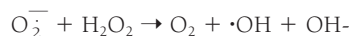
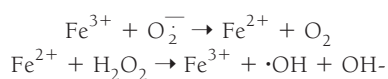


The Role of Iron in Diabetes and Its Complications

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The central importance of iron in the pathophysiology of disease is derived from the ease with which iron is reversibly oxidized and reduced. This property, while essential for its metabolic functions, makes iron potentially hazardous because of its ability to participate in the generation of powerful oxidant species such as hydroxyl radical (1). Oxygen normally accepts four electrons and is converted directly to water. However, partial reduction of oxygen can and does occur in biological systems. Thus, the sequential reduction of oxygen along the univalent pathway leads to the generation of superoxide anion, hydrogen peroxide, hydroxyl radical, and water (1,2). Superoxide and hydrogen peroxide appear to be the primary generated species. These species may then play a role in the generation of additional and more reactive oxidants, including the highly reactive hydroxyl radical (or a related highly oxidizing species) in which iron salts play a catalytic role in a reaction. This reaction is commonly referred to as the metal catalyzed Haber-Weiss reaction (1):



Because iron participates in the formation of reactive oxygen species, organisms take great care in the handling of iron. Indeed, iron sequestration in transport and storage proteins may contribute to antioxi-

dant defenses. It is now well established that oxidants can cause the release of catalytic iron (1); thus, a vicious cycle is initiated that leads to the formation of more reactive oxygen species.

In this review, we discuss the role tissue iron and elevated body iron stores play in causing type 2 diabetes and the pathogenesis of its important complications, particularly diabetic nephropathy and cardiovascular disease (CVD). In addition, we emphasize that iron overload is not a prerequisite for iron to mediate either diabetes or its complications. Important in its pathophysiology is the availability of so-called catalytic iron or iron that is available to participate in free radical reactions.

THE ROLE OF IRON IN THE INDUCTION OF DIABETES

Evidence that systemic iron overload could contribute to abnormal glucose metabolism was first derived from the observation that the frequency of diabetes is increased in classic hereditary hemochromatosis (HH). However, with the discovery of novel genetic disorders of iron metabolism, it is obvious that iron overload, irrespective of the cause or the gene involved, results in an increased incidence of type 2 diabetes. The role of iron in the pathogenesis of diabetes is suggested by 1) an increased incidence of type 2 diabetes in diverse causes of iron overload and 2) reversal or improvement in diabetes (glycemic control) with a reduction in iron load achieved using either phlebotomy or iron chelation therapy.

Recently, a link has been established between increased dietary iron intake, particularly eating red meat and increased body iron stores, and the development of diabetes. A causative link with iron overload is suggested by the improvement in insulin sensitivity and insulin secretion with frequent blood donation and decreased iron stores (3,4).

Although the exact mechanism of iron-induced diabetes is uncertain, it is likely, as discussed below, to be mediated by three key mechanisms: 1) insulin deficiency, 2) insulin resistance, and 3) hepatic dysfunction. An understanding of the pathogenic pathways of iron-induced diabetes is derived mainly from studies on animal models of hemochromatosis.

In a mouse model of hemochromatosis, iron excess and oxidative stress mediate apoptosis of pancreatic islets with a resultant decrease in insulin secretory capacity (5). Pancreatic islets have an extreme susceptibility to oxidative damage, perhaps because of the nearly exclusive reliance on mitochondrial metabolism of glucose for glucose-induced insulin secretion and low expression of the antioxidant defense system (6). A high expression of divalent metal transporter additionally predisposes them for more accumulation of iron than other cells (7) and potentiates the danger from iron-catalyzed oxidative stress.

In studies on thalassemic patients, insulin resistance is significantly increased (8,9). In human studies, McClain et al. (10) recently demonstrated a high prevalence of abnormal glucose homeostasis in individuals with hemochromatosis and examined possible mechanisms for this high prevalence. Using glucose tolerance tests, they demonstrated not only that insulin secretion is impaired but also that there is insulin resistance. The mechanisms for insulin resistance include the possibility of iron overload causing resistance directly or through hepatic dysfunction (11). In a study of patients with unexplained hepatic iron overload, most were found to be insulin resistant, which suggests a common etiologic link among hepatic iron, hepatic dysfunction, and insulin resistance (12) (Fig. 1).

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Abbreviations: CVD, cardiovascular disease; HCV, hepatitis C; HH, hereditary hemochromatosis; NTBI, non-transferrin-bound iron.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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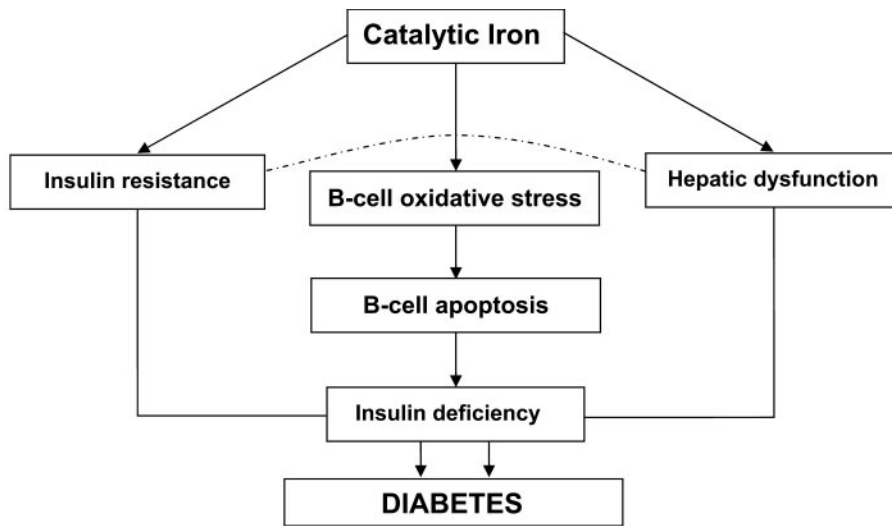


Figure 1—Pathogenic pathways for iron in induction of diabetes.

Epidemiology of type 2 diabetes in known iron overload conditions

Genetic iron overload syndromes and diabetes. Over 80% of cases of HH (type 1) result from a mutation in the gene encoding the hereditary hemochromatosis protein (HFE) (13) (Table 1). These mutations lead to an accumulation of iron in several tissues and present as a classic syndrome of hypogonadism, diabetes, liver disease, cardiomyopathy, and arthritis. In type 1 hereditary hemochromatosis, up to 60% of the affected patients develop diabetes (14,15). Diabetes appears to be a result of both insulin deficiency and resistance. Evidence for this is derived from studies in HH patients whose body iron stores were reduced with phlebotomy and/or iron chelation therapy, which resulted in improved glycemic control and 30–40% of patients achieving elimination of oral hypoglycemic therapy or a substantial reduction in dosage (15,16).

A similar increase in the incidence of type 2 diabetes is observed in other genetic iron overload syndromes that involve iron transport. For example, in

autosomal dominant hemochromatosis syndrome involving the iron transporter ferroportin, diabetes is present in up to 25% of patients (17). In juvenile hemochromatosis involving hemojuvelin mutations, 25% of patients were glucose intolerant (18). An increased incidence of diabetes is also seen in hereditary aceruloplasminemia, a condition where the lack of synthesis of apoceruloplasmin affects the distribution of tissue iron and leads to a progressive accumulation of iron (19). The levels of malondialdehyde and 4-hydroxynonenals, which are indicators of lipid peroxidation, are significantly elevated in the frontal lobe and putamen and suggest a pathogenic role for iron-mediated oxidative stress in end-organ manifestations of this syndrome (20).

Several recent reports focused on specific mutations such as C282Y, and its associations with diabetes extend these observations and suggest that even moderate elevations of body iron stores outside the setting of hereditary hemochromatosis can be associated with diabetes. The C282Y mutation particularly has been shown to be associated with elevated ferritin and transferrin saturation values (13). This genotype is found in patients with diverse endocrine problems including diabetes despite the absence of overt hereditary hemochromatosis (21). Acton et al. (22) evaluated the associations of diabetes with serum ferritin, transferrin saturation, and hereditary hemochromatosis mutations in the Hemochromatosis and Iron Overload Screening (HEIRS) Study. Serum ferritin concentrations were associated with dia-

betes at levels below those typically associated with hemochromatosis and iron overload. Fumeron et al. (23), in a prospective study, also reported a positive association between transferrin and ferritin with the onset of abnormalities and glucose metabolism. They argue that their results support a causative role of disordered iron metabolism in the onset of insulin resistance in type 2 diabetes.

Transfusional iron overload and diabetes. Transfusional iron overload is the most common cause of acquired iron overload and is typically seen in transfusion-dependent chronic hemolytic anemia such as β -thalassemia. Impaired glucose tolerance is often detected in the second decade of life. In a study of 80 transfusion-dependent β -thalassemic patients, diabetes was reported in 19.5% of patients and impaired glucose tolerance in 8.5% of patients. The risk factors for impaired glucose tolerance and type 2 diabetes found in that study were high serum ferritin and hepatitis C (HCV) infection (24). Insulin deficiency due to iron deposition in the interstitial pancreatic cells, with resultant excess collagen deposition and defective microcirculation (25) and insulin resistance (26), are the likely mechanisms for type 2 diabetes. Treatment with intravenous or oral chelation improves glucose tolerance in up to one-third of these patients, suggesting a causal role for iron (27,28). Preliminary evidence also suggests a direct role for iron-derived free radicals in mediating end-organ damage of diabetes in transfusional iron overload (29). In this study, patients with higher degrees of lipid peroxidation had an accelerated onset of diabetic nephropathy.

African iron overload and diabetes. Dietary iron overload is well described in South African tribal populations. It is ascribed to cooking food in African three-legged iron pots and eating acidic cereal, which increases iron absorption. However, a genetic predisposition has also been considered. Clinically, it behaves like hereditary hemochromatosis, and type 2 diabetes is a well known late manifestation (30). Similarly, autopsy studies of African Americans suggest that pathologic iron overload that cannot be linked to specific gene mutations occurs in 1% of these patients. This data has been confirmed in National Health and Nutrition Examination Survey (NHANES) II and III studies, where 0.9% of African Americans had markedly elevated transferrin saturations and 14% had intermediate eleva-

Table 1—Iron overload states and diabetes

Genetic iron overload
HH (C282Y and H63D mutations)
Ferroportin disease
Hemojuvelin mutation
Hereditary aceruloplasminemia
Mitochondrial iron overload
Friedreich's ataxia (frataxin mutation)
Transfusional iron overload
Hepatic iron overload
HCV
Porphyria cutanea tarda

tions of transferrin saturation (31). Pilot data from the Jackson Heart Study shows a positive correlation between serum ferritin and fasting glucose and A1C ($r = 0.15$), though no correlation has been found between transferrin saturation and glycemic status (32). Because of these inconsistencies and absence of specific data on iron chelation or phlebotomy to reverse diabetes or improve glycemic control, it is difficult to draw firm conclusions on the link between iron overload and diabetes in African Americans.

HCV, porphyria cutanea tarda, and diabetes. Recent studies have described not only a significantly increased risk for diabetes in patients with HCV infection (33,34) but also its associated conditions such as porphyria cutanea tarda. In porphyria cutanea tarda, a cutaneous condition associated with increased iron overload, up to 87% of patients were glucose intolerant (35). Further, HCV can adversely affect progression of kidney disease in patients with biopsy-proven diabetic nephropathy (36). HCV infection is well known to be associated with an accumulation of iron in the liver parenchyma. Many patients with chronic HCV infection often have elevated serum iron, transferrin saturation, and ferritin levels, and a few have severe hepatic iron overload (37,38). This might suggest that iron overload has a role in the pathogenic link between HCV and accelerated end-organ damage in diabetes.

Type 2 diabetes in mitochondrial iron overload. Friedreich's ataxia, an inherited neurodegenerative disease with a trinucleotide (GAA) hyperexpansion within the first intron of the Friedreich's ataxia gene (FRDA), is a classic disorder associated with mitochondrial iron accumulation. FRDA encodes for a protein called frataxin, which has a specific association with the mitochondrial inner membrane and is involved in the formation of Fe-S clusters. Friedreich's ataxia is associated with a high incidence of type 2 diabetes (39), suggesting a possible relation between mitochondrial iron accumulation leading to mitochondrial DNA damage and type 2 diabetes. Disruption of the frataxin gene in pancreatic β -cells causes diabetes following cellular growth arrest and apoptosis, paralleled by an increase in reactive oxygen species in islets (40). In turn, this leads to progressive damage to both mitochondrial and nuclear DNA (41). Noted occurrence of diabetes in other disorders of mitochondrial DNA, such as Wolfram Syndrome (42),

thiamine-dependent megaloblastic anemia (43), and specific disorders with mitochondrial mutation (tRNA) (44), support this conclusion. Absence of a similar association of type 2 diabetes with other disorders of mitochondrial iron overload, such as Hallervorden-Spatz disease, might be due to an organ-localized nature of iron overload in these conditions (45).

The role of iron in diabetes without overt iron overload

A relationship between high iron intake and high body iron stores outside the setting of genetic iron overload and type 2 diabetes is well recognized (46). Loma Linda University's Adventist Health Study was the first to report the association between meat intake and type 2 diabetes risk (47) that has since been consistently observed by several other studies (48,49). Numerous studies have confirmed that this association is related to the high heme content of meat and increased dietary heme intake (3,50–52). Similarly, high body iron stores have been linked to insulin resistance (53,54), metabolic syndrome (53,55–57), and gestational diabetes (58,59). Recently, Jiang et al. (60) carried out a nested case-control study within the Nurses' Health Study cohort. Among cases of incident diabetes, the mean concentration of serum ferritin was significantly higher compared with control subjects, and the mean ratio of transferrin receptors to ferritin was significantly lower. This relationship with markers of body iron stores persisted after correction for various other risk factors for diabetes, including markers of obesity and inflammation.

Jehn et al. (61) argue that the modest elevations in ferritin levels observed in diabetes may be a consequence or marker rather than the cause of impending insulin resistance and that elevated ferritin may not reflect elevated body iron stores or an intracellular labile iron pool that participates in oxidant injury. However, the common presence (59–92% of patients) of non-transferrin-bound iron (NTBI), a form of iron most susceptible to redox activity, in excess amounts in type 2 diabetes with a strong gradient for severity (62), and the preliminary evidence that reduction in body iron stores with bloodletting in type 2 diabetes results in improvement in glycemic control and insulin resistance (56,63), suggests a pathogenic role of iron in type 2 diabetes.

Blood donation and diabetes

As discussed, iron overload is common in patients outside the setting of known iron overload syndromes. Insulin resistance has been described in such patients (11,64), and iron-chelating agents and blood donations have been shown to decrease the development of diabetes in such patients (65,66). Interestingly, even in apparently healthy individuals, blood donation resulting in decreased iron stores has been associated with a low incidence of diabetes (66). Recent randomized studies (56) have demonstrated that iron stores influence insulin action, and following bloodletting over a 4-month period, insulin sensitivity improved. Finally, a low-iron diet improves cardiovascular risk profiles (67). Fernandez et al. (68) investigated the relationship between iron stores and insulin sensitivity in 181 men. Men who donated blood more than two times over a 5-year period were matched with nondonors. Blood donation was associated with increased insulin sensitivity and decreased iron stores. Additional and intriguing support to this association also comes from a study on patients with iron deficiency who exhibit a decreased incidence of diabetes.

THE ROLE OF IRON IN COMPLICATIONS OF DIABETES

The importance of protein glycation is well known in the pathogenesis of diabetic vascular complications. Transition metals also play a role in protein glycation induced by hyperglycemia. It has been shown that glycated proteins have a substantial affinity for the transition metals, and the bound metal retains redox activity and participates in catalytic oxidation (69). Thus, should similar glycochelates form *in vivo*, reactions mediated by the chelates could be involved in the vascular complications of diabetes (70). Desferoxamine causes a modest reduction in A1C. Also, *in vivo* conditions, treatment with desferoxamine has been shown to modestly reduce A1C levels in patients with non-insulin-dependent diabetes (71) and diabetic rats (72). In this section, we review additional clinical and epidemiological studies and pathogenic mechanisms that link iron to complications of diabetes.

The role of iron in diabetic nephropathy

Evidence linking iron to diabetic nephropathy includes 1) animal and epide-

miological studies, 2) studies in which an increased amount of iron has been shown in the kidneys of both animals (73,74) and humans (75) with kidney disease, 3) evidence for increased urinary iron in patients with diabetic nephropathy, and 4) the prevention of progression either by an iron-deficient diet or agents that bind and remove iron (chelators) (76–78).

Animal studies provide considerable evidence for the role of iron and oxidants in diabetic nephropathy (79–84). Oxidative stress from factors such as hyperglycemia, advanced glycation end products, and hyperlipidemia further contribute to the availability of intracellular iron that can generate and viciously worsen oxidative stress and renal damage. Iron content in the kidney has been shown to be increased in an animal model of diabetes (84), and urinary iron excretion is increased early in the course of diabetic renal disease in humans (83,85). There is considerable evidence that, once renal insufficiency develops, regardless of etiology, it tends to progress over time. This has been interpreted to indicate some common pathways for progression of kidney disease. Thus, lessons learned from other models of progression are likely to be relevant to diabetic nephropathy. In proteinuric models of kidney disease, iron accumulates within proximal tubule lysosomes. Nankivell et al. evaluated iron accumulation in kidney biopsies of patients with chronic kidney disease and proteinuria by energy-dispersive analysis. Compared with normal kidneys, iron accumulates in proximal tubular lysosomes in chronic kidney disease kidneys, and its accumulation correlates with the degree of proteinuria (73,75). Most importantly, the pathogenic role of iron in progression is indicated by the observation that progression can be prevented either by an iron-deficient diet or chelators (76–78). More specifically, in diabetes, a recent randomized trial involving 191 patients with diabetes, proteinuria, and a decreased glomerular filtration rate showed that a low-iron–available, carbohydrate-restricted, polyphenol-enriched diet compared with a standard protein-restricted diet had a renoprotective effect (67).

The role of iron in endothelial and vascular disease

The possibility that iron status has a role in CVD was postulated by J.L. Sullivan in 1981 (70). The man-woman ratio for median serum ferritin levels for ages 18–45

years is 3.8, which is similar to the increased risk for heart disease, with the reduced risk against heart disease in women ending with the onset of menopause. Epidemiologic studies in overt iron overload states such as transfusional iron overload and hemochromatosis have shown that the incidence of cardiac disease is increased (64) and that treatment with iron chelation improves cardiovascular outcome (27,86,87). Similarly, several studies have demonstrated a direct association between increased iron intake, body iron stores, and cardiovascular risk in the general population. Increased intake of heme iron is associated with increased cardiovascular events (88–91), and increased body iron stores are associated with myocardial infarction in a prospective epidemiological study (92). Additionally, varieties of cardiovascular risk factors are associated with iron overload and commonly cluster in the metabolic syndrome. Ramakrishnan et al. (91) have demonstrated this close relationship between iron stores and cardiovascular risk factors in women of reproductive age in the U.S. The association was seen with total cholesterol, triglycerides, diastolic blood pressure, and glucose, factors that often cluster in individual patients. Additional evidence of the role of iron can also be derived from studies on surrogate markers such as carotid atherosclerosis finding a positive association with iron stores (93). However, several other studies argue against an association between increased iron intake and body iron stores and cardiovascular risk (94–99). One possible reason for these conflicting data is the lack of precision of markers that were used to indicate iron load. In most of these studies, serum ferritin has been used as an indicator of iron load; however, serum ferritin also increases with a variety of inflammatory and stressful conditions. Similarly, other markers indicative of iron status in the body such as transferrin saturation are not reflective of total body iron stores or the presence of reactive forms of iron in blood. In fact, NTBI may be present in the serum even when transferrin is not fully saturated (62,100).

Pathologic mechanisms for iron in promoting vascular disease can be derived from cell culture studies, animal models, and human functional studies (vascular reactivity). In cell culture models, the addition of NTBI to human endothelial cell cultures increases surface expression of adhesion molecules

(101,102) and also increases monocyte adherence to the endothelium. These abnormalities can be corrected by the addition of iron chelators such as desferoxamine and dipirydy. Such an addition decreases expression of adhesion molecules and monocyte adherence (101–103). In human studies of end-stage renal disease patients, intravenous iron therapy has been shown to increase vascular and systemic oxidative stress (104–106), promote atherosclerosis (106), and increase the risk of arterial thrombosis (105). Further, intravenous iron has been shown to cause impaired flow-mediated dilatation in the brachial artery, a surrogate for endothelial dysfunction (107). Conversely, improvement in vascular reactivity after phlebotomy in patients with high-ferritin type 2 diabetes further supports these observations (108). Additionally, several recent studies on cardiovascular evaluation and outcome in high-frequency blood donors demonstrate improvement in surrogate markers of vascular health such as decreased oxidative stress and enhanced vascular reactivity when compared with low-frequency donors (107). However, conflicting data exists regarding the relationship between decreased iron stores from frequent blood donation and hard end points such as a decrease in cardiovascular events and mortality (66,109).

Plasma NTBI measures reactive forms of iron that result in increased oxidative stress and cell injury. A recent prospective study on the association of NTBI with cardiovascular events in postmenopausal women, the first of its kind, disappointingly showed no excess of CVD or acute myocardial infarction in patients with a highest tertile of NTBI compared with those with a lowest tertile of NTBI (110). The reason for negative results in this study might reside in the limitations of the study itself, including short follow-up, low event rates, and age of the population studied (49–70 years). Also, the NTBI assay method used yielded negative NTBI values in some patients and skepticism has been cast on this method before (111). Future studies using a reliable and precise NTBI measurement method to test its association with cardiovascular events will be very informative. Alternatively, better methods of measuring excess free/catalytic iron need to be developed and validated.

The beneficial effect of iron chelators on endothelial dysfunction suggests the role of iron in vascular disease. Impaired endothelial function as a result of in-

creased NAD(P)H oxidase-dependent oxidant generation was restored by desferoxamine (112). Furthermore, desferoxamine has been shown to prevent diabetes-induced endothelial dysfunction (113) and deficits in endoneural nutritive blood flow in streptozotocin-induced diabetic rats (113,114). Additionally, dexrazoxane, a chelating agent, has been shown to prevent homocysteine-induced endothelial dysfunction in healthy subjects.

Desferoxamine continues to be the most common iron chelator in use, but it has several limitations, including the need for parenteral administration, side effects, and cost. The availability of safe and effective oral iron chelators such as deferoxamine and deferasirox has made treatment of iron overload states more practical. These drugs are widely available outside the U.S. but have not yet been approved by the Food and Drug Administration. Yet another potential advantage of oral iron chelators is their ability to penetrate the cell membrane and chelate intracellular iron species (115). Randomized clinical trials of these agents are needed to determine their effectiveness in treating/preventing diabetes and its complications.

CONCLUSIONS

In summary, there is suggestive evidence that iron plays a pathogenic role in diabetes and its complications such as microangiopathy and atherosclerosis. Reliable and sensitive methods need to be developed to precisely measure the free/catalytic iron that participates in oxidative injury. Iron chelation therapy may present a novel way to interrupt the cycle of catalytic iron-induced oxidative stress and tissue injury and consequent release of catalytic iron in diabetes and to prevent diabetes-related complications.

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