Iron Studies

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Objectives

- Iron homeostasis
- Serum iron
- TIBC
- TSAT
- Serum ferritin
- IDA
- Haemachromatosis

Body iron metabolism

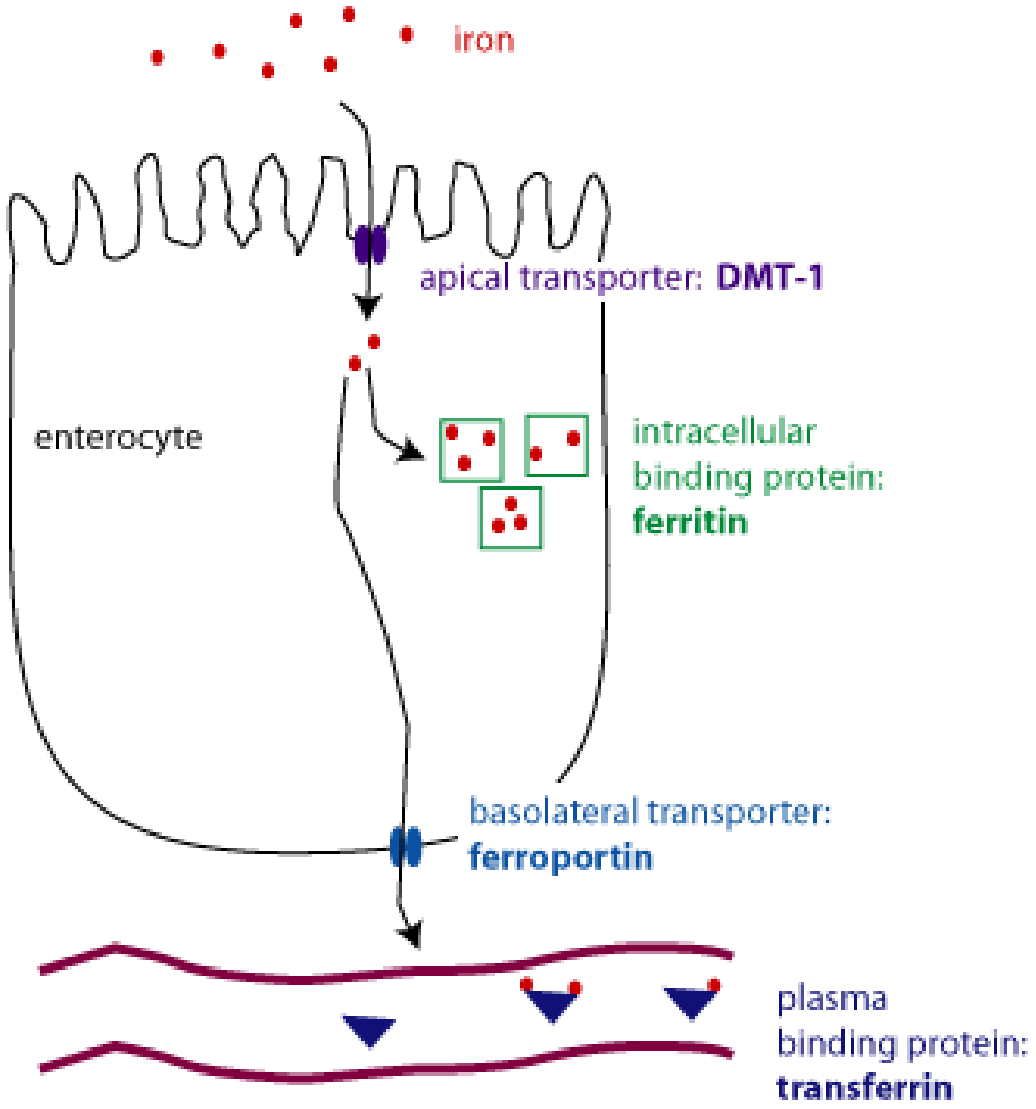
- Normal intake of iron 10-20 mg/d
- About 5-10% is absorbed
- Iron balance is regulated by gut absorption
- Limited capacity to alter the rate of iron excretion

Rate of absorption

- Rate of erythropoiesis
- State of iron stores

- Contents of diet
  - Vit C, red meat
  - Antacids, ppi, calcium, eggs, cocoa, coffee, tea, Phytates
- Chemical state of iron (Fe^{3+}<Fe^{2+})

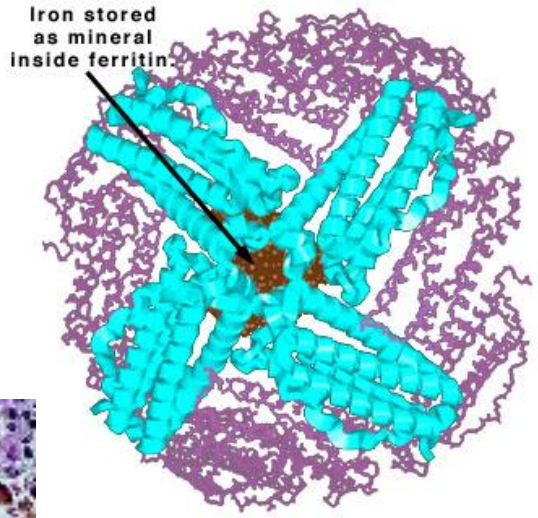
Absorption at the cellular level



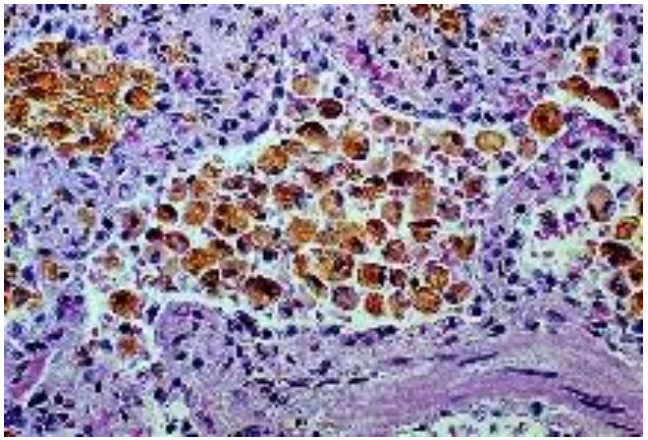
Transport, Storage

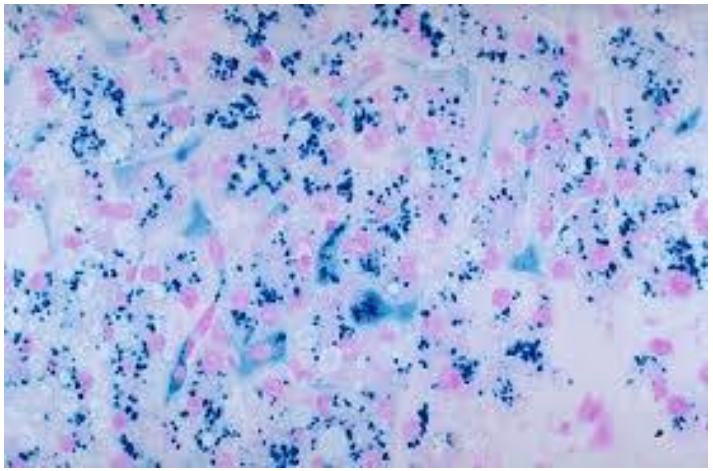
- Transported to plasma→transferrin→taken up by cells
    - Combine with haem→Hb
    - Stored as ferritin (or haemosiderin)

Iron Distribution



- Haemoglobin
- Storage iron
  - Ferritin
  - Haemosiderin
- Tissue iron
- Myoglobin





Excretion

- Cells desquamated (skin/intestine)
- Menstruation/pregnancy
- Iron in faeces is exogenous
- Urine excretion is negligible

Lab assessment of iron status

Serum iron

Serum ferritin

TIBC

Transferrin saturation

Serum iron

- Varies widely
- Diurnal variation (higher values in the morning)
- Menstrual cycle
- Pregnancy

Serum iron

- Levels change (without changes in iron stores) in
  - Acute infections ↓
  - Trauma ↓
  - Chronic inflammatory disorders (RA) ↓
  - Malignant diseases ↓

Analysis

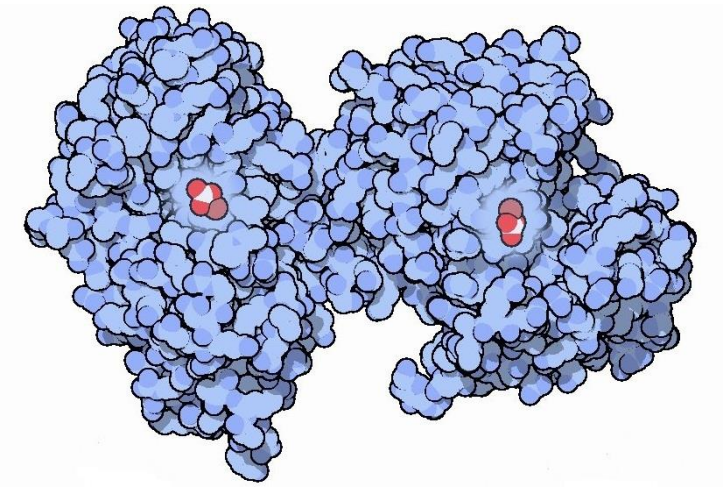
- Iron is released from transferrin by lowering the pH
- Reduced from Fe^{3+} to Fe^{2+}
- Complexed with a chromogen
  - Bathophenanthroline
  - Ferrozine
- Measurement of absorption

Analysis

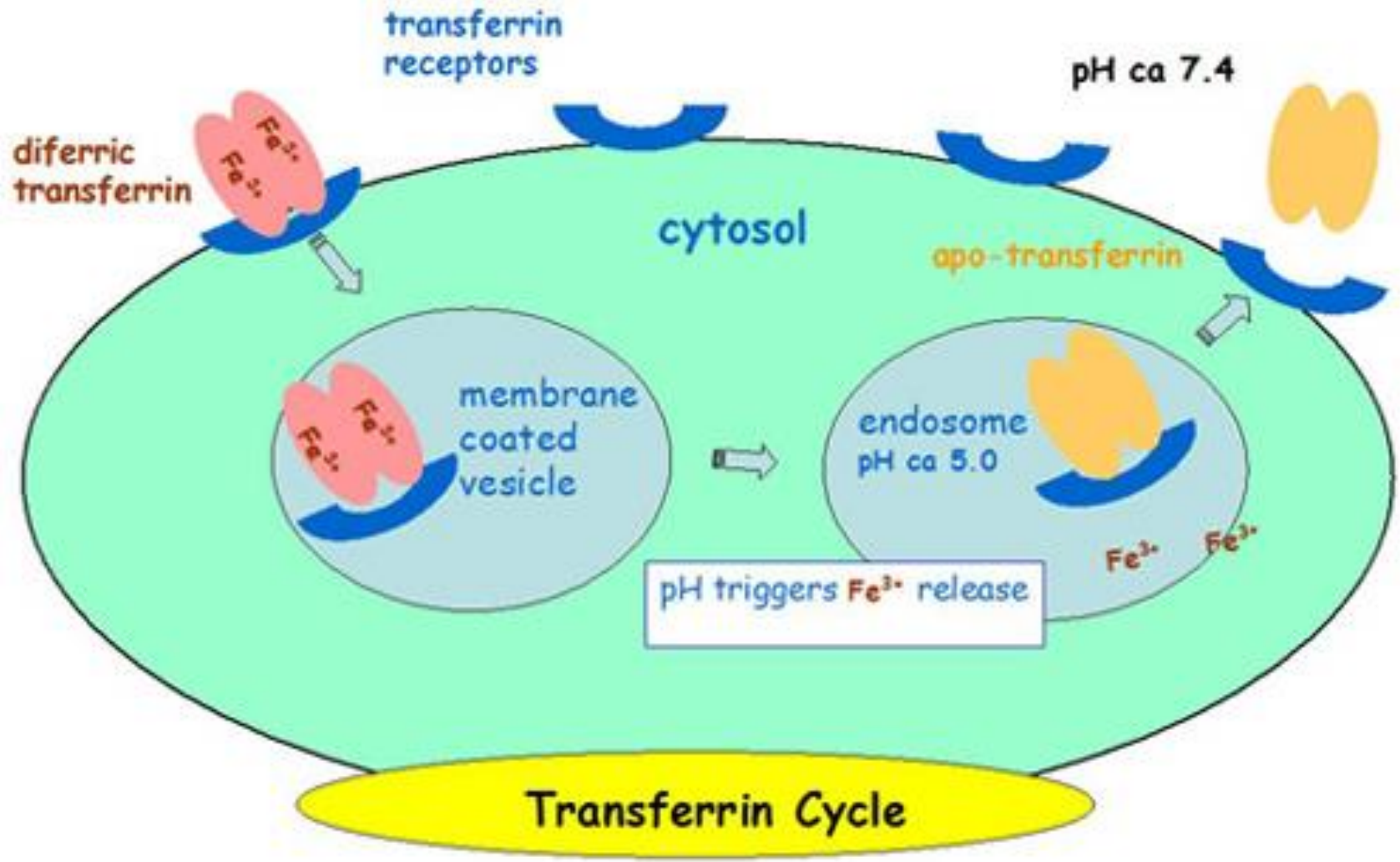
- Addition of acid to serum
  - Release iron from transferrin
  - Reduced from Fe^{3+} to Fe^{2+}
  - Precipitates serum proteins
- Interference
  - Haemolysis
  - Copper - thiourea

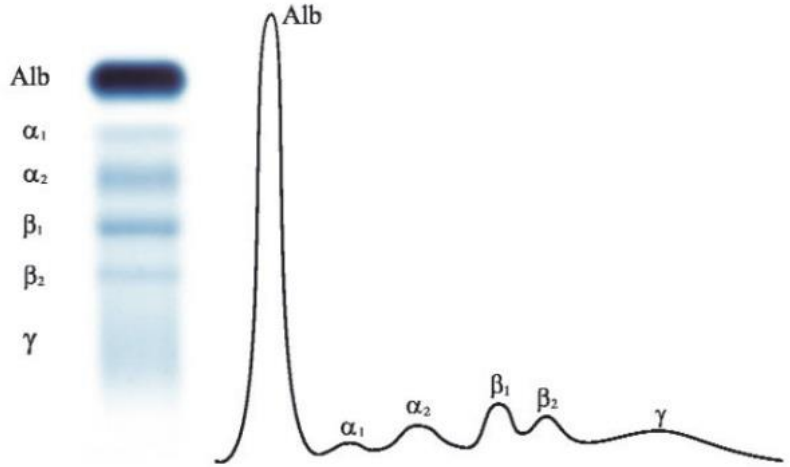
Transferrin

- Transferrin is the carrier protein which binds iron in serum.
- Binds iron very tightly but reversibly (apoT when not bound to iron)
- About 30% of the binding sites are occupied
- Increases in iron deficiency
- Decreases in
  - acute phase response
  - chronic liver disease



Transferrin





Albumin

α1-Antitrypsin

α1- Acid Glycoprotein

α2-Macroglobulin

Ceruloplasmin

Haptoglobins

α-Lipoproteins

Hemopexin

Transferrin

Plasminogen

β-Lipoprotein

C3

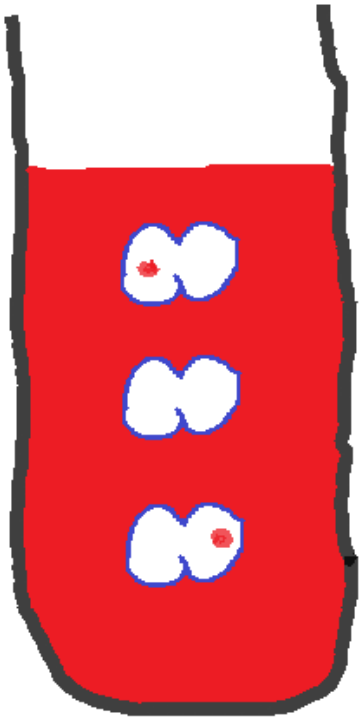
Fibrinogen

Immunoglobulins

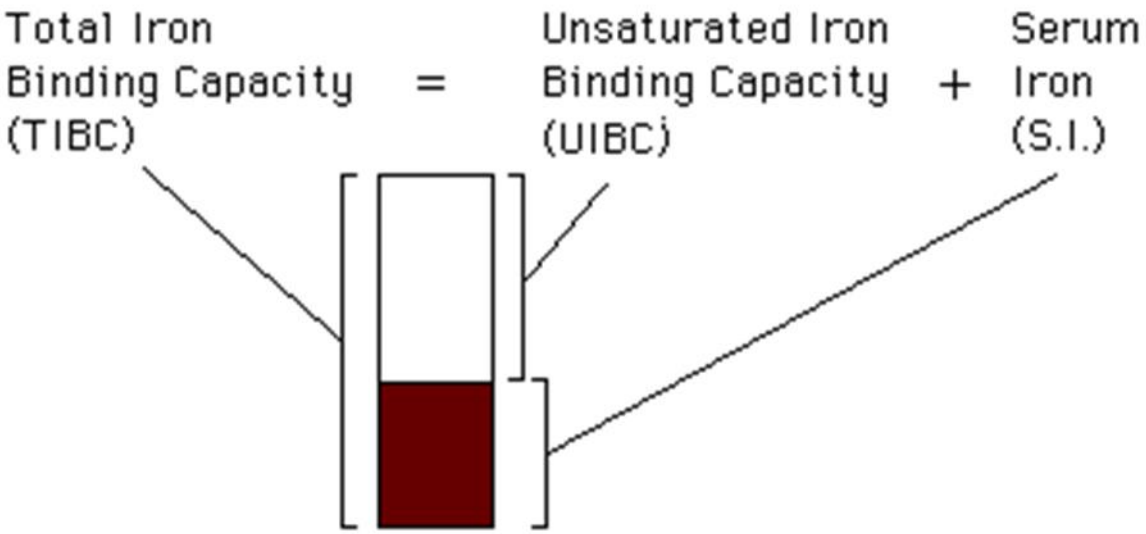
C Reactive protein

TIBC

- Indirect measure of transferrin
- Ability of transferrin to bind iron
- The maximum amount of iron that T can bind

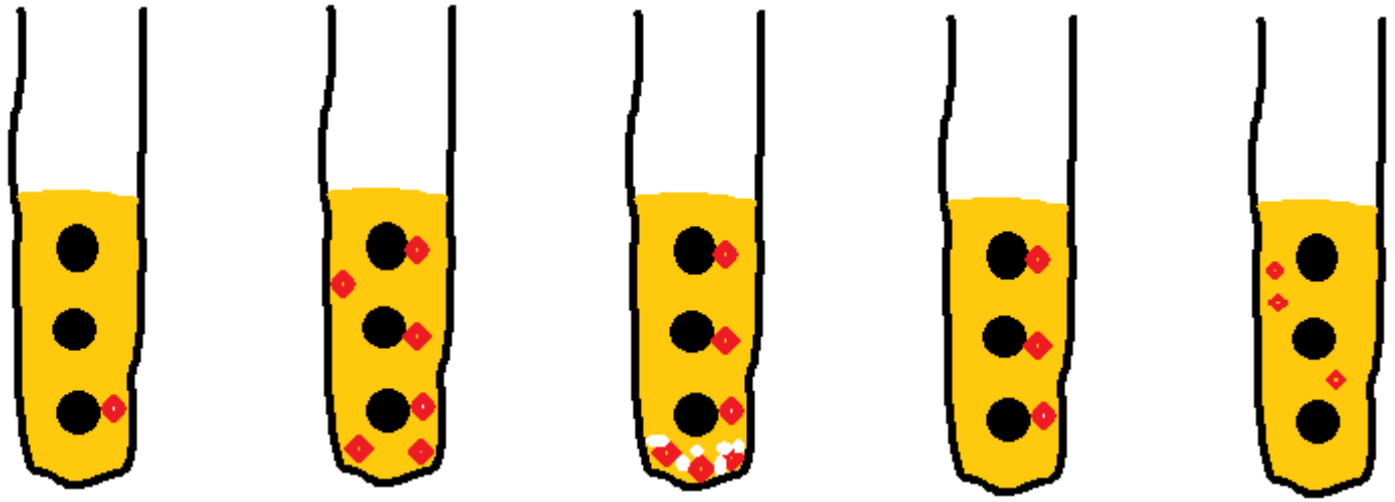


Serum Iron and Iron Binding Capacity



TIBC

- Analysis
  - Add sufficient Fe^{3+} to saturate iron binding sites
  - Then excess Fe^{3+} is removed ($MgCO_3$)
  - Measure iron



TIBC results interpretation

Condition	Serum iron	Transferrin & TIBC
Iron deficiency anaemia	Low	High
Anaemia of chronic disease	Low	Low
Pregnancy, OCP	Normal	High

Transferrin saturation

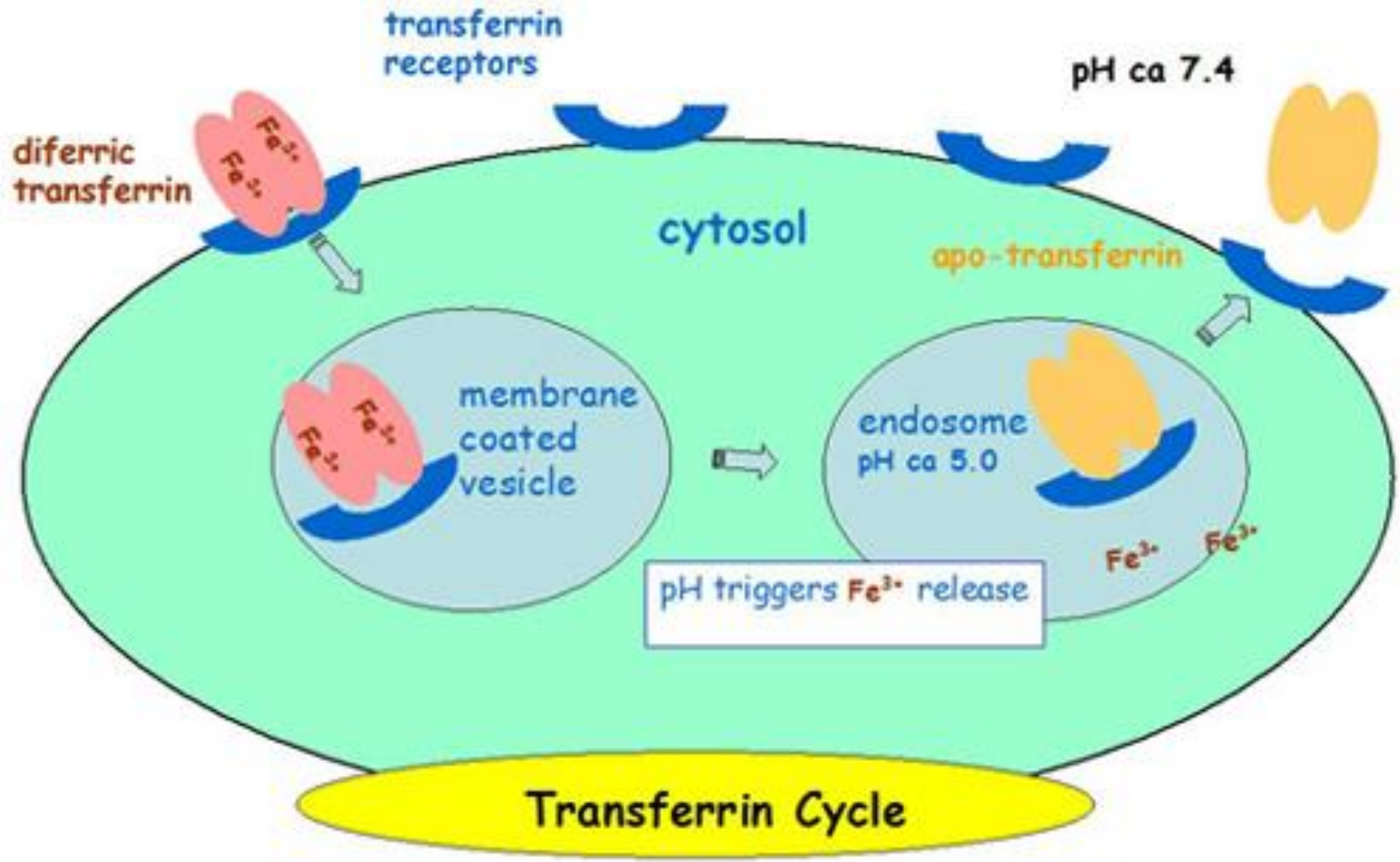
- (Serum iron/TIBC) x 100
- less than 20% - iron deficiency
- more than 50% - iron overload

- In inflammation??

Serum Transferrin Receptor

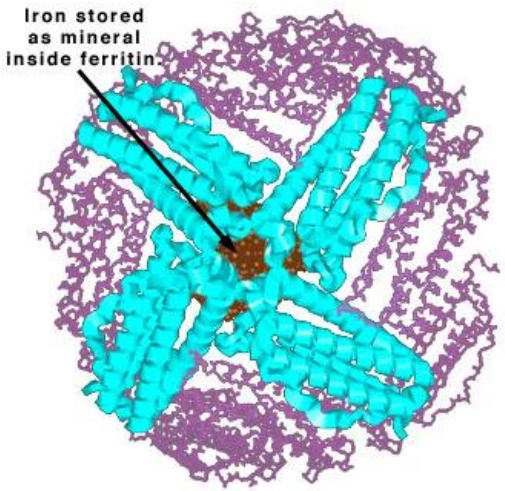
- Immature red cells in BM got lot of TR on their membrane.
- The number of TR ↑ses in iron def and ↓ses in iron OL
- This is seen in serum TR
- But it reflects the amount of erythropoietic activity rather than the iron status

Transferrin



Serum ferritin

- Consist of protein shell and an iron core
- Found in all cells in the body-hepatocytes, macs in BM
- Very little amount is in the serum. This amount is proportional to the total body stored iron



Serum ferritin

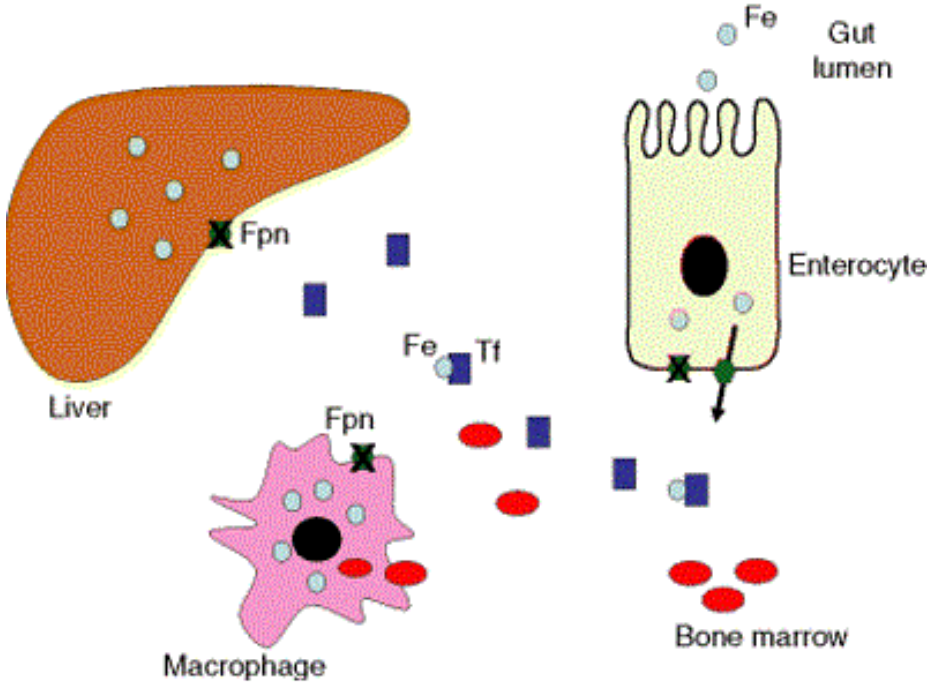
- Closely related to body iron stores
- Low level indicates depleted iron stores
  - Sensitive and specific indicator of iron deficiency
- High serum ferritin
  - 10% - iron overload
  - 90% - Chronic alcohol consumption, metabolic syndrome, obesity, diabetes, malignancy, infection and inflammatory conditions
  - normal STFR rules out iron overload

Ferritin analysis

- Immunoassay

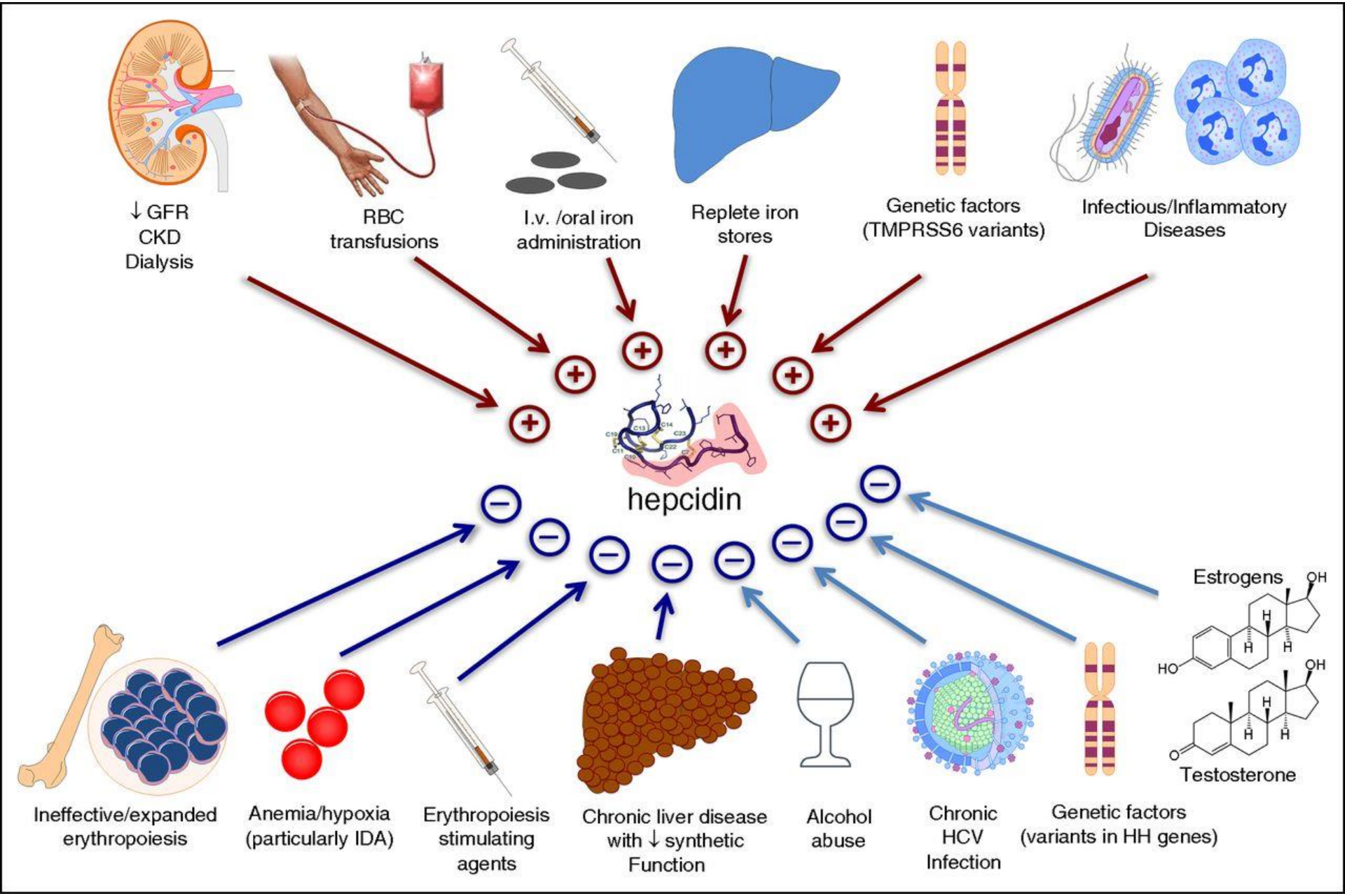
Ferroportin

- Transmembrane iron transporter
- Exports iron into the plasma from the duodenum, macrophages and hepatocytes
- Iron is then bound to transferrin



Hepcidin

- Hepcidin, produced in hepatocytes, is the key regulator of iron homeostasis
- It is a negative regulator of iron release from macrophages (red cell processing and iron recovery, iron store), hepatocytes (iron store) and enterocytes (iron absorption)


↓ GFR
CKD
Dialysis
RBC
transfusions
I.v. /oral iron
administration
Replete iron
stores
Genetic factors
(TMPRSS6 variants)
Infectious/Inflammatory
Diseases
hepcidin
Estrogens
Testosterone
Ineffective/expanded
erythropoiesis
Anemia/hypoxia
(particularly IDA)
Erythropoiesis
stimulating
agents
Chronic liver disease
with ↓ synthetic
Function
Alcohol
abuse
Chronic
HCV
Infection
Genetic factors
(variants in HH genes)

Action of Hepcidin

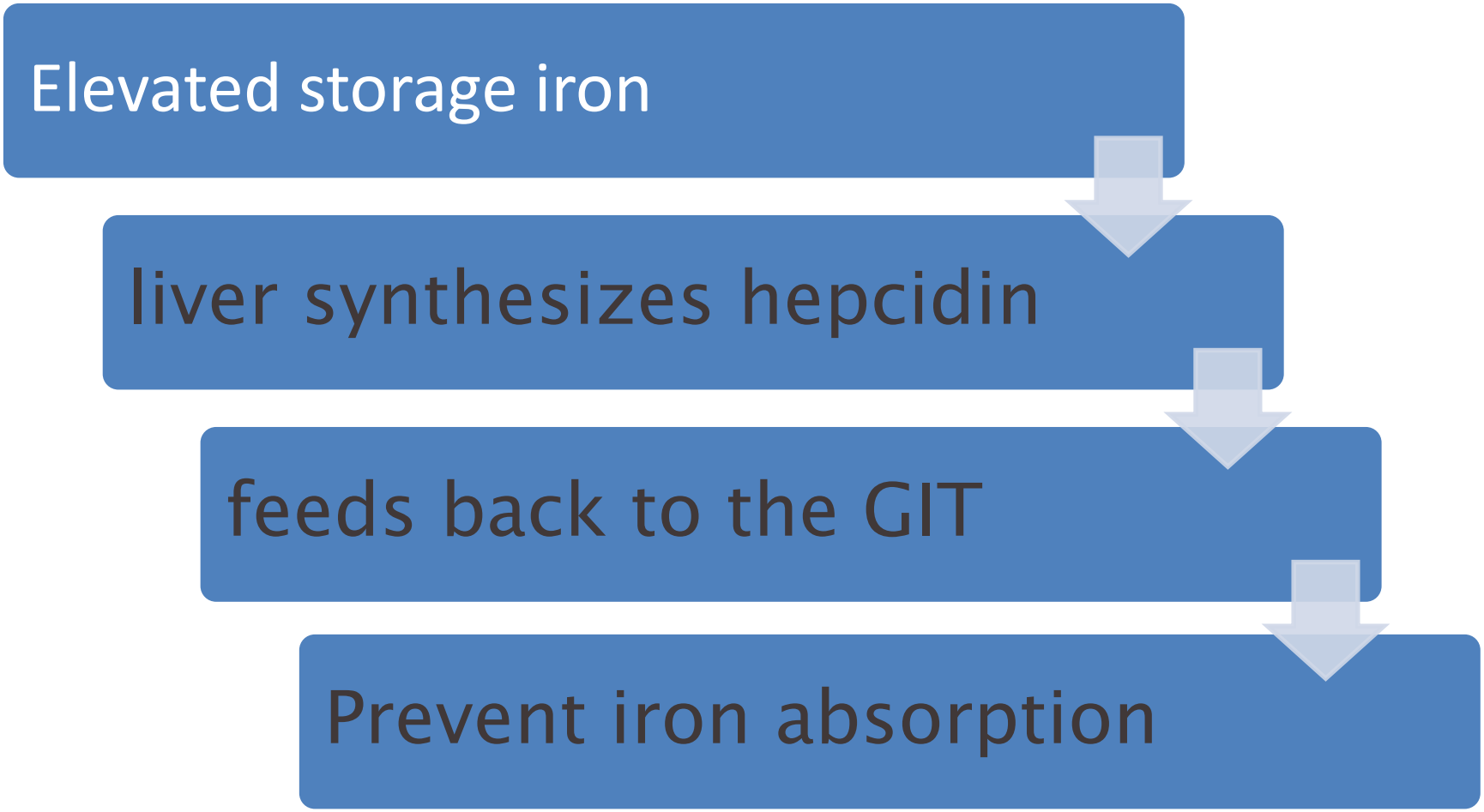
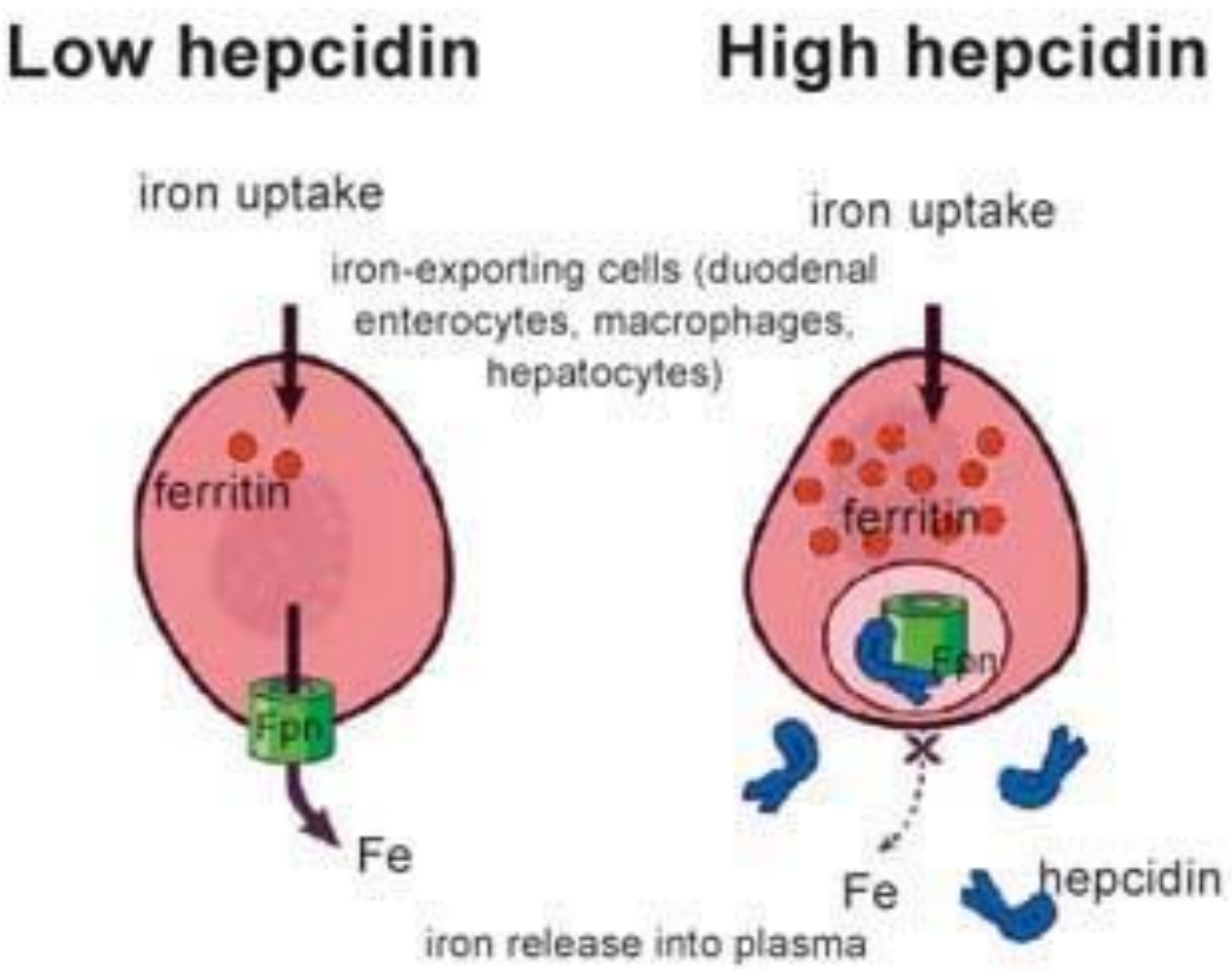


Figure 1 - Mechanism of hepcidin-mediated cellular iron regulation



Fpn = ferroportina (adaptado de Ganz [3]).

Anaemia of Chronic Disease

- Seen in chronic infection, chronic inflammation and malignancy
- Hb values typically 9.5-10.5 g/dL

	IDA	ACD
Serum iron	Low	Low
TIBC	High	Low
TSAT	Very low	Low
Ferritin	Low	High/Normal
ESR	Normal	High
CRP	Normal	High

Case 1

- 30 year old female presented with general symptoms of anaemia and her lab results are as follows:
  - Haemoglobin 10 g/L
  - Serum Iron 15 µmol/L (5-25µmol/L)
  - TRF 3.4 g/L (2.0-3.5g/L)
  - T SAT 15 % (5-35%)
  - Serum Ferritin 7 µg/L (20-200µg/L)

Case 2

- A 47 year old farmer presents with tiredness. His iron studies show the following abnormalities:
  - Serum Iron 40 µmol/L (5-30µmol/L)
  - TRF 2.2 g/L (2.0-3.2g/L)
  - T SAT 73 % (10-45%)
  - Serum Ferritin 128 µg/L (30-500µg/L)

Case 3

- A 57 year-old man who is a chronic alcoholic presents to his GP with tiredness. His LFTs show few abnormalities and his iron studies were also abnormal:
  - T.protein 67 g/L (63-80g/L)
  - Albumin 38 g/L (34-45g/L)
  - ALT 100 U/L (5-40 U/L)
  - AST 67 U/L (10-40 U/L)
  - GGT 344 U/L (5-50 U/L)
  - ALP 228 U/L (35-110 U/L)

Case 3 cont.

– Serum Iron	10 µmol/L	(5-30 µmol/L)
– TRF	2.3 g/L	(2.0-3.2 g/L)
– T SAT	25 %	(10-45 %)
– Serum Ferritin	711 µg/L	(30-500 µg/L)

Single best answer question 1

- Which condition is associated with the lowest percent saturation of transferrin?
  A. Haemochromatosis
  B. Anaemia of chronic disease
  C. Iron deficiency anaemia
  D. Non-iron deficiency anaemia
  E. Pregnancy

Single best answer question 2

- Which condition is most often associated with a high serum iron level?
  A. Nephrosis
  B. Chronic infection
  C. Polycythaemia vera
  D. Noniron deficiency anaemia
  E. Chronic inflammation

Single best answer question 3

- Which of the following is likely to occur first in iron deficiency anaemia?
  A. Decreased serum iron
  B. Increased TIBC
  C. Decreased serum ferritin
  D. Increased transferrin
  E. Decreased MCV

MCQ

- Which statement regarding iron studies is/are true or false?
  A. Ferritin is a beta globulin
  B. Serum iron levels begin to fall before the body stores become depleted
  C. A normal level of serum ferritin rule out iron deficiency
  D. The commonest cause of high serum ferritin is iron overload
  E. TIBC depends on serum iron and serum transferrin levels

Thank You