Hemochromatosis

Summer 2011
Outline

• Iron metabolism
• Hemosiderosis
• Pathogenesis of hemochromatosis
• Management of hemochromatosis
Iron Deficiency

• Among the most common nutritional disorders in the world
  – Even in industrialized countries, premenopausal women are commonly affected
• Evolutionary pressures have yielded iron metabolic pathways strongly biased toward iron retention
• Mechanisms to eliminate excess iron are limited
Iron Metabolism

• Shedding of epithelial cells results in losses of 1-2 mg/day
  – Additional losses with chronic bleeding from gastrointestinal, urinary, or genital tracts

• Normal Western diet contains 10-20 mg/day
  – Major source is heme contained in animal products (≈ 20% absorbed)
  – Minor source is inorganic iron found in vegetable products (< 5% absorbed)
Iron Metabolism

• Iron absorption
  – Occurs primarily in the duodenum
  – The villous enterocyte absorbs both heme and non-heme iron into a common cellular storage pool
    » Ferritin
  – From there...
    • If iron deficient: transferred to portal blood
      » Transferrin
    • If iron replete: exfoliated with duodenal mucosa
Iron Metabolism

• Iron absorption (duodenum)
  – Non-heme iron (< 5% absorbed)
    • Traverses the luminal and basal membranes of villous enterocytes
      – Luminal
        » Duodenal cytochrome B (ferrireductase)
        » Divalent metal transporter 1 (DMT1)
      – Basal
        » Ferroportin (cellular iron efflux channel)
        » Hephaestin iron oxidase
    • Absorption is variable and inefficient
  – Heme iron (≈ 20% absorbed)
    • Heme transporter (not yet well characterized)
Iron Metabolism

*Kumar, 2008*
Iron Metabolism

• Hepcidin
  – Small circulating peptide important in the regulation of iron absorption
  – Synthesized and released by the liver in response increased hepatic iron
  – Blocks transfer of iron from enterocyte to bloodstream by inhibiting ferroportin
  – Therefore, as hepcidin levels rise, iron becomes trapped within duodenal epithelium and is lost with exfoliation
Iron Metabolism

• Anemia of chronic disease
  – Associated with…
    • Chronic microbial infections (e.g., osteomyelitis)
    • Chronic immune disorders (e.g., rheumatoid arthritis)
    • Neoplasms (e.g., carcinomas of the lung and breast)
  – Characterized by low serum iron despite adequate storage iron
    • Low serum iron saturation
    • Low serum TIBC (i.e., transferrin)
      – Negative acute phase reactant
    • High serum ferritin
      – Positive acute phase reactant
Iron Metabolism

• Role of *hepcidin* in anemia of chronic disease
  – Inflammatory mediators (IL-6) stimulate hepatic hepcidin production
  – Hepcidin inhibits *ferroportin* function in bone marrow macrophages
  – Erythroid precursors are denied access to iron required for heme production
  – End result is a mild normocytic, normochromic OR microcytic, hypochromic anemia
    • May mimic iron deficiency
Iron Metabolism

• Total iron body content
  – Men: 3-6 grams
  – Women: 2-3 grams

• Two major compartments
  – Functional compartment (80%)
  – Storage compartment (20%)
### Iron Distribution in Healthy Young Adults (mg)

<table>
<thead>
<tr>
<th>Pool</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>3450</td>
<td>2450</td>
</tr>
<tr>
<td>Functional</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>2100</td>
<td>1750</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>300</td>
<td>250</td>
</tr>
<tr>
<td>Enzymes</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Storage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferritin, hemosiderin</td>
<td>1000</td>
<td>400</td>
</tr>
</tbody>
</table>

*Kumar, 2008*
Iron Metabolism

• Storage compartment (20%)
  – Free iron is toxic, therefore it is bound to storage protein
  – Ferritin
    • Complex of ferric salt and the protein, *apoferritin*
    • Found in *mononuclear phagocytic cells* in all tissues
    • Highest levels found in spleen, bone marrow, skeletal muscle, and liver
Iron Metabolism

• Hepatic iron storage
  – Mononuclear phagocytic (Kupffer cell) storage
    • Iron derived from red blood cell breakdown
      – Similar to other organs (spleen, bone marrow, etc.)
  – Parenchymal (hepatocyte) storage
    • Primary site of iron storage
    • Iron derived from plasma *transferrin*
Iron Metabolism

• Transferrin
  – Iron binding protein synthesized in the liver
  – Major function is to transport iron from the gut to the bone marrow for Hb synthesis
    • Immature RBCs possess high-affinity transferrin receptors (CD71); take in iron by receptor-mediated endocytosis
  – Generally measured indirectly, as the total serum iron binding capacity (TIBC)
  – A negative acute phase reactant
    • Therefore TIBC is low in anemia of chronic disease
    • Along with high ferritin (a positive acute phase reactant)
Iron Metabolism

*McPherson, 2007*
“Iron Studies”

• **Serum Ferritin**
  – Levels generally correlate with body iron stores
    • Iron deficiency: ferritin < 50 mcg/L
    • Iron overload: ferritin > 1000 mcg/L
  – A *positive* acute phase reactant!

• **TIBC (indirect measure of transferrin)**
  – Normal: 300 - 360 mcg/dL
  – A *negative* acute phase reactant

• **Serum iron (bound to transferrin)**
  – Normal: 100 - 120 mcg/dL
  – Subject to diurnal and dietary fluctuations

• **Iron saturation (of transferrin)**
  – Normal: ~1/3
Iron Metabolism

*Kumar, 2008*
Hemosiderin

*Kumar, 2008
Hemosiderin

- Golden yellow-to-brown, granular pigment formed by *ferritin* when there is a local or systemic excess of iron
  - Localized hemosiderosis (e.g., hemorrhage)
  - Systemic hemosiderosis (e.g., hemochromatosis)
- Normally, trace amounts found in mononuclear phagocytes of the bone marrow, spleen, and liver
- Confirmation by Prussian blue histochemical stain
  - Colorless ferrocyanide converted by ferric storage iron to blue ferric cyanide
Systemic Hemosiderosis

- Hemosiderin-laden macrophages found outside of the bone marrow, spleen, and liver

- Progressive iron accumulation leads to hemosiderin deposition in parenchymal cells throughout the body
  - Principally the liver, pancreas, heart, and endocrine organs
Hemochromatosis

- Lifelong iron accumulation (systemic hemosiderosis) leading to symptomatic organ damage
- Clinical presentation before age 40 is rare

- Two forms
  - Hereditary (primary hemochromatosis)
  - Acquired (secondary hemochromatosis)
    - Preferable to use the term “systemic hemosiderosis”
Secondary Hemochromatosis

- **Parenteral intake (transfusions) for many conditions**
  - Hemodialysis (long-term), aplastic anemia, sickle cell disease, myelodysplastic syndromes, leukemias, etc.
- **Oral intake**
  - Excess iron supplementation, Bantu siderosis
- **Ineffective erythropoiesis with increased erythroid activity**
  - Beta thalassemia, sideroblastic anemia, myelodysplastic syndromes, pyruvate kinase deficiency, etc.
- **Chronic liver disease**
  - Chronic alcoholic liver disease, porphyria cutanea tarda, HBV and HCV infections
HFE Protein

- **β2 Microglobulin**
- **Extracellular**
- **Intracellular**
- **Plasma membrane**

**Heavy chain**

- **α1**
- **α2**
- **α3**

**Disulfide bonds (S-S)**

**Sites of interest**

- **His63Asp**
- **Cys282Tyr**

*Courtesy Dr. Fan*
Hereditary Hemochromatosis

• The HFE gene
  – Located on the short arm of chromosome 6 (6p21.3)
  – Encodes a 343 amino acid protein that regulates intestinal absorption of iron
  – Most common mutation
    • Guanine (G) to adenine (A) substitution at nucleotide 845 [G845A], resulting in a...
    • Cysteine (C) to tyrosine (Y) substitution at amino acid 282 [C282Y]
      – An inactivating mutation found in up to 90% of patients diagnosed with hereditary hemochromatosis
Hereditary Hemochromatosis

• The HFE gene (cont.)
  – The other common mutation
    • Histidine (H) to aspartate (D) substitution at amino acid 63 [H63D]
    • H63D homozygotes and C282Y/H63D compound heterozygotes typically have less iron accumulation than C282Y homozygotes
  – Mutations in other genes involved in iron metabolism (e.g., transferrin receptor, ferroportin, etc.) have also been implicated
    • Hepcidin gene (HAMP) mutations may cause a particularly severe form of hereditary hemochromatosis (juvenile hemochromatosis)
Hereditary Hemochromatosis

Type 1 (HFE-related)
- C282Y homozygous
- C282Y/H63D compound heterozygous
- Other HFE mutations

Type 2 (juvenile hemochromatosis)
- A. Hemojuvelin (HJV) mutations
- B. Hepcidin (HAMP) mutations

Type 3 (TFR2 mutations)

Type 4 (ferroportin mutations)
- A. Loss-of-function
- B. Gain-of-function

*Olynyk, 2008*
Hereditary Hemochromatosis

HFE Mutation Detection

- PCR amplification of segments flanking the C282Y and H63D mutation sites
- Followed by allele specific restriction enzyme digestion and gel visualization

*www.ub.edu*
Hereditary Hemochromatosis

- **Epidemiology**
  - C282Y mutation in populations of predominantly northern European descent
    - Frequency of homozygosity 0.45% (1 in 220)
    - Frequency of heterozygosity 11% (1 in 9)
  - Penetrance only 20-30% in homozygotes
    - Making mutational analysis impractical as a screening tool on a population level
  - Iron accumulation may occur in heterozygotes, but rarely to a clinically significant degree
  - Males predominate (5 to 7:1)
    - Physiologic iron loss (menstruation, pregnancy) is protective in females
Hereditary Hemochromatosis

• Pathogenesis
  – Normal
    • Total body iron tightly regulated (2-6 grams in adults) with limited daily iron loss matched by GI absorption
  – Hereditary hemochromatosis
    • Regulation of GI absorption is lost, leading to net accumulation (0.5-1.0 grams per year)
    • Manifestations of disease appear after 20 grams of storage iron have accumulated
Hereditary Hemochromatosis

• Pathogenesis
  – HFE mutations impair cell membrane interactions between the *HFE protein* and *transferrin receptor*
  – This interferes with signaling pathways in *at least* two critical areas…
    • Enterocyte iron absorption
    • Hepatocyte hepcidin synthesis
Hereditary Hemochromatosis

*Kumar, 2008*
Hereditary Hemochromatosis

*Olynyk, 2008*
Hereditary Hemochromatosis

• Pathogenesis
  – Iron toxicity
    • Iron catalyzed free radical reactions
      – Lipid peroxidation (disrupts cell membranes)
      – DNA fragmentation
        » Predisposition to hepatocellular carcinoma?
    • Stimulation of collagen formation (fibrosis)
Hereditary Hemochromatosis

• Major pathologic features
  – Hemosiderin deposition
    • In order of decreasing amount: liver, pancreas, myocardium, endocrine glands (pituitary, adrenal, thyroid, parathyroid), joints, and skin
  – Classic triad
    • Cirrhosis
    • Diabetes mellitus
    • Bronzing of the skin
Hereditary Hemochromatosis

• Liver
  – Histopathology
    • First evident as hemosiderin deposition in the cytoplasm of periportal hepatocytes followed by progressive deposition toward the centrolobular area
    • Fibrous tissue expands the portal tracts, ultimately resulting in lobules enveloped by portal-to-portal septa
      – Micronodular pattern of cirrhosis
    • Inflammation is characteristically mild
  – Gross pathology
    • Dense, intensely pigmented (chocolate brown) liver
Hereditary Hemochromatosis
Hereditary Hemochromatosis

- Pancreas
  - Hemosiderin deposition in both acinar and islet cells
  - Diffuse interstitial fibrosis and parenchymal atrophy
    - Exocrine and endocrine pancreatic insufficiency
- Heart
  - Hemosiderin deposited within myocardial fibers
  - Interstitial fibrosis
    - Cardiomyopathy, arrhythmia (more common in juvenile forms of hereditary hemochromatosis)
Hereditary Hemochromatosis

• Skin
  – Characteristic slate-gray color
    • Hemosiderin deposition in dermal macrophages and fibroblasts (minor factor)
    • Increased epidermal melanin production, particularly in sun exposed areas (major factor)

• Joints
  – Hemochromatosis arthropathy
    • Typically involves the 2nd and/or 3rd MCP joints

• Testes
  – Atrophy
    • No significant hemosiderin deposition
    • Derangement of the hypothalamic-pituitary axis
Hereditary Hemochromatosis

• Diagnosis
  – Iron studies
    • Elevated transferrin saturation is the earliest marker
    • Serum ferritin > 1000 mcg/L often indicates significant hepatic fibrosis
      » Serum ferritin is a positive acute phase reactant!
      » Secondary causes of iron overload must be excluded!
  – Genetic testing
  – Liver biopsy
    • Indicated when serum ferritin > 1000 mcg/L or with elevated hepatic enzymes (AST, ALT, etc.)
Hemochromatosis

- Biochemical determination of hepatic iron content
  - Normal
    - Less than 1000 µmol/g dry liver weight
  - Hereditary hemochromatosis (C282Y homozygotes)
    - Greater than 2000 µmol/g dry liver weight
      » Typically greater than 10,000 µmol/g dry liver weight
  - Hepatic iron index (HII)
    - Iron concentration (µmol/g) divided by age (years)
    - HII > 1.9 in hereditary hemochromatosis
  - Less useful in the current era of genetic testing
Hemochromatosis

• Treatment
  – Therapeutic phlebotomy
  – Iron chelation medications
    • Deferoxamine (subcutaneous infusion)
    • Deferasirox (oral)
  – Proton pump inhibitors
    • Inhibit absorption of non-heme iron
      – DMT1 (divalent metal transporter 1) on the luminal surface of the enterocyte requires proton co-transport
      – Decreased solubility at higher pH
Hemochromatosis

Table 2. Dietary management recommendations for persons with hemochromatosis

<table>
<thead>
<tr>
<th>Recommendation</th>
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</thead>
<tbody>
<tr>
<td>Avoid supplemental iron.</td>
</tr>
<tr>
<td>Consume red meats in moderation.</td>
</tr>
<tr>
<td>Consume ethanol in moderation. *</td>
</tr>
<tr>
<td>Limit supplemental vitamin C to 500 mg daily.</td>
</tr>
<tr>
<td>Use mineral supplements for specific deficiencies only. †</td>
</tr>
<tr>
<td>Do not consume raw shellfish.</td>
</tr>
<tr>
<td>Do not consume cooked seafood contaminated with seawater drippings.</td>
</tr>
<tr>
<td>Avoid contact of seawater with cuts or other open skin lesions.</td>
</tr>
</tbody>
</table>

*This recommendation applies to persons without liver abnormalities. Persons with evidence of liver injury such as elevated serum concentrations of hepatic enzymes or hepatomegaly should consume little or no ethanol. Persons with cirrhosis should abstain from consuming ethanol.

†Nonferrous metals including cobalt, zinc, manganese, and chromium share absorptive pathways with iron; excess zinc and manganese are retained in the liver.

*Adams, 2010
Therapeutic Phlebotomy

• The first successful treatment for iron overload, first published in 1952*, and remains the mainstay of treatment to this day

• Rationale
  – Stimulation of erythropoiesis mobilizes iron from storage sites
    • The success of phlebotomy depends upon normal erythropoiesis

*Davis, 1952
Therapeutic Phlebotomy

• Benefits
  – The major goal of therapy is to prevent cirrhosis (and hepatocellular carcinoma)
  – Alleviates the malaise that affects some hemochromatosis patients (mechanism unknown)
  – Normal life expectancy is achieved among most hemochromatosis patients who initiate therapeutic phlebotomy prior to developing cirrhosis
Therapeutic Phlebotomy

• Initiation
  – Serum ferritin > 1000 mcg/L
    • Phlebotomy recommended
  – Serum ferritin 200-1000 mcg/L
    • Phlebotomy acceptable depending on patient preferences and clinical judgment
      – Coexisting liver disease
Therapeutic Phlebotomy

- General Performance
  - Weekly removal of one unit of blood (450-500 mL)
    - Results in the loss of 200-250 mg of iron
  - Phlebotomy *held* if hemoglobin < 11 g/dL
  - Additional factors may delay phlebotomy
    - Concerning symptoms: chest pain, dyspnea, etc.
    - Abnormal vitals signs: hypotension, tachycardia, etc.
  - Concomitant administration of salt-containing beverage to maintain plasma volume
Therapeutic Phlebotomy

• Monitoring
  – Serum ferritin
    • Measure monthly (or after 4 phlebotomy sessions)
      – Measure every 1-2 weeks once ferritin < 200 mcg/L
  – Transferrin saturation
    • A sensitive indicator of hemochromatosis, but…
    • Not as useful for the purposes of monitoring therapy
      – Day to day values fluctuate
      – Often remains elevated even when ferritin levels are subnormal
Therapeutic Phlebotomy

• End points of therapy
  – Treatment until serum ferritin ≈ 50 mcg/L
    • Value in the lower reference range consistent with minimal storage iron

• Absorption of non-heme iron is markedly increased by phlebotomy therapy once ferritin falls below 50 mcg/L
  – Absorption of *heme* iron is relatively stable in C282Y homozygotes, regardless of iron stores
Therapeutic Phlebotomy

Figure 1. A clinical guide to phlebotomy management of HFE C282Y homozygotes. IO indicates iron overload; SF, serum ferritin; and TS, transferrin saturation.

*Adams, 2010
Erythrocytapheresis

- Advantages (v. phlebotomy)
  - Removes more erythrocytes per session while sparing plasma proteins, coagulation factors, and platelets
  - Achieves iron depletion more rapidly
  - Potentially useful in patients with poor peripheral venous access (via a central line)

- Disadvantages
  - Requires an apheresis machine and skilled technician
  - Requires larger bore needle to accommodate flow and pressure requirements
  - Cost?
Hemochromatosis

• In 2001, the FDA issued a policy of variances for collecting blood from hemochromatosis donors (Title 21, CFR 640.120)
  – Allowed transfusion of blood from hemochromatosis donors that met known safety standards
  – Eliminated financial incentives for hemochromatosis donors to falsify responses to blood bank screening questionnaires
  – Mandated that blood banks accepting blood for transfusion from any hemochromatosis patient provide phlebotomy therapy without charge to all hemochromatosis patients
  – Authorized blood banks to perform phlebotomy therapy according to the prescribing physician’s written orders about frequency and Hb/Hct limits
Hemochromatosis

• Hemochromatosis patient as voluntary blood donors (cont.)
  – Many blood banks have declined to obtain the required variances
  – Ethical principles
    • No person with hemochromatosis has the right to insist that his or her blood be used for transfusion
    • No person with hemochromatosis is obligated to donate therapeutic phlebotomy blood for transfusion
  – In Canada, blood from hemochromatosis patients whose blood meets other safety criteria has been used for transfusion since 1991
References

• Hepcidin expression results in which of the following actions…
  – Increased iron absorption
  – Decreased iron absorption
  – Retention of macrophage iron
  – Release of macrophage iron
Question

• Hepcidin expression results in which of the following actions…
  – Increased iron absorption
  – **Decreased iron absorption**
  – Retention of macrophage iron
  – Release of macrophage iron

*Hepcidin inhibits the *ferroportin* iron efflux channel in both duodenal enterocytes and bone marrow macrophages.*