

Cross-Talk Between Iron Metabolism and Diabetes

José Manuel Fernández-Real, Abel López-Bermejo, and Wifredo Ricart

Emerging scientific evidence has disclosed unsuspected influences between iron metabolism and type 2 diabetes. The relationship is bi-directional—iron affects glucose metabolism, and glucose metabolism impinges on several iron metabolic pathways. Oxidative stress and inflammatory cytokines influence these relationships, amplifying and potentiating the initiated events. The clinical impact of these interactions depends on both the genetic predisposition and the time frame in which this network of closely related signals acts. In recent years, increased iron stores have been found to predict the development of type 2 diabetes while iron depletion was protective. Iron-induced damage might also modulate the development of chronic diabetes complications. Iron depletion has been demonstrated to be beneficial in coronary artery responses, endothelial dysfunction, insulin secretion, insulin action, and metabolic control in type 2 diabetes. Here, we show that iron modulates insulin action in healthy individuals and in patients with type 2 diabetes. The extent of this influence should be tested in large-scale clinical trials, searching for the usefulness and cost-effectiveness of therapeutic measures that decrease iron toxicity. The study of individual susceptibility and of the mechanisms that influence tissue iron deposition and damage are proposed to be valuable in anticipating and treating diabetes complications. *Diabetes* 51:2348–2354, 2002

It is increasingly recognized that iron influences glucose metabolism, even in the absence of significant iron overload. In the general population, body iron stores are positively associated with the development of glucose intolerance, type 2 diabetes (1–7), and gestational diabetes (8,9). Among U.S. adults, men with newly diagnosed diabetes had an odds ratio (OR) of 4.94 (95% confidence interval [CI] 3.05–8.01) and women had an OR of 3.61 (2.01–6.48) of having elevated ferritin concentrations (6). These figures are especially remarkable when considering the increased prevalence of elevated iron stores in the healthy, free-living U.S. elderly population (28% of men and 12.2% of women showed high iron stores in a recent study) (10).

From the Unit of Diabetes, Endocrinology and Nutrition, University Hospital of Girona “Dr Josep Trueta,” Girona, Spain.

Address correspondence and reprint requests to J.M. Fernández-Real, Unitat de Diabetes, Endocrinologia i Nutrició, Hospital de Girona “Dr Josep Trueta,” Avinguda de França s/n, 17007 Girona, Spain. E-mail: endocrino@htrueta.scs.es.

Received for publication 12 February 2002 and accepted in revised form 30 April 2002.

BP, binding protein; CI, confidence interval; IRE, iron-regulatory element; IR-HIO, insulin resistance-associated hepatic iron overload; OR, odds ratio.

Frequent blood donations, leading to decreased iron stores, have been demonstrated to reduce postprandial hyperinsulinemia in healthy volunteers (11), to improve insulin sensitivity (12), and to constitute a protective factor for the development of type 2 diabetes (13). Phlebotomy was followed by decreases in serum glucose, cholesterol, triglycerides and apoprotein B (14), and by improvement in both β -cell secretion and peripheral insulin action in patients with type 2 diabetes (15). A significant impact of tissue iron excess on systemic effects of diabetes is suggested by recent reports in which iron appears to influence the development of diabetic nephropathy and vascular dysfunction. In this sense, intravenous administration of deferoxamine resulted in improved coronary artery responses to cold stress testing in type 2 diabetic subjects (16) and in amelioration of endothelial dysfunction in subjects with coronary heart disease (17).

All these observations suggest that iron is more intimately linked to human pathophysiology than previously thought. In fact, iron metabolism is closely associated with the clinical presentation of numerous systemic diseases (18). Tissue iron excess contributes to produce and amplify the injury caused by free radicals as well as to modulate various steps involved in the inflammatory lesion.

In this article, we summarize the relationships between iron, insulin resistance, and type 2 diabetes and discuss the therapeutic and clinical implications of reducing body iron.

Iron stores are associated with insulin sensitivity, insulin secretion, and type 2 diabetes

Iron and insulin sensitivity. Iron stores, expressed as serum ferritin concentration, have been proposed to be a component of the insulin-resistance syndrome. Indeed, the concentration of circulating ferritin was significantly associated with centrally distributed body fatness as well as with several other measurements of obesity (19). In the apparently healthy general population, serum levels of ferritin were also positively correlated with baseline serum glucose and with the area under the curve for glucose during the glucose oral tolerance test (20,21). In gestational diabetes, both BMI and serum ferritin levels were found to be independent predictors of 2-h glucose during an oral glucose tolerance test (8,9). Ferritin levels also correlated with diastolic arterial blood pressure, even after adjustment for BMI. Of note is the beneficial effect of blood letting, a means of reducing iron stores, in the treatment of resistant hypertension (22) and in posttransplant hypertension associated with erythrocytosis (23). Serum ferritin concentration was also directly associated

with uric acid (another component of the insulin resistance syndrome) and inversely related with HDL cholesterol and the HDL₂-to-HDL₃ ratio (21).

Insulin resistance itself, assessed by either the euglycemic clamp (24) or the minimal model (25,26), was found to be associated with total body iron stores, even in the presence of normal glucose tolerance. Dmochowski et al. (25) reported that serum concentrations of ferritin were negatively correlated with insulin sensitivity ($r = -0.58$) in subjects with hemosiderosis. Cavallo-Perin et al. (26) reported that insulin sensitivity, which correlated closely with iron overload ($r = -0.70$), was reduced by 40% in thalassemia patients. Insulin resistance also appeared to be closely linked to total body iron stores in the general population (21). Serum ferritin levels could be a useful marker of insulin resistance beyond a given threshold (20,21). In the study by Toumainen et al. (20), the increase in serum insulin concentrations was clearly apparent in the upper two quintiles of ferritin levels. In a different study, the correlation between circulating ferritin and insulin resistance was only observed in the upper two quartiles of ferritin levels (21). Below this threshold, the potential tissue effects of siderosis would be negligible.

Some comments on the specificity of serum ferritin as an indicator of iron stores seem necessary. The relationship between serum ferritin and histochemical assessment of stainable tissue iron contributes to define threshold values for serum ferritin, indicating exhausted, small, normal, ample, and increased iron stores. However, the barrier between "normal" and "small" or "ample" iron stores is not well defined and remains controversial. Approximately 10% of type 2 diabetic patients with high ferritin levels had transferrin saturations greater than normal (40%). On the other hand, serum ferritin should be cautiously evaluated in patients with type 2 diabetes, because it may falsely indicate "normal iron stores." It should not be ignored that chronic inflammation could contribute, to some extent, to increased ferritin concentration (see below).

Iron and β -cell function. Recent in vitro studies have shown that H-ferritin mRNA is four- to eightfold higher in rat islets treated with 20 mmol/l glucose than in islets treated with 1 mmol/l glucose (27). The potential reason for the increased ferritin in the β -cell is that ferritin exhibits antioxidant properties and the β -cell is particularly sensitive to oxygen radicals. This high amount of ferritin can explain why iron is preferentially retained in the β -cell. In fact, iron deposition in islets, albeit variable, is restricted to β -cells (28).

An increase in β -cell mass was demonstrated in a small number of nondiabetic or mildly diabetic patients with iron overload (28). In agreement with this increase in β -cell mass, raised basal and stimulated C-peptide secretion were observed in type 2 diabetic patients with increased serum ferritin concentration. Furthermore, significantly lowered C-peptide secretion was found after phlebotomy-induced iron depletion, suggesting increased β -cell insulin sensitivity (15).

Iron overload and type 2 diabetes. Five additional pieces of scientific evidence favor the hypothesis that iron plays a role in type 2 diabetes. First, increased prevalence of hemochromatosis was found among unselected patients with type 2 diabetes. Phelps et al. (29) and Conte et al. (30)

reported that diabetes confers increased risk for hereditary hemochromatosis, which was 2.4% and 1.34% higher in Australian and Italian populations, respectively.

This evidence, however, is not always consistent. The recent characterization of *HFE* has allowed a more direct study of the prevalence of its mutations in type 2 diabetes. Homozygosity for the C282Y change is generally associated with clinically evident hereditary hemochromatosis (83% of hemochromatosis patients are YY homozygotes). Compound heterozygotes for H63D mutation (C282Y/H63D) succumb to the disease, although with reduced penetrance. An increased frequency of C282Y mutations in subjects with type 2 diabetes has been described in some studies (31–32). Notwithstanding, at least four additional studies reported no significant differences in the prevalence of C282Y mutations between patients with type 2 diabetes and control subjects of Caucasian origin (33–36). In the Spanish population, the frequency of the H63D mutation was significantly higher in type 2 diabetic subjects (36). The H63D mutation is also associated with other nonclassical conditions of iron overload. On the other hand, it is interesting to mention that genetic hemochromatosis contributed to 1% of late-onset type 1 diabetes (37).

Second, frequent blood donations, leading to decreasing iron stores, have been demonstrated to constitute a protective factor for the development of diabetes (13). This finding is especially important given the high prevalence of increased iron stores in the general population of western countries (10) and the observation that increased iron stores appear to predict an increased incidence of type 2 diabetes (5). In experimental models, the incidence of diabetes was reduced from 78 to 22% at 120 days of age after serial blood withdrawals in the BB rat (38).

Third, a recent randomized study also suggests that iron stores may influence insulin action in type 2 diabetes (15). In this report, 28 type 2 diabetic patients with increased serum ferritin concentration and negative for C282Y mutation of hereditary hemochromatosis were randomized to blood letting (three 500 ml phlebotomies at 2-week intervals) or to observation. Insulin secretion and sensitivity were tested at baseline and at 4 and 12 months thereafter. The two groups were matched for age, BMI, pharmacological treatment, and chronic diabetes complications (15). Baseline glycated hemoglobin and insulin sensitivity were not significantly different between the two groups. A statistically significant increase in insulin sensitivity was observed in the blood-letting group (from 2.30 ± 1.81 to 3.08 ± 2.55 $\text{mg} \cdot \text{dl}^{-1} \cdot \text{min}^{-1}$ at 4 months to 3.16 ± 1.85 $\text{mg} \cdot \text{dl}^{-1} \cdot \text{min}^{-1}$ at 12 months, $P = 0.045$) in contrast to patients subjected to observation in whom insulin sensitivity did not significantly change (from 3.24 ± 1.9 to 3.26 ± 2.05 $\text{mg} \cdot \text{dl}^{-1} \cdot \text{min}^{-1}$ at 4 months to 2.31 ± 1.35 $\text{mg} \cdot \text{dl}^{-1} \cdot \text{min}^{-1}$ at 12 months). Accordingly, blood HbA_{1c} decreased significantly only in the blood-letting group at 4 months (mean differences -0.61 , 95% CI -0.17 to -1.048 , $P = 0.01$).

Fourth, a novel syndrome of hepatic iron overload has been described that associates hyperferritinemia with normal transferrin saturation and is not linked to the HLA-A3 antigen, a common marker for hereditary hemochromatosis (39). This condition is known as insulin

resistance-associated hepatic iron overload (IR-HIO) and combines abnormalities in iron metabolism (isolated hyperferritinemia with normal transferrin saturation), steatohepatitis, and the insulin resistance syndrome (obesity, hyperlipidemia, abnormal glucose metabolism, and hypertension) (39–41). In IR-HIO, iron overload occurs in both hepatocytes and sinusoid cells, being higher in the latter cells in 45% of cases, a finding seen in only 3% of subjects with hemochromatosis (42). Approximately two-thirds of these patients develop steatosis, whereas the remaining third show isolated signs of inflammation (42). Thus, these patients are at high risk for developing liver fibrosis, a complication observed in 60% of all cases, even in the presence of moderate iron overload. In contrast, liver fibrosis affects only 33% of patients with hemochromatosis. Because patients with IR-HIO are prone to experience significant tissue damage and because this can be prevented with simple and inexpensive therapies (i.e., phlebotomy), higher awareness in order to diagnose the disease has been suggested.

It cannot be ruled out, however, that IR-HIO is the same process of iron overload-related insulin resistance that associates liver steatosis and fibrosis in susceptible patients. It is important to recognize that in one study, liver iron stores were found within the normal range in patients with type 2 diabetes (43) in contrast to other studies (29,30). Under this assumption, IR-HIO would be at one end of the spectrum of iron overload-related insulin resistance.

Fifth, insulin resistance features are frequently seen in patients chronically infected with the hepatitis C virus. In these subjects, BMI, elderliness, iron stores, and family history of diabetes and advanced liver fibrosis were found to predict the development of diabetes (44).

Interacting pathways linking glucose and iron metabolism

A. Insulin influences iron metabolism. Insulin is an anabolic hormone that stimulates the cellular uptake of many nutrients, including hexoses, amino acids, cations and anions. Intestinal absorption of nonheme iron is tightly regulated in keeping with the body requirements, and absorption of iron is minimal when body iron stores are normal. Absorption of heme iron (largely provided by red meat in western countries) does not appear to be dependent on body iron content. In the steady state, circulating iron is bound to transferrin and is taken up from the blood by a high-affinity specific transferrin receptor. The transferrin-receptor complex is internalized by endocytosis and released into a nonacidic cellular compartment, where it can be used in the synthesis of essential cellular components. Insulin is known to cause a rapid and marked stimulation of iron uptake by fat cells, redistributing transferrin receptors from an intracellular membrane compartment to the cell surface (45). Insulin is also responsible for the increased ferritin synthesis in cultured rat glioma cells (46). Importantly, transferrin receptors have been shown to colocalize with insulin-responsive glucose transporters and insulin-like growth factor II receptors in the microsomal membranes of cultured adipocytes, suggesting that regulation of iron uptake by insulin occurs in parallel with its effects on glucose transport (47).

B. Iron influences glucose metabolism. Reciprocally, iron influences insulin action. Iron interferes with insulin inhibition of glucose production by the liver. Hepatic extraction and metabolism of insulin is reduced with increasing iron stores, leading to peripheral hyperinsulinemia (48). In fact, the initial and most common abnormality seen in iron overload conditions is liver insulin resistance (49). There is some evidence that iron overload also affects skeletal muscle (50), the main effector of insulin action.

C. Oxidative stress influences both glucose and iron metabolism. Oxidative stress induces both insulin resistance [by decreasing internalization of insulin (51)] and increased ferritin synthesis.

Iron is intimately linked to oxidative stress. Iron participates, through the Fenton reaction, in the formation of highly toxic free radicals, such as hydroxide and the superoxide anion, which are capable of inducing lipid peroxidation. For iron to act as a prooxidant agent, it must be in its free form. Iron can be released from ferritin by the action of reducing agents that convert Fe^{3+} into Fe^{2+} (52). Glycation of transferrin decreases its ability to bind ferrous iron (53) and, by increasing the pool of free iron, stimulates ferritin synthesis. Glycated holotransferrin is additionally known to facilitate the production of free oxygen radicals, such as hydroxide, that further amplify the oxidative effects of iron (53).

The fraction of nonused and highly toxic iron is stored as ferritin molecules in order to be neutralized. Apoferritin, the protein fraction of ferritin, is spatially folded to create a central groove that holds oxidized iron molecules [Fe^{3+}]. Apoferritin is a high-molecular weight (450 kDa) multimeric protein (24 subunits of heavy and light chains) that exhibits exquisite high capacity for iron storage (4,500 mol iron per mole of ferritin). Synthesis of apoferritin is induced at both the transcriptional and posttranscriptional levels by the presence of free iron. The increase in Fe^{2+} downregulates the affinity of iron-regulatory element (IRE) binding protein (BP) for its IRE binding site in the 5' region of ferritin mRNA, leading to increased ferritin translation.

The heavy chain in the apoferritin molecule exerts ferroxidase activity, catalyzing the oxidation of Fe^{2+} into Fe^{3+} , which prevents iron-induced cyclic red-ox reactions that would spread and amplify the oxidative damage. This activity occurs under aerobic conditions, allowing the storage of intracellular iron. When concentrations of antioxidants are low, the reducing potential and anaerobiosis progressively increase, facilitating a rapid release of iron from ferritin. Additionally, the ferroxidase activity in the heavy chain is downregulated in this setting, decreasing the incorporation of iron into ferritin. The overall result of oxidative reactions is an increase in the availability of free iron from the ferritin molecule as well as from other molecules undergoing degradation, such as the heme group. These events, in turn, can enhance and amplify the process of generation of free radicals, causing cellular and tissue damage. The oxidative stress also downregulates the affinity of IRE for IRE-BP. Thus, ferritin can act both as a source of iron, which induces oxidative stress, and as a mechanism that protects against iron toxicity (54).

Hyperferritinemia is present in 6.6% of unselected

patients with type 2 diabetes (55). Serum concentrations of ferritin are usually increased in poorly controlled type 1 and type 2 diabetic subjects, and ferritin has been shown to predict HbA_{1c} independently of glucose (21), probably reflecting increased oxidative stress. Short-term improvement in glycemic control is followed by variable decreases in serum ferritin concentration.

D. Cytokines influence iron and glucose metabolism.

Cytokines simultaneously cause an increase in transferrin receptors on the cell surface, favoring tissue deposition of iron (56) and insulin resistance (57).

In summary, a scenario can be envisioned in which the physiological action of insulin leads to increased uptake of different nutrients and iron. Any factor causing hyperinsulinemia (weight gain, aging, repeated usual-life infections, or periodontitis) amplifies this process, determining increased deposition of iron, which in the long-term worsens insulin resistance.

Clinical and therapeutical implications of iron depletion

A. Iron depletion and diabetic metabolic control.

There are historical notes regarding iron overload conditions that can be helpful in delineating the effects of iron depletion (58–60). Phlebotomy was first used in the treatment of hemochromatosis in the 1950s. Interestingly, diabetic metabolic control improved in 35–45% of patients with hemochromatosis after iron depletion (58). In 1969, Williams et al. (59) showed that diabetic patients treated with phlebotomy required less insulin than similar patients during the prephlebotomy period. In 1972, Dymock et al. (60) reported a significant reduction in total daily insulin dosage following phlebotomy.

Facchini (11) found significant reductions in insulin concentrations 1 month after performing a 550-ml phlebotomy in healthy volunteers. It has also been suggested that the increased insulin sensitivity observed in vegetarian subjects might be related to their low-iron diet. Recently, as stated above, blood letting of 1,500 ml has been demonstrated to improve insulin sensitivity and to decrease C-peptide secretion in type 2 diabetic subjects who were negative for common hemochromatosis mutations but had increased serum ferritin concentration (15).

Iron chelators also seem beneficial in optimizing diabetic metabolic control. In 1989, Cutler (61) administered deferoxamine to nine type 2 diabetic patients with hyperferritinemia who were negative for the most common hemochromatosis haplotypes. Major improvement in the metabolic control was observed in seven patients, and parallel reductions in baseline concentrations of glucose, triglycerides, and glycated hemoglobin were observed. Treatment with either insulin or oral hypoglycemic agents could be discontinued in some patients (61). Subsequently, it was reported that subcutaneous deferoxamine caused an improvement in glycated hemoglobin in another nine patients (62), although serum concentrations of C-peptide after glucose or arginine infusion did not improve significantly (62).

B. Iron depletion and chronic diabetes complications. Macrovascular disease. The general effect of catalytic iron is to convert poorly reactive free radicals, such as H₂O₂, into highly reactive ones, such as OH⁻ and O₂. Free radicals and other oxidation by-products are well

known factors that impair the mechanisms of vasodilatation (63) and cause endothelial depletion of endogenous antioxidants, such as ascorbic acid (64). Iron chelation blocks oxidation of LDL, and iron released from heme and ferritin favors oxidation of this lipoprotein (65). Increased iron availability is, theoretically, expected to contribute to macrovascular disease because iron has an adverse effect on endothelium (66) and accelerates the development of atherosclerosis (67). In fact, ferritin gene expression increases in the course of atherosclerotic plaque formation (68). Notwithstanding, studies performed in experimental models offer conflicting conclusions. For instance, an iron-deficient diet has been found to reduce atherosclerotic lesions (69), whereas iron overload led to a decrease in atherosclerosis (70) in the same animal model. It should be noted, however, that the possible influence of obesity, insulin resistance, or diabetes has not been explored in experimental iron-modified atherosclerosis. Moreover, humans are the only species in which genetic iron overload has been described to induce significant parenchymal damage (71).

In subjects with hemochromatosis, medium-sized arteries are characterized by an eccentric hypertrophy and decreased distensibility that are partially reversible after iron depletion (72). These findings seem to be linked to iron-induced fibrogenesis, determining an increased total collagen content in arteries from these patients. There is also some evidence for iron-dependent growth of arterial wall tissue: iron chelation by deferoxamine inhibits vascular smooth muscle cell proliferation (73).

Long-term use of the modified iron chelator hydroxyethyl starch conjugated-deferoxamine prevented endothelial dysfunction associated with experimental diabetes (74). In type 2 diabetic patients, coronary artery responses to cold stress testing improved substantially after deferoxamine administration (16). Similarly, iron chelation was shown to ameliorate the endothelial dysfunction of patients with coronary heart disease (17).

Improvement of nitroglycerine-induced vasodilatation was also observed following phlebotomy in type 2 diabetic patients in a preliminary study. The improvement in vascular reactivity paralleled the decrease in serum transferrin saturation, total hemoglobin (markers of circulating iron), and blood glycated hemoglobin (75). These observations suggest that diabetic vascular dysfunction seems partially reversible and that the circulating compartment acts as a reservoir of transition metals that directly affects vascular function (76). Increased hemoglobin, an iron-enriched protein, is deleterious for endothelial function, as normal blood vessels exposed to total and glycated hemoglobin are known to experience impaired vascular relaxation (77).

In the general population, the relationship between iron and atherosclerosis is, however, controversial (78). Thus, some animal experimental data regarding the effect of manipulating iron stores on atherosclerosis and human data showing improvement of vascular structure and function following iron depletion (16,17,72,75) are both consistent with the theory that iron contributes to the development of vascular disease. However, the current epidemiological data associating iron stores with either

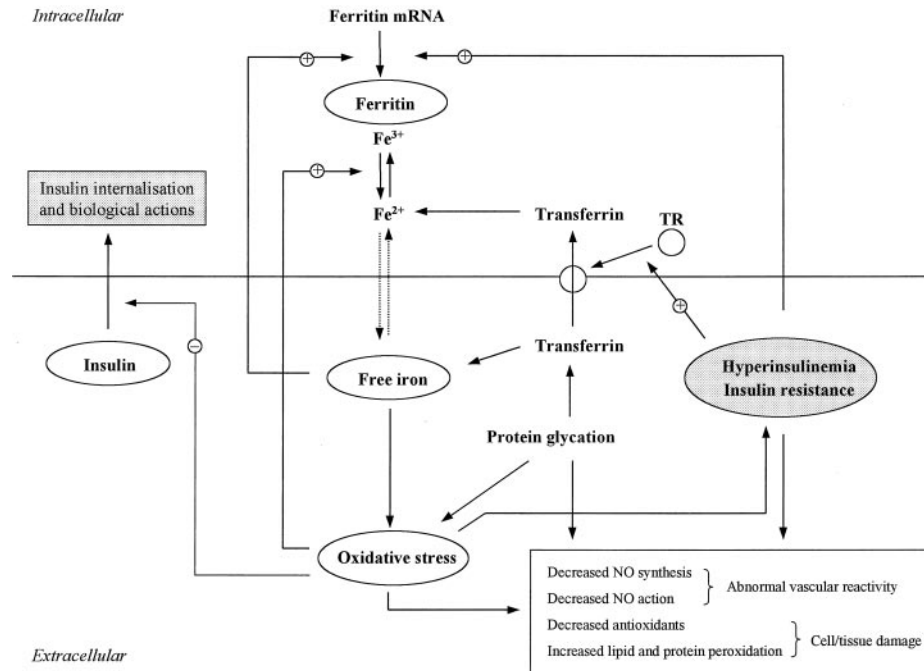


FIG. 1. Schematic representation of iron interactions with insulin resistance and oxidative stress. Insulin influences iron metabolism. Insulin stimulates ferritin synthesis and facilitates iron uptake by the cell through the translocation of transferrin receptors from the intracellular compartment to the cell surface. Conversely, iron influences glucose metabolism. Iron is a potent prooxidant that increases the cell oxidative stress, causing inhibition of insulin internalization and actions, results in hyperinsulinemia and insulin resistance. Free iron also exerts a positive feedback on ferritin synthesis, while oxidative stress increases the release of iron from ferritin. The increased oxidative stress and insulin resistance cause endothelial and tissue damage. Protein glycation, as seen in diabetes, further amplifies these abnormalities stimulating iron release from transferrin, increasing the cell oxidative stress and directly causing endothelial and tissue damage. NO, nitric oxide; TR, transferrin receptor; (+), stimulation; (-), inhibition; dotted lines, possible trafficking or iron through the cell membrane.

atherosclerosis or coronary heart disease (reviewed in 78) do not fully support this hypothesis.

Diabetic nephropathy. An early development and accelerated course of diabetic nephropathy has been observed in iron-loaded patients with β -thalassemia, a condition with recognized iron overload (79). Increased proximal tubular lysosomal iron concentration has been observed in patients with diabetic nephropathy (80). These observations are pertinent to the finding that mutations for hereditary hemochromatosis appeared to predict the development of diabetic nephropathy (32).

Diabetic neuropathy. Deferoxamine administration restored motor and sensory nerve conduction velocity and improved nerve blood flow in experimental studies (76). The possible extrapolation of these findings to human diabetes has not been explored.

CONCLUSIONS

The central role of iron in biology is illustrated by the fact that this is the fourth most abundant element in Earth's crust as well as the transition element most abundant in living organisms. Iron has additionally proven to be fundamental in the selection imposed by evolution, given its close relationship with oxygen. Although losses of this metal are only a tenth of those found in any other given mammal, iron regulation is maintained within very narrow limits in humans (71).

Our biochemistry and physiology are tuned to life conditions that existed before the advent of agriculture some 10,000 years ago. Hunter-gatherer societies obtained more than 56–65% of their subsistence from animal foods

(81). Meat eaters, with a typical high protein and low-carbohydrate diet, have a significant higher plasma concentration of iron (82) and concomitant insulin resistance in the liver and peripheral tissues (83). It is plausible that the survival advantage of both iron and high protein-induced insulin resistance in our ancestral line was that the little available glucose from carbohydrate consumption was preserved for brain function and reproductive fetal/placental/mammary tissues (84). Nowadays, with increased life expectancy, this protective mechanism has become detrimental, with iron promoting both insulin resistance and increased oxidative stress.

In the last decades, the impact of transition metals, in general, and iron, in particular, on human physiology has begun to be elucidated. Because iron is a first-line prooxidant, it contributes to regulate the clinical manifestations of numerous systemic diseases, including diabetes and atherosclerosis. Iron regulation of the cell oxidative stress can explain, at least in part, its close association with abnormalities in insulin sensitivity (see Figure 1 for a summary of iron interactions with insulin sensitivity).

The clarification of the mechanisms that regulate this interaction are proposed to contribute to improve the management of diabetes and to anticipate its possible complications. Here, we show that iron modulates insulin action in healthy individuals and in patients with type 2 diabetes. The extent of this influence should be tested in large-scale clinical trials, searching for the usefulness and cost-effectiveness of therapeutic measures that decrease iron toxicity. Of paramount importance will be the definition of "normal body iron stores" and the establishment of

early therapeutical interventions. Simple and inexpensive therapies, such as blood letting and iron chelators, are emerging as alternative and effective treatments for insulin resistance.

It will also be necessary to explore whether important elements of iron metabolism are altered in diabetes, namely the transporters DMT1, ferroportin, and MTP1, which are critical in intestinal absorption and entry of iron into the circulation, and haephastin, which oxidizes Fe^{2+} to Fe^{3+} during this process (85). Interestingly, certain genes appear to be simultaneously involved in iron balance, inflammation, and glucose responsiveness, suggesting a link between these pathways and type 2 diabetes (85).

REFERENCES

- Medalie JH, PapieR CM, Goldbourt U, Herman JB: Major factors in the development of diabetes mellitus in 10,000 men. *Arch Intern Med* 135:811–817, 1975
- Wilson PW, McGee DL, Kannel WB: Obesity, very low density lipoproteins and glucose intolerance over fourteen years: the Framingham Study. *Am J Epidemiol* 114:697–704, 1981
- Catalano C, Muscelli E, Quiñones A, Baldi S, Ciociaro D, Seghieri G, Ferrannini E: Reciprocal association between insulin sensitivity and the hematocrit in man (Abstract). *Diabetes* 45 (Suppl. 2):323A, 1996
- Wannamethee SG, Perry IJ, Shaper AG: Hematocrit and risk of NIDDM. *Diabetes* 45:576–579, 1996
- Salonen JT, Tuomainen T-P, Nyyssönen K, Lakka H-M, Punnonen K: Relation between iron stores and non-insulin-dependent diabetes in men: case-control study. *Br Med J* 317:727–730, 1999
- Ford ES, Cogswell ME: Diabetes and serum ferritin concentration among U.S. adults. *Diabetes Care* 22:1978–1983, 1999
- Barbieri M, Ragno E, Benvenuti E, Zito GA, Corsi A, Ferrucci L: New aspects of the insulin resistance syndrome: impact on haematological parameters. *Diabetologia* 44:1232–1237, 2001
- Lao TT, Tam K-F: Maternal serum ferritin and gestational impaired glucose tolerance. *Diabetes Care* 20:1368–1369, 1997
- Lao TT CP, Tam KF: Gestational diabetes mellitus in the last trimester: a feature of maternal iron excess? *Diabet Med* 18:218–223, 2001
- Fleming DJ, Jacques PF, Tucker KL, Massaro JM, D'Agostino RB, Wilson PWF, Wood RJ: Iron status of the free-living, elderly Framingham Heart Study cohort: an iron-replete population with a high prevalence of elevated iron stores. *Am J Clin Nutr* 73:638–646, 2001
- Facchini FS: Effect of phlebotomy on plasma glucose and insulin concentrations. *Diabetes Care* 21:2190, 1998
- Hua NW, Stoohs RA, Facchini FS: Low iron status and enhanced insulin sensitivity in lacto-ovo vegetarians. *Br J Nutr* 86:515–519, 2001
- Ascherio A, Rimm EB, Giovannucci E, Willett WC, Stampfer MJ: Blood donations and risk of coronary heart disease in men. *Circulation* 103:52–57, 2001
- Bofill C, Joven J, Bages J, Vilella E, Sans T, Cavallé P: Response to repeated phlebotomies in patients with non-insulin-dependent diabetes mellitus. *Metabolism* 43:614–620, 1994
- Fernández-Real JM, Peñarroja G, Castro A, García-Bragado F, Hernández I, Ricart W: Blood letting in high-ferritin type 2 diabetes: effects on insulin sensitivity and β -cell function. *Diabetes* 51:1000–1004, 2002
- Nitenberg A, Ledoux S, Valensi P, Sachs R, Antony I: Coronary microvascular adaptation to myocardial metabolic demand can be restored by inhibition of iron-catalyzed formation of oxygen free radicals in type 2 diabetic patients. *Diabetes* 51:813–818, 2002
- Duffy SJ, Biegelsen ES, Holbrook M, Russell JD, Gokce JK Jr: Iron chelation improves endothelial function in patients with coronary artery disease. *Circulation* 103:2799–2804, 2001
- Guillen C, McInnes IB, Kruger H, Brock JH: Iron, lactoferrin and iron regulatory protein activity in the synovium: relative importance of iron loading and the inflammatory response. *Ann Rheum Dis* 57:309–314, 1998
- Gillum RF: Association of serum ferritin and indices of body fat distribution and obesity in Mexican American men: the Third National Health and Nutrition Examination Survey. *Int J Obes Rel Metab Dis* 25:639–645, 2001
- Tuomainen T-P, Nyyssönen K, Salonen R, Tervahauta A, Korpela H, Lakka T: Body iron stores are associated with serum insulin and blood glucose concentrations. *Diabetes Care* 20:426–428, 1997
- Fernández-Real JM, Ricart W, Arroyo E, Balança R, Casamitjana R, Cabrero D: Serum ferritin as a component of the insulin resistance syndrome. *Diabetes Care* 21:62–68, 1998
- Zidek W, Tenschert W, Karoff C, Vetter H: Treatment of resistant hypertension by phlebotomy. *Klin Wochenschr* 63:762–764, 1985
- Barenbrock M, Spieker C, Rahn KH, Zidek W: Therapeutic efficiency of phlebotomy in posttransplant hypertension associated with erythrocytosis. *Clin Nephrol* 40:241–243, 1993
- Merkel PA, Simonson DC, Amiel SA, Plewe G, Sherwin RS, Pearson HA: Insulin resistance and hyperinsulinemia in patients with thalassemia major treated by hypertransfusion. *N Engl J Med* 318:809–814, 1988
- Dmochowski K, Finegood DT, Francombe W, Tyler B, Zinman B: Factors determining glucose tolerance in patients with thalassemia major. *J Clin Endocrinol Metab* 77:478–483, 1993
- Cavallo-Perin P, Pacini G, Cerutti F, Bessone A, Condo C, Sacchetti L: Insulin resistance and hyperinsulinemia in homozygous β -thalassemia. *Metabolism* 44:281–286, 1995
- MacDonald MJ, Cook JD, Epstein ML, Flowers CH: Large amount of (apo)ferritin in the pancreatic insulin cell and its stimulation by glucose. *FASEB J* 8:777–781, 1994
- Rahier JR, Loozen S, Goebbels RM, Abraham M: The hemochromatotic human pancreas: a quantitative immunohistochemical and ultrastructural study. *Diabetologia* 30:5–12, 1987
- Phelps G, Chapman I, Hall P, Braund W, Mackinnon M: Prevalence of genetic haemochromatosis among diabetic patients. *Lancet* 2:233–234, 1989
- Conte D, Manachino D, Colli A, Guala A, Aimo G, Andreoletti M: Prevalence of genetic hemochromatosis in a cohort of Italian patients with diabetes mellitus. *Ann Intern Med* 128:370–373, 1998
- Kwan T, Leber B, Ahuja S, Carter R, Gerstein H: Patients with type 2 diabetes have a high frequency of the C282Y mutation of the hemochromatosis gene. *Clin Invest Med* 21:251–257, 1998
- Moczulski DK, Grzeszczak W, Gawlik B: Role of hemochromatosis C282Y and H63D mutations in HFE gene in development of type 2 diabetes and diabetic nephropathy. *Diabetes Care* 24:1187–1191, 2001
- Frayling T, Ellard S, Grove J, Walker M, Hattersley AT: C282Y mutation in HFE (haemochromatosis) gene and type 2 diabetes. *Lancet* 351:1933–1934, 1998
- Braun J, Donner H, Plock K, Rau H, Usadel KH, Badenhop K: Hereditary hemochromatosis mutations (HFE) in patients with type II diabetes mellitus. *Diabetologia* 41:983–984, 1998
- Dubois-Laforgue D, Caillat-Zucman S, Djilali-Saiah I, Larger E, Mercadier A, Boitard C: Mutations in HFE, the hemochromatosis candidate gene, in patients with NIDDM. *Diabetes Care* 21:1371–1372, 1998
- Fernández-Real JM, Vendrell J, Baiget M, Gimferrer E, Ricart W: C282Y and H63D mutations of the hemochromatosis candidate gene in type 2 diabetes. *Diabetes Care* 22:525–526, 1999
- Ellervik C, Mandrup-Poulsen T, Nordestgaard BG, Larsen LE, Appleyard M, Frandsen M, Petersen P, Schlichting P, Saermark T, Tybjaerg-Hansen A, Birgens H: Prevalence of hereditary hemochromatosis in late-onset type 1 diabetes mellitus: a retrospective study. *Lancet* 358:1405–1409, 2001
- Yale JF, Grose M, Seemayer TA, Marliss EB: Diabetes prevention in BB rats by frequent blood withdrawal started at a young age. *Diabetes* 37:327–333, 1988
- Moirand R, Mortaji A, Loréal O, Paillard F, Brissot P, Deugnier Y: A new syndrome of liver iron overload with normal transferrin saturation. *Lancet* 349:95–97, 1997
- Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M: Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes* 50:1844–1850, 2001
- Mendler MH, Turlin B, Moirand R, Jouanolle AM, Sapey T, Guyader D, Le Gall JY, Brissot P, David V, Deugnier Y: Insulin resistance-associated hepatic iron overload. *Gastroenterology* 117:1155–1163, 1999
- Turlin B, Mendler MH, Moirand R, Guyader G, Guillygomarc'h A, Deugnier Y: Histological features of the liver in insulin resistance-associated iron overload. *Am J Clin Pathol* 116:263–270, 2001
- Dinneen SF, Silverberg JD, Batts KP, O'Brien PC, Ballard DJ, Riza RA: Liver iron stores in patients with non-insulin-dependent diabetes mellitus. *Mayo Clin Proc* 69:13–15, 1994
- Petit JM, Bour JB, Galland-Jos C, Minello A, Verges B, Guiguet M: Risk factors for diabetes mellitus and early insulin resistance in chronic hepatitis C. *J Hepatol* 35:279–283, 2001
- Davis RJ, Corvera S, Czech MP: Insulin stimulates cellular iron uptake and causes the redistribution of intracellular transferrin receptors to the plasma membrane. *J Biol Chem* 261:8708–8711, 1986
- Yokomori N, Iwasa Y, Aida K, Inoue M, Tawata M, Onaya T: Transcrip-

- tional regulation of ferritin messenger ribonucleic acid levels by insulin in cultured rat glioma cells. *Endocrinology* 128:1474–1480, 1991
47. Tanner LJ, Lienhard GE: Localization of transferrin receptors and insulin-like growth factor II receptors in vesicles from 3T3-L1 adipocytes that contain intracellular glucose transporters. *J Cell Biol* 108:1537–1545, 1989
 48. Niederau C, Berger M, Stremmel W, Starke A, Strohmeyer G, Ebert R: Hyperinsulinemia in non-cirrhotic haemochromatosis: impaired hepatic insulin degradation? *Diabetologia* 26:441–444, 1984
 49. Dandona P, Hussain MAM, Varghese Z, Politis D, Flynn DM, Hoffbrand AV: Insulin resistance and iron overload. *Ann Clin Biochem* 20:77–79, 1983
 50. Shafer AI, Cheron RG, Dluhy R, Cooper B, Gleason RE, Soeldner JS: Clinical consequences of acquired transfusional iron overload in adults. *N Engl J Med* 304:319–324, 1981
 51. Bertelsen M, Ånggard EE, Carrier MJ: Oxidative stress impairs insulin internalization in endothelial cells in vitro. *Diabetologia* 44:605–613, 2001
 52. Reif DW: Ferritin as a source of iron for oxidative damage. *Free Rad Biol Med* 12:417–427, 1992
 53. Fujimoto S, Kawakami N, Ohara A: Nonenzymatic glycation of transferrin: decrease of iron-binding capacity and increase of oxygen radical production. *Biol Pharm Bull* 18:396–400, 1995
 54. Juckett MB, Balla J, Balla G, Jessurun J, Jacob HS, Vercellotti GM: Ferritin protects endothelial cells from oxidized low density lipoprotein in vitro. *Am J Pathol* 147:782–789, 1995
 55. O'Brien T, Basset B, Burray DM, Dinneen S, O'Sullivan DJ: Usefulness of biochemical screening of diabetic patients for hemochromatosis. *Diabetes Care* 13:532–534, 1990
 56. Hirayama M, Kohgo Y, Kondo H, Shintani N, Fujikawa K, Sasaki K, Kato J, Niitsu Y: Regulation of iron metabolism in HepG2 cells: a possible role for cytokines in the hepatic deposition of iron. *Hepatology* 18:874–880, 1993
 57. Fernandez-Real JM, Vayreda M, Richart C, Gutierrez C, Broch M, Vendrell J, Ricart W: Circulating interleukin 6 levels, blood pressure, and insulin sensitivity in apparently healthy men and women. *J Clin Endocrinol Metab* 86:1154–1159, 2001
 58. Davis WD, Arrowsmith WR: The treatment of haemochromatosis by massive venesection. *Ann Intern Med* 39:723–734, 1953
 59. Williams R, Smith PM, Spicer EJF, Barry M, Sherlock S: Venesection therapy in idiopathic haemochromatosis: an analysis of 40 treated and 18 untreated patients. *Q J Med* 149:1–16, 1969
 60. Dymock IW, Cassar J, Pyke DA, Oakley WG, William SR: Observations on the pathogenesis, complications and treatment of diabetes in 115 cases of hemochromatosis. *Am J Med* 203–210, 1972
 61. Cutler P: Deferoxamine therapy in high-ferritin diabetes. *Diabetes* 38:1207–1210, 1989
 62. Redmon JB, Pyzdrowski KL, Robertson RP: No effect of deferoxamine therapy on glucose homeostasis and insulin secretion in individuals with NIDDM and elevated serum ferritin. *Diabetes* 42:544–549, 1993
 63. Graier WF, Simecek S, Kukovetz WR, Kostner GM: High D-glucose-induced changes in endothelial Ca²⁺/EDRF signaling are due to generation of superoxide anions. *Diabetes* 45:1386–1395, 1996
 64. Yue DKS, McLennon S, Fisher E, Heffernan S, Capogreco C, Ross GR: Ascorbic acid status and polyol pathway in diabetes. *Diabetes* 38:257–261, 1989
 65. Abdalla DSP, Campa A, Monteiro MP: Low density lipoprotein oxidation by stimulated neutrophils and ferritin. *Atherosclerosis* 97:149–159, 1992
 66. Lekakis J, Papamicheal C, Stamatelopoulous K, Cimponeriu A, Voutsas A, Vemmos K, Mavrikakis M, Stamatelopoulous S: Hemochromatosis associated with endothelial dysfunction: evidence for the role of iron stores in early atherogenesis. *Vasc Med* 4:147–148, 1999
 67. Araujo JA, Romano EL, Brito BE, Parthe V, Romano M, Bracho M, Montano RF, Cardier J: Iron overload augments the development of atherosclerotic lesions in rabbits. *Arterioscler Thromb Vasc Biol* 15:1172–1180, 1995
 68. Pang J, Jiang MJ, Chen YL, Wang FW, Wang DL, Chu SH: Increased ferritin gene expression in atherosclerotic lesions. *J Clin Invest* 97:2204–2212, 1996
 69. Lee TS, Shiao MS, Pan CC, Chau LY: Iron-deficient diet reduces atherosclerotic lesions in apoE-deficient mice. *Circulation* 99:1222–1229, 1999
 70. Kirk EA, Heinecke JW, LeBoeuf RC: Iron overload diminishes atherosclerosis in apoE-deficient mice. *J Clin Invest* 107:1545–1553, 2001
 71. Finch C: Regulators of iron balance in humans. *Blood* 84:1697–1702, 1994
 72. Failla M, Giannattasio C, Piperno A, Vergani A, Grappiolo A, Gentile G, Meles E, Mancina G: Radial artery wall alterations in genetic hemochromatosis before and after iron depletion therapy. *Hepatology* 32:569–573, 2000
 73. Porreca E, Ucchino S, Di Febbo C, Di Bartolomeo N, Angelucci D, Napolitano AM, Mezzetti A, Cuccurullo F: Antiproliferative effect of desferrioxamine on vascular smooth muscle cells in vitro and in vivo. *Arterioscler Thromb Vasc Biol* 14:299–304, 1994
 74. Pieper GM, Siebeneich W: Diabetes-induced endothelial dysfunction is prevented by long-term treatment with the modified iron chelator, hydroxyethyl starch conjugated-deferoxamine. *J Cardiovasc Pharmacol* 30:734–738, 1997
 75. Fernández-Real JM, Peñarroja G, Castro A, García-Bragado F, Ricart W: Phlebotomy improves vascular dysfunction in high ferritin type 2 diabetes mellitus (Abstract). *Diabetologia* 43 (Suppl. 1):A54, 2000
 76. Cameron NE, Cotter MA: Effects of an extracellular metal chelator on neurovascular function in diabetic rats. *Diabetologia* 44:621–628, 2001
 77. Angulo J, Sánchez-Ferrer CF, Peiró C, Marín J, Rodríguez-Mañas L: Impairment of endothelium-dependent relaxation by increasing percentages of glycosylated human hemoglobin: possible mechanisms involved. *Hypertension* 28:583–592, 1996
 78. Valko MX JJ: Iron, atherosclerosis, and ischemic heart disease. *Arch Intern Med* 159:1542–1548, 1999
 79. Loebstein R, Lehotay DC, Luo X, Bartfay W, Tyler B, Sher GD: Diabetic nephropathy in hypertransfused patients with β -thalassemia: the role of oxidative stress. *Diabetes Care* 21:1306–1309, 1998
 80. Nankivell BJ, Tay YC, Boadle RA, Harris DC: Lysosomal iron accumulation in diabetic nephropathy. *Ren Fail* 16:367–381, 1994
 81. Cordain L, Brand-Miller J, Eaton S, Mann N, Speth J, Holt S: Plant-animal subsistence ratios and macronutrient energy estimations in world-wide hunter-gatherer diets. *Am J Clin Nutr* 71:682–692, 2000
 82. Mann N, Li D, Dudman N, Guo X, Elsworth C, Kelly F, Wilson A, Abedin L, Sinclair AJ: The effect of diet on plasma homocysteine concentration in healthy male subjects. *Eur J Clin Nutr* 53:895–899, 1999
 83. Rossetti L, Rothman DL, DeFronzo RA, Schulman GI: The effect of dietary protein on in vivo insulin action and liver glycogen repletion. *Am J Physiol* 257: E212–E219, 1989
 84. Mann N: Dietary lean red meat and human evolution. *Eur J Nutr* 39:71–79, 2000
 85. Fleming RE, Sly WS: Mechanisms of iron accumulation in hereditary hemochromatosis. *Annu Rev Physiol* 64:663–680, 2002