Bach1 deficiency protects pancreatic β-cells from oxidative stress injury

Running title: Protective effects of Bach1 deficiency in pancreatic β-cells

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Abstract

BTB and CNC homology 1 (Bach1) is a transcriptional repressor of anti-oxidative enzymes, such as heme oxygenase-1 (HO-1). Oxidative stress is reportedly involved in insulin secretion impairment and obesity-associated insulin resistance. However, the role of Bach1 in the development of diabetes is unclear. HO-1 expression in the liver, white adipose tissue and pancreatic islets was markedly up-regulated in Bach1-deficient mice. Unexpectedly, glucose and insulin tolerance tests showed no differences in obese wild-type (WT) and obese Bach1-deficient mice, after high-fat diet loading for 6 weeks, suggesting minimal roles of Bach1 in the development of insulin resistance. In contrast, Bach1 deficiency significantly suppressed alloxan-induced pancreatic insulin content reduction and the resultant glucose elevation. Furthermore, TUNEL-positive cells in pancreatic islets of Bach1-deficient mice were markedly decreased, by 60%, compared with those in WT mice. HO-1 expression in islets was significantly up-regulated in alloxan-injected Bach1-deficient mice, while expression of other anti-oxidative enzymes, catalase, superoxide dismutase and glutathione peroxidase, was not changed by either alloxan administration or Bach1 deficiency. Our results suggest that Bach1 deficiency protects pancreatic β-cells from oxidative stress-induced apoptosis and that the enhancement of HO-1 expression plays an important role in this protection.
Type 2 diabetes is caused by complex interactions between insulin resistance in peripheral tissues and impaired insulin secretion from pancreatic $\beta$-cells (47). Exacerbation of oxidative stress (19), as well as endoplasmic reticulum stress (50) and hypoxic conditions (44), play key roles in the progressive deterioration of pancreatic $\beta$-cells during the development of diabetes. Pancreatic $\beta$-cells are a direct target of oxidative stress-mediated effects. Oxidants have been shown to negatively affect mitochondrial action in pancreatic $\beta$-cells, leading to impaired insulin secretion (25). However, pancreatic islets have very low intrinsic anti-oxidant enzyme capacity as compared with other tissues (8), making islets more vulnerable to oxidative stress than other tissues. Therefore, reducing oxidative stress and enhancing the anti-oxidative system may both be beneficial strategies for preserving $\beta$-cell function.

On the other hand, oxidative stress also plays crucial roles in obesity-associated insulin resistance (46). Obesity increases reactive oxygen species (ROS) production via NADPH oxidase activation in adipose tissue (6). Excessive generation of ROS impairs glucose uptake into muscle (24) and adipose tissues (37), and is involved in the pathogenesis of hepatic steatosis (36), collectively leading to systemic insulin resistance. In addition, oxidative stress directly causes dysregulated production of adipocytokines, such as adiponectin, plasminogen activator inhibitor-1 and monocyte chemotactic protein-1 (6), resulting in deterioration of insulin sensitivity. Thus, oxidative stress is closely related to obesity-associated insulin resistance, and thereby contributes to the vicious cycle leading to the development of metabolic syndrome.

Transcription factor BTB and CNC homology 1 (Bach1) is a basic region-leucine zipper (bZip) transcription factor that suppresses oxidative stress responses (33). Bach1 forms a heterodimer with small Maf oncoproteins and binds to Maf-recognition elements (MAREs) on the genome, thereby inhibiting transcription of oxidative stress-responsive genes, including heme oxygenase-1 (HO-1) (30), NAD(P)H quinone oxidoreductase 1 (2) and thioredoxin reductase 1 (10). Expression of these genes is very low under normal conditions due the dominant binding of Bach1 to MAREs. Once cells are subjected to oxidative stress, Bach1 loses its DNA-binding activity and is exported out of the nuclei. Instead of Bach1, NF-E2 related factor (Nrf) 2, another bZip transcription factor, rapidly accesses and binds to MAREs, resulting in activation of the transcription of oxidative stress-responsive
genes (38). Indeed, in Bach1-deficient mice, HO-1 is constitutively expressed at higher levels in many tissues, indicating that Bach1 plays a major role in negative regulation of HO-1 expression (39).

It is particularly noteworthy that pharmacological enhancement of the HO-1 pathway reportedly exerts protective effects against the development of diabetes via several mechanisms (1). Up-regulation of HO-1 protects pancreatic \( \beta \)-cells from oxidative injury induced by high glucose concentrations, resulting in preservation of insulin secretion \textit{in vitro} (48). Administration of an HO-1 inducer decreases pancreatic superoxide contents, which in turn results in suppression of \( \beta \)-cell loss in NOD mice, a well-established model of type 1 diabetes (22). In addition to its effects on insulin secretion, HO-1 induction suppresses fat accumulation (21, 29) and insulin resistance (28) in obese rodents.

Previous studies have revealed Bach1-deficient mice to be protected from a wide range of pathological conditions, including myocardial infarction (51), atherosclerosis (45), steatohepatitis (12), intestinal injury (9), lung injury (43), keratinocyte differentiation (31) and neural tissue damage (16). Thus, Bach1 deficiency may protect mice against oxidative tissue damage. These findings prompted us to hypothesize that ablation of Bach1 would be a promising approach to preventing the development of insulin resistance and insulin secretion impairment, and ultimately diabetes. Therefore, in this study, we examined the effects of Bach1 deficiency on glucose metabolism in two different murine models of diabetes, i.e. alloxan-induced pancreatic \( \beta \)-cell loss and high-fat diet (HFD)-induced insulin resistance.
Methods

Animals
Animal studies were conducted in accordance with the institutional guidelines for animal experiments at Tohoku University. Bach1-deficient mice were backcrossed with C57BL/6J mice (39). Wild-type (WT) and Bach1-deficient mice were kept on a 12-h light/dark cycle with free access to food and sterile water. For the purpose of oxidative stress loading of pancreatic β-cells, a 50 mg/kg dose of alloxan (2,4,5,6-tetraoxypyrimidine) (Sigma-Aldrich, St. Louis, MO, USA) was injected intravenously into fasted 8-week-old mice (49). In detail, a 10 mg/ml alloxan/citrate solution was prepared with ice cold 0.05M citrate buffer (pH 4.3) immediately prior to injection. In the control group of mice, the same volume of citrate buffer was injected intravenously. Mice with HFD-induced obesity were obtained by 18-week feeding of a HFD (32% safflower oil, 33.1% casein, 17.6% sucrose and 5.6% cellulose) (14) beginning at 6 weeks of age.

Analysis of glucose metabolism
Blood glucose was assayed with Antsense-III (Horiba Industry, Kyoto, Japan). Plasma insulin concentrations were determined using an ELISA kit (Morinaga, Tokyo, Japan). Glucose tolerance tests were performed on fasted (10 h, daytime) mice. The mice were given glucose (1 or 2 g/kg of body weight) intraperitoneally, followed by measurement of blood glucose levels. Insulin tolerance tests were performed on ad libitum-fed mice. The mice were intraperitoneally injected with human regular insulin (0.5 or 0.75 units/kg of body weight; Eli Lilly, Kobe, Japan), followed by measurement of blood glucose levels (7).

Quantitative RT-PCR-based gene expression
Quantitative RT-PCR was performed as previously described (13). The relative amount of mRNA was calculated employing GAPDH mRNA as the invariant control. The oligonucleotide primers are presented in Table 1.

Histological analysis
The pancreases from WT and Bach1-deficient mice were fixed with 10% formalin, embedded in paraffin, and sectioned. Sections were stained with hematoxylin and eosin (HE).

Laser micro-dissection
Coronal cryostat sections (10 μm) of the pancreas were placed on PEN-coated slides (Leica Microsystems, Wetzler, Germany). Laser micro-dissection
was carried out on a Leica AS LMD (Leica Microsystems). Immediately after micro-dissection of each islet, the samples were stored at -80 °C until RNA purification (15).

**Apoptosis detection**

At 24 hours after alloxan injection, DNA fragmentation associated with apoptosis was detected in situ by the addition of nucleotides to free 3’ hydroxyl groups in DNA. Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining was performed using an in situ cell death detection kit (Roche, Mannheim, Germany). The results are expressed as the number of TUNEL-positive cells per islet, as reported previously.

**Pancreatic insulin contents**

Pancreases were suspended in cold acid ethanol (1.5% HCl in 75% ethanol) and minced with scissors, and left at −20°C for 48 hours, with sonication every 24 hours. Insulin contents in the acid ethanol supernatant were determined with an ELISA kit (Morinaga) (41).

**Statistical Analysis**

All statistical analyses were performed with the Statistical Package for the Social Sciences version 15.0 (SPSS Japan Inc, Tokyo, Japan). All data were tested for normality by the Kolmogorov-Smirnov test. When data were normally distributed, the statistical significance of differences was assessed using the unpaired t test and one-way ANOVA followed by Tukey post hoc analyses. When data were not normally distributed, the statistical significance of differences was judged based on P values using the Mann-Whitney U-test.
Results

*Bach1 deficiency exerted minimal effects on glucose metabolism under standard feeding conditions*

First, we measured body weights and blood glucose levels until 12 weeks of age, followed by analysis of glucose metabolism under standard feeding conditions. Body weights were similarly increased and fasting blood glucose levels were also similar in WT and Bach1-deficient mice (Figure 1A). In addition, neither blood pressure nor plasma insulin differed significantly between the two groups at 12 weeks of age (Figure 1B). Furthermore, glucose and insulin tolerance tests revealed that Bach1 deficiency affected neither systemic glucose tolerance nor insulin sensitivity (Figure 1C, D). Histological findings with HE staining revealed no apparent differences in pancreatic islet morphology between WT and Bach1-deficient mice (Figure 1E). In addition, pancreatic insulin contents were also similar in Bach1-deficient and WT mice (Figure 1F). Thus, Bach1 deficiency had no significant effects on systemic glucose metabolism under standard feeding conditions.

In Bach1-deficient mice, HO-1 is constitutively expressed at very high levels in many tissues, such as the liver, lungs and heart (39). We next examined expression of HO-1 in organs/tissues responsible for glucose metabolism. As shown in Figure 1G, HO-1 expression was markedly increased not only in the liver, but also in pancreatic islets and white adipose tissue (WAT).

*Bach1 deficiency minimally impacts obesity and insulin resistance*

Pharmacological HO-1 up-regulation reportedly prevents the development of diabetes by both maintaining pancreatic β-cell function (48) and decreasing adiposity and insulin resistance (21). Therefore, we next examined metabolic effects of Bach1 deficiency on insulin resistance in models of HFD-induced obesity. First, WT and Bach1-deficient mice were started on a HFD at 18 weeks of age. In Bach1-deficient mice, body weights were similarly increased and blood glucose was elevated, during the 6 weeks of HFD feeding, as compared to WT mice (Figure 2A). Furthermore, neither glucose tolerance nor systemic insulin sensitivity (Figure 2B) differed between WT and Bach1-deficient mice after 6-week HFD loading. Fasting insulin levels were similarly increased during the 18 weeks of HFD feeding in the two groups (Fig. 2C). These findings suggest minimal roles of Bach1 in the development of obesity and insulin resistance.
**Bach1 deficiency suppressed alloxan-induced pancreatic β-cell injury**

Next, to examine the effects of Bach1 deficiency on pancreatic β-cell injury induced by oxidative stress, we administered alloxan to Bach1-deficient mice at 8 weeks of age. Alloxan is an oxidative stress agent that is relatively specific to pancreatic β-cells and its administration is widely accepted as a model of diabetes caused by β-cell loss (18). When a 50mg/kg dose of alloxan was administered to WT mice intravenously, fasting blood glucose gradually rose, reaching approximately 200 mg/dl on day 6 after starting alloxan administration (Figure 3A). In contrast, fasting blood glucose levels were significantly lower in Bach1-deficient than in WT mice. In addition, glucose tolerance tests on day 6 after starting alloxan administration revealed that glucose elevation after a glucose load was consistently suppressed in Bach1-deficient mice as compared with WT mice (Figure 3B). Furthermore, pancreatic insulin contents were significantly higher, by approximately 2-fold, in Bach1-deficient than in WT mice (Figure 3C). Thus, Bach1 deficiency protected mice from alloxan-induced injury of pancreatic β-cells.

Oxidative stress is one of the most important causes of pancreatic β-cell death during the development of diabetes (19), and many studies have shown relationships between β-cell loss and ROS accumulation-induced pancreatic β-cell apoptosis (35). Therefore, to determine the effect of Bach1 on alloxan-induced β-cell apoptosis, we measured the number of TUNEL-positive cells in pancreatic islets 24 hours after alloxan administration. At this time point, pancreatic islet sizes were similar in the two groups of mice (Figure 4A, C, E, G, J). TUNEL-positive pancreatic β-cells were quite rare in both types of mice in the absence of alloxan injection (Figure 4B, F). Although TUNEL-positive cells were clearly detected in islets of both types of alloxan-treated mice (Figure 4D, H), the number of TUNEL-positive cells in Bach1-deficient mice was markedly decreased, being 60% less than in WT mice (Figure 4I). These findings indicate that Bach1 deficiency protected pancreatic β-cells from oxidative stress-induced apoptosis.

In addition, using the laser micro-dissection procedure, we evaluated mRNA expression of genes related to oxidative stress. Alloxan administration-induced HO-1 up-regulation in islets was enhanced in Bach1-deficient mice (Figure 4K). In contrast, expression of other anti-oxidative enzymes, e.g. catalase, superoxide dismutase (SOD) and glutathione peroxidase (GPx), showed no significant changes in response...
to either alloxan administration or Bach1 deficiency (Figure 4K). These findings, taken together, suggest that enhancement of HO-1 expression plays a major role in the anti-oxidant effect of Bach1 deficiency. In addition, expression of p22phox and gp91phox, both of which are subunits of NADPH oxidase, were decreased in pancreatic islets of alloxan-treated Bach1-deficient mice as compared with those of alloxan-treated WT mice (Figure 4L). These findings suggest that decreased ROS production is also involved in pancreatic β-cell protection in Bach1-deficient mice.
Discussion

The present study showed the effect of Bach1 deficiency on β-cell protection against acute loading of a chemical oxidant, alloxan, which reportedly induces apoptosis in pancreatic β-cells. Bach1 deficiency preserved insulin content and suppressed apoptosis of pancreatic islet cells, resulting in amelioration of alloxan-induced hyperglycemia. Thus, Bach1 in pancreatic β-cells is involved in oxidative stress-induced β-cell dysfunction and apoptosis, which may underlie the development of both type 1 and type 2 diabetes. In the present study, Bach1 deficiency decreased the alloxan-induced apoptosis of pancreatic islet cells. This protective effect against oxidative stress resulted in suppression of hyperglycemia in Bach1-deficient mice. These results indicate that Bach1 deficiency-induced HO-1 up-regulation prevented pancreatic β-cell deterioration induced by oxidative stress.

A putative mechanism underlying Bach1 deficiency-induced protection of pancreatic β-cells is HO-1 up-regulation. HO-1 has been identified as a ubiquitous stress protein up-regulated in many cell types by various stimulants, including hemolysis, inflammatory cytokines, oxidative stress and heat shock (26). HO-1 expression is induced by the Nrf2 system, which is suppressed in the absence of oxidative stress stimuli (17). HO-1 is the rate-limiting enzyme in the heme degradative pathway that catalyzes the oxidation of heme into biliverdin, carbon monoxide (CO) and free iron. CO as well as biliverdin and its metabolite, bilirubin, have anti-oxidant activities in vivo (32). Thus, HO-1 plays a crucial role in protection from oxidative stress (5). Induction or transgenic overexpression of HO-1 reportedly renders pancreatic islets resistant to apoptosis in islet transplantation models (34) and NOD mice (11). Administration of an HO-1 inducer decreases pancreatic superoxide contents, leading to suppression of β-cell reduction in NOD mice (22). In the present study, we observed that Bach1 deficiency induced constitutive expression of HO-1 in pancreatic islets. In addition, alloxan-induced HO-1 up-regulation was markedly promoted in Bach1-deficient mice. In contrast, other anti-oxidative enzymes were not significantly up-regulated in pancreatic islets by either Bach1 deficiency or alloxan administration. Taken together, these observations suggest that HO-1 plays important roles in the antioxidant effects of Bach1 deficiency. Since p22phox expression is reportedly up-regulated by ROS (3), the observed p22phox downregulation suggests decreased ROS levels in pancreatic islets of Bach1-deficient mice. Thus, anti-oxidant
effects induced by Bach1 deficiency may further suppress ROS production. In
addition, expression of gp91phox, which reportedly plays a major role in ROS
production in pancreatic β cells (23), was downregulated in pancreatic islets of
Bach1-deficient mice. Although the mechanism whereby Bach1 deficiency suppresses
gp91phox expression is unclear, suppression of ROS production by downregulation of
NADPH oxidase may also contribute to the pancreatic β cell protection mediated by
Bach1 deficiency. Thus, Bach1 inhibition by pharmacological strategies is a potential
therapeutic target for pancreatic β cell protection against oxidative stress, although
pharmacological reagents which suppress Bach1 expression and/or function have not
as yet been developed.

Chemical HO-1 inducers reportedly improve insulin resistance in murine
obesity models (21, 28, 29). Unexpectedly, however, Bach1 deficiency had no impact
on either insulin resistance or obesity even after long term HFD loading. Thus, in
contrast to acute induction of HO-1, congenital prolonged high expression of HO-1
might have less impact on insulin sensitivity. HO-1 is an inducible protein and is
thought to act as a defense mechanism against acute oxidative injuries, such as
inflammation, ischemia and radiation. Because the cytoprotective function of HO-1 is
manifested by reaction products such as bilirubin and CO, amounts of the substrates
heme and/or oxygen could be the limiting factor in HO-1 function (42). It is possible
that depletion of free heme (i.e., not bound to proteins) due to constitutive HO-1
expression would cancel the effects on insulin resistance in Bach1-deficient mice.
This may also explain the differential effects of Bach1 deficiency on acute injury of β
cells and chronic development of insulin resistance. In addition, differences in HO-1
expression levels may contribute to the diversity of effects in various tissues/organs.
According to an HO-1 transgenic study, low levels (2- to 5-fold) of HO-1
overexpression are protective while moderate levels (10- to 15-fold) do not modify
the cell injury caused by hyperoxia, and high-level (15-fold or more) overexpression
actually exacerbates the damage (40). In the present study, the HO-1 expression levels
in Bach1-deficient mice were increased 12-fold in the liver and 30-fold in white
adipose tissue, while the increase was 6-fold in pancreatic islets. Thus, constant and
markedly elevated HO-1 expression may deplete the necessary heme substrate,
resulting in minimal effects of Bach1 deficiency on metabolism. It has recently been
reported that sustained expression of HO-1 in cultured cells leads to profound changes
to cellular iron homeostasis (20). Such adaptive changes may also suppress the
protective function of HO-1. Alternatively, a pathway(s) other than HO-1 may exacerbate HFD-induced insulin resistance in Bach1-deficient mice, thereby cancelling out the beneficial effects of HO-1. For instance, Bach1 was reported to inhibit p53 transcriptional activity (4). Overexpression of p53 in adipose tissue reportedly causes insulin resistance via elevated inflammatory responses (27).

Collectively, chronic HO-1 elevation showed minimal effects on the development of insulin resistance induced by high-fat feeding. Thus, the proper levels and duration of HO-1 induction are important for the protection of cells from oxidative stress. Application of a chemical HO-1 inducer clinically, especially for insulin resistance, would require close attention to the levels and duration of HO-1 expression.

In conclusion, our results indicate Bach1 deficiency to have preventive effects against oxidative stress-induced β-cell injury. In addition to the numerous beneficial effects observed in Bach1-deficient mice, these findings provide clinically applicable evidence that Bach1 deficiency protects against the development of diabetes, making it a possible treatment target for diabetes prevention.
**Acknowledgements**

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**Disclosure Statement**

None


17. Kobayashi M, and Yamamoto M. Molecular mechanisms activating the


Figure Legends

Figure 1

**Bach1 deficiency exerted minimal effects on glucose metabolism under standard feeding conditions**

A, Body weight and fasting blood glucose of WT (open circles, n=12) and Bach1-deficient (closed circles, n=12) mice from 6 to 12 weeks of age. B, Blood pressure at 8 weeks of age in WT (white bar, n=16) and Bach1-deficient (black bar, n=8) mice. Fasting plasma insulin at 12 weeks of age in WT (white bar, n=12) and Bach1-deficient (black bar, n=12) mice. C, Intraperitoneal glucose (2mg/kg) tolerance tests at 10 weeks of age in WT (open circles, n=7) and Bach1-deficient (closed circles, n=7) mice. D, Insulin (0.5 units/kg) tolerance tests at 12 weeks of age in WT (open circles, n=9) and Bach1-deficient (closed circles, n=8) mice. E, HE staining of pancreatic islets of WT (left) and Bach1-deficient (right) mice at 12 weeks of age. F, Insulin contents adjusted by pancreatic weights of WT (white bar, n=8) and Bach1-deficient (black bar, n=8) mice at 12 weeks of age. G, Quantitative RT-PCR for HO-1 of pancreatic islets at 8 weeks of age (n=4 each), and in the liver and WAT at 12 weeks of age (n=8 each). WT (white bar) and Bach1-deficient (black bar). Data are presented as means ± SEM.

Figure 2

**Bach1 deficiency minimally impacts obesity and insulin resistance**

WT and Bach1-deficient mice were fed a HFD starting at 6 weeks of age. A, Body weights and fasting blood glucose values of WT (open circles, n=6-8) and Bach1-deficient (closed circles, n=8-12) mice from 6 to 24 weeks of age (n=8, each). B, Intraperitoneal glucose (2mg/kg) tolerance tests at 12 weeks in WT (open circles, n=8) and Bach1-deficient (closed circles, n=7) mice, and insulin (0.75 units/kg) tolerance tests at 13 weeks of age in WT (open circles, n=5) and Bach1-deficient (closed circles, n=5) mice. C, Fasting plasma insulin from 6 to 24 weeks of age in WT (white bar, n=6-8) and Bach1-deficient (black bar, n=8-12) mice. Data are presented as means ± SEM.

Figure 3

**Bach1 deficiency suppressed alloxan-induced hyperglycemia and reduced pancreatic insulin contents.**
Alloxan or citrate buffer (control) was intravenously injected into WT and Bach1-deficient mice at 8 weeks of age. A, Fasting blood glucose 1 hour before, and 2 and 6 days after 50mg/kg alloxan injection. WT (open circles n=30), Bach1-deficient (closed circles n=32), and WT control mice (open square, n=5). B, Intraperitoneal glucose (1mg/kg) tolerance tests 6 days after 50mg/kg alloxan injection into WT (open circles, n=19) and Bach1-deficient (closed circles n=20), as well as WT control (open squares, n=2) mice. C, Insulin content 7 days after 50mg/kg alloxan injection into WT (white bar, n=9) and Bach1-deficient (black bar, n=7), as well as WT control (gray bar, n=3) mice.

Figure 4

Suppression of apoptosis of pancreatic islet cells and up-regulation of HO-1 expression in alloxan-injected Bach1-deficient mice

Alloxan or citrate buffer (control) was injected into 8-week-old WT and Bach1-deficient mice. A to H, HE staining and TUNEL staining of pancreases taken 24 hours after injection of 50mg/kg alloxan. Paired adjacent slices were used for HE and TUNEL staining. A&B, WT control, C&D alloxan-injected WT, E&F Bach1-deficient control, G&H, alloxan-injected Bach1-deficient mice. I&J, Numbers of TUNEL-positive cells per islet slice and mean islet areas in alloxan-injected mice. The average islet count was 12 per slice for WT and 14 per slice for Bach1-deficient mice. WT (white bar n=36) and Bach1-deficient islets (black bar n=42) were measured. K, Quantitative RT-PCR for HO-1, catalase, SOD and GPx of pancreatic islets 2 hours after alloxan or citrate buffer injection. WT control (left white bar, n=4), Bach1-deficient control (left black bar, n=4), alloxan-injected WT (right white bar, n=5) and alloxan-injected Bach1-deficient mice (right black bar, n=6) were studied. L, Quantitative RT-PCR for p22 phox and gp91phox of pancreatic islets 2 hours after alloxan. Alloxan-injected WT (right white bar, n=4) and alloxan-injected Bach1-deficient mice (right black bar, n=6) were studied. Data are presented as means ± SEM.
Figure 1

A) Body Weight (g)

B) Fasting Blood Glucose (mg/dl)

C) Blood Glucose (mg/dl)

D) Blood Glucose Level (% of Initial Level)

E) Images of WT and Bach1 KO tissues

F) Insulin Content (μg/g)

G) HO-1/GAPDH mRNA ratio

WT KO WT KO WT KO

* **
Figure 2

A

Body Weight (g) vs. weeks

Fasting Blood Glucose (mg/dl) vs. weeks

B

Blood Glucose (mg/dl) vs. min

Blood Glucose Level (% of Initial Level) vs. min

C

Fasting Plasma Insulin (ng/ml) vs. time

WT KO

6w (0w HFD)

12w (6w HFD)

16w (10w HFD)

24w (18w HFD)
Figure 3

A

Fasting Blood Glucose (mg/dl)

WT Alloxan (-)  WT Alloxan (+)  KO Alloxan (+)

0 2 6 days

B

Blood Glucose (mg/dl)

WT Alloxan (-)  WT Alloxan (+)  KO Alloxan (+)

0 15 30 60 120 min

C

Insulin Content (μg/g)

WT  WT  KO

Alloxan (-)  (interaction)
Figure 4

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I

![Graph showing TUNEL positive cells per islet section](image)

J

![Graph showing islet area (×10^6 μm^2)](image)
Figure 4

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K

HO-1/GAPDH mRNA Ratio

Catalase/GAPDH mRNA Ratio

SOD/GAPDH mRNA Ratio

GPx/GAPDH mRNA Ratio

L

p22phox/GAPDH mRNA Ratio

gp91phox/GAPDH mRNA Ratio
Table 1

The oligonucleotide primers used in Quantitative RT-PCR

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