Iron homeostasis and its disorders

From anemias to iron overload

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Disclosures

Financial Relationship (prior 12 months)	Commercial Interest
Grant/Research Support	NIH
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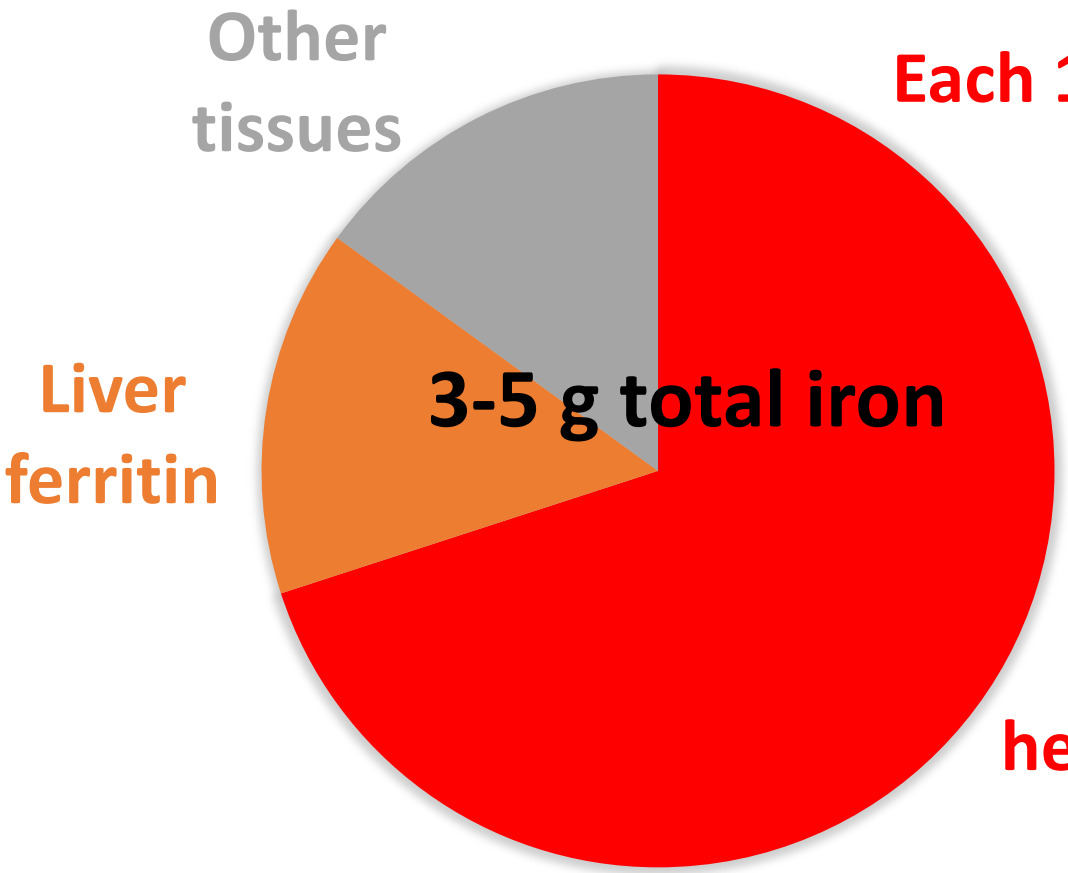
Globally, iron disorders are common

- Too little iron:
  - **Iron deficiency anemia** (IDA): 1.2 billion affected people in 2016
    - IDA is among the five greatest causes of years lived with disability
    - Concentrated among women and children in low- and middle-income countries
    - Iron supplementation is problematic because of interactions with endemic infections, chronic inflammation and complex nutrient deficiencies
  - **Anemia of inflammation/anemia of chronic disease:** iron maldistribution is a major pathogenic factor
- Too much iron:
  - **Hemochromatosis:** genotype is common (0.1-1%) in northern Europe and countries with northern European immigrants
  - **Hemoglobinopathies and other genetic anemias:** many patients develop iron overload, with or without regular transfusions

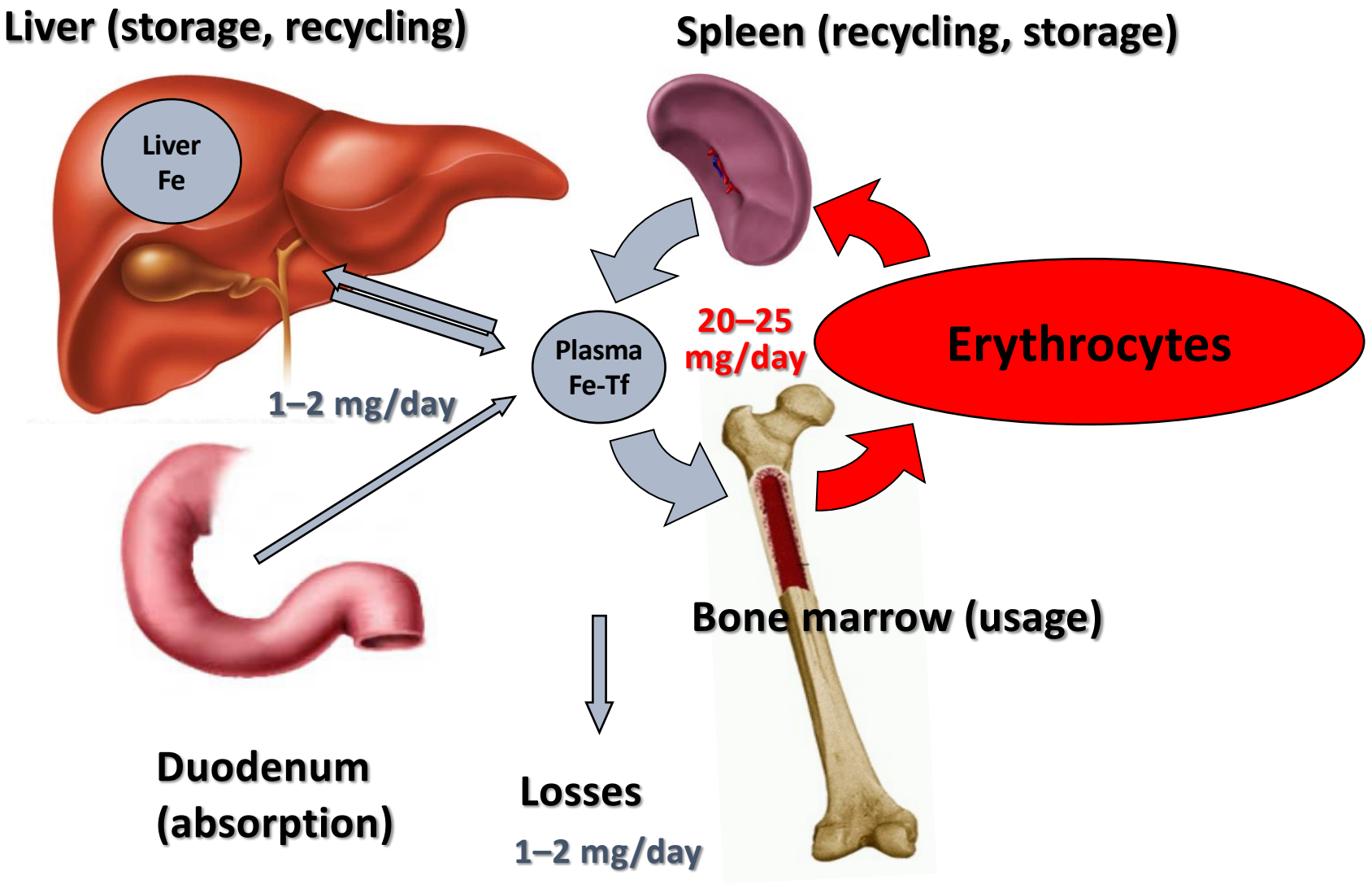
Iron is required for erythropoiesis



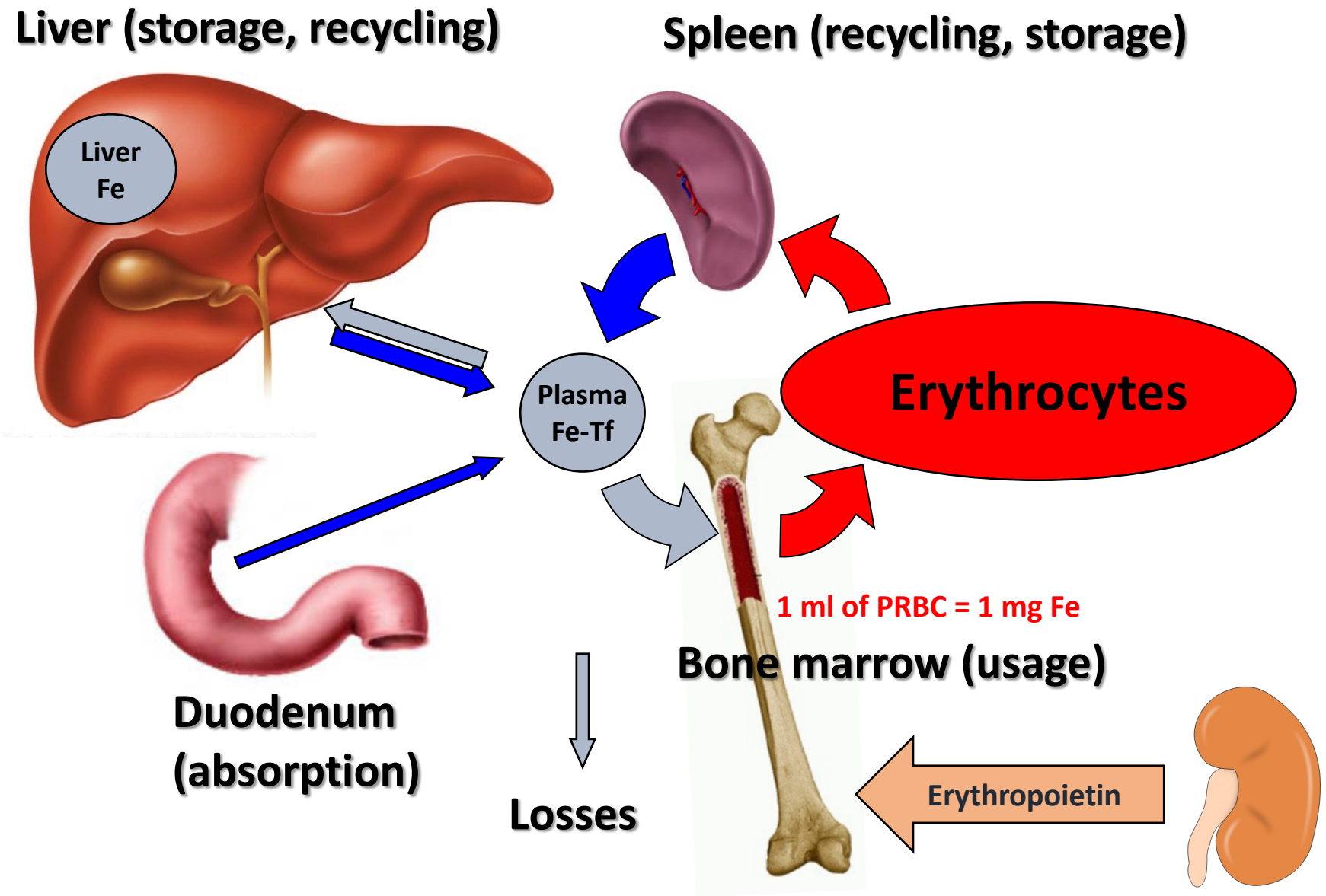
Each 1 ml of packed RBC ≈ 1 mg of iron

Iron economy of the body

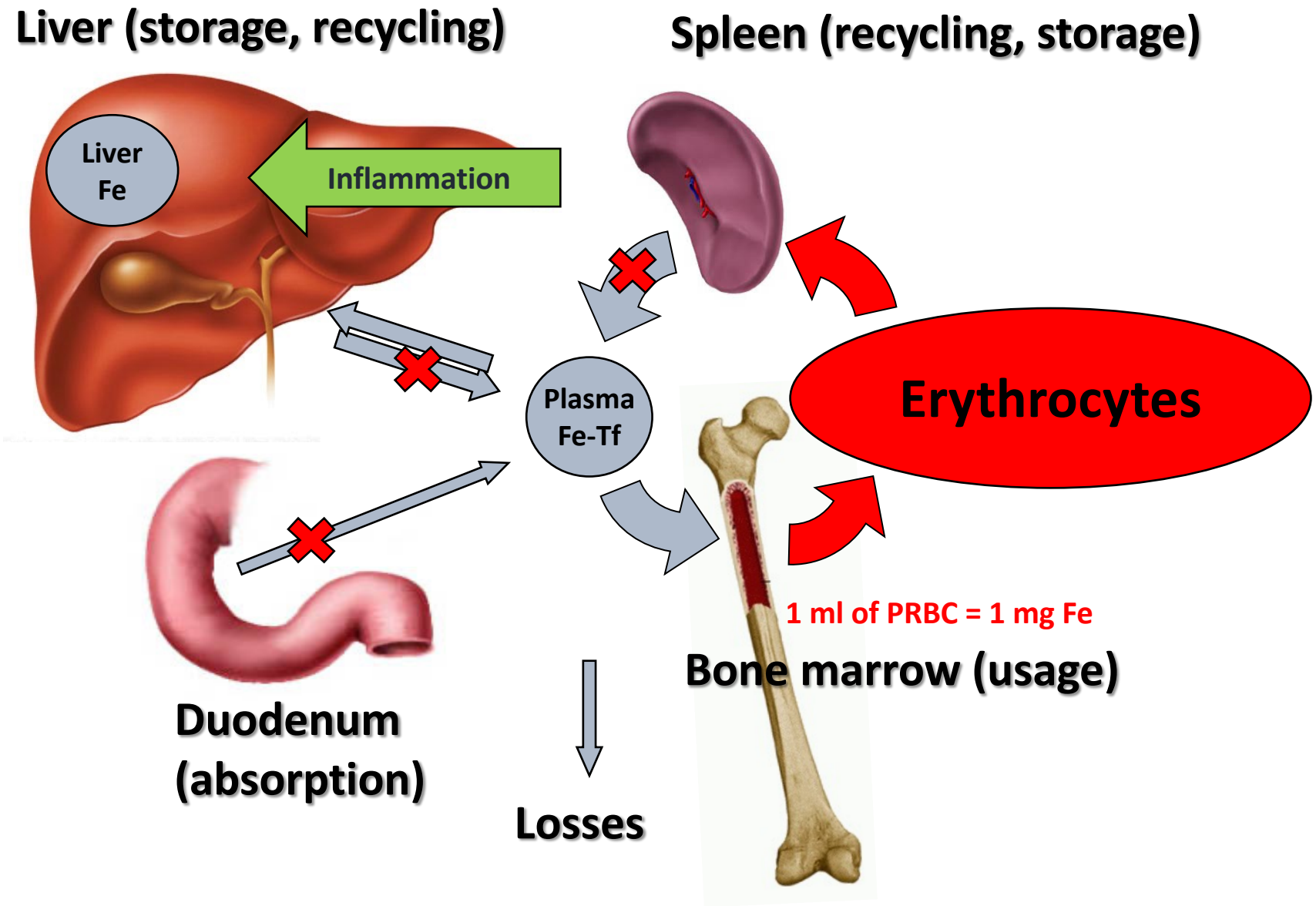
Plasma iron concentrations 10-30 μM, body iron content 3-4 g, plasma iron only 3-4 mg total



During erythropoietic stimulation



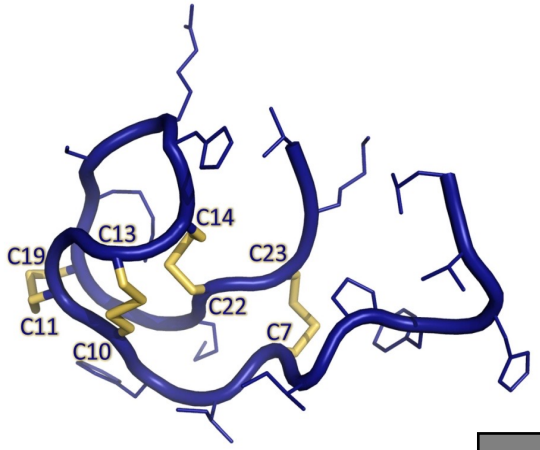
During infection/inflammation



Molecular Basis of Iron Homeostasis

Hepcidin—an iron-regulatory peptide hormone

- Secreted by hepatocytes as a 25 aa peptide
- Regulates intestinal iron absorption and the distribution of iron to tissues



Jordan et al. JBC 2009

DTHFPICIFCCGCCHRSKCGMCCKT

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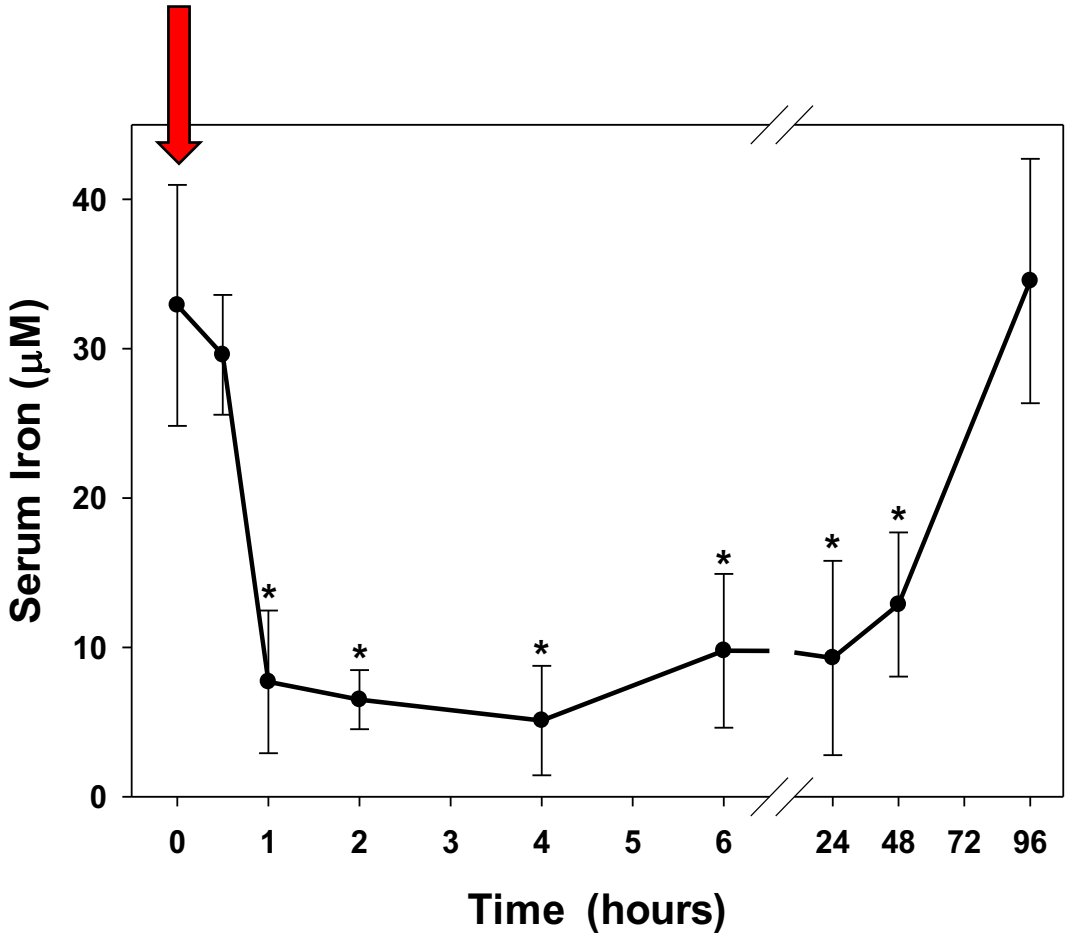
Hepcidin peptide lowers plasma iron

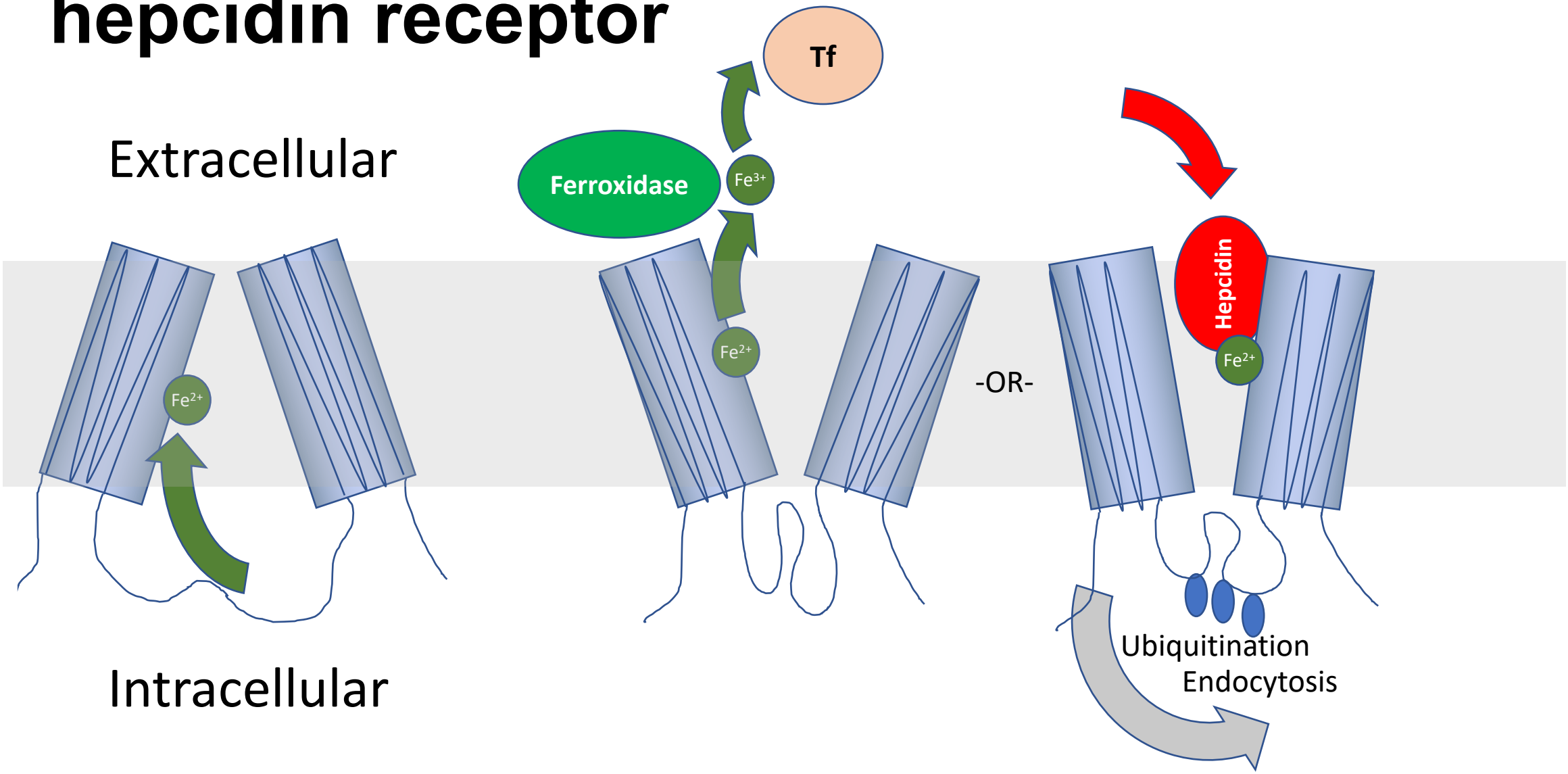
Synthetic hepcidin 50 μg IP/mouse



Mnemonic: hepcidin is to iron like insulin is to glucose ☺

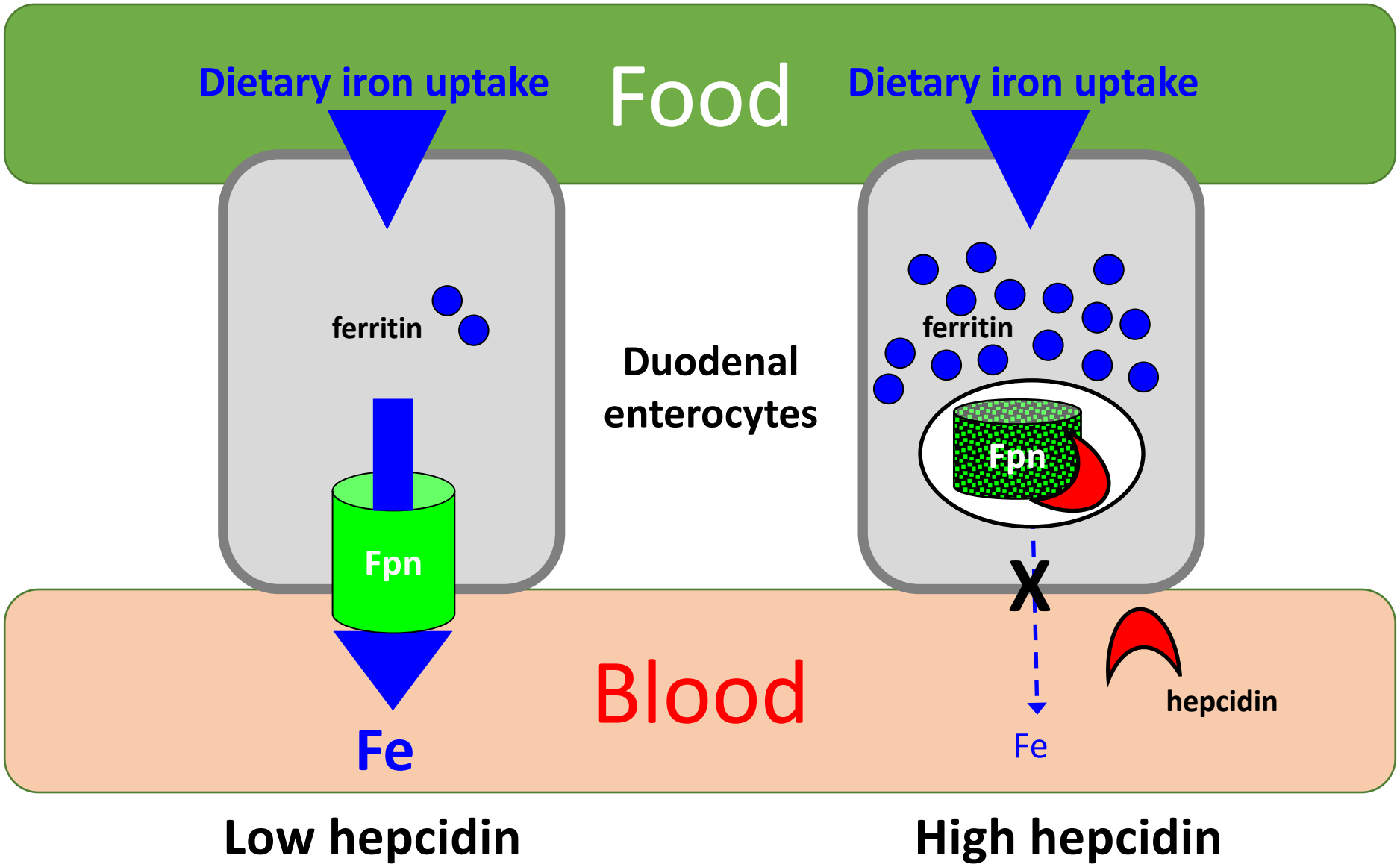
Rivera et al. Blood 2005

Ferroportin – the iron exporter and hepcidin receptor

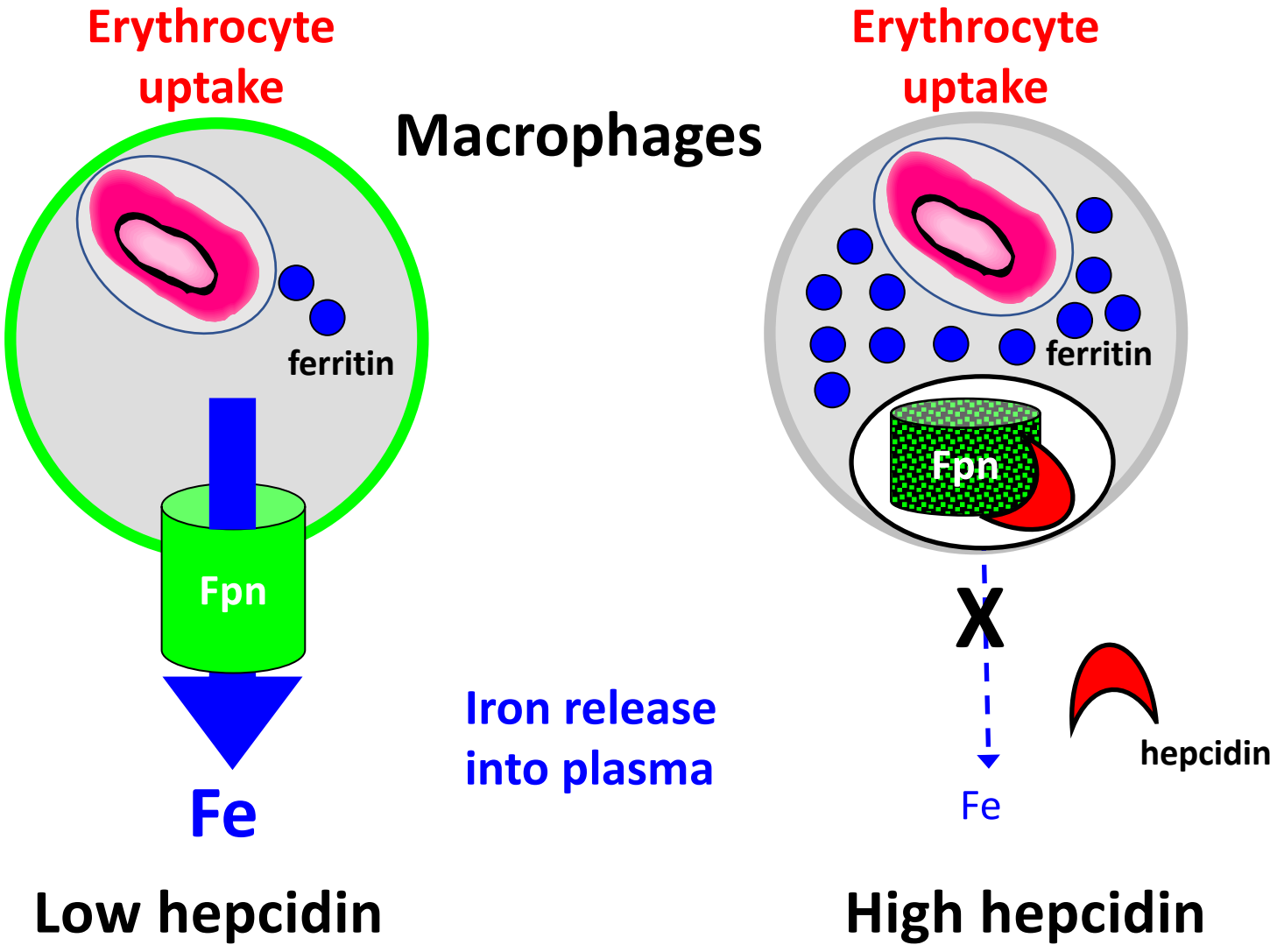


Nemeth et al. Science 2004, Qiao et al. Cell Metabolism 2012, Deshpande Nature Comm. 2018, Aschemeyer Blood 2018, Billesbolle Nature 2020

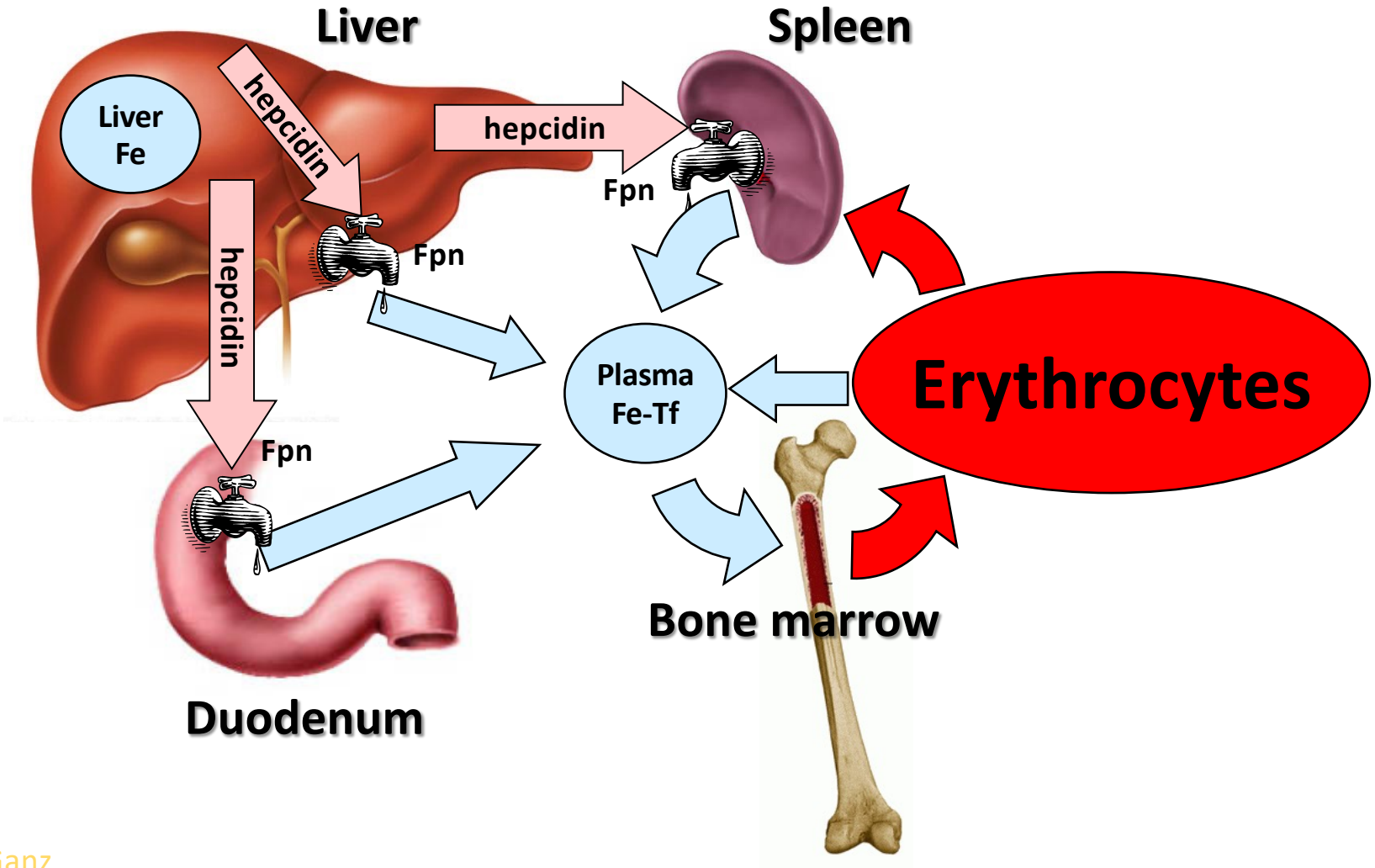
Regulation of intestinal iron absorption



Regulation of erythrocyte iron recycling



Hepcidin regulates dietary iron absorption and influx to plasma



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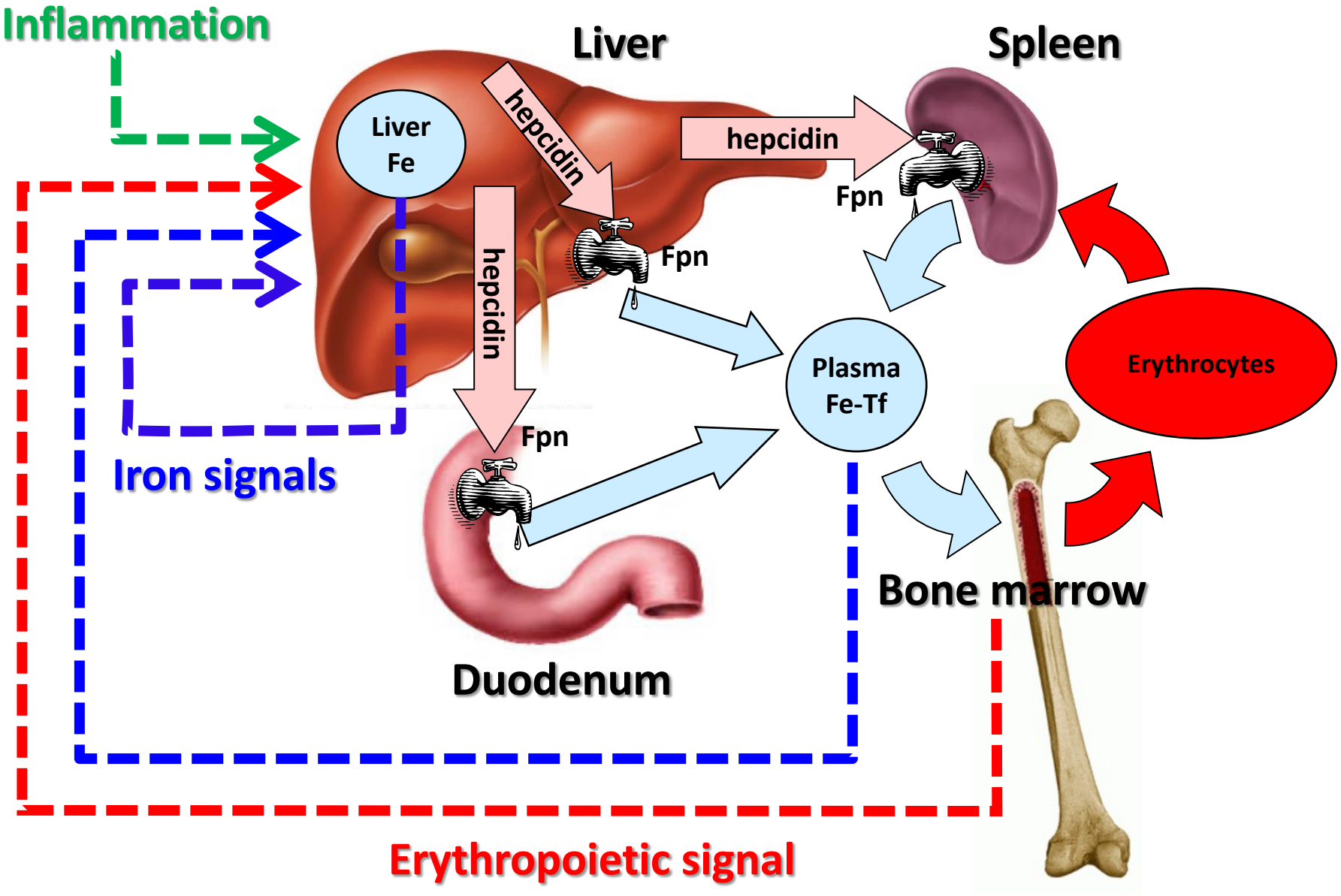
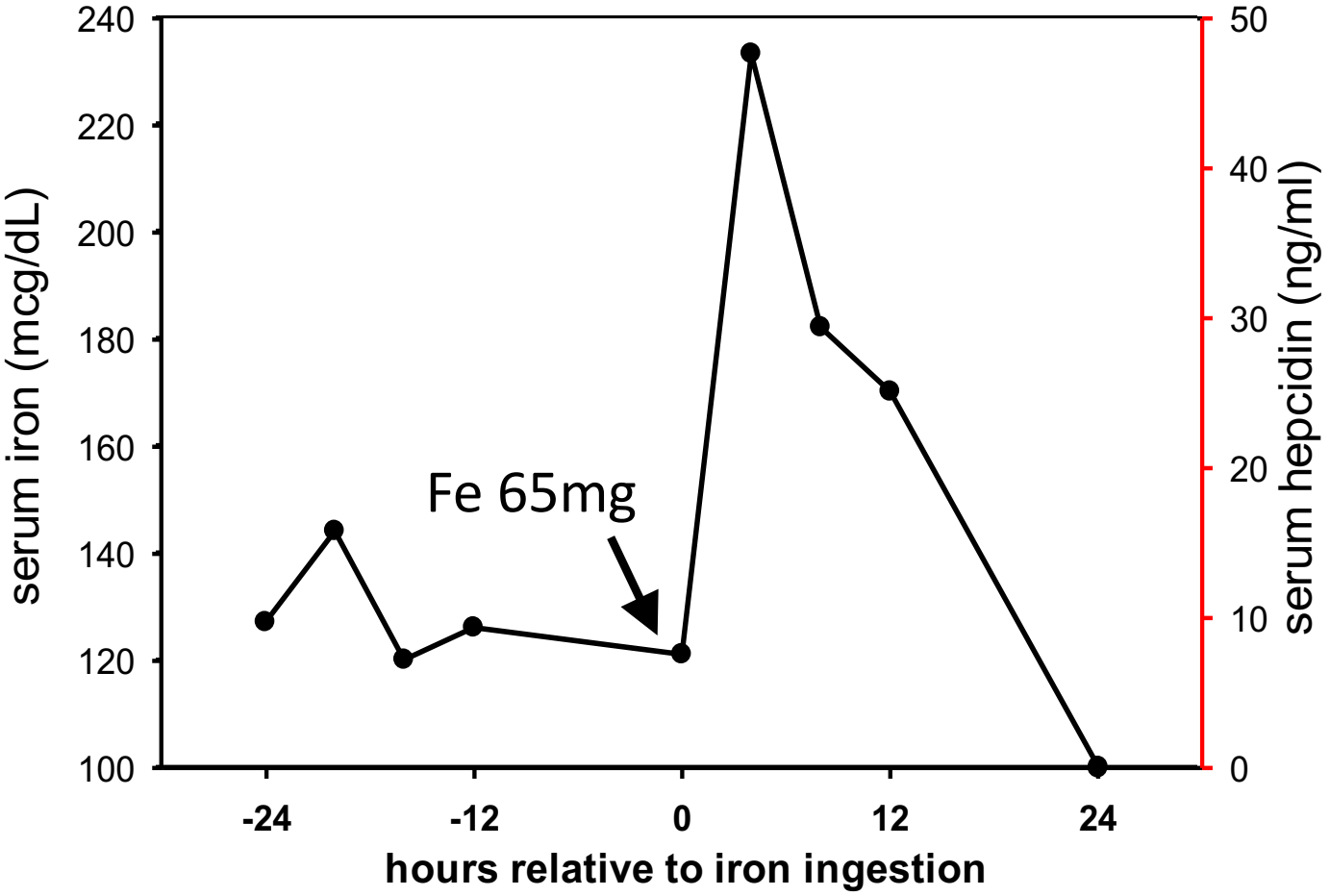
Signals regulating hepcidin

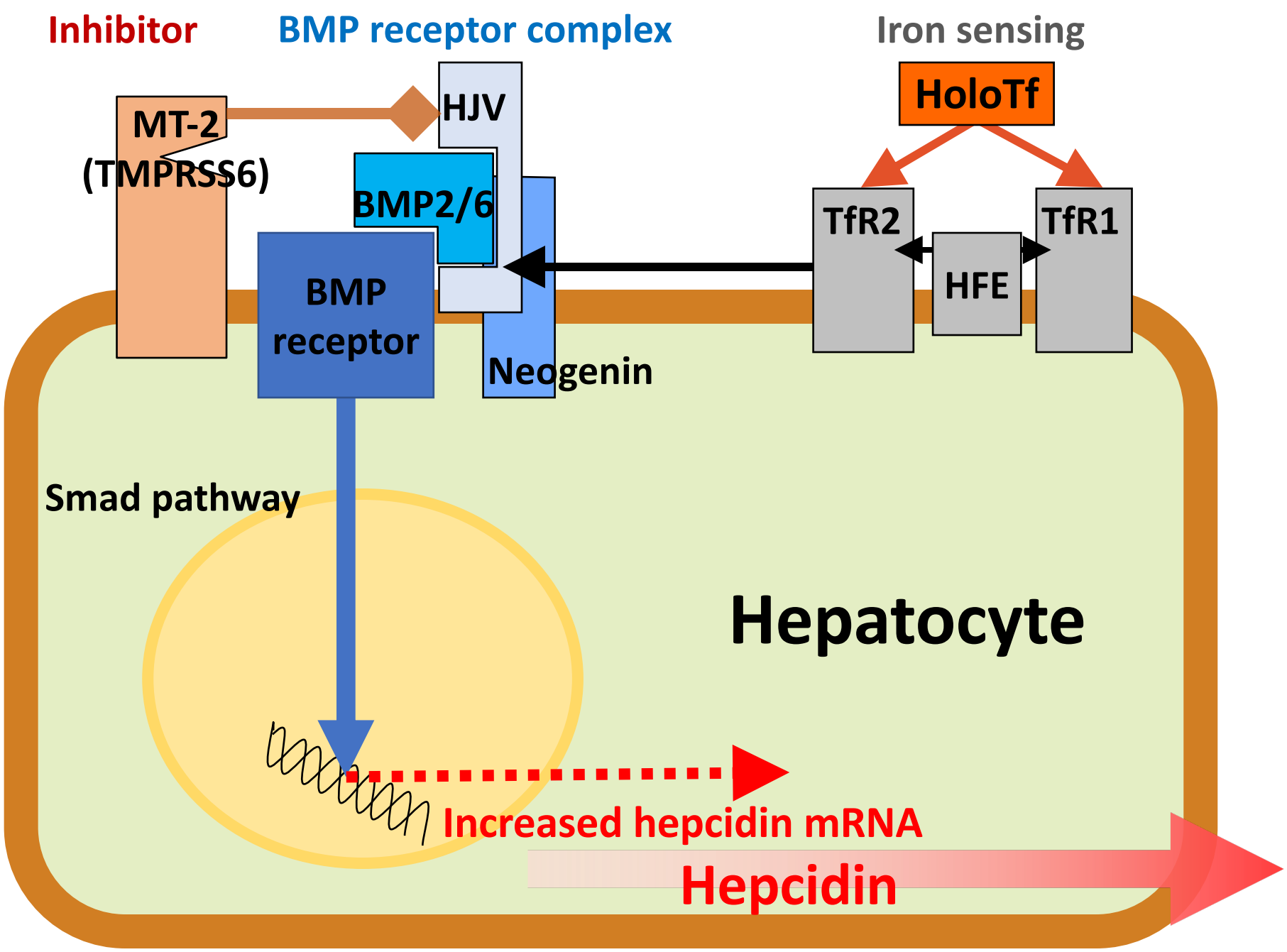


Hepcidin Regulation by Iron: Health and Disease

Hepcidin response to oral iron



Ganz et al, *Blood* 2008


Inhibitor
BMP receptor complex
Iron sensing
MT-2
(TMPRSS6)
HJV
BMP2/6
BMP
receptor
Neogenin
HoloTf
TfR2
HFE
TfR1
Smad pathway
Hepatocyte
Increased hepcidin mRNA
Hepcidin

Patient presentation: Too much iron…

- A 26-year-old woman, admitted to a Brazilian university hospital with a recent history of diabetic ketoacidosis, arthralgia involving small and medium size joints, hair loss, constipation and amenorrhea
- Discrete hyperpigmentation on face, limbs and abdomen, ferritin 8377 μg/ml (13-150), transferrin saturation 105% (20-45), serum iron 47 μM (7-27), hemoglobin 12.1 g/dl, hyperglycemia, very low estradiol, FSH, LH, and free T4 with "normal" TSH. Hepatic enzymes elevated.
- Percutaneous liver biopsy: marked iron deposition, mostly in hepatocytes but no fibrosis
- Tests for HFE mutations (C282Y/H63D) were negative.
- Presumptive clinical diagnosis: juvenile hemochromatosis

Santiago de Sousa Azulay R. et al.: Novel mutation in the hemojuvelin gene (*HJV*)… Am J Case Rep, 2020; 21: e923108

Liver biopsy

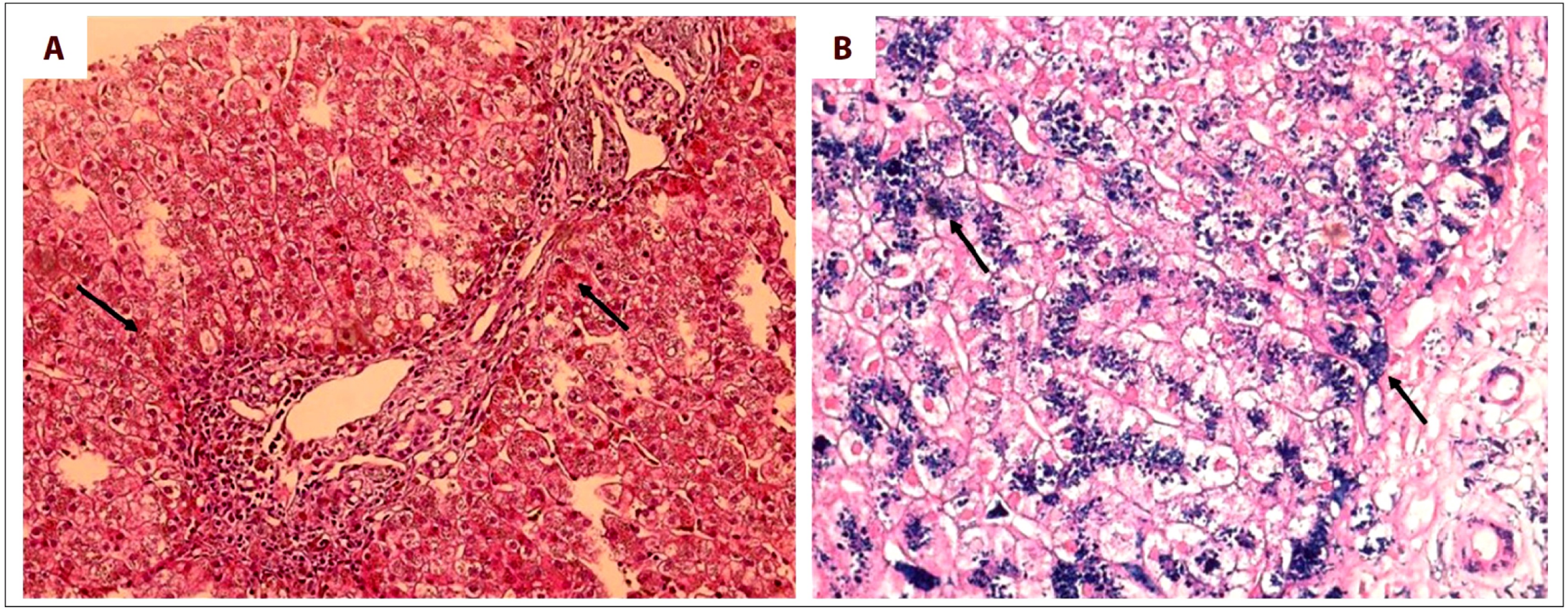
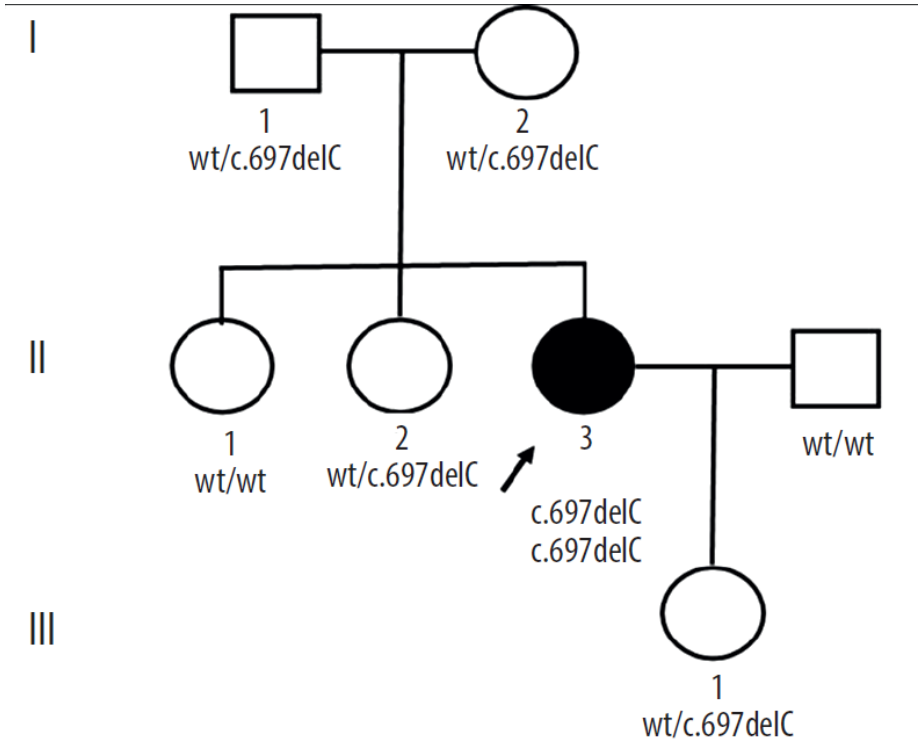


Figure 1. Photomicrography of hepatic hemochromatosis. (**A**) Hepatic tissue with preserved lobular architecture and presence of golden brown granular deposits within hepatocytes (indicated by the black arrow) (hematoxylin and eosin 200×); (**B**) Perl's stain showing bluish iron deposits in hepatocyte cytoplasm (indicated by the black arrow) (400×).

Too much iron…

- MRI: iron deposition in the liver, pancreas and anterior pituitary
- Genetic analysis: homozygous mutation in the hemojuvelin gene, predicted as pathogenic

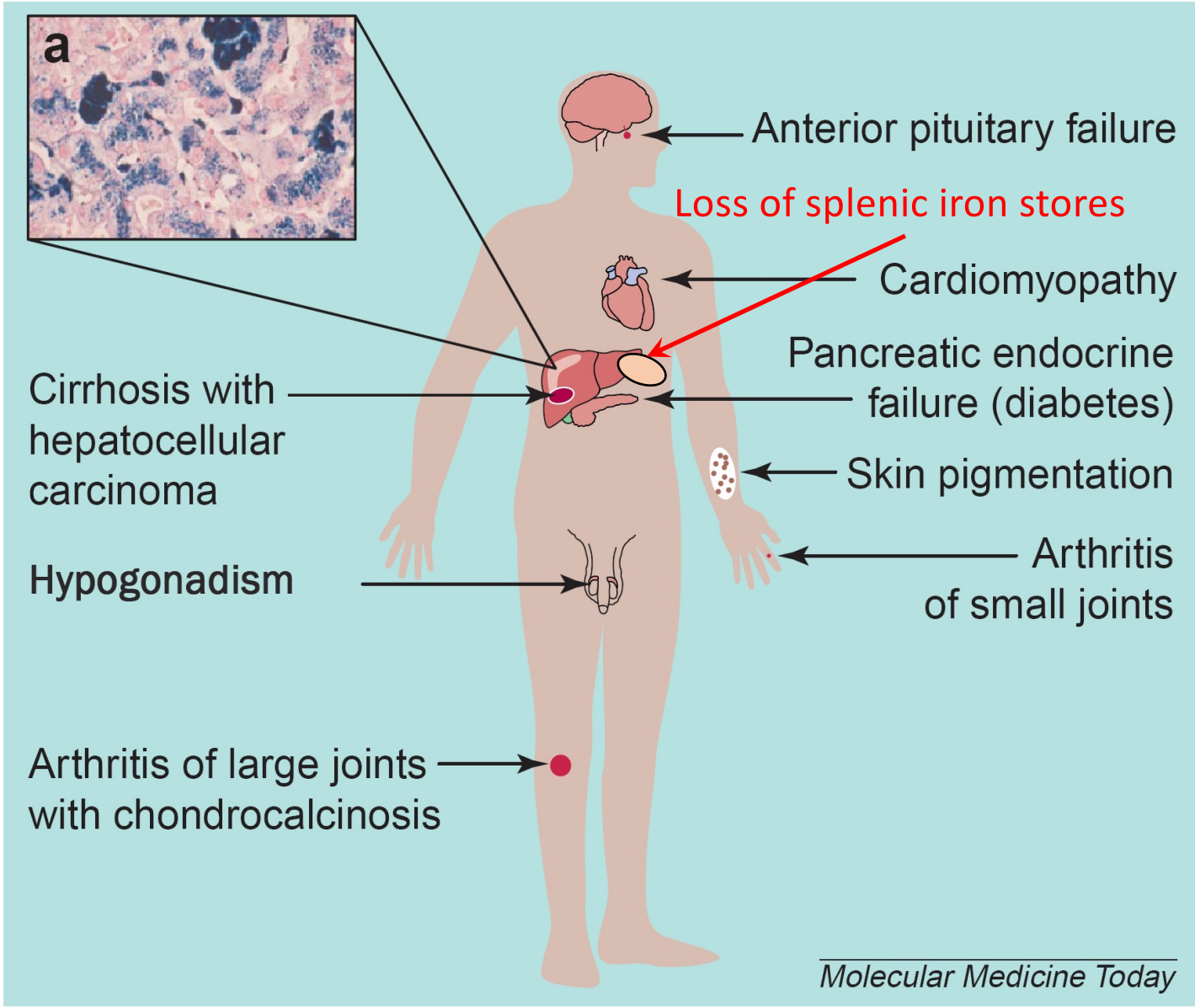


Too much iron…

- Phlebotomy, iron chelation and hormone replacement
- After 1 year, supraventricular tachycardia
- 4 months later she returned with dyspnea and lower limb edema
- Echocardiographic examination: moderate dilation of the cardiac chambers, significant left systolic and moderate right systolic dysfunction and pulmonary hypertension
- A cardiac MRI showed significant iron deposition in the myocardium, chamber dilation, and global left ventricular dysfunction and a discrete enlargement of the left atrium

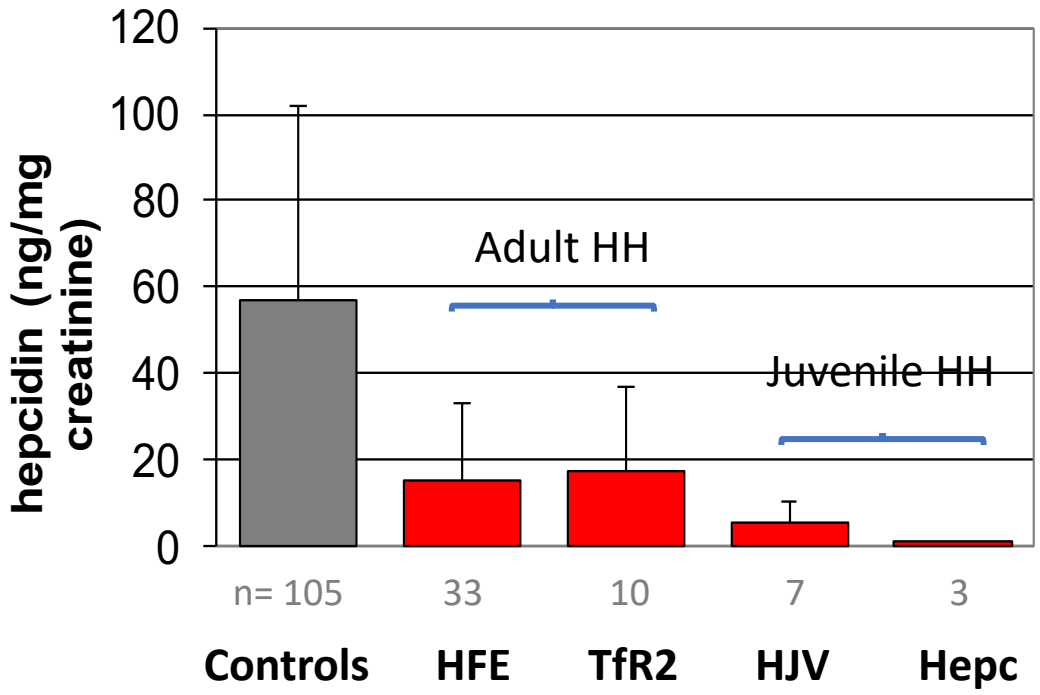
(= Not adequately treated by iron chelation and phlebotomy)

Hemochromatosis (“hereditary hemochromatosis”)



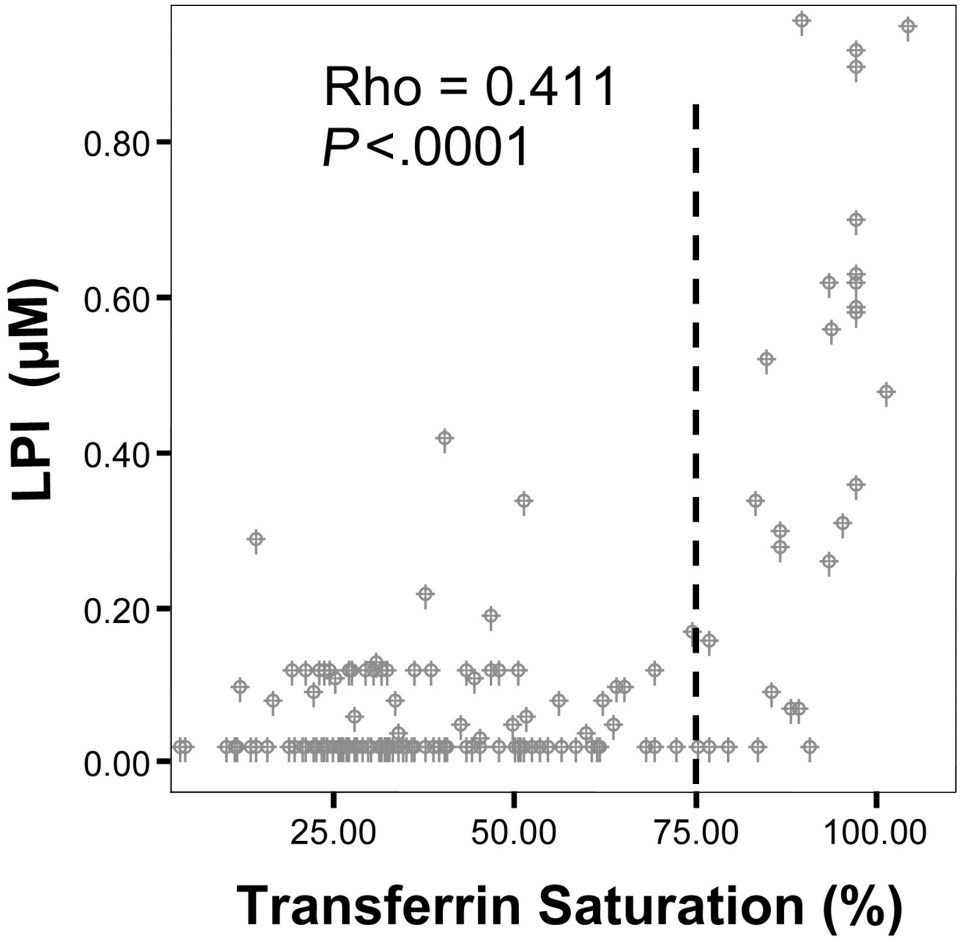
Molecular basis of hemochromatosis

- **Hepcidin deficiency**
  - mutations in *Hamp* (hepcidin gene) or its regulators (*HFE, TfR2* and *HJV*)
- **Resistance to hepcidin (rare but instructive)**
  - mutations in the hepcidin receptor ferroportin



Non-transferrin bound iron (NTBI) vs transferrin saturation

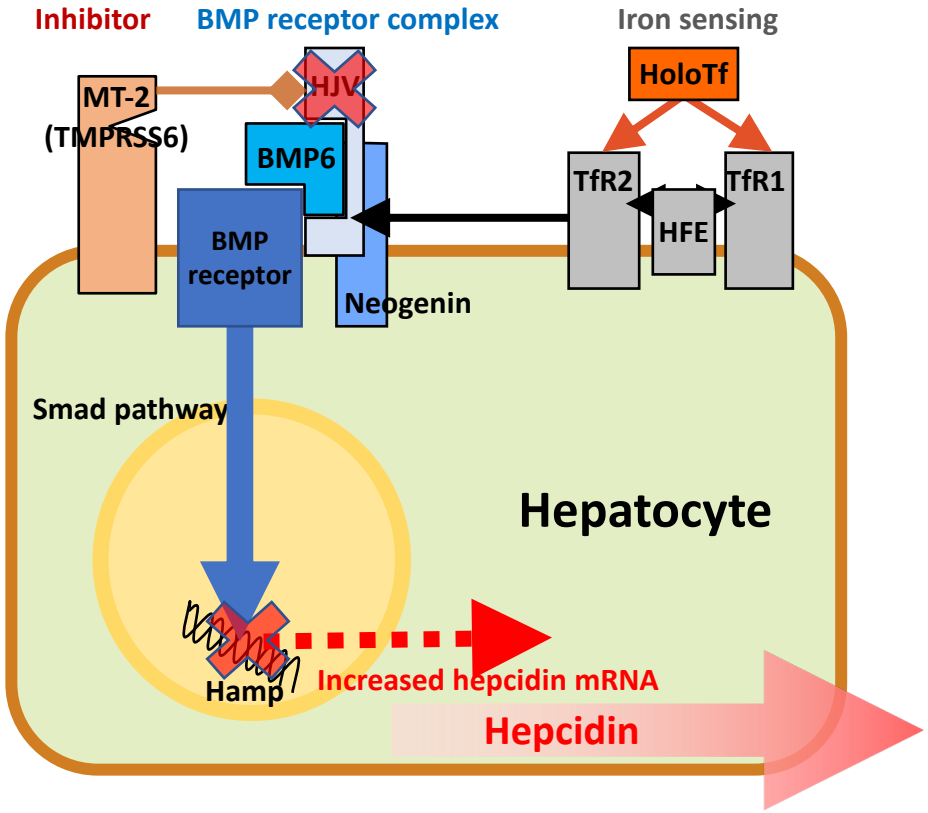
- Fe bound to citrate, acetate, albumin
- Seen when transferrin is (nearly) saturated with Fe
- NTBI is selectively taken up by the liver and other parenchymal tissue and causes injury
- Labile plasma iron (LPI) is the redox-active portion of NTBI
- LPI is detectable when Tsat >75%



Caroline Le Lan et al. Blood 2005;105:4527-4531

Juvenile hemochromatosis genetics and pathophysiology

- Autosomal recessive
- Very low serum hepcidin concentrations
- Only homozygotes or compound heterozygotes affected
- A great variety of mutations have been described and their severity varies
- Onset of clinical problems before the age 21 is common (range 10-30 yrs)
- Two genes: hemojuvelin(HJV, rare) and hepcidin (Hamp, very rare)



Criteria for the diagnosis of hemochromatosis

Necessary criteria

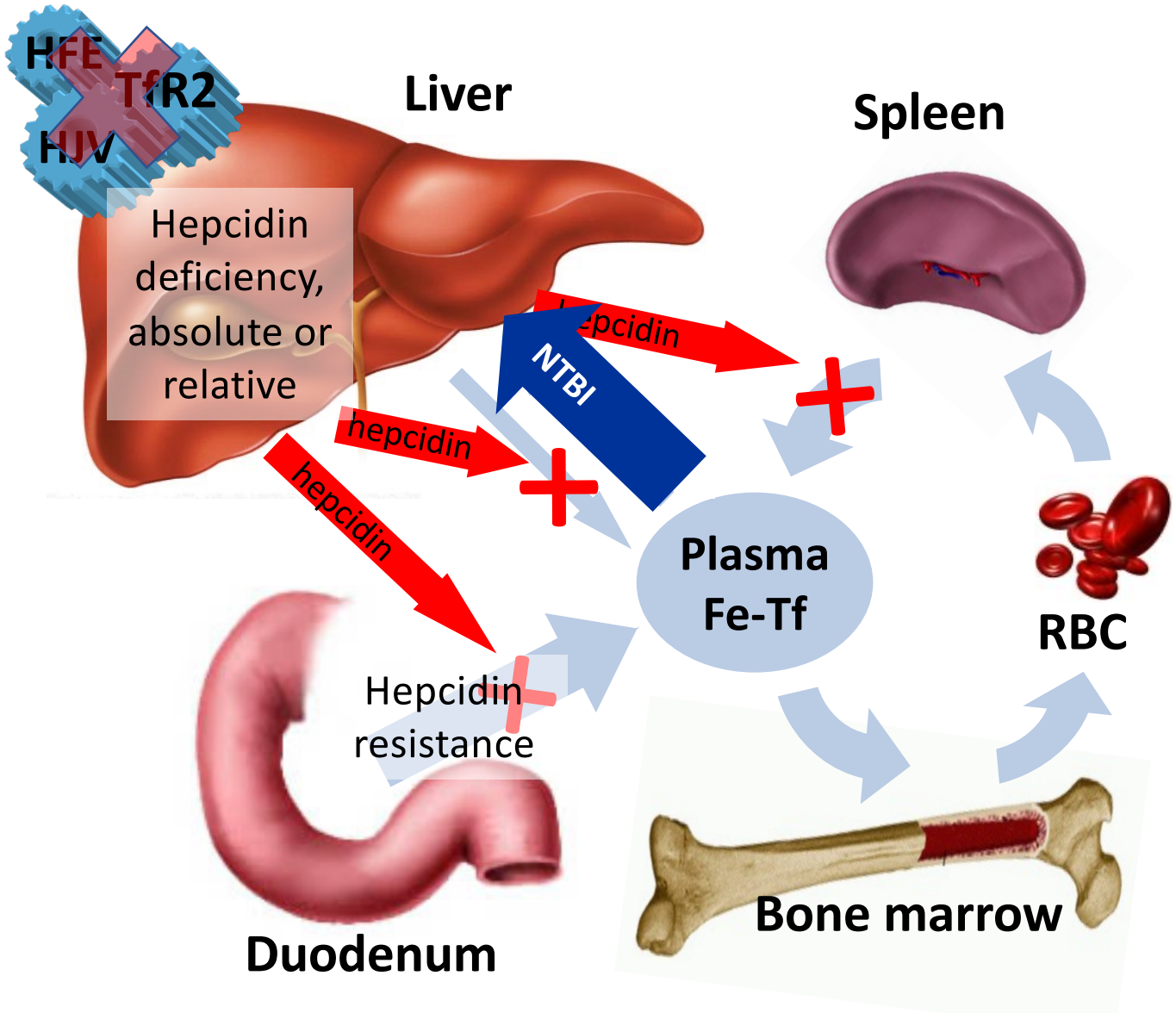
+ TSAT >45%
+ S-Ferritin >200 µg/L (females) or >300 µg/L (males)
+ Evidence of liver iron overload (MRI and/or biopsy)
+ Absence of acquired risk factors for hepcidin deficiency (e.g. alcohol abuse or end-stage liver disease) and iatrogenic iron overload (e.g. regular transfusions)
+ Absence of hematological signs of a primary red blood cell disorder, such as anemia (i.e. Hb>120 g/L in females, >130 g/L in males) and/or reticulocytosis

Signs and/or symptoms associated with iron overload may or may not be present

- skin pigmentation, asthenia
- persistent increase of aminotransferases, hepatomegaly, cirrhosis, hepatocellular carcinoma
- joint pain, arthritis, chondrocalcinosis, reduced bone mineral density
- diabetes mellitus, hypopituitarism, hypoparathyroidism, hypogonadotropic hypogonadism
- cardiomyopathy, heart failure, cardiac arrhythmias

Domenico Girelli et al. Hemochromatosis classification: update and recommendations by the BIOIRON Society. *Blood* 2021; blood.2021011338. doi: https://doi.org/10.1182/blood.2021011338

Hemochromatosis

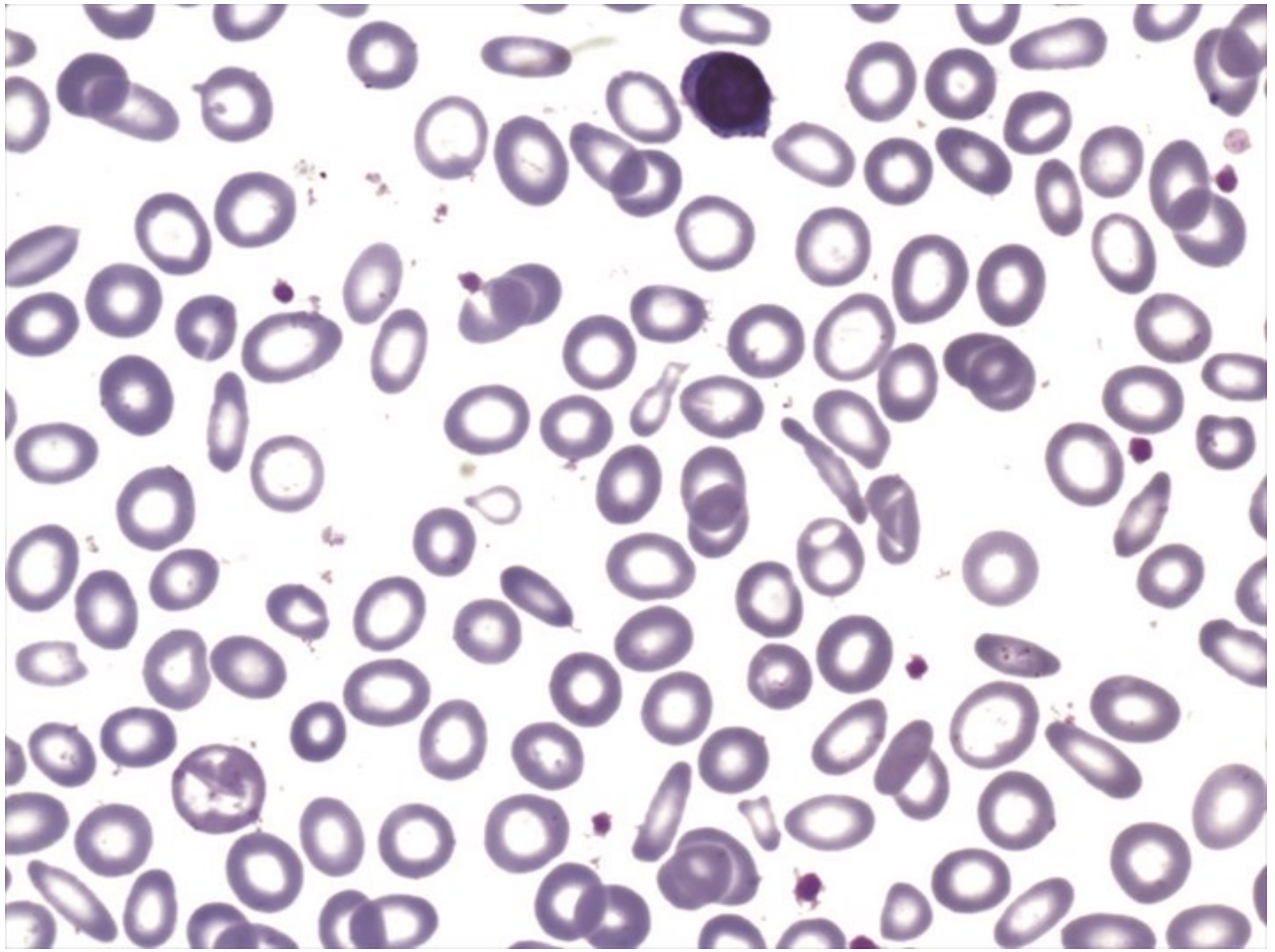


Patient presentation: Too little iron…

- A 14-year old boy: genetic evaluation for anemia
- Unrelated healthy parents, anemia at 18 months: Hb 6 g/dL, MCV 47 fl, serum iron < 5 µM
- Serum iron was consistently low (<5 µM), transferrin saturation < 5%, serum ferritin in the lower normal range
- Oral iron supplementation did not improve Hb or serum iron
- At age 7, iron absorption assessed by administration of oral ferrous iron at 3 mg/kg followed by measurement of serum iron at 3 hours. Defective iron absorption: serum iron rose only 13 µmol/L (>18 µmol/L expected)
- Duodenal biopsy showed normal mucosa
- Presumptive clinical diagnosis: Iron-refractory iron deficiency anemia
- Given intravenous iron sucrose 100 mg weekly x 3. Serum ferritin rose from 11 µg/L to 109 µg/L and hemoglobin rose from 6.8 g/dL before the treatment to a maximum of 9.8 g/dL
- Microcytosis with pencil cells and hypochromia persisted. Bone marrow mildly hypocellular with no sideroblasts. Iron was present in macrophages.

Modified from Guillem et al. BLOOD 112, 2008

Blood smear



Karin Finberg

Too little iron…

14 y/o boy with iron-refractory iron-deficiency microcytic anemia since 1.5 years of age

Table 1. Biologic parameters of the patient and of his relatives at the time of the present study (2008)

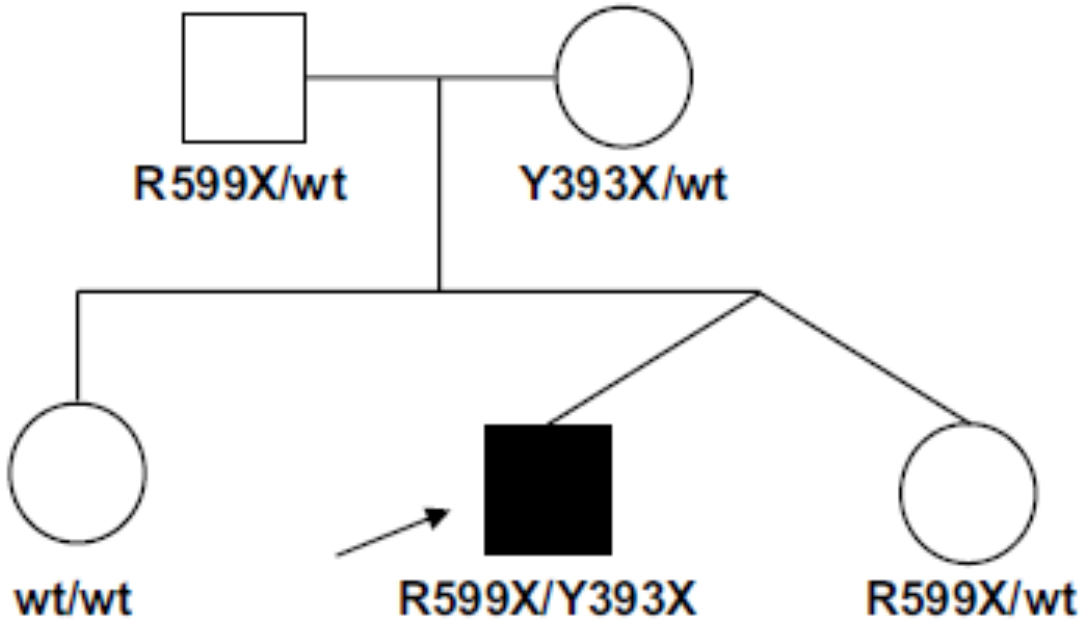
	Normal values	Patient	Father	Mother	Twin sister	Elder sister
Age, y		14	48	47	14	16
Hb, g/L	120-155	86	151	127	144	142
Hematocrit	0.36-0.45	0.30	0.44	0.38	0.43	0.42
MCV, fL	80-90	54.3	85.7	85.8	86	79.1
Reticulocytes, 10^9/L	50-100	30	ND	ND	ND	ND
Iron status						
Serum iron (μmol/L)	14.5-26.0	2.44	22	14.5	12	19
Serum ferritin (μg/L)	30-300 (M), 20-150 (F)	9	88	10.4	9	20.2
Soluble transferrin receptors (mg/L)	0.83-1.76	6.52	1.44	1.52	1.6	1.17
Plasma hepcidin (ng/mL)	*29-254 (M), *16-288 (F)	70	191	24	66	86

ND indicates not determined; M, male; F, female.
*5% to 95 % range (T. Ganz, G. Olbina, D. Girelli, E. Nemeth, M.W., unpublished data).

In severe iron deficiency, hepcidin concentration should be unmeasurable, so it is inappropriately high

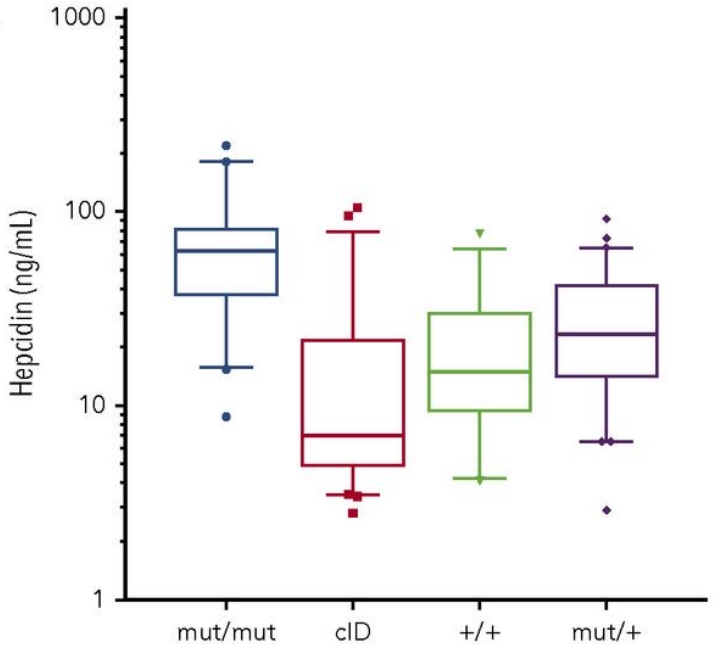
Too little iron…

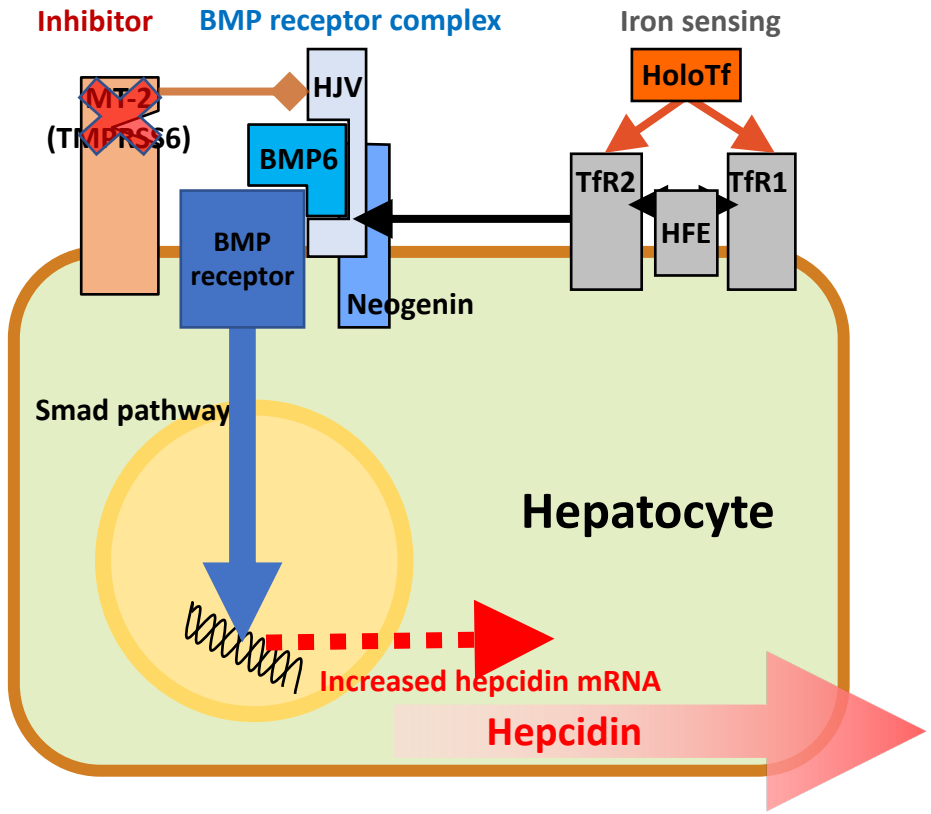
- TMPRSS6 exome sequencing



Iron-refractory iron deficiency anemia

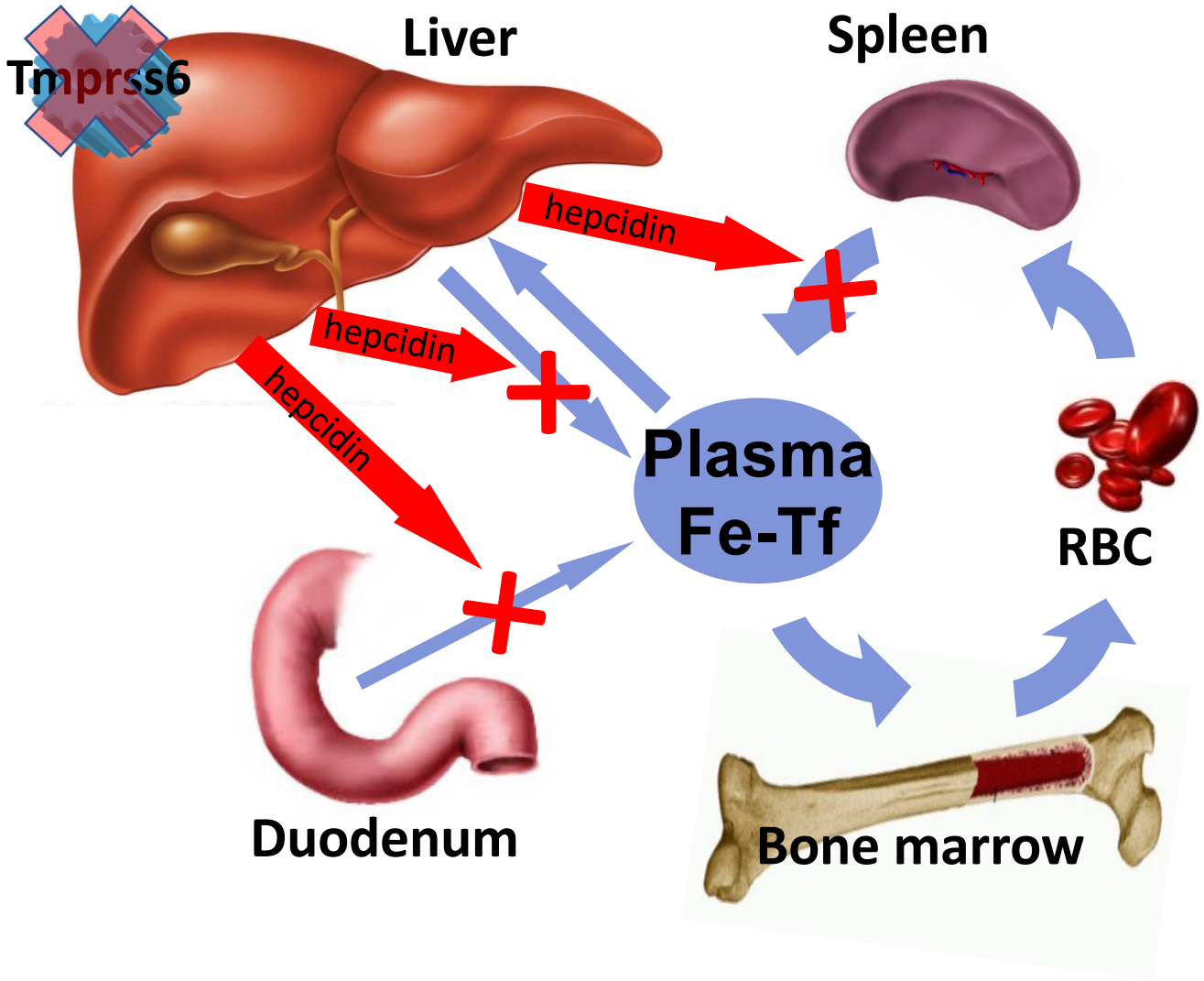
- Mutations in TMPRSS6/MT-2 usually affecting both copies of the gene
- Loss of inhibition of hepcidin transcription leads to excess hepcidin compared to common iron deficiency





Heeney MM, Guo D, De Falco L, et al. Blood. 2018;132(4):448-452.

Iron-refractory iron deficiency anemia



Erythropoiesis and Iron Homeostasis

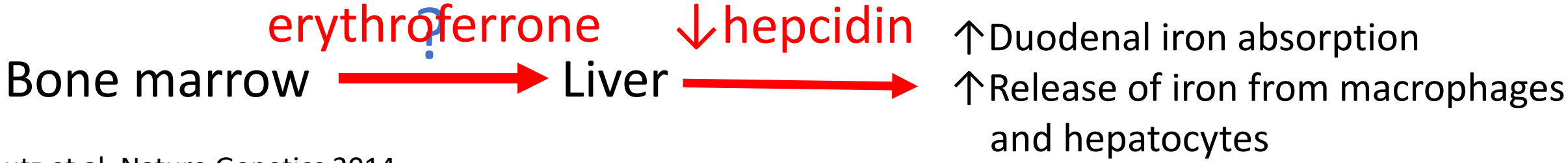
Patient presentation: why too much iron?

- A 61-year-old man with the diagnosis of β-thalassemia, referred for:
  - mild elevation of serum transaminases (2x upper normal)
  - elevated serum ferritin (1249 µg/L) and transferrin saturation (92%)
- Transfused only during splenectomy when 33 years old, 3 PRBC
- Total bilirubin: 3.61 mg/dL; conjugated: 2.21 mg/dL, hemoglobin 9.8 g/dL
- Ultrasound: enlarged liver with nodularity
- MRI: liver iron concentration of 12.7 mg Fe/g dw, indicating moderate to severe iron overload
- Tests for viral and autoimmune hepatitis were negative
- No hx of alcohol and his body mass index was normal

Pinto et al. Int. J. Mol. Sci 2020 Nov 20;21(22):8771

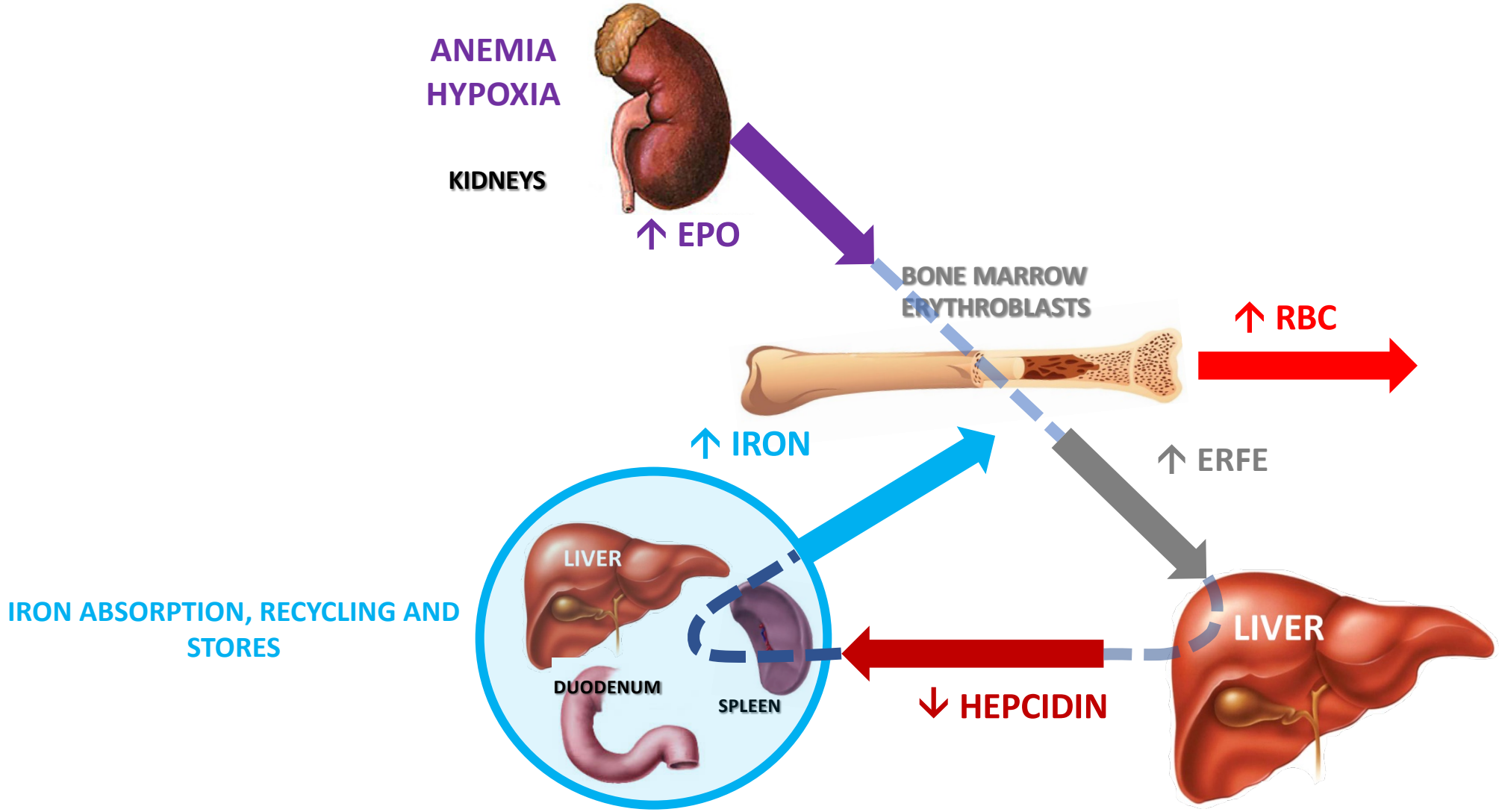
Erythroid regulator = erythroferrone (Erfe)

- Production of each 1 ml of red blood cells requires 1 mg of iron
- Intestinal iron absorption is upregulated when red blood cell production increases
- Hepcidin is suppressed by erythropoietin by a mechanism that depends on functional marrow (Pak 2006)
- We searched for a new hormone secreted by the marrow:
  - Induced by erythropoietin
  - Suppresses hepcidin
- **Protein hormone secreted by erythroid precursors: erythroferrone**

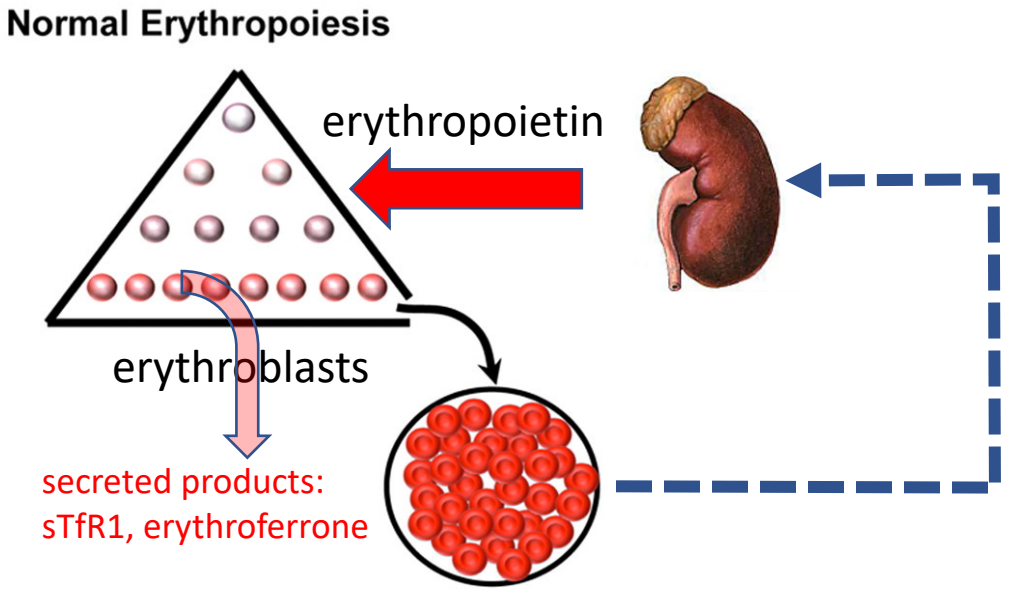


Kautz et al. Nature Genetics 2014

Physiological role of ERFE in iron regulation



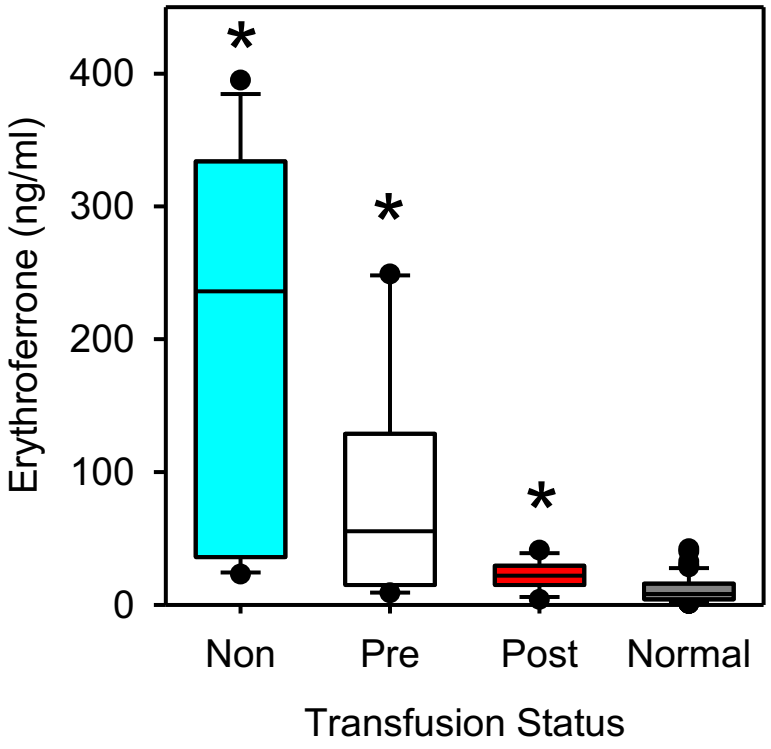
Ineffective erythropoiesis

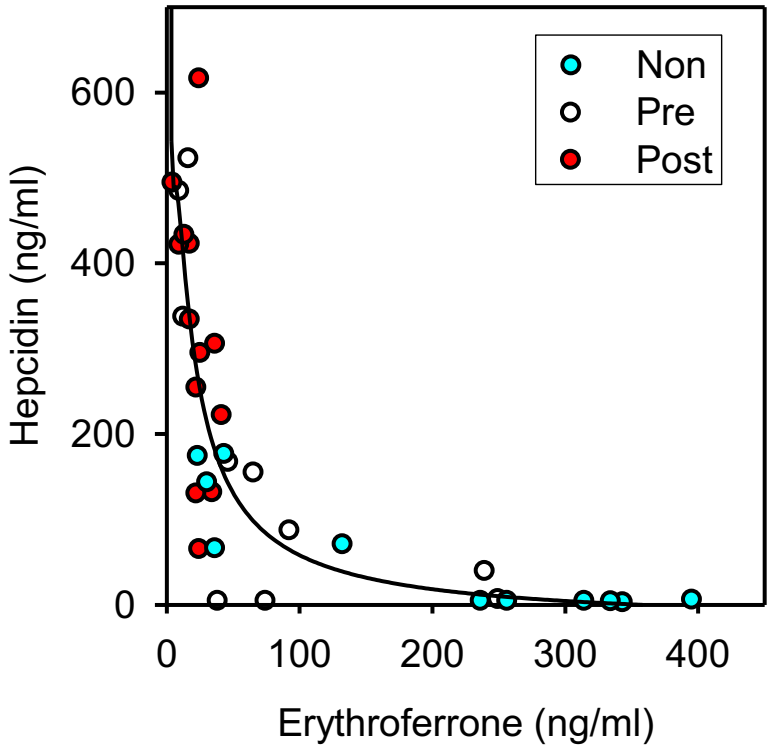


Patients with anemias from ineffective erythropoiesis develop lethal iron overload even when not transfused

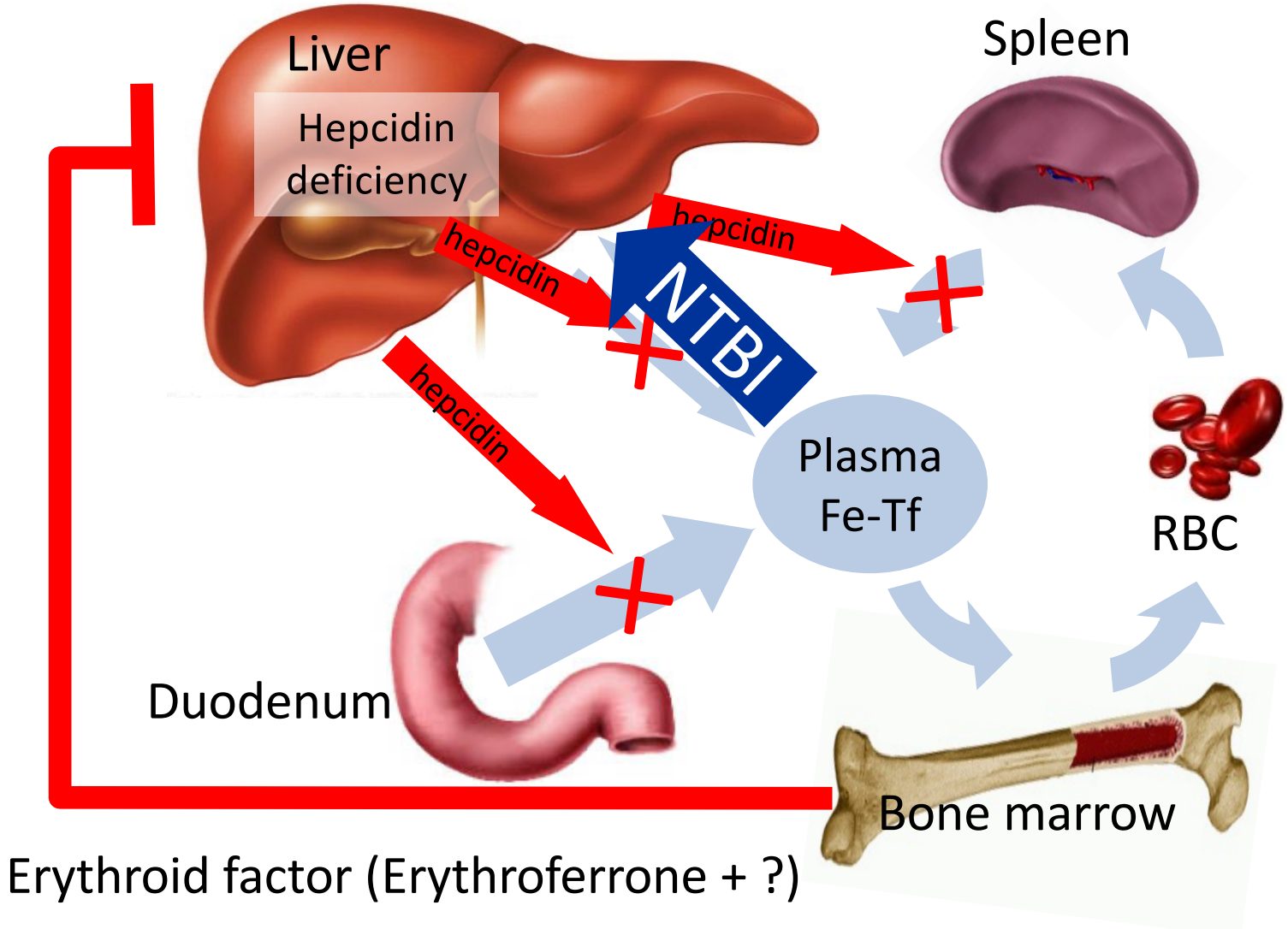
- Diseases: β-thalassemia intermedia, pyruvate-kinase deficiency, congenital dyserythropoietic anemias, sideroblastic anemia,…
- Hyperabsorption of iron phenocopies hereditary hemochromatosis = “iron-loading anemias”
- Hepcidin is suppressed considering the severity of iron overload
- Overproduction of Erfe by exuberantly proliferating erythroblasts suppresses hepcidin

In human β-thalassemia, serum Erfe inversely correlates with hepcidin





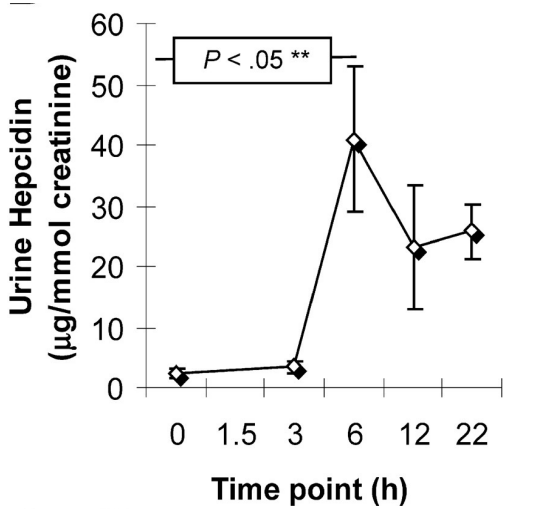
Iron-loading anemias



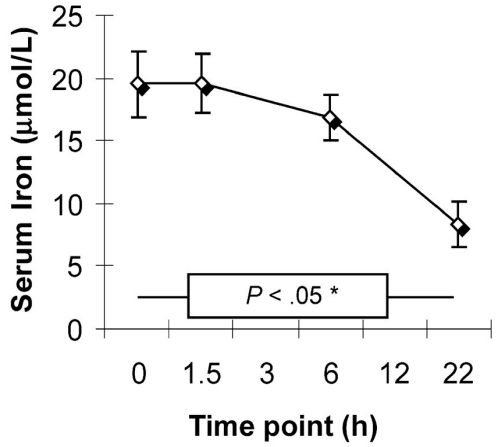
Infection/Inflammation and Iron Homeostasis

In human volunteers, hepcidin is increased by LPS and IL-6, decreasing plasma iron

LPS injection



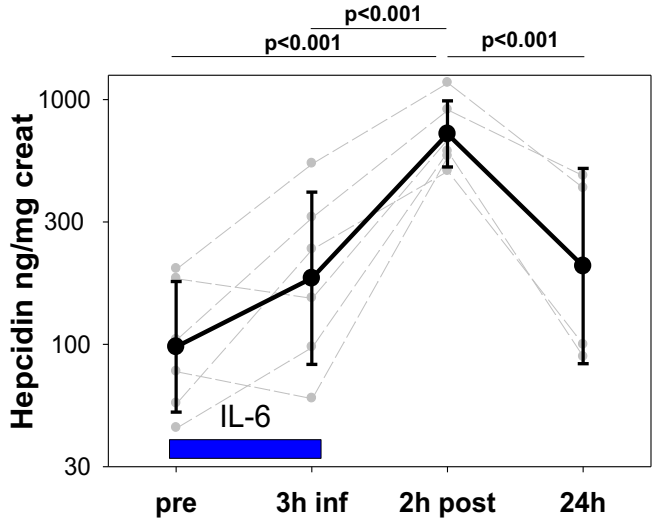
Hepcidin

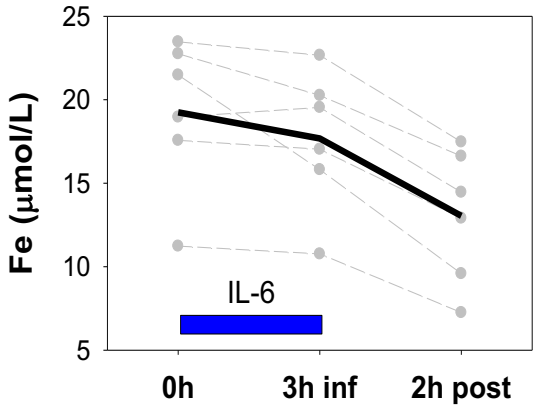


Serum iron

Kemna et al. Blood 2005

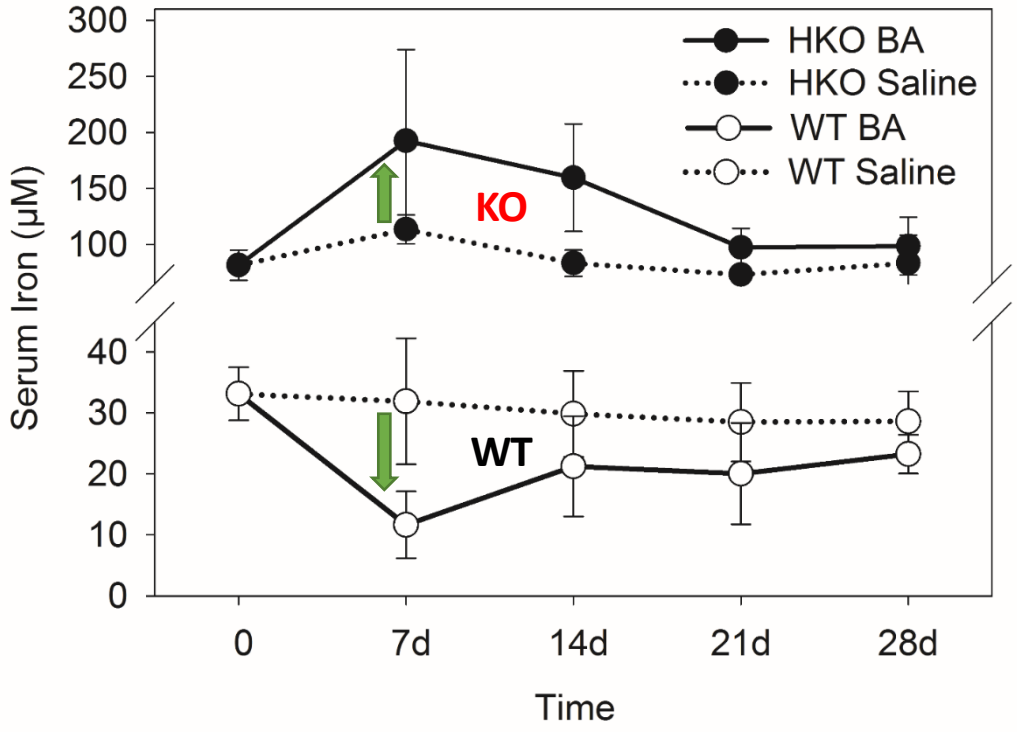
IL-6 infusion





Nemeth et al. JCI 2004

Without hepcidin, inflammation may increase plasma iron



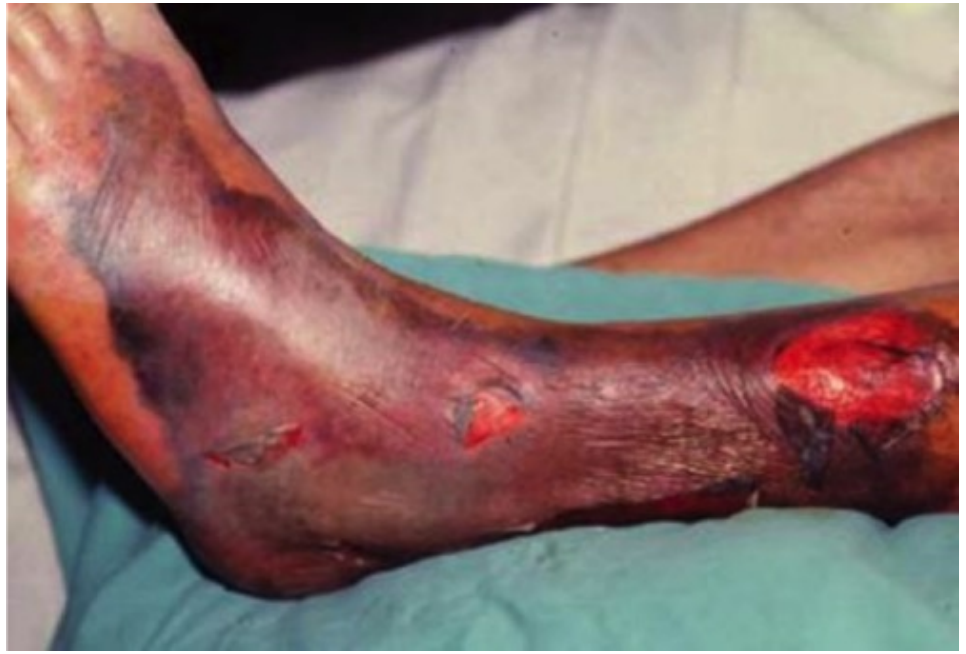
- Erythropoietic suppression = ↓utilization of Fe from plasma
- ↑destruction of erythrocytes and other cells = ↑ delivery of Fe to plasma

Kim et al. Blood 2014

Patient presentation: why susceptible to infection?

- A previously healthy 59-year old woman ate three raw clams at a restaurant. Two days later, she was hospitalized, her left leg amputated, required multiple operations to save her arm

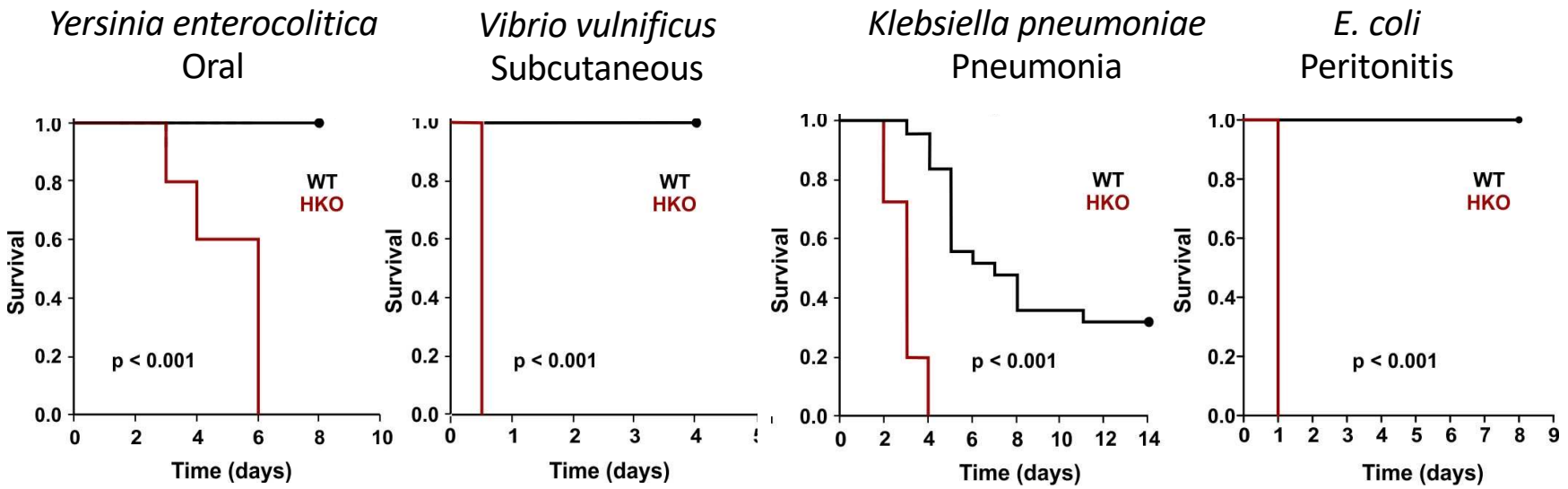
- Blood and wound cultures: *Vibrio vulnificus*
- She was subsequently advised she had an iron overload disorder - hemochromatosis - and to never eat raw shellfish



UNITED STATES COURT OF APPEALS FOR THE THIRD CIRCUIT No. 17-2243

Hepcidin deficiency promotes susceptibility to Gram-negative pathogens

WT or iron-loaded HKO mice infected with YE (10^8 cfu/mouse), VV (10^4 cfu/mouse), KP (10^3 cfu/mouse) or EC (10^4 cfu/mouse), monitored survival



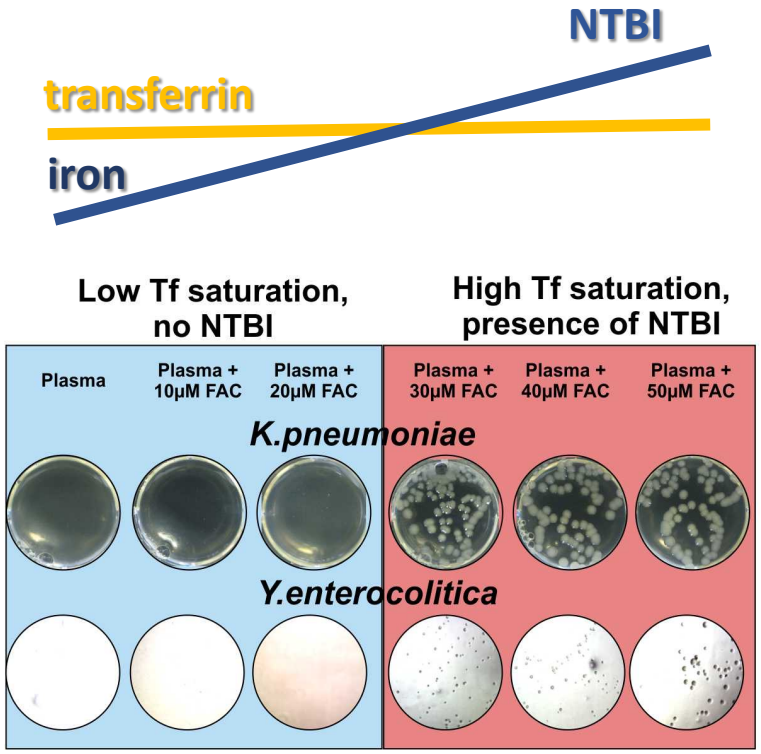
In all of these infections:

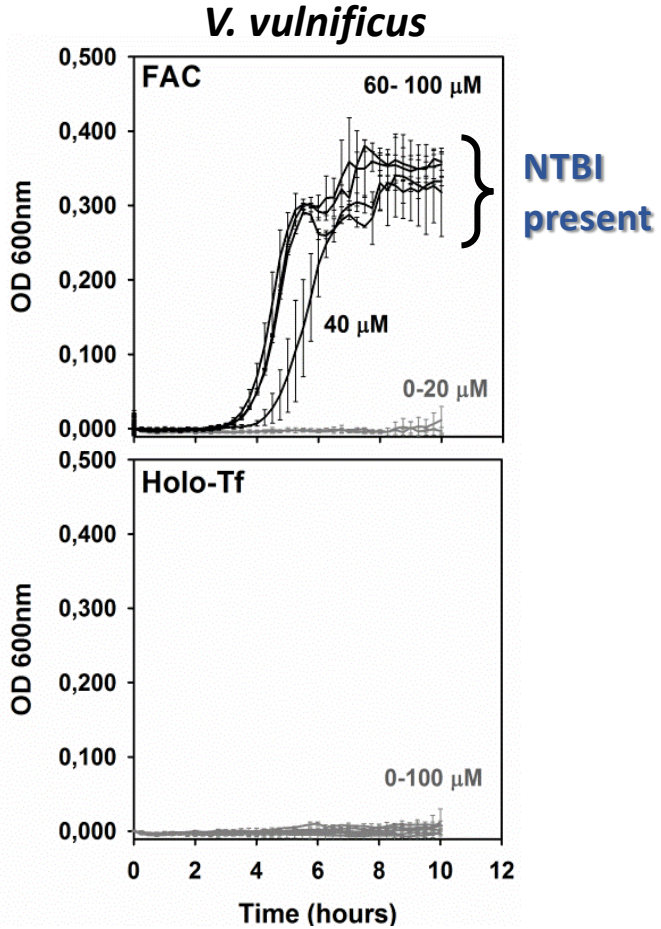
- minihepcidin prevents mortality in HKO mice
- improves outcomes in HKO mice if given early enough during infection

Michels, *JCI Insight 2017;* Stefanova, *Blood 2017;* Stefanova, *Infection and Immunity* 2018

NTBI promotes rapid growth of siderophilic bacteria in plasma

Human plasma supplemented with increasing concentration of iron (ferric ammonium citrate) to saturate transferrin and generate NTBI





What is the function of hepcidin in innate immunity?

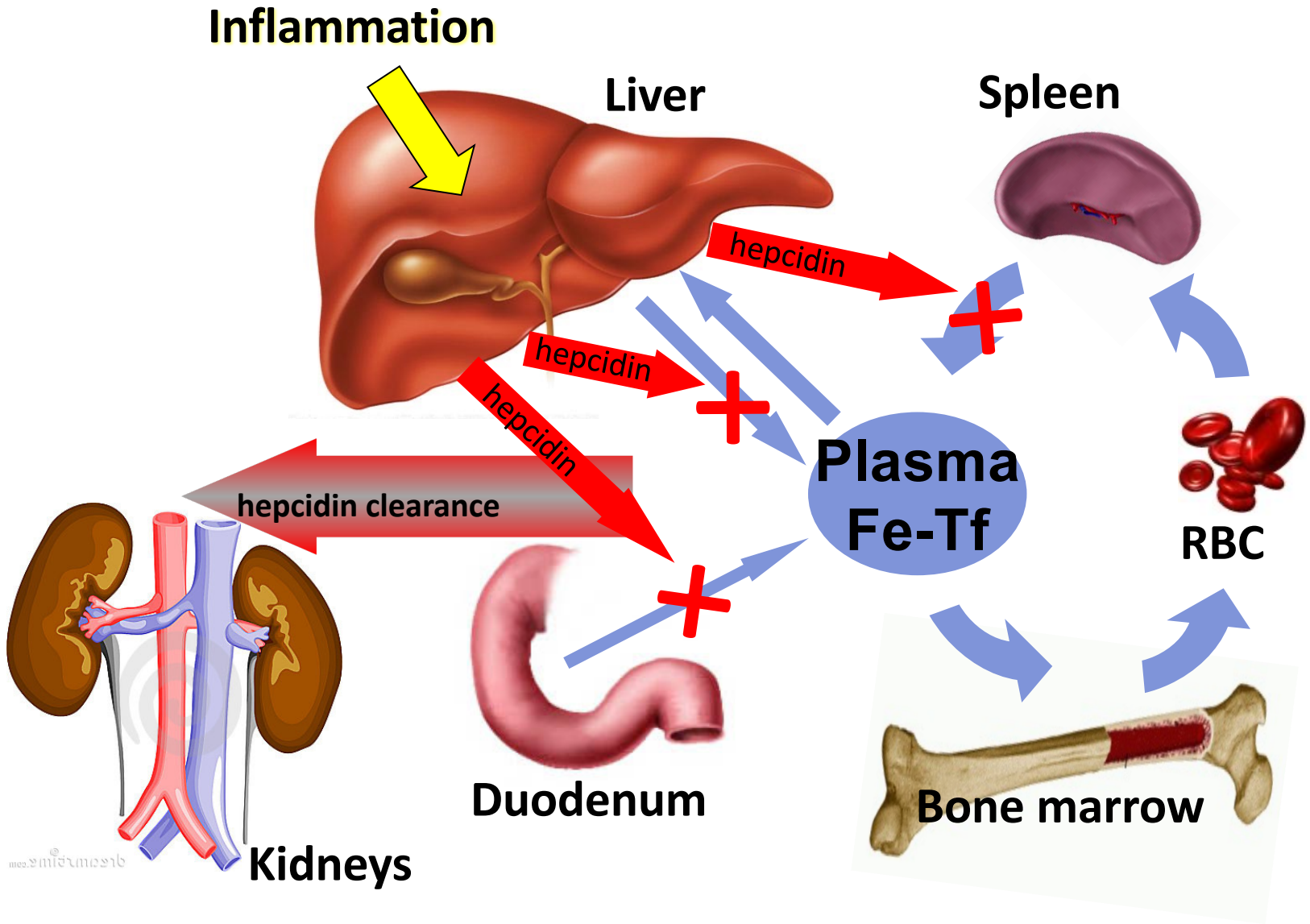
New hypothesis:

- During infection, macrophages phagocytize increased number of erythrocytes and necrotic cells and could release more iron
- Inflammatory cytokines inhibit erythropoiesis, decreasing iron consumption
- Hepcidin induction early during infection prevents the release of iron from macrophages, saturation of transferrin and the formation of nontransferrin-bound iron species (NTBI)
- NTBI (mainly ferric citrate) promotes microbial growth
- Hepcidin inhibits microbial growth by blocking the production of NTBI

Anemia of inflammation
= “Anemia of chronic disease”

- **Hepcidin-dependent effects**
  - Inflammatory cytokines increase hepcidin and cause Fe trapping in macrophages
  - ↓Fe restricts hemoglobin synthesis and erythropoiesis is inhibited
- **Hepcidin-independent effects**
  - Shortened erythrocyte lifespan
  - Direct suppression of erythropoiesis by cytokines

Iron-restricted anemias



Summary

- The absorption and distribution of iron in the body is regulated by an endocrine system consisting of the hormone hepcidin and its receptor/iron transporter ferroportin
- The production of hepcidin by hepatocytes is feedback-regulated by plasma iron concentrations and by the iron stores in the liver
- Genetic disorders of the iron-regulatory system can cause hepcidin excess resulting in iron-restrictive anemia or hepcidin deficiency producing iron-overload diseases
- Erythropoiesis is closely coupled to iron regulation through the production of the erythroid hormone erythroferrone
- In anemias with ineffective erythropoiesis, hepcidin suppression by erythroferrone can cause iron overload and phenocopy hereditary hemochromatosis
- Inflammation induces hepcidin and reduces extracellular iron (especially NTBI) as part of an innate immune response to infections
- During infection/inflammation, high concentrations of hepcidin contribute to anemia of inflammation (anemia of chronic disease)


GOOD BYE
THANK YOU!