Elevated reactive oxygen species (ROS) are linked to insulin resistance and islet dysfunction. Manganese superoxide dismutase (SOD2) is a primary defense against mitochondrial oxidative stress. To test the hypothesis that heterozygous SOD2 deletion impairs glucose-stimulated insulin secretion (GSIS) and insulin action, wild-type (sod2+/+) and heterozygous knockout mice (sod2+/-) were fed a chow or high-fat (HF) diet, which accelerates ROS production. Hyperglycemic (HG) and hyperinsulinemic-euglycemic (HI) clamps were performed to assess GSIS and insulin action in vivo. GSIS during HG clamps was equal in chow-fed sod2+/- and sod2+/+ but was markedly decreased in HF-fed sod2+/-.

Remarkably, this impairment was not paralleled by reduced HG glucose infusion rate (GIR). Decreased GSIS in HF-fed sod2+/- was associated with increased ROS, such as superoxide ion. Surprisingly, insulin action determined by HI clamps did not differ between sod2+/- and sod2+/+ of either diet. Since insulin action was unaffected, we hypothesized that the unchanged HG GIR in HF-fed sod2+/- was due to increased glucose effectiveness. Increased GLUT-1, hexokinase II, and phospho-AMPK protein in muscle of HF-fed sod2+/- support this hypothesis.

We conclude that heterozygous SOD2 deletion in mice, a model that mimics SOD2 changes observed in diabetic humans, impairs GSIS in HF-fed mice without affecting insulin action.
sensing signals that disrupt β-cell function (9), the role of O$_2^-$ and SOD2 in β-cell dysfunction is unknown.

The role of SOD2 in the regulation of muscle insulin sensitivity also remains a point of contention. Both mitochondrial O$_2^-$ and H$_2$O$_2$ serve as metabolic sensors functionally linking the mitochondrial redox state of a cell to insulin sensitivity (1,2). There is some debate as to whether increased SOD2 expression has beneficial or deleterious effects on muscle insulin sensitivity since SOD2 decreases O$_2^-$ but increases H$_2$O$_2$. Reduced O$_2^-$ has been shown to improve insulin action in muscle (1), and elevated H$_2$O$_2$ links excess fat intake to insulin resistance (2).

Mice with a heterozygous knockout of SOD2 (sod2$^{-/-}$), a model that mimics SOD2 changes observed in diabetic humans (10), were used to study the role of this key mitochondrial O$_2^-$ scavenging enzyme in insulin secretion and insulin sensitivity. Complete SOD2 knockout in mice is lethal (11,12). In the current study, the hypothesis that sod2$^{-/-}$ mice have impaired GSIS and insulin action was tested in vivo. For this purpose validated hyperglycemic (HG) and hyperinsulinemic-euglycemic (HI) clamps coupled with isotopic tracer techniques were used to assess insulin secretion and action in conscious, unrestrained stress-free mice.

**RESEARCH DESIGN AND METHODS**

**Mouse Models**

Wild-type (sod2$^{+/+}$) and sod2$^{-/-}$ C57BL/6J mice were fed chow (LabDiet 5001; www.labdiet.com/Products/StandardDiets/index.html) or a high-fat (HF) diet (F3282, Bio-Serv; www.bio-serv.com/product/HFPellets.html) for 16 weeks. The calorie breakdown was 29% protein, 13% fat, and 58% carbohydrate for the chow diet and 15% protein, 59% fat, and 26% carbohydrate for the HF diet. Noticeably, the HF diet is also a low-protein, low-carbohydrate diet compared with the chow diet. Body composition was determined by nuclear magnetic resonance. Vanderbilt and East Carolina Mouse Models and Insulin Secretion and Action Research Committees approved all animal procedures.

**In Vivo HG and HI Clamp Protocol**

Mice had catheters implanted in the left carotid artery and the right jugular vein for blood sampling and intravenous infusion, respectively, 5 days prior to the study (13,14). HG and HI clamps were performed on 5-h fasted mice (13). The clamp procedure used in these studies is unique in that it has been validated from a number of standpoints, including stability of blood glucose over time, absence of stress, insulin measurements, and absence of a hematocrit fall (13). For HG clamps, arterial glucose was clamped at 250 mg/dL using a variable glucose infusion rate (GIR). GIR is an index of glucose tolerance during the HG clamp. Plasma insulin and C-peptide were determined during clamps. For HI clamps [3-3H]glucose was primed (2.4 μCi) and continuously infused (basal 0.04 μCi/min; clamp 0.12 μCi/min) into the jugular vein catheter. At time 0, infusion of insulin (4 mU/kg/min) was started and continued for 155 min. Blood was sampled at 80–120 min for the determination of plasma [3-3H]glucose specific activity. HI clamp insulin was measured at t = 100 and 120 min. At 120 min, 2[14C]deoxyglucose (13 μCi) was administered intravenously. Blood samples were taken thereafter for plasma 2[14C]deoxyglucose measurements. After the last sample, mice were anesthetized and tissues were excised and freeze clamped.

**Plasma and Tissue Sample Processing**

Plasma insulin and C-peptide were determined by ELISA or radioimmunoassay (Millipore). Glycogen was measured in gastrocnemius and liver extracts (15). 3H and 14C radioactivity was determined by liquid scintillation counting (16). Glucose appearance (Ra) and disappearance (Rd) rates were calculated using non-steady-state equations (17). Endogenous (primarily hepatic) glucose appearance (endoRa) was determined by subtracting the GIR from total Ra. The glucose metabolic index (Rg) was calculated as previously described (18).

**Ex Vivo Islet Perfusion and Static Incubation**

**Islet Isolation, Function Assay, and Islet Area Measurement**

We detected no sex effects in vivo, thus islets were isolated only from male mice for the ex vivo measurements. Mouse islets were isolated as described (19) and cultured in RPMI 1640 containing 10% FBS and 5 mmol/L glucose at 37°C overnight. GSIS was assessed by perifusion and static culture using size-matched islets and normalized to islet equivalents. Two secretagogues, glucose (16.7 mmol/L) and 3-isobutyl-1-methylxanthine (IBMX; 100 μmol/L) were used during perifusion. For static GSIS, islets were cultured in 3 mmol/L glucose for 2 h and then 20 mmol/L glucose for an additional 2 h. Insulin in the culture medium was determined by ELISA. Islet size was assessed with MetaMorph version 7.7 (Universal Imaging) on 324–926 islets/genotype (20).

**Quantitative RT-PCR**

RNA was extracted from isolated islets (Ambion). RNA integrity number was >8. Quantitative PCR was performed using TaqMan primers/probes (Applied Biosystems). Data were normalized to 18s, and relative changes in mRNA expression were calculated by the comparative ΔCt method (19,20). Minimum Information for Publication of Quantitative Real-Time PCR Experiments guidelines was followed for quantitative RT-PCR experiments (21).

**Western Blotting**

SOD2 expression in muscle was measured in mitochondria isolated from gastrocnemius (Imgenex). SOD2 expression in islets was measured in isolated islet extracts. Akt, GLUT-1, hexokinase II, and AMPK were measured in gastrocnemius homogenate as described previously (22). The antibodies used were SOD2 antibody (Assay Designs),
RESULTS

**Basal Characteristics of the sod2<sup>−/−</sup> Mice**

Chow-fed sod2<sup>+/+</sup> and sod2<sup>−/−</sup> mice had comparable body weight, percent body fat, and basal 5-h fasting arterial glucose and insulin (Table 1). HF feeding increased body weight, percent body fat, and arterial glucose and insulin equally in both genotypes. Arterial nonesterified fatty acid was not affected by genotype or diet (Table 1).

Deletion of one SOD2 allele resulted in a 50% reduction in SOD2 protein in the islets (Fig. 1A) and a 60% reduction in muscle mitochondria (Fig. 1B). HF feeding had no effects on SOD2 protein in islets or muscle (Fig. 1A and B). Consistent with protein measurements, SOD2 activity in muscle mitochondria was decreased by 60% in sod2<sup>−/−</sup> mice relative to sod2<sup>+/+</sup> mice (Fig. 1C).

**In Vivo Insulin Secretion (HG Clamp)**

HG and HI clamps were performed in both male and female mice. No sex effect was detected. Therefore, data from both sexes were combined. Arterial glucose was 250 mg/dL during the clamp (Fig. 2A). GIR was slightly higher in the chow-fed mice compared with the HF-fed mice, but genotype had no effect on GIR (Fig. 2B). In the chow-fed mice, arterial insulin and C-peptide was comparable between genotypes (Fig. 2C and D). However, both insulin and C-peptide were markedly decreased in the HF-fed sod2<sup>−/−</sup> compared with HF-fed sod2<sup>+/+</sup>, reflecting a marked reduction in insulin secretion (Fig. 2E and F).

**Ex Vivo Insulin Secretion (Isolated Islets)**

The in vivo findings in GSIS were recapitulated in isolated islets ex vivo. Islets isolated from chow-fed sod2<sup>+/+</sup> and sod2<sup>−/−</sup> mice had identical insulin peaks in response to glucose and IBMX (Fig. 3A). Insulin peak response to glucose in the HF-fed sod2<sup>−/−</sup> mice was smaller compared with that in the HF-fed sod2<sup>+/+</sup> mice during the islet perfusion (Fig. 3B). The quantification of the area under the peak showed a 30% reduction in sod2<sup>−/−</sup> mice (Fig. 3C). Impaired insulin secretion in HF-fed sod2<sup>−/−</sup> mice was specific to glucose stimulation, as IBMX-stimulated insulin secretion was identical in HF-fed sod2<sup>+/+</sup> and sod2<sup>−/−</sup> (Fig. 3D). To confirm the differences seen with islet perfusion, insulin secretion during static incubation of islets with glucose was assessed. Since differences in GSIS were only observed in the HF-fed mice, static incubation of

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**Table 1** — Basal (5-h fasted) characteristics of sod2<sup>−/−</sup> mice

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Chow sod2&lt;sup&gt;+/+&lt;/sup&gt;</th>
<th>Chow sod2&lt;sup&gt;−/−&lt;/sup&gt;</th>
<th>HF sod2&lt;sup&gt;+/+&lt;/sup&gt;</th>
<th>HF sod2&lt;sup&gt;−/−&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>25 ± 1</td>
<td>24 ± 1</td>
<td>34 ± 1†</td>
<td>35 ± 1†</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>9 ± 1</td>
<td>8 ± 0.4</td>
<td>30 ± 1†</td>
<td>28 ± 2†</td>
</tr>
<tr>
<td>Arterial glucose (mg·dL&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>123 ± 3</td>
<td>131 ± 4</td>
<td>152 ± 6†</td>
<td>155 ± 6†</td>
</tr>
<tr>
<td>Insulin (ng·mL&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>0.7 ± 0.1</td>
<td>0.9 ± 0.2</td>
<td>2.0 ± 0.2†</td>
<td>2.4 ± 0.3†</td>
</tr>
<tr>
<td>Nonesterified fatty acid (mmol/L)</td>
<td>0.72 ± 0.04</td>
<td>0.75 ± 0.07</td>
<td>0.75 ± 0.06</td>
<td>0.67 ± 0.03</td>
</tr>
</tbody>
</table>

All data are expressed as mean ± SEM. n = 9–11, †P < 0.05 compared with chow with the same genotype.
islets was performed in the HF-fed mice. Insulin did not differ between HF-fed sod2<sup>+/+</sup> and sod2<sup>+/−</sup> mice at 3 mmol/L glucose, but was decreased in the islets of HF-fed sod2<sup>+/−</sup> mice at 20 mmol/L glucose compared with sod2<sup>+/+</sup> mice (Fig. 3E).

Reduction in GSIS in isolated islets of HF-fed sod2<sup>+/−</sup> mice was associated with increased ROS levels determined by the ROS indicator dichlorofluorescein diacetate (Fig. 4A). Protein carbonyl groups and TBARS were also higher in the islets from HF-fed sod2<sup>+/−</sup> mice (Fig. 4B and C). Superoxide concentration determined by DHE staining was increased by HF feeding and heterozygous deletion of SOD2 independently (Fig. 4D). The reduction in GSIS was not associated with changes in gene expression of proteins involved in the insulin secretory pathways (Fig. 4E). Furthermore, impaired GSIS in the HF-fed sod2<sup>+/−</sup> mice was not associated with changes in islet size and areas (Fig. 4F).

**In Vivo Insulin Action (HI Clamp)**

Since the HG GIR was the same between HF-fed sod2<sup>+/+</sup> and sod2<sup>+/−</sup> mice despite the marked reduction in arterial insulin in the sod2<sup>+/−</sup> mice, we tested whether insulin action might be increased in these mice. The HI clamp was used to assess insulin action in vivo. Arterial glucose was 150 mg/dL during the HI clamp in all groups (Fig. 5A). Infusion of insulin increased arterial insulin to a similar extent in all mice (Chow sod2<sup>+/+</sup> 2.4 ± 0.3 ng/mL; chow sod2<sup>+/−</sup> 3.0 ± 0.5 ng/mL; HF sod2<sup>+/+</sup> 3.0 ± 0.2 ng/mL; HF sod2<sup>+/−</sup> 3.1 ± 0.2 ng/mL). GIR was lower in HF-fed mice relative to chow-fed mice as expected, but genotype did not affect GIR (Fig. 5B). EndoRa was not different between groups in the basal or insulin-clamped state (Fig. 5C). Basal Rd was not different between groups (Fig. 5D). Rd during the HI clamp in sod2<sup>+/+</sup> and sod2<sup>+/−</sup> mice was not different on a chow diet and was decreased to a similar extent by HF feeding (Fig. 5D). Rg in gastrocnemius, superficial vastus lateralis, and heart was similar between genotypes on both chow and HF diets (Fig. 5E). Diaphragm Rg was slightly decreased in chow-fed sod2<sup>+/−</sup> mice compared with chow-fed sod2<sup>+/−</sup> mice but was the same between genotypes on HF diet (Fig. 5E). Changes in muscle insulin signaling after the HI clamp were consistent with metabolic flux measurements observed during the HI clamp. Phosphorylated Akt (p-Akt), total Akt, and p-Akt/Akt ratio were decreased by HF feeding (Fig. 5F). Consistent with similar muscle Rg, p-Akt, total Akt, and p-Akt/Akt ratio did not differ between sod2<sup>+/+</sup> and sod2<sup>+/−</sup> mice, independent of diet (Fig. 5F). These results show that a 50% SOD2 reduction does not influence insulin action. Since HG GIR was the same in HF-fed sod2<sup>+/+</sup> and sod2<sup>+/−</sup> mice even though arterial insulin was reduced in sod2<sup>+/−</sup> mice and insulin action was not increased, one can deduce that heterozygous SOD2 deletion increases glucose effectiveness, the ability of glucose to facilitate its own disposal (24).

**Glucose Effectiveness During the HG Clamp**

Expression of GLUT-1, hexokinase II, and AMPK and activation of AMPK (p-AMPK/AMPK ratio), which are associated with glucose effectiveness, were increased in the HF-fed sod2<sup>+/−</sup> mice as compared with sod2<sup>+/+</sup> mice in muscle excised after the HG clamp (Fig. 6A–C). Muscle
glycogen was not different between the HF-fed sod2+/+ and sod2+/- mice (Fig. 6D), but liver glycogen was higher in the HF-fed sod2+/- mice (Fig. 6E). The latter finding is again consistent with an increase in glucose effectiveness.

Muscle Redox State
GSH/GSSG was decreased in muscle of sod2+/+ mice by HF feeding (Fig. 7A), suggesting a shift to a more oxidized redox environment. sod2+/- significantly decreased muscle GSH/GSSG in chow-fed mice and caused no further decrease in HF-fed mice (Fig. 7A). Protein carbonyl groups were not different between chow-fed sod2+/- and sod2+/+ mice but tended to be increased in HF-fed sod2+/- mice (P = 0.07) (Fig. 7B). Muscle TBARS were not affected by genotype or diet (Fig. 7C).

Muscle mitochondrial H2O2 level was measured during both succinate- and palmitoylcarnitine-supported state-4 respiration. Succinate increased H2O2 level in a dose-dependent manner (Fig. 7D). HF feeding caused an elevation in H2O2 level in the muscle of sod2+/- mice (Fig. 7D and E). sod2+/-, however, had no effect on either succinate- or palmitoylcarnitine-supported H2O2 level in mice on either diet (Fig. 7D and E).

DISCUSSION
SOD2 is a primary defense against oxidative stress, since ROS are largely generated on the matrix side of the inner mitochondrial membrane (25). In the current study, we used a mouse model with genetically reduced SOD2 levels, so as to increase mitochondrial O2−. The putative role of SOD2 or mitochondrial O2− in regulation of GSIS and insulin action was tested using glucose clamp techniques that are unique in that they do not require restraint, severing of the tail, or mouse handling. Our results demonstrate for the first time that sod2+/- causes dramatic impairments in GSIS in mice fed HF but not in lean mice. The observation of reduced GSIS in HF-fed sod2+/- mice in vivo is also evident in isolated islets. Decreased GSIS is likely attributable to increased O2− in islets of knockout mice. We conclude that SOD2 reduction impairs GSIS when mitochondrial ROS is further elevated by HF diet and that this combination exceeds the adaptive capacity of the β-cells. Surprisingly, sod2+/- does not affect insulin action. The ability of insulin to suppress hepatic glucose production and to stimulate glucose utilization is not sensitive to the changes in SOD2 in the current study.

Pancreatic β-cells are particularly vulnerable to oxidative stress due to low antioxidant enzyme expression (26). It is suggested that an imbalance between excess ROS production and limited antioxidant enzymes damages secretory capacity and viability of β-cells (26,27). Our results showing that a 50% reduction in islet SOD2 dramatically decreases GSIS both in vivo and in isolated islets...
of the HF-fed mice are consistent with these previous studies, suggesting that SOD2, the primary mitochondrial O$_2^-$ scavenger, is critical in regulating the redox state and therefore the secretory capacity of $\beta$-cells. Decreased GSIS in HF-fed $sod2^{+/2}$ mice is associated with increased O$_2^-$.

One could conclude that SOD2 reduction increases mitochondrial O$_2^-$, which contributes to elevated ROS and impaired GSIS. However, SOD2 converts O$_2^-$ to H$_2$O$_2$. A reduction in SOD2 could potentially decrease mitochondrial and cytosolic H$_2$O$_2$ in islets. Although H$_2$O$_2$ has been shown to be involved in sensing signals disrupting $\beta$-cell functions (9,28), it has also been shown that H$_2$O$_2$ derived from glucose metabolism serves as an important metabolic signaling molecule for insulin secretion (29). Therefore, the contribution of potential changes in H$_2$O$_2$ on impaired GSIS in HF-fed $sod2^{+/2}$ mice cannot be ruled out. In the presence of HF feeding, both excessive O$_2^-$ and H$_2$O$_2$ could negatively impact GSIS. Therefore one might predict that a combination of SOD2 and catalase, which converts H$_2$O$_2$ to O$_2$ and H$_2$O, would have the most antioxidative effect.

Mitochondrial dysfunction and oxidative stress have been implicated in the metabolic deterioration of $\beta$-cells, yet the mechanisms are not fully understood. It has been shown that transient exposure of $\beta$-cells to 200 $\mu$mol/L H$_2$O$_2$ decreases the secretory response to glucose, accompanied by reduced oxygen consumption, glucose-induced ATP generation, subunits of the respiratory chain, and expression of genes responsible for mitochondrial biogenesis (30). These findings suggest that mitochondrial dysfunction is potentially contributing to the impaired GSIS resulted from oxidative stress. Mitochondrial dysfunction is normally associated with increased ROS formation (31). While elevated ROS directly damage and oxidize DNA, protein, and lipids, ROS can also activate a number of stress-sensitive pathways that have been linked to decreased insulin secretion (3). These include activation of several key inflammatory pathways such as NFkB, p38 MAPK, JNK, and Janus kinase/STAT pathways and islet-enriched transcription factors (28). Activation of the NFkB and JNK/STAT pathways has been associated with ROS-mediated pancreatic $\beta$-cell death and therefore decreased GSIS (32). In addition to apoptosis, ROS has also been shown to decrease insulin gene expression by downregulating the expression of transcription factor Pdx-1 (26,28). However, here we show that the HF-fed $sod2^{+/2}$ mice had normal expression of genes that encode proteins involved in the regulation of insulin secretion. In addition, islet size is expanded equally in HF-fed $sod2^{+/2}$ and $sod2^{+/+}$ mice. These results suggest that the decreased islet secretory capacity of the SOD2 knockout mice is due to a defect in

![Figure 3](image-url)

**Figure 3**—Ex vivo GSIS as assessed by islet perfusion and static incubation. A and B: Isolated islets were perfused with 16.7 mmol/L glucose and 16.7 mmol/L glucose plus IBMX. C and D: Areas under the insulin peaks in response to glucose and IBMX stimulation were quantified. E: Isolated islets were cultured and incubated with either 3 or 20 mmol/L glucose, and insulin was measured in the culture medium. Data are represented as mean ± SEM. $n = 4-8$ males for all groups. *P < 0.05 compared with $sod2^{+/+}$ with the same diet/treatment. IEQs, islet equivalents.
the regulation of insulin secretion (e.g., glucose sensing, insulin exocytosis) rather than insulin biosynthesis or β-cell mass.

Heterozygous SOD2 deletion had no effects on insulin action either in healthy lean mice or HF diet-induced obese mice. This was despite a decrease in muscle GSH/GSSG, an indication of overall cellular redox state, in chow-fed sod2<sup>+/−</sup> mice. Hoehn et al. (1) reported that sod2<sup>+/−</sup> mice had impaired glucose tolerance on a chow diet. Our results show that these effects on glucose tolerance are not due to changes in insulin action. The interpretation of glucose tolerance testing is complicated, as glycemic excursions can be due to a number of factors (33), including insulin secretion, insulin clearance, and glucose effectiveness (34). Although we did not see a defect in insulin secretion in the chow-fed sod2<sup>+/−</sup> mice at

![Figure 4](image-url)
an arterial glucose of 250 mg/dL, a higher arterial glucose concentration may have provoked insulin secretory defects, thereby contributing to the impaired glucose tolerance. Nevertheless, our results are consistent with the data of Hoehn et al. (1) that showed that overexpression of SOD2 in L6 cells had no effect on insulin action and provide evidence that SOD2 reduction is not a critical determinant of muscle insulin action.

The effect of H$_2$O$_2$ on muscle glucose uptake is controversial. H$_2$O$_2$ has been reported to induce an insulin signaling defect in skeletal muscle cells (35). This defect is associated with a selective loss of insulin receptor substrate-1 and insulin receptor substrate-2 proteins, in part related to a p38 MAPK-dependent mechanism (36). Chronic elevation of mitochondrial H$_2$O$_2$ has been shown to link excess fat intake to insulin resistance in both rodents and humans (2). On the other hand, low levels of H$_2$O$_2$ are shown to be required for normal cellular function and intracellular signaling (8). In the current study, we found that mitochondrial H$_2$O$_2$ level, however, was not altered by the reduction of SOD2 in the muscle. These results are consistent with previous studies that showed that muscle mitochondrial H$_2$O$_2$ level was not affected by SOD2 overexpression (37), suggesting that even a 50–60% decrease in SOD2 expression in muscle mitochondria is not rate limiting for converting O$_2$ to H$_2$O$_2$. Although the enzymatic activity of SOD1, the cytosolic isoform of SOD and glutathione peroxidase, remains unchanged in the chow-fed sod$_2^{+/+}$ mice (11), compensations from other antioxidant mechanisms, especially during HF feeding, are possible.

Consistent with the H$_2$O$_2$ data, further measurements of the muscle redox state showed that GSH/GSSG, protein carbonyl groups, and TBARS levels were not different...
between muscles of HF-fed sod2−/− and sod2+/+ mice. These results further suggest that muscle is resistant to the changes in redox state caused by a 60% reduction of SOD2. The unchanged redox state in the muscle may explain the lack of changes in insulin sensitivity in the HF-fed sod2−/− mice. In contrast, pancreatic β-cells are particularly vulnerable to such a reduction, possibly due to low antioxidant enzyme expression (26). Total ROS, O₂⁻, protein carbonyl groups, and TBARS were increased in the islets of HF-fed sod2−/− mice. These differences in the oxidative damage status between muscle and islets provide mechanistic insight for the observed phenotype differences between tissues.

Surprisingly, muscle protein carbonyl groups level was found to be decreased in the HF-fed sod2−/− mice compared with chow-fed sod2+/+ mice. Despite high uncertainty, one possible postulation is that the HF diet contains lower protein and carbohydrate content compared with the chow diet. These differences in protein and carbohydrate content between diets may create unforeseen effects on redox status of protein in muscle. Moreover, these differences in diets may also have direct impacts on insulin secretion and action. Fortunately, the concluding results of the current study emphasizes a genetic effect rather than a diet effect. Therefore metabolic effects caused by the differences in nutritional profile of diets shall remain the same between genotypes.

Hyperglycemia per se exerts direct effects on glucose homeostasis, independent of changes in insulin or other hormonal signals (38). This phenomenon is referred to as "glucose effectiveness." The loss of glucose effectiveness in type 2 diabetes contributes importantly to hyperglycemia (24). Interestingly, HF-fed sod2−/− mice exhibited increased glucose effectiveness during hyperglycemia. This was related to increased GLUT-1, hexokinase II expression, and activation and expression of AMPK. These proteins involved in
glucose utilization may serve to compensate for the defects in insulin secretion. While this article was in preparation, Muscogiuri et al. (39) reported that mice with SOD1 deletion have glucose intolerance secondary to impairments in β-cell function rather than defects in insulin sensitivity. These results are consistent with our findings and, when combined with the current study, highlight a critical role of SOD enzymes in regulating insulin secretion. However, there exist important differences in the role for SOD1 and SOD2 in insulin secretion. Our study specifically targeted SOD2 with its mitochondrial ROS scavenging capacity, while the study of Muscogiuri et al. (39) targeted the cytosolic compartment of the cell where SOD1 resides. Our results show that the level of SOD2 and mitochondrial O$_2^-$/H$_2$O$_2$ are more critical in the impairment of insulin secretion seen with heterozygous deletion of SOD2 (with 50% remaining protein and activity of SOD2). Furthermore, the activity of SOD1 and other antioxidant enzymes regulate the transcription of key β-cell genes such as Pdx1, insulin, GLUT-2, and glucokinase (40–42). However, our results show that mitochondrial SOD2 does not affect the mRNA of these genes. These results indicate that SOD1 and SOD2 impact insulin secretion by distinctive mechanisms.

In conclusion, these data demonstrate that SOD2 reduction dramatically decreases pancreatic β-cell secretory capacity under dietary model of insulin resistance without affecting insulin action. These studies show for the first time that pancreatic islets are particularly sensitive to a 50% reduction of SOD2, yet skeletal muscle is not. Our mouse model of heterozygous SOD2 knockout mimics changes observed in diabetic humans (10,43,44) and thus is relevant to human disease. Our results suggest that efforts to target mitochondrial antioxidant enzymes may be beneficial in β-cell dysfunction and insulin resistance. Identification of tissue-specific therapeutic targets is particularly important because of the different sensitivity of tissues to changes in redox state.

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**Author Contributions.** L.K. performed experimental design, researched data, contributed to discussion, and wrote the manuscript. C.D. performed experimental design, researched data, contributed to discussion, and reviewed and edited the manuscript. M.E.L., J.S.B., W.H.M., S.M., F.D.J., C.S.T., C.-T.L., C.G.R.P., and...
E.J.A. researched data and reviewed the manuscript. P.D.N., D.H.W., and A.C.P. performed experimental design, reviewed data, contributed to discussion, and reviewed and edited the manuscript. All authors approved the final version of this manuscript. L.K. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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**References**


44. Marchetti P, Del Guerra S, Marselli L, et al. Pancreatic islets from type 2 diabetic patients have functional defects and increased apoptosis that are ameliorated by metformin. J Clin Endocrinol Metab 2004;89:5535–5541