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IRON OVERLOAD: Causes and Consequences

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INTRODUCTION

Iron overload, long considered a rarity, is now recognized as a common disorder of iron metabolism. In the United States, a genetically determined form of iron loading, the homozygous state for hereditary hemochromatosis, is believed to affect as much as 0.5% of the population, or a million individuals or more (1, 2). For comparison, iron deficiency anemia among adult men is less than half as prevalent according to an interpretation of data collected in the second United States National Health and Nutrition Examination Survey, NHANES II (3). In the United States, additional thousands of individuals have iron overload associated with refractory and transfusion-

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dependent anemias such as thalassemia major. From a global perspective, thalassemia major and related conditions are now a serious public health problem in many parts of the world. A recent review by a Working Group of the World Health Organization (4) has estimated that worldwide over 100,000 individuals are born annually with thalassemic syndromes who are at risk for iron loading. In sub-Saharan Africa, a special form of dietary iron overload resulting from intake of iron in brewed beverages is also a public health concern. The causes and consequences of the various forms of iron overload are described here, along with summaries of recent developments in the detection and measurement of iron excess and of current therapeutic approaches to the management of these disorders.

IRON METABOLISM

The concentration of iron in the human body is normally about 40–50 mg Fe/kg body weight; women typically have lower and men higher amounts. Most of this iron, about 30 mg Fe/kg, is contained within circulating red cells as hemoglobin; an additional 5–6 mg Fe/kg is present in tissues throughout the body in functional form in a variety of heme compounds (myoglobin, cytochromes), enzymes with iron-sulfur complexes, and other iron-dependent enzymes. The remainder of the iron (5 mg Fe/kg in women, 10–12 mg Fe/kg in men) is stored as ferritin and hemosiderin in the liver, bone marrow, spleen, and muscle, serving as a readily available reserve in the event of blood loss. Only a small fraction of the body iron (about 3 mg) circulates in plasma, carried by the iron transport protein, transferrin.

Iron balance is normally regulated by controlling iron absorption; iron stores and iron absorption are reciprocally related so that as stores decline absorption increases. The rate of erythropoiesis is also a major determinant of iron absorption, with increased erythropoietic activity linked to enhanced iron absorption. Iron is absorbed through the upper intestine under the regulation of the intestinal mucosa. The amount and bioavailability of dietary iron, lumenal pH and motility, and other factors influence but do not regulate absorption. The means whereby iron is transferred from the lumen to mucosal cells remain unknown. In some animals a lumenal apotransferrin seems to act as a shuttle for iron, but there is no evidence of a physiological role for transferrin in the absorption of iron in humans (5). The body lacks any effective means to excrete excess iron. Iron exchange is limited so that an adult man absorbs and loses only about 0.01 mg Fe/kg/day. A woman of child-bearing age loses slightly more iron because of menstrual blood flow and in balance absorbs a larger amount so that iron exchange is about 0.015 mg Fe/kg/day.

Storage iron is usually present in roughly equal amounts in the macro-

phages of the reticuloendothelial system, in hepatic parenchymal cells, and in skeletal muscle. The concentration of storage iron in skeletal muscle is low (15–30 μg/g), but because of the large mass present (about 30,000 g in an adult man), about one third of the body reserve is normally found in muscle. With iron overload, muscle storage iron is increased but not as much as are hepatic or bone marrow stores; the muscle storage iron seems relatively nonmiscible (6). Reticuloendothelial cell iron is derived almost entirely from phagocytosis of senescent erythrocytes or defective developing red cells, with the exception of the iatrogenic source of parenteral iron preparations. The phagocytized iron is recycled to plasma transferrin and then transported to the erythroid marrow for use in hemoglobin production. Normally, only a small portion of the transferrin iron enters hepatocytes, which can also derive iron from methemalbumin, hemoglobin-haptoglobin, and heme-hemopexin complexes formed after intravascular hemolysis. Ferritin is also taken up by liver parenchymal cells. In the normal individual, the overall extent of iron exchange by hepatocytes is much less (about one fifth) than that by the reticuloendothelial cells (7).

CAUSES OF IRON OVERLOAD

The causes of iron overload are summarized in Table 1. In adults and children, iron overload may be produced by an increased absorption of dietary iron, by parenteral administration of iron, or both. An increased absorption of iron may be the result (*a*) of an inappropriately elevated uptake from a diet with normal amounts of iron, as occurs in hereditary hemochromatosis, the iron-loading anemias, and other conditions; or (*b*) of consumption of large amounts of bioavailable iron, as is found in some regions in Africa and, possibly, with prolonged ingestion of medicinal iron. Parenteral iron loading is produced by repeated blood transfusion or, less often, by injections of therapeutic iron preparations. Iron overload has also been recognized in the neonate and infant, presumably as the result of a disturbance in the regulation of maternal-fetal iron balance, but the pathogenesis of such disorders is still uncertain. Rarely, sequestration of iron, as occurs in pulmonary hemosiderosis, may lead to a focal iron overload.

The magnitude, rate, and distribution of iron accumulation influence the onset and severity of complications and differ for the various disorders described below. Estimates of the body burdens associated with clinical manifestations in iron overload are shown in Table 2. Because the magnitude of the body iron accumulation is only one factor affecting iron toxicity, these estimates are approximate and exceptions must be anticipated. The distribution of the excess iron between relatively benign reticuloendothelial sites and potentially toxic parenchymal locations, internal redistribution of iron,

Table 1 Causes of iron overload

I. Increased iron absorption
A. From diets with normal amounts of bioavailable iron
1. Hereditary (HLA-linked) hemochromatosis
2. Iron-loading anemias (refractory anemias with hypercellular erythroid marrow)
3. Chronic liver disease (cirrhosis, portacaval shunt)
4. Porphyria cutanea tarda
5. Congenital defects (atransferrinemia and other disorders)
B. From diets with increased amounts of bioavailable iron
1. African dietary overload
2. Kaschin-Beck (Urov) disease (?)
3. Medicinal iron ingestion (?)
II. Parenteral iron overload
A. Transfusional iron overload
B. Inadvertant iron overload from therapeutic injections
III. Neonatal iron overload
A. Hereditary tyrosinemia
B. Zellweger's cerebrohepatorenal syndrome
C. Neonatal hemochromatosis
IV. Focal seqestration of iron
A. Idiopathic pulmonary hemosiderosis
B. Hallervorden-Spatz syndrome
C. Renal hemosiderosis

amounts of circulating non-transferrin-bound iron, ascorbate status, and other factors also influence the extent of tissue damage. The specific clinical consequences of toxic iron accumulation are described after considering the clinical characteristics of the various forms of iron overload.

Increased Iron Absorption

Iron overload as a result of increased iron absorption may be produced either by (*a*) a regulatory abnormality permitting an absorption that is inappropriately high for the level of body iron stores from a diet with normal amounts of bioavailable iron, or (*b*) ingestion of such large amounts of bioavailable iron that the regulatory mechanism is overwhelmed. Since the molecular events governing the normal absorptive mechanism are unknown, it is not surprising that the metabolic defects responsible for disorders resulting from increased iron absorption remain obscure.

INCREASED ABSORPTION FROM DIETS WITH NORMAL AMOUNTS OF BIOAVAILABLE IRON

Hereditary (HLA-linked) hemochromatosis Hereditary hemochromatosis is an iron-loading disorder with an autosomal recessive mode of inheritance; the

Table 2 Body iron stores and clinical manifestations in iron overload

Condition	Body iron stores[a] (mg Fe/kg body weight)		
	"Safe"	"Symptomatic"	"Lethal"
Normal	5–12	—	—
Homozygous hereditary hemochromatosis	<50	<50–>200	<200–>400
Untransfused iron-loading anemia	<100	<100–>400	<400–>1000
Chronic liver disease	<25–50	—	—
Porphyria cutanea tarda	<20	<20–>50	-
African dietary iron overload	<100	<100–>400	<400–>1000
Transfusion-dependent anemia	<400	<400–>1000	<1000–>2000

[a] Approximate estimates with considerable overlap between the ranges shown.

hemochromatosis locus is less than one centimorgan (corresponding to a recombination frequency of < 1%) from the HLA-A locus on the short arm of chromosome 6 (8–10). The frequency of a hemochromatosis gene in various countries was recently reported to be 5–7%, with corresponding frequencies of 10% or more for heterozygotes and of 0.25–0.5% for homozygotes (1, 2, 11, 12). Previous reports of an apparently dominant inheritance pattern are now considered to be explicable by heterozygote-homozygote matings (13). In some populations, a linkage with HLA-A3 has been identified (14).

In homozygotes for hereditary hemochromatosis, iron absorption is inappropriately high at any level of body iron, and a chronic positive iron balance results with progressive, predominantly parenchymal cell overload and damage, first in the liver (15, 16) but later in pancreas, skin, joints, endocrine organs, heart, and other tissues. Reticuloendothelial macrophages in the bone marrow, spleen, and liver initially may have normal or even decreased amounts of storage iron (17) although later in the course of hemochromatosis stores in these sites may also increase. Symptomatic expression is usually delayed until middle or late life when the body iron burden is 100–200 mg Fe/kg body weight; accumulations of as little as 200–400 mg Fe/kg may be lethal (Table 2)(18). Environmental factors, such as dietary iron content and alcohol use, affect the expression of the disease; the male predominance in symptomatic patients is presumably explained by the relative protection provided to women during the reproductive years by iron losses during menstruation and pregnancy.

The classical clinical tetrad of hemochromatosis consists of hepatomegaly, skin pigmentation, diabetes mellitus, and hypogonadism, although not all these features occur in each patient. Cardiac dysfunction, other endocrinopathies, arthropathy, and occasionally neurological and psychological abnormalities may also develop. Mortality from hemochromatosis appears to be preventable if patients are diagnosed and excess body iron removed

by phlebotomy therapy before the development of hepatic cirrhosis (19). Although the usual presentation of hemochromatosis is in the older patient, hemochromatosis may also occur in young people, with cardiac dysfunction and hypogonadism as the presenting manifestations (20, 21); it is uncertain whether hemochromatosis in the young is a different genetic disorder or a more severe form of the adult disease.

In heterozygotes for hereditary hemochromatosis, the disorder is only partially expressed: biochemical abnormalities develop in about 25% of those affected. No deleterious consequences of the minor iron load that may be found in simple heterozygotes have been reported (22–24). By contrast, heterozygotes who inherit or acquire certain other disorders may develop clinical manifestations similar to those seen in the homozygous state. Reports have now appeared of increased iron loading in patients heterozygous for hereditary hemochromatosis in combination with idiopathic refractory sideroblastic anemia (25), with hereditary spherocytosis (26), with abnormal hemoglobins with increased oxygen affinity (116), and possibly, with chronic hemodialysis (27–30). Iron loading has not been observed in heterozygotes for hemochromatosis with beta-thalassemia minor (31).

Iron-loading anemias The iron-loading anemias are a group of refractory disorders, usually with hypercellular marrows and erythroid expansion associated with ineffective erythropoiesis, in which massive iron overload may develop that is not accounted for by red cell transfusions. These refractory anemias include thalassemia major and intermedia, hemoglobin E–beta thalassemia, a variety of sideroblastic anemias, congenital dyserythropoietic anemias, pyruvate kinase deficiency, and a number of anemias associated with blocks in the incorporation of iron into hemoglobin (32). In untransfused patients with iron-loading anemias, increased erythroid activity with ineffective erythropoiesis results in an increased iron demand, and intestinal iron absorption may increase as much as tenfold to 0.1 mg/kg/day. The excess absorbed iron is mainly deposited in parenchymal sites—initially in the hepatocyte but eventually in the pancreas, heart, and other organs—with a magnitude and pattern similar to that found in hereditary hemochromatosis. In untransfused patients, fatal levels have not been determined with precision, but are likely to lie between those of homozygous hereditary hemochromatosis and transfusion-dependent thalassemia major, i.e. 400–1000 mg Fe/kg (Table 2). As noted above, the iron accumulation in some cases of sideroblastic anemia may be determined by the presence of a hemochromatosis allele (25), but in others the increased iron absorption seems to be associated with the extent of erythroid activity. The magnitude of iron overload is independent of the degree of anemia; major iron loads may accumulate without severe anemia in patients with sideroblastic anemia, congenital dyserythropoietic anemia, and thalassemia intermedia (33–35).

Chronic liver disease Chronic liver disease, including alcoholic cirrhosis and, possibly, portacaval shunting, may be associated with modest iron overload. Patients with alcoholic liver disease and iron overload are neither heterozygous nor homozygous for hereditary hemochromatosis (9, 12). The cause of iron accumulation is unknown, but increased absorption related to (*a*) ineffective erythropoiesis associated with alcohol-related folate and sideroblastic abnormalities and (*b*) a saturated transferrin with alcohol ingestion resulting in preferential hepatic iron deposition have been suggested as possible factors (36). The body iron is characteristically increased to only 25–50 mg Fe/kg (Table 2). In the liver, iron deposits are found predominantly in Kupffer rather than parenchymal cells (32). Hepatic iron concentrations, when corrected for the age of the patient, appear to distinguish patients with alcoholic siderosis from those with hereditary hemochromatosis (37).

Porphyria cutanea tarda Porphyria cutanea tarda is a hepatic porphyria in which the liver produces an excessive amount of acetate-substituted porphyrins that circulate to the skin. Because porphyrins are potent photosensitizers, the sun-exposed skin is particularly susceptible to pathologic changes; increased skin fragility, altered pigmentation, hypertrichosis, and scleroderma-moid thickening result. Biochemically, porphyria cutanea tarda is characterized by deficient activity of uroporphyrinogen decarboxylase, the enzyme that sequentially decarboxylates uroporphyrinogen to form coproporphyrinogen. The hepatic enzyme is present but has reduced substrate affinity and increased susceptibility to inhibition by iron (38). Two forms of porphyria cutanea tarda have been described: an autosomal dominant familial variety with decreased uroporphyrinogen decarboxylase activity in red cells, and another type with normal erythrocyte enzyme activity. In clinically affected patients, the liver typically contains increased amounts of storage iron; the body iron is usually modestly increased to 20–50 mg Fe/kg (39, 40, 41). In some individuals, the increased iron seems related to alcoholic cirrhosis, but frequently no cause for increased iron absorption is found (42). Clinically, the role of iron in the symptomatic expression of porphyria cutanea tarda is clearly demonstrated by the therapeutic response of patients to phlebotomy therapy. Phlebotomy results in clinical and biochemical remission of the disease, while repletion of iron exacerbates the disease in previously phlebotomized patients.

Congenital defects associated with iron overload Congenital atransferrinemia is a rare, probably autosomal recessive, disorder characterized by the absence of transferrin, the plasma iron transport protein. A microcytic, hypochromic anemia unresponsive to iron therapy is present, but infusion of transferrin stimulates erythropoiesis and improves the hemoglobin concentration (43). Iron deposits are found in the liver, heart, thyroid, kidneys, and

pancreas but are scant in the spleen and absent in the bone marrow. Other rare congenital defects of unknown etiology but characterized by hypochromic microcytic anemia in association with hepatic parenchymal iron deposition have also been described (44, 45).

INCREASED ABSORPTION FROM DIETS WITH LARGE AMOUNTS OF BIOAVAILABLE IRON

African iron overload Iron overload has been clearly shown to result from excess dietary iron only in sub-Saharan Africa, where it has been described in nine countries (46). Although the prevalence and magnitude of iron accumulation have been reported to be decreasing in urban residents (47), a recent study in the rural areas, where more than 80% of the population lives, found evidence of a continued high prevalence of iron overload (46). The excess dietary iron is derived from a traditional fermented maize beverage that is home-brewed from locally grown crops in steel drums. A substantial portion of the iron in the beverage is in the reduced ionic state and is easily absorbed from the gastrointestinal tract. The body iron burden may equal or exceed that found in patients with hereditary hemochromatosis and reach 400–1000 mg Fe/kg (Table 2). Iron deposition is prominent in both reticuloendothelial tissues and in hepatic parenchymal cells; after hepatic cirrhosis develops, iron is also found in the pancreas, thyroid, adrenal, and heart (48).

In African dietary iron overload, bone marrow iron stores rise in proportion to the total body iron, while in hereditary hemochromatosis marrow stores do not show a comparable increase (49). Most of the clinical complications encountered with hereditary hemochromatosis may develop in patients with African dietary overload and, in addition, ascorbic acid deficiency and osteoporosis may occur (50). Apparently, no data on the prevalence of the hemochromatosis allele in African blacks have been reported and its role, if any, in African dietary iron overload is uncertain.

Kaschin-Beck (Urov) disease Kaschin-Beck disease, also known as Urov disease, is a disorder characterized by skeletal deformities and widespread hemosiderosis that is endemic in eastern Siberia, Manchuria, and northern China. Early investigators attributed this condition to the high iron content of drinking water, but the bone and joint deformities of Kaschin-Beck disease have not been observed in any other form of iron overload (51). Some Soviet investigators have suggested that a chronic fungal infection with *Fusaria sporotrichiella,* acquired by ingesting contaminated grain, is the cause (52), while others have called attention to a "decreased content of calcium and rise in the content of strontium, iron, manganese, lead, zinc and silver" in the bones (53). The etiology of this disorder and its relationship, if any, to iron remain obscure.

Medicinal iron ingestion The production of iron overload in normal individuals by prolonged medicinal iron ingestion has not been definitively demonstrated. Case reports of normal individuals with long histories of iron ingestion have provided conflicting evidence (32) and the possible effect of a hemochromatosis allele in combination with iron ingestion has not been examined. In contrast, there is no doubt that oral medicinal iron can add to the body burden of patients with iron-loading disorders.

Parenteral Iron Overload

TRANSFUSIONAL IRON OVERLOAD Iron overload in patients with refractory anemia may be the consequence of repeated blood transfusion, of excessive absorption of dietary iron, or of a combination of both. In patients with thalassemia major (Cooley's anemia) death in infancy from anemia can be averted by a regular transfusion program, which, if adequate, allows for normal growth and development during the first decade of life. Without treatment, growth slows, liver disease, diabetes and other endocrine disturbances develop, and death owing to iron loading of the myocardium occurs toward the end of the second decade, typically as a result of cardiac dysfunction (54). The course of patients with other severe congenital anemias such as the Blackfan-Diamond syndrome is nearly identical to that seen with thalassemia major. Transfusion-dependent anemia that appears later in life (aplastic anemia, pure red cell aplasia, hypoplastic or myelodysplastic disorders, anemia of chronic renal failure) eventually has a similar prognosis (55).

In patients on a high transfusion regimen, erythropoiesis is suppressed and iron absorption may be near normal, but each unit of transfused red cells contains 200–250 mg of iron. Most patients with thalassemia major require 200–300 ml/kg/year of blood, an amount equivalent to 0.25–0.40 mg Fe/kg/day. Initially the transfused iron is held within macrophages in the reticuloendothelial system, but as more accumulates parenchymal deposition and damage develop. In transfused patients with ineffective erythropoiesis and combined parenchymal and reticuloendothelial iron deposition (thalassemia major), levels below about 400 mg Fe/kg have been suggested to be "safe," levels of 750 mg Fe/kg to be toxic (with growth failure and endocrinopathy), and levels of 1000 mg Fe/kg or more to be lethal (Table 2) (54). Transfused patients with aplastic marrows (aplastic anemia, red cell aplasia) and primarily reticuloendothelial iron may tolerate up to 2000 mg Fe/kg (18).

Transfusional iron overload may also occur in patients with sickle cell disease who are chronically transfused for the prevention of recurrent complications such as central nervous system disorders, severe infections, incapacitating painful crises, and other problems. Although partial exchange transfusions, combining transfusion with phlebotomy, may retard iron

accumulation, this form of therapy is often impractical when red cells must be given for prolonged periods of time. Excessive iron stores have been found in patients with sickle cell anemia or sickle cell thalassemia who have received numerous red cell transfusions (56–58). Iron stores in some of these patients are near or at levels that would be expected to produce the pattern of organ damage found during the second decade in patients with thalassemia major.

IRON OVERLOAD FROM THERAPEUTIC INJECTIONS Parenteral administration of therapeutic iron preparations to patients with anemias unresponsive to iron therapy may make an inadvertent iatrogenic contribution to iron overload. Patients with refractory microcytic anemias are at the greatest risk of this avoidable form of iron loading, but the problem has also been recognized in some chronically hemodialyzed patients. With hemodialysis, iron losses have been estimated to be as great as 1.5 to 2.0 g/year, owing to blood remaining in the dialysis apparatus, blood taken for laboratory tests, and other blood loss (59). To prevent the development of iron deficiency, iron supplements are often routinely given to these patients. Because of doubts about adequate intestinal iron absorption, injectable iron has been recommended, but Gokal et al (60) showed that the use of parenteral supplementation could lead to potentially dangerous iron overload. To avoid this complication, most dialysis units now use supplemental oral iron.

Neonatal Iron Overload

Parenchymal iron overload has been described in certain rare or uncommon neonatal metabolic disorders. In hereditary tyrosinemia (hypermethioninemia), a moderate iron accumulation in hepatocytes, but not in other organs, occurs in association with hepatic cirrhosis, renal abnormalities, pancreatic islet hyperplasia, and a peculiar "fishy" odor (61).

In Zellweger's cerebrohepatorenal syndrome, a fatal autosomal recessive disorder, prominent parenchymal iron deposits are found in the liver (usually with cirrhosis), spleen, kidney, and lungs; deficiencies in several iron-containing enzymes have been identified in the liver, kidney, brain, and skeletal muscle. Abnormal facies, hypotonia, and polycystic kidneys characterize this disorder (62).

The massive iron deposition seen with neonatal hemochromatosis in hepatic parenchymal cells (with cirrhosis) and, to a lesser extent, in the heart and endocrine organs has a morphologic appearance recalling that of adult HLA-linked hemochromatosis. This rapidly fatal disorder has been described in only 18 infants, but lack of recognition may create a falsely low impression of its prevalence (62, 63). One infant with neonatal hemochromatosis has successfully undergone liver transplantation (R. Sokol, personal communication).

Focal Sequestration of Iron

Idiopathic pulmonary hemosiderosis presents the paradox of an increased whole-body iron content with decreased iron stores in the liver and bone marrow. The excess iron is sequestered in the lung in pulmonary macrophages after repeated episodes of alveolar hemorrhage and is not available for use elsewhere (64).

Hallervorden-Spatz syndrome is a rare autosomal recessive neurological disorder, clinically manifest in childhood or early adulthood by progressive dystonia, spasticity and dementia and pathologically characterized by iron deposition in the basal ganglia (65, 66). The role of iron in the pathogenesis of the condition is unknown.

Renal hemosiderosis may develop in conditions with chronic hemoglobinuria but apparently has no deleterious effects on kidney structure or function (32).

CONSEQUENCES OF IRON OVERLOAD

Whatever the cause of iron accumulation, the clinical consequences are influenced by the magnitude, rate, and distribution of iron overload. Since the body lacks any effective means to excrete excess iron, when the burden exceeds the body's capacity for safe storage, the result is widespread damage to the liver, heart, pancreas, and other organs. Although clinical experience provides evidence that tissue injury is iron-induced, the specific pathophysiologic mechanisms underlying these toxic effects are poorly understood. After reviewing proposed mechanisms for the deleterious actions of iron, the manifestations of iron toxicity in specific organ systems are described.

Mechanisms of Iron Toxicity

Although many organs may be damaged by iron, most of the available information about iron toxicity has been derived from clinical and experimental studies of the effect of iron on the liver. Several mechanisms have been proposed whereby excess hepatic iron could cause cellular injury with resultant fibrosis and cirrhosis (Figure 1). One hypothesis suggests that increased lysosomal fragility is responsible for cellular injury in iron overload. Increased membrane fragility has been demonstrated in vitro in hemosiderin-laden secondary lysosomes isolated from liver biopsy specimens from patients with iron overload (67); this increase returns to normal in patients with hereditary hemochromatosis in whom excess iron is removed by phlebotomy therapy. The proposed mechanism for cellular injury is that iron-induced lipid peroxidation produces lysosomal rupture, which causes hydrolytic lysosomal enzymes to leak into the cytosol, damages subcellular

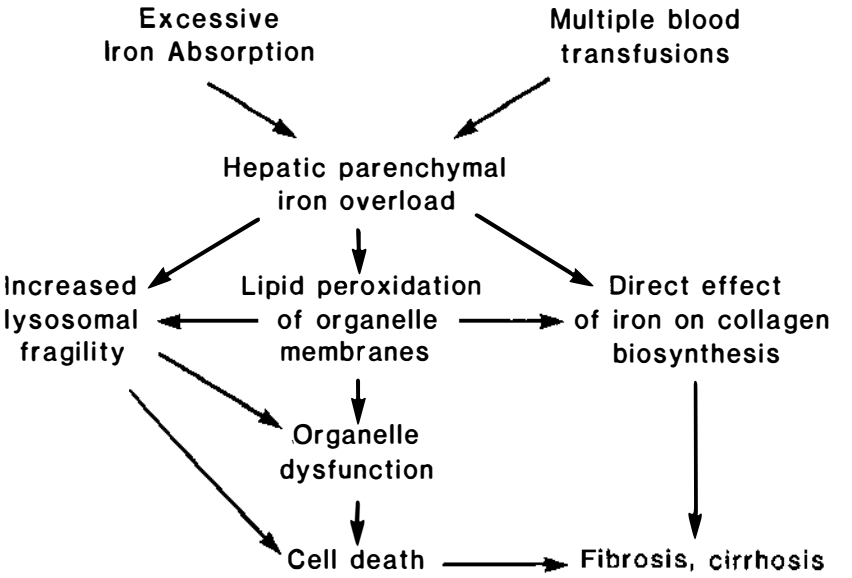

Figure 1 Postulated mechanisms of liver injury in chronic iron overload.

organelles, and ultimately leads to cell death (68). Peroxidative injury to isolated hepatic lysosomes by iron salts has been demonstrated (69, 70), but the relevance of laboratory observations of increased lysosomal fragility to hepatic fibrosis is still uncertain.

Iron-induced peroxidation of membrane lipids of subcellular organelles other than lysosomes, such as mitochondria and microsomes, leading to functional insufficiency with subsequent cell injury and death is an alternative possibility that is not necessarily incompatible with the lysosomal theory. Whole-animal studies have shown that injections with iron salts or ferric nitrilotriacetate increase alkane expiration, an index of lipid peroxidation, although the site of the peroxidation is uncertain (71, 72). Evidence of iron-induced lipid peroxidation in hepatic mitochondria and microsomes has been found in studies using rats with chronic dietary iron overload (73); subsequent studies have shown associated functional abnormalities both in hepatic mitochondria and microsomes (74, 75). In these experiments, both organelle lipid peroxidation and the associated dysfunction were dependent on the concentration of hepatic nonheme iron, with a "threshold" of 3000–4000 μg Fe/g liver (wet weight; normal $<$ 200 μg Fe/g). These biochemical findings may be related to the further observation that animals maintained at hepatic iron concentrations above this threshold for several months have developed hepatic fibrosis (76). The biochemical form of intracellular iron responsible for initiating these hepatotoxic manifestations has not been identified.

Other mechanisms for iron-induced hepatic damage have been suggested. Iron-stimulated collagen biosynthesis has been proposed as a possible explanation for fibrosis that does not necessarily require prior iron-mediated cellular damage (77). Iron-induced damage to nucleic acids could produce cell injury and be a cofactor or cause of neoplasia (78). The toxic effects of non-transferrin-bound iron (plasma iron not complexed with transferrin) have also been considered as potentially responsible for both hepatic damage (79) and iron loading (80) as well as for injury to other tissues (81, 82). These postulated explanations for iron-induced injury are not mutually exclusive and tissue damage may well be the result of multiple mechanisms, including some at present unknown.

Manifestations of Iron Toxicity in Specific Organ Systems

The consequences of the various forms of iron overload resulting from increased absorption or parenteral administration are ultimately similar, consisting of widespread iron-induced damage to the liver, heart, pancreas, and other organs. The specific patterns of tissue injury are described below.

LIVER DISEASE Hepatotoxicity is the most consistent finding in patients with iron overload. Whether derived from increased absorption or parenteral administration, massive deposition of iron in hepatic parenchymal cells eventually produces fibrosis and, ultimately, cirrhosis. The *amount* of hepatocyte iron is a critical determinant of liver injury. For patients with hereditary hemochromatosis (83, 19), African dietary iron overload (47, 84), and transfusional iron overload (85, 86), (*a*) the magnitude of parenchymal iron overload is related to the occurrence and extent of liver damage, and (*b*) removal of excess iron by phlebotomy or chelation produces clinical improvement. The importance of the hepatic storage iron concentration and the duration of exposure to the iron has been demonstrated in hereditary hemochromatosis (37). In the absence of coexistent alcoholic liver disease, fibrosis or cirrhosis usually does not occur until the hepatic storage iron reaches a concentration of 4000–5000 μg Fe/g liver (wet weight). An almost identical threshold for hepatic fibrosis was previously demonstrated for African dietary iron overload (48, 84). In unchelated patients with thalassemia major, the apparent threshold concentration for the development of fibrosis is nearly twice this level, or about 10,000 μg Fe/g liver (wet weight). Whether this higher threshold is the result of the initial reticuloendothelial localization of transfused iron, of a shorter duration of exposure to high storage iron concentrations, or of other factors is uncertain, but a quantitative relationship between hepatic iron excess and toxicity is still present. The threshold concentration for patients with aplastic anemia or red cell aplasia may be even higher (18).

In a recent series, the two major causes of death in patients homozygous for hereditary hemochromatosis were complications of liver cirrhosis and hepatocellular carcinoma; patients with the greatest risk of death were those with the largest body iron burdens (19). All liver cancers developed in cirrhotic livers; to date there have been no published reports of the development of a liver cancer in a noncirrhotic patient with hemochromatosis. Importantly, the results of this study suggest that early diagnosis of hemochromatosis in a noncirrhotic stage and phlebotomy therapy can return the patient's life expectancy to normal and may also prevent the late development of liver cancer. Chelation therapy in transfusional iron overload has also been shown to forestall the development of liver disease and other complications (54).

CARDIAC DISEASE Iron-related cardiomyopathy with heart failure, arrhythmias, or both is the most common cause of death in patients with transfusional iron overload (54). Patients with untreated hereditary hemochromatosis may also develop congestive heart failure, sometimes complicated by arrhythmias; the prevalence of cardiac complications has been lower in recent series (10, 19) than in earlier studies, perhaps because of earlier diagnosis. Young patients with hereditary hemochromatosis may present with a particularly severe form of cardiac disease. Whatever the etiology of the iron overload, cardiac failure is resistant to standard therapy, fatal unless iron is removed, and should be regarded as an emergency (87). In patients with hereditary hemochromatosis the efficacy of phlebotomy therapy in reversing cardiac failure and arrhythmias has been repeatedly demonstrated (32); the initial use of both phlebotomy and chelation therapy in patients with cardiac involvement has also been advocated (87). Chelation therapy is also beneficial in preventing the development of cardiac complications in transfusional iron overload (88).

DIABETES MELLITUS AND OTHER ENDOCRINE ABNORMALITIES Diabetes mellitus is a common complication of hereditary hemochromatosis that also occurs in both transfusional and African dietary iron overload. In a recent study of patients with hereditary hemochromatosis, 71% of cirrhotic patients and 20% of noncirrhotic patients were diabetic (19). Most patients achieve control with diet or oral hypoglycemic agents; in those requiring insulin, control is usually not difficult. All of the complications of diabetes, including retinopathy, nephropathy, neuropathy, and vascular disease, have been described in the syndrome complicating hereditary hemochromatosis (89). Degenerative changes have been described in the pancreatic islet cells, but no direct pathogenetic relationship has been demonstrated between iron deposition and islet cell dysfunction. Pancreatic exocrine function is typically normal despite degenerative changes in acinar cells and some fibrotic

changes. The effect of phlebotomy therapy on diabetes in hereditary hemochromatosis is variable; insulin dependence is usually not eliminated by removal of iron but some patients are able to decrease their daily dose of insulin (19, 83).

In transfusional iron overload, disturbances of growth and sexual maturation are common (54). Abnormalities in both pituitary and end-organ function have been reported in hereditary hemochromatosis: gonadotropin and prolactin deficiencies are prevalent (90, 91). Hypogonadism may be either hypogonadotrophic or due to primary testicular failure. Impotence in male patients may antedate other manifestations of hereditary hemochromatosis by as much as 5–10 years. The endocrine abnormalities that occur with hereditary hemochromatosis do not seem to improve with phlebotomy therapy (83).

SKIN PIGMENTATION Iron overload is frequently associated with excessive skin pigmentation classically described as a "bronze" coloration, most often in sun-exposed areas, but the changes may be mild and escape clinical detection. In patients with severe iron overload, a prominent slate-grey discoloration of the skin may be speckled with small pigment-free areas, giving the appearance of "reverse freckling" (54). The bronze appearance is attributed to melanin, but the small amounts of iron deposited in the basal layers of the epidermis and in the sweat glands may contribute to the greyish pigmentation. In transfusional iron overload, chelation therapy produces a rapid lightening of the skin, often within the first 4–6 weeks of treatment.

ARTHROPATHY Arthropathy is a common complication of hereditary hemochromatosis that may precede other clinical manifestations and be the presenting feature of the disease. The pathogenesis of the joint changes is unknown. The clinical syndromes seen with hereditary hemochromatosis include chondrocalcinosis, a hypertrophic arthritis involving the knees and the metacarpophalangeal and proximal interphalangeal joints, and a polyarthritis most often affecting the wrists, the metacarpophalangeal and proximal interphalangeal joints, the knees, and hips. Phlebotomy does not seem to improve the symptoms related to the joints; arthritic complaints may develop during phlebotomy therapy or even after the completion of iron removal (32). In transfusional iron overload, arthropathy is uncommon but has been reported (92).

ASCORBIC ACID DEFICIENCY AND OSTEOPOROSIS Ascorbic acid deficiency is frequently observed in African dietary iron overload, and frank scurvy may develop. The large deposits of parenchymal iron seem to accelerate the catabolism of ascorbic acid in patients whose diets typically contain marginal amounts of vitamin C. In turn, the deficiency of ascorbic acid

promotes the development of osteoporosis, apparently by inhibiting osteogenesis and by producing other disturbances in bone metabolism. Osteoporosis is also found in some patients with hereditary hemochromatosis (32).

Ascorbic acid deficiency also occurs in transfusional siderosis and may diminish the iron excretion produced by chelation therapy with desferrioxamine, probably because a lack of ascorbate inhibits the mobilization of iron from reticuloendothelial cells (93). Administration of ascorbic acid can enhance desferrioxamine-induced iron excretion but carries the risk of a possible internal redistribution of iron from relatively benign storage sites in reticuloendothelial cells to a potentially toxic pool in parenchymal cells. Internal redistribution of iron may explain the abrupt development of cardiac failure observed in several patients with thalassemia major shortly after beginning treatment with ascorbic acid (94). Although the evidence is anecdotal, large doses of ascorbic acid may be hazardous in some patients with iron overload. Ascorbic acid deficiency may actually have a protective role in some patients with severe iron overload (95).

RISK OF INFECTION The effect of iron excess on the risk of infection remains uncertain; infection is not a frequent complication of any of the iron overload syndromes. Both because iron is an essential nutrient for microorganisms and because blood with saturated transferrin loses its bacteriostatic property in vitro, the hyperferremia, saturated transferrin, circulating non-transferrin-bound iron, and increased tissue iron levels found in iron overload might predispose to infection. Several clinical reports have suggested that an increased availability of iron might be pathogenetically related to infections with certain organisms, including *Vibrio vulnificus, Listeria monocytogenes, Yersinia enterocolitica, Escherichia coli* and *Candida* species (96–99). In addition, septicemia might account for the occasional reports of acute abdominal pain and shock in patients with hereditary hemochromatosis (32). Despite these observations, there is no clear evidence that iron availability and the degree of transferrin saturation have a substantial influence on infectious risk in the presence of intact cellular and humoral immunity.

MEASUREMENT AND MANAGEMENT OF IRON OVERLOAD

Fortunately, means for both the early detection and treatment of iron overload are now available and, with appropriate therapy, many of the pathological effects of iron excess can be avoided.

Detection and Monitoring of Iron Overload

Both indirect and direct means of assessing body iron stores are available. The most helpful indirect means of detecting iron overload are measurements of

the serum ferritin and transferrin saturation; elevated values can identify individuals who need evaluation with a definitive test to confirm an increase in body storage iron. The results of these tests will be abnormal in most homozygotes for hemochromatosis (24, 87). Unfortunately, both these indirect tests lack sensitivity and specificity and must be used with an awareness of their limitations.

In an apparently small number of patients with hereditary hemochromatosis, serum ferritin levels may not be elevated even with a large total body iron load and, even when abnormal, may greatly underestimate the magnitude of the iron excess (7, 16, 100–102). In heavily iron-overloaded subjects, it has been suggested that the correlation between serum ferritin and iron stores results from a "fortuitous addition of the effects of iron levels on ferritin synthesis and the effect of cell damage on ferritin release from the liver." Furthermore, "a simple relationship between serum ferritin and iron stores cannot be assumed when ferritin concentrations exceed 4000 μg/l or in patients who have received more than 100 units of transfused blood" (103).

Elevated values for the transferrin saturation (greater than 70–80%) suggest parenchymal iron overload (18) but do not quantitatively reflect body iron stores. The lability of the transferrin saturation, especially in early hemochromatosis, should be recognized. There are thus conditions, such as liver disease, in which the serum ferritin concentration and transferrin saturation may be elevated without an increase in body iron stores. In addition, one fourth to one third of those heterozygous for hemochromatosis may have abnormal serum ferritin or transferrin concentrations (12). Other indirect indicators of iron status, such as measurement of the urinary iron excretion after injection of desferrioxamine, cannot be relied upon for detection of the early stages of hereditary hemochromatosis.

In patients with elevated serum ferritin and transferrin saturation, a direct measure of body storage iron is needed. In families where a proband homozygous for hereditary hemochromatosis has been identified, HLA typing permits identification of relatives at risk for iron loading but does not indicate the extent of iron loading. Because no HLA type is a certain marker of hemochromatosis, typing is of no help in ascertaining whether an isolated individual has hemochromatosis. Bone marrow aspiration and biopsy provide a direct measure of reticuloendothelial iron stores but cannot be used for the diagnosis of hereditary hemochromatosis because bone marrow iron may be normal or decreased even in the presence of massive parenchymal overload. Liver biopsy is the definitive test for assessing iron deposition and tissue damage, permitting histochemical determination of the cellular distribution of iron, pathological examination of the extent of injury, and quantitative determination of the hepatic iron concentration. The age-adjusted hepatic iron concentration can be used to judge the risk of fibrosis and, if needed, to distinguish among patients with alcoholic liver disease with iron loading, with

heterozygous, and with homozygous forms of hereditary hemochromatosis (37). Quantitative phlebotomy in patients undergoing therapeutic venesection can provide an accurate retrospective determination of the amount of stored iron that can be mobilized for hemoglobin formation. In summary, measurements of serum ferritin and transferrin saturation are imperfect but useful procedures for detecting and monitoring iron overload. Liver biopsy with chemical iron determination provides the most quantitative direct means generally available for assessing iron status.

The limitations and risks of available methods for assessing iron status have prompted a search for noninvasive direct means of measuring body iron. Computed tomography (CT) theoretically should detect excessive tissue iron deposition by an increase in tissue x-ray absorption coefficients. However, if the usual single-energy technique is used, the wide variation in normal liver x-ray attenuation values obscures differences owing to iron (104, 105) unless massive overload is present (106). Mitnick et al (107) suggested that the CT density of the liver related to the degree of hepatic fibrosis and cirrhosis rather than the amount of iron in transfused patients with thalassemia major. A dual-energy technique using computed tomography is more promising (108), but the clinical usefulness of this approach has not yet been determined. Magnetic resonance spectroscopy has been able to detect iron overload in rats (109), but magnetic resonance imaging (MRI) scanners currently available for human use have not yet achieved a sufficiently homogenous magnetic field to make this technique quantitatively useful. Studies using nuclear resonant scattering of x-rays (NRS) must be carried out near a nuclear reactor and seem unlikely to be generally applicable (110). Diagnostic x-ray spectrometry is a technique that can estimate dermal iron content (111), but the relationship to total body iron load has not been clearly established. Magnetic susceptometry uses the unusual paramagnetic property of ferritin and hemosiderin to measure storage iron. The technique requires the use of a specially designed superconducting quantum interference device (SQUID) magnetic susceptometer. The results of noninvasive magnetic determinations and of the chemical analysis of hepatic tissue obtained by biopsy are quantitatively equivalent when body iron stores are normal or increased (112, 113). In effect, the susceptometric method provides an automated, safe, and noninvasive magnetic "biopsy" of liver ferritin and hemosiderin iron that is well accepted by patients. Magnetic susceptometry has been used in a research setting for several years, and a commercial version of the SQUID susceptometer is under development to make the device more generally available.

Management of Iron Overload

PREVENTION African dietary iron overload is a preventable disease. A recent survey suggests that this form of iron loading is still prevalent in rural

sub-Saharan Africa and is a public health concern (46). Education and community efforts to alter the patterns of preparation and consumption of traditionally brewed beverages could greatly influence the incidence and severity of this disorder.

PHLEBOTOMY Phlebotomy is the standard therapy for hereditary hemochromatosis and may be useful in the treatment of African dietary iron overload; infrequently, patients with iron-loading anemias have hemoglobin concentrations high enough to permit venesection. Weekly phlebotomy of 500 ml (200–250 mg Fe) is continued until storage iron has been depleted. For heavily loaded patients, a period of 2–3 years of weekly phlebotomy may be required to eliminate all excess iron. The time required for removing storage iron may be important prognostically: in a recent study survival was markedly reduced in patients who could not be depleted of iron during the initial 18 months of venesection therapy because of a large iron excess (19). After complete removal of the iron load, lifelong maintenance therapy is needed, usually requiring phlebotomy of 500 ml every 3–4 months. In contrast to the beneficial effect of phlebotomy in hereditary hemochromatosis, a controlled study, reported only in abstract form, demonstrated no improvement in patients with alcoholic liver disease and iron overload who were treated with venesection (114). Although phlebotomy therapy for African dietary iron overload would be expected to be beneficial, the efficacy of venesection has not been adequately examined. The single preliminary study available reported encouraging results (115).

CHELATION In the majority of patients with refractory anemia, the severity of the anemia precludes phlebotomy therapy as a means of removing toxic accumulations of iron. Treatment with a chelating agent capable of sequestering iron and permitting its excretion from the body is the only other therapeutic approach now available. In patients with hereditary hemochromatosis and cardiac failure, a combination of phlebotomy and chelation therapy has been recommended (86). Desferrioxamine B, a naturally occurring trihydroxamic acid produced by *Streptomyces pilosus,* was first introduced 25 years ago and is the only iron-chelating agent now in clinical use. Desferrioxamine given orally is poorly absorbed, and to be effective the drug must be given by subcutaneous or intravenous infusion using a small portable syringe pump, ideally for 12 hours each day. Compliance with this regimen may be difficult, particularly for adolescents with thalassemia major who may be at greatest risk for the lethal complications of iron overload. Despite these problems, a number of studies in the past decade have shown that regular chelation therapy with desferrioxamine can prevent organ damage and improve survival in transfusion-dependent patients with thalassemia major and other disorders. These studies have thereby validated iron chelation as a therapeutic approach

to iron overload (87). The development of an iron chelator that is effective when given orally would permit more general application of chelation therapy for iron overload.

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