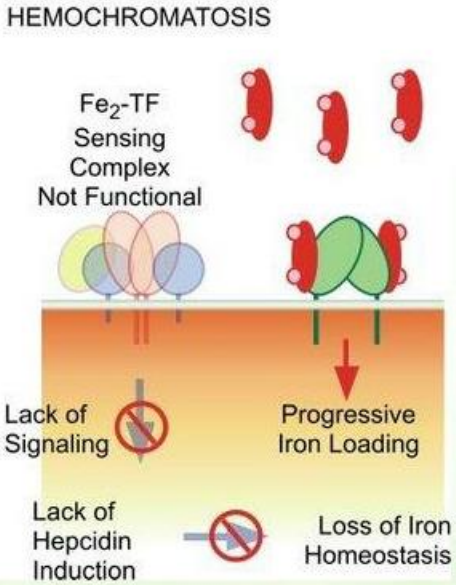




Serum Fe2+ -TF competes with HFE for binding to TFR1. Increased serum transferrin saturation results in the dissociation of HFE from TFR1.

HFE binds TFR2 and induces a intracellular signaling that stimulates hepcidin production.





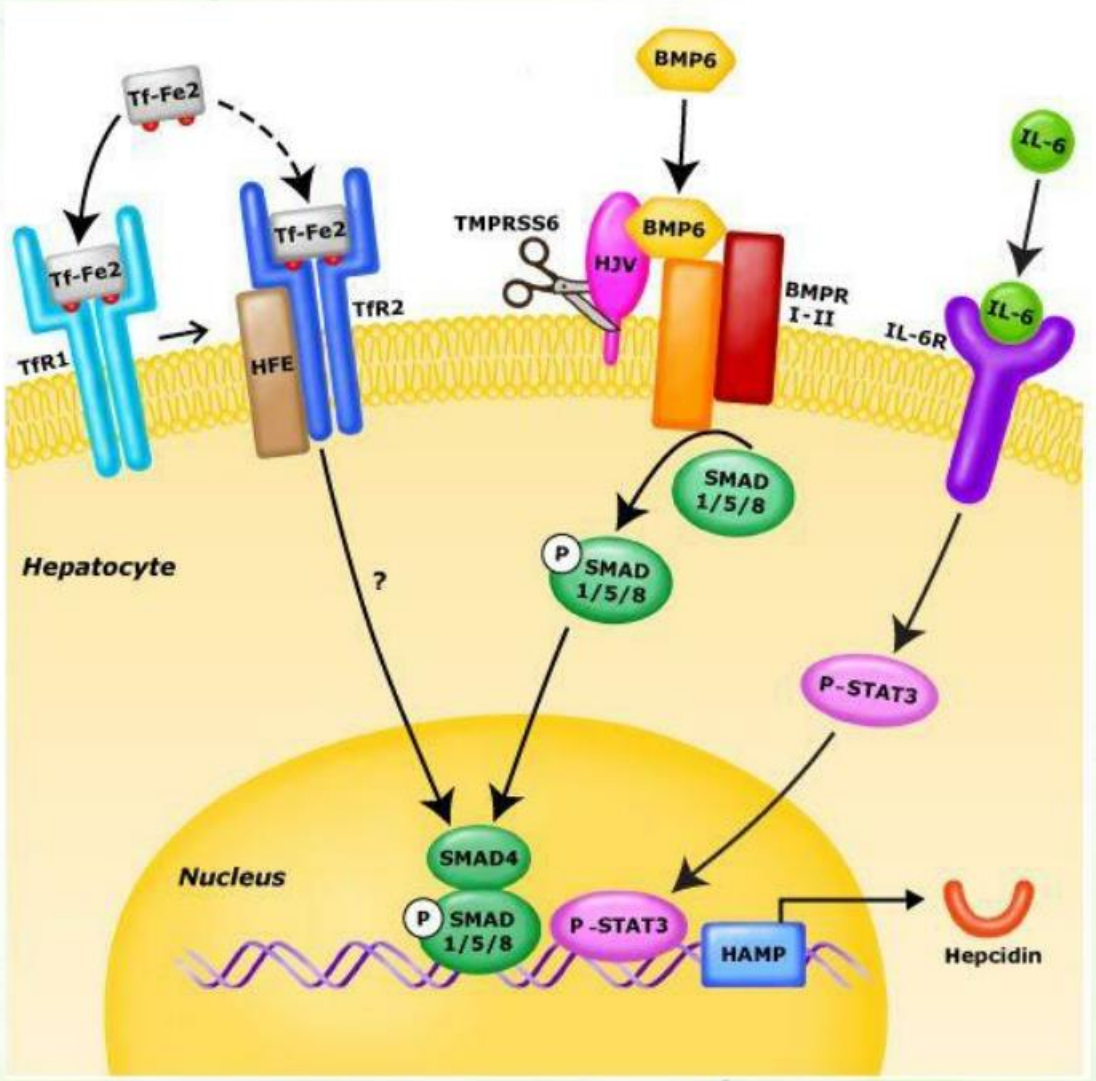
Mutation or absence of HFE or TFR2 prevents formation of a functional iron sensor and signal transduction effector complex leading to dysregulation of systemic iron homeostasis increased intake of iron into the system

Juvenile hemochromatosis

Symptoms appear at a young age

- Type 2A hereditary hemochromatosis
  - *AKA* HFE2 (HJV)-dependent hereditary hemochromatosis
- Mutations in HJV gene, which encodes the protein “hemojuvelin”, account for the majority of JH.
- Normal HJV upregulates expression of hepcidin.

- Type 2B is also juvenile hemochromatosis, but is caused by mutations in hepcidin gene.

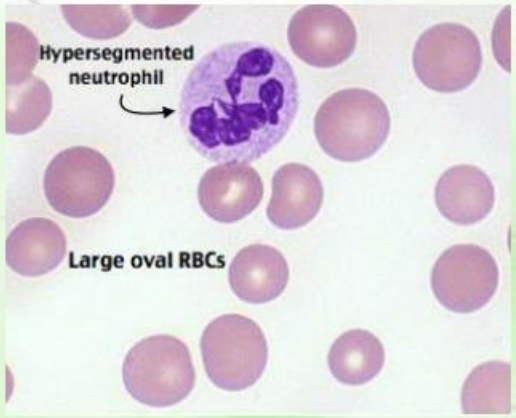


Iron-deficiency anemia

Anemia is a very common diseases worldwide especially in poor countries

- 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.

Cells cannot synthesize DNA and, hence, cannot divide and megaloblasts accumulate.



NUTRITIONAL ANEMIAS

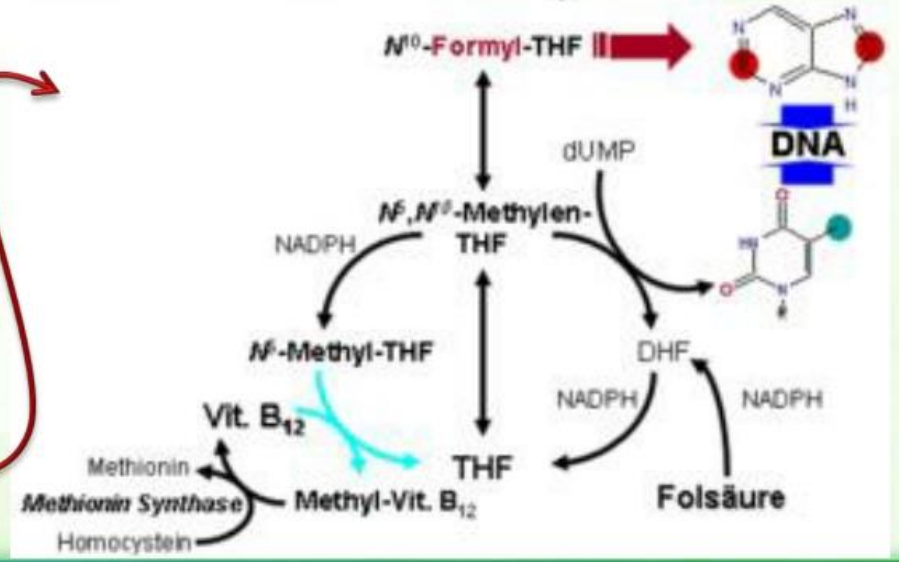
MICROCYTIC (MCV < 80)
- Deficiency in iron
- Deficiency in copper
- Deficiency in pyridoxine

NORMOCYTIC (MCV = 80–100)
- Protein-energy malnutrition

MACROCYTIC (MCV > 100)
- Deficiency in vitamin B_{12}
- Deficiency in folate

Folate is important for producing nucleotides and Vit B12 is important for regenerating folate

Folate is not regenerated

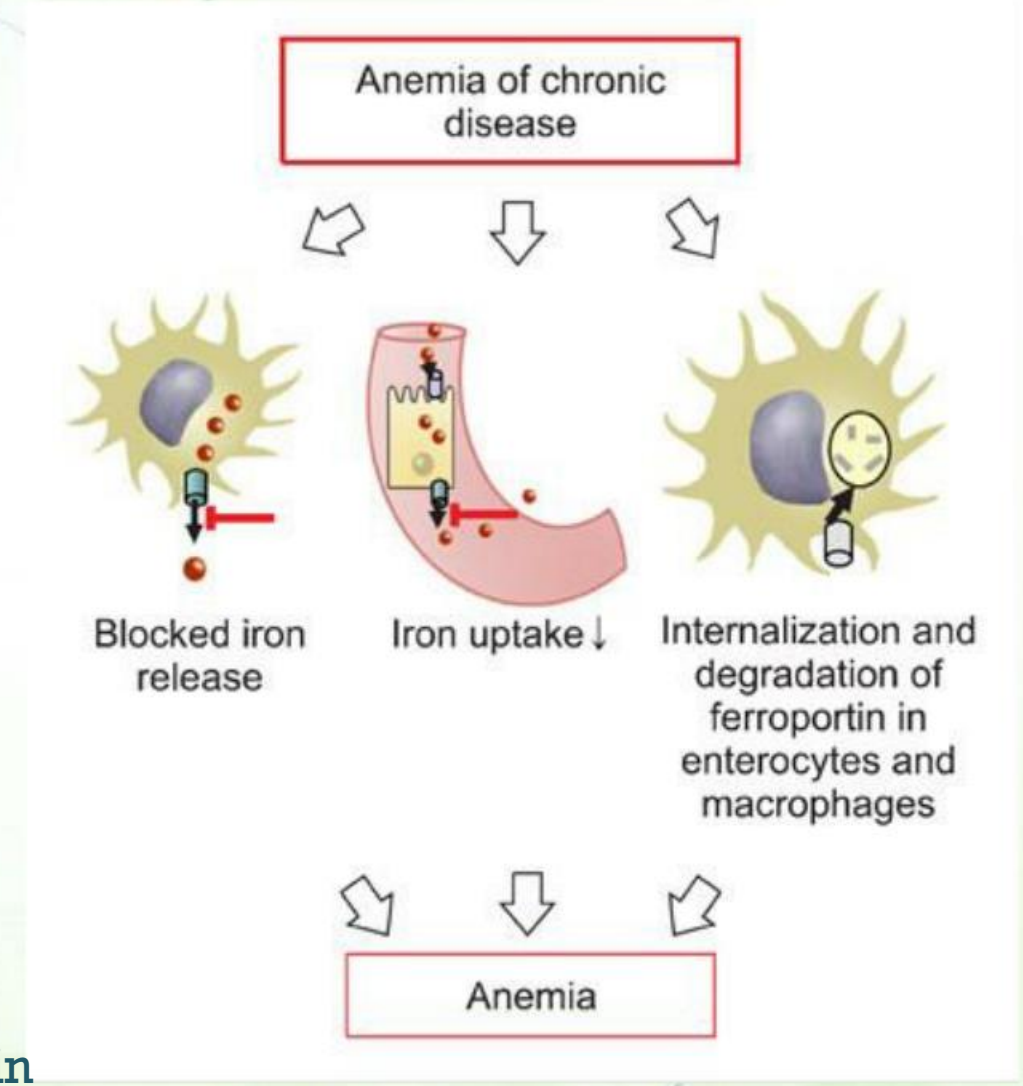


Anemia of chronic disease

Anemia can be common in more industrialized nations such as USA & Europe

- Causes: chronic kidney disease, chronic infections and chronic inflammatory diseases
- Inflammatory cytokines → increased hepcidin production by hepatocytes → downregulation of ferroportin expression in major iron-exporting cells such as macrophages, duodenal enterocytes, and hepatocytes → decreased enteric iron absorption and, perhaps more importantly, to increased iron retention within splenic macrophages and hepatocytes.

That would result in lack of production of hemoglobin and that would result eventually in anemia



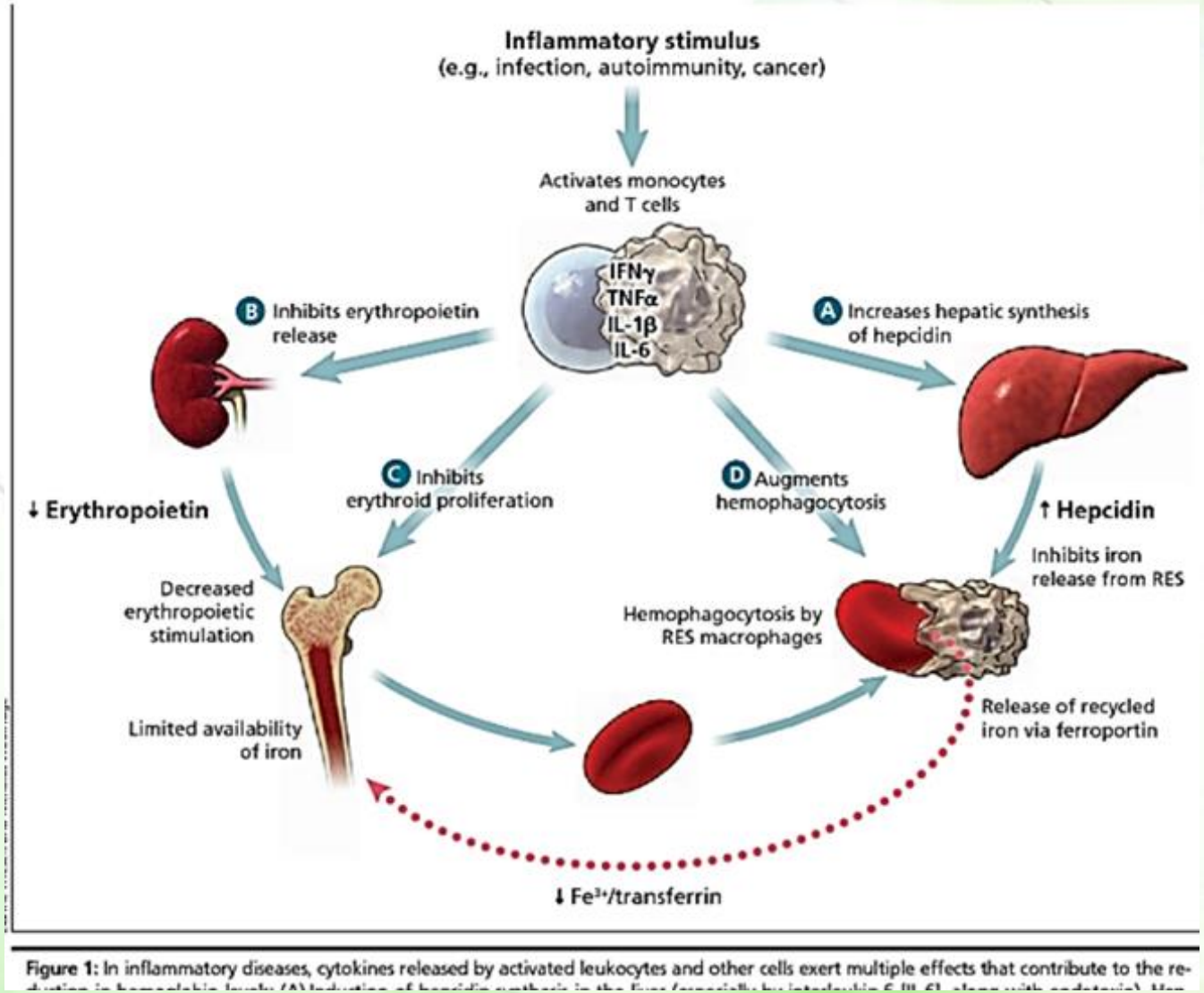


Figure 1: In inflammatory diseases, cytokines released by activated leukocytes and other cells exert multiple effects that contribute to the re-

There are other consequences of chronic inflammation: (inflammatory cytokines):

- Increased production of hepcidin
- Inhibition of erythropoietin release from the kidneys and that would result in decreased erythropoietic stimulation and production of RBCs
- They can inhibit erythroid proliferation
- They can augment hemo-phagocytosis and this causes the inability to release iron from the system