### Genetic Disruption of SOD1 Gene Causes Glucose Intolerance and Impairs β-cell Function

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**Key Words:**
- Oxidative Stress
- Type 2 Diabetes
- SOD 1
- Insulin Resistance
- Insulin Secretion
Genetic Disruption of SOD1 Gene Causes Glucose Intolerance and Impairs β-cell Function

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Running title: Disruption of SOD 1 impairs glucose metabolism

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ABSTRACT

Oxidative stress has been associated with insulin resistance and type 2 diabetes. However, it is not clear whether oxidative damage is a cause or a consequence of the metabolic abnormalities present in diabetic subjects. The goal of this study was to determine whether inducing oxidative damage through genetic ablation of superoxide dismutase 1 (SOD1) leads to abnormalities in glucose homeostasis. We studied SOD1 null mice and wild type littermates. Glucose tolerance was evaluated with intraperitoneal glucose tolerance tests. Peripheral and hepatic insulin sensitivity was quantitated with the euglycemic hyperinsulinemic clamp. β-cell function was determined with the hyperglycemic clamp and morphometric analysis of pancreatic islets. Genetic ablation of SOD1 caused glucose intolerance, which was associated with reduced in vivo β-cell insulin secretion and decreased β-cell volume. Peripheral and hepatic insulin sensitivity were not significantly altered in SOD1 null mice. High fat diet caused glucose intolerance in WT mice, but did not further worsen the glucose intolerance observed in standard chow fed SOD1 null mice. Our findings suggest that oxidative stress per se does not play a major role in the pathogenesis of insulin resistance and demonstrate that oxidative stress caused by SOD1 ablation leads to glucose intolerance secondary to β-cell dysfunction.

Keywords: oxidative stress, insulin resistance, insulin secretion, SOD 1, type 2 diabetes
INTRODUCTION

Markers of oxidative stress are elevated in insulin resistant (obese and type 2 diabetic) subjects. For example, the concentrations of isoprostanes, one of the best characterized markers of oxidative damage, are elevated in plasma and urine of subjects with T2DM (1). Deficiencies in antioxidant defenses also have been described in diabetes, including lower levels of ascorbate, glutathione and superoxide dismutase (2). Lower concentrations of reduced glutathione have been documented in neutrophils and monocytes while reduced concentrations of ascorbate have been found in both diabetic plasma and mononuclear cells (2). Collectively, these data suggest that oxidative stress may play a role in the pathogenesis of insulin resistance and diabetes.

Despite substantial information indicating that insulin resistant states are associated with increased oxidative damage (3-5), it remains unclear whether these changes are the cause or consequence of the unbalanced metabolic state. To examine if increased oxidative stress leads to impaired glucose homeostasis, we studied mice which are null for superoxide dismutase (SOD) 1, the gene that encodes for the antioxidant enzyme copper zinc (CuZn) SOD. This enzyme, which mainly is located in the cytosol, converts superoxide to H$_2$O$_2$ and O$_2$. We hypothesized that the oxidative damage caused by the absence of SOD1 would impair glucose tolerance and insulin sensitivity.

RESEARCH DESIGN AND METHODS

Animal model. We studied male SOD1 null (-/-) mice between the ages of 3 and 4 months. These mice were generated by Dr. Charles Epstein's laboratory at the University of California San Francisco and have been described previously (6). Our group has characterized this animal model extensively and demonstrated that these mice have no detectable CuZn SOD activity in skeletal muscle, whereas the activities of Mn SOD (SOD2), glutathione peroxidase 1 (GPX1), and catalase are unchanged (7). These mice have elevated F$_2$ isoprostanes levels in muscle and plasma, and oxidative damage to proteins, lipids and DNA (7).
Maintenance of mice. Animals were housed in an animal room maintained at 23 °C with a 12 h light/12 h dark cycle and fed standard laboratory chow and water ad libitum. All procedures were approved by the Institutional Animal Care and Use Committee of the UTHSCSA.

Intraperitoneal glucose tolerance test (GTT). Fasted SOD1 null mice (n=12) and wild type (WT) (n=11) littermates were injected intraperitoneally with dextrose (2 g/kg). Glucose level was measured in tail blood at times 0, 30, 60, 90 and 120 min using an automatic glucose meter (Roche Diagnostics, Indianapolis, IN).

Hyperinsulinemic-euglycemic clamp studies. Insulin clamp studies were performed on 7 SOD1 null and 7 WT mice. Three to five days prior to the insulin clamp, a catheter was inserted into the right atrium of the heart through the jugular vein as previously described (8). A 90 min euglycemic hyperinsulinemic (18 mU/kg.min) clamp was performed in fasted mice, as described (8).

Hyperglycemic clamp. Six SOD1 null and 6 WT mice had a catheter inserted into the right atrium and 3-5 days later underwent a hyperglycemic clamp to evaluate β-cell function. At time 0, a glucose bolus (0.75 g/kg) was injected through the catheter, tail blood glucose concentration was measured every 5-10 min, and a variable infusion of 20% glucose was administered to maintain plasma glucose concentrations at ~ 300 mg/dl. Blood samples were collected from the tail at times -10, 0, 5, 10, 15, 20, 30, 50, 70 and 90 min for the measurement of plasma insulin concentration (Crystal Chem, Downers Grove, IL).

Histology and immunohistochemistry. Six SOD1 null and 6 WT type mice were sacrificed with an overdose (150 mg/kg) of pentobarbital and pancreatic tissues were collected. The tissue was fixed in 10% neutral-buffered formalin and paraffin embedded. 5 µm-thick sections were stained with H&E. For immunohistochemistry, 3 µm-thick sections, after endogenous peroxidase activity inhibition, were incubated with the primary antibody (anti-insulin mouse, clone AE9D6, Biogenex Laboratories, San Ramon, CA) at 4°C for 18–20 hours, followed by the avidin-biotin complex procedure (9). Immunoreactions were developed using 0.03% 3, 3′diaminobenzidine tetrahydrochloride. Morphometric analysis was performed using the Computer Assisted Stereology
Toolbox (CAST) 2.0 system from Olympus (Ballerup, Denmark) and using the stereology fundamentals on pancreatic sections randomly collected from the pancreas (10). The operator was blinded to the genotype? status of the mice. Each field was selected randomly using the CAST meander sampling. We analyzed approximately 168 fields per section and in each field point counting of total pancreatic tissue and islets was performed at the magnification of 100X in order to calculate the relative β-cell volume. Relative β cell volumes were calculated using the following formulas: (i) relative β-cell volume/pancreas: (IP/TP) x 100, where IP = points that hit β-cells in the insulin stain and TP = total pancreas points and (ii) relative β-cell volume/islet: (II/TPI) x 100, where II = points that hit insulin positive cells and TPI = total points that hit the islet. Total pancreatic points averaged 4497 per slice per mice.

**Glucose-stimulated insulin secretion (GSIS).** SOD1 null and WT mice were anesthetized with pentobarbital and pancreatic islets isolated using a modification of the method of Gotoh et al. (11). Briefly, pancreas ducts were infused with collagenase (rodent LiberaseTM Roche, Indianapolis, IN), followed by Histopaque (Sigma, St Louis, MO, USA) gradient separation and handpicking to ensure high purity. To measure insulin secretion, two replicate experiments were done for each animal with 6 animals tested individually per group. Insulin secretion was measured in static conditions by sequential incubation in basal, stimulatory glucose and IBMX. After overnight culture, islets were washed twice with Krebs-Ringer bicarbonate buffer (KRB) containing16 mM HEPES and 0.1 % BSA, pH 7.4 (KRB-HEPES) and preincubated for 1 h in 1 ml KRB-HEPES in 24 well dishes at 37°C with 5% CO2 with 2.8 mM glucose as previously described (12). Media was then changed and, after an additional hour in KRB-HEPES 2.8 mM, islets were removed for determination of basal insulin release and then incubated with 16.8 mM glucose for one hour. This was followed by a third incubation with 16.8 mM glucose plus IBMX (1mM). Islets were washed twice with KRB-HEPES 2.8 mM glucose every time secretion media was changed. Supernatants were frozen until assayed in duplicate with Insulin Rat (EIA) kit (ALPCO, Salem, NH). Islets were
collected and sonicated for a total of 30 seconds and stored at -20°C until assayed for DNA and insulin content.

**High fat feeding protocol.** At approximately 4 months of age, a second cohort of WT and SOD1 null mice were assigned to a standard diet or a high fat defined diet (45% kCal from fat, D12451, Test Diets) for 12 weeks (n=4 per group). These mice then were subjected to intraperitoneal GTTs (1.5 g dextrose/kg) and body composition measurement by Quantitative Magnetic Resonance imaging using an EchoMRI 3-in-1 composition analyzer (Echo Medical Systems, Houston, TX).

**Mitochondrial H$_2$O$_2$ release.** Mitochondria were purified from freshly collected whole hind-limb skeletal muscle as described previously (13). H$_2$O$_2$ production by muscle mitochondria was measured using the Amplex red (Molecular Probes, Eugene OR)–horseradish peroxidase method, as described (14). Amplex red reagent was made in ROS buffer (125 mM KCl, 10 mM HEPES, 5 mM MgCl2, 2 mM K2HPO4, pH 7.44) with 77.8 µM AmplexRed, 1 unit/ml of HRP and 37.5 units/ml of SOD (to convert all superoxide into H$_2$O$_2$). All assays were performed in black 96-well plates at 37°C and fluorescence was measured using a Fluoroskan Ascent type 374 multiwell plate reader (Labsystems, Helsinki, Finland) reading at an excitation wavelength of 545 nm and an emission wavelength of 590 nm. Each assay utilized one reaction well containing buffer only and another containing buffer with mitochondria to estimate the mitochondria H$_2$O$_2$ release without substrate. Mitochondria complex substrates/inhibitors were added at following concentrations: glutamate/malate (5 mM), succinate (10 mM), rotenone (1 µM).

**Measurement of ATP production.** ATP production by isolated mitochondria was measured using a luminometric assay (ATP Bioluminescence Assay CLS II; Roche), as described (15).

**Measurement F$_2$-isoprostanes and 4-hydroxynonenal (4-HNE)-modified proteins.** F$_2$-isoprostanes and 4-HNE are accurate indicators of lipid peroxidation. Muscle F$_2$-isoprostanate content was performed by chromatography–mass spectrometry as previously described by our group (16). Protein-bound 4-HNE was determined by immunoblotting of 75 µg skeletal muscle
protein separated by SDS-PAGE and incubated with primary anti-4 HNE antibody from Abcam (Cambridge, Mass).

**Neuroglycopenia induction.** WT and SOD1 null mice (n=4 per group) received an intraperitoneal injection of 2-deoxyglucose (2DG; 500 mg/kg) (17). Plasma glucose (Roche glucose meter) and glucagon (ELISA kit, ALPCO) concentration were measured before and 30 min after 2DG administration. 2DG, by competitive inhibition of glucose utilization, produces a state of intracellular neuroglycopenia with resultant activation of the autonomic nervous system (17), followed by glucagon release and hyperglycemia.

**Antioxidant enzyme activity assays.** SOD and GPX activity were measured using assay kits for each from Trevigen (Gaithersburg, MD). Muscle (quadriceps) and fat (epigonadal) were homogenized in provided 1X assay buffer with the addition of protease inhibitors. For each assay, 40 µg protein from each tissue homogenate was used and all samples were assayed in duplicate. Enzyme activity was calculated based on manufacturer’s directions.

**Immunoblotting.** Muscle (quadriceps) tissue was homogenized in a modified RIPA homogenization buffer with the addition of protease and phosphatase inhibitors. 75 µg protein of each sample was separated by SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) for immunobloting. Phospho-Akt (ser473) and total Akt antibodies were from Cell Signaling (Danvers, MA).

**Statistical analysis.** Data are expressed as mean ± SE. Statistical evaluation was performed by Student's two-tailed t test or two-way ANOVA followed by the Fisher's post hoc test for multiple comparisons. A \( P<0.05 \) was considered statistically significant. Statistical analysis was performed using SigmaStat software.

**RESULTS**

**General characteristics of SOD1 null mice.** Consistent with our previous findings (7), the body weight of SOD1 null mice was significantly reduced (18.7 ± 2.55 in SOD1 null vs. 24.8 ± 0.62 g in WT; \( P<0.05 \)). The fasting plasma insulin concentrations tended to be lower in SOD1 null mice vs.
WT (150 ± 3.42 vs. 277 ± 37.4 µUI/ml; \(P=0.05\)). Fasting glucose was not significantly different between WT and SOD1 null mice (159 ±9.64 vs. 119 ± 15.9 mg/dl; \(P=\text{NS}\)).

**Glucose tolerance.** As shown in figure 1, ablation of SOD1 caused a significant impairment in glucose tolerance. The blood glucose concentration was significantly elevated at 30 and 60 min after glucose administration.

**Insulin sensitivity.** We conducted euglycemic hyperinsulinemic clamps to examine the effect of SOD1 ablation on peripheral and hepatic insulin sensitivity. There was a tendency (\(P=0.13\)) for a 20% reduction in insulin-stimulated glucose disposal (Rd) in SOD1 null mice (figure 2A). Basal endogenous (primarily hepatic) glucose production (EGP) and insulin-suppression of EGP were similar in both groups (figure 2 B).

**Insulin secretion in vivo.** Blood glucose concentrations were rapidly increased and maintained at about ~300 mg/dl during the hyperglycemic clamp. The plasma insulin concentration during the 1st phase and second phase of insulin secretion were significantly reduced in SOD1 null vs. WT mice (figure 3).

**Morphometric analysis of islets.** Morphometric examination was performed in 8 SOD1 and 9 WT mice (figure 4). \(\beta\)-cell volume accounted for 0.98 ± 0.17 % of the pancreatic area in WT and 0.73 ± 0.18 % in SOD1 null mice (\(P=0.2\)) (figure 5A). The volume of the islets occupied by \(\beta\)- cells was lower in SOD1 null vs. WT mice (64 ± 3.3 vs. 78 ± 3.0 % of islet volume; \(P<0.05\)) (figure 5B). Furthermore, SOD1 null mice showed a reduced average islet size of 8490 ± 685.5 µm\(^2\) compared with 9235 ± 1541 µm\(^2\) in WT animals, although this difference did not reach statistical significance (figure 5C).

**GSIS assay.** As shown in Table 1, \(\beta\) cells were able to normally secrete insulin in response to hyperglycemia *in vitro*.

**2DG challenge.** Plasma glucagon and glucose concentrations before and after 2DG administration were similar between WT and SOD1 groups (Supplemental figures 1A and 1B).
Effects of high fat feeding. The high fat diet increased adipose tissue mass in both groups of mice, though to a smaller degree in SOD1 null mice (figure 6A). Consistent with a prior study from our own group (7), deletion of SOD1 caused a decrease in lean mass, and this decline was evident in animals fed standard and high fat diets (Figure 6A). Similar to the first cohort examined (figure 1), SOD1 mice on normal chow from the second cohort had impaired glucose tolerance (figures 6B and 6C) and decreased plasma insulin concentration (figure 6D). As expected, the high fat diet caused glucose intolerance in WT mice. Notably, in SOD1 null mice the high fat diet did not further worsen the glucose intolerance observed in standard chow fed animals (figures 6B and 6C).

As expected, muscle from SOD1 null mice on the standard diet had increased mitochondrial H$_2$O$_2$ emissions (figure 7A) and oxidative stress, as evidenced by elevated muscle F$_2$-isoprostane content (figure 7B) and 4-HNE (figure 7C). High fat diet increased oxidative damage in WT mice but did not alter mitochondrial H$_2$O$_2$ emissions (figures 7A-C). Interestingly, the high fat diet did not further alter ROS production and markers of oxidative stress in the SOD1 null mice. No alterations in mitochondrial ATP production were observed in muscle from SOD1 null mice placed on the standard and high fat diets (Supplementary figure 2). As predicted, total SOD activity was significantly reduced in muscle and adipose tissue of SOD1 null mice (Supplementary figure 3). Interestingly, muscle in SOD1 null mice had almost no total SOD activity whereas adipose tissue retained ~1/3 total SOD activity. This suggests that the greatest contribution to total SOD activity in muscle is provided by SOD1. GPX activity was not affected by the lack of SOD1. In addition, high fat feeding did not significantly alter SOD in either WT or SOD1 null mice, nor did it alter GPX activity in either genotype. These data demonstrate that the lack of SOD1 does not lead to compensatory effects on the other antioxidant enzymes and suggests that increased oxidative stress with high fat feeding is not due directly to reduced antioxidant defense.

**DISCUSSION**

Consistent with our hypothesis, deletion of SOD1 resulted in glucose intolerance. This suggests that oxidative damage plays a role in the glucose metabolic abnormalities in type 2
diabetic subjects. Surprisingly, the glucose intolerance seen in SOD1 null mice was not the result of significantly reduced insulin sensitivity in periphery (muscle) tissues or the liver. This prompted us to examine whether abnormalities in β-cell function could explain the glucose intolerance. Indeed, the results from the hyperglycemic clamp demonstrated a significantly blunted insulin secretion response, a defect which was in part explained by a reduction in β-cell mass.

The effect of oxidative stress on insulin sensitivity and peripheral (muscle) glucose disposal is controversial (18-20). Elevations in plasma glucose and free fatty acids are thought to increase ROS levels (18, 21), which in turn activate inflammation signaling pathways such as mitogen activated protein kinases (MAPK) (22) and nuclear factor κB (23). The activation of these inflammation cascades is thought to cause insulin resistance, which accounts for > 80% of peripheral glucose disposal (24). However, a beneficial effect of oxidative stress on muscle glucose disposal also has been reported, and attributed to the activation of the phosphatidylinositol 3-kinase signaling pathway (19-20).

We hypothesized that SOD1 null mice would be significantly insulin resistant compared to WT animals due to excess ROS production and decreased lean body mass (7). Nonetheless, we did not observe a significant reduction in peripheral insulin sensitivity in SOD 1 null mice, although it is possible that the study might have been underpowered to detect subtle alterations in peripheral insulin action. In addition, we did not see significant differences in Akt phosphorylation (Supplementary figure 4). The minimal effect caused by SOD1 ablation on insulin sensitivity was unexpected, considering that oxidative stress has been implicated in the pathogenesis of insulin resistance and diabetes (18, 21). On the other hand, an important role for ROS on the maintenance of normal muscle function increasingly is now being recognized. For example, muscle contraction increases ROS production, an effect that might play a role in the adaptations to exercise training (25). Consistent with this, increased activity of GPX activity leads to the development of insulin resistance in mice (26) due to a reduction in H₂O₂ and resultant inhibition of protein tyrosine phosphatase (27).
One might speculate that SOD1 ablation per se is not sufficient to cause a pathologic state in peripheral tissues. Yet, our group has demonstrated that SOD1 null mice fed a standard diet exhibit sarcopenia and reduced physical performance (7), presumably resulting from pathologic oxidative stress. We also fed the SOD1 null mice a diet with a high fat content to examine whether potential alterations in glucose metabolism would become apparent by high fat feeding. Interestingly, the glucose intolerance caused by the high fat diet was not significantly worsened by deletion of SOD1. The lack of additive effect of SOD1 ablation and high fat feeding on glucose intolerance suggests that oxidative stress could be an important mechanism by which high fat feeding causes glucose intolerance.

The present results demonstrate that SOD1 null mice manifest defects in both 1\textsuperscript{st} and 2\textsuperscript{nd} phase of insulin secretion. Accordingly, several studies have shown that pancreatic β-cells are a target of oxidative stress in type 2 diabetes (28-30). A reduced expression of antioxidant enzymes in β-cells, including catalase and GPX1, predisposes these cells to ROS damage (31-32). Multiple pathogenic factors may contribute to the impairment of insulin secretion \textit{in vivo} in SOD1 null mice. SOD1 regulates the expression of pancreatic and duodenal homeobox (Pdx1), a transcription factor necessary for pancreatic development and β-cell maturation, and stimulates the expression of multiple pancreatic genes, including insulin, glucose transporter 2, and glucokinase 5 (33). Reducion of SOD1 decreases H3 acetylation and H3K4 methylation of the Pdx1 promoter region, resulting in decreased Pdx1 mRNA and protein levels (34). In addition, lack of SOD1 causes a decrease in FOXA2 mRNA protein, which binds to the promoter/enhancer region of the Pdx1 gene and augments its expression \textit{in vivo} (34). Thus, changes induced by knocking out SOD1 can impair insulin secretion that already is compromised by the decrease in β-cell mass and low expression of Pdx1.

In the present study we demonstrate impaired insulin secretion \textit{in vivo}. Yet, we did not observe any difference in glucose-stimulated insulin secretion \textit{in vitro}. This may indicate that the environment in which the β-cells reside, rather than an intrinsic β-cell defect, is responsible for the
impaired in insulin secretion. Because SOD1 null mice also display neuropathologic changes (35), we evaluated autonomic input to the pancreatic islets in vivo by measuring glucagon release (and consequent hyperglycemia) after 2DG-induced neuroglycopenia. Nonetheless, the response was similar in WT and SOD1 null mice. Because removal of the islets from the body and in vitro perfusion could result in a “wash out” of the toxic effect of ROS, it is possible that deletion of SOD1 alters the levels of one or more systemic factors that impair in vivo insulin release. Future studies will be aimed at identifying and characterizing potential systemic factors that impair β-cell function in this animal model of elevated oxidative stress.

In conclusion our results demonstrate that the absence of SOD1 is associated with the development of impaired glucose tolerance that results from impaired β-cell function and reduced β-cell mass. Insulin-stimulated glucose disposal is minimally affected by genetic ablation of SOD1.

Acknowledgements

NM is the guarantor of the manuscript. HVR, GM, ABS, ML, RGM, CAM, BB, RLR and SR carried out the experiments. GM, ABS, and NM drafted the manuscripts. NM, AG, RAD and GW revised the data. NM conceived the study, participated in its design and coordination.

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Table 1. *In vitro* glucose-stimulated insulin secretion and islet insulin content. Data are means ± SE

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<th>Glucose Stimulated Insulin Secretion</th>
<th>2.6 mM Glc (pg Insulin/hr/ng DNA)</th>
<th>16.8 mM Glc (pg Insulin/hr/ng DNA)</th>
<th>16.8 mM Glc + IBMX 1 mM (pg insulin/h/ng DNA)</th>
<th>Islet Insulin content (ng insulin/ng DNA)</th>
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<td>WT</td>
<td>3 ± 0.7</td>
<td>48 ± 11</td>
<td>938 ± 107</td>
<td>29 ± 3</td>
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<tr>
<td>SOD1null</td>
<td>3 ± 0.4</td>
<td>49 ± 11</td>
<td>1020 ± 85</td>
<td>25 ± 2</td>
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FIGURE LEGENDS

**Figure 1.** Glucose tolerance in SOD1 null mice. Blood glucose concentration was measured after the administration of intraperitoneal glucose in WT and SOD1 null mice. Data are means ± SE. *P<0.05

**Figure 2.** Insulin-stimulated whole-body glucose disposal determined with the euglycemic-hyperinsulinemic clamp technique (A). Endogenous glucose production rate (B). Data are means ± SE

**Figure 3.** Insulin secretion *in vivo* during a hyperglycemic clamp. Data are means ± SE. *P<0.05

**Figure 4.** Representative microscopic images of insulin, glucagon, and somatostatin immunostaining of pancreatic islets.

**Figure 5.** Morphometric analysis of pancreatic islets. β-cell volume expressed as the percentage of the pancreas (A), percentage of the islets (B), and islet size (C). Data are means ± SE. *P<0.05.

**Figure 6.** Average fat (gray) and lean (white) mass for mice in indicated diet group (A); *P<0.05 between genotypes for indicated diet; Glucose tolerance curve in low (LF) and high (HF) fat fed mice (B). Area under the curve calculated from glucose tolerance tests (C); *P<0.05 vs. WT on low fat diet. Plasma insulin concentration (D); *P<0.05 vs. WT mice from corresponding diet assignment. Data are means ± SE.

**Figure 7.** H₂O₂ production of isolated skeletal muscle mitochondria with different (or no) mitochondrial substrates; *P<0.05 between genotypes for indicated diet (A). F₂-isoprostanes content in muscle (B); *P<0.05 vs. WT from corresponding diet. 4-HNE adducts in muscle (C); *P<0.05 vs. WT on low fat diet. Data are means ± SE.

**Supplementary Figure 1.** Plasma glucagon (A) and blood glucose (B) concentration before and after 2DG challenge. Data are means ± SE.

**Supplementary Figure 2.** H₂O₂ production of isolated skeletal muscle mitochondria with different mitochondrial substrates. Data are means ± SE.

**Supplementary Figure 3.** Total SOD (A) and GPX (B) activity in skeletal muscle and adipose tissue. Data are means ± SE.

**Supplementary Figure 4.** Representative blots and quantification of phospho-Akt and total Akt in muscle from low fat and high fat fed WT and SOD1 null mice. Data in quantification are means ± SE.
Figure 1

Blood glucose (mg/dl) vs. Minutes after injection for WT and SOD1 KO groups. The graph shows a significant increase in blood glucose for both groups immediately after injection, with WT showing a higher initial peak and SOD1 KO reaching a higher peak with a subsequent decrease. The asterisk (*) indicates a significant difference between the two groups at certain time points.
Figure 2

A.

Whole body glucose disposal (mg/kg/min)

WT
SOD1 KO

B.

EGP (mg/kg/min)

WT
SOD1 KO

Basal
Clamp
Figure 3

Plasma Insulin (µU/ml) vs. Time (min) for WT and SOD1 KO.
Figure 4

INSULIN  GLUCAGON  SOMATOSTATIN

SOD 1 KO

WT
Figure 5

A. 

\[ \text{β cell volume (}\%\text{ of pancreas)} \]

- WT
- SOD1 KO

B. 

\[ \text{β cell volume (}\%\text{ of islets)} \]

- WT
- SOD1 KO

C. 

\[ \text{Islet size (μm}^2\text{)} \]

- WT
- SOD1 KO
Figure 6

A. 

![Graph showing mass distribution between WT and SOD1 KO mice on low and high fat diets.](image)

B. 

![Graph showing blood glucose levels over time after injection for WT and SOD1 KO mice on low and high fat diets.](image)

C. 

![Graph showing area under the curve for WT and SOD1 KO mice on low and high fat diets.](image)

D. 

![Graph showing insulin levels for WT and SOD1 KO mice on low and high fat diets.](image)
Figure 7

A.

H$_2$O$_2$ production (pmol/min/mg)

State 1  Succ/Rot  Glut/Mal

0  20  40  60  80  100  120  140

WT LF  WT HF  SOD1 KO LF  SOD1 KO HF

B.

F$_2$-isoprostanes (ng/g tissue)

Low Fat  High Fat

0.0  0.5  1.0  1.5  2.0  2.5  3.0  3.5

WT  SOD1 KO

C.

4-HNE (AU)

Low Fat  High Fat

0  5  10  15  20  25

WT  SOD1 KO
Supplementary Figure 2

ATP production (nmol/min/mg)

Succ/Rot Glut/Mal

WT LF
WT HF
SOD1 KO LF
SOD1 KO HF
Supplementary Figure 3

A.

**MUSCLE**

![Graph](image1)

**ADIPOSE**

![Graph](image2)

B.

![Graph](image3)

![Graph](image4)
Supplementary Figure 4

For Peer Review Only

<table>
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<tr>
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<th>Low fat</th>
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<th>High fat</th>
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Low fat High fat

p-AKT/total AKT (AU)

WT  SOD1 KO

0.0 0.1 0.2 0.3 0.4

p-AKT/total AKT (AU)

WT  SOD1 KO

0.0 0.1 0.2 0.3 0.4

Low fat High fat

Bar graph showing p-AKT/total AKT (AU) for WT and SOD1 KO in low and high fat conditions.