Iron Regulation Gone Wrong

Stephanie Blackburn, MHS, MLS(ASCP)CM

Objectives

- Describe the function of proteins involved in iron regulation.
- Explain the development of iron deficiency.
- Evaluate cases involving iron deficiency and hemochromatosis.

Case Study

- 2-year old female
- Checkup with pediatrician for wellness visit
- Identified with anemia
  - Microcytic, hypochromic
  - Low serum iron

Case study

- Otherwise, healthy; eats well
- Treatment
  - Iron supplements
- Non-responsive to treatment after adequate time interval

Overview of iron regulation

Iron is highly reactive

- It is able to bind and release oxygen in hemoglobin

However…

Free radicals = oxidizing agents

- It is toxic because it causes oxidation of proteins, lipids, DNA via the Fenton reaction

Well-balanced diet

- Sufficient iron to meet daily requirements
- 10% of 10-20 mg of iron absorbed
  - This balances the normal daily loss of 1-2 mg
- Efficiency of iron absorption increases to 20% when there is a greater need

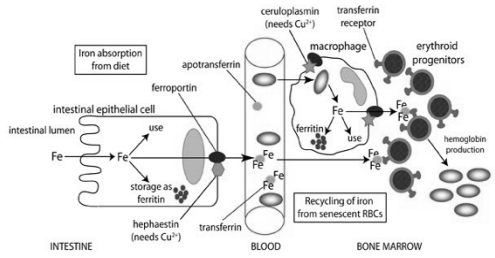
7

Tight regulation

- Only mechanism for iron regulation is via intestinal absorption from diet
  - Increase absorption when body needs iron
  - Decrease absorption when body has adequate iron needs, to avoid having too much

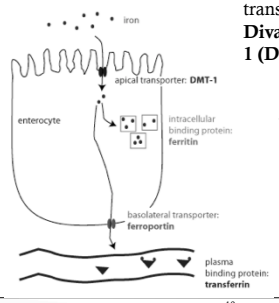
8

Iron kinetics



9

When the body needs iron

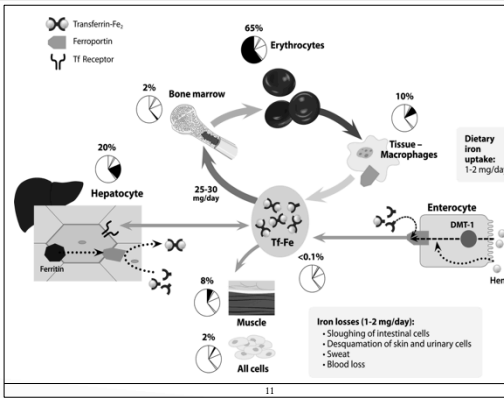


Iron is carried transferred across the intestinal epithelial cells by the transport protein **Divalent metal transporter 1 (DMT-1)**

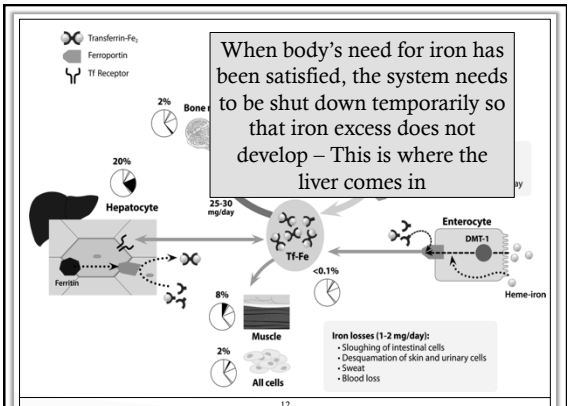
Iron is transported to bloodstream by the membrane protein **ferroportin**

In bloodstream, iron binds to the transport glycoprotein **transferrin**

10



11



12

Regulation of iron absorption

Short-term increase in dietary iron is not readily absorbed

↓

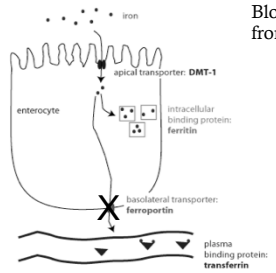
Mucosal cells have accumulated iron and "block" additional uptake

13

When liver senses that there is enough iron in the body, it produces a protein called **hepcidin**

14

What does hepcidin do?



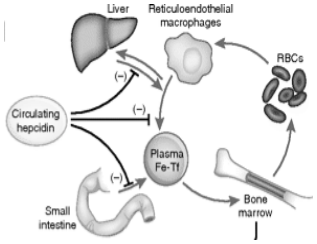
Blocks absorption of iron from enterocyte into blood

Leads to degradation of ferroportin

As body iron stores decline, hepcidin diminishes & ferroportin becomes active again, transporting iron into circulation

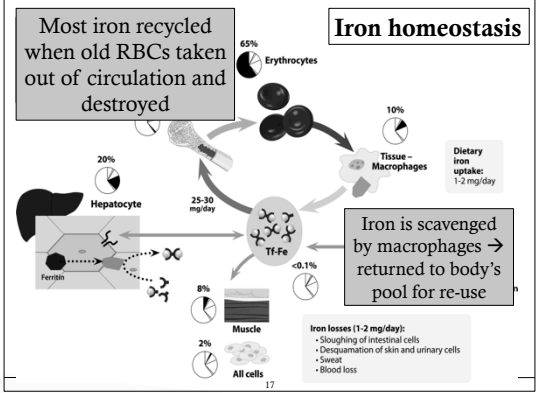
15

Hepcidin also regulates release of iron from macrophages and hepatocytes via ferroportin in their membranes AND decreases iron absorption



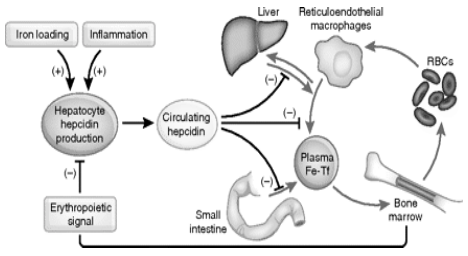
16

Most iron recycled when old RBCs taken out of circulation and destroyed



Iron is scavenged by macrophages → returned to body's pool for re-use

17



Iron homeostasis is closely regulated via intestinal absorption

18

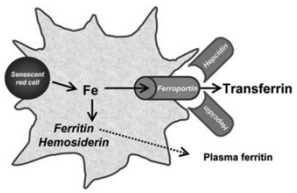
Effects of diet on iron absorption

- Increase iron absorption
  - Citrate & ascorbate
- Decrease iron absorption
  - Tannates
- Iron in heme found in meats → more readily absorbed

19

Iron storage

- Storage iron occurs in two forms:
  - Ferritin
  - Hemosiderin



20

Ferritin

- Protein that acts as holding vessel
- Contains iron we do not presently need
- Produced by nearly every cell in body
- Largest amount in brain and liver

21

Hemosiderin

- Too much ferritin → changes to hemosiderin
- Contains ferric oxide
- Small amount → NORMAL
- Large amount → HARMFUL
  - Accumulates in cells of heart, liver, lungs, pancreas, CNS, etc.

22

How can iron deficiency develop?

23

Intake does not keep up with need

Inadequate diet

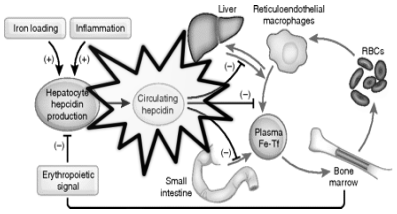
Excessive loss

Increased need

Impaired absorption

24

Let's take a closer look at hepcidin

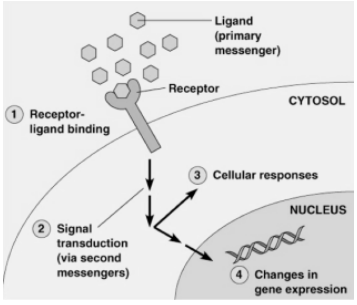


25

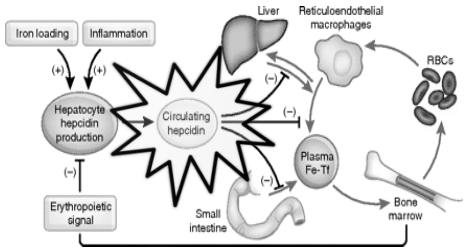
A brief review of cell biology…

26

How do cells receive and respond to messages from the outside?



27



Hepcidin production is the expression of the hepcidin gene as a result of signal transduction in hepatocytes

28

Remember…

- As hepcidin rises:
  - Ferroportin decreases
  - Iron absorption decreases

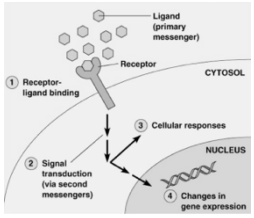
29

There are two pathways to hepcidin production in hepatocytes:

1. Ligand with information about how much iron is circulating

AND

1. Ligand with information about how much iron in storage



2. Membrane receptors receive the message & transfer information inside of the cell

3. Second messenger carries information into nucleus

4. Hepcidin gene is upregulated

Results in increased plasma hepcidin and diminished ferroportin activity → decreased iron absorption

30

↑ Iron = ↑ Hepcidin = ↓ Ferroportin

Iron absorption & recycling slows

↓ Iron = ↓ Hepcidin = ↑ Ferroportin

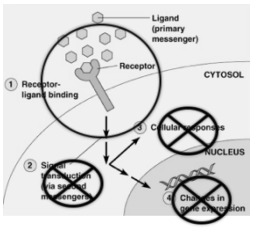
Iron absorption & recycling increase

31

What could possibly go wrong?

32

What happens if any of these proteins' genes have a mutation and the proteins are non-functional?



Hepcidin cannot be produced and ferroportin is constantly active

↓

Iron is continuously absorbed

↓

This is hemochromatosis or iron overload

33

Hemochromatosis

- In most cases, mutations of iron regulatory proteins prevent production of hepcidin
- Results in continuous iron absorption at the intestines due to continual ferroportin activity

34

Hereditary hemochromatosis

- Autosomal recessive
- Mutation in *HFE* gene is most common
- Other mutations affecting iron absorption: TFR2 & HJV

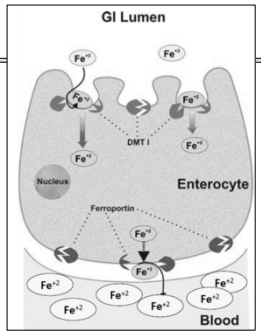
35

Ferroportin mutation

Hepcidin produced like it is supposed to, trying to decrease iron absorption

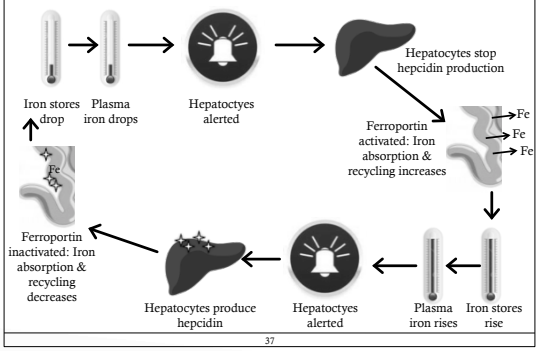
↓

Enterocyte does not respond due to the mutation and iron is continually absorbed



36

Back to "normal" iron absorption…



37

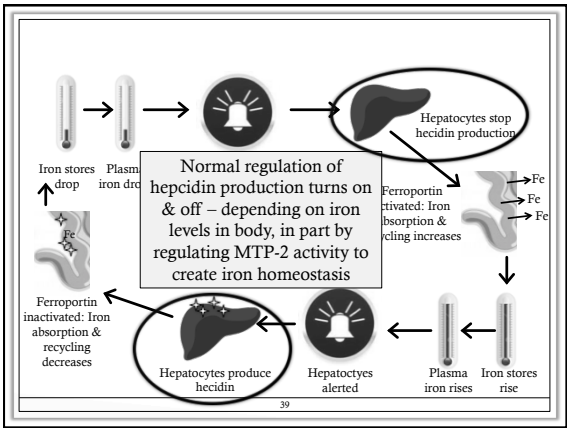
Martriptase-2 (MTP-2)

- Protein that cleaves HJV
- Responds to hypoxia & amount of iron in storage

↓Iron = ↑MTP-2

Dec hepcidin → Active ferroportin → Inc iron absorption

38

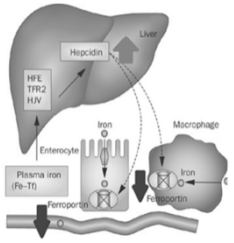


39

Most cases of hemochromatosis are due to mutation of HFE

Treatment!

If MTP-2 can be suppressed, then HJV will be active, and more hepcidin can be produced to reduce iron absorption



40

More on iron deficiency

(And our iron deficient patient)

41

Martriptase-2 mutation

- HJV will not be cleaved → always active
- Constantly increased hepcidin
- Constantly inactive ferroportin
- Persistent decrease iron absorption & recycling

Iron Refractory Iron Deficiency Anemia (IRIDA)

42

Iron Refractory Iron Deficiency Anemia (IRIDA)

- GI ferroportin appears primarily affected
  - Still some macrophage ferroportin activity
- Results in IDA
- Called "iron refractory" because the IDA does not typically respond to oral iron supplements

43

IRIDA: Lab results

- Marked microcytic, hypochromic anemia
- Low serum iron
- Normal TIBC (usually)
- Low transferrin saturation
- Normal/increased serum ferritin
- Low reticulocyte count

44

Special test for IRIDA

- Urine hepcidin
  - Elevated levels
- In typical IDA, urine hepcidin is VERY low so that ferroportin is active as the body tries to absorb all it can

45

IRIDA

- Anemia not present at birth
  - Iron had been transferred from mother to baby → this is like having IV iron
- Due to impaired absorption → IDA shows up shortly after birth
- Delay of onset → distinguishes IRIDA from inherited mutations of other iron related proteins

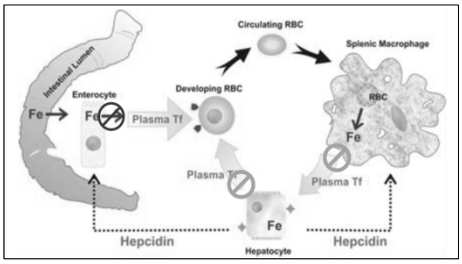
46

IRIDA Treatment

- Parenteral iron
  - Macrophage take up the iron and export it to plasma
  - Slow process
- Anemia improved, but not fully corrected
- Serum ferritin remains normal/slightly increased
- FUTURE: anti-hepcidin Ab or hepcidin gene suppression

47

True iron deficiency vs. IRIDA



48

Back to the initial cases study

- 2-year old, seemingly healthy female
- Diagnosed with IDA
  - Treated with oral iron supplements
- No response to treatment
- Further tests revealed **IRIDA**

49

Other causes of hereditary IDA

50

Case Study #2

- 3 mos. old female with gastroenteritits
- Siblings and parents are healthy

Hgb: 4 g/dL	(RR: 11-14 g/dL)
MCV: 71 fL	(RR: 80-100 fL)
MCH: 23 pg	(RR: 28-32 pg)
MCHC: 33%	(RR: 32-36%)
Retics: 0.5%	(RR: 0.5-2%)

- Bone marrow – erythroid hyperplasia, absent iron in RBCs

51

Case Study #2

- Treated with transfusions, iron, and folate supplements
- Lab results at 6 mos. old:

Hgb: 9.4 g/dL	(RR: 11-14 g/dL)
Normal iron studies	
Normal hgb electrophoresis	

52

Case Study #2

- At the age of 3 years transferrin levels were measured:
  - 24 mg/dL (RR: 200-300 mg/dL)
- Parents transferrin levels:
  - Father: 109 mg/dL
  - Mother: 169 mg/dL

53

Case Study #2

- At the age of 9 years:

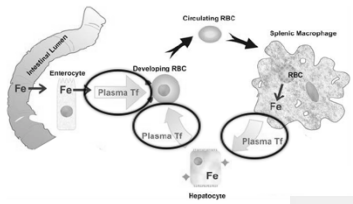
Hgb: 4.5 g/dL	(RR: 11-14 g/dL)
MCV: 62 fL	(RR: 80-100 fL)
MCH: 17 pg	(RR: 28-32 pg)
MCHC: 28%	(RR: 32-36%)
Retics: 0.5%	(RR: 0.5-2%)
Ferritin: 837 ng/mL	(RR: 9-90 ng/mL)

54

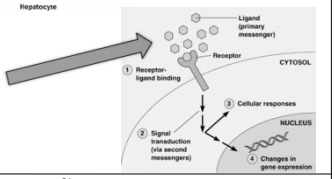
This is a case of Atransferrinemia

- Disease develops related to iron accumulation
  - Similar to hemochromatosis
  - Affects life span
- Autosomal recessive inheritance
- In 2013, 16 cases reported from 14 families

55



Transferrin is the ligand that senses how much iron is circulating

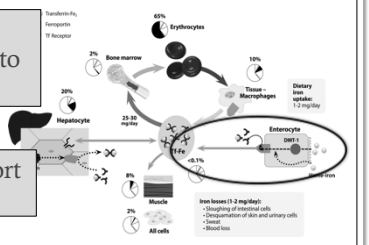


56

What happens without transferrin?

Normal iron absorption into enterocytes

Normal export to plasma



57

But…

- There is not transferrin present to bind to the iron in plasma
- In its ionic form, the iron is absorbable by most cells
  - No regulation of this acquisition
  - Results in massive iron overload in tissues
- RBCs cannot absorb ionic iron, resulting in iron deficiency anemia

58

Treatment for atransferrinemia

- Removal of iron (via RBCs) with phlebotomies
- Plasma transfusion to provide transferrin

59

Case Study #3

- 20 year-old female
- Transfusions:
  - Shortly after birth
  - 8 more during infancy
  - Anytime hemoglobin dropped below 7 g/dL
- Bone marrow: erythroid hyperplasia, decreased hemoglobinization of precursors, no sideroblasts

60

Case Study #3

- Current lab findings:

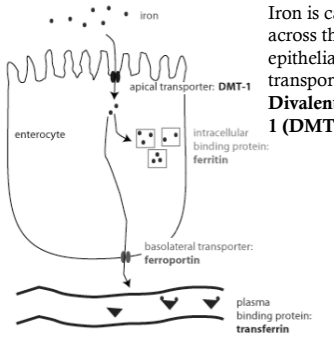
Hgb: 7.4 g/dL	(RR: 12-15.5 g/dL)
MCV: 54 fL	(RR: 80-90 fL)
MCH: 15 pg	(RR: 26-31 pg)
MCHC: 28.5%	(RR: 32-36%)
Retics: 2.1%	(RR: 0.5-3%)
Estimated reticulocyte production index = 0.6%	
Serum iron: Increased	
TIBC: Normal	
Ferritin: High normal	
sTfR: 41.5mg/L	(RR: 1.9-4.4 mg/L)

61

This is a case of DMT-1 deficiency

- Cells unable to properly use iron
  - Iron overload observed in tissues
- Anemia present at birth
  - DMT-1 present in placenta
  - RBCs need DMT-1 to make hemoglobin
- Autosomal recessive inheritance
- 3 affected families

62



Iron is carried transferred across the intestinal epithelial cells by the transport protein **Divalent metal transporter 1 (DMT-1)**

63

DMT-1

- Transports iron across intestinal epithelial cells
- Intracellular iron trafficking to mitochondria
  - ★RBCs
  - Hepatocytes
  - Macrophages
- Iron transport in the placenta

64

Lab findings in DMT-1 deficiency

- Microcytic, hypochromic anemia with polychromasia
- High serum iron
- Normal TIBC
- Increased transferrin saturation
- Increased ferritin
- Increased soluble TfR
- Normal/low hepcidin

65

Treatment for DMT-1 deficiency

- Transfusions & EPO
  - EPO increases number of poorly hemoglobinized RBCs
- Oral or IV iron are ineffective

66

Making the differential diagnosis

67

Lab testing

- Classic iron studies
- Ferritin
- Soluble (serum) transferrin receptor
  - Iron deficient cells make more transferrin receptors
  - Inc sTfR = iron deficiency in cells
- Hepcidin (Rarely)

68

In summary

- Often only think about IDA
- The regulation of iron is dependent upon several proteins
  - Mutations are possible and will affect its function
- Mutations have been recognized that cause decrease hepcidin
  - Leads to over absorption of iron → iron overload (hemochromatosis)

69

In summary

- Maritriptase-2 mutation: inc hepcidin & IRIDA
  - Rare
  - Likely underdiagnosed
- Iron transport mutations: Atransferrinemia & DMT-1 deficiency
  - Super rare
  - Iron accumulation

70

Sources & References

- De falco, L. et al. (2013). Iron defractory iron deficiency anemia. *Hematologica, 98*(6), 845-853.
- Doig, K. (2018) Newest pieces in the iron puzzle and the mutations leading to anemias. ASCLS National Meeting.
- Europena Network for Rare and Congenital Anemias. https://www.enerca.org/anaemias/
- Priwitzerova, M. et al. (2004). Severe hypochromic microcytic anemia caused by a congenital defect of the iron transport pathway in erythroid cells. *Blood, 103*, 3991-3992.
- Shamsian, B.S., et al. (2009). Severe hypochromic microcytic anemia in a patient with congenital atransferrinemia. *Pediatric Hematology and Oncology, 26*(5). 356-362.

71

Any questions?

72