IN THE NAME OF GOD

Targeting Transferrin Receptor 2: A Novel "Erythropoiesis-Stimulating Approach"

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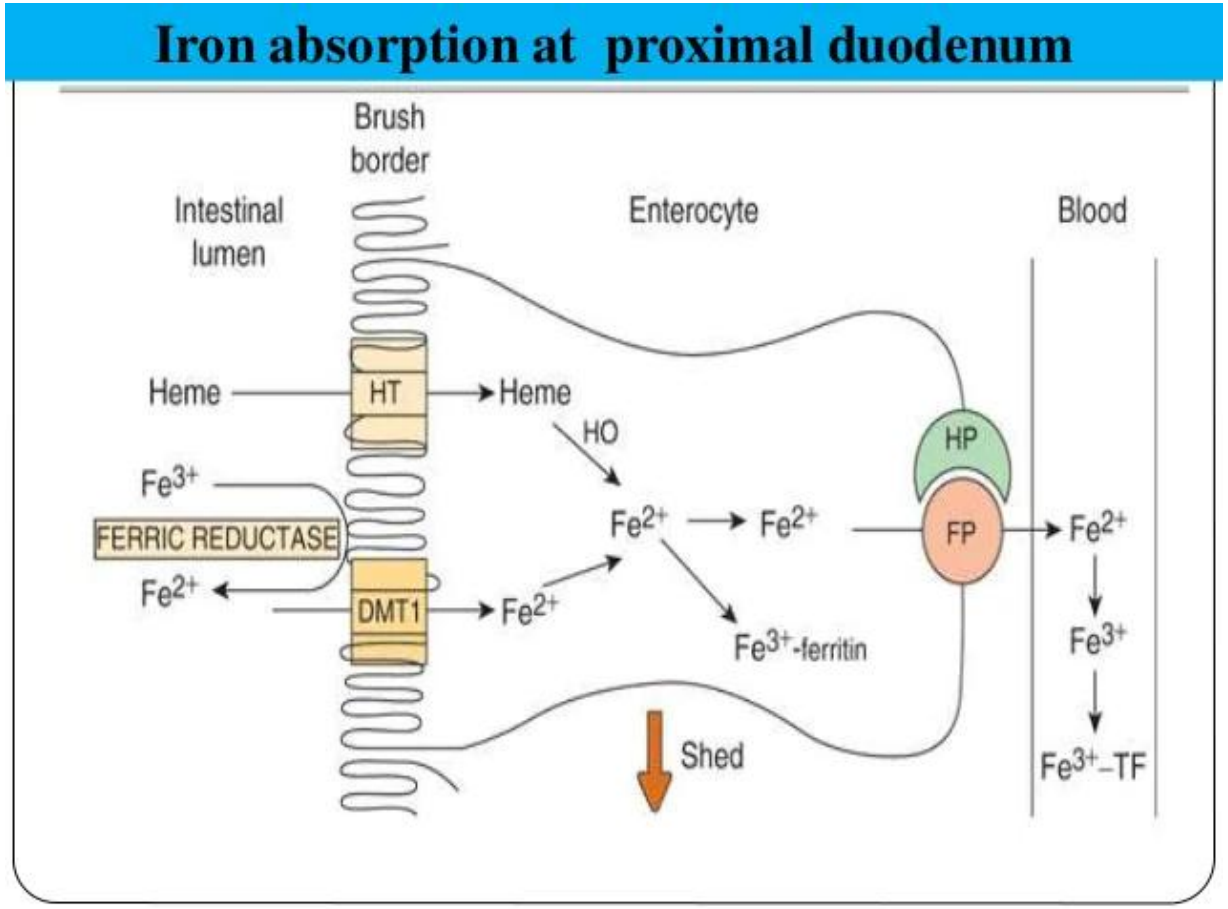
Iron Metabolism

- Iron plays vital roles in the human body including
- Enzymatic processes,
- Oxygen-transport via hemoglobin
- Immune response.

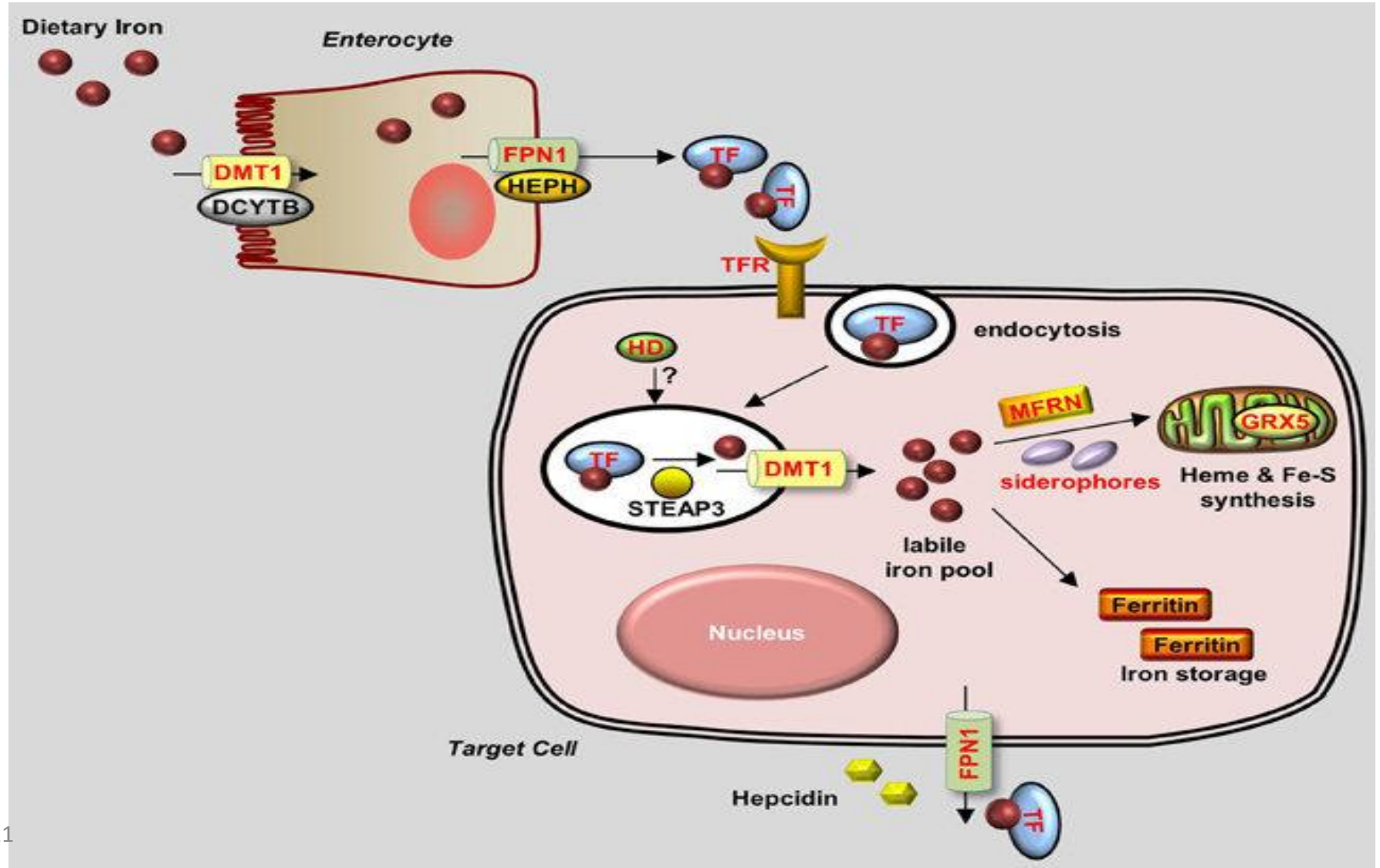
Iron Metabolism

- Most of the iron requirements are provided by recycling the iron present in senescent erythrocytes and the release of iron from storage sites .(95%)
- In addition, there is no physiological mechanism to regulate iron excretion.
- It is lost from the
- ***desquamation of intestinal epithelial cells*,**
- ***skin cells***
- ***and blood loses***

IRON METABOLISM



IRON METABOLISM



Cellular iron regulation

- **Iron import**
- Most cell types take up iron primarily through receptor-mediated endocytosis via transferrin receptor 1 (TFR1), transferrin receptor 2 (TFR2) and GAPDH.
- TFR1 has a 30-fold higher affinity for transferrin-bound iron than TFR2 and thus is the main player in this process.
- The higher order multifunctional glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) also acts as a transferrin receptor.

Cellular iron regulation

- *Once absorbed or recycled*, iron binds in the plasma to the iron carrier, transferrin (Tf), which is essential for efficient iron transfer to target cells.
- ***Binding to transferrin receptor 1 (TFR1),***
- transferrin delivers iron to cells through the endosomal cycle, which is crucial for erythroblasts, muscle cells, B- and T-lymphocytes,

Cellular iron regulation

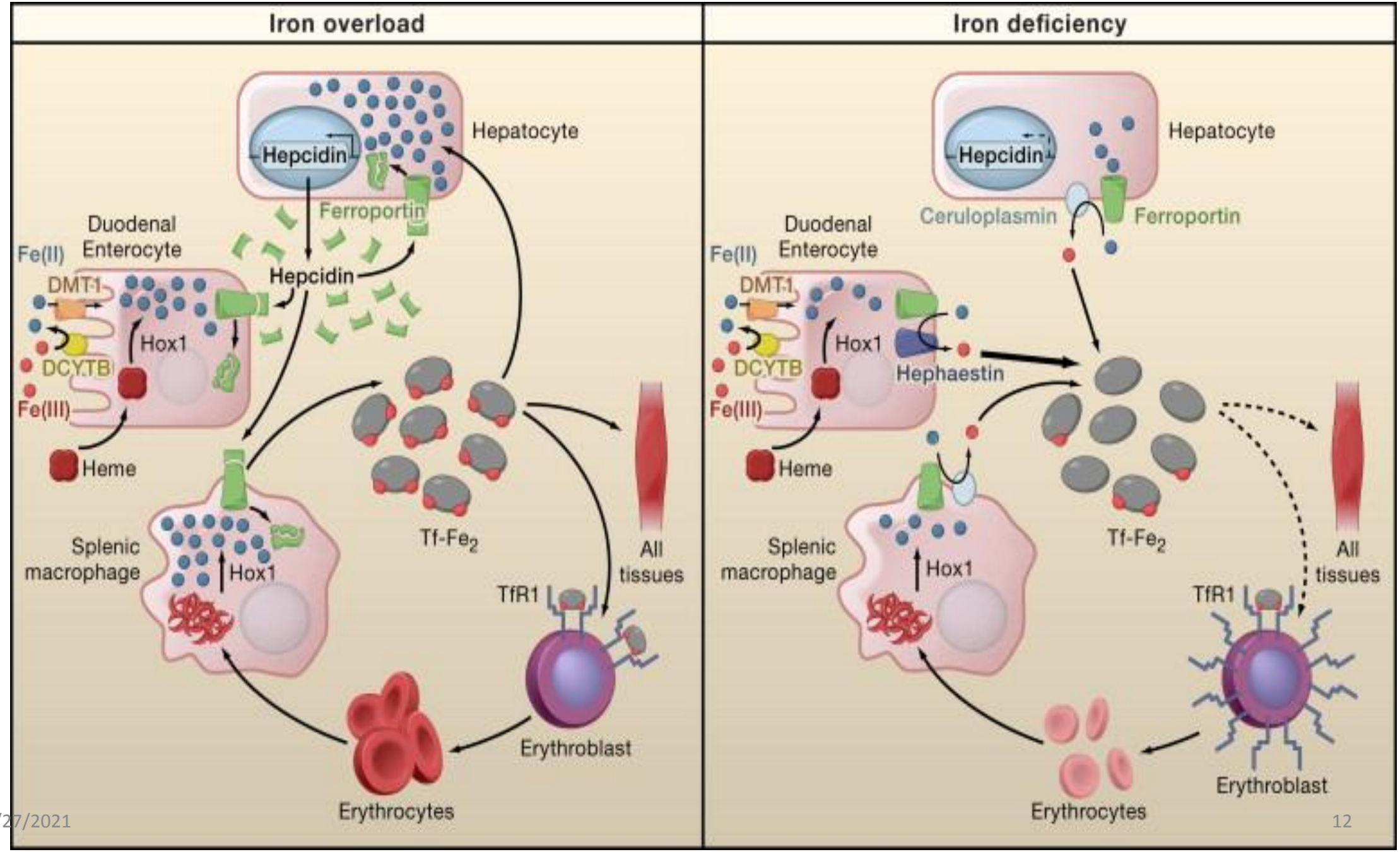
- ***By binding to transferrin receptor 2 (TFR2),***
- whose expression is restricted to hepatocytes and erythroblasts and which has lower binding affinity than TFR1,
- **In high plasma iron concentration, transferrin binds to TFR2, upregulates hepcidin in hepatocytes**,
- and reduces EPO responsiveness in erythroid cells, where TFR2 binds EPO receptor.
- *In iron, deficiency occurs opposite regulation* .

Cellular iron regulation

- TfR2 is expressed in immature erythroid cells, where it interacts with the *erythropoietin receptor, stabilizing it on the cell surface* .
- In mice, conditional inactivation of *TfR2* in the bone marrow causes erythrocytosis with normal erythropoietin levels.
- TfR2 modulates the erythropoietin sensitivity of erythroblasts by sensing iron deficiency through decreased diferric transferrin.
- Through its sensor activity, TfR2 may coordinate erythropoiesis with hepcidin synthesis .

Cellular iron regulation

- A *circulating acute-phase protein produced in the liver*, **hepcidin**, **is the key regulator of iron metabolism.**
- Its purpose is to maintain adequate systemic iron levels.
- Hepcidin is thought to decrease the absorption of iron in the doudenum by downregulating the expression of apical divalent metal transporter 1 (DMT1) in the enterocyte.
- Indeed, hepcidin promotes the internalization of ferroportin into the cell for its degradation, and thus preventing iron form exiting into the circulation from enterocytes, macrophages or other iron stores.
- *Iron overload increases hepcidin levels, whereas iron deficiency reduces its concentrations .*


Iron overload
Hepcidin
Hepatocyte
Ferroportin
Duodenal Enterocyte
Fe(II)
DMT1
Hepcidin
Hox1
DCYTB
Fe(III)
Heme
Splenic macrophage
Hox1
Tf-Fe2
All tissues
TfR1
Erythroblast
Erythrocytes
Iron deficiency
Hepcidin
Hepatocyte
Ceruloplasmin
Ferroportin
Duodenal Enterocyte
Fe(II)
DMT1
Hox1
DCYTB
Hephaestin
Fe(III)
Heme
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All tissues
TfR1
Erythroblast
Erythrocytes

Anemia in CKD

- Anemia is a common complication in chronic kidney disease (CKD), and is associated with a reduced quality of life, and an increased morbidity and mortality.
- The mechanisms involved in anemia associated to CKD are diverse and complex.
- Patients are most commonly managed with oral or intravenous iron supplements and with erythropoiesis stimulating agents (ESA). ***However, these treatments have associated risks, and sometimes are insufficiently effective.***

Anemia in CKD

- Nonetheless, in the last years, there have been some remarkable advances in the treatment of CKD-related anemia, which have raised great expectations.
- On the one hand, a novel family of drugs has been developed: the hypoxia-inducible factor prolyl hydroxylase inhibitors (HIF-PHIs).
- These agents induce, among other effects, an increase in the production of endogenous EPO, improve iron availability and reduce hepcidin levels.
- On the other hand, recent clinical trials have elucidated important aspects of iron supplementation, which may change the treatment targets in the future.

Anemia in CKD

- Absolute and relative iron deficiency are frequent conditions in CKD patients.
- Blood losses, for instance, due to blood left in the hemodialysis circuit are common.
- In addition, the “uremic” state and other comorbidities causing inflammation prevent from an adequate intestinal iron absorption and from the release of iron from the body stores.
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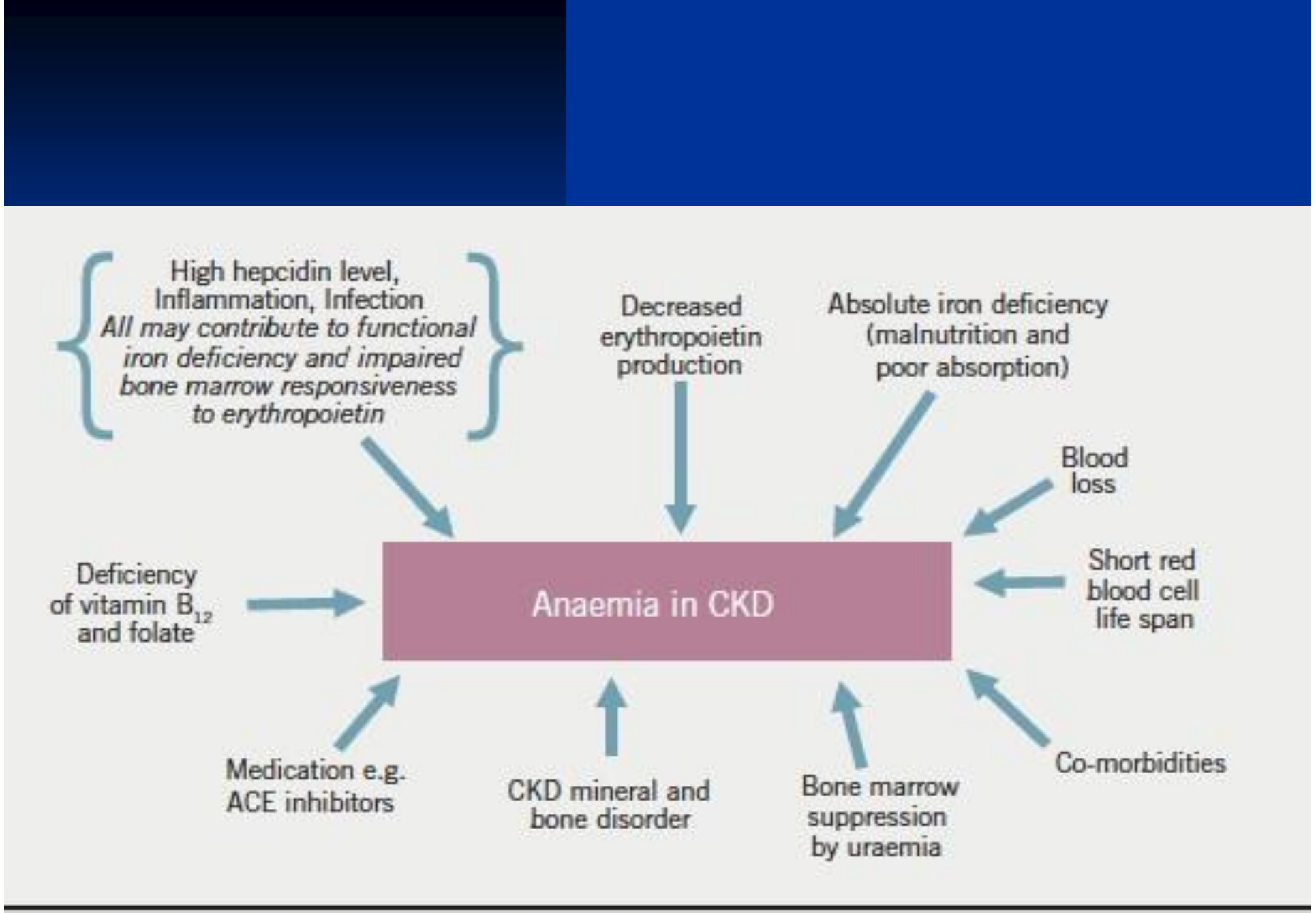
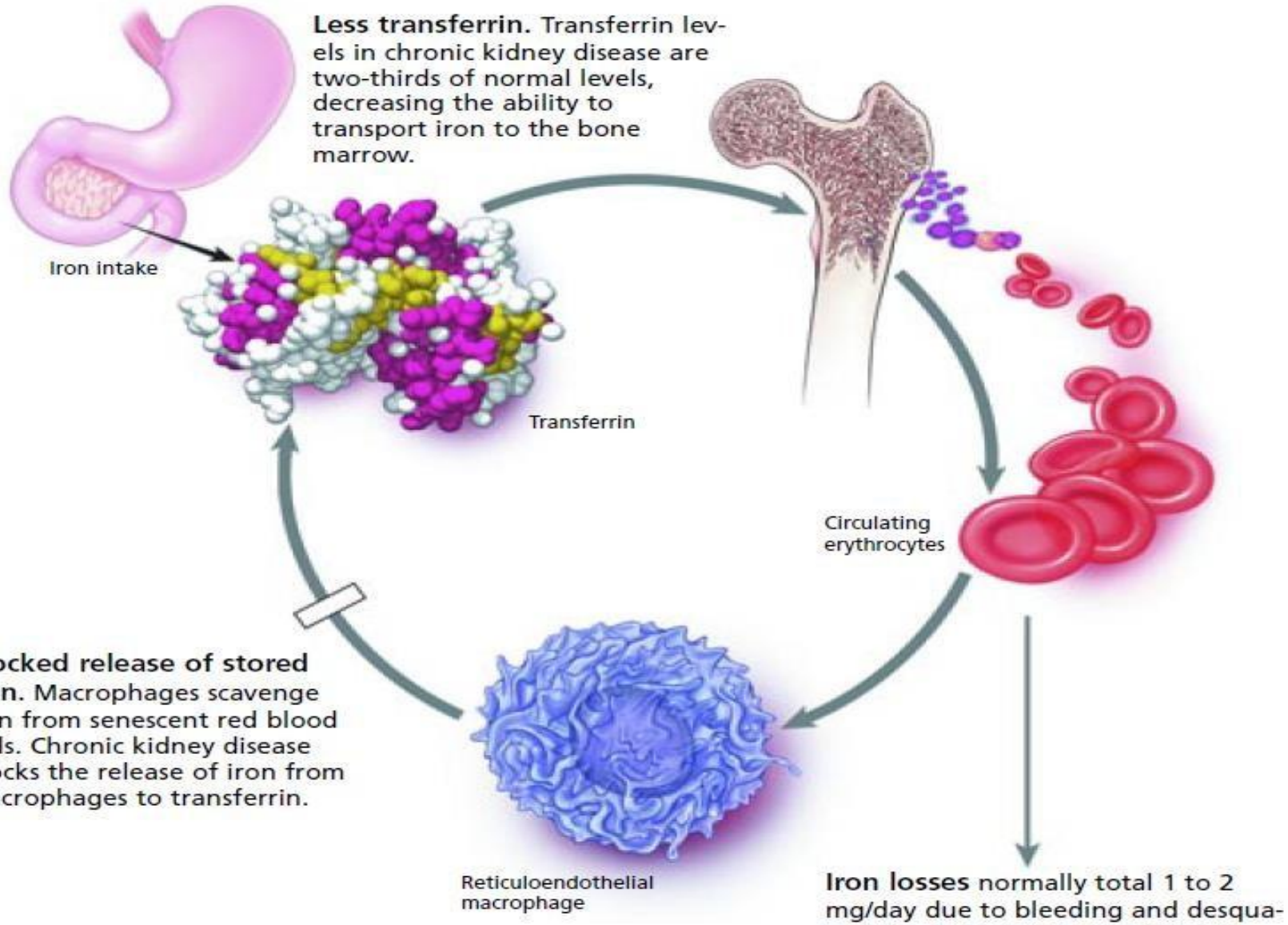
Anemia in CKD

- ***Proinflammatory cytokines* contribute to a *functional iron deficiency* in several ways:**
- They stimulate the hepatic synthesis of hepcidin,
- they induce the expression of DMT1 in macrophages,
- and induce the expression of ferritin, and inhibit that of ferroportin.
- They also promote the *uptake of iron bound to transferrin into macrophages, via the transferrin receptor .*
- Moreover, hepcidin is eliminated by kidney and its clearance is reduced as eGFR declines.
- All these mechanisms favor intracellular iron storage, *limiting the availability of iron in CKD.*



Adapted from Agarwal AK. *J A Med Dir Assoc* 2006;**7**:S7–S12

Iron homeostasis is perturbed in patients with chronic kidney disease. The ability to provide enough iron for erythropoiesis is disrupted at several points in the iron cycle for reasons not yet clear. Thus, patients receiving erythropoiesis-stimulating agents will require iron supplementation, often intravenously, in order to produce additional red blood cells.



Iron Metabolism in Chronic Kidney Disease

- The central role in iron metabolism regulation plays an *increased level of hepcidin,*
- *decreasing FPN on cell membranes and thus diminishing duodenal iron absorption and acquirement from stores in RES.*
- Hepcidin expression and production are stimulated by *iron treatment,*
- *inflammation, uremic milieu, and fibroblast growth factor 23 (FGF23),*
- *and, at the same time, its removal is impaired due to decreased glomerular filtration rate (GFR).*
- Both *erythropoietin and erythroferrone play suppressive effect on hepcidin production.*

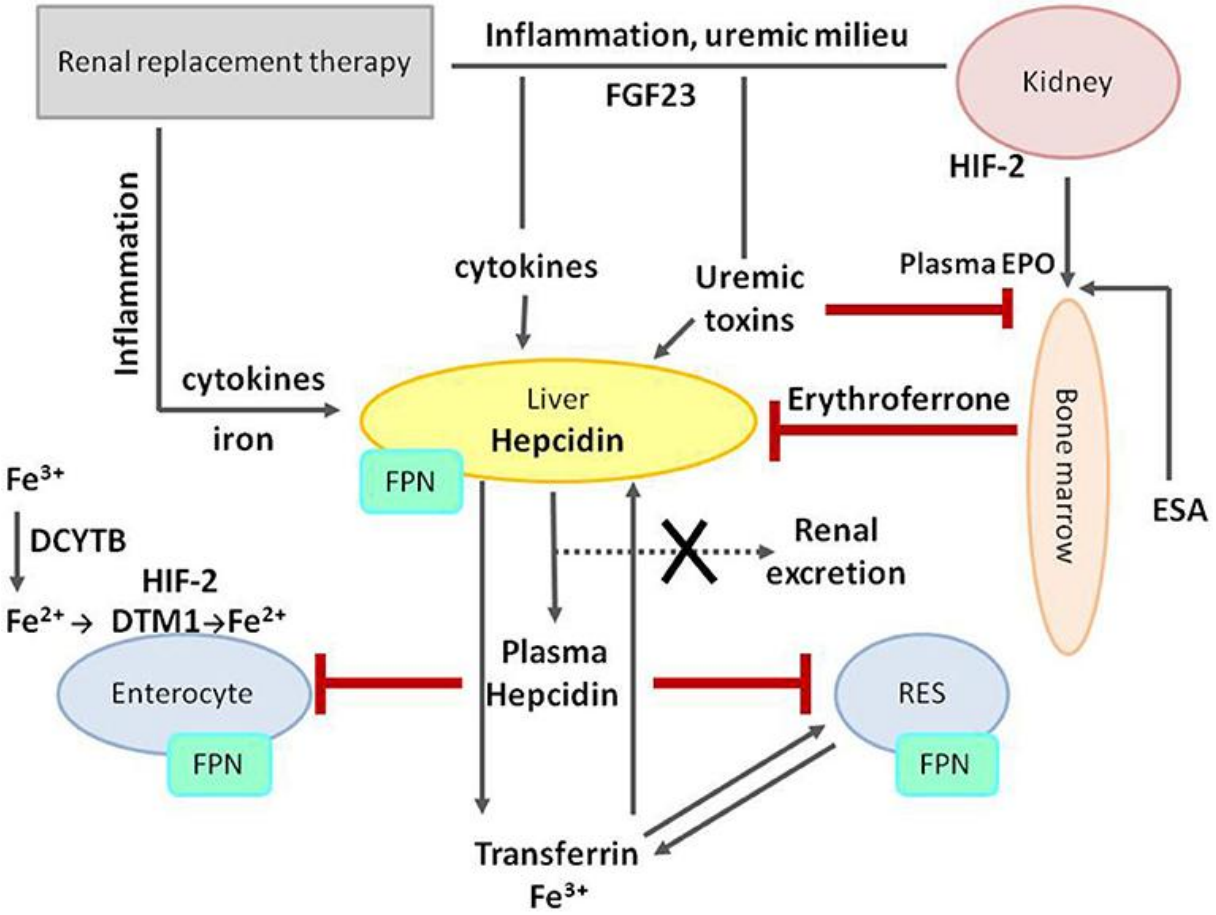
Erythropoiesis in Chronic Kidney Disease

- **Hepcidin–Ferroportin Axis—A Key Regulator of Iron Balance in Chronic Kidney Disease**
- In general, downregulation of ferroportin and excess hepcidin promote iron restriction and limit its absorption, availability for recycling, and delivery to erythroid precursors for hemoglobin synthesis .
- Ferroportin mediates all major iron flows, and the loss of ferroportin from cell surfaces decreases the delivery of iron from cells to blood plasma.

Erythropoiesis in Chronic Kidney Disease

- Due to this export block, absorbed dietary iron cannot be transferred from duodenal enterocytes to plasma, is retained in the cells, and then lost during shedding.
- In addition to this mechanism, in CKD patients, iron absorption may be impaired due to insufficient dietary supply and the use of phosphate binders typically used to treat hyperphosphatemia.
- The loss of ferroportin blocks the release of recycled iron from macrophages, enhances retention, and hinders mobilization from stores in the presence of increased demands.
- Parenteral iron administration, often used in patients with CKD, stimulates hepcidin expression and may paradoxically worsen iron restriction.

Iron Metabolism in Chronic Kidney Disease



Erythropoiesis in Chronic Kidney Disease

- In CKD, ***the mechanism of regulation of EPO secretion in response to the hypoxic stimulus remains fairly long functional***, and EPO levels may be normal or even slightly increased, however inappropriately low relative to the degree of anemia
- Along with the loss of renal parenchyma, the efficiency of EPO production gradually decreases, and its deficiency becomes more pronounced.
- Moreover, increasing levels of kynurenine (product of L-tryptophan) and indoxyl sulfate (uremic toxin) are observed in uremia, aryl hydrocarbon receptor nucleus translocator (ARNT) is activated and competes with HIF-2α to prevent its binding to HIF-β, and EPO production is decreasing

Erythropoiesis in Chronic Kidney Disease

- In addition, excess iron reduces HIF-2α and EPO expression in the event of EPO deficiency
- These factors simultaneously stimulate expression and production of hepcidin, a critical factor in iron dysregulation in CKD.

- The fact that anemia in CKD develops even despite of elevated EPO levels may also result from peripheral EPO hyporesponsiveness or resistance caused by inflammation or secondary hyperparathyroidism

Blood (2019) 134 (Supplement 1): 956.

- **Targeting Transferrin Receptor 2: A Novel "Erythropoiesis-Stimulating Approach" in a Model of Anemia of Chronic Kidney Disease**

Targeting Transferrin Receptor 2: A Novel "Erythropoiesis-Stimulating Approach" in a Model of Anemia of Chronic Kidney Disease

- The current treatment, based on erythropoiesis-stimulating agents (ESA), is far from optimal, because of potential off-target side effects.
- The same holds true for HIF-stabilizers tested in phase 3 clinical trials.
- For this reason, the identification of agents that selectively boost erythropoiesis would be of great benefit.

Targeting Transferrin Receptor 2: A Novel "Erythropoiesis-Stimulating Approach" in a Model of Anemia of Chronic Kidney Disease

- Transferrin Receptor 2 (TFR2) is a protein expressed in hepatocytes, where it modulates iron homeostasis activating hepcidin production, and in erythroid cells, where it acts as a partner of erythropoietin (EPO) receptor decreasing EPO signaling.
- *Tfr2* deletion in the liver causes iron overload due to low hepcidin levels, while its deletion in erythroid compartment enhances erythropoiesis through increased EPO

Targeting Transferrin Receptor 2: A Novel "Erythropoiesis-Stimulating Approach" in a Model of Anemia of Chronic Kidney Disease

- **Results**
- BM *Tfr2*deletion, increasing EPO sensitivity of erythroid cells, improves erythropoiesis and anemia until iron levels remains adequate.

Targeting Transferrin Receptor 2: A Novel "Erythropoiesis-Stimulating Approach" in a Model of Anemia of Chronic Kidney Disease

- Given the TFR2 restricted expression, its inactivation would enhance EPO responsiveness selectively in erythroid cells, minimizing the risk of side effects.
- Targeting erythroid *Tfr2* might be considered a novel "erythropoiesis-stimulating approach", likely applicable to other forms of anemia due to insufficient EPO stimulation and response.


In God we trust