HEMATO LYMPHATIC SYSTEM

BIOCHEMISTRY

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Hello again, in this sheet we're going to talk about iron metabolism and some iron related diseases.

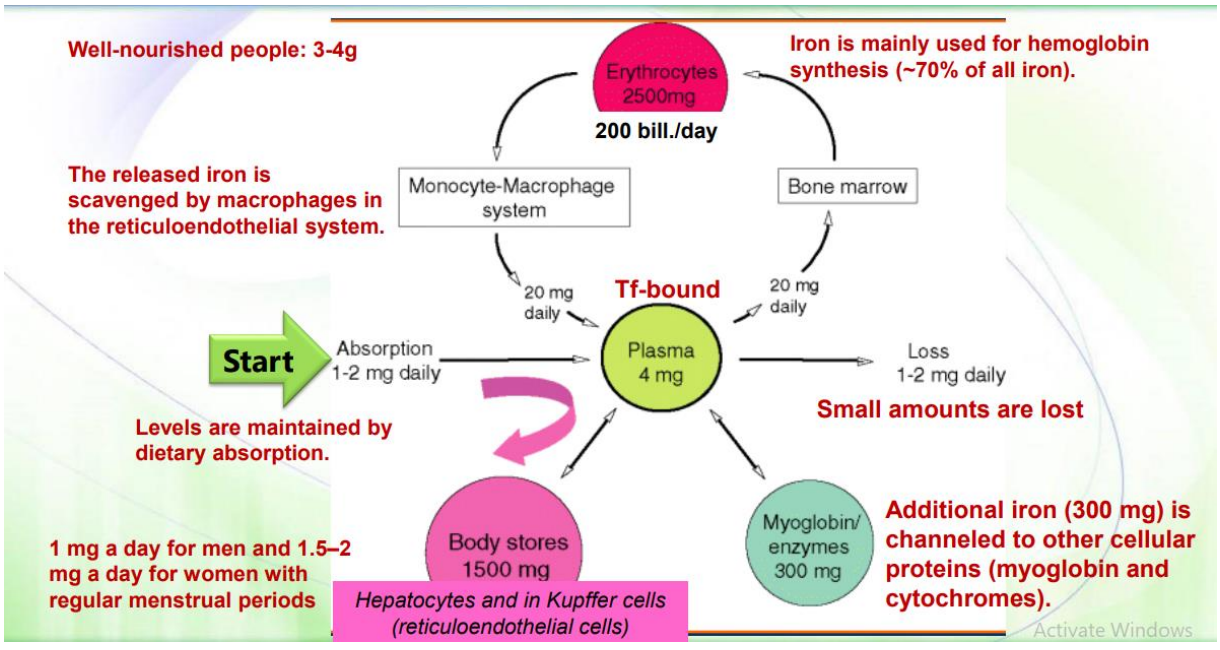
Iron metabolism: as we all know, iron extinct in two oxidation states: ferric (Fe^{3+}), ferrous (Fe^{2+}).

- **Iron has great importance for our body:**

- ♣ It's a prosthetic group of several enzymes such as redox cytochromes and the P450 class of detoxifying cytochromes.
- ♣ Iron can be potentially toxic due to its ability to form radicals which causes tissue damage, so that we can't find iron as a free molecule, its always combined with other molecules.

What is the life cycle of iron in our bodies?

- ♣ Normal people have 3-4 grams of iron in their bodies.
- ♣ 1-2 mg of iron are absorbed daily and its equal to the daily iron loss. (Women need more Iron than men and that is due to blood loss during menstrual cycle).
- ♣ most of the Iron is used for HB synthesis (about 70%, which is equal to 2500mg of Iron).
- ♣ A lot of RBCs die daily releasing their content of haemoglobin, then Iron is sequestered by cells of the reticuloendothelial system, specifically, macrophages.
- ♣ part of the released Iron will immediately bind to transferrin in plasma (4mg).
- ♣ Most of the released Iron will be recycled by cells of the bone marrow to produce RBC's.
- ♣ Some Iron is stored in hepatocytes and Kupffer cells (reticuloendothelial cells) as a body store (1500 mg).
- ♣ About 300mg of Iron is used in myoglobin and cytochromes synthesis.



Iron absorption:

=> under neutral or alkaline PH conditions (duodenum): iron found in ferric state Fe^{3+}

=> under acidic PH conditions (stomach): iron found in the ferrous state Fe^{2+}.

For absorption iron must be in the ferrous state Fe^{2+}

Fe^{3+} reductase → Fe^{2+}

- **Steps of iron absorption:**

1. **First non-heme iron:**

   As Iron reaches the small intestine, it gets converted from the ferrous state to the ferric state (because the small intestine is alkaline). In order to get absorbed, it must be converted back to the ferrous state. This is done by a ferric reductase enzyme on the enterocytes brush border, DCYTB (duodenal cytochrome B), which reduces Fe^{3+} to Fe2+. After that, Divalent metal transporter 1 (DMT1) transports Iron into the cell.

   Note: DMT-1 can transport other metal ions such as zinc, copper, cobalt, manganese, cadmium, and lead.

   So, from where we got this electron to reduce Fe^{3+} to Fe^{2+}?

   1. It comes from vitamin C so, it extracts an electron from vitamin C in the cytosol and this electron then can move to the outward portion of the enzyme and iron can then be reduced and that's why vitamin C is considered an important supplement for iron metabolism or absorption.
   2. From heme itself.

2. **Second heme iron:**

   Heme comes mainly from eating meat so, heme can be taken up by anthracites through a transporter or a carrier protein known as heme carrier protein.

   Once heme gets inside the cell, an enzyme known as heme oxygenase would release Iron inside the cell,

   Note: heme oxygenase is also found in other cells such as macrophages. It releases Iron from heme.

3. **Fates of iron:**

   1. Storage: Iron can be stored by binding to ferritin.

- Each ferritin complex can store about 4500 Iron (Fe3+) ions.
- If Iron is stored in the intestinal cells (bound to ferritin), it would be lost as these cells keep shedding off from the tip of the villus into feces, so iron is eliminated from the body (this is called mucosal block).

Note:
Iron to be metabolized → Fe^{2+}
Iron to be stored → Fe^{3-}

In order to take an advantage from iron, it has to be transported rather being stored in the intestinal cells.

2. Transport:

- Iron is transported out of the intestinal cells via a basolateral transporter (channel) known as ferroprotein.
- Once Iron leaves intestinal cells, it must be oxidized back to the ferric state (Fe3+), and that is done by an enzyme known as ferroxidase (aka iron oxidase or hephaestin).
- A similar enzyme can be found in the plasma protein ceruloplasmin to produce ferric iron (Fe3+).
- After that 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).

Intestine-related iron metabolism disorders:

so, there are conditions that result in abnormally low level of iron in the body and these conditions are related to the intestine itself, and these conditions are:

1. Iron Malabsorption:
   There is a problem in absorbing iron which car result from:
   - Gastrectomy (total or partial)
   - Celiac disease (villous atrophy)
   - Crohn's disease
   - Helicobacter pylori
2. Intestinal haemorrhage (gastrointestinal-mediated iron loss):
   - Gastric cancer
   - Ulcers: mainly caused by helicobacter pylori
   - Inflammatory bowel disease
   - Hookworm infection

Prosperities of transferrin:

Apo-transferrin can bind several metals, but ferric, not ferrous, Iron has highest affinity forming ferro-transferrin. (When Iron is bound covalently to transferrin it's a holoprotein, and when it's not bound to Iron it's an apoprotein).

- Each Transferrin molecule contains two Iron binding sites.
- 1/9 of the transferrin molecules have Iron bound at both sites.
- 4/9 of the transferrin molecules have Iron bound at one site
- 4/9 of the transferrin molecules have no Iron bound.

When iron exceeds the normal level → all transferrin molecules become saturated -→ as a result, iron can't bind to transferrin and this free iron is called non-transferrin-bound iron (NTBI).

- Cells in need of iron display a receptor on the cell surface and it's known as transferrin receptor, specifically transferrin receptor I.
- This transferrin receptor can bind to two iron atoms (diferric transferrin)
- The affinity of the receptor to diferric transferrin is higher than monoferric transferrin
- Mono ferric transferrin has higher affinity than apotransferrin.

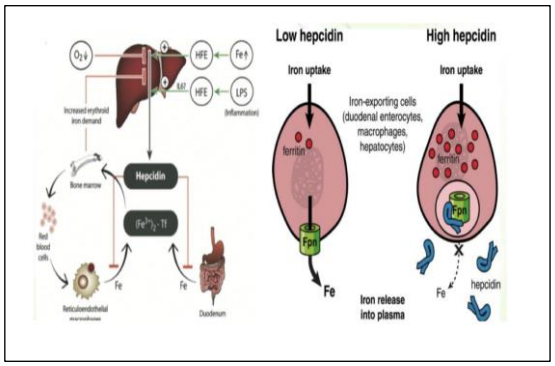
After binding to transferrin, how its transported to tissues?

- Once the transferrin binds to receptor, it will activate receptor mediated endocytosis → invagination (formation of a vesicle) → transforms to early endosomal vesicle →which transforms to late endosomal vesicle.
- The late endosomal vesicle has lower PH than the early one, this acidity allow iron to be released from the transferrin receptor.
- Once iron is released it gets reduced by a reductase called STEEP3.
- Then, iron can be transported out of the late endosomal vesicles into the cytosol and can be stored into ferritin.
- Finally, receptor-transferrin complex recycled to the plasma membrane to bind with another transferrin molecule.

Regulation of protein function

Hepcidin (the main iron sensor and regulator**)**:

- Hepcidin is a peptide hormone composed of 25 amino acids, secreted from liver
- It regulates iron level:
    1. When iron level increases and in case of inflammation, hepcidin secretion increases —> reduction of iron absorption
    2. When iron levels are low, hepcidin release is suppressed —> iron absorption increases.



But what does hepcidin exactly do?

Hepcidin inhibits iron absorption by two ways:

1. **By regulating ferroprotein:**
   High levels of Iron —> increased release of hepcidin —> Hepcidin binds to ferroportin and induces internalization of ferroportin inside cells into lysosome, then ferroportin will be degraded inside these lysosomes—> the amount of ferroportin that exists in the basolateral membrane of intestinal cells will be reduced —> so Iron will be stored inside intestinal cells and will be lost as these cells shed.
2. **By regulating DMT1:**
   Hepcidin inhibits the presentation of the Iron transporters (e.g., DMT1) in intestinal membranes —> Iron absorption is decreased by intestinal cells.

Remember that DMT1 exist in the apical portion of intestinal cells and it's responsible for absorbing Iron into intestinal cells.

Regulation of hepcidin:

It's regulated by 4 mechanisms:

The 1st mechanism:

We said that transferrin binds to a receptor **Transferrin receptor (TFR),** in fact there are 2 receptors, **Transferrin receptor-1 (TFR-1) and Transferrin receptor-2 (TFR-2)**

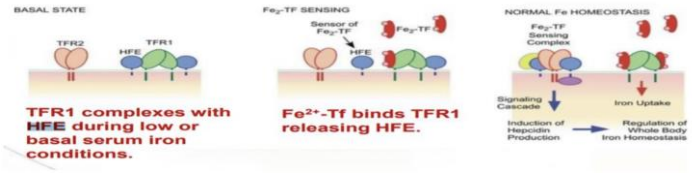
NOW we have 2 scenarios:

1. When there is no transferrin bound to Iron (BASAL STATE):

In the basal state (when there is no Iron bound to Transferrin), the transferrin Receptor-1 (TFR1) is bound to a protein known as HFE (HFE stands for hemochromatosis), and Transferrin Receptor-2 (TR2) is not bound to anything.

2. When there is transferrin bound to Iron (Fe2-TF Sensing):

transferrin (Fe2-TF) binds to TFR1-HFE complex, inducing the dissociation of HFE from TFR1, HFE protein will then bind to Transferrin Receptor-2 (TFR2), this induces a signal transduction cascade that results in the stimulation of hepcidin synthesis, resulting in a decreased iron absorption.



The 2nd mechanism

- This mechanism is mediated by a hormone called bone morphogenic protein 6 (BMP 6). The only function known for BMP-6 is the regulation of iron in the body.
- BMP-6 binds to a receptor on the surface of hepatocytes called BMP-6 Receptor (BMPR), inducing signal transduction that results in the production of hepcidin.
- This receptor activates a signal transduction pathway mediated by transcriptional regulatory protein called SAMD —> inducing the expression of hepcidin.
- The BMPR is associated with a protein known as Hemojuvelin (HJV), this protein is important for the function of BMPR and the stimulation the production of hepcidin. So, if HJV protein is mutated (not functional), this results in a decreased production of hepcidin.
- BMPR is degraded by a protease known as TMPRSS6, this protein is important for the entry of Corona virus to our cells.

The 3rd mechanism:

This mechanism is regulated by IL-6.

Remember: inflammation induces the production of Hepcidin by IL-6.

IL-6 binds to a receptor on hepatocytes (IL-6 receptor)—> that induces a signal transduction —> increased production of hepcidin from hepatocytes.

It's a protective mechanism in our body against pathogens. How??

Many pathogens rely on Iron (remember, iron is needed for growth and replication of pathogens), so our body tries to protect itself through decreasing the availability of iron by increasing the production of hepcidin which results in a decreased iron absorption.

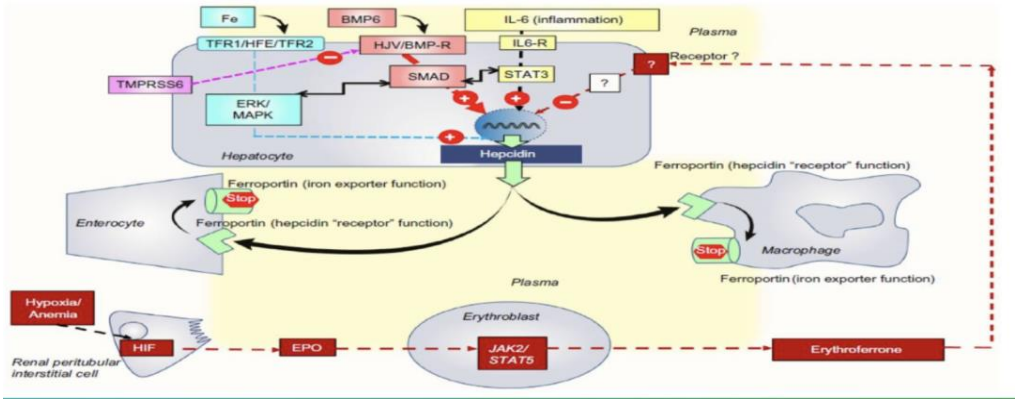
The 4th mechanism: hormonally mediated

• This mechanism is mediated by hypoxia and anemia.

• Hypoxia stimulates the production of erythropoietin from the kidneys.

• Erythropoietin stimulates the production of another hormone from erythroblast cells known as erythroferrone.

• Erythroferrone will bind to an unknown receptor on hepatocytes surface, this induces signal transduction to BLOCK (inhibit) the production of hepcidin, because we need iron to produce more and more hemoglobin to overcome the effect of hypoxia.



Post-transcriptional regulation of expression:

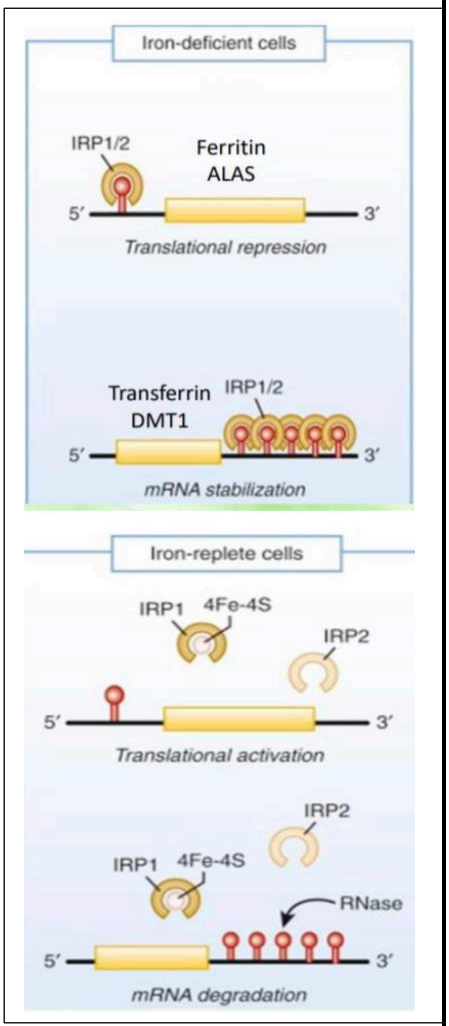
• The proteins critical for iron metabolism (e.g., ferritin & transferrin) have regions called **iron response elements (IRE)** in their mRNA molecule, these elements are the sites where **iron-responsive element-binding proteins (IRP-1/2)** can bind to. We have 2 possible positions of iron response elements at the mRNA, one at the 5' UTR for the regulation of **ferritin** (in macrophages) and **ALAS** (ALA synthase) translation, the other one is located at the 3' UTR for the regulation of **transferrin** and **DMT1** translation.

↳ untranslated regions

1. **Low iron state:**

A. IRP binds to the iron response element (IRE) on 5' UTR, repressing the translation and production of ferritin and ALAS. There is no iron, so, no need to produce storage sites for iron (ferritin).

B. IRP binds the IRE on the 3' UTR to stabilize the mRNA and increase the production of transferrin and DMT1.



2. High iron state:

A. Iron prevents the IRP from binding to the IRE on the 5' UTR, which leads to the production of ferritin and ALAS, we remove the block.

B. Iron prevents the IRP from binding to the IRE on the 3' UTR, which leads to the destabilization and degradation of the mRNA, therefore transferrin and DMT1 are not expressed, no need to absorb and transport excess iron because iron is toxic.

JUST A FEW SMALL PAGES LEFT TO STUDY, refill your coffee and let's continue

➢ IRON RELATED DISEASES:

1. Hemochromatosis (HC):

- It 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 result in tissue damage and organ failure.
- **HFE** is a major histocompatibility complex (**MHC**) class-1 gene.
- **HC** is more common in males than females because females lose iron in menstruation and childbirth.

➢ **The classes of hereditary hemochromatosis:**

autosomal recessive

1. Type1 (hemochromatosis protein, HFE-dependent) Most common
2. Type2A [HFE2 (HJV) dependent]
3. Type2B (hepcidin, HAMP-dependent)
4. Type3 (TfR2-, TfR2-dependent)
5. Type4 (ferroprotein independent) → following autosomal domenant pattern

- The normal total body iron stores may range from **2 to 6 gm**, but persons with **HC** have much greater stores, they **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**.
- Excess hemosiderin leads to cellular dysfunction and damage.

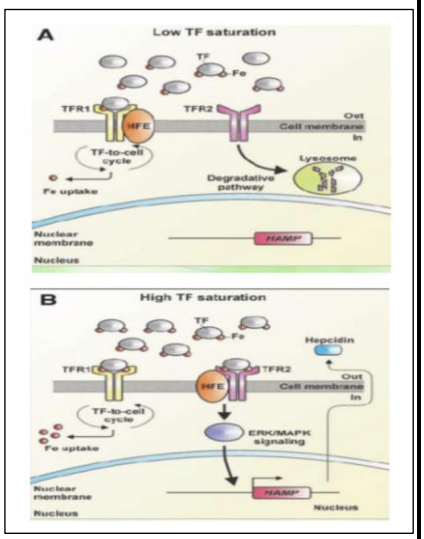
➢ **Affected organs and conditions:**

1. Liver (hepatic fibrosis)
2. Pancreas (diabetes mellitus)
3. Joints (arthropathy)
4. Skin (pigmentation)
5. Heart (cardiomyopathy)
6. Gonadotrophin-secreting cells (hypo-gonadotrophic hypogonadism)

➢ NORMALLY:

• At low iron concentration (low TF saturation):
TFR1 is bound to HFE protein, there's little or no iron in the system, so transferrin cannot bind to TFR1, as a result, the iron uptake is so minimal.

• At high iron concentration (high TF saturation):
Transferrin will be saturated, it binds to TFR1 releasing HFE, which then binds to TFR2 inducing signal transduction, this increases the production of hepcidin.



Now we are going to talk in detail about **type 1** and **type 2A:**

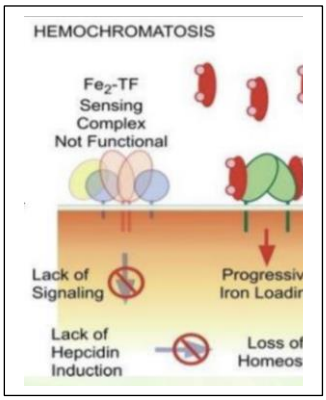
❖ Type 1 (HFE-dependent)

•The primary cause of hemochromatosis.

• Mutation or absence of HFE (e.g.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 signal transduction, hepcidin won't be produced.

• This results in continuous entry of iron into the system.



❖ Type 2A (Juvenile hemochromatosis or HFE2 (HJV)-dependent HC):

• It's a very rare, severe juvenile form of hemochromatosis that is due to a homozygous deletion of the gene for hepcidin.

• NORMALLY: HJV protein binds to and regulates the function of BMPR, so HJV protein up-regulates the expression of hepcidin.

• Mutations in HJV gene, which encodes the protein "hemojuvelin", result in a defective **BMPR** —> decreases the ability of hepatocytes to produce hepcidin —> excessive absorption of iron accounts for the majority of JH.

2. Anemia:

Anemias are characterised 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.

• Folic acid is important to produce nucleotides for DNA replication. Its deficiency means cells can't replicate, so they become larger.

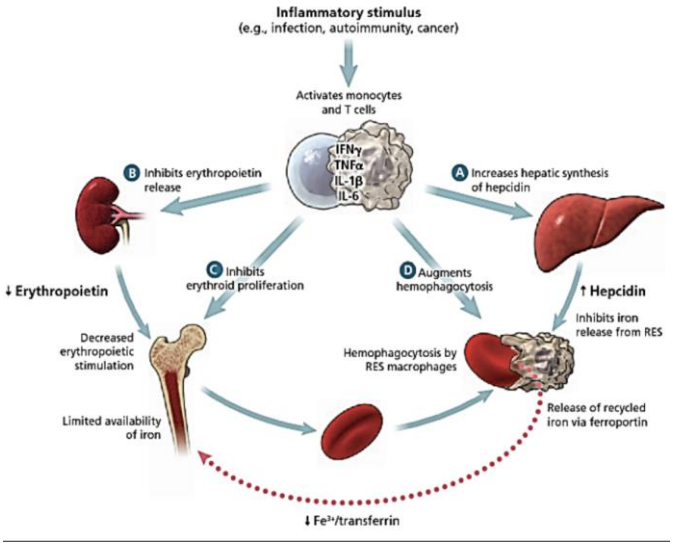
• Vitamin B12 is necessary for folate's regeneration.

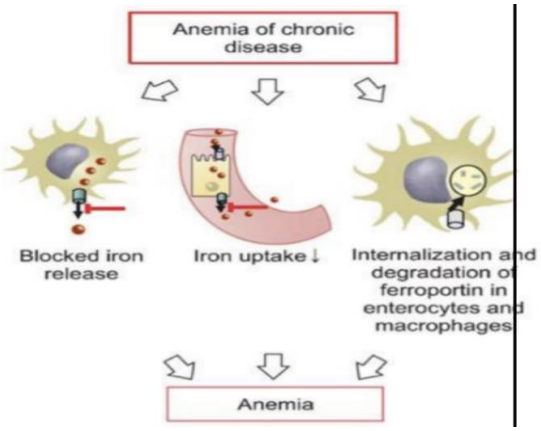
• Folic acid and/or vitamin B12 deficiency causes inability to synthesise DNA and, as a result, the cells cannot divide and megaloblasts accumulate.

Important note: we said that anemia is common in developing countries, but it also common in industrialise countries like US, Europe.

❖ **Anemia of Chronic diseases:**

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





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