بسم الله الرحمن الرحيم

Biochemistry

﴿ وَقُل رَّبِّ أَدْخِلْنِي مُدْخَلَ صِدْقٍ وَأَخْرِجْنِي مُخْرَجَ صِدْقٍ وَاجْعَل لِّي مِن لَّدُنكَ سُلْطَانًا نَّصِيرًا ﴾

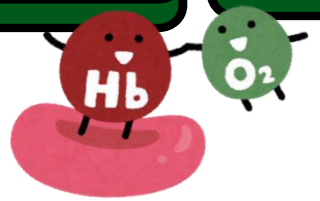
ربنا آتنا من لدنك رحمة وهيئ لنا من أمرنا رشدًا

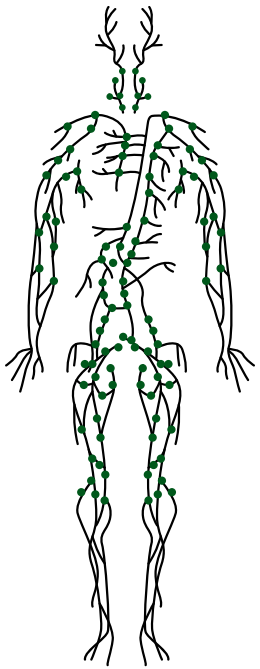
MID | Lecture #5

Metabolism of iron

Written by: Leen Mamoon

Reviewed by: Leen Mamoon





Metabolism of iron

- Prof. Mamoun Ahram

Hematopoietic-lymphatic system

اللهم إني أسألك فهم النبيين، وحفظ المرسلين، وإلهام الملائكة المقربين، اللهم اجعل لساني عامرًا بذكرك،
وقلبي بخشيتك، وسري بطاعتك، إنك على كل شيء قدير.

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

- 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

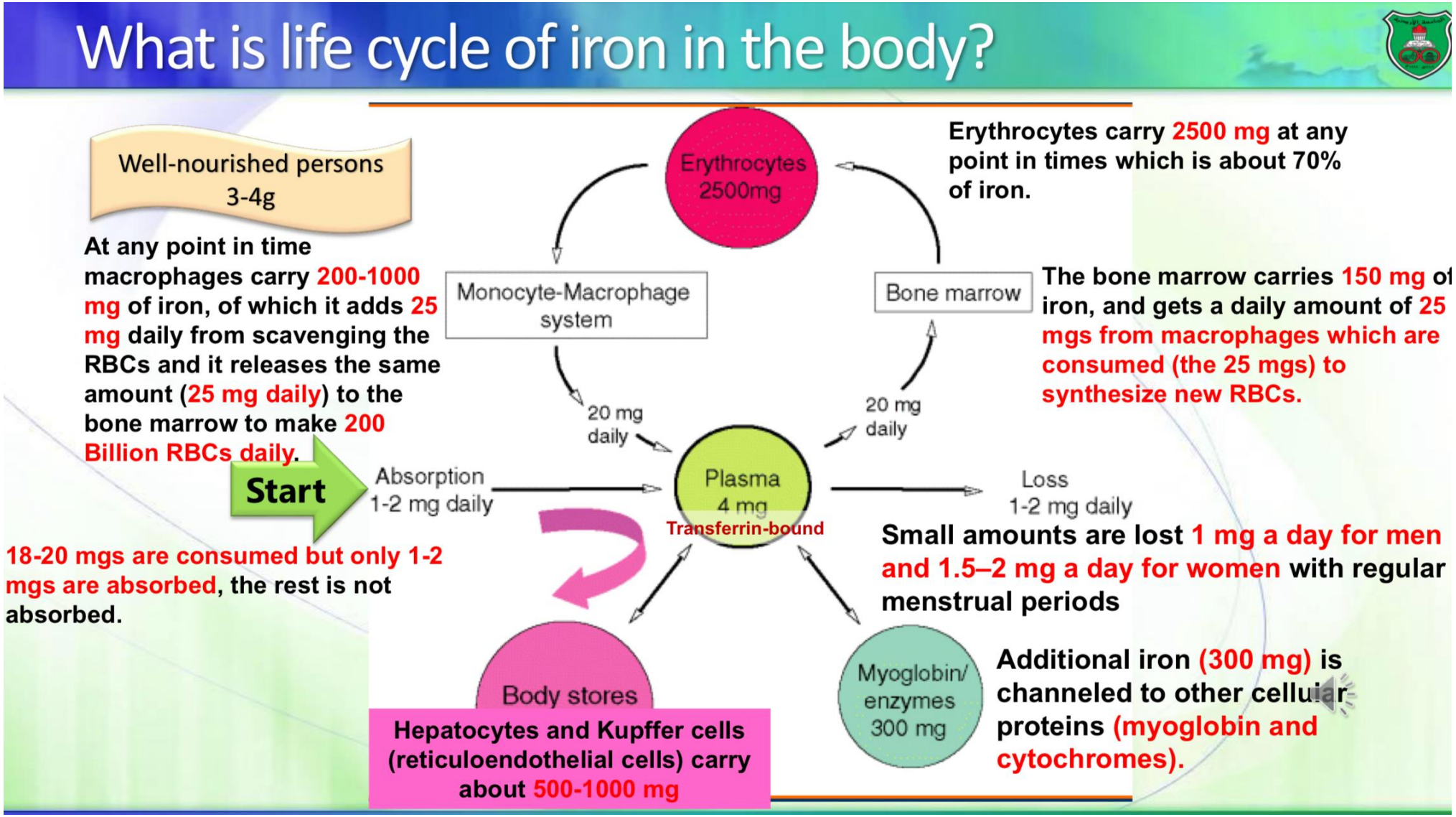
- • Within the body, iron exists in two oxidation states: ferrous (Fe^{2+}), or the ferric (Fe^{3+}) which binds to anions, water and peroxides forming insoluble compounds.
- • It is also present in the prosthetic group of several enzymes such as redox cytochromes and the P450 class of detoxifying cytochromes.
- • Iron is important for metabolism and oxygen
- transport. Yet…
- • Iron can be potentially toxic due its ability to form free radicals when reacting with H_2O_2

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\bullet}$$

  - Solution: iron is not free.

- ✓ It will be explained in the next two slides.

What is life cycle of iron in the body?



What is life cycle of iron in the body?

- The human body contains approximately **3-4 grams** of iron, which represents a significant amount for a trace element:

1. Erythrocytes (Red Blood Cells, RBCs)
Iron content: **~2.5 g (70%)**

- ✓ The majority of body iron is present in hemoglobin within RBCs, derived from approximately **200 billion** circulating RBCs.

2. Monocyte-Macrophage System
Iron content: **~200-1000 mg**

- ✓ At any given time, macrophages carry approximately **200-1000 mg** of iron.
- ✓ These cells play a central role in iron recycling **by scavenging senescent red blood cells (RBCs).**
- ✓ Each day, macrophages acquire about **25 mg of iron from degraded RBCs and simultaneously release the same amount (25 mg/day) into the plasma.**
- ✓ This recycled iron is then **utilized by the bone marrow** to support the production of approximately **200 billion** new RBCs daily.

What is life cycle of iron in the body?

3. Bone Marrow

Iron content: **~150 mg**

- ✓ The bone marrow receives a daily supply of **~25 mg** of iron from the **monocyte-macrophage system.**
- ✓ This iron is immediately utilized to synthesize new red blood cells (RBCs).

4. Tissue Stores (Hepatocytes and Kupffer Cells)

Iron content: **~500-1000 mg**

- ✓ Iron release from these stores is **slower** than from the **monocyte-macrophage system**

5.Enzymes /Myoglobin & cytochrome

Iron content: **~300 mg**

6. Plasma (Transported by Transferrin)

Iron content: **~4 mg**

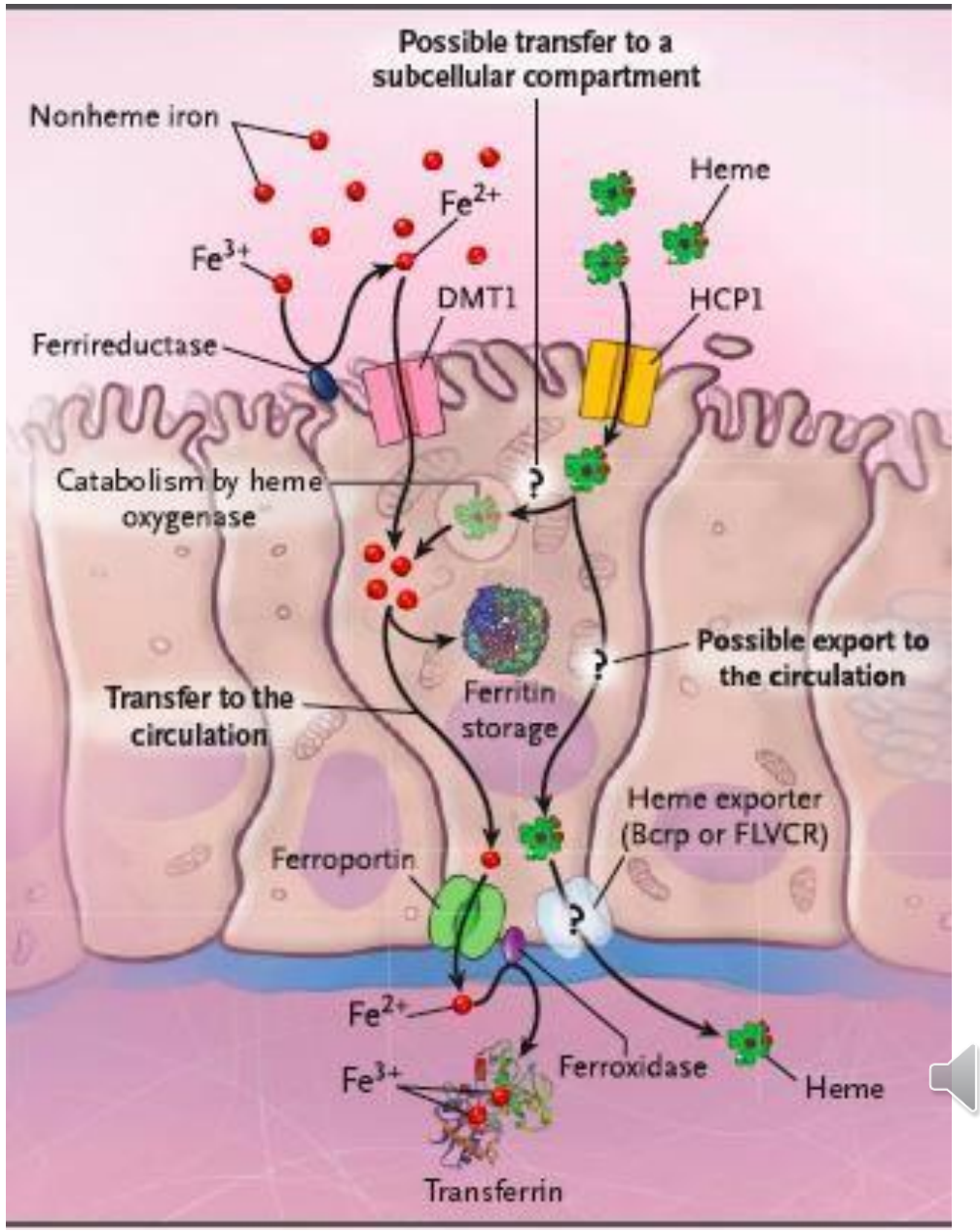
- ✓ A very small but crucial fraction for transporting iron between stores and the bone marrow.

- ➢ Dietary iron absorption: **~1-2 mg/day**
- ➢ Daily iron requirement for erythropoiesis and other functions: **~18-20 mg/day**
- ➢ Small amount are lost **1mg** a day for men and **1.5-2mg** a day for women with a regular menstrual period.
- ➢ **the total body iron remains relatively constant.**

Iron absorption

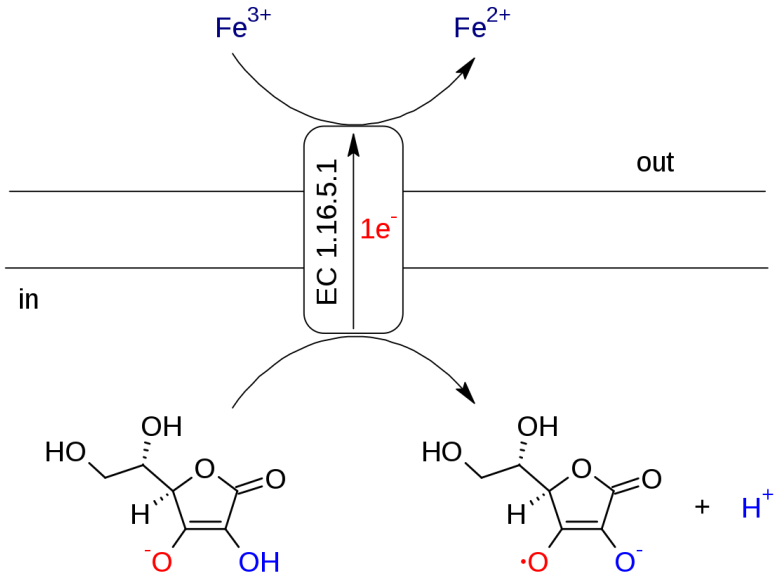
State of iron

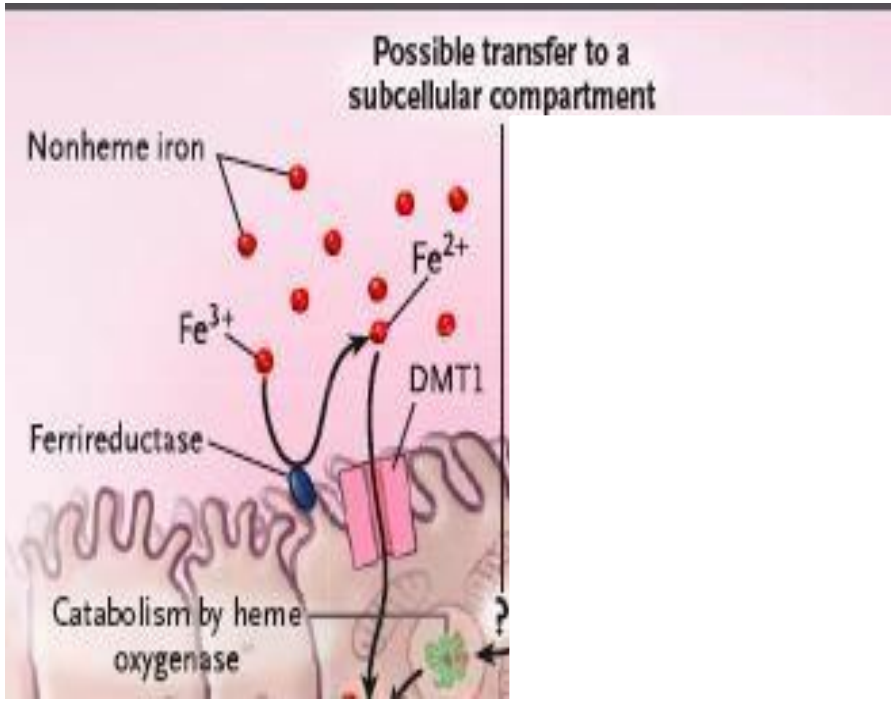
- Under conditions of neutral or alkaline pH, iron is found in the ferric Fe^{3+} state and, at acidic pH, in the ferrous Fe^{2+} state.
  - In the stomach, iron will be in the ferrous state.
  - In the duodenum, iron is in the ferric state.
- However, to be absorbed, dietary iron must be in its ferrous Fe^{2+} form.
- Plant based iron is in the Fe+3 state while animal based is in the Fe+2 state.
- Although plant iron is converted to Fe+2 in the stomach, it is still not as efficiently absorbed.
  - ✓ **Animal-derived iron is better absorbed than plant-derived iron.**



Site of absorption

- Ferrireductase enzyme on the enterocytes' brush border reduces Fe^{3+} to Fe^{2+} in a vitamin C-dependent reaction, where Vitamin C supplies the electron.
- 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.

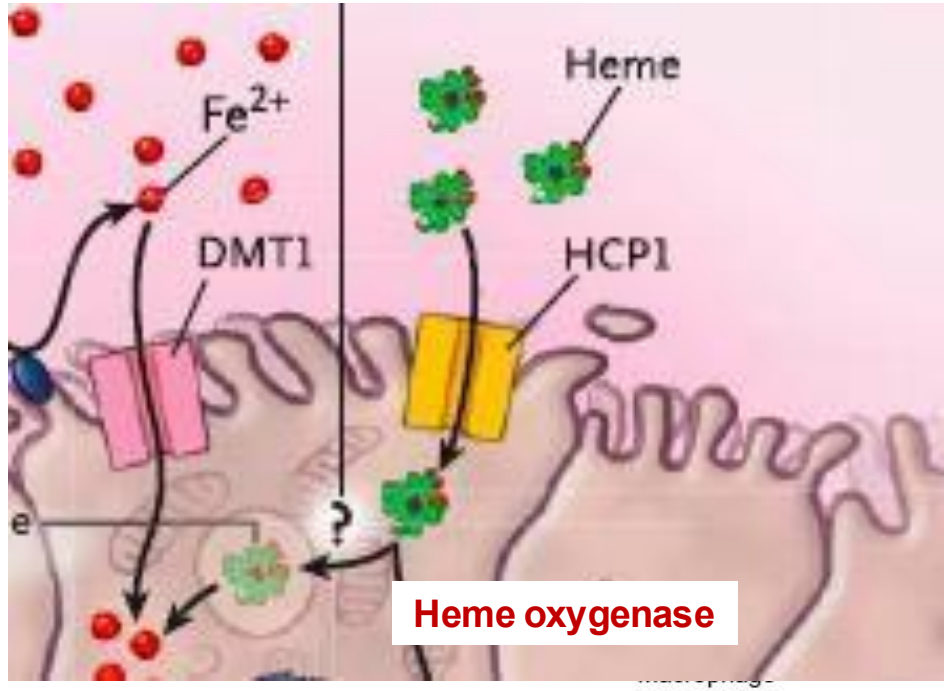




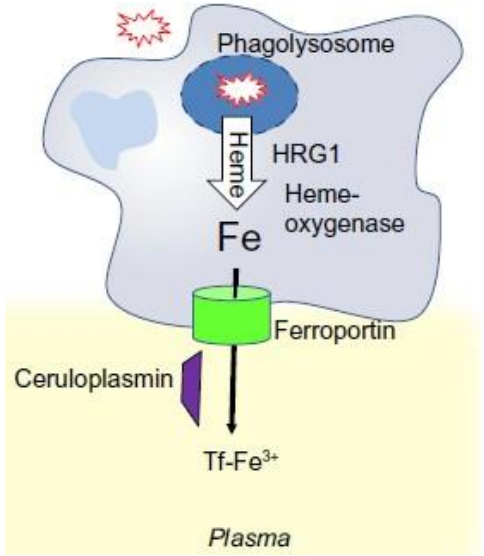
Heme as a source of iron

- 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 other cells such as macrophages, heme oxygenase also extracts iron from heme.

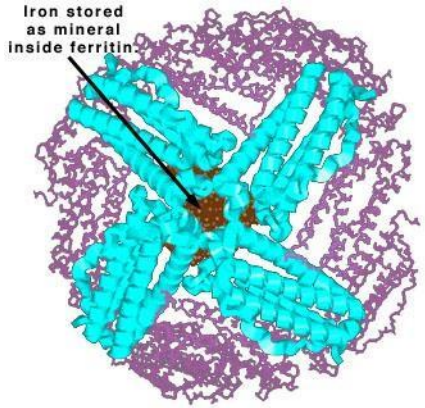
Proton pump-inhibiting drugs such as omeprazole greatly reduce iron absorption.



- ✓ **Omeprazole (or other proton pump inhibitors) reduces stomach acidity by inhibiting hydrogen ion secretion.**
- ✓ **Lower stomach acidity impairs the conversion of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+})**



Fates of iron

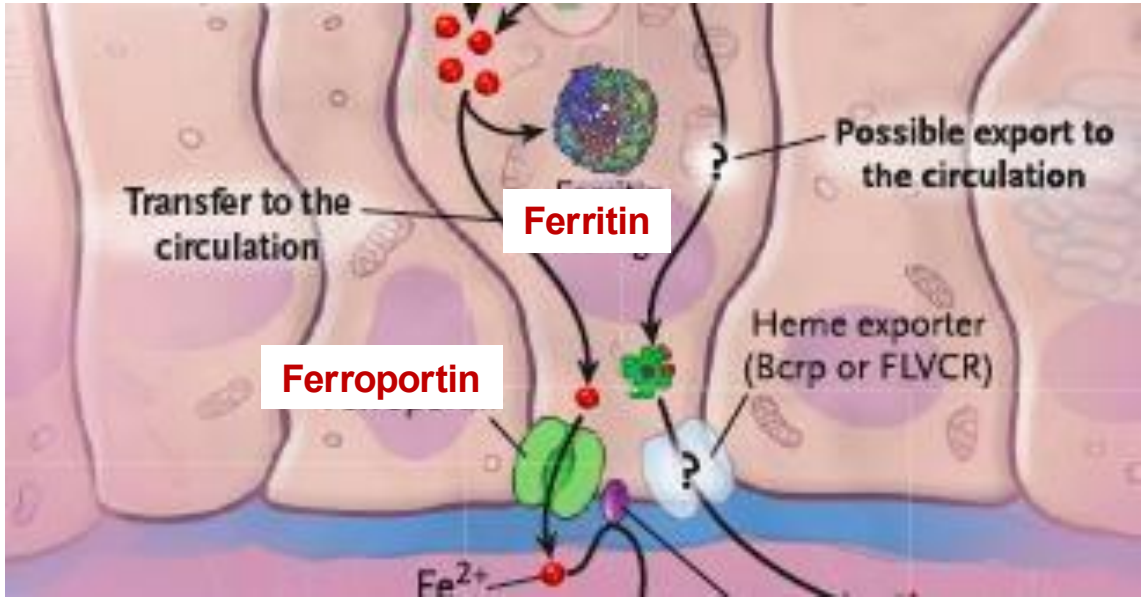


Fate 1: storage

- Cells can store iron as ferritin.
  - Each Ferritin complex can store about 4500 iron ions. Ferritin is present in all storage locations including liver cells, macrophages..
- But, if cells are sloughed off from the tip of the villus into feces before absorption, iron is eliminated from the body.

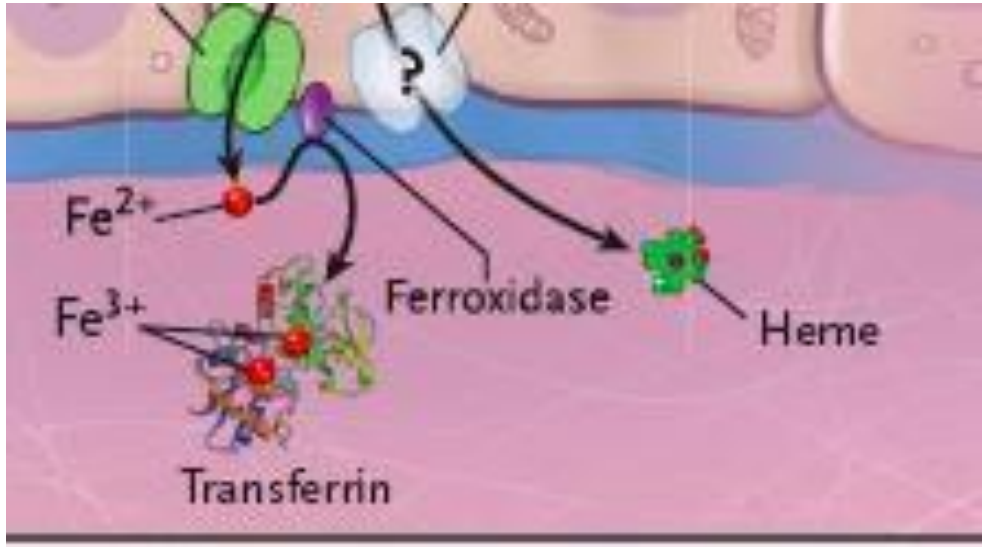
Fate 2: Transport

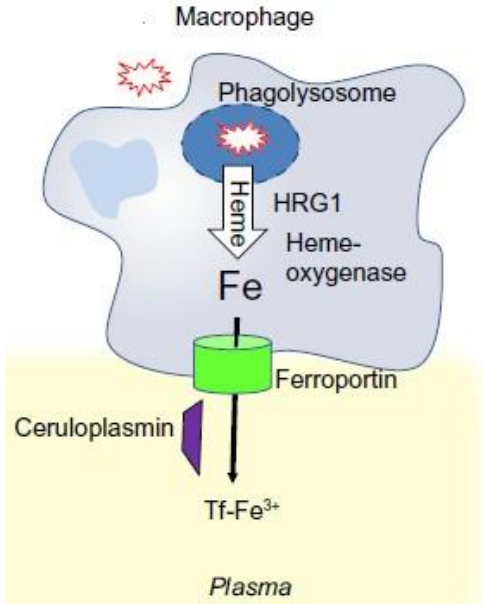
- Iron is transported out via a basolateral transporter known as ferroportin, which is distributed throughout the body on all cells.



Ferroxidase and transferrin

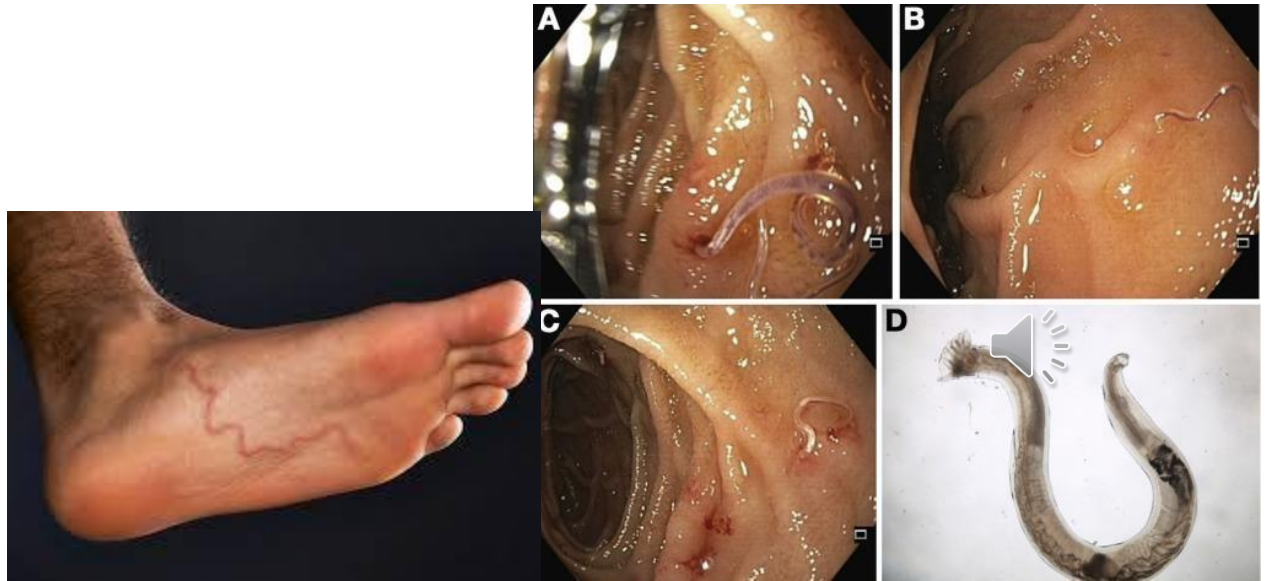
- Once iron leaves the intestinal cells, an iron oxidase, known as hephaestin or ferroxidase, converts iron from the ferrous state to the ferric state. If stored as Fe+2 it would be active and may participate in non desirable reactions, thus it is stored as Fe+3.
  - Nonintestinal cells use the plasma protein ceruloplasmin to oxidize iron.
- Iron is rapidly bound to transferrin, an iron-binding protein of the blood that delivers iron to liver cells and from liver cells for storage to other tissues via receptor-mediated endocytosis.





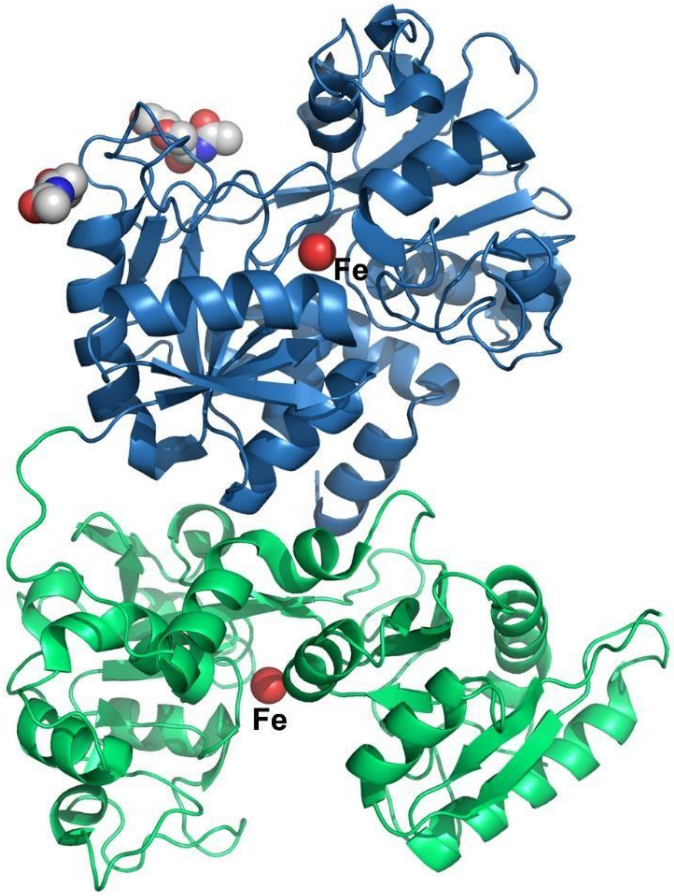
Intestine-related iron metabolism disorders

- Iron malabsorption
  - Gastrectomy (total or partial)
  - Celiac disease (villous atrophy)
  - Crohn’s disease
  - Helicobacter pylori
- Intestinal hemorrhage (gastrointestinal-mediated iron loss)
  - Gastric cancer
  - Ulcers
  - Inflammatory bowl disease
  - Hookworm infection



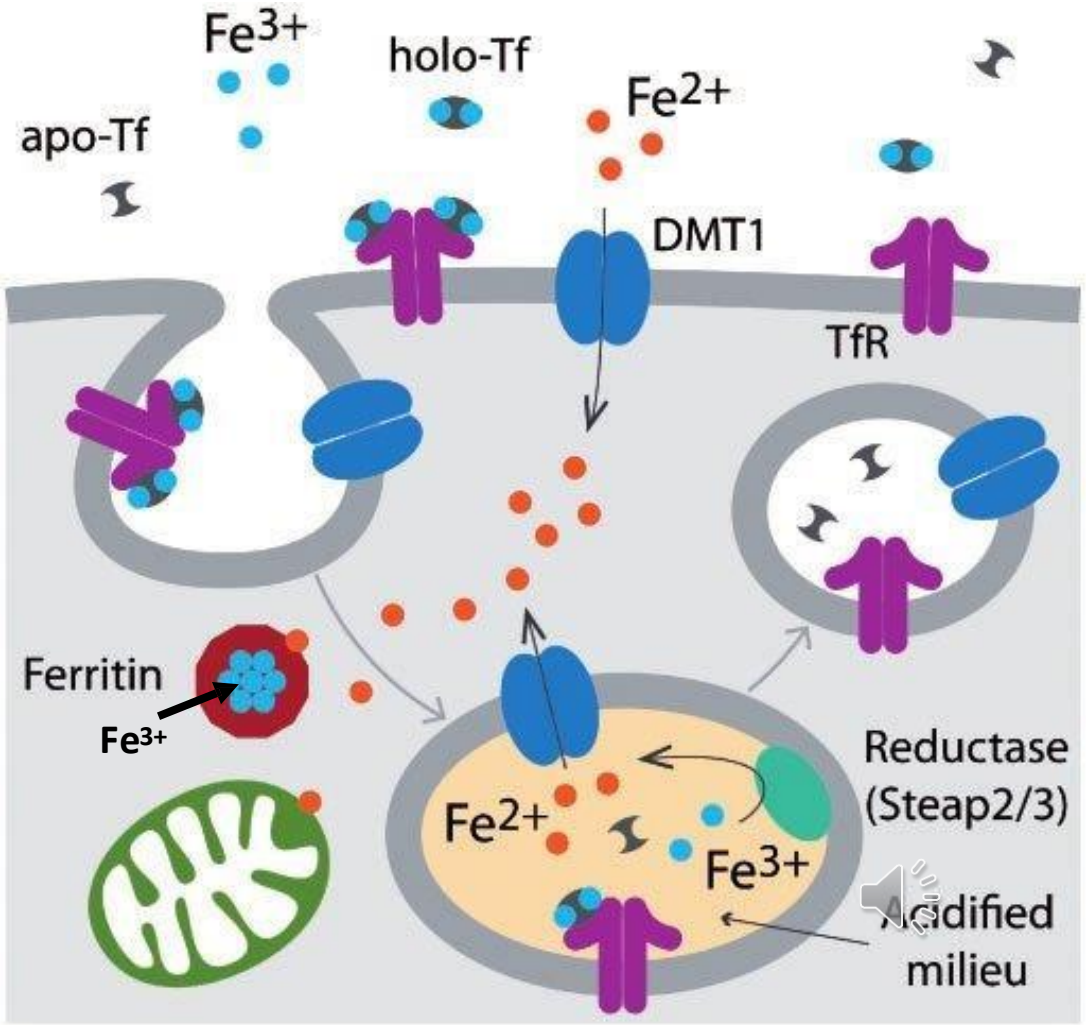
Properties of transferrin

- Apotransferrin can bind several metals, but ferric, not ferrous, iron has the highest affinity forming ferrotransferrin.
- Transferrin contains two sites that bind ferric irons:
  - *iron-binding sites of transferrin are normally only about 1/3 saturated with iron.*
- When iron exceeds normal levels, non-transferrin-bound iron (NTBI) appears.



Receptor-mediated endocytosis

- Ferrotransferrin binds to a transferrin receptor (TfR) on the surface of cells triggering endocytosis into early endosomes (pH of 6.0).
- Early endosomes are transformed into late endosomes (pH of 5.0) where Fe^{3+} atoms dissociate, get reduced into Fe^{2+} by the ferrireductase STEAP3, and are transported into the cytosol via DMT1.
  - STEAP3 depends on vitamin C.
- 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



Summary

➢ **Absorption in the Duodenum**

- **Fe^{3+}** must be reduced to **Fe^{2+}** to be absorbed.
- ***Ferrireductase*** enzyme on enterocytes' brush border reduces **$Fe^{3+} \rightarrow Fe^{2+}$** (vitamin C-dependent).
- Transport into enterocytes:

✓ Non-heme iron (**Fe^{2+}**): via ***DMT1 (Divalent Metal Transporter 1).***

✓ Heme iron: via ***Heme Carrier Protein 1 (HCP1)*** → iron released by ***Heme Oxygenase-1 (HO-1).***

- Stored as **ferritin**.

➢ **Transport to Blood**

- Exported via **ferroportin** on the basolateral membrane.
- Oxidized from **$Fe^{2+} \rightarrow Fe^{3+}$** by ***hephaestin/ferroxidase*** (in intestine) or ***ceruloplasmin*** (other tissues).
- Binds **transferrin** (Tf) in plasma for transport.

➢ **Transport in Blood**

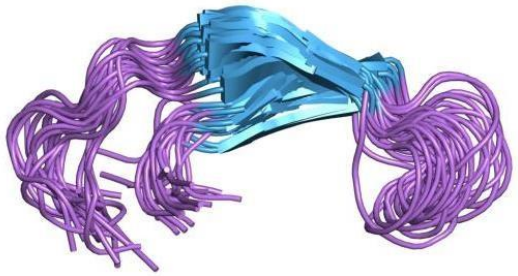
- **Transferrin** (Tf): binds **Fe^{3+}** → delivers to tissues via receptor-mediated endocytosis.
- Iron-binding: transferrin is normally 1/3 saturated.

➢ **Cellular Uptake**

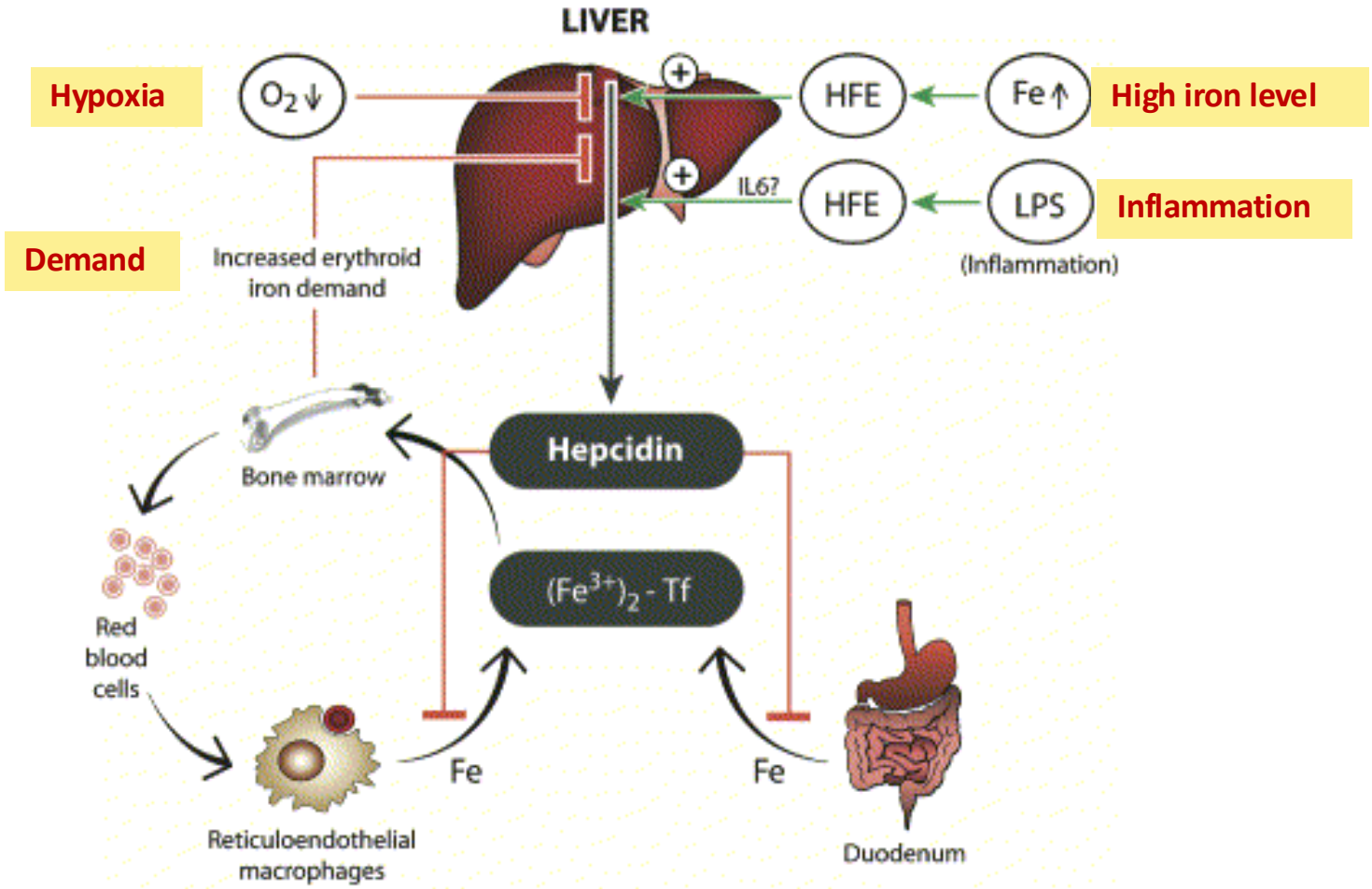
- **Transferrin receptor** (TfR) binds diferric transferrin → endocytosis into early endosomes (pH 6.0).
- Late endosomes (pH 5.0) , **Fe^{3+}** dissociates from **transferrin**.
- Reduced to **Fe^{2+}** by ***STEAP3 ferrireductase*** (vitamin C-dependent).
- Transported into cytosol via ***DMT1***.
- **TfR** and **apotransferrin** recycled to cell surface.

Regulation of iron in the body

Hepcidin (iron sensor)

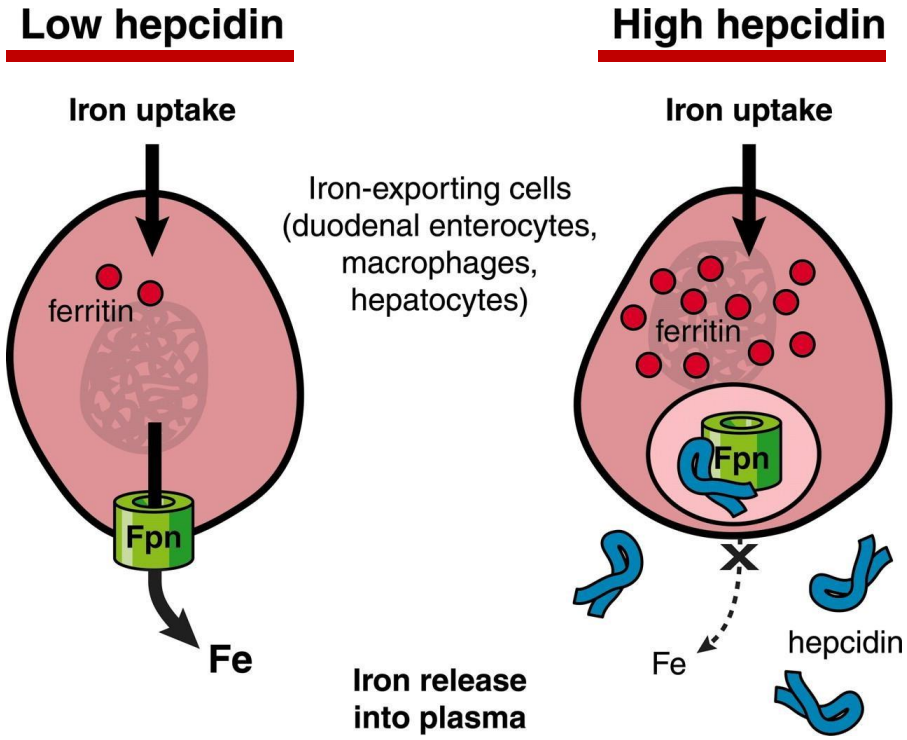


- Hepcidin is a peptide hormone (25 amino acids) secreted by the liver and it **reduces** iron levels.
- When iron level increases and in cases of inflammation, hepcidin secretion increases.
- When iron levels are low, there is high iron demand, or hypoxia, its release is suppressed.



How does hepcidin reduce iron levels in the body?

- Hepcidin binds to the basolateral iron transporter ferroportin inducing ferroportin internalization and degradation.
  - This results in higher iron storage.
    - Iron is eliminated in sloughed off intestinal cells.
    - Iron is not released from macrophages.
- Hepcidin also inhibits the presentation of the iron transporters (e.g. DMT1) in intestinal membranes decreasing iron absorption.

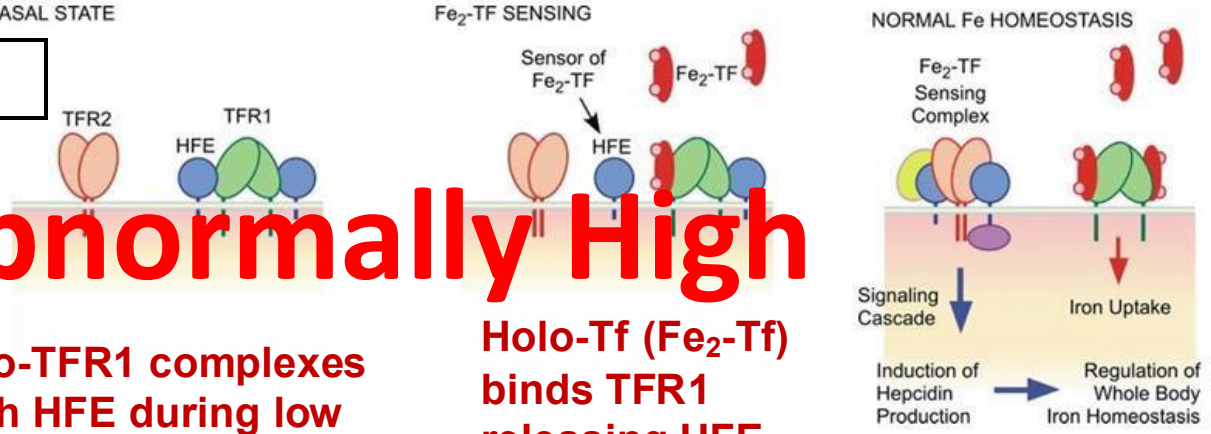


What happens when iron increases

✓ **It will be explained in the next four slides.**

- Release of hepcidin through

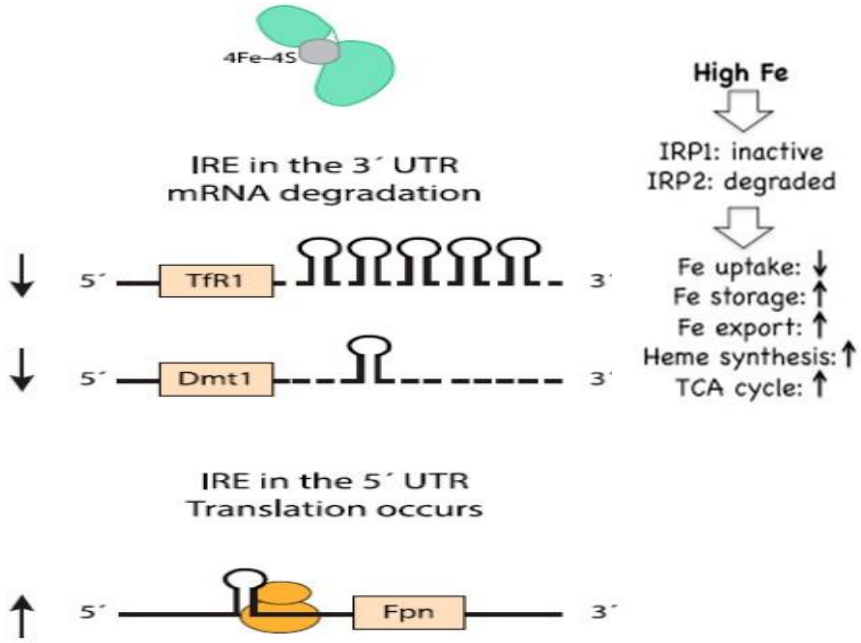
Normally High ① Abnormally High

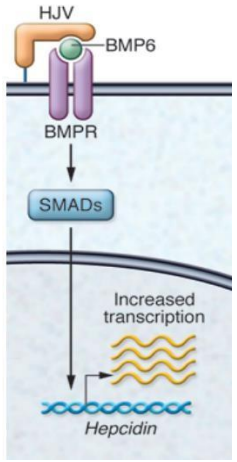


Apo-TFR1 complexes with HFE during low or basal serum iron conditions.

Holo-Tf (Fe_2-Tf) binds TFR1 releasing HFE.

HFE binds to TFR2 sending a signaling cascade to increase hepcidin production





② High intracellular iron binds to bone morphogenic protein (BMP6), which binds to its receptor (BMPR). BMPR is associated with Hemojuvelin (HJV), thus upon binding to BMP6, both proteins start a cascade of a signaling pathway inside the cell to upregulate hepcidin production.

③ At the transcriptional mRNA level:

A- In the 3 UTR of TfR1 and Dmt1, the binding of iron to IRP removes it from the IRE in both the 3 end and the 5 end. This causes TfR1 and DMT1 mRNA to become unstable and downregulated which reduces iron uptake.

B- In the 5 UTR region of Fpn the IRP is removed allowing the Ferroportin to be expressed probably to allow iron circulation. In case of too much iron, hepcidin downregulates ferroportin to prevent overload of iron in other tissues.

1) What happens when iron increases?

When there's too much iron (Fe) circulating in the blood, the liver senses this and increases hepcidin production.

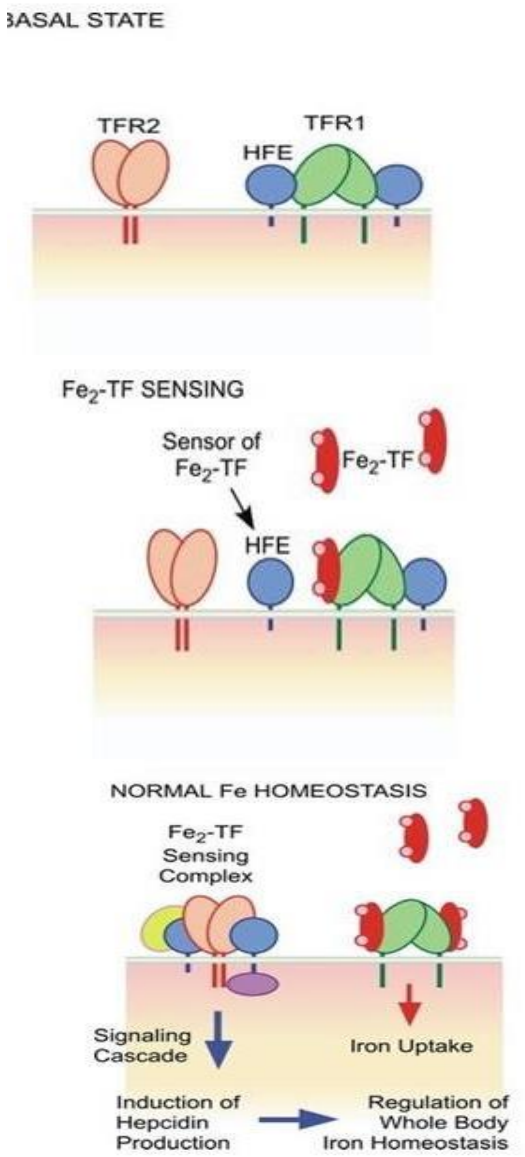
- **How that signal travels?**

- When **iron** levels are **high** → transferrin becomes more saturated (diferric transferrin = Fe2-Tf) which binds strongly to **Transferrin Receptor 1 (TfR1)** on hepatocytes (liver cells).
- Normally, **HFE** protein is attached to **TfR1** But when **Fe2-Tf** binds to **TfR1** → **HFE** is displaced (released).

لما يزيد الحديد في الدم، الـ Fe2–Transferrin يدخل وينافس HFE على موقع الارتباط)

- The free **HFE** now binds to **Transferrin Receptor 2 (TfR2)** on the hepatocyte surface.

- **TfR2-HFE** complex acts like a signal sensor for hepcidin production



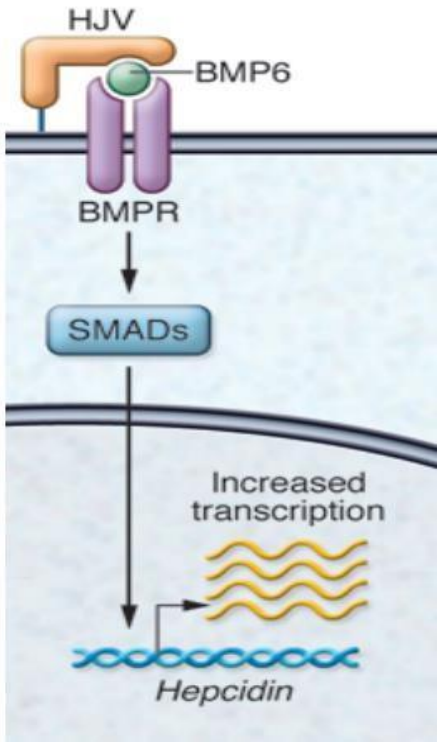
2) What happens when iron increases?

1. When intracellular iron increases, liver produces more **BMP6 (Bone Morphogenetic Protein 6).**

2. **BMP6** binds to its receptor – **BMPR (Bone Morphogenetic Protein Receptor).**

3. BMPR forms a complex with a helper protein called **Hemojuvelin (HJV).**

4. This **BMP6-BMPR-HJV** interaction triggers an intracellular signaling inside the cell **to upregulate hepcidin production.**



3) What happens when iron increases?

➢ Both **Transferrin Receptor 1 (TfR1)** and **Divalent Metal Transporter 1 (DMT1)** have **Iron-Responsive Elements (IREs)** in their **3′ untranslated regions (3′ UTR).**

- Normally, when **Iron Regulatory Proteins (IRPs)** are bound to these **IREs,** they stabilize the **mRNA**, allowing more translation → ↑ **iron uptake.**

✓ *But when iron is high:*

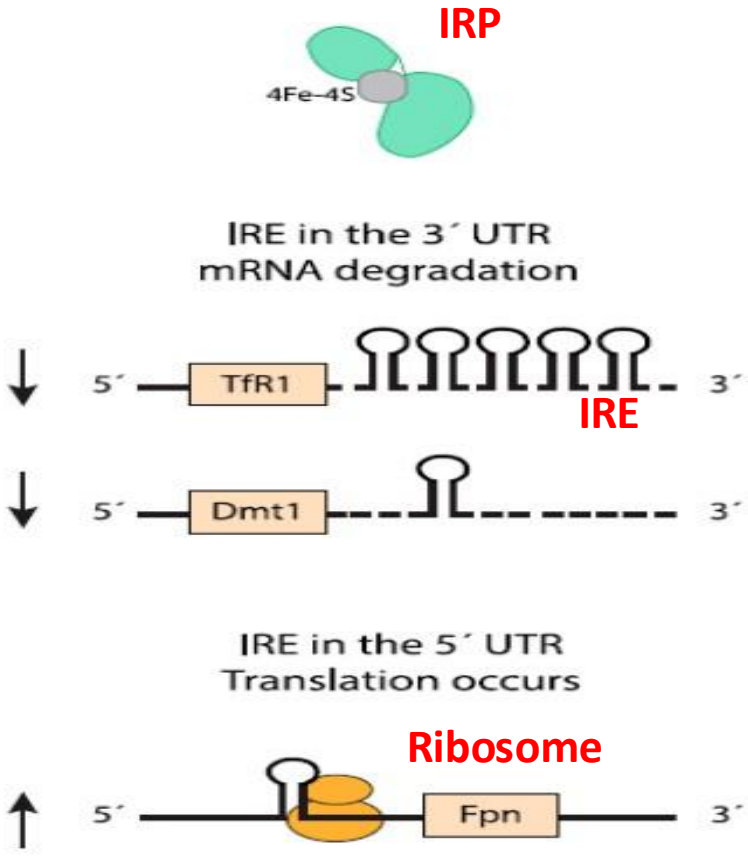
- Iron binds **IRP** → **IRP** detaches from **IRE** → **mRNA** becomes unstable → degraded.
- Result: ↓ **TfR1** and ↓ **DMT1** → ↓ iron uptake into the cell.

➢ **Ferroportin** has its **IRE** at the **5′ untranslated region (5′ UTR).**

When **IRP** is bound (*in low iron*), it blocks translation of **Fpn**.

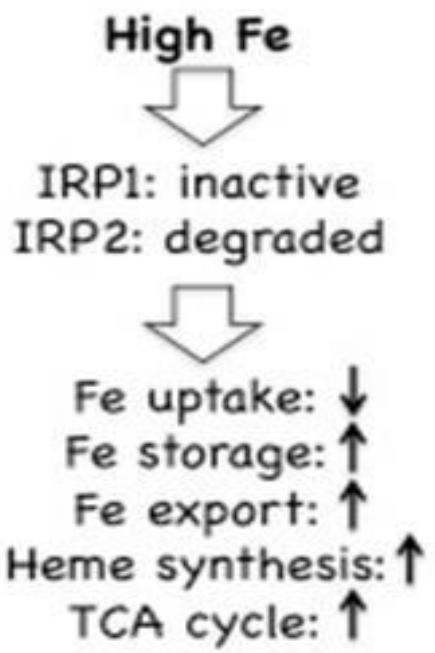
- When *iron is high*, **IRP** detaches → translation proceeds → ↑ **ferroportin** expression → more iron can exit the cell into circulation.

الجسم عنده الحديد كافي، فيسمح بخروج الحديد لتوصيله للأنسجة الأخرى



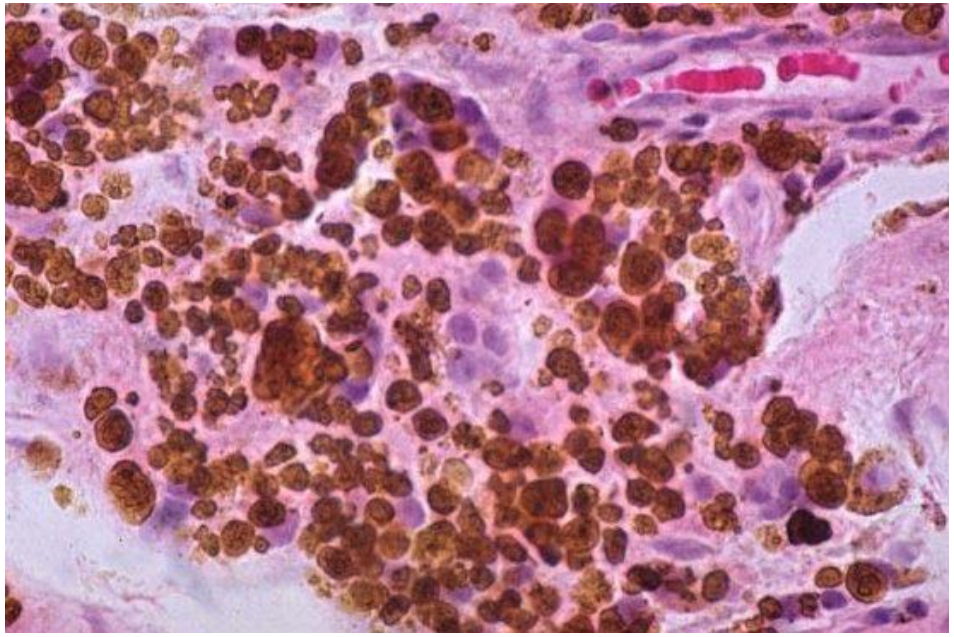
What happens when iron increases?

- In conditions of iron overload, the first and second regulatory pathways (abnormal) are primarily activated, whereas in moderate increases of iron, the third pathway (normal) predominantly regulates iron homeostasis.



Hemosiderin

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



Affected organs and conditions

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

Disease of high iron overload Hereditary hemochromatosis

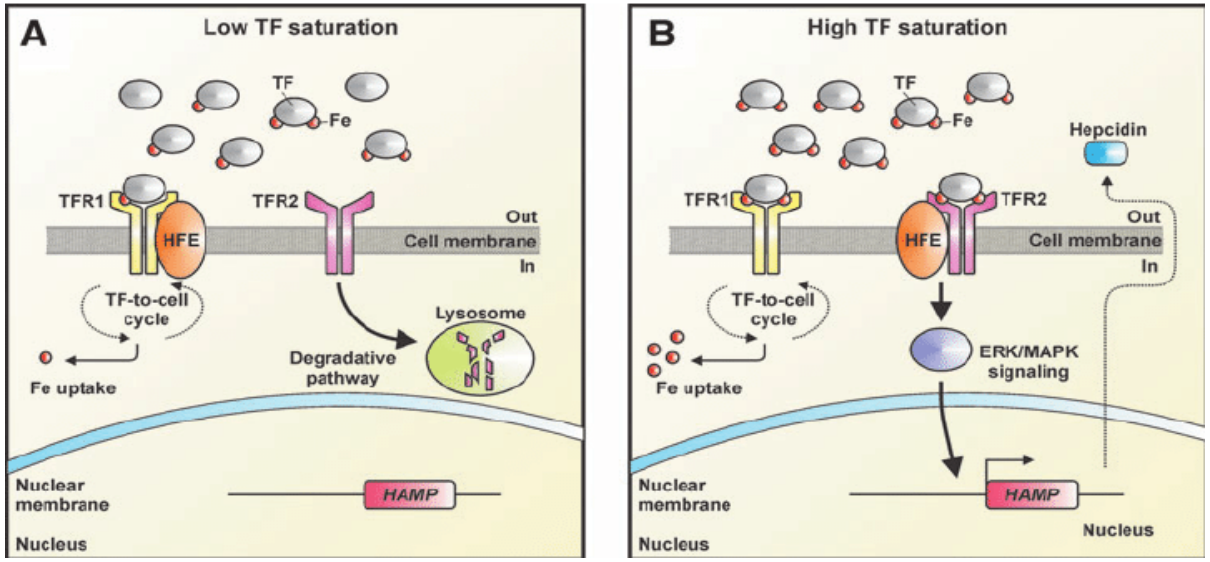
- It is a group of disorders in iron metabolism that is characterized by excess iron absorption, saturation of iron-binding proteins and deposition of hemosiderin in the tissues.
- The primary cause of hemochromatosis is the inheritance of an autosomal recessive allele designated as HFE (type I or primary 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 (HJV-dependent)
- Type 2B (hepcidin-dependent)
- Type 3 (TfR2-dependent)
- Type 4 (ferroportin-dependent)
  - Autosomal dominant disorder

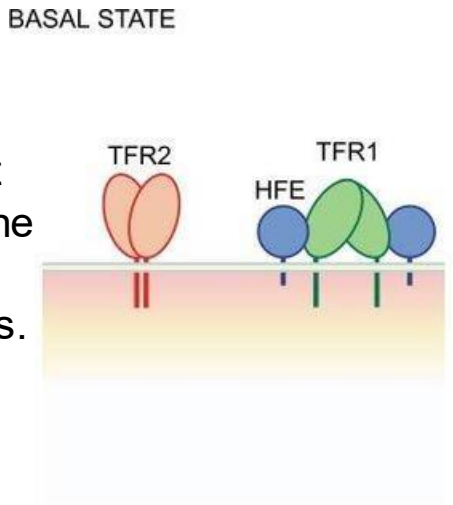
Regulation of transferrin receptor

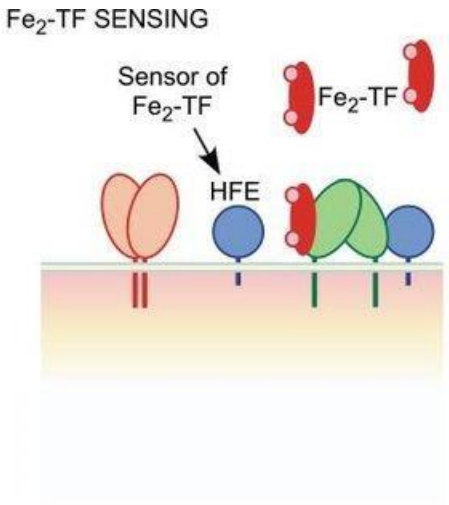
- HFE is a major histocompatibility complex (MHC) class-1 gene.
- Normal HFE complexes with TfR1 reducing iron transfer into cells.
- Mutated HFE has a reduced presence on membrane and/or lack of interaction with Tfr1, leading to the loss of inhibition of transferrin receptor, and, therefore, increased iron uptake and storage.



Mechanism of action

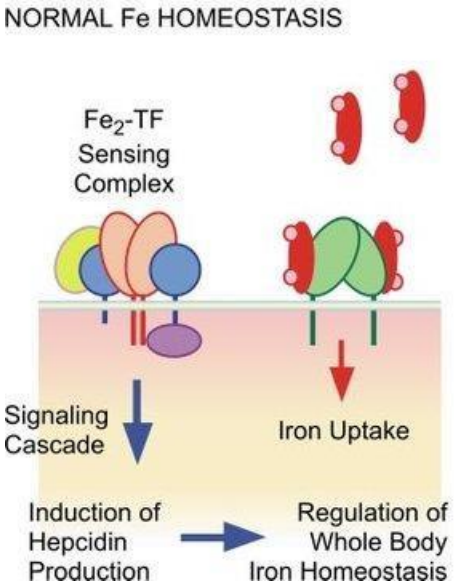
TFR1 exists as a complex with HFE at the plasma membrane during low or basal serum iron conditions.

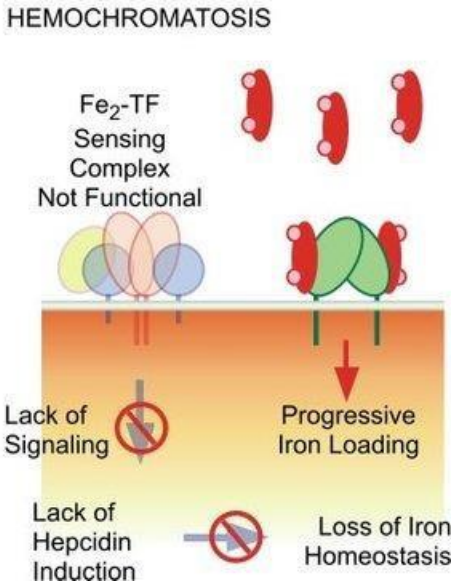




Serum Fe2 -TF competes with HFE for binding to TFR1. Increased serum transferrin saturation results in the dissociation of HFE from TFR1.

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



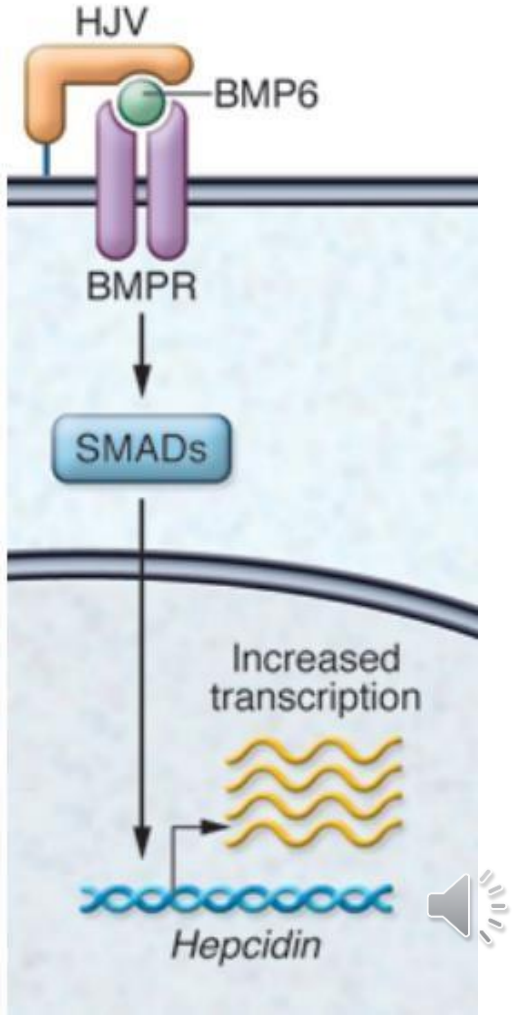


Mutation or absence of HFE or TFR2 prevents formation of a functional iron sensor and signal transduction effector complex leading to dysregulation of systemic iron homeostasis

Juvenile hemochromatosis

- 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.



What happens in case of low iron

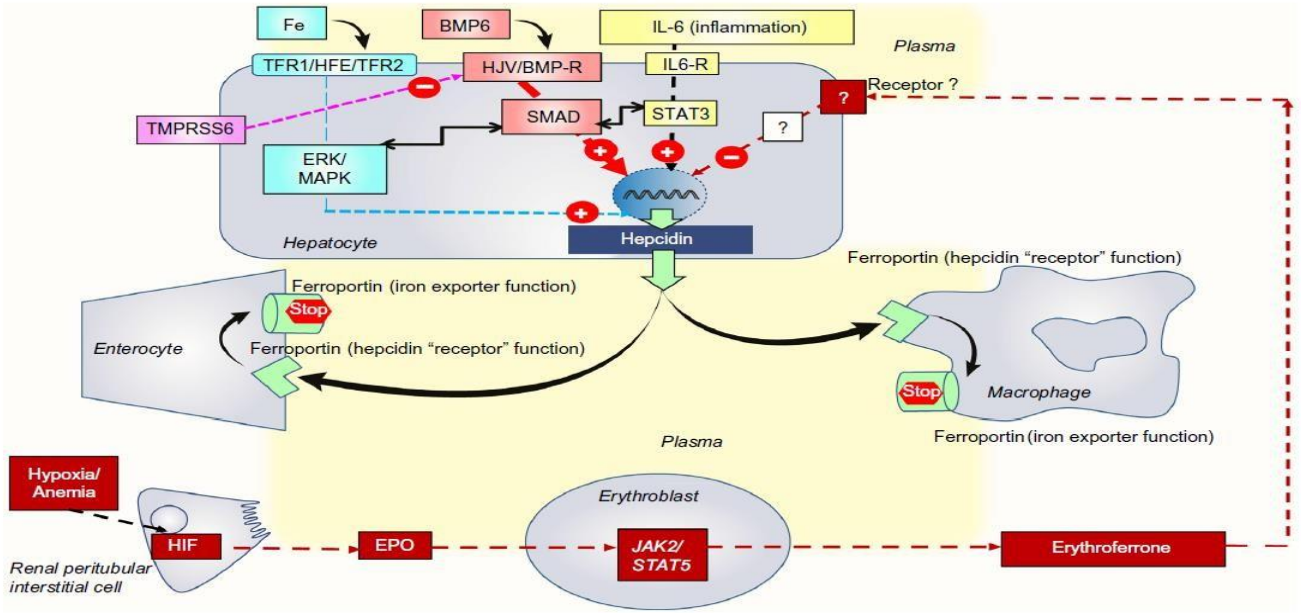
- 1. The expression of EPO (erythropoietin) by the kidney. EPO stimulates the synthesis of erythroferrone which is a protein produced by growing RBCs, thus inhibiting the synthesis of hepcidin.

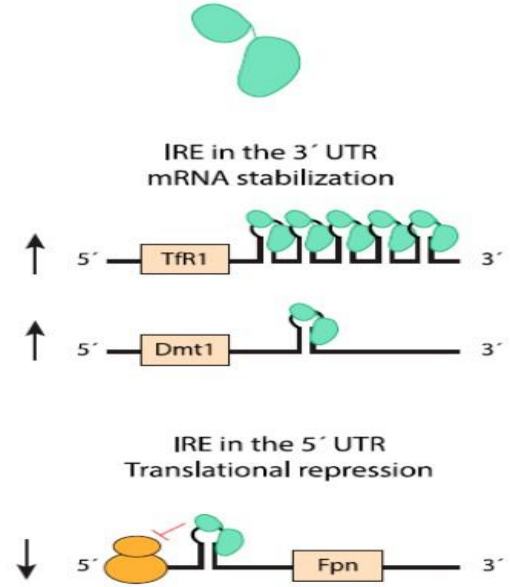
- 2. At transcriptional level there is a sequence in the DNA called Iron response element (IRE) that binds to iron response protein (IRP). The binding of this protein to IRE at the 3 UTR causes stabilization of the mRNA allowing it to be translated. Thus:
  A- Upregulation of TfR1 to increase iron absorption since it functions in engulfing TF-Fe+2 complex.
- B- Upregulation of DMT1 which functions in absorbing heme directly
- C- Downregulation of Ferroportin production to prevent loss of iron. The binding of IRP to the 5 UTR causes downregulation of the protein as it prevents its translation.

Low Fe

IRP1: active
IRP2: active

Fe uptake: ↑
Fe storage: ↓
Fe export: ↓
Heme synthesis: ↓
TCA cycle: ↓



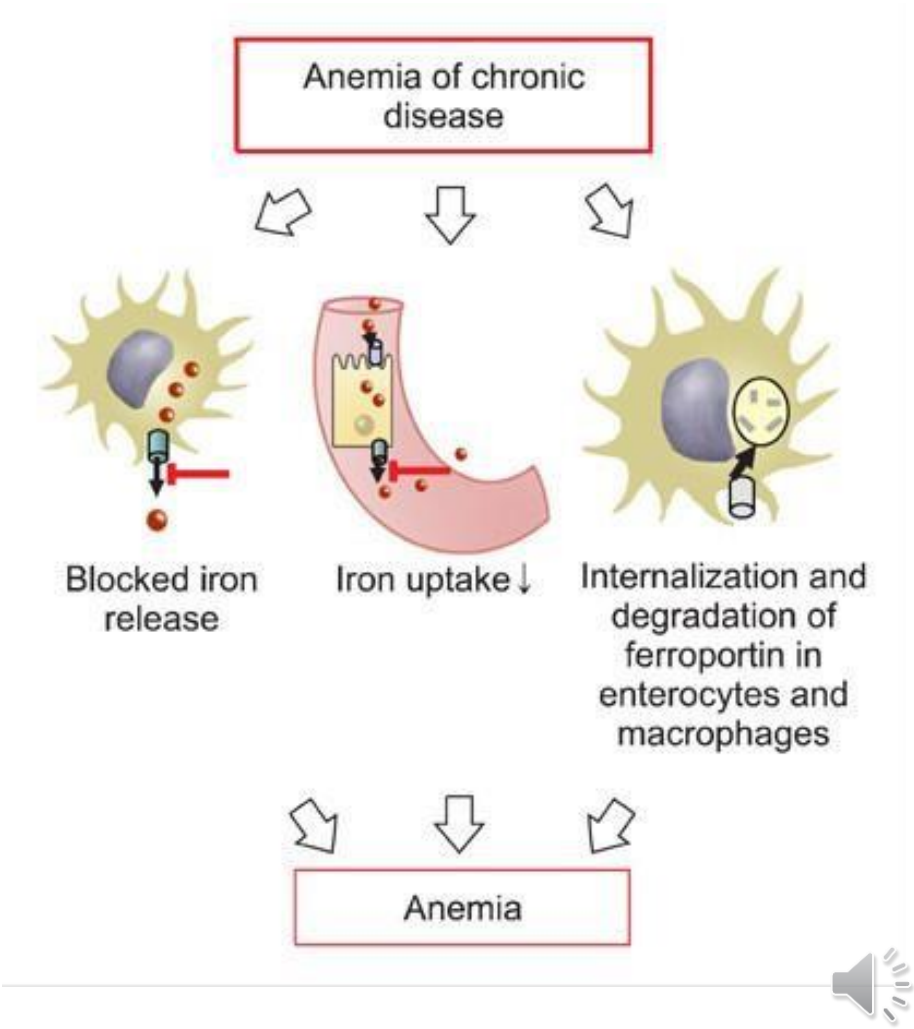


IRP–IRE Regulation: Low vs High Intracellular Iron

Protein	IRE Location	Low Iron (IRP bound)	High Iron (IRP detached)	Physiological Effect
TfR1	3′ UTR	↑ mRNA stability → ↑ TfR1	↓ mRNA stability → ↓ TfR1	Uptake of transferrin-bound iron ↑ when low, ↓ when high
DMT1	3′ UTR	↑ mRNA stability → ↑ DMT1	↓ mRNA stability → ↓ DMT1	Absorption of divalent metals and heme ↑ when low, ↓ when high
Ferroportin (Fpn)	5′ UTR	Translation blocked → ↓ Fpn	Translation proceeds → ↑ Fpn	Iron export ↓ when low iron, ↑ when high iron

What happens in case of chronic inflammation

- Inflammation causes the release **Inflammatory cytokines, including IL6, which iduces the expression of hepcidin.**
- 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 → Anemia

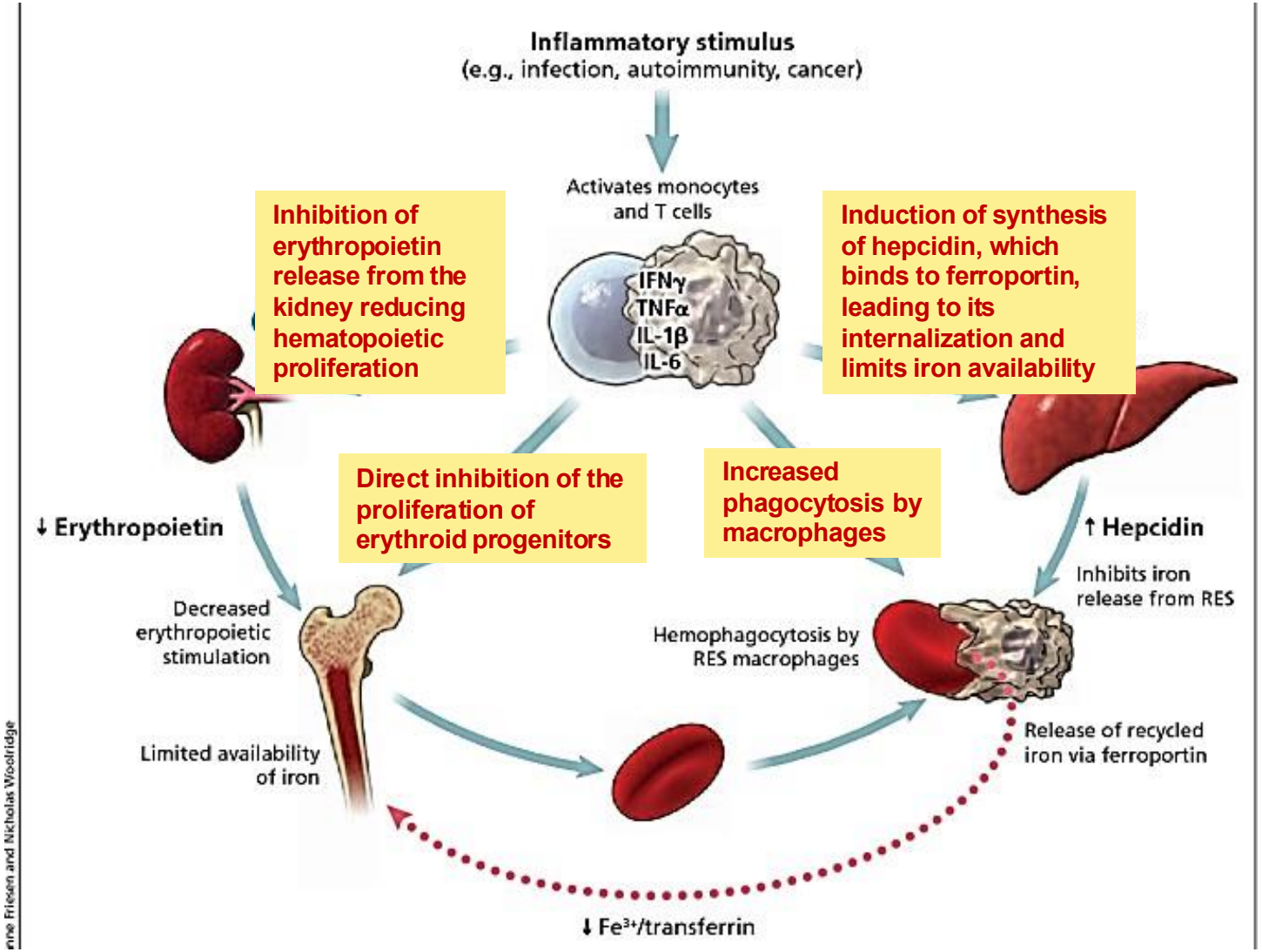


Additional molecular consequences of chronic inflammation

Besides IL6 release which causes the release of hepcidin leading to anemia, inflammation also:

1 decreases erythropoietin release from kidneys, which causes a decline of proliferation of erythroid progenitors.

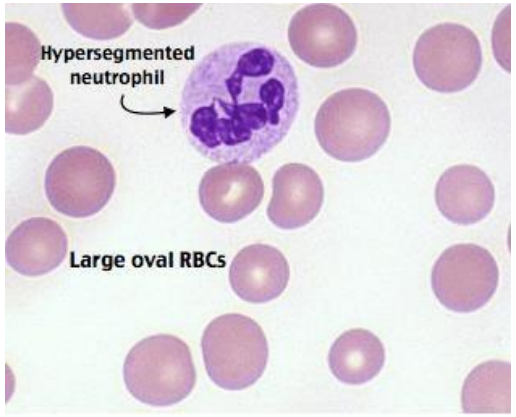
2 Increases phagocytosis of RBCs and reduces the release of the iron from the phagocytosed cells as Ferroportin is reduced.



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.

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



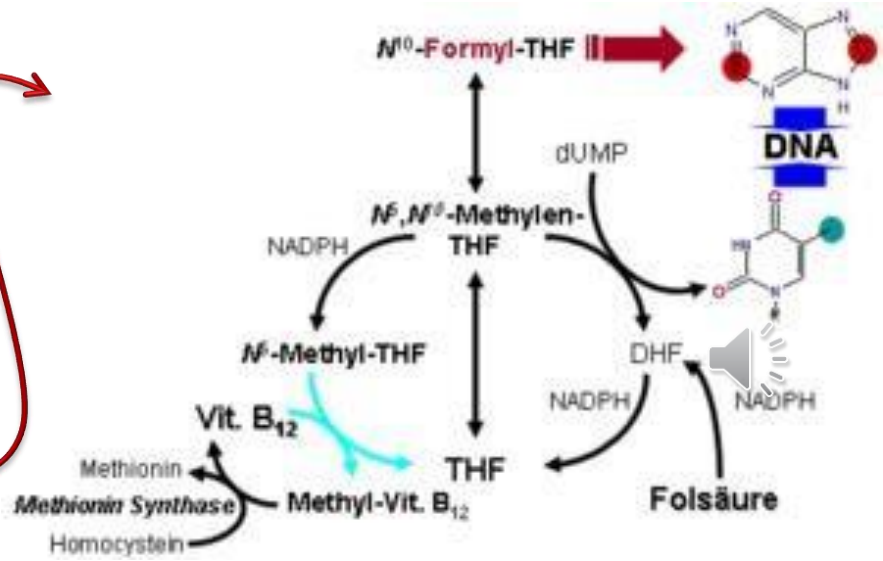
NUTRITIONAL ANEMIAS

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 not regenerated

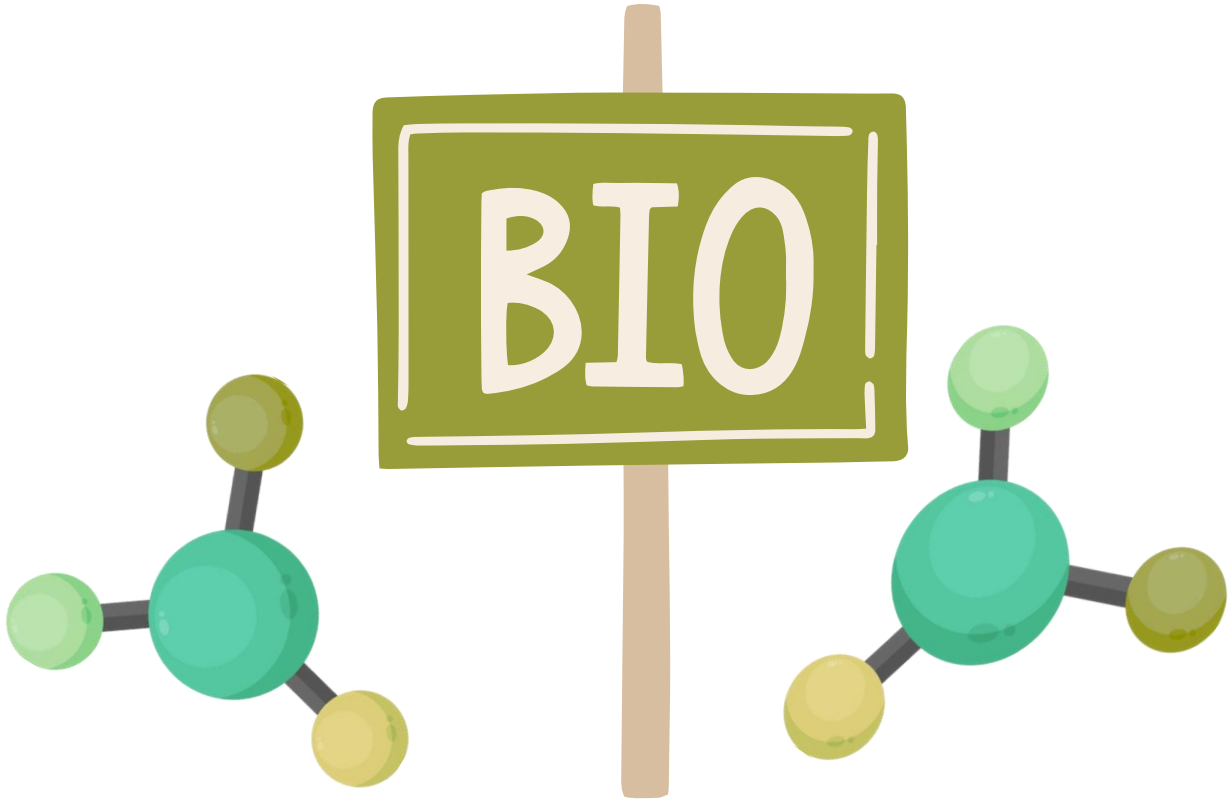


Other issues associated with chronic inflammation

- Inflammatory cytokines → increased hepcidin production by hepatocytes → downregulation of ferroportin expression in major iron-exporting cells such as macrophages, duodenal enterocytes, and hepatocytes → perhaps more importantly, increased iron retention within splenic macrophages and hepatocytes→ Hemosiderin.

Biochemistry Quiz #5

اللهم اجعلني عبداً صالحاً تحبه وترضى عنه وامسح على قلبي برحمة منك



For any feedback, scan the code or click on

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			