Iron, sideropenic anemia, iron overload

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Human body contains 3-4 g of iron

Hemoglobin contains about **2.5** grams of iron

Major part of body iron is used for **hemoglobin** synthesis

1 liter of blood = 0.5 gram of iron

Iron has an unique regulation of its metabolism, which is **different from all other biometals**

- **There is no special pathway for iron excretion**

  - **Iron excretion cannot be regulated**

- **Iron balance is determined solely at the level of iron uptake from the diet**

Iron excretion is not regulated, but, at a small scale, it occurs:

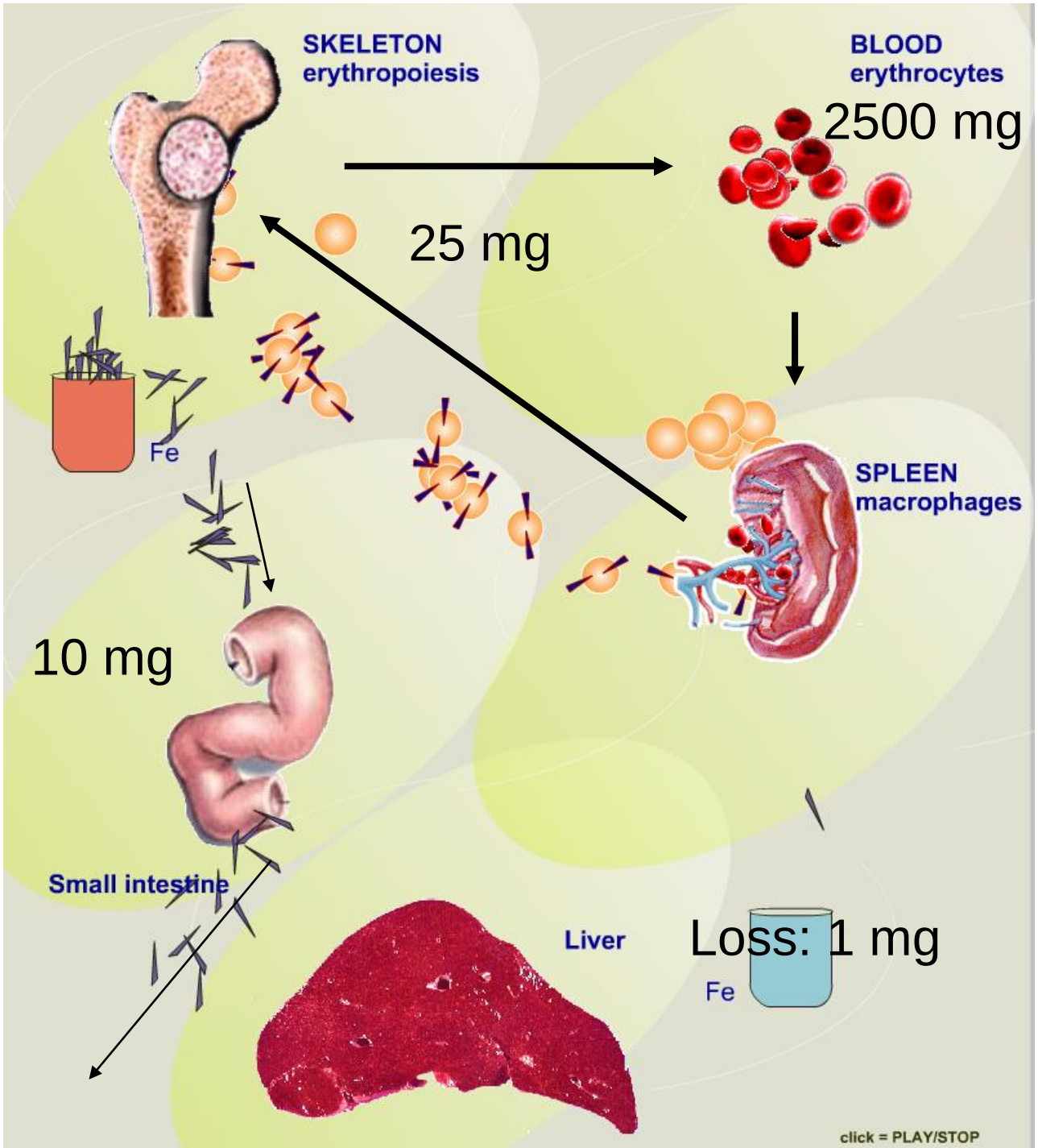
- 1 mg of iron is lost daily from the body by *nonspecific* pathways (loss of small quantities of blood and loss of dead cells)

- In women, additional ~30 mg of iron is lost monthly by menstruation – iron stores are generally larger in men than in women

Gender has a major effect on iron homeostasis:

- Males tend to accumulate iron during their lifetime
- Females have generally low iron stores

Iron metabolism:


SKELETON
erythropoiesis
BLOOD
erythrocytes
2500 mg
25 mg
Fe
SPLEEN
macrophages
10 mg
Small intestine
Liver
Loss: 1 mg
Fe
click = PLAY/STOP

Key organs participating in iron metabolism:

- **Bone marrow**: Production of new erythrocytes
- **Spleen**: Degradation of senescent erythrocytes
- **Small intestine**: Iron uptake from the diet
- **Liver**: Storage of excess iron, control of iron metabolism

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- The two faces of iron:
- Iron is necessary for life
- Excess iron leads to organ damage by the formation of free radicals

Consequences of iron overload:

Ferrous (2+) iron:dangerous if free, **forms free radicals**

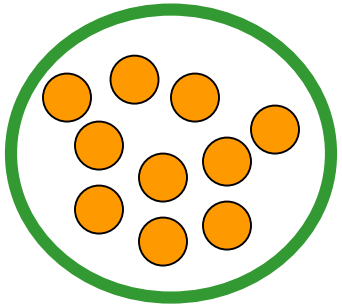
Iron overload leads to organ damage

Since free iron is toxic, it must be always bound to proteins

Iron-containing proteins

- Ferritin - iron storage protein
- Transferrin: iron transport protein
- Ribonucleotide Reductase: important in DNA synthesis

Ferritin: iron storage protein
In healthy men, it stores approximately 1 gram of iron in the liver



Vilém Laufberger, 1937: Sur la cristallisation de la **ferritine**.
Bull Soc Chim Biol., 19, p.1575

Ferritin is an intracellular iron storage protein

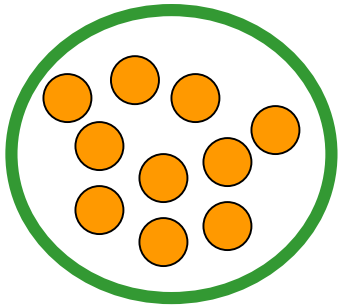
(stores iron in hepatocytes, macrophages…)

Plasma (serum) ferritin

- **Reflects the amount of body iron stores**
- Normal range: about 100 µg/litre
- 10 µg/litre and less: insufficient iron stores
- 1000 µg/litre and more: severe iron overload
- **Plasma ferritin has no role at all in iron metabolism!**

Transferrin

- Transports iron in the blood
- Contains only 2 atoms of iron
- Transferrin is the only source of iron for hemoglobin



Ferritin

Inside the cell,
stores iron

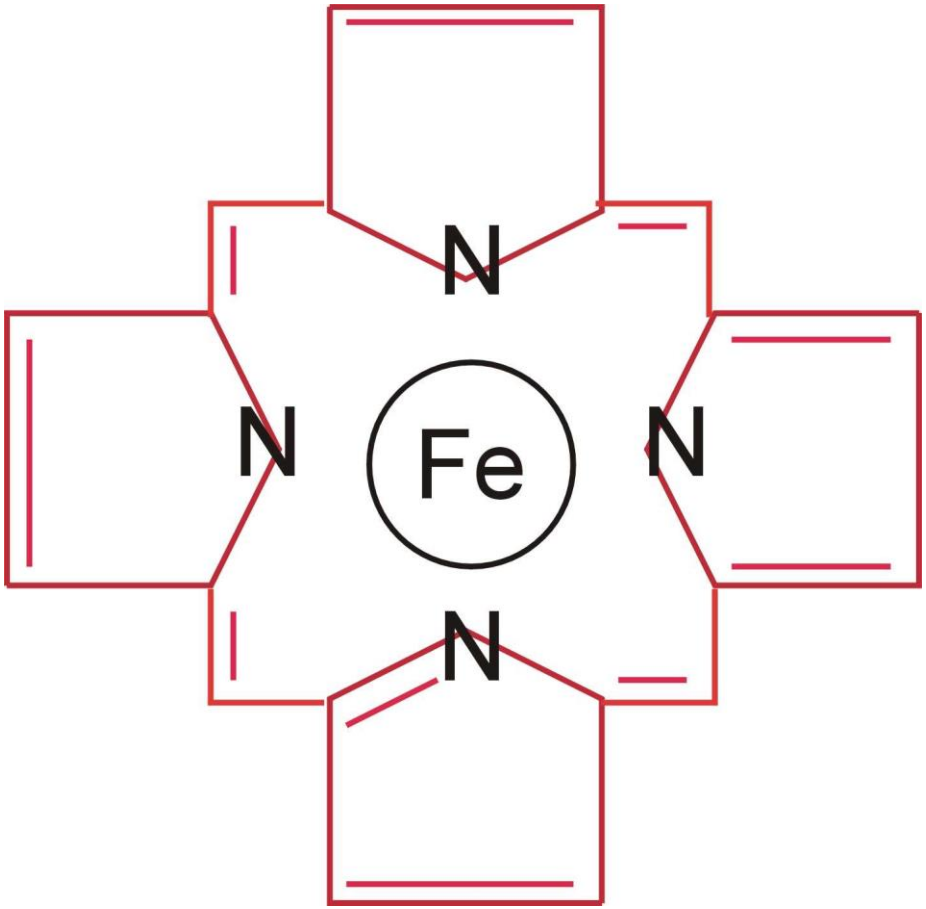
Transferrin

In plasma,
transports iron

- Both Ferritin (iron storage protein) and Transferrin (iron transport protein) contain iron in the form of simple inorganic ferric iron

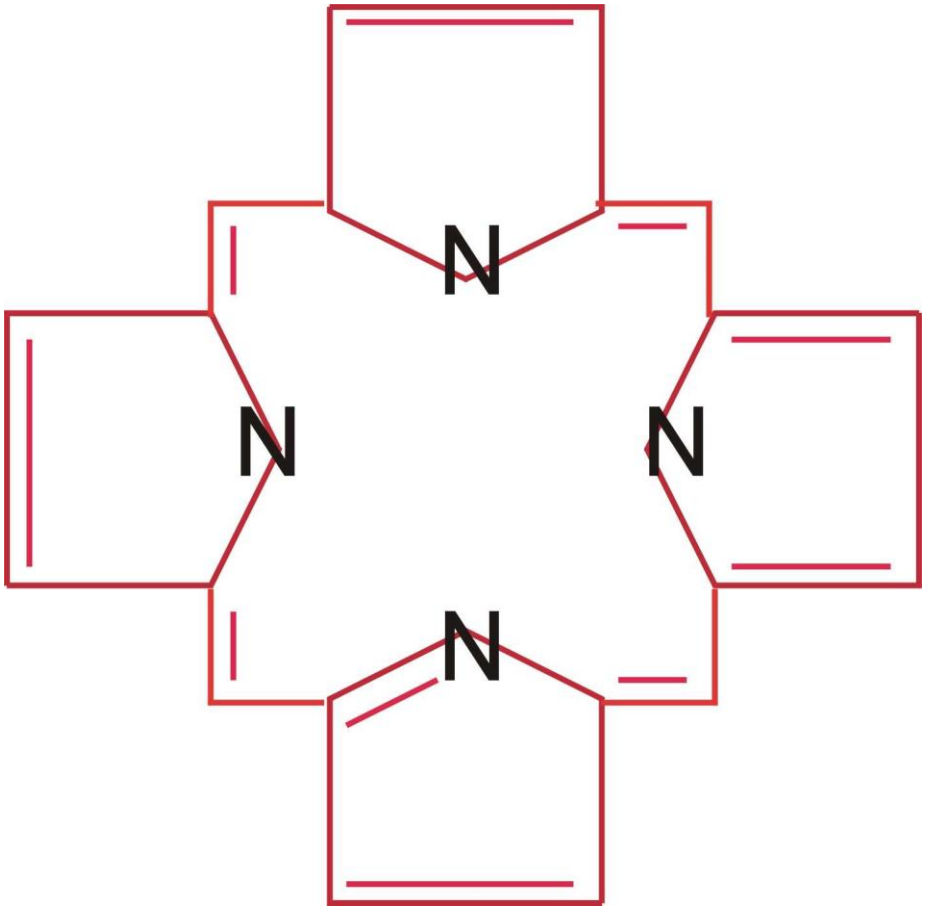
- Many other proteins contain iron in the form of **Heme**

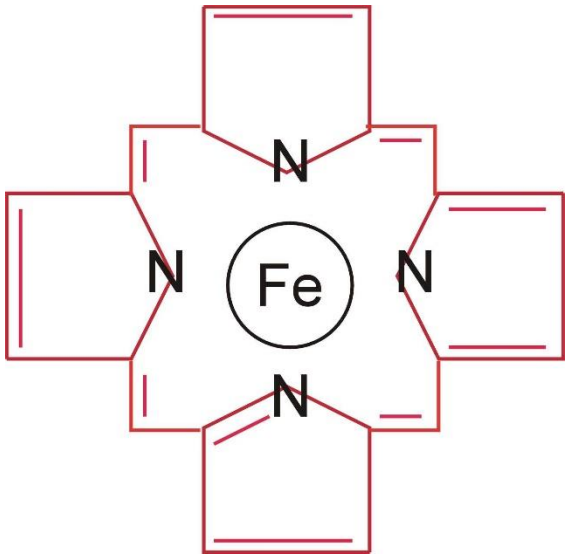
Hemoproteins: contain iron in the form of **heme**



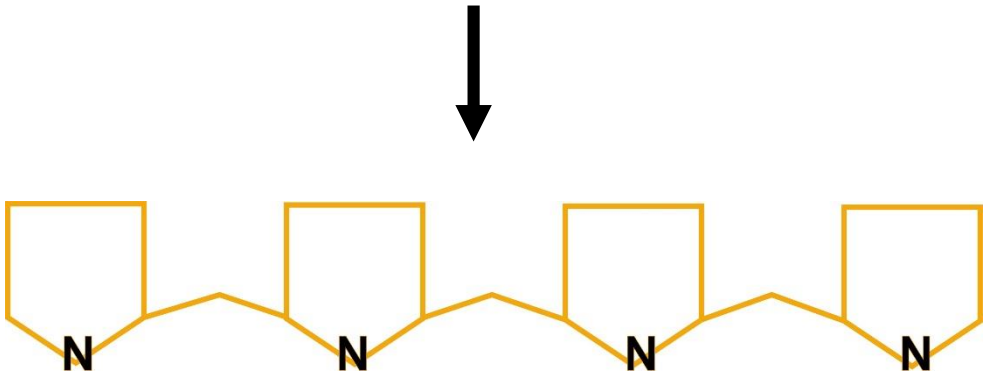
Heme: iron inserted in a tetrapyrrole ring

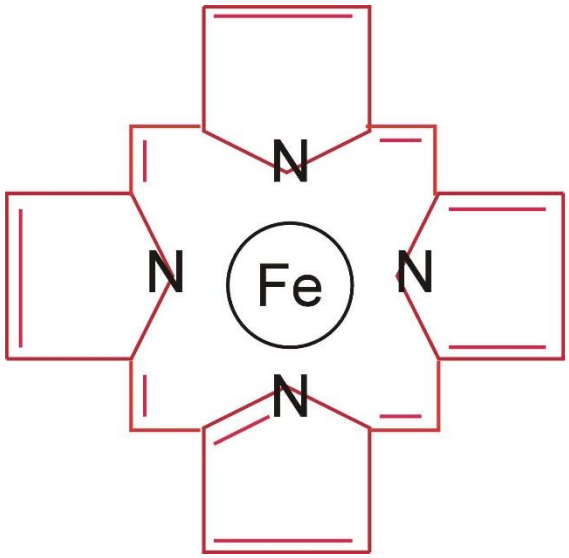
Porhyrin: a tetrapyrrole ring with conjugated double bonds



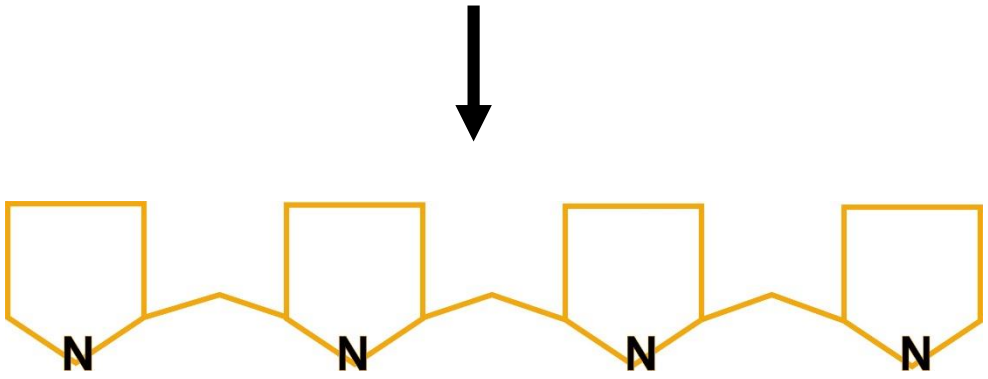


Heme





Heme



Bilirubin

Heme: iron + porphyrin
Heme-containing proteins: *Hemoproteins*

- Hemoglobin
- Myoglobin
- Mitochondrial Cytochromes
- Cytochrome P450

Disorders of iron metabolism

1) Decreased amount of iron in the body:

IRON DEFICIENCY ANEMIA

2) Increased amount of iron in the body: typically in

HEMOCHROMATOSIS

Iron deficiency anemia

most common anemia worldwide, one of the most common diseases worldwide

- Menstruation, pregnancy and birth deplete iron stores, iron deficiency anemia is therefore more common in women than in men

- Causes: **Chronic bleeding**
  - Lack of iron in the diet

- **If iron deficiency anemia is encountered, the patient should always be checked for chronic blood loss**

Iron deficiency anemia:

- Anemia: mild to moderate (approx 100 g Hb/l).
- **WHO Cutoff: 130 g/litre men, 120 g/litre women**

- Microcytosis (~ 75 fl)
- **range 80-100 fl**

- Hypochromia (MCH ~ 230 g/l, range 280-340)

- Decreased RBC (~ 4 mil/µl, range 4.2 - 5.7)

- Decreased reticulocytes (should be increased!)

- Iron Deficiency Anemia: **Hypoproliferative, Microcytic Hypochromic Anemia**

Iron deficiency anemia:

1) More common in females than in males

2) Always check for chronic blood loss!

Hereditary Hemochromatosis

Characterised by excessive absorption of iron from the gut

Since excess iron can not be excreted, the result is

iron overload

Consequence of hemochromatosis:

- Iron accumulates in hepatocytes (more than 10 g of iron in the liver, healthy man about 1 g)
- Free radicals damage hepatocytes, resulting in liver cirrhosis and hepatocellular carcinoma

Pathophysiology of **hereditary hemochromatosis:**

Mutation of genes which control iron uptake from the gut.

- 1996: Identification of the *HFE* gene

- *HFE* hemochromatosis:
- autosomal recessive mode of inheritance

- ***HFE* mutation: Most common mutation of any known gene in white population**

- one person in 10 carries a *HFE* gene mutation

Theoretically: One in 400 of Czech inhabitants: homozygous for *HFE* mutation

Fortunately:

Low penetrance of clinically manifest hemochromatosis in homozygotes

Diagnosis of hemochromatosis:

- Typical patient: 50 year + old male
- Fatigue, joint pain, and **hepatomegaly**
- High Ferritin (~1000 µg/litre)
- Liver iron (biopsy or NMR)

Therapy of hemochromatosis:

Removal of excess iron from the body

Therapy of hemochromatosis:

Phlebotomy (removal of 0.5 l of blood once a week):

Decrease of iron in the circulation leads
to iron mobilisation from stores

Phlebotomy is a relatively simple procedure to remove excess iron from the body

Causes of iron overload:

- Primary: a mutation which increases iron absorption from the gut
- Secondary: adverse effect of :
- REPEATED TRANSFUSIONS

Secondary iron overload:

- Transfusion dependent anemias, for example

β-thalassemia major

- Excess iron **must** be removed, otherwise patients will die of heart failure caused by free radicals

- Therapy: **iron chelators**

Iron in the diet:

Best source: Heme iron. One portion of beef: 2 mg Fe

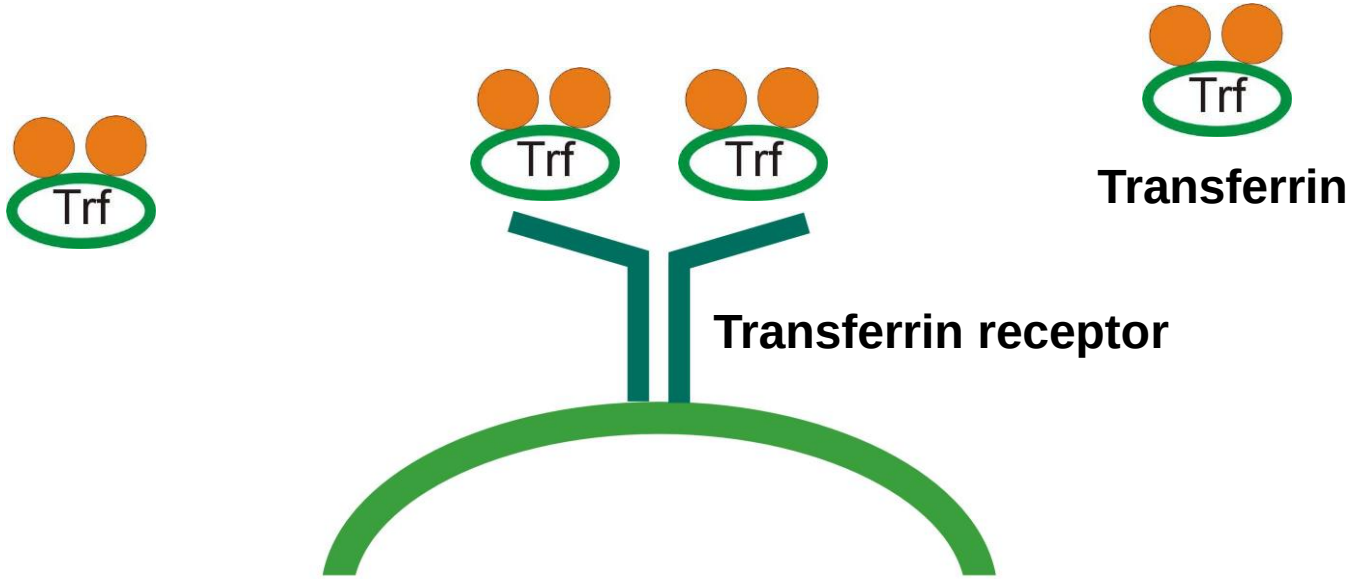
Regulation of iron metabolism

- At the whole body level: by iron UPTAKE

- At the cell level: by both uptake and EXCRETION

Transferrin uptake

Transferrin receptor



Cells which need iron express high number of **transferrin receptors** on their surface

Erythroblasts: nucleated cells present in bone marrow, extremely high concentration of transferrin receptors

Reticulocytes: no nucleus, but active hemoglobin synthesis, still relatively high concentration of transferrin receptors

Erythrocytes: no nucleus, no ribosomes,
no hemoglobin synthesis,
loss of transferrin receptors on cell surface

Transferrin receptor expression is regulated at the level of transferrin receptor mRNA stability:

Lack of iron stabilises mRNA for transferrin receptor

A nice example of **posttranscriptional** regulation of gene expression (about 1985)

Regulation of iron metabolism

Before 2000: The most studied process has been

IRON UPTAKE BY THE CELL

New (2001) look at iron metabolism:

Iron metabolism is regulated mainly at the level of

IRON EXPORT FROM THE CELL

Iron is transported from the cell by

FERROPORTIN

a relatively recently (2001) discovered iron export protein

Which cells must be able to export iron?

- Macrophages: they must recycle about 25 mg daily from old erythrocytes
- Enterocytes (endothelial cells from the small intestine): daily uptake and export of about 1 mg of iron from the diet
- Hepatocytes: they are able to mobilise stored iron from ferritin if iron is needed

2001: Revolution in the look at iron metabolism:

Discovery of HEPCIDIN

Hepcidin: "iron regulatory hormone"

Hepcidin is produced in the liver, is transported in the blood stream, and

BLOCKS IRON EXPORT FROM THE CELL
(by degrading cell surface ferroportin)

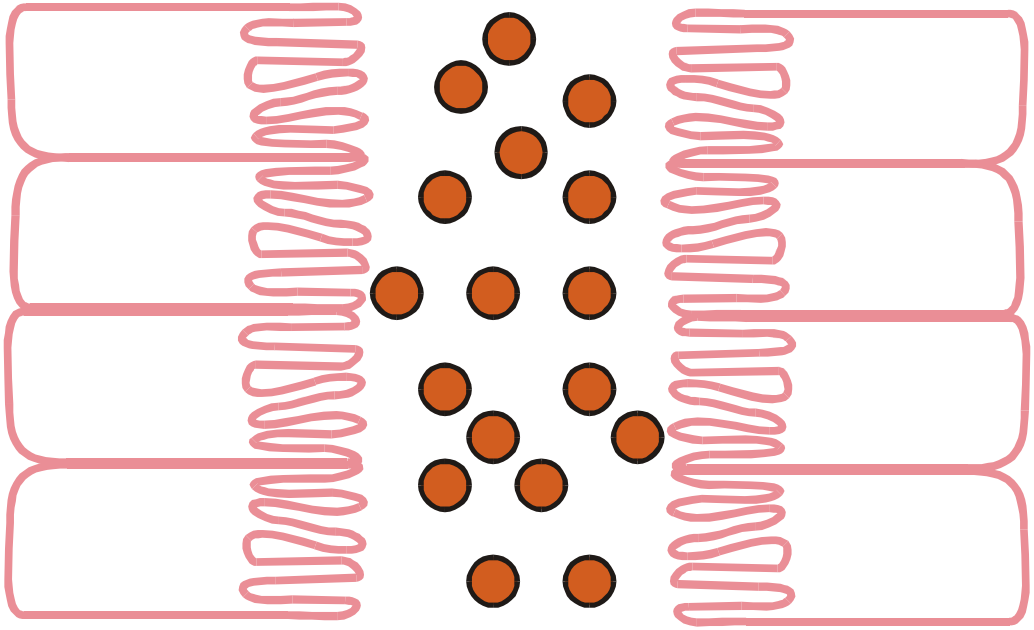
Please remember:

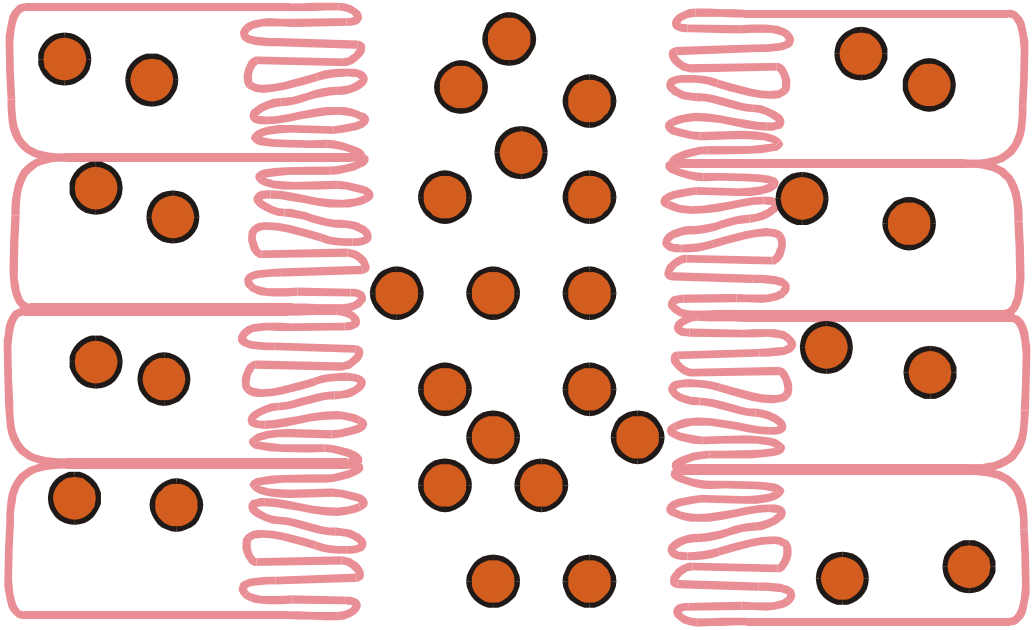
- **HEPCIDIN BLOCKS IRON EXPORT FROM THE CELL**

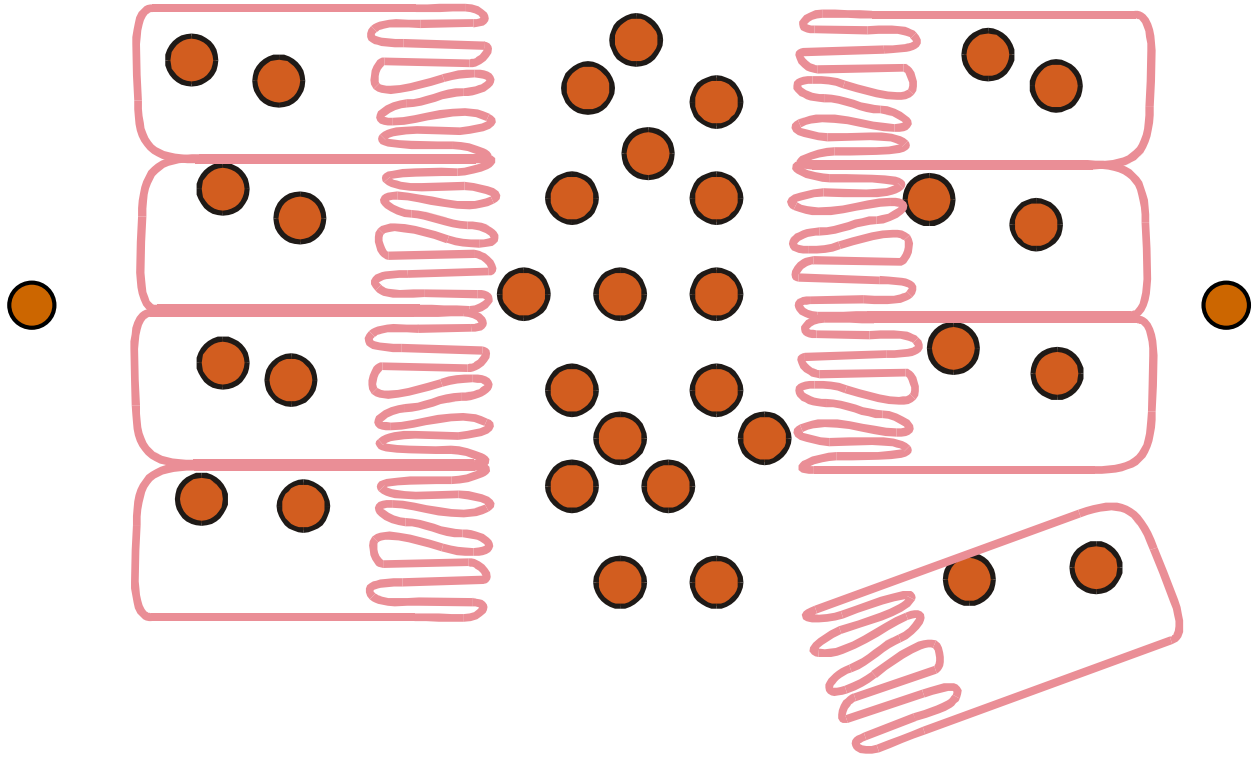
Hepcidin blocks iron export from:

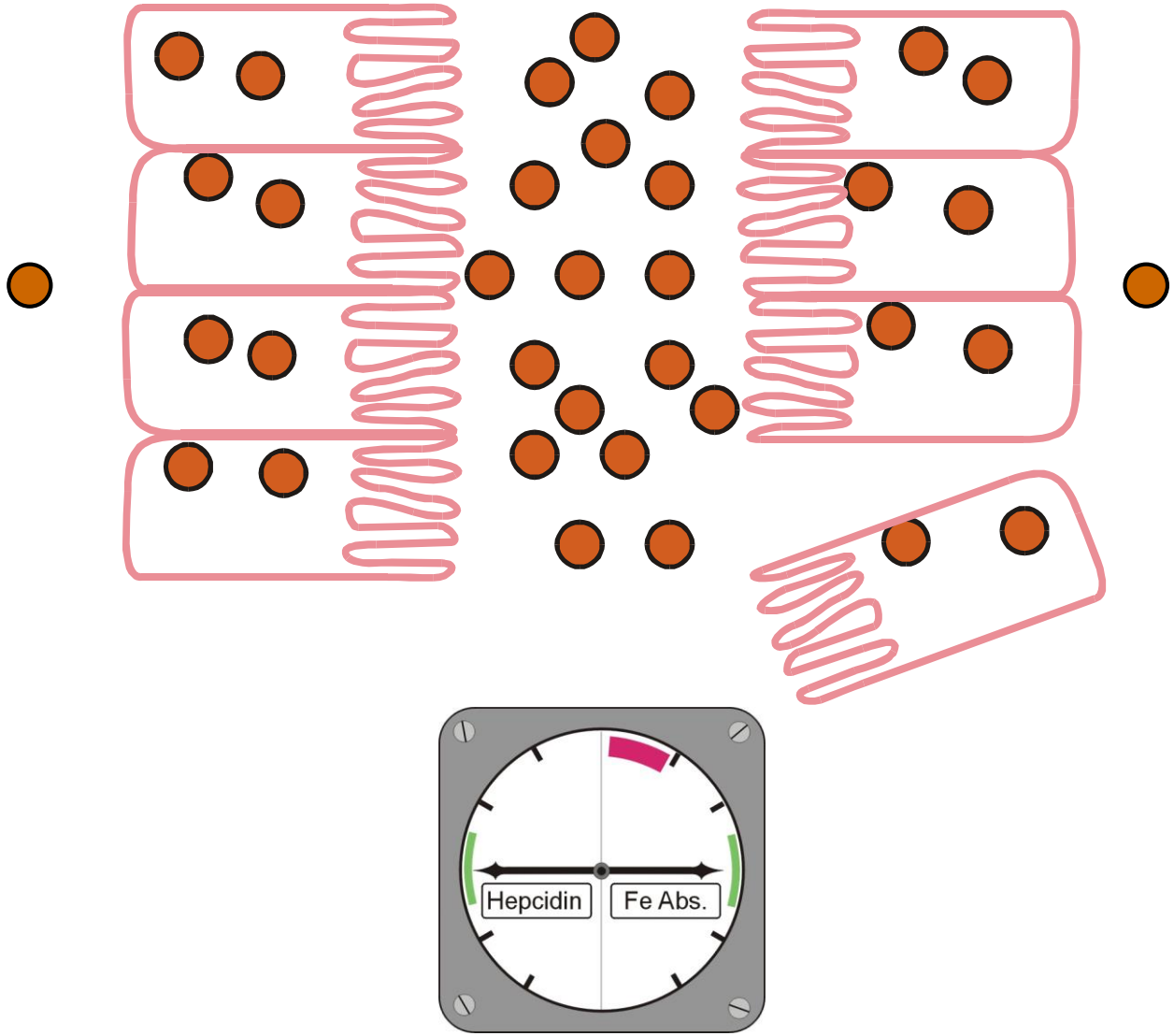
MACROPHAGES

ENTEROCYTES IN THE SMALL INTESTINE

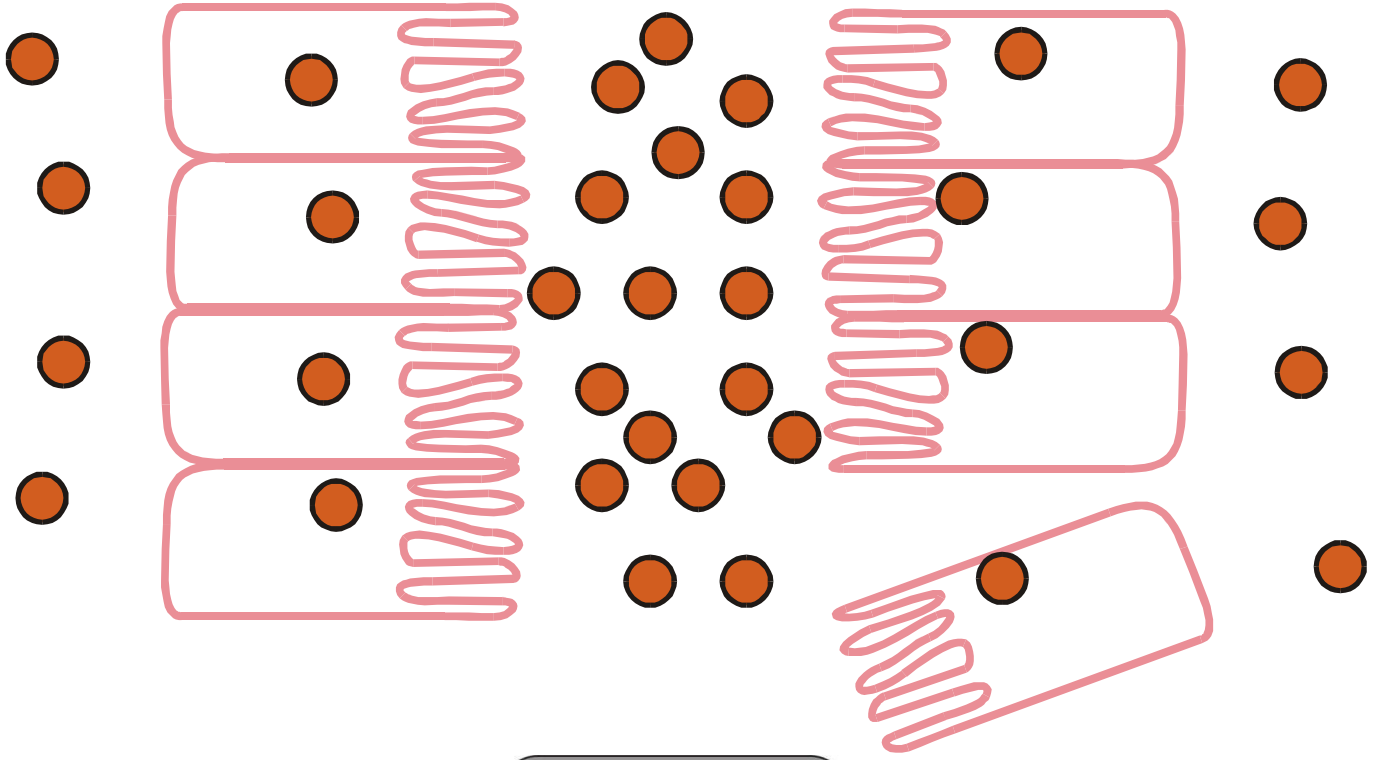


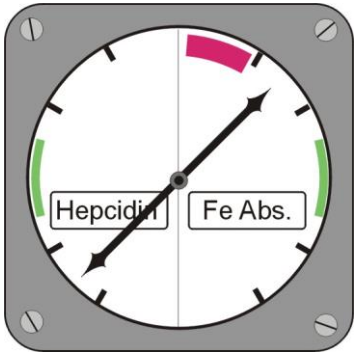




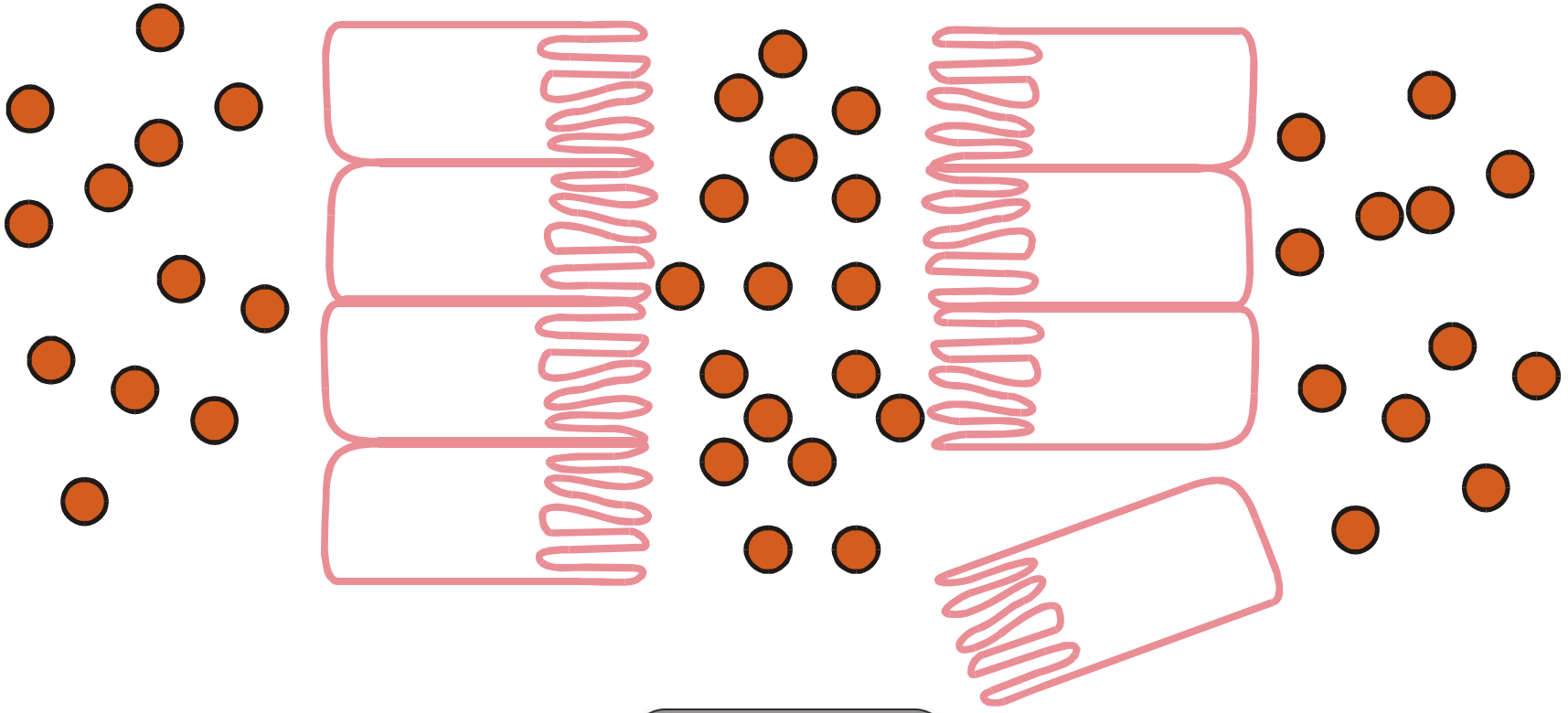

Hepcidin
Fe Abs.

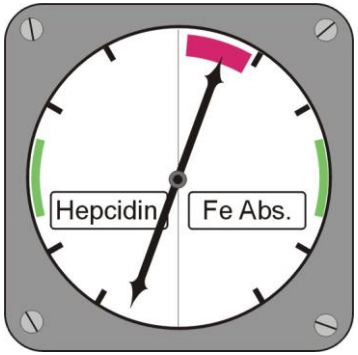
HFE hemochromatosis



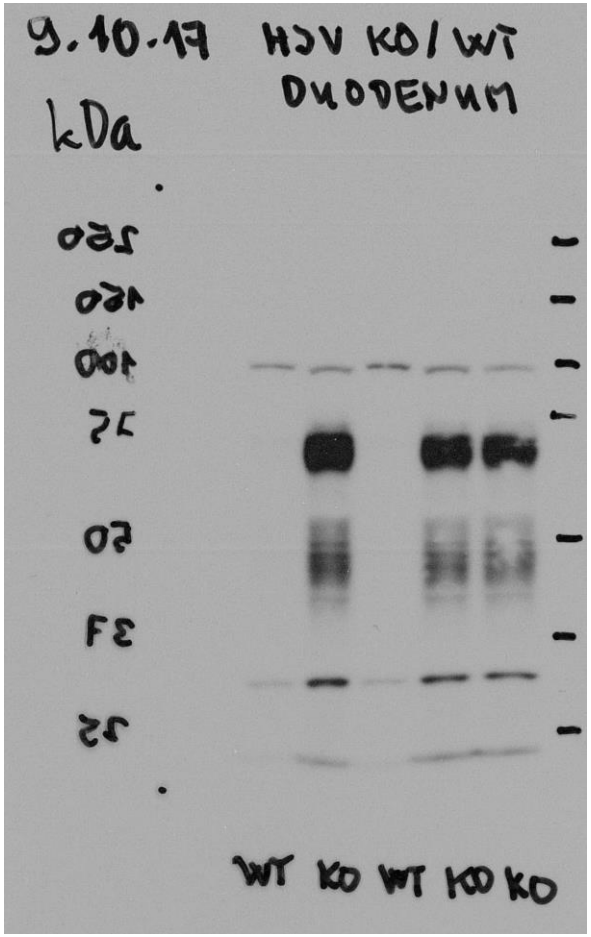


Juvenile hemochromatosis





Western blot analysis of duodenal **ferroportin** expression in control („wild type“) mice and hemojuvelin „knock out“ mice



Pathophysiology of hereditary hemochromatosis

All hereditary hemochromatosis subtypes display **decreased hepcidin levels**

Decreased hepcidin allows more iron to be exported from the enterocytes into blood

Decreased hepcidin allows more iron to enter from the gut

Proof of the hepcidin theory (2004):

Extremely severe form of hemochromatosis
(juvenile hemochromatosis)
is caused by mutation of the hepcidin gene

As of 2018:

Four genes whose mutations decrease hepcidin in humans:

HFE (1996)
TFR2 (1999)
Hepcidin (2001)
Hemojuvelin (2004)

Regulation of hepcidin expression:

- Hepcidin is produced in hepatocytes in relation to body iron balance, in small intestine it blocks iron uptake
- Iron overload increases hepcidin expression
- Iron deficiency decreases hepcidin expression
- Increased erythropoiesis decreases hepcidin expression
- **(Vokurka M, Necas E et al (2006): Hepcidin mRNA content in mouse liver is regulated by the rate of erythropoiesis)**

- Hepcidin is regulated by:
  - Iron
  - Erythropoiesis
  - Inflammation

Hepcidin expression dramatically increases during inflammation

Formally, hepcidin is an **acute phase protein**

(a protein synthesised in the liver, whose synthesis is increased during inflammation)

Hepcidin: **Hep**atic bacteri**cid**al prote**in**

Hepcidin has antibacterial properties

Hepcidin demonstrates the strong connection between

iron metabolism and defence against pathogens

Bacteria need iron for their ribonucleotide reductase
(DNA synthesis)

Host needs iron for his antibacterial enzymes
(Nitric oxide synthase and others)

Bacteria and host compete for free iron

Anemia of Chronic Disease:
(also known as **Anemia of Inflammation)**

Second most common anemia

Iron stores are normal (normal ferritin), but iron is unable to leave macrophages and transit to the bone marrow (low transferrin saturation) :

A problem with iron export from macrophages

Pathophysiology of Anemia of chronic disease

- Inflammatory mediators increase hepcidin

- Hepcidin keeps iron in macrophages

(by degrading macrophage ferroportin)

Pathophysiology of both hemochromatosis and anemia of chronic disease can be easily explained by the action of **hepcidin**.

Iron summary:

1) There is no regulated pathway for iron excretion

2) Iron metabolism is influenced by gender:
Males tend to accumulate iron, females tend to lose iron

3) The most common cause of iron deficiency is chronic blood loss

4) Iron metabolism is regulated by hepcidin

Hepcidin summary:

1) Hepcidin is released from the liver according to body iron status: iron overload increases hepcidin, iron deficiency and accelerated erythropoiesis decrease hepcidin expression.

2) Hepcidin blocks iron export from macrophages and enterocytes.

3) Hepcidin is regulated by iron, erythropoiesis and inflammation

Thank you for your attention. Questions: jkri@lf1.cuni.cz