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, Deborah Jones, Scott Gabrielsen, Jingyu Huang, Judith A. Simcox, Bai Luo, Yudi Soesanto, Hugh Rienhoff, E. Dale Abel, and Donald A. McClain

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

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We have previously reported that adult humans with iron overload from hereditary hemochromatosis (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 and antidiabetic and that vary both with environmental (diet) and genetic influences.
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 chelation. 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 FBS0701 (FerroKin BioSciences, San Carlos, CA), a magnesium salt of (S)-3´-(OH)-DPDT polyether iron chelator (3). Drug was dosed at 10 mg/kg, provided once a day, mixed in 100 mg of peanut butter. Controls received peanut butter alone.

**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-steadated animals. Tail vein blood (3 μl) was sampled for glucose determination (Glucometer 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 chamor. 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 μg/ml 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.

**RESULTS**

Prevention of diabetes in ob/ob mice by a low-iron diet, independent of changes in weight. We fed ob/ob mice chow containing either 35 or 500 mg/kg iron, the latter being “normal” chow. Ob/ob mice were fed normal chow containing 500 mg/kg iron until 4–6 wk of age, at which time they were either maintained on that diet or given a chow containing 35 mg/kg iron. At the time of the change in diet, most of the mice had already developed diabetes. At the time of initiation of these diets, the mice were given an IPGTT. They were distributed into the test groups such that the areas under the glucose curve (AUCG) were equivalent between the groups at the start of the test period. After 4 wk, the IPGTT was repeated. The glucose tolerance of the group on normal chow (95% carbohydrate and 500 mg/kg iron) had worsened significantly, whereas the group on the 35 mg/kg iron diet (also 95% carbohydrate) had normal glucose tolerance, better than at the beginning of the study and equivalent to the wild-type background strain, C57BL6 (Fig. 1A). The glucose values at each time point (Fig. 1A) and the AUCG (not shown) were all significantly different between the pretest results and after 1 mo of either normal chow (higher glucose values and AUCG; P < 0.0001) or low-iron chow (lower glucose and AUCG; P < 0.0001). Fasting hyperinsulinemia was seen in all of the ob/ob mice compared with the wild-type C57BL6 mice, but whereas the ob/ob mice on normal chow approximately tripled their insulin levels from 30 to 60 days, the mice on low iron maintained the same insulin levels as at the start of the study (Fig. 1B). The results in Fig. 1, A and B, were reflected in significantly improved insulin resistance indices in the low-iron diet group, as assessed by the homeostasis model of insulin resistance (HOMA-IR), compared with both ob/ob mice on normal chow and their own levels at the start of the low-iron diet (Fig. 1C). After 30 days on the 500 mg/kg normal iron chow, the insulin resistance had resulted in a compensatory increase in insulin secretion measured as an increase in β-cell function assessed by the homeostasis model (HOMA-B) (P < 0.01; Fig. 1D). HOMA-B was even greater in the ob/ob mice on low iron (P < 0.01 compared with normal chow, P < 0.0001 compared with prediet).

We also assessed the ability of a diet even lower in iron (2–6 mg/kg) to protect ob/ob mice from diabetes. On this diet, the AUCG during IPGTT was significantly better than in the mice on normal chow but was slightly inferior to that observed on the 35 mg/kg diet (AUCG 35 ± 9% higher on the very low-iron diet compared with 35 mg/kg, P < 0.05; data not shown). In addition, the mice on the very low-iron diet became mildly anemic (Table 1), so the very low-iron diet was not studied further. Importantly, however, mice on the 35 mg/kg iron diet did not exhibit significant changes in hematocrit or red blood cell indices compared with mice on the 500 mg/kg iron diet (Table 1). The mice on the 35 mg/kg diet did exhibit a 43% decrease in hepatic iron (P < 0.05) and a 12% decrease in serum ferritin (P < 0.05) compared with mice on the 500 mg/kg diet (Table 1). The effects of iron on glucose tolerance are not attributable to changes in insulin clearance, since the ratio of C-peptide to insulin did not change as a function of iron (Table 1).

In this study, the ob/ob mice on low iron gained slightly less weight than the mice on normal chow, but they remained significantly obese compared with the wild-type C57BL6 mice (Fig. 1E). Because body weight is a major determinant of
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 was 0.35, but there was no such relationship for the ob/ob mice on higher iron. Shown are means ± SE; n = 12–20/group. In A, all glucose values differ significantly among the 3 groups of ob/ob mice; P < 0.001. In B and C, *P < 0.01 compared with prediet levels in the ob/ob mice on normal chow; †P < 0.01 compared with ob/ob mice on normal chow.

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>
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<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 adipocytokine</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 than 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 was as low as the mice on the 35 mg/kg iron pellet chow (Fig. 1), whereas the mice on the higher-iron
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 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-

![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 (C) 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·d⁻¹ 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.](http://ajpendo.physiology.org/10.1152/ajpendo.00448.2009)
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, \( \text{HOMA-B} \) 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 \( \text{ob/ob} \) mouse. This is associated with improvements in both \( \beta \)-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 \( \text{ob/ob} \) mice treated with an iron chelator exhibited normal glucose tolerance despite significant obesity that did not differ from untreated control \( \text{ob/ob} \) mice.

We have reported previously that iron impairs \( \beta \)-cell function at least partially by inducing oxidant stress and impairing mitochondrial function \((7, 23)\). Maintenance of \( \beta \)-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 the AUC\(_G\) to assess \( \beta \)-cell function. Low fasting glucose values in the treated mice precluded the obtaining of meaningful HOMA-B measurements. We have used both homeostasis model (HOMA-B) calculations and ratios of stimulated insulin to the AUC\(_G\) to assess \( \beta \)-cell function. Low fasting glucose values in the treated mice precluded the obtaining of meaningful HOMA-B measurements.
was performed. 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-dependent effects of iron in promoting fatty acid oxidation 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 protects 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.

Table 2. Effect of dietary iron content on glucose tolerance in ob/ob or wild-type C57BL6 mice eating normal or high-fat chow

<table>
<thead>
<tr>
<th></th>
<th>Normal Chow, mg/kg iron</th>
<th>High-Fat Chow, mg/kg iron</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>35</td>
<td>350</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35</td>
</tr>
<tr>
<td><strong>Ob/ob</strong></td>
<td></td>
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</tr>
<tr>
<td>HOMA-B</td>
<td>808 ± 71</td>
<td>567 ± 38*</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>77.0 ± 4.9</td>
<td>68.0 ± 11.1</td>
</tr>
<tr>
<td>AUCO, g·min⁻¹·dl</td>
<td>37.1 ± 2.8</td>
<td>40.0 ± 1.9</td>
</tr>
<tr>
<td>Weight, g</td>
<td>57.4 ± 1.2</td>
<td>58.7 ± 1.7</td>
</tr>
<tr>
<td><strong>C57BL6</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA-B</td>
<td>76.0 ± 4.8</td>
<td>67.4 ± 3.6</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.9 ± 0.5</td>
<td>2.9 ± 0.4</td>
</tr>
<tr>
<td>AUCO, g·min⁻¹·dl</td>
<td>27.3 ± 1.4</td>
<td>34.2 ± 2.2*</td>
</tr>
<tr>
<td>Weight, g</td>
<td>28.9 ± 0.4</td>
<td>25.5 ± 1.6</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>38.4 ± 1.9</td>
<td>42.8 ± 1.8</td>
</tr>
<tr>
<td>Islet insulin, mg/islet</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Results are means ± SE; n = 10 (ob/ob) or 5 (C57BL6) mice/group. HOMA-B, homeostasis model assessment of β-cell function; HOMA-IR, homeostasis model assessment of insulin resistance; AUCO, area under the glucose curve. At 30 days of age, mice were placed on the indicated chows: 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 was performed. *P < 0.05 for 35 compared with 350 mg/kg chow within types of chow. †P < 0.05 for high-fat compared with normal chow of the same iron content.

β-cell mass and desensitization of glucose-stimulated insulin secretion with iron overload (23).

Mitochondrial dysfunction was prominent in the hemochromatosis model of iron overload, and consistent with those results, iron chelation significantly increased ATP production in mitochondria of the ob/ob mice on the higher-iron chow. The mechanism could not be attributed to an overall increase in oxygen consumption capacity but rather an improved coupling 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 of iron 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 on high-fat but not normal chow, resulting in relative protection from obesity and obesity-induced insulin resistance. That such effects of iron are operative in the results reported herein is demonstrated by the fact that iron restriction is less effective in protecting mice on high-fat diets (Table 2). This is likely related to AMPK- and adiponectin-dependent effects of iron on promoting fatty acid oxidation 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 protects 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 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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