Dietary iron restriction or iron chelation protects from diabetes and loss of β-cell function in the obese (ob/ob lep−/−) mouse

Robert C. Cooksey,1,2* Deborah Jones,1* Scott Gabrielsen,1 Jingyu Huang,1 Judith A. Simcox,1 Bai Luo,1 Yudi Soesanto,1 Hugh Rienhoff,3,4 E. Dale Abel,1 and Donald A. McClain1,2

1Departments of Medicine and Biochemistry, University of Utah School of Medicine; 2Veterans Affairs Medical Center, Research Service, Salt Lake City, Utah; 3FerroKin BioSciences, San Carlos; and 4The Children’s Hospital Oakland Research Institute, Oakland, California

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Cooksey RC, Jones D, Gabrielsen S, Huang J, Simcox JA, Luo B, Soesanto Y, Rienhoff H, Abel ED, McClain DA. Dietary iron restriction or iron chelation protects from diabetes and loss of β-cell function in the obese (ob/ob lep−/−) mouse. Am J Physiol Endocrinol Metab 298: E1236–E1243, 2010. First published March 30, 2010; doi:10.1152/ajpendo.00220.2010.—Iron overload can cause insulin deficiency, but in some cases this may be insufficient to result in diabetes. We hypothesized that the protective effects of decreased iron would be more significant with increased β-cell demand and stress. Therefore, we treated the ob/ob mouse model of type 2 diabetes with an iron-restricted diet (35 mg/kg iron) or with an oral iron chelator. Control mice were fed normal chow containing 500 mg/kg iron. Neither treatment resulted in iron deficiency or anemia. The low-iron diet significantly ameliorated diabetes in the mice. The effect was long lasting and reversible. Ob/ob mice on the low-iron diet exhibited significant increases in insulin sensitivity and β-cell function, consistent with the phenotype in mouse models of hereditary iron overload. The effects were not accounted for by changes in weight or feeding behavior. Treatment with iron chelation had a more dramatic effect, allowing the ob/ob mice to maintain normal glucose tolerance for at least 10.5 wk despite no effect on weight. Although dietary iron restriction preserved β-cell function in ob/ob mice fed a high-fat diet, the effects on overall glucose levels were less apparent due to a loss of the beneficial effects of iron on insulin sensitivity. Beneficial effects of iron restriction were minimal in wild-type mice on normal chow but were apparent in mice on high-fat diets. We conclude that, even at “normal” levels, iron exerts detrimental effects on β-cell function that are reversible with dietary restriction or pharmacotherapy.

WE HAVE REPORTED PREVIOUSLY THAT ADULT HUMANS WITH IRON OVERLOAD FROM HEREDITARY HEMOCROMATOSIS (HH) HAVE A HIGH PREVALENCE OF DIABETES (22%) AND IMPAIRED GLUCOSE TOLERANCE (31%) (29). The impaired glucose tolerance of HH is associated with decreased insulin secretory capacity (29). A mouse model of HH with targeted deletion of the gene most commonly mutated in hemochromatosis, Hfe, shares the same phenotype (7). In humans, the impaired insulin secretory capacity is reversed with phlebotomy therapy (1).

Increased iron stores are also significantly associated with typical type 2 diabetes (reviewed in Ref. 10). In the National Health and Nutrition Examination Survey cohort, the odds ratios for newly diagnosed diabetes were 4.94 for men and 3.61 for women who had elevated serum ferritin levels compared with those with normal ferritin (14). A more recent study shows an even higher relative risk, 7.4-fold (15), comparable in magnitude with the risk engendered by obesity (25). The increased diabetes risk in the general population is not accounted for by hemochromatosis (13). Similar relationships between iron and diabetes risk are seen in several other populations, including Europeans, African-Americans, gestational diabetics, prediabetics, and individuals with transfusional iron overload (2, 22, 26, 34, 35, 37). Iron has similar associations with other aspects of the metabolic syndrome (16, 20, 21, 31).

Conversely, low iron stores are associated with a lower risk of diabetes. In relatively small and/or short-term studies of non-HH subjects, phlebotomy improved insulin sensitivity and glycemia in both nondiabetic subjects (8) and type 2 diabetics with high ferritin (11). These results are concordant with rat studies in which animals with iron deficiency anemia are more insulin sensitive than controls (5). Phlebotomy in iron-overloaded individuals also improves hypertriglyceridemia (4), vascular reactivity (12), and markers of nonalcoholic steatohepatitis (9).

The phenotype related to iron overload in HH differs from that caused by pure dietary iron excess. This is likely based upon the differing tissue distributions of iron in HH vs. dietary excess, which are based on the dysregulation of the iron channel ferroportin in HH (24, 36). Although HH is associated with decreased insulin secretory capacity, the insulin deficiency is compensated partially by increased insulin sensitivity both in humans (29) and the mouse model (7). However, iron overload in the general non-HH population is associated with insulin resistance and the metabolic syndrome. This suggests that iron may have complex and multiple effects on metabolism and diabetes risk. We have shown, for example, that in pancreatic β-cells excess iron impairs mitochondrial function and glucose-stimulated insulin secretion (7, 23, 29). High levels of iron in skeletal muscle, on the other hand, favor fatty acid oxidation through a mechanism dependent on AMP-activated protein kinase (AMPK) resulting in protection from obesity and insulin resistance when mice are fed a high-fat diet (Ref. 19 and Huang J and McClain D, unpublished observations). Finally, high levels of iron in adipose tissue downregulate serum adiponectin (Huang J and McClain D, unpublished observations), which would be predicted to increase diabetes risk. Thus, the integrated effect of iron on metabolism is mediated by complex tissue-specific effects that are both profound antidiabetic and that vary both with environmental (diet) and genetic influences.

* Both of these authors contributed equally to this work.

Address for reprint requests and other correspondence: D. A. McClain, Div. of Endocrinology, 30 N. 2030 East, Salt Lake City, UT 84132 (e-mail: Donald.mcclain@hsc.utah.edu).
We hypothesized that the effects of iron on β-cell function would be most evident when the demand on insulin secretion is greatest. For example, in an obese animal the risk of β-cell failure is high, and under these conditions the role of iron could be unmasked. Therefore, we used a mouse model of type 2 diabetes, the ob/ob (Lep−/−), exposed to a low-iron diet or iron chelators to modulate iron exposure. Consistent with our hypothesis, we find that this low-iron diet in the ob/ob mouse protects against the development of diabetes. Even more dramatic results are seen with pharmacological treatment using an iron chelator. The reduced levels of iron sufficient to induce protection do not result in anemia. The protective effect is reversible with a resumption of a normal iron diet. Finally, the effect of low iron is mediated by beneficial effects on both insulin secretion and insulin sensitivity.

**EXPERIMENTAL PROCEDURES**

**Animals.** Leptin-deficient ob/ob mice bred on the C57BL/6j genetic background were purchased from the Jackson Laboratory. Five diets were used during the course of this study. Mice were routinely maintained in our animal facility on high-carbohydrate normal chow with an iron content ranging from 350 to 600 mg/kg (8656; Harlan Teklad, Madison, WI). We also used defined high-carbohydrate diets with very low iron (4–6 mg/kg, TD 80396; Harlan Teklad), 35 mg/kg iron (TD TD94045; Harlan Teklad), and 500 mg/kg iron (TD04404; Harlan Teklad). The high-fat diet containing 45 kcal% as fat and 35–50 mg/kg iron (D12451; Research Diets) was supplemented with carboxyl iron to create a higher-iron, high-fat diet. Procedures were approved by the Institutional Animal Care and Use Committee of the University of Utah.

**Iron chelator treatment.** Mice were treated with FBSO701 (FerroKin BioSciences, San Carlos, CA), a magnesium salt of (S)-3′-(OH)-DADFT polyether iron chelator (3). Drug was dosed at 10 mg/kg, BioSciences, San Carlos, CA), a magnesium salt of (S)-3′-(OH)-DADFT polyether iron chelator (3). Drug was dosed at 10 mg/kg, provided once a day, mixed in 100 mg of peanut butter. Controls with very low iron (4–6 mg/kg, TD 80396; Harlan Teklad), 35 mg/kg iron (TD TD94045; Harlan Teklad), and 500 mg/kg iron (TD04404; Harlan Teklad). The high-fat diet containing 45 kcal% as fat and 35–50 mg/kg iron (D12451; Research Diets) was supplemented with carboxyl iron to create a higher-iron, high-fat diet. Procedures were approved by the Institutional Animal Care and Use Committee of the University of Utah.

**Intraperitoneal glucose tolerance testing and insulin determination in vivo.** Experimental animals were fasted for 6 h, after which time glucose (1 g/kg body wt) was administered intraperitoneally to non-seatedated animals. Tail vein blood (3 μl) was sampled for glucose determination (Glucomer Elite; Bayer, Tarrytown, NY) before and 5, 15, 30, 60, 90, and 120 min after glucose administration. Tail vein blood (50 μl) was also collected before and 30 min after the start of the intraperitoneal glucose tolerance test (IPGTT) for insulin determination using the Sensitive Rat Insulin Kit (Linco Research, St. Charles, MO). Homeostasis model assessments were calculated as described (28).

**Mitochondrial oxygen consumption.** Oxygen consumption in cardiac muscle mitochondria was measured using a fiber-optic oxygen sensor (Ocean Optics, Orlando, FL) (6). Cardiac muscle tissue was suspended in 2.0 ml of 120 mM KCl, 3 mM HEPES, 5 mM KH₂PO₄, 1 mM EGTA, and 1 mg/ml free fatty acid BSA, pH 7.2. Respiration was measured at 25°C in a sealed glass chamber. Oxygen consumption was measured with 5 mM glutamate plus 2 mM malate as substrates, in the basal state (state 2), following the addition of 1 mM ADP (state 3), and in the presence of 1 μM oligomycin to block ATP synthesis (state 4). Respiratory control ratios are the ratio of states 3 and 4.

**Indirect calorimetry.** Mice were studied for 3 consecutive days in a four-chamber-open circuit Oxymas system (Comprehensive Laboratory Animal Monitoring System; Columbus Instruments, Columbus, OH) to measure oxygen consumption and carbon dioxide production.

**Statistical procedures.** Descriptive statistics are represented as average ± SE. The Student t-test (2-tailed) was used to compare differences between groups. ANCOVA was used to test whether two regression lines represented independent populations.
glucose tolerance status, we investigated whether those differences in weight might account for their improved glucose tolerance. This was not the case (Fig. 1F). The average HOMA-IR value for the cohort of ob/ob mice on low iron within the weight range of 40–45 g (17.4 ± 2.3) differed significantly from that of the ob/ob mice on normal chow within that same weight range (142.5 ± 25.1, P < 0.0001). The correlation of HOMA-IR to weight was significant for the ob/ob mice on low iron (r² = 0.35, P < 0.01), whereas the correlation was not significant for the ob/ob mice on normal chow.

Improved serum triglyceride and fatty acid levels in ob/ob mice with dietary iron restriction. Consistent with the improved diabetic status of the ob/ob mice on lower iron diets, the mice on 35 mg/kg chow also exhibited a 40% decrease in serum triglycerides (P < 0.01) and a trend toward lower serum free fatty acid levels (20%, P = 0.13; Table 1).

**Table 1. Effects of dietary iron content on red blood cells and serum chemistries in ob/ob mice**

<table>
<thead>
<tr>
<th>Dietary Iron Content, mg/kg</th>
<th>2–6</th>
<th>35</th>
<th>500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit, %</td>
<td>47.7 ± 0.9*</td>
<td>54.6 ± 3.1</td>
<td>54.2 ± 1.3</td>
</tr>
<tr>
<td>Hemoglobin, g/dl</td>
<td>17.1 ± 0.5†</td>
<td>17.8 ± 0.6</td>
<td>19.0 ± 0.1</td>
</tr>
<tr>
<td>Mean corpuscular volume, fl</td>
<td>50.2 ± 0.17</td>
<td>51.0 ± 0.8</td>
<td>51.9 ± 0.4</td>
</tr>
<tr>
<td>Hepatic iron, mmol/mg protein</td>
<td>2.4 ± 0.5*</td>
<td>7.4 ± 0.8†</td>
<td>13.0 ± 1.8</td>
</tr>
<tr>
<td>Serum ferritin, mg/l</td>
<td>ND</td>
<td>0.90 ± 0.12†</td>
<td>1.02 ± 0.03</td>
</tr>
<tr>
<td>Serum adiponectin, mg/dl</td>
<td>ND</td>
<td>9.4 ± 1.0</td>
<td>8.6 ± 0.7</td>
</tr>
<tr>
<td>Serum triglycerides, mg/dl</td>
<td>ND</td>
<td>59 ± 6†</td>
<td>98 ± 4</td>
</tr>
<tr>
<td>Free fatty acids, mM</td>
<td>ND</td>
<td>57.6 ± 5.9</td>
<td>73.0 ± 8.1</td>
</tr>
<tr>
<td>C-peptide/insulin ratio</td>
<td>ND</td>
<td>1.75 ± 0.54</td>
<td>1.27 ± 0.46</td>
</tr>
</tbody>
</table>

Results are means ± SE; n = 6–8 mice/group, ND, not determined. At 30 days of age, mice were placed on normal chow (low fat) containing the indicated concentrations of iron. After 60–90 days, blood was taken for analysis. *P < 0.05 compared with 35 and 500 mg/kg diets; †P < 0.05 compared with 500 mg/kg diet.
switched from 500 to 35 mg/kg iron chow (group 1; Fig. 2A, □), their glucose tolerance status improved significantly within 7 days, and the protective effect persisted for ≥90 days. The mice on low iron that were switched to normal chow (group 2; Fig. 2A, △) had a somewhat slower worsening of their glucose tolerance status, becoming less glucose tolerant than group 1 17 days after the switch [P = not significant (NS)] and significantly worse at 120 days. At the termination of the study, the AUCG values for group 1 on low iron did not differ significantly from the AUCG value of group 2 measured at 30 days when that group had also been on low iron and vice versa.

The AUCG values at the end of the study are shown in Fig. 2B, compared with wild-type mice on the two diets. Wild-type C57BL6 mice on low iron showed a modest (16%) but not statistically significant trend toward improved glucose tolerance. Consistent with the data in Fig. 1, ob/ob mice on low iron weighed slightly less that those on normal chow, but both groups of ob/ob mice weighed significantly more than the wild types (Fig. 2C).

Verification that the effects of the different diets are attributable only to iron content. Because the above diets might have differed in other trace metals and in the relative amounts of carbohydrate derived from cornstarch vs. other sources, we sought to verify that the effects of the diets on insulin sensitivity could be isolated to iron. Therefore, we ground the low-iron diet (35 mg/kg iron) and added to it powdered carbonyl iron to match it to the normal chow iron content of 500 mg/kg. The mice on the higher-iron diet had significantly worse glucose tolerance after 3 (data not shown) and 6 wk on the diets (Fig. 3A). The AUCG was 31% higher in the high-iron group (P = 0.02). The weights of the mice did not differ on these two diets (Fig. 3B), but their weights were significantly lower than the mice on the normal pellet chow for an equivalent time (see Fig. 2), suggesting that the mice did not eat as much of the powdered food. Consistent with this, the AUCG for the low-iron group were as low as the mice on the 35 mg/kg iron pellet chow (Fig. 1), whereas the mice on the higher-iron

Fig. 2. Reversibility and duration of effects of low iron on glucose tolerance. Ob/ob mice aged 4 wk had glucose tolerance testing performed, after which they were randomized to either the low-iron or nl chow diets. After retesting 4 wk later, the diets of the 2 groups were reversed, with repeat glucose tolerance testing performed 7, 17, and 90 days after the switch. A: areas under the glucose curve (AUCG) for the 2 groups over time (n = 5–12/group, *P < 0.05 for the difference between the 2 groups at the indicated times). B: The AUCG values for the groups at the end of the study compared with wild-type mice of the same age on the 2 diets (*P < 0.01 for the low-iron group compared with the same strain on nl chow). C: weights of the mice at the end of the study (*P < 0.01 for the low-iron group compared with the same strain on nl chow).

Fig. 3. Mice on powdered diets containing 35 mg/kg iron exhibit better glucose tolerance than mice fed the same powdered diet supplemented with carbonyl iron to 500 mg/kg. Ob/ob mice (10/group) were placed on the powdered diet containing 35 mg/kg iron, with or without added carbonyl (elemental) iron, to total 500 mg/kg. Glucose tolerance testing was performed at 6 wk (A), at which time the mice were also weighed (B). Individual glucose values are significantly different at 30, 60, and 120 min.
powdered food had lower glucose excursions than the cohort on higher iron (normal) pellet chow (see Fig. 2).

Insulin secretion does not fail in the face of obesity in mice on the low-iron diet. The data in Fig. 1 demonstrate a significant effect of the low-iron diet on both the insulin resistance and β-cell function indices HOMA-IR and -B. Our earlier data in a mouse model of hemochromatosis had also demonstrated that excess iron in β-cells resulted in oxidant stress and loss of insulin secretory capacity (23). Therefore, we investigated further the relationship between β-cell function and glucose tolerance over time. Ob/ob mice exhibited a significant increase in insulin levels 30 min after intraperitoneal glucose challenge between the ages of 30 and 45–60 days, consistent with their increasing weight and insulin resistance (Fig. 4, left solid line). However, as their diabetes status worsened between 60 and 90 days, insulin levels during the IPGTT declined. Also declining was the ratio of this insulin level to the AUCG (the insulin/AUC value is multiplied by 100 to facilitate visualization on the same graph; Fig. 4, left dotted line), an indication of their increasing inability to fully compensate for insulin resistance. In the ob/ob mice on low iron, in contrast, in the period between starting the low-iron diet on 30 and 45–60 days, insulin levels increased only slightly in the face of markedly improved glucose tolerance (see Figs. 1 and 3), resulting in a significant increase in the ratio of insulin during the IPGTT to the AUCG. These results are consistent with the decreased HOMA-IR and increased HOMA-B levels documented in Fig. 1. In the low-iron group, nearly normal glucose tolerance was maintained in the interval from 60 to 90 days (see Fig. 3), but at the expense of increased insulin secretion (Fig. 4, right solid line, as the animals aged and increased in weight. Thus the ratio of insulin to AUCG increased markedly (Fig. 4, right dotted line), demonstrating an improved capacity to maintain insulin secretion in the face of increased metabolic demand, obesity, and insulin resistance compared with the mice on normal chow.

Iron chelation also protects from diabetes and preserves β-cell function in ob/ob mice. As an independent means of assessing the effects of iron on diabetes in ob/ob mice, we treated them with an oral iron chelator at a dose of 10 mg/day for ≤10.5 wk, with glucose tolerance testing performed at 3- to 4-wk intervals. AUCG trended downward after 3 wk of therapy and was decreased by 67% at 6.5 wk (P = 0.0001; Fig. 5A), with the effect being maintained at 10.5 wk (57% decrease, P < 0.001). Similar effects were seen with fasting glucose values, and in fact, the levels of fasting glucose in the treated mice were not in the diabetic range (all P < 0.05; Fig. 5B). Weights did not differ between the control and chelator-treated animals (Fig. 5C). Insulin levels were assessed at 6.5 wk and were significantly lower in the chelator-treated mice (P < 0.05; Fig. 5D). The low fasting glucose values precluded HOMA-B calculations, but as with the cohort restricted in dietary iron (Fig. 4), the chelator-treated group exhibited an improved ratio of insulin to the AUCG (P = 0.03; Fig. 5E). HOMA-IR values were also significantly lower in the treated group (Fig. 5F). No differences were noted in the red blood cell counts or red cell indices (not shown), and in fact the chelated group trended toward having higher hematocrits (50.4 ± 3.6 vs. 42.4 ± 2.5%, P = NS). In calorimetry cages, we documented that chelator-treated mice ate as much as the control mice and had higher rates of oxygen consumption, carbon dioxide production, and heat generation, with no change in the respiratory exchange ratio (Fig. 5G). Mitochondrial function was assessed in cardiac muscle, because the measurements in islets would require prohibitive numbers of mice. Oxygen consumption was similar between the two groups, although the chelator-treated mice had a significant increase in ATP production (66%, P < 0.05; Fig. 5H) and in the ratio of ATP production to oxygen consumption (49%, P < 0.05).

**Diminished protective effect of low iron in ob/ob mice on a high-fat diet.** Low iron also protected β-cell function in ob/ob mice on a high-fat diet (HOMA-B, P < 0.001; Table 2) to a degree similar to the mice on normal chow (Fig. 1). Because higher tissue iron levels also favor fatty acid oxidation and protect from high-fat-induced glucose intolerance (Huang J and McClain D, unpublished observations), we also predicted that the beneficial effects of low iron on insulin sensitivity and overall glucose disposal would not be seen to the same degree on high fat. Ob/ob mice on high fat exhibited a trend toward increased insulin resistance on the lower-iron (35 mg/kg) diet (P = NS), in contrast to the mice on the normal, high-carbohydrate chow containing 500 mg/kg iron (Fig. 1). Consistent with the counterbalancing effects of low iron on improving β-cell function but worsening insulin resistance, there was no significant difference in overall glucose tolerance between the groups, as shown by the AUCG. Weights also did not differ between the ob/ob mice on high fat/low iron compared with high fat/normal iron (Table 2).

Low iron improves glucose tolerance in wild-type C57BL6 mice on normal but not high-fat chow and protects β-cell function on high-fat chow. On the basis of the above results, we hypothesized that the effect of the lower-iron diet would be less evident in mice not stressed by excessive caloric intake and then only on normal (low-fat, high-carbohydrate) chow. Mice on normal chow with lower iron content (35 mg/kg) exhibited a modest 20% reduction in area under the glucose curve compared with normal chow with 350 mg/kg iron (P < 0.05; Table 2). This occurred despite a trend (13%, P = NS) toward increased weight. Neither HOMA-IR, HOMA-B, se-

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**Fig. 4.** Maintenance of insulin secretion in the face of obesity in the ob/ob mice on low-iron chow. From the intraperitoneal glucose tolerance test studies described in Fig. 3, the insulin level 30 min after glucose challenge and the AUCG values were calculated for mice on 500 mg/kg iron chow (●) and mice on reduced (35 mg/kg) iron chow (□). Insulin levels (solid lines) are presented as ng/ml. The AUCG values were g·min⁻¹·dl over the 120-min study. The ratios of insulin to AUCG (dashed lines) were multiplied by 100 to allow visualization on the same graphing scale. Shown are means ± SE; n = 5–12/group. For the insulin values, error bars are within the data points and thus not visible. *P < 0.001 for low-iron group vs. normal chow; ‡P < 0.01 within groups for change from previous value.
rum triglycerides, nor free fatty acids (not shown) differed in these two groups of mice. On a high-fat diet, the effect of iron on glucose tolerance was the opposite of that seen in mice on the high-carbohydrate chow. On high-fat and higher-iron chow, mice had a trend toward lower weight and improved glucose tolerance, consistent with data in other models of iron overload (Huang J and McClain D, unpublished observations). However, despite the improved glucose tolerance in the mice on higher-iron, high-fat chow, HOMA-B levels were 55% lower than in mice on the lower-iron chow (P < 0.01) and with insulin content per islet decreased by 37% (P < 0.01).

**DISCUSSION**

We have presented data demonstrating that decreasing iron levels through either dietary iron restriction or systemic iron chelation therapy dramatically improves the glycemia of the diabetes-prone, leptin-deficient (ob/ob) mouse. This is associated with improvements in both β-cell function and insulin sensitivity. The effects are stable over time and reversible, and they occur at levels of iron that are sufficient to maintain normal hematopoiesis. The effects are not explained by weight; for example, the ob/ob mice treated with an iron chelator exhibited normal glucose tolerance despite significant obesity that did not differ from untreated control ob/ob mice.

We have reported previously that iron impairs β-cell function at least partially by inducing oxidant stress and impairing mitochondrial function (7, 23). Maintenance of β-cell function is likely a factor in the current results, as evidenced by the fact that mice with lower iron regulate insulin levels more appropriately as a function of glycemia. We have made use of both homeostasis model (HOMA-B) calculations and ratios of stimulated insulin to glucose levels measured as the AUCG. Although neither of these values is completely validated as a measurement of β-cell function in mice, they provide an overall index of insulin responses to glucose levels, and they agree with assessments of islet insulin content (Table 2). Our results are consistent with the results in mouse models of hemochromatosis wherein β-cell function was analyzed by perifusion and immunohistochemistry, revealing both loss of...
normal chow (low fat) or high-fat chow containing the indicated concentrations of iron. After 45 days the mice were weighed, and glucose tolerance testing (Table 2). This is likely related to AMPK- and adiponectin-action is less effective in protecting mice on high-fat diets reported herein is demonstrated by the fact that iron restriction of oxygen consumption to ATP production. The precise mechanism underlying this mitochondrial effect of chelator therapy is under investigation.

The effects of iron as a prooxidant are not likely to be the entire explanation of its effects on insulin secretion. Iron is not simply a toxin but also plays a key role in physiological regulation of metabolism. In yeast, for example, iron is a key regulator of fuel choice and the AMPK-mediated response to glucose exhaustion (18). In multicellular organisms, iron and heme are involved in multiple processes, including signaling circadian rhythmicity (38), transcriptional regulation (30), translational regulation (17), and hypoxia responses (33), to name but a few. Likewise, “oxidant stress” itself plays a role beyond toxicity in physiological and adaptive signaling (27).

As further evidence of the role in physiological regulation of metabolism, groups including ours have shown that iron affects metabolism in a wide-reaching fashion well beyond the scope of preserving insulin secretory capacity. We have shown that iron affects insulin sensitivity through complex mechanisms involving AMPK-mediated skeletal muscle glucose uptake, hepatic glucose cycling, and adipokine production (Ref. 19 and Huang J and McClain D, unpublished observations). Specifically, iron promotes fatty acid oxidation such that high-iron mice are hypermetabolic at the expense of glucose oxidation (Huang J and McClain D, unpublished observations) and possibly other interactions of fat and iron metabolism (32). The effect of iron on altering triglyceride and fatty acid metabolism likely plays a role in the insulin-sensitizing effects of low iron, but it is likely not the sole mechanism because of the lack of significant effects of iron on these parameters in wild-type C57 mice. In summary, iron exerts pleiotropic effects across several tissues. In all models, low iron promotes β-cell function, but iron’s promotion of fatty acid oxidation translates to effects on obesity and metabolism that vary with diet. These effects are seen in both wild-type and ob/ob mice (Table 2), suggesting that the effects are not specific to that genetic model and not leptin dependent.

These results have significant clinical implications for human type 2 diabetes. As reviewed briefly in the introduction, increased iron stores are a significant risk factor for type 2 diabetes (2, 10, 14, 15, 22, 26, 34, 35, 37) and other aspects of the metabolic syndrome (16, 20, 21, 31). The clinical manipulation of iron stores, including by pharmacological means, may influence the natural history of diabetes and provide additional tools for the clinical management of the disease. Importantly, the chelator-treated mice described in this report maintained normal hematopoiesis and serum ferritin levels similar to untreated controls. These results have significant clinical implications for human type 2 diabetes. As reviewed briefly in the introduction, increased iron stores are a significant risk factor for type 2 diabetes (2, 10, 14, 15, 22, 26, 34, 35, 37) and other aspects of the metabolic syndrome (16, 20, 21, 31). The clinical manipulation of iron stores, including by pharmacological means, may influence the natural history of diabetes and provide additional tools for the clinical management of the disease. Importantly, the chelator-treated mice described in this report maintained normal hematopoiesis and serum ferritin levels similar to untreated controls. These data suggest that similar effects might be observed in patients with high “normal” iron stores as reflected by serum ferritin. Phlebotomy of research subjects with high-normal serum ferritin in the early stages of diabetes to reduce total body iron stores is underway to test this hypothesis.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).
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