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Review Article

Medical Progress

DISORDERS OF IRON METABOLISM

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IRON has the capacity to accept and donate electrons readily, interconverting between ferric (Fe^{2+}) and ferrous (Fe^{3+}) forms. This capability makes it a useful component of cytochromes, oxygen-binding molecules (i.e., hemoglobin and myoglobin), and many enzymes. However, iron can also damage tissues by catalyzing the conversion of hydrogen peroxide to free-radical ions that attack cellular membranes, proteins, and DNA. Proteins sequester iron to reduce this threat. Iron ions circulate bound to plasma transferrin and accumulate within cells in the form of ferritin. Iron protoporphyrin (heme) and iron-sulfur clusters serve as enzyme cofactors. Under normal circumstances, only trace amounts of iron exist outside these physiologic sinks, although stored iron can be mobilized for reuse. Iron balance is tenuous; both iron deficiency and iron overload are deleterious. Disorders of iron homeostasis are among the most common diseases of humans.

PHYSIOLOGY OF IRON TRANSPORT

Distribution of Iron

The distribution of iron in tissue is shown in Figure 1. Adult men normally have 35 to 45 mg of iron per kilogram of body weight.¹ Premenopausal women have lower iron stores as a result of their recurrent blood loss through menstruation. More than two thirds of the body's iron content is incorporated into hemoglobin in developing erythroid precursors and mature red cells. Uptake of erythroid iron is highly dependent on receptor-mediated endocytosis of diferric transferrin bound to transferrin receptors (the transferrin cycle, Fig. 2). Each erythrocyte contains a billion atoms of iron; at normal rates of turnover,

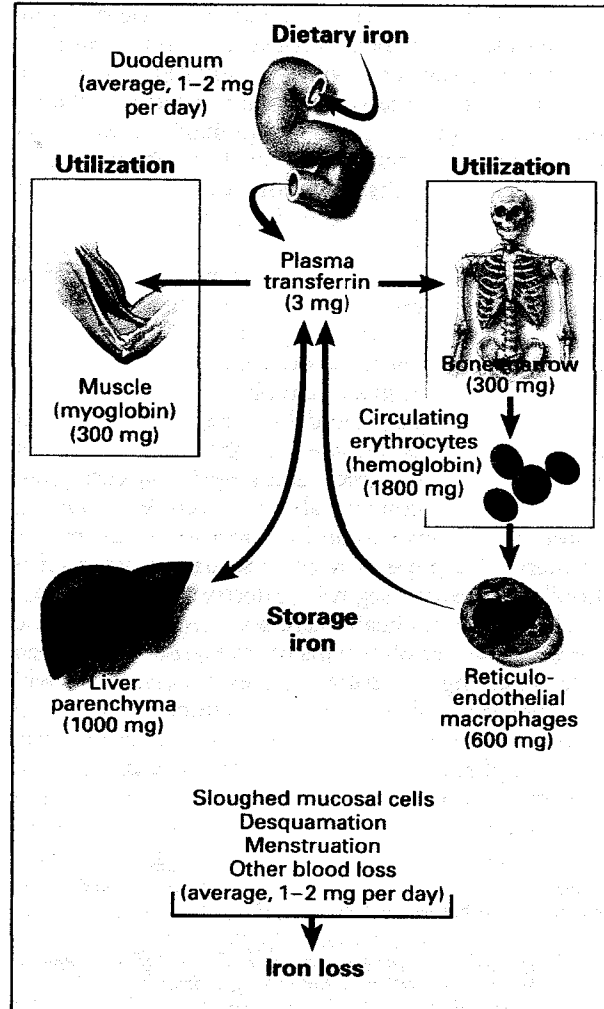


Figure 1. Distribution of Iron in Adults.
 In the balanced state, 1 to 2 mg of iron enters and leaves the body each day. Dietary iron is absorbed by duodenal enterocytes. It circulates in plasma bound to transferrin. Most of the iron in the body is incorporated into hemoglobin in erythroid precursors and mature red cells. Approximately 10 to 15 percent is present in muscle fibers (in myoglobin) and other tissues (in enzymes and cytochromes). Iron is stored in parenchymal cells of the liver and reticuloendothelial macrophages. These macrophages provide most of the usable iron by degrading hemoglobin in senescent erythrocytes and reloading ferric iron onto transferrin for delivery to cells.

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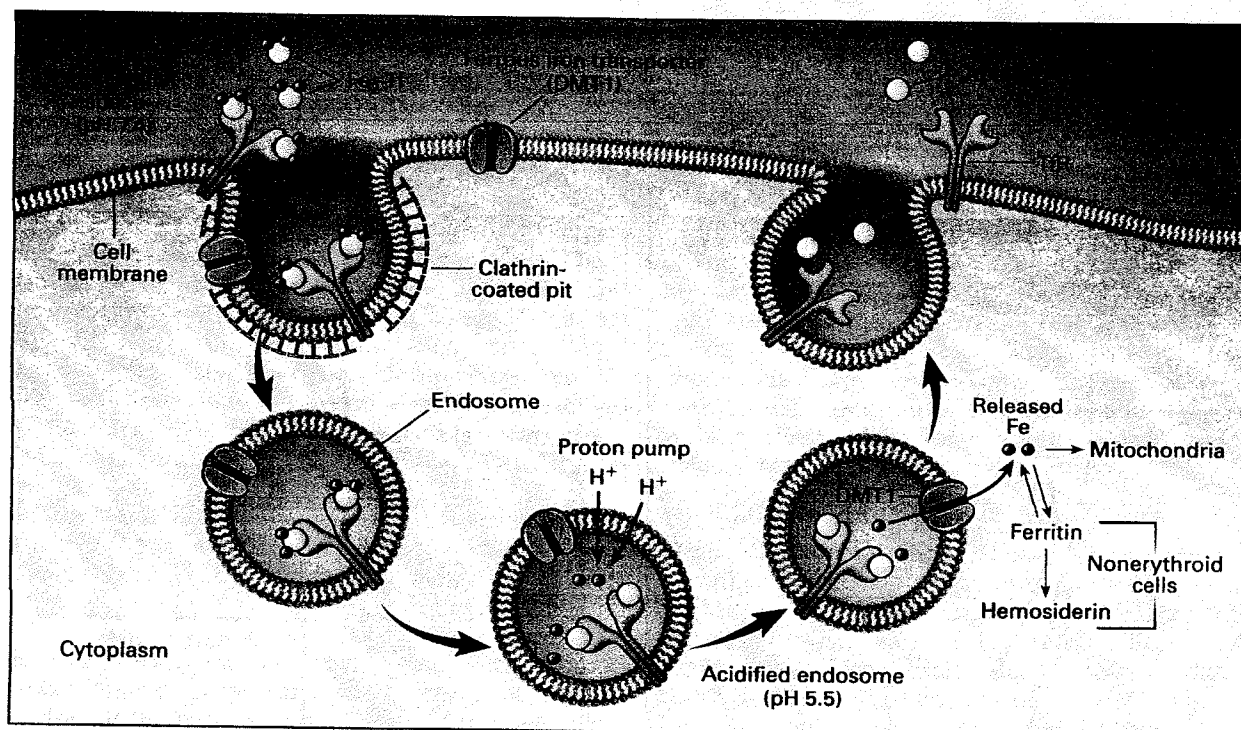


Figure 2. The Transferrin Cycle.

Iron-laden transferrin ($\text{Fe}_2\text{-Tf}$) binds to transferrin receptors (TfR) on the surface of erythroid precursors. These complexes localize to clathrin-coated pits, which invaginate to form specialized endosomes.² A proton pump decreases the pH within the endosomes, leading to conformational changes in proteins that result in the release of iron from transferrin. The iron transporter DMT1 moves iron across the endosomal membrane, to enter the cytoplasm.³ Meanwhile, transferrin (Apo-Tf) and transferrin receptor are recycled to the cell surface, where each can be used for further cycles of iron binding and iron uptake. In erythroid cells, most iron moves into mitochondria, where it is incorporated into protoporphyrin to make heme. In nonerythroid cells, iron is stored as ferritin and hemosiderin.

this concentration corresponds to the incorporation of 2×10^{20} atoms of iron per day.⁴ Consequently, anemia is the cardinal sign of iron deficiency.

Most of the remaining body iron is found in hepatocytes and reticuloendothelial macrophages, which serve as storage depots. The liver has first-pass access to dietary nutrients and can readily take up an amount of circulating iron that exceeds the binding capacity of plasma transferrin. Reticuloendothelial macrophages ingest senescent red cells, catabolize hemoglobin to scavenge iron, and load the iron onto transferrin for reuse. This process is indispensable; the erythron alone has a daily requirement of about 20 mg of iron,⁵ but only 1 to 2 mg of iron normally enters the body each day through the intestine.

Regulation of Iron Absorption

Although the amount of iron extracted from the diet is small, the regulation of the intestinal absorption of iron is critical because humans have no physiologic pathway for excretion. Duodenal crypt cells sense the iron requirements of the body and are programmed by that information as they mature into

absorptive enterocytes. Enterocytes lining the absorptive villi close to the gastroduodenal junction are responsible for all iron absorption. Iron must pass from the gut lumen through the apical and basolateral membranes of the enterocyte to reach the plasma (Fig. 3). Iron obtained from food is not bound to transferrin, and there is no role for transferrin within the lumen of the intestine. Instead, the low pH of gastric effluent helps dissolve ingested iron and provides a proton-rich milieu. This facilitates enzymatic reduction of ferric iron to its ferrous form by a brush-border ferrireductase.⁶ Divalent metal transporter 1 (DMT1; formerly called Nramp2 or DCT1) is a protein that transfers iron across the apical membrane and into the cell through a proton-coupled process.^{7,8} DMT1 is not specific to iron; it can transport a wide variety of divalent metal ions, including manganese, cobalt, copper, zinc, cadmium, and lead.⁸

Heme iron is taken up by a separate process that is not well characterized. Inside the absorptive enterocyte, iron has two possible fates: it may be stored as ferritin, or it may be transferred across the basolateral membrane to reach the plasma. These are not

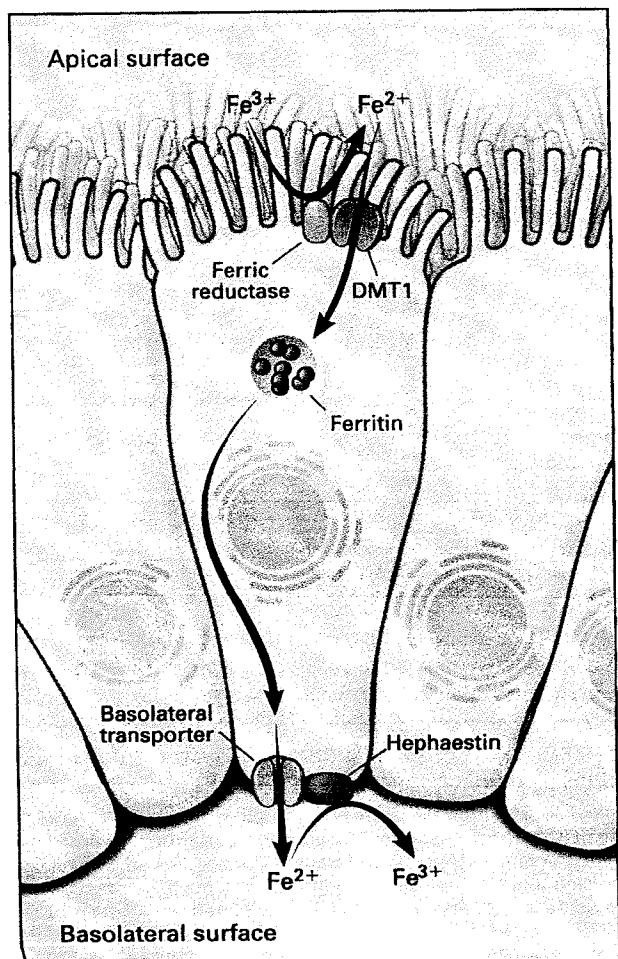


Figure 3. Iron Transport across the Intestinal Epithelium. Iron must cross two membranes to be transferred across the absorptive epithelium. Each transmembrane transporter is coupled to an enzyme that changes the oxidation state of iron. The apical transporter has been identified as DMT1. It acts in concert with a type of ferrireductase activity that has not yet been cloned. The basolateral transporter has not yet been identified. This transporter requires hephaestin, a ceruloplasmin-like molecule, for the transfer of iron to the plasma. On the basis of its structure, hephaestin is presumed to be a form of ferroxidase. In this diagram, hephaestin is depicted at the basolateral surface of the cell, although it has not yet been established that it functions in that location. Iron within enterocytes is stored as ferritin.

mutually exclusive, and the determining factor is probably an iron absorption "set point" that was established when the enterocyte developed from a crypt cell. Iron that remains in the form of ferritin as the enterocyte completes its limited life cycle will be sloughed with the senescent cell and will leave the

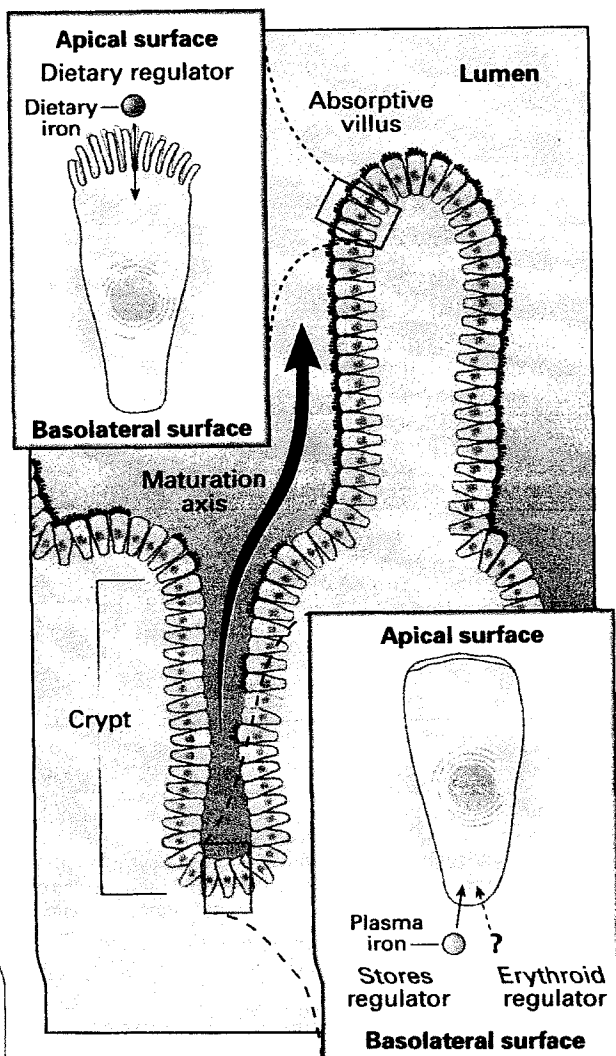


Figure 4. Regulation of the Absorption of Intestinal Iron. The iron-absorbing cells of the duodenal epithelium originate in the intestinal crypts and migrate toward the tip of the villus as they differentiate (maturation axis). Absorption of intestinal iron is regulated by at least three independent mechanisms. First, iron absorption is influenced by recent dietary iron intake (dietary regulator). After a large dietary bolus, absorptive cells are resistant to iron uptake for several days. Second, iron absorption can be modulated considerably in response to body iron stores (stores regulator). Third, an unidentified signal communicates the state of bone marrow erythropoiesis to the intestine (erythroid regulator). When red-cell production in the bone marrow is accelerated because of ineffective erythropoiesis, absorption of intestinal iron is increased. This process occurs even when there is systemic iron overload.

body through the gastrointestinal tract. This process represents an important mechanism of iron loss.

The basolateral enterocyte iron transporter has not been definitively identified, but a recently described protein, Ireg1, is a likely candidate.⁹ Genetic studies in mice have shown that the basolateral transporter

requires an accessory protein, a multicopper protein called hephaestin.¹⁰ Hephaestin is similar to plasma ceruloplasmin and is presumed to function as a ferroxidase. As will be discussed below, ceruloplasmin also has an important role in iron metabolism.

The absorption of intestinal iron is regulated in several ways (Fig. 4). First, it can be modulated by the amount of iron recently consumed in the diet, a mechanism referred to as the dietary regulator. For several days after a dietary iron bolus, absorptive enterocytes are resistant to acquiring additional iron. This phenomenon has previously been called "mucosal block."¹¹ This blocking action probably results from the accumulation of intracellular iron, leading the enterocyte to believe that its set-point requirements have been met. It may occur even in the presence of systemic iron deficiency.

A second regulatory mechanism also senses iron levels but responds to total body iron, rather than dietary iron. This mechanism has been termed the stores regulator.¹² It is capable of changing the amount of iron absorbed to a limited extent: iron absorption is modulated by a factor of only two to three in iron-deficient states as compared with iron-replete states.⁵ Although the molecular details of the stores regulator are not known, it probably acts at the level of crypt-cell programming, in response to the saturation of plasma transferrin with iron. Experiments in animals suggest that the levels of the apical transporter, DMT1, are altered in response to changes in body iron stores.⁸

The third regulatory mechanism, known as the erythropoietic regulator,¹² does not respond to iron levels at all. Rather, it modulates iron absorption in response to the requirements for erythropoiesis. The erythropoietic regulator has a greater capacity to increase iron absorption than the stores regulator.¹² It is logical that the erythron should have some influence on the rate of intestinal iron absorption, since most of the body iron is used for erythropoiesis. Yet how it accomplishes this is unknown. The erythropoietic regulator probably involves a soluble signal that is carried by plasma from the bone marrow to the intestine.

It is well documented that, in addition to iron-deficiency anemia, several other anemic states may lead to increased absorption of dietary iron. These conditions include the thalassemia syndromes, congenital dyserythropoietic anemias, and sideroblastic anemias. Strikingly, many other forms of anemia that are characterized by similar rates of erythropoiesis do not stimulate intestinal iron absorption. These disorders include hereditary spherocytosis, autoimmune hemolytic anemia, and sickle cell anemia. Thus, hyperproliferative anemias can be divided into two classes: those that stimulate iron absorption and those that do not. The two types can be differentiated in a simple way. The types that stimulate iron absorption have

in common the fact that erythroid cells are destroyed near the site of their development within the bone marrow (a situation known as ineffective erythropoiesis). The types that do not stimulate iron absorption involve the destruction of cells in the periphery. The importance of the site of destruction is not well understood. However, cells destroyed before their release from the bone marrow are less mature than circulating erythrocytes, suggesting that the soluble erythropoietic regulator is a molecule derived from precursor forms of erythrocytes, rather than later forms.

Iron absorption increases in response to acute hypoxia. It is not known whether the hypoxic signal is transduced through one of the regulatory pathways discussed above or through an independent mechanism.

DISEASES OF IRON DEFICIENCY

The clinical effects of iron deficiency have been described in the medical literature dating back to the Middle Ages, in fascinating accounts of a disorder called chlorosis. Chlorosis, resulting from iron deficiency in adolescent girls, peaked in incidence during the Victorian era.¹³ Although iron was not universally given to treat chlorosis, the disease disappeared as a clinical entity before World War II. However, iron deficiency remains an important public health problem today. In 1997, Looker et al. reported that 3 percent of American toddlers and 2 to 5 percent of American teenage girls are sufficiently iron-deficient to have anemia.¹⁴ More than half a billion people worldwide have adverse effects as a result of iron deficiency.

Iron-Deficiency Anemia

The human body prioritizes the use of iron in several ways. During development, the fetus draws iron away from its mother for itself. After birth, the erythron has relative priority as compared with other tissues. Red-cell production is unperturbed until iron stores are depleted, as reflected by low serum ferritin levels. When the stores have been used up, the iron saturation of transferrin decreases and patients begin to show evidence of iron-deficient erythropoiesis. The first biochemical clues of iron deficiency are increased levels of free protoporphyrin and zinc protoporphyrin in erythrocytes. The levels of soluble transferrin receptor, a protein-cleavage product that is present in plasma, increase when the lack of iron limits the production of new red cells. Frank anemia with microcytosis is detected later. A decreased reticulocyte hemoglobin level is a useful early indicator of iron-deficient erythropoiesis and may be superior to other laboratory measures in this respect.¹⁵

The symptoms and signs of iron deficiency are partially explained by the presence of anemia. They include pallor, fatigue, poor exercise tolerance, and decreased work performance. However, there also appears to be a direct effect of iron deficiency on the

central nervous system. In young children, measurable cognitive abnormalities may develop.¹⁶ In both children and adults, pica — a bizarre behavioral symptom that is highly characteristic of severe iron deficiency — can develop.¹⁷ Pica is characterized by the inappropriate consumption of nonnutritive substances; it disappears with iron treatment. Severe, long-standing iron deficiency may also be associated with koilonychia and the Plummer-Vinson syndrome, but these conditions are very rare in clinical practice in the United States.

Causes of iron deficiency are easy to understand when one accepts the fact that there is no physiologic pathway for iron excretion. Iron deficiency will result from any condition in which dietary iron intake does not meet the body's demands. For this reason, rapidly growing children and premenopausal women are at highest risk. Worldwide, dietary insufficiency as a cause of iron deficiency is usually secondary to intestinal blood loss resulting from parasitosis. In such cases, dietary intake is unable to keep up with chronic losses. A comprehensive list of the causes of iron deficiency is shown in Table 1.

Congenital and acquired abnormalities of the intestinal epithelium can also result in iron deficiency. Congenital defects in iron metabolism are fascinating but are poorly understood on a molecular level. Hypotransferrinemia, also called atransferrinemia, is a condition in which little or no plasma transferrin is produced. This rare disorder leads to severe iron-deficiency anemia accompanied by parenchymal iron overload.¹⁸⁻²¹

A distinct group of patients with normal plasma transferrin levels have iron-deficiency anemia that is unresponsive to oral iron therapy and incompletely responsive to parenteral iron therapy.^{22,23} Bannerman²⁴ made note of the similarity between patients with this condition and mutant mice that are now known to have an abnormality in the iron transporter DMT1.⁷ However, no mutations in the gene encoding DMT1 have been found in humans thus far.²⁵ It is possible that such patients have defects in other iron-transport steps.

Several forms of iron salt are used to treat iron deficiency. Remarkably, however, the treatment used by the 19th-century French physician Blaud²⁶ (ferrous sulfate) is still as effective as any other oral therapy. Perseverance is the cornerstone of successful treatment; it takes several months of replacement therapy to replenish body iron stores. Some patients have difficulty tolerating iron salts, because these substances tend to cause gastrointestinal distress. Liquid iron-salt preparations, given to young children, may cause permanent staining of the teeth. These problems can be circumvented by the use of an oral iron-polysaccharide complex. Both iron salts and the iron-polysaccharide preparation are inexpensive.

Infants and toddlers need relatively more iron

TABLE 1. CAUSES OF IRON DEFICIENCY.

Inadequate absorption
Poor bioavailability
Antacid therapy or high gastric pH
Excess dietary bran, tannin, phytates, or starch
Competition from other metals (e.g., copper or lead)
Loss or dysfunction of absorptive enterocytes
Bowel resection
Celiac disease
Inflammatory bowel disease
Intrinsic enterocyte defects
Increased loss
Gastrointestinal blood loss
Epistaxis
Varices
Gastritis
Ulcer
Tumor
Meckel's diverticulum
Parasitosis
Milk-induced enteropathy of early childhood
Vascular malformations
Inflammatory bowel disease
Diverticulosis
Hemorrhoids
Genitourinary blood loss
Menorrhagia
Cancer
Chronic infection
Pulmonary blood loss
Pulmonary hemosiderosis
Infection
Other blood loss
Trauma
Excessive phlebotomy
Large vascular malformations

than adults to support their rapid growth. Normal, full-term infants have a generous iron endowment at birth, totaling about 75 mg per kilogram.²⁷ Premature infants, infants of mothers with diabetes mellitus, and infants who are small for gestational age have substantially smaller iron stores than normal, full-term infants.²⁸ Stores are rapidly depleted, however, even in normal children, and there is little margin in iron balance. For that reason, iron-fortified infant formulas have been widely used since the early 1970s. There are no known contraindications to feeding with iron-fortified formulas and no apparent side effects.²⁸ Although a small proportion of infants have a genetic predisposition to iron overload later in life (see below), the amount of iron given in infant feedings should be inconsequential. All formula consumed by infants in the first year of life should contain 4 to 12 mg of iron per liter; "low iron" formulas containing less than 4 mg of iron per liter should not be given. Breast-fed infants receive adequate iron in a highly bioavailable form, and breast-feeding is recommended by the Committee on Nutrition of the American Academy of Pediatrics.²⁸

Patients who cannot tolerate or absorb oral iron,

those with severe iron deficiency who are not compliant with oral treatment, and those with profound iron deficits may benefit from parenteral iron therapy. Iron dextran is given intravenously, because intramuscular administration frequently leads to complications. Although there are rare cases of anaphylaxis, this treatment is generally safe and effective, particularly if the patient tolerates a test dose given before the replacement dose. Although the manufacturer recommends administering a maximum of 2 ml (100 mg) per day, most clinicians find that there is no problem in giving an entire replacement dose at one time.²⁹ Regimens for intravenously administered iron differ from those for orally administered iron. The correct dose can easily be determined with the use of a calculator provided by the manufacturer on the Internet (at <http://www.infed.com/calcltor.htm>). Intravenous iron dextran is taken up rapidly by reticuloendothelial macrophages, where it can be processed and loaded onto transferrin without toxic effects.

Anemia of Chronic Inflammation

Anemia of chronic inflammation, also known as anemia of chronic disease, has some features in common with iron-deficiency anemia. Iron-deficient erythropoiesis results from a defect in iron recycling. As a result, reticuloendothelial iron is plentiful in bone marrow macrophages, but this iron is not available to erythroid precursors. In patients with anemia of chronic inflammation, there appears to be a defect in the freeing of iron from macrophages, the loading of iron onto plasma transferrin, or both. Characteristic laboratory findings include low serum iron levels, low serum iron-binding capacity, increased serum ferritin, and normocytic or slightly microcytic erythrocytes. In contrast to patients with iron-deficiency anemia, those with anemia of chronic inflammation do not have elevated levels of serum transferrin receptor.³⁰ The pathophysiology of anemia of chronic inflammation is not understood, but the condition probably evolved as a cytokine-mediated defense against microbial pathogens. It effectively leads to the withholding of iron from microbes as well as from erythroid precursors.³¹ Mild anemia may be a relatively small price to pay for the attenuation of infection. The only effective treatment for anemia of chronic inflammation is correction of the underlying disorder.

DISEASES OF IRON OVERLOAD

Iron overload usually presents in one of two characteristic patterns. In cases in which erythropoiesis is normal but the plasma iron content exceeds the iron-binding capacity of transferrin (e.g., in cases of hereditary hemochromatosis), iron is deposited in parenchymal cells of the liver, the heart, and a subgroup of endocrine tissues. In contrast, when iron overload results from the increased catabolism of erythrocytes (e.g., in cases of transfusional iron overload), iron

accumulates in reticuloendothelial macrophages first and only later spills over into parenchymal cells. Parenchymal iron loading is particularly dangerous, because it leads to tissue damage and fibrosis. The reticuloendothelial system is generally a safe sink for iron; reticuloendothelial macrophages keep it sequestered, even after rather large doses (e.g., after the administration of parenteral iron dextran). If left untreated, however, both forms of iron overload progress to parenchymal deposition and organ damage.

Hereditary Hemochromatosis

Classic hereditary hemochromatosis is the most prevalent monoallelic genetic disease in whites. It was first described in 1865 as a clinical triad of glycosuria, cirrhosis, and hyperpigmentation of the skin.³² Von Recklinghausen later established that these clinical features were due to iron deposition and coined the term hemochromatosis.³³ In 1976, Simon et al. discovered that the genetic predisposition for hemochromatosis cosegregated with the HLA-A3 allele, indicating that the defective gene was closely linked to the human major histocompatibility complex.³⁴ This critical finding paved the way for positional cloning of the hemochromatosis gene 20 years later.³⁵

The majority of patients with hereditary hemochromatosis are descended from a common Celtic ancestor who lived 60 to 70 generations ago.³⁶ They carry a unique missense mutation (C282Y) that alters a major-histocompatibility-complex class I-like protein designated HFE.³⁵ On the basis of data from blood donors, it is estimated that as many as 1 in 10 white Americans carries at least one allele with this mutation.³⁷ Clinically significant iron overload usually develops in patients who are homozygous for this mutation. A subgroup of heterozygous persons is also affected. Several other polymorphisms have been found in the gene encoding the HFE protein, but their clinical significance is unclear.^{33,38-40} At least one of these mutations, H63D, is probably deleterious when it is present as the second allele in persons who are heterozygous for C282Y.^{41,42}

HFE forms a heterodimer with beta₂-microglobulin. This heterodimer is expressed on the surface of many cells, including duodenal crypt cells and macrophages. The C282Y mutation alters the conformation of the HFE protein and interferes with its function. In the three years since this finding was first reported, the crystal structure of HFE has been identified, and it has been shown to form a high-affinity complex with the transferrin receptor.⁴³⁻⁴⁸ Nonetheless, it remains unclear how HFE regulates iron absorption. Insight may come from mouse strains that have been engineered to carry mutated *Hfe* genes, serving as models in which to study the pathophysiology of iron loading in hemochromatosis.^{49,50}

Although there is no doubt that the C282Y mutation causes hemochromatosis, there is a broad spec-

trum of clinical presentations in both persons homozygous for C282Y and those who are heterozygous. A small proportion of C282Y homozygotes have no clinical or biochemical evidence of iron overload. This finding indicates that other genetic and environmental factors must affect the phenotypic expression of the mutation.⁴²

Patients with hemochromatosis regularly absorb two to three times as much dietary iron as normal persons. Most do not have symptoms until adulthood, although the saturation of serum transferrin is usually increased by adolescence. Hemochromatosis should be suspected when the serum transferrin saturation exceeds 50 percent in premenopausal women and 60 percent in men and postmenopausal women. Excess iron is deposited in parenchymal cells of the liver, heart, pancreas, pituitary gland, and parathyroid gland. Early symptoms are nonspecific; they include fatigue, arthralgia, erectile dysfunction, and increased skin pigmentation. As the disease progresses, tender hepatomegaly develops and leads to liver fibrosis and cirrhosis. There is an increased incidence of hepatocellular carcinoma after substantial damage to the liver has occurred. Iron deposition in the heart causes cardiomyopathy that is usually congestive but may be restrictive or associated with pericarditis and arrhythmias. Associated types of endocrinopathy include diabetes mellitus, hypopituitarism, hypogonadism, and hypoparathyroidism. Patients with hemochromatosis are more susceptible than others to infection, particularly with *Vibrio vulnificus*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Salmonella enteritidis* serotype typhimurium, *Klebsiella pneumoniae*, *Escherichia coli*, *Rhizopus arrhizus*, and mucor species.

Liver biopsy is the gold standard for quantifying iron. The hepatic iron concentration typically exceeds 80 μmol per gram of liver, dry weight, resulting in a hepatic iron index of more than 1.9 mmol per kilogram per year (the hepatic iron index is the ratio of the hepatic iron concentration to the age of the patient in years). Iron overload may also be assessed by quantitative phlebotomy to the point of iron depletion. The diagnosis can be confirmed by direct mutation analysis of the *HFE* gene. Homozygosity for the C282Y mutation plus biochemical evidence of iron overload makes the diagnosis of hemochromatosis indisputable.

The availability of a genetic test for hemochromatosis has fueled controversy about the benefits of screening for the disease. The test is simple, and the disease is highly prevalent and treatable. However, important disadvantages must also be considered. There is concern, particularly in the United States, that persons known to be homozygous for the C282Y mutation would face discrimination from health and life insurers. Furthermore, the test is not always predictive; some persons who are homozygous for C282Y never have adverse effects resulting from iron overload.⁴²

and some patients with genetic iron overload do not have mutations in the *HFE* gene.⁵¹

The treatment of hemochromatosis has not changed substantially since 1950.⁵² Therapeutic phlebotomy is safe, effective, and inexpensive. Each 450 to 500 ml of blood contains 200 to 250 mg of iron. Ideally, therapy is begun before symptoms develop, when the serum ferritin level exceeds 200 μg per liter in nonpregnant, premenopausal women or 300 μg per liter in men and postmenopausal women.⁵³ Typically, phlebotomy is performed at a rate of 1 unit of blood per week until the patient has mild hypoferritinemia. Thereafter, it is continued as needed to keep the serum ferritin level below 50 μg per liter. On average, men require phlebotomy three to four times per year, and women require it one to two times per year.⁵³ When phlebotomy is instituted before end-stage organ damage has occurred, patients can have a normal life expectancy and quality of life. Even if begun later, phlebotomy can improve constitutional symptoms, relieve hepatomegaly and liver tenderness, and protect joints from arthritis. However, endocrine abnormalities and liver fibrosis, once they have developed, usually do not resolve.⁵³

In addition to performing phlebotomy, it is prudent to advise patients with hemochromatosis to modify their diets. They should avoid iron supplementation and restrict their intake of vitamin C, since vitamin C facilitates the absorption of iron. In addition, they should limit their consumption of red meat (a rich source of heme iron) and alcohol. It is wise for such patients to avoid raw shellfish, because several cases of fatal infection with *V. vulnificus* have been reported in patients with hemochromatosis.⁵³

Hemochromatosis Not Attributable to Mutations in *HFE*

A subgroup of patients with hereditary hemochromatosis, indistinguishable from those described above, do not have mutations in *HFE*, and their disease does not appear to be linked to the HLA complex.⁵¹ The genetic basis for their condition has not yet been determined.

African Iron Overload

Iron overload is not limited to persons of European descent. A distinct iron-loading disorder is prevalent in Africa, affecting up to 10 percent of some rural populations.⁵⁴ Formerly termed Bantu siderosis, African iron overload results from a predisposition to iron loading that is exacerbated by excessive intake of dietary iron.⁵⁵ It is particularly problematic among Africans who drink a traditional beer brewed in nongalvanized steel drums. Although the disorder was once attributed to dietary excess, serious iron overload does not develop in all beer drinkers, and not all patients with iron overload consume excessive amounts of the beer. Investigators have concluded that heterozygosity for an unidentified iron-loading

gene confers susceptibility; homozygous persons may be more severely affected.⁵⁶ African iron overload is not due to mutations in the *HFE* gene and is not linked to the HLA locus.^{55,57}

The pattern of iron deposition among persons with African iron overload differs from that among those with hereditary hemochromatosis.⁵⁸ Among the former, there is marked iron loading of Kupffer's cells as well as hepatocytes, resembling the pattern seen in patients with transfusional siderosis and suggesting a defect in erythroid iron recycling. Cirrhosis, occasionally complicated by hepatocellular carcinoma, is the predominant organ manifestation. Cardiomyopathy and diabetes are less common. Although serum ferritin levels are elevated, the transferrin saturation does not always reflect the true extent of iron overload in these patients. Patients with African iron overload are probably more susceptible than others to infection, and they have an increased incidence of tuberculosis.^{59,60}

Clinically significant iron overload may also occur in Americans of African descent,⁶¹⁻⁶³ although such persons rarely have mutations in the *HFE* gene.⁶⁴ It has been suggested that the mutation associated with African iron overload was brought to the United States primarily through the slave trade.⁶¹ The true incidence of siderosis among African Americans is not yet known. The results of epidemiologic studies may be confounded by the fact that the defective gene has not yet been identified and by the presence of coexisting conditions, such as various types of hemoglobinopathy, viral hepatitis, or alcoholism. If black Americans have the same disorder as patients with African iron overload, the transferrin saturation may not be elevated and it may be less useful as a screening tool for these patients than for patients with hemochromatosis.⁶⁵ Nonetheless, it seems prudent to consider a diagnosis of iron overload in black patients and to measure serum ferritin in those with pertinent clinical findings.

Juvenile Hemochromatosis

In rare instances, iron overload develops in a pattern resembling that of hereditary hemochromatosis but at a greatly accelerated rate. Several Italian families with multiple affected members have been particularly well characterized.⁶⁶ Their disorder has been termed juvenile hemochromatosis. Perhaps because of the young age of these patients, or perhaps because of the rate of iron loading, they are more likely to present with cardiomyopathy and endocrinopathy than with severe liver disease. Patients with this disorder typically die of heart failure before their 30th birthdays. The genetic basis of juvenile hemochromatosis is unknown. The *HFE* gene has been ruled out as a possible locus, and juvenile hemochromatosis maps to human chromosome 1q.^{67,68} It is reasonable to speculate that the product of the juvenile hemochroma-

tosis gene participates in the same regulatory pathway as the *HFE* gene.

Neonatal Hemochromatosis

Neonatal hemochromatosis is a fulminant disease characterized by massive hepatic iron loading and liver failure in the perinatal period.⁶⁹ Like other iron-overload disorders, neonatal hemochromatosis is characterized by the accumulation of iron in the myocardium and pancreatic acinar cells.^{70,71} The pathophysiology of this disorder is poorly understood, and it is not yet known whether iron loading is the primary problem or secondary to some other insult to developing hepatocytes.^{72,73} Rare familial cases have been reported, in some of which there was consanguinity, but the inheritance pattern has not been clearly defined.^{74,75} Unaffected siblings and parents do not have evidence of iron loading,^{70,76} and there is no genetic linkage to the HLA complex.⁷⁵ Liver transplantation is the primary treatment, but it is often unsuccessful.⁷⁷⁻⁷⁹

Aceruloplasminemia

In 1995, two groups described patients with progressive extrapyramidal signs, cerebellar ataxia, dementia, and diabetes mellitus, which were associated with low serum ceruloplasmin levels resulting from mutations in the ceruloplasmin gene.^{80,81} This disorder, aceruloplasminemia, is distinct from Wilson's disease, in which low serum ceruloplasmin levels result from a copper-transport defect. Ceruloplasmin has ferroxidase activity, which is involved in the release of iron from cells.⁸² Accordingly, patients with aceruloplasminemia have accumulation of iron in neural and glial cells of the brain (particularly the basal ganglia and dentate nucleus), hepatocytes, and pancreatic islet cells.⁸³ Aggressive chelation with deferoxamine may halt the progression of these complications.⁸⁴ Treatment with plasma or ceruloplasmin concentrate may be helpful. However, the decision about therapy must take into account the difficulty of moving proteins across an intact blood-brain barrier. Phlebotomy is unlikely to be helpful and may exacerbate hypochromic microcytic anemia, which develops in some patients as a result of their inability to recycle iron efficiently through the reticuloendothelial system.

In addition to aceruloplasminemia, there are several other iron-loading disorders that lead to degenerative neurologic conditions, including Hallervorden-Spatz disease and Friedreich's ataxia. Descriptions of these disorders are beyond the scope of this review.

Transfusional Siderosis

Long-term transfusion therapy is now a routine, life-saving treatment for patients with intractable anemia resulting from thalassemia, bone marrow failure, or aggressive treatment of cancer. In many centers, it is also used for patients with serious complications

of sickle cell disease. As discussed earlier, there is no mechanism for iron excretion. Repeated transfusion leads to rapid iron loading, because each unit of blood contains 200 to 250 mg of iron and can cause what is known as transfusional siderosis. Since this iron is derived from red cells, reticuloendothelial macrophages become iron-loaded before parenchymal tissue cells. However, in transfusional siderosis iron is ultimately deposited in the same sites as in other iron-overload disorders (hepatocytes, the myocardium, and endocrine tissues). Cardiomyopathy is more prominent in patients with transfusional iron overload than in those with hemochromatosis, probably because of rapid iron loading. The body iron burden is best determined by quantitative liver biopsy or magnetic-susceptibility measurement⁸⁵; measurement of serum ferritin and magnetic resonance imaging are less accurate methods. Phlebotomy is usually not a treatment option for patients with transfusional siderosis, because of their underlying diseases. Iron overload must be treated by chelation therapy. At present, the only option that is widely available is deferoxamine administered by continuous infusion. The goal of chelation is to maintain a hepatic iron burden of less than 15 mg per gram of liver, dry weight.⁸⁶ Oral chelators are under development, but to date none are as effective or safe as deferoxamine.

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REFERENCES

- Bothwell TH, Finch CA. Iron metabolism. Boston: Little, Brown, 1962.
- Aisen P, Wessling-Resnick M, Leibold EA. Iron metabolism. *Curr Opin Chem Biol* 1999;3:200-6.
- Fleming MD, Romano MA, Su MA, Garrick LM, Garrick MD, Andrews NC. Nramp2 is mutated in the anemic Belgrade (b) rat: evidence of a role for Nramp2 in endosomal iron transport. *Proc Natl Acad Sci U S A* 1998;95:1148-53.
- Andrews NC, Bridges KR. Disorders of iron metabolism and sideroblastic anemia. In: Nathan DG, Orkin SH, eds. *Nathan and Oski's hematology of infancy and childhood*. 5th ed. Vol. 1. Philadelphia: W.B. Saunders, 1998:423-61.
- Cook JD, Barry WE, Hershko C, Fillet G, Finch CA. Iron kinetics with emphasis on iron overload. *Am J Pathol* 1973;72:337-43.
- Riedel H-D, Remus AJ, Fitscher BA, Stremmel W. Characterization and partial purification of a ferrereductase from human duodenal microvillus membranes. *Biochem J* 1995;309:745-8.
- Fleming MD, Trenor CC III, Su MA, et al. Microcytic anaemia mice have a mutation in Nramp2, a candidate iron transporter gene. *Nat Genet* 1997;16:383-6.
- Gunshin H, Mackenzie B, Berger UV, et al. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature* 1997;388:482-8.
- McKie AT, Wehr K, Simpson RJ, Peters TJ, Hentze MW, Farzaneh F. Molecular cloning and characterisation of a novel duodenal-specific gene implicated in iron absorption. *Biochem Soc Trans* 1998;26:S264.
- Vulpe CD, Kuo YM, Murphy TL, et al. Hephaestin, a ceruloplasmin homologue implicated in intestinal iron transport, is defective in the sla mouse. *Nat Genet* 1999;21:195-9.
- Hahn PF, Bale WF, Ross JF, Balfour WM, Whipple GH. Radioactive iron absorption by the gastro-intestinal tract: influence of anemia, anoxia, and antecedent feeding distribution in growing dogs. *J Exp Med* 1943;78:169-88.
- Finch C. Regulators of iron balance in humans. *Blood* 1994;84:1697-702.
- Guggenheim KY. Chlorosis: the rise and disappearance of a nutritional disease. *J Nutr* 1995;125:1822-5.
- Looker AC, Dallman PR, Carroll MD, Gunter EW, Johnson CL. Prevalence of iron deficiency in the United States. *JAMA* 1997;277:973-6.
- Brugnara C, Zurakowski D, DiCanzio J, Boyd T, Platt O. Reticulocyte hemoglobin content to diagnose iron deficiency in children. *JAMA* 1999;281:2225-30.
- Pollitt E. Iron deficiency and cognitive function. *Annu Rev Nutr* 1993;13:521-37.
- Moore DF Jr, Sears DA. Pica, iron deficiency, and the medical history. *Am J Med* 1994;97:390-3.
- Heilmeyer L, Keller W, Vivell O, et al. Congenital transferrin deficiency in a seven-year old girl. *German Med Mon* 1961;6:385-9.
- Goya N, Miyazaki S, Kodate S, Ushio B. A family of congenital atransferrinemia. *Blood* 1972;40:239-45.
- Hamil RL, Woods JC, Cook BA. Congenital atransferrinemia: a case report and review of the literature. *Am J Clin Pathol* 1991;96:215-8.
- Hayashi A, Wada Y, Suzuki T, Shimizu A. Studies on familial hypotransferrinemia: unique clinical course and molecular pathology. *Am J Hum Genet* 1993;53:201-13.
- Buchanan GR, Sheehan RG. Malabsorption and defective utilization of iron in three siblings. *J Pediatr* 1981;98:723-8.
- Hartman KR, Barker JA. Microcytic anemia with iron malabsorption: an inherited disorder of iron metabolism. *Am J Hematol* 1996;51:269-75.
- Bannerman RM. Of mice and men and microcytes. *J Pediatr* 1981;98:760-2.
- Galanello R, Cau M, Melis MA, Deidda F, Cao A, Cazzola M. Studies of NRAMP2, transferrin receptor and transferrin genes as candidate genes for human hereditary microcytic anemia due to defective iron absorption and utilization. *Blood* 1998;92:Suppl 1:669a. abstract.
- Blaud P. Sur les maladies chlorotiques et sur un mode de traitement spécifique dans ces affections. *Rev Med Fr Etrang* 1832;45:357-67.
- Oski FA, Naiman JL, eds. Hematologic problems in the newborn. 3rd ed. Vol. 4 of Major problems in clinical pediatrics. Philadelphia: W.B. Saunders, 1982:32-3.
- American Academy of Pediatrics, Committee on Nutrition. Iron fortification of infant formulas. *Pediatrics* 1999;104:119-23.
- Auerbach M, Witt D, Toler W, Fierstein M, Lerner RG, Ballard H. Clinical use of the total dose intravenous infusion of iron dextran. *J Lab Clin Med* 1988;111:566-70.
- Ferguson BJ, Skikne BS, Simpson KM, Baynes RD, Cook JD. Serum transferrin receptor distinguishes the anemia of chronic disease from iron deficiency anemia. *J Lab Clin Med* 1992;119:385-90.
- Jurado RL. Iron, infections, and anemia of inflammation. *Clin Infect Dis* 1997;25:888-95.
- Trousseau A. Glycosurie: diabete sucre. *Clin Med Hotel Dieu Paris* 1865;2:663-98.
- von Recklinghausen FD. *Über haemochromatose*. *Tageblatt Versammlung Dtsch Naturforsch Artze Herdelbert* 1889;62:324-5.
- Simon M, Bourel M, Faucher R, Genetet B. Association of HLA-A3 and HLA-B14 antigens with idiopathic haemochromatosis. *Gut* 1976;17:332-4.
- Feder JN, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet* 1996;13:399-408.
- Ajioka RS, Jorde LB, Gruen JR, et al. Haplotype analysis of hemochromatosis: evaluation of different linkage-disequilibrium approaches and evolution of disease chromosomes. *Am J Hum Genet* 1997;60:1439-47.
- Edwards CQ, Griffen LM, Goldgar D, Drummond C, Skolnick MH, Kushner JP. Prevalence of hemochromatosis among 11,065 presumably healthy blood donors. *N Engl J Med* 1988;318:1355-62.
- Barton JC, Sawada-Hirai R, Rothenberg BE, Acton RT. Two novel missense mutations of the HFE gene (I105T and G93R) and identification of the S65C mutation in Alabama hemochromatosis probands. *Blood Cells Mol Dis* 1999;25:147-55.
- Mura C, Raguene O, Ferec C. HFE mutations analysis in 711 hemochromatosis probands: evidence for S65C implication in mild form of hemochromatosis. *Blood* 1999;93:2502-5.
- Wallace DF, Dooley JS, Walker AP. A novel mutation of HFE explains the classical phenotype of genetic hemochromatosis in a C282Y heterozygote. *Gastroenterology* 1999;116:1409-12.
- Bacon BR, Olynyk JK, Brunt EM, Britton RS, Wolff RK. HFE gen-

- otype in patients with hemochromatosis and other liver diseases. *Ann Intern Med* 1999;130:953-62.
42. Olynyk JK, Cullen DJ, Aquilia S, Rossi E, Summerville L, Powell LW. A population-based study of the clinical expression of the hemochromatosis gene. *N Engl J Med* 1999;341:718-24.
43. Lebron JA, Bennett MJ, Vaughn DE, et al. Crystal structure of the hemochromatosis protein HFE and characterization of its interaction with transferrin receptor. *Cell* 1998;93:111-23.
44. Parkkila S, Waheed A, Britton RS, et al. Association of the transferrin receptor in human placenta with HFE, the protein defective in hereditary hemochromatosis. *Proc Natl Acad Sci U S A* 1997;94:13198-202.
45. Feder JN, Penny DM, Irinki A, et al. The hemochromatosis gene product complexes with the transferrin receptor and lowers its affinity for ligand binding. *Proc Natl Acad Sci U S A* 1998;95:1472-7.
46. Eisenstein RS. Interaction of the hemochromatosis gene product HFE with transferrin receptor modulates cellular iron metabolism. *Nutr Rev* 1998;56:356-8.
47. Waheed A, Parkkila S, Saarnio J, et al. Association of HFE protein with transferrin receptor in crypt enterocytes of human duodenum. *Proc Natl Acad Sci U S A* 1999;96:1579-84.
48. Roy CN, Penny DM, Feder JN, Enns CA. The hereditary hemochromatosis protein, HFE, specifically regulates transferrin-mediated iron uptake in HeLa cells. *J Biol Chem* 1999;274:9022-8.
49. Zhou XY, Tomatsu S, Fleming RE, et al. HFE gene knockout produces mouse model of hereditary hemochromatosis. *Proc Natl Acad Sci U S A* 1998;95:2492-7.
50. Levy JE, Montross LK, Cohen DE, Fleming MD, Andrews NC. The C282Y mutation causing hereditary hemochromatosis does not produce a null allele. *Blood* 1999;94:9-11.
51. Pietrangelo A, Montosi G, Totaro A, et al. Hereditary hemochromatosis in adults without pathogenic mutations in the hemochromatosis gene. *N Engl J Med* 1999;341:725-32.
52. Davis WD Jr, Arrowsmith WR. The effect of repeated bleeding in hemochromatosis. *J Lab Clin Med* 1950;36:814-5. abstract.
53. Barton JC, McDonnell SM, Adams PC, et al. Management of hemochromatosis. *Ann Intern Med* 1998;129:932-9.
54. Gordeuk VR. Hereditary and nutritional iron overload. *Baillieres Clin Haematol* 1992;5:169-86.
55. Gordeuk V, Mukiibi J, Hasstedt SJ, et al. Iron overload in Africa: interaction between a gene and dietary iron content. *N Engl J Med* 1992;326:95-100.
56. Moyo VM, Mandishona E, Hasstedt SJ, et al. Evidence of genetic transmission in African iron overload. *Blood* 1998;91:1076-82.
57. McNamara L, MacPhail AP, Gordeuk VR, Hasstedt SJ, Rouault T. Is there a link between African iron overload and the described mutations of the hereditary hemochromatosis gene? *Br J Haematol* 1998;102:1176-8.
58. Gangaidzo IT, Moyo VM, Saungweme T, et al. Iron overload in urban Africans in the 1990s. *Gut* 1999;45:278-83.
59. Gordeuk VR, McLaren CE, MacPhail AP, Deichsel G, Bothwell TH. Associations of iron overload in Africa with hepatocellular carcinoma and tuberculosis: Strachan's 1929 thesis revisited. *Blood* 1996;87:3470-6.
60. Moyo VM, Gangaidzo IT, Gordeuk VR, Küre CF, Macphail AP. Tuberculosis and iron overload in Africa: a review. *Cent Afr J Med* 1997;43:334-9.
61. Barton JC, Edwards CQ, Bertoli LF, Shroyer TW, Hudson SL. Iron overload in African Americans. *Am J Med* 1995;99:616-23.
62. Wurapa RK, Gordeuk VR, Brittenham GM, Khiyami A, Schechter GP, Edwards CQ. Primary iron overload in African Americans. *Am J Med* 1996;101:9-18.
63. Bacr D. Hereditary iron overload and African Americans. *Am J Med* 1996;101:5-8.
64. Monaghan KG, Rybicki BA, Shurafa M, Feldman GL. Mutation analysis of the HFE gene associated with hereditary hemochromatosis in African Americans. *Am J Hematol* 1998;58:213-7.
65. Gordeuk VR, McLaren CE, Looker AC, Hasselblad V, Brittenham GM. Distribution of transferrin saturations in the African-American population. *Blood* 1998;91:2175-9.
66. Camaschella C, Roetto A, Cicilano M, et al. Juvenile and adult hemochromatosis are distinct genetic disorders. *Eur J Hum Genet* 1997;5:371-5.
67. Camaschella C, Fargion S, Sampietro M, et al. Inherited HFE-unrelated hemochromatosis in Italian families. *Hepatology* 1999;29:1563-4.
68. Roetto A, Totaro A, Cazzola M, et al. Juvenile hemochromatosis locus maps to chromosome 1q. *Am J Hum Genet* 1999;64:1388-93.
69. Knisely AS. Neonatal hemochromatosis. *Adv Pediatr* 1992;39:383-403.
70. Goldfischer S, Grotsky HW, Chang CH, et al. Idiopathic neonatal iron storage involving the liver, pancreas, heart, and endocrine and exocrine glands. *Hepatology* 1981;1:58-64.
71. Blisard KS, Bartow SA. Neonatal hemochromatosis. *Hum Pathol* 1986;17:376-83.
72. Witzleben CL, Uri A. Perinatal hemochromatosis: entity or end result? *Hum Pathol* 1989;20:335-40.
73. Hoogstraten J, de Sa DJ, Knisely AS. Fetal liver disease may precede extrahepatic siderosis in neonatal hemochromatosis. *Gastroenterology* 1990;98:1699-701.
74. Danks D, Bodian M. A genetic study of neonatal obstructive jaundice. *Arch Dis Child* 1963;38:378-90.
75. Driscoll SG, Hayes AM, Levy HL. Neonatal hemochromatosis: evidence for autosomal recessive transmission. *Am J Hum Genet* 1988;43: Suppl:A232. abstract.
76. Dalhoj J, Kiaer H, Wiggers P, Grady RW, Jones RL, Knisely AS. Iron storage disease in parents and sibs of infants with neonatal hemochromatosis: 30-year follow-up. *Am J Med Genet* 1990;37:342-5.
77. Rand EB, McClenathan DT, Whittington PE. Neonatal hemochromatosis: report of successful orthotopic liver transplantation. *J Pediatr Gastroenterol Nutr* 1992;15:325-9.
78. Lund DP, Lillehei CW, Kevy S, et al. Liver transplantation in newborn liver failure: treatment for neonatal hemochromatosis. *Transplant Proc* 1993;25:1068-71.
79. Sigurdsson L, Reyes J, Kocoshis SA, Hansen TW, Rosh J, Knisely AS. Neonatal hemochromatosis: outcomes of pharmacologic and surgical therapies. *J Pediatr Gastroenterol Nutr* 1998;26:85-9.
80. Yoshida K, Furihara K, Takeda S, et al. A mutation in the ceruloplasmin gene is associated with systemic hemosiderosis in humans. *Nat Genet* 1995;9:267-72.
81. Harris ZL, Takahashi Y, Miyajima H, Serizawa M, MacGillivray RTA, Gitlin JD. Aceruloplasminemia: molecular characterization of this disorder of iron metabolism. *Proc Natl Acad Sci U S A* 1995;92:2539-43.
82. Frieden E, Hsieh HS. Ceruloplasmin: the copper transport protein with essential oxidase activity. *Adv Enzymol Relat Areas Mol Biol* 1976;44:187-236.
83. Morita H, Ikeda S, Yamamoto K, et al. Hereditary ceruloplasmin deficiency with hemosiderosis: a clinicopathological study of a Japanese family. *Ann Neurol* 1995;37:646-56.
84. Miyajima H, Takahashi Y, Kamata T, Shimizu H, Sakai N, Gitlin JD. Use of desferrioxamine in the treatment of aceruloplasminemia. *Ann Neurol* 1997;41:404-7.
85. Brittenham GM, Farrell DE, Harris JW, et al. Magnetic-susceptibility measurement of human iron stores. *N Engl J Med* 1982;307:1671-5.
86. Olivieri NF. The β -thalassemias. *N Engl J Med* 1999;341:99-109.