Iron homeostasis – a balancing act

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Disturbances in iron homeostasis are common causes of human disease

**Iron deficiency**
- Iron deficiency anemia
  - IRIDA (genetic), nutritional deficiency
- Anemia of chronic disease
  - (chronic infection, inflammation, cancer)

**Hematopoieses**
- Normal
- Iron-deficient

**Cell growth**

**Iron overload**
- Hereditary Hemochromatosis
- Ineffective erythropoiesis
  - (e.g. Thalassemias, iron-loading anemia)
- Common acquired diseases
  - (e.g. chronic liver disease, diabetes, Alzheimer, Parkinson)

**Damage of cells and tissues**
- "Oxidative stress"
  - (Fenton-type redox chemistry)

**Oxygen transport**
**DNA synthesis**
**Respiration**
Regulation of iron homeostasis

Systemic: The organism maintains serum iron levels by regulating dietary iron absorption and iron release from storage tissues (e.g. macrophages)

Cellular: Each cell regulates its iron uptake and subcellular distribution in autonomous manner
Body iron homeostasis: an almost perfect recycling system

- Bone marrow: ~300mg
- Red blood cells: ~1800mg
- Reticuloendothelial macrophages: ~600mg
- Liver: ~1000mg
- Other cells and tissues: ~400mg
- Fe$_2$$^{3+}$ - Tf: ~3mg
- Iron loss: 1-2mg/day
- Duodenum: 1-2mg/day

The Hepcidin/Ferroportin regulatory system controls systemic iron homeostasis.

Hepcidin, a 25-aa peptide hormone, regulates iron homeostasis by controlling the expression of Ferroportin (Fpn) in various tissues.

- **Liver**: Hepcidin is produced in the liver in response to hypoxia, inflammation, and infection, which increase iron levels.
- **Bone marrow**: Increased erythropoiesis leads to higher iron demand, triggering hepcidin production.
- **Reticuloendothelial macrophages**: Iron is transported from the bone marrow to macrophages, where it is degraded and recycled.
- **Duodenum**: Iron absorption is regulated by hepcidin, which binds to Ferroportin on enterocytes, preventing iron uptake.

Hepcidin interacts with Ferroportin (Fpn) to modulate iron transport and regulate systemic iron homeostasis.

Hepcidin-mediated control of iron export

HEPCIDIN ↓
Iron export into the plasma

Iron-exporting cell (e.g. duodenal enterocyte, macrophage)

HEPCIDIN ↑
Cellular iron retention due to FPN internalization and degradation
Hepcidin injection rapidly reduces serum iron levels

Hepcidin reduces serum iron by 80% within 60 minutes

Rivera et al
Blood 2005
How is hepcidin synthesis regulated?

- **Iron levels**
  - e.g. Hereditary Hemochromatosis

- **Hypoxia and increased erythropoiesis**
  - e.g. secondary Hemochromatosis (iron-loading anemias)

- **Inflammation and infection**
  - e.g. anemia of inflammation
How is hepcidin synthesis regulated?

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… he observed

- tiredness und petulance
- stomach pain
- pain in the joints
- susceptibility for infections
- impotence
- heart failure
- diabetes
- bronze colouring of the skin
• frequent iron overload disorder (carrier frequency 1:8)
• increased iron absorption
• and iron deposition in the liver, heart and endocrine organs
• complications: liver cirrhosis and cancer, heart failure, endocrinopathy

Jordan et al., 2009
Hereditary Hemochromatosis

Iron accumulation in organs

HFE, TfR2, HJV sense systemic iron levels and control Hepcidin expression via the BMP/SMAD signalling pathway

Muckenthaler, Blood 2014
Mutations in HFE, HJV and TfR2 (proteins mutated in HH) disrupt the formation of a hepatic iron-sensing complex that regulates BMP/SMAD signalling and hepcidin levels to maintain iron homeostasis

Vujic-Spacic et al., Cell Metabolism 2008
Vujic-Spacic et al., BBA 2012
Corradini et al. 2009
Kautz et al., Blood 2009
Hereditary Hemochromatosis is a wide-spread disorder in the caucasian population...
How is hepcidin synthesis regulated?

- **iron levels**
  e.g. Hereditary Hemochromatosis

- **hypoxia and increased erythrophoiesis**
  e.g. disorders with ineffective erythrophoiesis (iron-loading anemias)

- **inflammation and infection**
  e.g. anemia of inflammation
CFU-E = colony-forming unit erythroid (Erythrozyten-Vorläuferzellen der myeloischen Hämatopoese).
Disorders of ineffective or dysplastic erythropoiesis

Haemoglobinopathies (e.g. thalassaemic syndromes)

- Common causes of ineffective erythropoiesis → thalassaemia major and intermedia
- Imbalances of α, β-globin chains → increased apoptosis during erythroblast maturation
- Iron overload is a well-recognized complication

Sideroblastic anaemias

- Erythropoiesis characterized by mitochondrial iron accumulation in a ring around the nucleus
- Caused by mutations in genes controlling mitochondrial iron metabolism

Dyserythropoietic anaemias (e.g. pyruvate kinase deficiency)

- Defective glycolysis results in erythroblast apoptosis and peripheral blood haemolysis

Myelodysplastic syndrome (MDS)

- BM failure, peripheral blood cytopenias, iron overload, reduced survival
- Expansion or evolution of the abnormal clone to AML can occur
Ineffective erythropoiesis

- Ineffective erythropoiesis frequently results from mutations in genes that control erythropoiesis

- Insufficient or malfunctioning erythrocytes are produced that have an impaired capacity to transport oxygen
Disorders with ineffective erythropoiesis are hallmarked by iron overload.
How is hepcidin synthesis regulated?

- **iron levels**
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- **inflammation and infection**
  e.g. anemia of inflammation
Iron plays a central role in host-pathogen interactions.

Pathogens require iron for proliferation and full virulence.

Hypoferremia is a major host defense strategy that causes AI.
Accidental reactivation of Tb by iron
True and False Chlorosis, Lectures in Clinical Medicine
Armand Trousseau, Paris 1872

“When a very young physician, I was called to see the wife of an architect, a pale woman, presenting every appearance of chlorosis: I prescribed large doses of iron. There was a complete change: the young woman acquired a ravenous appetite, and an unwonted vivacity: but her gratitude and my delight did not last long. The restored colour of the cheek became every evening after dinner more ardent than it had been when she was in good health. A short cough supervened; and in less than a month there appeared signs of phthisis which nothing could impede.”

“The first case of galloping consumption which I had to deplore occurred under nearly similar circumstances. A girl of fifteen fell into a state of anaemia which I considered chlorosis. I administered ferruginous remedies, which rapidly restored her to florid health: and although there was nothing in the family history to lead me to fear the coming calamity, she was simultaneously seized with hemoptysis and menorrhagia, and died two months afterwards”

“Gentlemen, I am constrained to impute to iron some of the evil consequences which I had to deplore”
Chronic infections and inflammation cause hypoferremia

- Reduced serum Fe levels
- Limited Fe for bacterial growth
- IL6
- IL1b
- Others
- ANAEMIA associated with chronic infections, autoimmune disorders and malignancies
Interaction network of putative hepcidin regulators

DAVID analysis
(90 enriched, nonredundant functional terms)
-> screen coherence and high quality

DAVID and STRING
(functional connections)
Regulation of hepcidin expression is integrated into signaling pathways that respond to growth-stimulating and nutrient-rich conditions.

Important clinical implications for liver stress conditions, including liver regeneration, viral and alcohol hepatitis, HCC and diabetes that involve the activation of the Ras/MAPK and mTOR pathways.
Failure of the Hepcidin-Ferroportin axis causes fatal iron overload in mice
Sandro Altamura
Mutations in ferroportin cause disease
BMDM from the FPNC326S mice are hepcidin resistant

Altamura et al., Cell Metabolism (2014)
Increased serum iron levels hallmark FPN(C326S) mice

Plasma iron (8-weeks old)

<table>
<thead>
<tr>
<th>SFBC (mg/dl)</th>
<th>wt/wt</th>
<th>wt/C326S</th>
<th>C326S/C326S</th>
</tr>
</thead>
</table>
| m
| n=6  | 100   | 200   | 300          |
| f
| n=6  | 150   | 250   | 350          |

Transferrin saturation (8-weeks old)

<table>
<thead>
<tr>
<th>Tf saturation %</th>
<th>wt/wt</th>
<th>wt/C326S</th>
<th>C326S/C326S</th>
</tr>
</thead>
</table>
| m
| n=6  | 40    | 60       | 80          |
| f
| n=6  | 80    | 100      | 120         |

Plasma ferritin (8 weeks old)

<table>
<thead>
<tr>
<th>Ferritin ng/L</th>
<th>wt/wt</th>
<th>wt/C326S</th>
<th>C326S/C326S</th>
</tr>
</thead>
</table>
| m
| n=6  | 50    | 100      | 150         |
| f
| n=3  | 150   | 200      | 250         |

Altamura et al., Cell Metabolism (2014)
Hepatic iron overload in FPNC326S mice

Altamura et al., Cell Metabolism (2014)
Splenic macrophages are iron-deficient in FPN C326S mice

Altamura et al., Cell Metabolism (2014)
FPNC326S mice show pancreatic iron overload

SLC40A1(wt/wt)  SLC40A1(C326S/C326S)

Perls staining

Altamura et al., Cell Metabolism (2014)
Pancreatic iron overload is associated with oxidative stress and elevated plasma lipase levels

Pancreatic non-heme iron content (24 weeks old)

Pancreatic oxidative stress (24 weeks old)

Lipase (24 weeks old)

Altamura et al., Cell Metabolism (2014)
Degenerated pancreatic tissue in FPNC326S mice

SLC40A1( wt/wt )

SLC40A1 ( C326S/C326S )
FPNC326S mice die around 35 weeks of age

Altamura et al., Cell Metabolism (2014)
Is death of the SLC40A1(C326S/C326S) mice related to the iron overload?

Is pancreatic failure the cause of death?
Weight loss can be rescued by a low iron diet and pancreatic enzyme replacement

Altamura et al., Cell Metabolism (2014)
Death of the FPNC326S mice is prevented by a low iron diet.
Disruption of the hepcidin/ferroportin axis causes ...

- Severe iron overload in vital organs
- Failure of the exocrine pancreas, wasting and eventual death
- This represents the first mouse model of fatal dietary iron overload.

Outlook:
Work is in progress to investigate pathologies related to iron overload and/or iron deficiency in various tissues e.g. lung, vasculature and kidney
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