The effect of pancreatic beta cell-specific SIRT6 deficiency on insulin secretion

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The effect of pancreatic beta cell-specific SIRT6 deficiency on insulin secretion

Directed by Professor Eun Seok Kang

The Doctoral Dissertation
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy

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June 2016

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June 2016

ACKNOWLEGEMENTS

I would like to express my deepest gratitude towards my supervisor, professor Eun Seok Kang. As a mentor, professor Kang gave me great opportunities and has supported, encouraged, and guided me throughout the course of my research. I am also deeply grateful to professor Chul Hoon Kim and professor Hyangkyu Lee for their critical advice, inspiration, and kind support as I worked to finish this thesis. I would also like to extend my great appreciation to professor Je-Wook Yu and professor Ho-Seong Kim for providing me with important advice during the course of my research.

Lastly, I would like to express my special appreciation to my parents for their endless love, constant encouragement, and great support. Also, I would like to thank my little brother, Yoon Han Kim, for always giving me warm encouragement. This thesis is dedicated to my family and to all those who supported me in any respect during the completion of the project.

June 2016

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ABSTRACT

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Sirtuin 6 (SIRT6), a NAD^+-dependent deacetylase, is known to play a key role in glucose metabolism. In a previous study, mice with SIRT6 deficiency in pancreatic beta cells showed impaired glucose-stimulated insulin secretion (GSIS) with mitochondrial defects and aberrant calcium flux in beta cells. However, the effects of beta cell-specific SIRT6 in glucose metabolism and the precise mechanism of insulin synthesis and the secretion pathway in pancreatic beta cells remain uncertain. To investigate the role of SIRT6 in pancreatic beta cells, we produced pancreatic beta cell-specific SIRT6 knockout mice. Pancreatic beta cell-specific SIRT6 knockout mice developed glucose intolerance with impaired GSIS without morphological change in islets. Isolated islets from beta cell-specific SIRT6 knockout mice secreted ~40% less insulin upon glucose stimulation, mainly in the

second phase of GSIS. Depletion of SIRT6 in beta cells revealed a remarkable reduction in *Insulin-1* mRNA expression level under glucose stimulation (60 min). Furthermore, we found that exenatide, a glucagon-like peptide-1 (GLP-1) receptor agonist, increased *Insulin-1* mRNA expression level and partially restored the impairment of glucose tolerance in beta cell-specific SIRT6 knockout mice. Altogether, these findings indicate that pancreatic beta cell-specific SIRT6 plays a critical role in insulin synthesis under glucose stimulation which is related to both GSIS and glucose tolerance, and SIRT6 deficiency may contribute to beta cell dysfunction and to mechanisms leading to diabetes.

Key words: sirtuin 6, diabetes, pancreatic beta cell, insulin, glucose metabolism

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I. INTRODUCTION

Sir2 family proteins, called sirtuins in yeast, regulate gene silencing, DNA repair, and longevity as histone deacetylases.[1-3] In mammals, there are seven sirtuins (SIRT1-7) genes with diverse cellular functions including genomic stability, DNA repair, energy metabolism, stress resistance, aging, and tumorigenesis.[4-6]

SIRT6, found in the nucleus and tightly bound to chromatin, serves as a NAD^+-dependent deacetylase of histone H3 lysine 9 (H3K9) and H3 lysine 56 (H3K56).[7-11] Therefore, histone deacetylation results in the transformation of chromatin and in decreased chromatin accessibility, ultimately regulating the dynamic binding of DNA repair factors to chromatin, telomeric modulation, and gene expression.[11,12] In

addition, SIRT6 deacetylates non-histone substrates, including forkhead box O1 (FoxO1) and general control of amino acid synthesis protein 5 (GCN5).[13,14]

SIRT6 plays a key role in glucose and lipid metabolism.[13-16] In a diabetic state hepatic SIRT6 activity was markedly reduced, and this reduction was related to the increased activation of the gluconeogenic gene expression of peroxisome proliferator-activated receptor-γ coactivator 1α (PGC 1α), phosphoenolpyruvate carboxykinase (PEPCK), and glucose-6-phosphatase (G6Pase) when compared with the non-diabetic state.[13] In addition, increased hepatic SIRT6 expression suppressed gluconeogenic gene expression and caused a significant decrease in the serum glucose levels of fasted diabetic mice without altering liver insulin/AKT pathway signalling. In human non-alcoholic fatty liver samples, SIRT6 levels are significantly lowered compared with normal controls, as liver-specific absence of SIRT6 causes increased glycolysis, triglyceride synthesis, and fatty liver formation.[16] Moreover, a recent study revealed that pancreatic beta cell-specific SIRT6 regulated cytosolic calcium dynamics, mitochondrial function, and glucose-stimulated insulin secretion.[17] However, the specific role played by beta cell-specific SIRT6 in insulin synthesis and secretion has not been elucidated.

Therefore, the present study focused on generating beta cell-specific SIRT6 knockout mice in order to investigate beta cell-specific SIRT6 effects on aspects of glucose metabolism, including insulin synthesis and secretion. Ultimately, a better understanding of beta cell-specific SIRT6 would promote new therapeutic approaches for patients with type 2 diabetes mellitus.

II. MATERIALS AND METHODS

1. Animals

A. Generation of pancreatic beta cell-specific *Sirt6* knockout mice

We generated mice with SIRT6 expression exclusively in beta cell of pancreas by crossing B6.Cg-Tg(Ins2-Cre)25Mgn/J (RIP-Cre) mice from the Jackson Laboratory with *Sirt6* floxed (*Sirt6*$^{Tm1.1Cxd}$/J; control) mice[16] to generate *Sirt6* heterozygous mice (β*Sirt6*$^{+/-}$:RIP-Cre^{+}; β*Sirt6* heterozygous) and then backcrossing β*Sirt6* heterozygous mice with *Sirt6* floxed mice to generate beta cell-specific *Sirt6* knockout mice (β*Sirt6*$^{-/-}$; β*Sirt6*KO). Mice carrying a recombinant allele of *Sirt6* were identified by polymerase chain reaction (PCR) using primers P1 (5'-GCT AAT GGG AAC GAG ACC AA-3') and P3 (5'-GCG TCC ACT TCT CTT TCC CTG-3'), which detect a fragment of 524 bp (Figure 1).

B. Animal study

The animals were given free access to regular chow and water, and were maintained at a temperature of 21 ± 2 ℃ and a humidity of 60 ± 10% on 12-hour light/dark cycles. We used 4- to 16-week-old male and female mice. Body weights were measured once a week over the course of the experimental period. Blood glucose levels were measured weekly after 18 hours of overnight fasting. After 16 weeks, the animals were euthanized, and blood was collected via heart puncture. All animal studies were approved by the Animal Care and Use Committee of the Yonsei University College of Medicine (No. 2013-0265-4).

C. Oral glucose tolerance, insulin tolerance, and pyruvate tolerance test

To perform the oral glucose tolerance test, animals were administered 40% glucose (2 g/kg body weight) bolus orally following 18 hours of fasting, and blood samples were collected via tail nick at 0, 15, 30, 60, 90, and 120 minutes after glucose administration. Insulin tolerance test was performed by intraperitoneal injection of human regular insulin (0.75 units/kg) (Sigma-Aldrich, Oakville, ON, Canada) following 3 hours of fasting. Blood samples were obtained from the tail vein before and 15, 30, 60, 90, and 120 minutes after insulin administration. Pyruvate tolerance test was performed in mice fasted for 18 hours via intraperitoneal injection of 2 g/kg sodium pyruvate (Sigma-Aldrich, Oakville, ON, Canada). Blood samples were collected from the tail vein before and 15, 30, 60, 90, and 120 minutes after pyruvate administration. Glucose concentrations were measured using an Accu-Chek Performa® glucometer (Boehringer-Mannheim, Indianapolis, IN, USA).

D. Intraperitoneal glucose tolerance test

To assess the effect of glibenclamide and exenatide on the intraperitoneal glucose tolerance test in control and β*Sirt6*KO mice, glibenclamide (1.5 mg/kg, diluted in ethanol) or exenatide (10 nM/kg, diluted in saline) was intraperitoneally injected following 18 hours of fasting, and vehicle-treated group was intraperitoneally injected with the same volume of ethanol or saline, respectively. Animals were intraperitoneally administered 20% glucose (2 g/kg body weight) 30 minutes after the administration of glibenclamide or exenatide, and blood samples were collected via tail nick at 0, 15, 30, 60, 90, and 120 minutes after glucose administration.

2. Glucose-stimulated insulin secretion in isolated pancreatic islets

Pancreatic islets were isolated from β*Sirt6*KO mice at 12 weeks of age using the collagenase digestion technique.[18] In brief, pancreases were inflated with an isolation buffer (10×Hank's buffered salt solution, 10 mM 4-2-hyroxyethyl-1-piperazine ethane sulfonic acid [HEPES], 1 mM $MgCl_2$, 5 mM glucose, pH 7.4) containing 0.375 mg/ml collagenase (Sigma, St Louis, MO, USA) via the pancreatic duct after clamping off its entry site to the duodenum. The inflated pancreases were then removed, incubated in a 37℃ water bath for 15 min, and shaken vigorously. Islets were separated from acinar tissue by discontinuous gradient purification after a series of washes, and then hand-picked using a microscope. Handpicked islets were cultured overnight 24 hours in RPMI media containing 2 mM L-glutamine, penicillin/streptomycin, and 10% fetal bovine serum (FBS; Gibco, Invitrogen, Carlsbad, CA, USA) at 37℃ in a humidified incubator containing 5% CO2 and 95% air. The islets were then preincubated in Krebs-Ringer bicarbonate (KRB) buffer (115 mM NaCl, 4.7 mM KCl, 2.6 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 5 mM $NaHCO_3$, 10 mM HEPES, 0.2% BSA [pH 7.4]) for 30 minutes at 37℃. Islets of similar size were handpicked into triplicate groups of 35 or 50 islets from each mouse and incubated with a 1 ml KRBH buffer containing 15 mM glucose for 1 hour at 37℃. The supernatant was collected 0, 5, 15, 30, and 60 minutes after incubation and secreted insulin was measured by enzyme-linked immunosorbent assay (ELISA) using mouse insulin as a standard (Merck Millipore, Darmstadt, Germany). When investigating the effect of glibenclamide or exenatide on insulin secretion and gene mRNA expression, 600 nM glibenclamide or 100 nM exenatide was administered 30 minutes before glucose administration.

3. RNA isolation and real-time polymerase chain reaction (RT-PCR) analysis

Total RNA was isolated from the cells by using the RNeasy Mini Kit (#74104; Qiagen, Valencia, CA, USA) and then reverse transcribed into complementary DNA (cDNA) using 2 μg of total RNA with QuantiTect Reverse Transcription kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The cDNA was then amplified in the Takara Thermal Cycler Dice® Real-Time system (Otsu, Shiga, Japan) using SYBR Premix Ex Taq (Takara, Shiga, Japan) under the following cycling conditions: 40 cycles of 95 ℃ for 5 seconds, 58 ℃ for 10 seconds, and 72 ℃ for 20 seconds. The average of triplicate assays was calculated and, target gene expression was normalized to that of the glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*). The following sets of primers were used for RT-PCR (Table 1).

Table 1. Primer sequences used in this study

Primers	Sequences	
Insulin-1	Forward	5’-GGA CCC ACA AGT GGA ACA AC-3’
	Reverse	5’-GCT GGT AGA GGG AGC AGA TG-3’
RhoGDI	Forward	5′-CGG GTG AAC AGA GAG ATC GT-3′
	Reverse	5′-CTC CAT GGG TGT CAG GAA CT-3’
Munc18c	Forward	5’-CTG AGA AAG GAT CGG TCT GC-3’
	Reverse	5’-ACA ATA CGG CCA CTC TTT GG-3’
Cdc42	Forward	5’-GGA CCC ACA AGT GGA ACA AC-3’
	Reverse	5’-GCT GGT AGA GGG AGC AGA TG-3’
Pak1	Forward	5′-TGA ACC GCT GTC TTG AGA TG-3’
	Reverse	5’-TCT TGG TTG CCT CTT TTG CT-3’
Gapdh	Forward	5’-GGC ATT GCT CTC AAT GAC AA-3’
	Reverse	5’-ATG TAG GCC ATG AGG TCC AC-3’

Gadph, glyceraldehyde 3-phosphate dehydrogenase; Pak1, p21-activated kinase1; RhoGDI, Rho Guanosine Diphosphate-dissociation Inhibitor.

4. Islet morphometry

Pancreases from 16 week-old male mice were fixed with 10% formalin (neutralized buffer), paraffin embedded, and longitudinally sectioned at 5 μm thickness and 100 μm intervals for hematoxylin and eosin (H&E) staining. Using ImageJ software (Wayne Rasband, Research Services Branch, National Institute of Mental Health, Bethesda, MD, USA), the relative islet area as a proportion of the total stained pancreatic section and the number of islets were determined in pancreatic sections captured at 1.45× magnification with a microscope.

5. Statistical analyses

All data were expressed as mean ± standard error of the mean (SEM). Student's t test was used to compare the control and βSirt6KO mice. A *p*-value of < 0.05 is considered statistically significant. Data analysis was carried out using Prism 5.0 software (GraphPad Software, La Jolla, CA, USA) and PASW Statistics version 20.0 for Windows (SPSS Inc., Chicago, IL, USA).

III. RESULTS

1. Pancreatic beta cell-specific knockout results in impaired glucose tolerance

We found that control and β*Sirt6*KO mice gradually grew in body weight during the study, and no significant difference was noted between the two groups in either gender (Figure 2). Blood glucose concentration after 18 hours of fasting did not differ between control and *Sirt6*KO mice in either male or female mice during the period from 4 to 16 weeks of age (Figure 3). We performed oral glucose tolerance tests (OGTTs) after 18 hours of fasting at 4, 8, 12, and 16 weeks of age. β*Sirt6*KO mice showed markedly higher levels of blood glucose 30 minutes after glucose load than control mice starting at 4 weeks of age in both genders (Figure 4). Insulin tolerance tests (ITTs) were performed on 15-week-old mice to assess systemic insulin resistance, and the area under the curve (ITT-AUC) did not differ between control and β*Sirt6*KO mice (Figure 5). Pyruvate tolerance tests (PTTs) and AUC analysis also showed no significant difference between control and β*Sirt6*KO mice (Figure 6). Therefore, ITTs and PTTs demonstrated that β*Sirt6*KO mice had similar whole-body insulin sensitivity and hepatic gluconeogenesis to control mice.

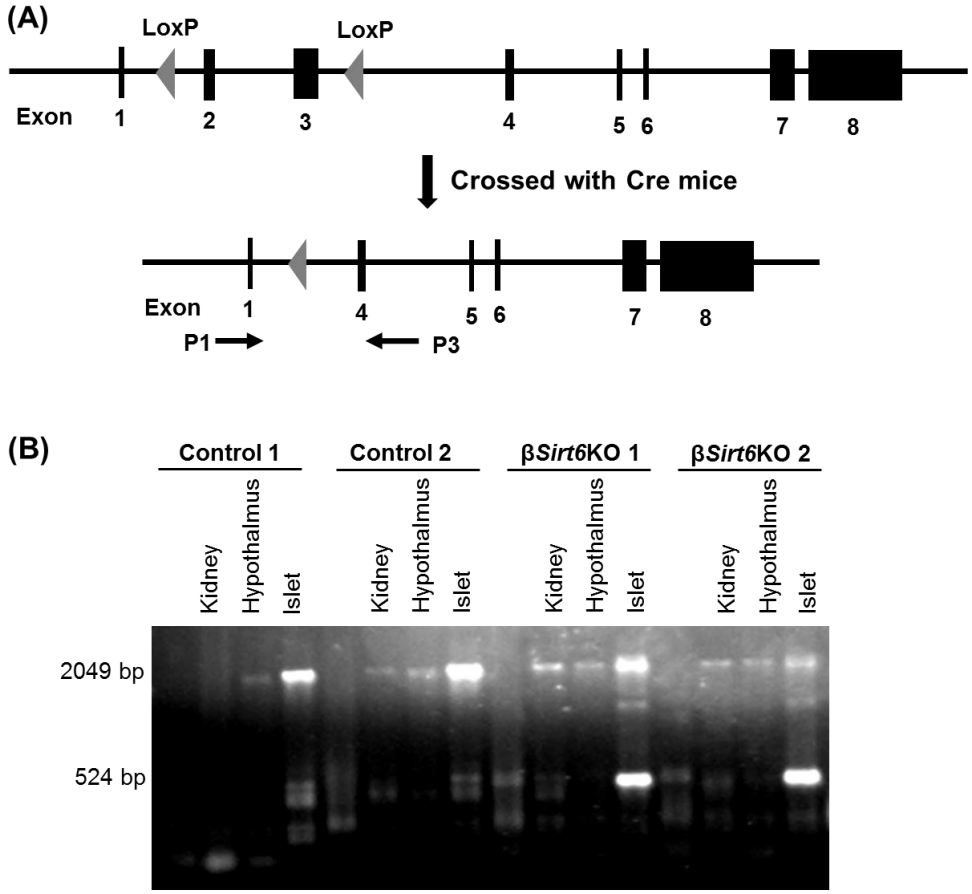

Figure 1. Generation of β cell-specific SIRT6 knockout mice. (A) Schematic diagram of *Sirt6* gene with loxP sites and primers. A loxP site was inserted upstream of exon 2, and another loxP site was inserted downstream of exon 3. Cre-medicated recombination resulted in deleting exon 2 and exon 3 of *Sirt6* gene. (B) Qualitative PCR analysis using primer pair which detects 524 bp Cre-deleted bands in isolated islets from β*Sirt6*KO mice.

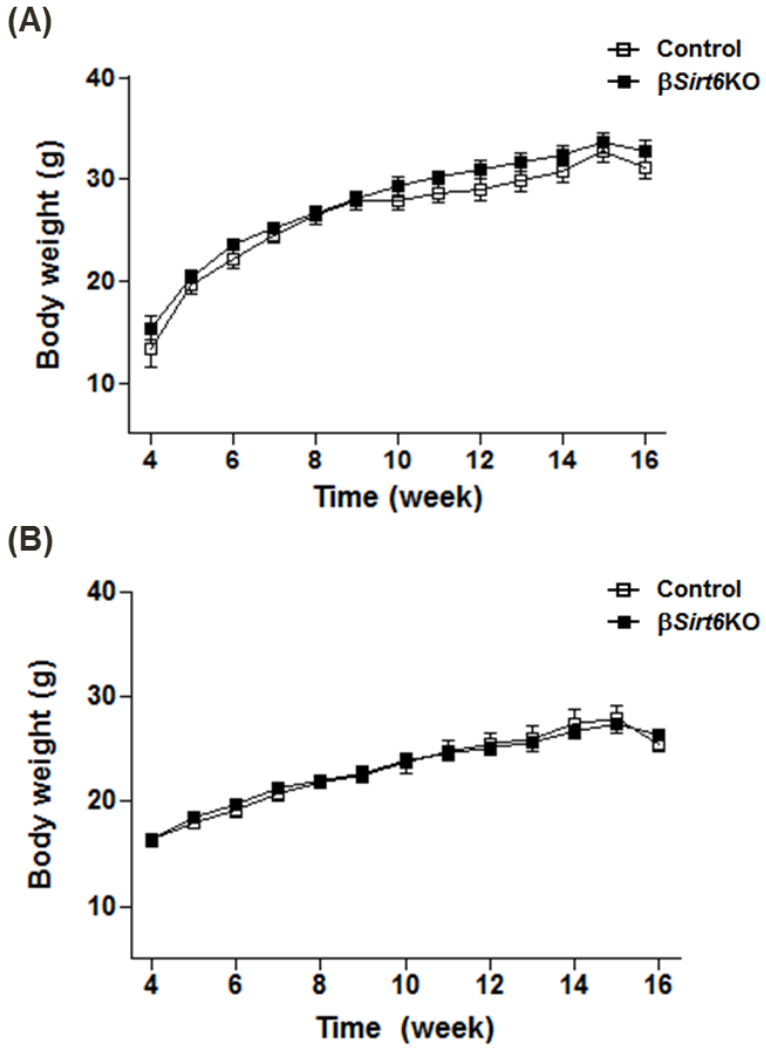

Figure 2. Body weight of (A) male and (B) female β*Sirt6*KO mice and littermates between 4 and 16 weeks of age. n = 10 each.

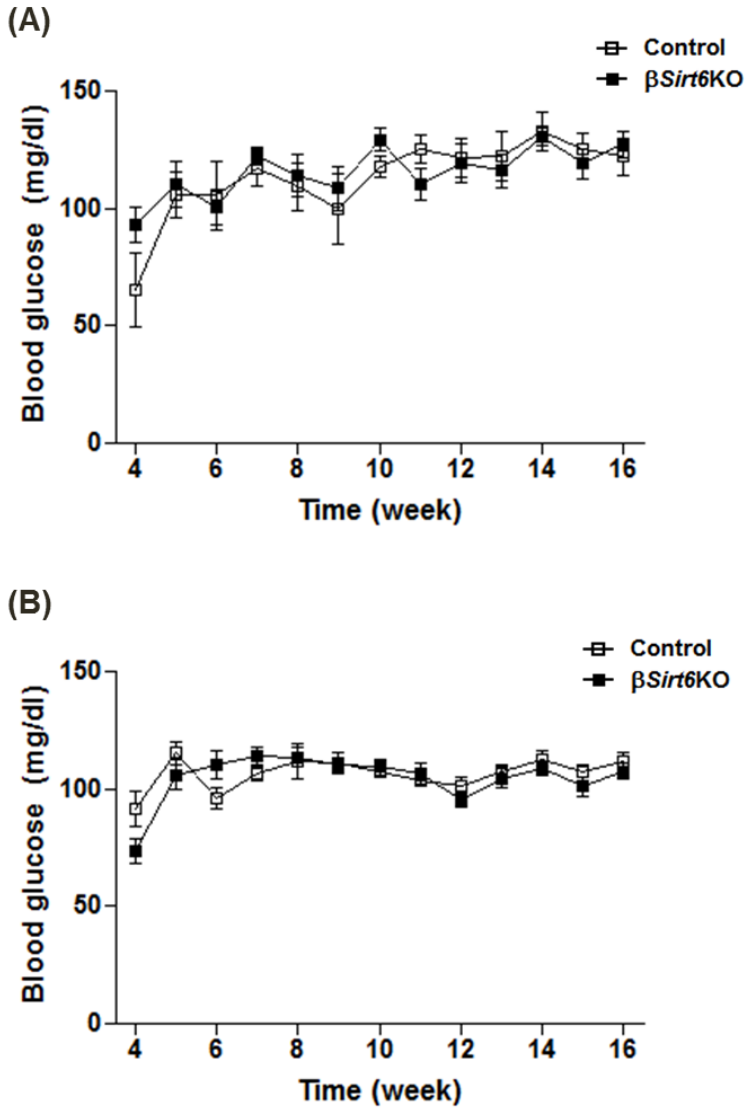

Figure 3. Fasting blood glucose levels after 18 hours of fasting in (A) male and (B) female mice. n = 10 each.

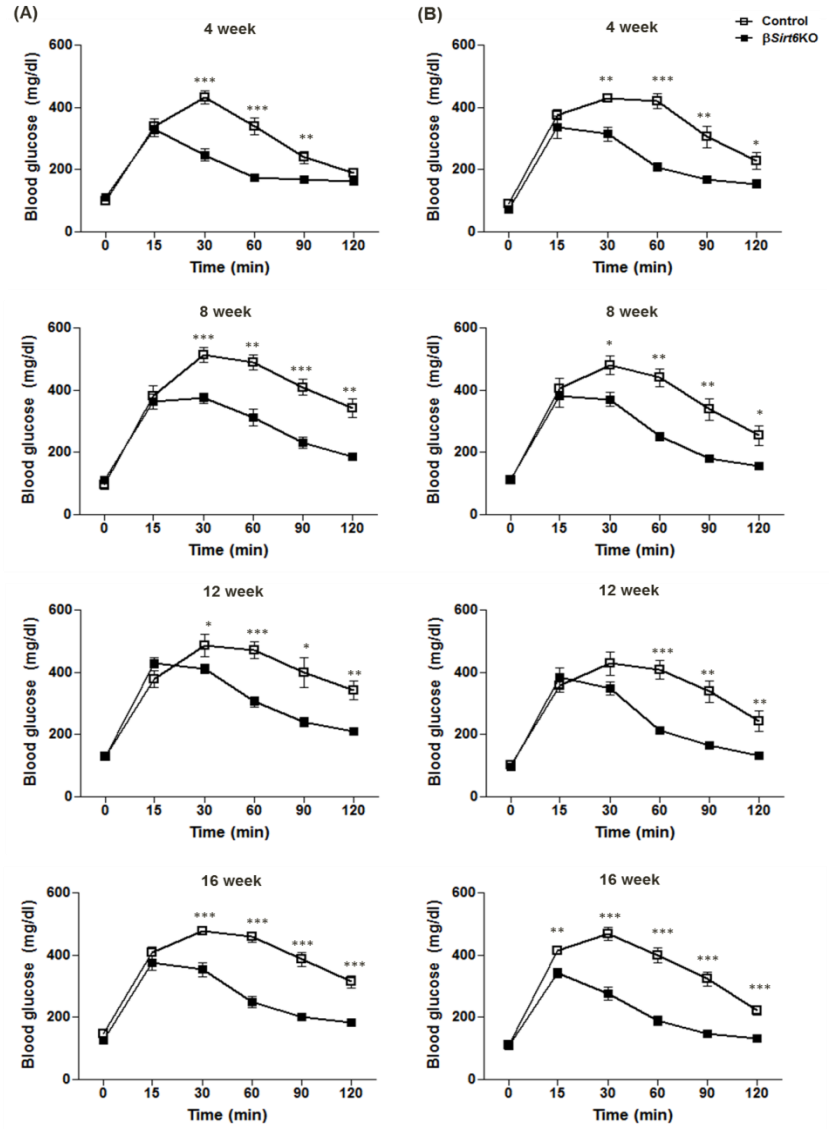

Figure 4. Oral glucose tolerance test after 18 hours of fasting in 4-, 8-, 12-, and 16-week-old (A) male and (B) female mice. Serum glucose levels after an oral administration of glucose (2 g/kg body weight). (A) n = 15-20 for male mice. (B) n = 29-30 for female mice. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ (*, comparison between control and β*Sirt6*KO mice).

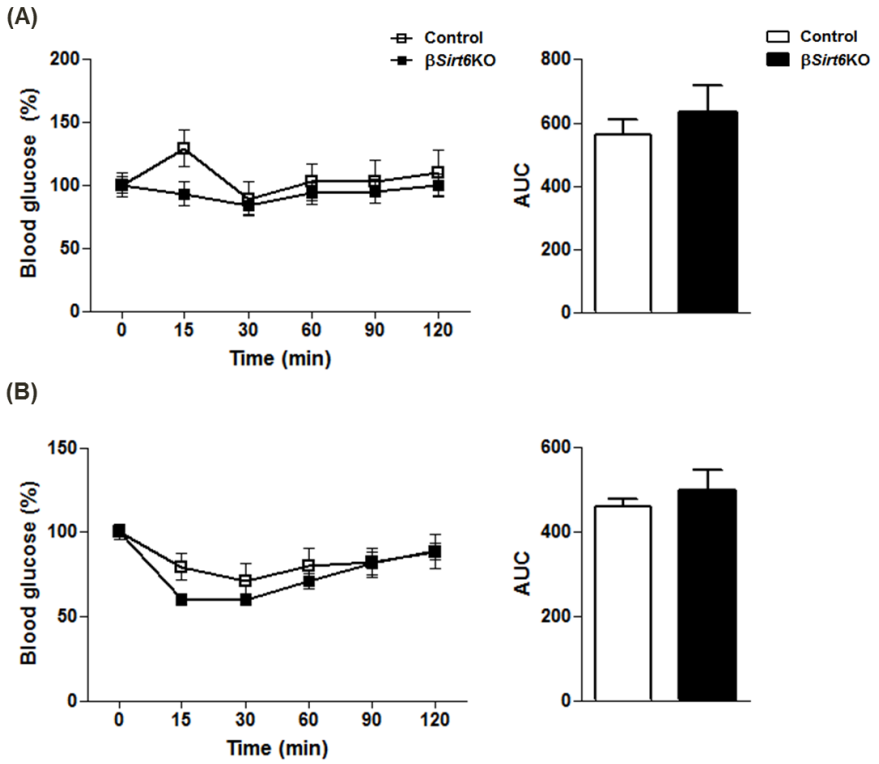

Figure 5. Insulin tolerance test and area under the curve (ITT-AUC) after 3 hours of fasting in 15-week-old (A) male and (B) female mice. Serum glucose levels after intraperitoneal injection of human regular insulin (0.75 units/kg) in 3-hour fasted 15-week-old mice. (A) n = 15 for male mice (control, n = 10; β*Sirt6*KO, n = 5). (B) n = 10 each (control, n = 10; β*Sirt6*KO, n = 10).

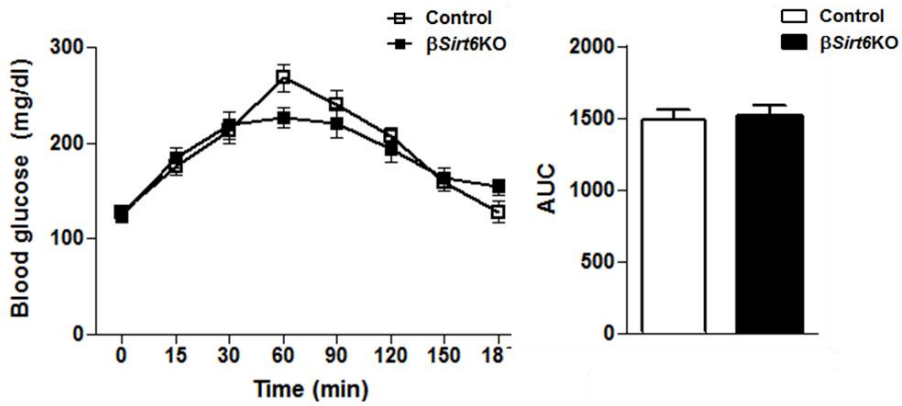

Figure 6. Pyruvate tolerance test and area under the curve (PTT-AUC) in 15-week-old male mice. Serum glucose levels after intraperitoneal injection of 2 g/kg sodium pyruvate in 18-hour fasted 15-week-old male mice (control, n = 9; β*Sirt6*KO, n = 10).

2. Effect of SIRT6 knockout on islet morphology

We conducted morphological analysis of pancreatic islets in β*Sirt6*KO mice to exclude effect on pancreatic morphometry as a cause of the impaired glucose homeostasis. The islet area relative to the total area of the pancreas and the number of islets in 16-week-old mice were measured. We found no significant difference in relative islet area and the number of islets in β*Sirt6*KO mice compared with control mice (Figure 7). Therefore, these findings showed that the *in vivo* defects in glucose homeostasis were independent of islet morphometry.

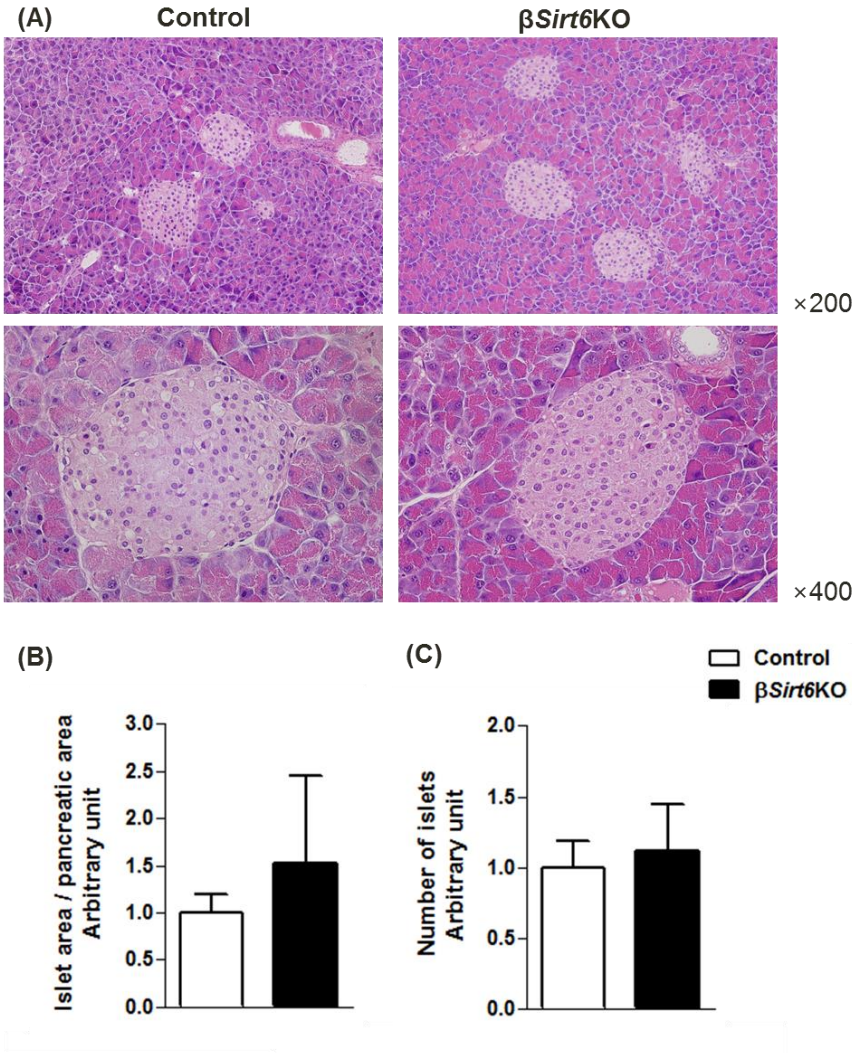

Figure 7. Morphometric analysis of islets in β*Sirt6*KO mice. (A) Pancreas sections stained with hematoxylin and eosin, (B) relative islet area, (C) number of islet in 16-week-old male control and β*Sirt6*KO mice (control, n = 4; β*Sirt6*KO, n = 3).

3. Defective glucose-stimulated insulin secretion in SIRT6 knockout beta cell

We next measured serum insulin levels *in vivo* following OGTTs in order to determine the function of islets in mice at 8, 12, and 16 weeks of age. In both male and female β*Sirt6*KO mice, we observed blunted levels of insulin following OGTTs beginning at 8 weeks of age when compared with control mice (Figure 8). We also performed glucose-stimulated insulin secretion (GSIS) analysis *ex vivo* in response to 15 mM glucose in static cultures with isolated islets from 12-week-old male mice. β*Sirt6*KO islets showed a slight increase of insulin secretion from baseline at 5 minutes following glucose stimulation, but further increases in insulin levels were not exhibited from 15 minutes to 60 minutes following glucose stimulation, whereas insulin levels continued to increase in control islets (Figure 9). This significant difference in insulin levels began to appear at 15 minutes following glucose stimulation between control and β*Sirt6*KO islets. Also, insulin levels of β*Sirt6*KO islets reached 60% of those of control islets at 60 minutes after glucose stimulation. Collectively, GSIS was significantly impaired in β*Sirt6*KO islets when compared with control islets.

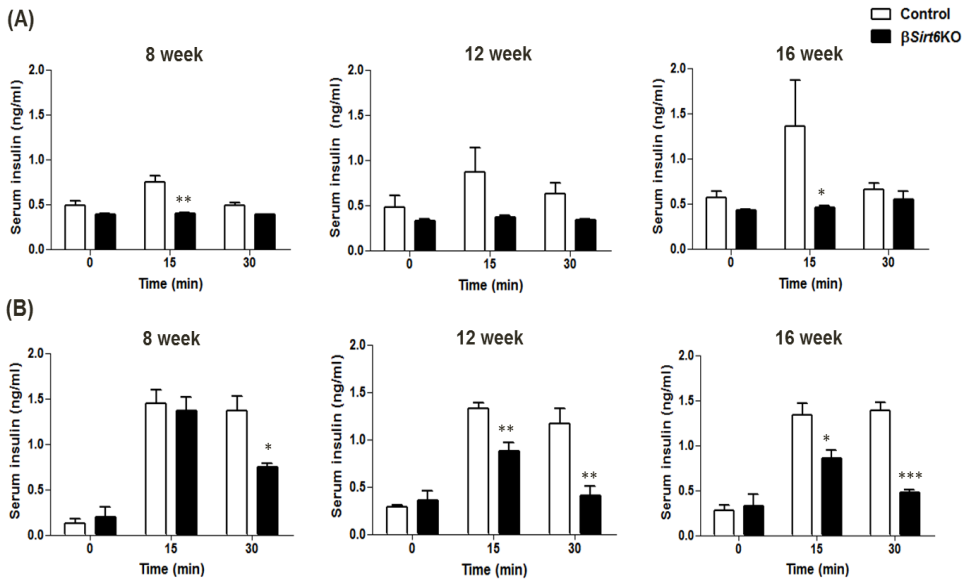

Figure 8. Defective glucose-stimulated insulin secretion *in vivo* in mice lacking SIRT6 in beta cell. Serum insulin levels after oral administration of glucose (2 g/kg body weight) in 18-hour fasted 8-, 12-, and 16-week-old (A) male and (B) female mice were determined by ELISA (n = 4 each). *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ (*, comparison between control and β*Sirt6*KO mice).

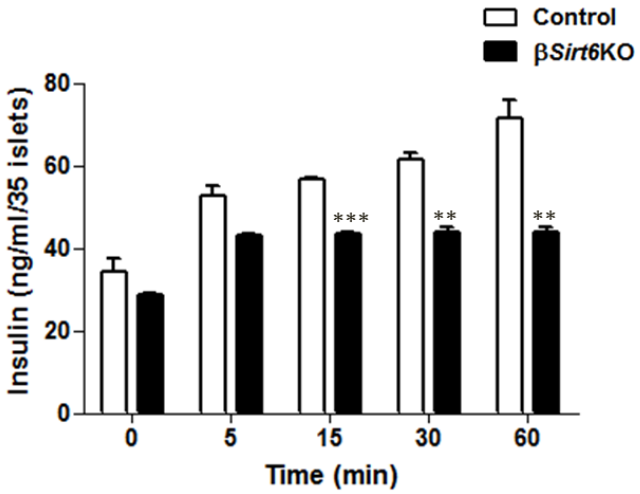

Figure 9. Defective glucose-stimulated insulin secretion *ex vivo* from beta cell-specific SIRT6 knockout islets. Insulin secretion in 60-minute static incubation experiments at 15 mM glucose from isolated islets of 12-week-old control and β*Sirt6*KO mice (control, n = 3; β*Sirt6*KO, n = 4). Recordings were made from each group of 35 islets of each mouse. $^{**}p < 0.01$ and $^{***}p < 0.001$ (*, comparison between control and β*Sirt6*KO mice).

4. Defective insulin gene expression in mice lacking SIRT6 in pancreatic beta cell

To further assess the GSIS defect in β*Sirt6*KO islets, we examined gene expression related to both insulin synthesis and insulin granule exocytosis under glucose-stimulated conditions. Before and after 60 minutes following administration of 15 mM glucose, *Insulin-1* mRNA and other gene expressions involved in insulin granule exocytosis in the first or the second phase of GSIS such as *Syntaxin 1* for the first phase, and *Cdc42, Rho Guanosine Diphosphate-dissociation Inhibitor (RhoGDI), p21-activated kinase1* (*Pak1),* and *Munc18c* mRNAs for the second phase were quantified by RT-PCR from islets isolated from male control mice and β*Sirt6*KO mice at 12 weeks of age. In control islets, *Insulin-1* mRNA levels increased 3.7-fold 60 minutes after glucose stimulation when compared with levels before stimulation (Figure 10). However, no increase in *Insulin-1* mRNA levels was shown in β*Sirt6*KO islets 60 minutes after glucose stimulation; in fact, a significant loss of *Insulin-1* mRNA was noted in β*Sirt6*KO islets 60 minutes after glucose stimulation when compared with control islets. Before glucose stimulation, mRNA levels of *Syntaxin1,* which is specific to insulin granule exocytosis in the first phase of GSIS, were lower in β*Sirt6*KO islets compared to control islets, but no significant difference was revealed (Figure 11). *Cdc42* mRNA relative levels of control and β*Sirt6*KO islets were similar and mRNA levels of *RhoGDI*, which suppresses the pathway of insulin granule exocytosis in the second phase of GSIS, were slightly but not significantly higher before glucose stimulation when compared with control islets. Before glucose stimulation, *Pak1* and *Munc18c* mRNA expression levels in β*Sirt6*KO islets tended to be lower than in control islets, but a significant difference was noted only in *Munc18c* mRNA levels. We also observed a tendency for *Munc18c* mRNAs to decrease at 60 minutes after glucose stimulation in β*Sirt6*KO islets relative to control islets, but this decrease was not significant (Figure 12). Taken together, these results show that deletion of SIRT6 in pancreatic beta cells

causes a remarkable decrease in *Insulin-1* gene expressions in response to glucose stimulation, and it may contribute to impaired GSIS *ex vivo* and glucose intolerance *in vivo*.

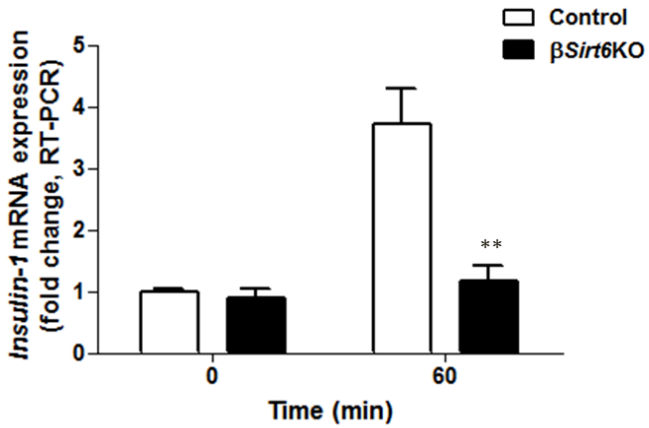

Figure 10. Reduction in *Insulin-1* gene mRNA in response to glucose in β*Sirt6*KO islets. Isolated islets from 12-week-old control and β*Sirt6*KO mice were treated with 15 mM glucose in 60-minute static incubation experiments. Expression levels of *Insulin-1* mRNA from islets of control and β*Sirt6*KO mice were quantitated by RT-PCR (n = 6 each for 0 min, n = 4 each for 60 min). Recordings were made from each group of 50 islets of each mouse. All mRNA levels were normalized to *Gapdh* mRNA. $^{**}p < 0.01$ (*, comparison between control and β*Sirt6*KO mice).

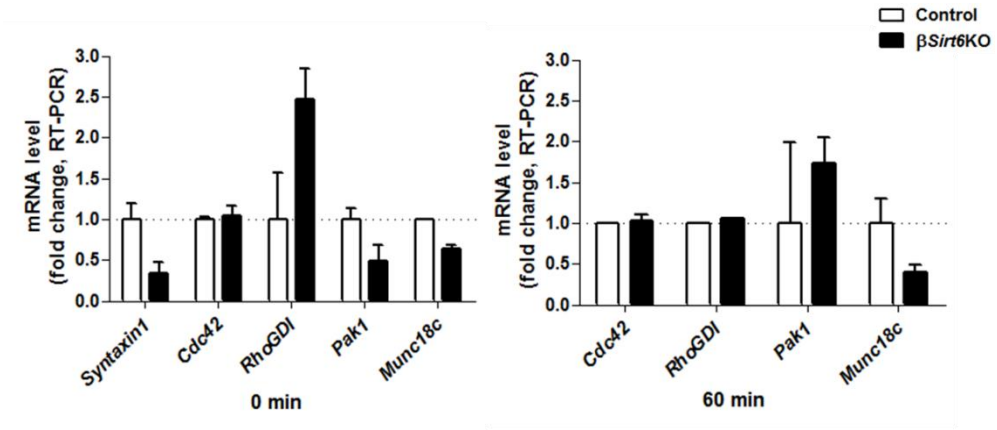

Figure 11. mRNA expression accounting for insulin granule exocytosis during glucose stimulation in β*Sirt6*KO islets. Isolated islets from 12-week-old control and β*Sirt6*KO mice were treated with 15 mM glucose in 60-minute statin incubation experiments. Expression levels of *Syntaxin1* mRNA for insulin granule exocytosis of the first phase, and *Cdc42, RhoGDI, Pak1, Munc18c* for that of the second phase of GSIS from control and β*Sirt6*KO islets were quantitated by RT-PCR (n = 3-4 each for 0 min, n = 1-4 each for 60 min). Recordings were made from each group of 50 islets of each mouse. All mRNA levels were normalized to *Gapdh* mRNA. ***p* < 0.01 (*, comparison between control and β*Sirt6*KO mice).

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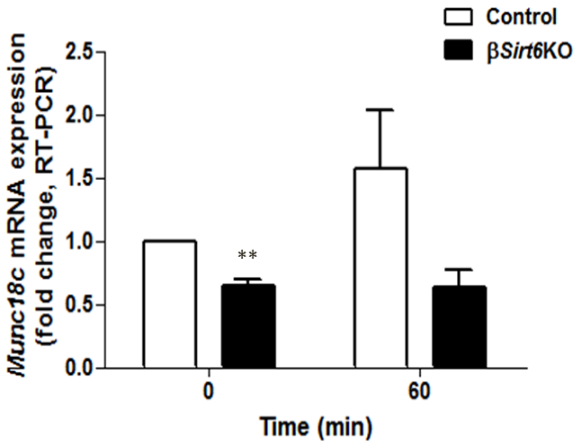

Figure 12. mRNA expression of *Munc18c* in response to glucose stimulation in β*Sirt6*KO islets. Isolated islets from 12-week-old control and β*Sirt6*KO mice were treated with 15 mM glucose in 60-minute statin incubation experiments. Expression levels of *Munc18c* mRNA from islets of control and β*Sirt6*KO mice were quantitated by RT-PCR (n = 2-3 each for 0 min, n = 4 each for 60 min). Recordings were made from each group of 50 islets of each mouse. All mRNA levels were normalized to *Gapdh* mRNA. ***p* < 0.01 (*, comparison between control and β*Sirt6*KO mice).

5. Effect of glibenclamide and exenatide on glucose tolerance and insulin secretion in mice lacking SIRT6 in pancreatic beta cells

Next, to determine whether the impairment of GSIS in β*Sirt6*KO islets is dependent on either insulin synthesis or secretion in response to glucose stimulation, we investigated the effects of glibenclamide, a type of sulfonylurea, and exenatide, a glucagon-like peptide-1 (GLP-1) receptor agonist, on pancreatic beta cell-specific SIRT6 knockout mice. Glibenclamide is known to affect insulin secretion, and exenatide is known to affect both insulin synthesis and secretion. As shown in Figure 13 (A), glibenclamide treatment 30 minutes before glucose administration by intraperitoneal glucose tolerance tests (IPGTTs) *in vivo* revealed that glibenclamide reduced levels of glucose 15 minutes after administration, but after 60 minutes no effect was noted. We also measured insulin secretion *ex vivo* using 60-minute static incubation experiment with 15 mM glucose from isolated islets (Figure 13 (B)). In this experiment glibenclamide treatment increased insulin concentration in β*Sirt6*KO islets for the first 15 minutes of incubation, after which insulin concentrations stayed constant. Next, when we performed IPGTTs after exenatide treatment, exenatide treatment improved glucose tolerance in β*Sirt6*KO mice from shortly after glucose administration until 120 minutes following glucose administration (Figure 14). Moreover, we found that exenatide treatment reduced glucose levels in β*Sirt6*KO mice to the levels of control mice. Therefore, the improvement of impaired glucose tolerance of β*Sirt6*KO mice may be attributed to the effect of insulin synthesis rather than insulin secretion, due to the fact that it was affected by exenatide treatment and not by glibeclamide treatment.

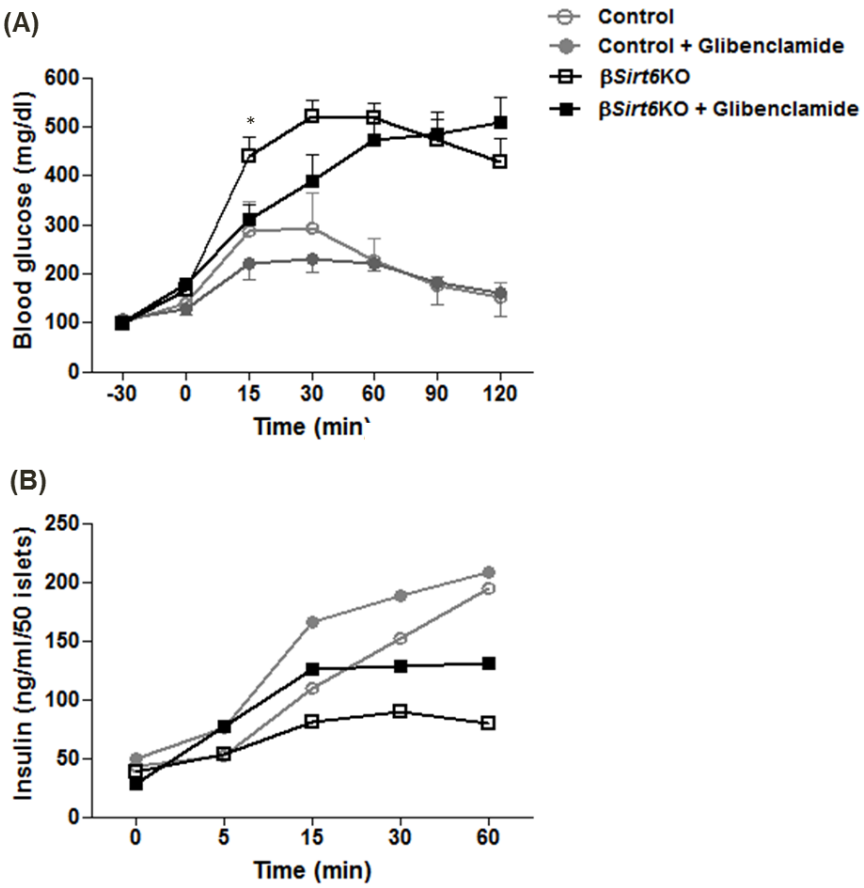

Figure 13. Effect of glibenclamide on glucose tolerance and insulin secretion in β*Sirt6*KO mice. (A) Intraperitoneal glucose tolerance test following glibenclamide treatment of 12-week-old male mice. After 18 hours of fasting, mice were intraperitoneally injected with 1.5 mg/kg of glibenclamide 30 minutes before intraperitoneal glucose injection (n = 4 each). *$p < 0.05$ (*, comparison between βSirt6KO and βSirt6KO + glibenclamide). (B) Isolated islets from 12-week-old control and β*Sirt6*KO mice treated with 15 mM glucose for 60 minutes. 600 nM glibenclamide was administered 30 minutes before glucose administration. Insulin levels were determined by ELISA.

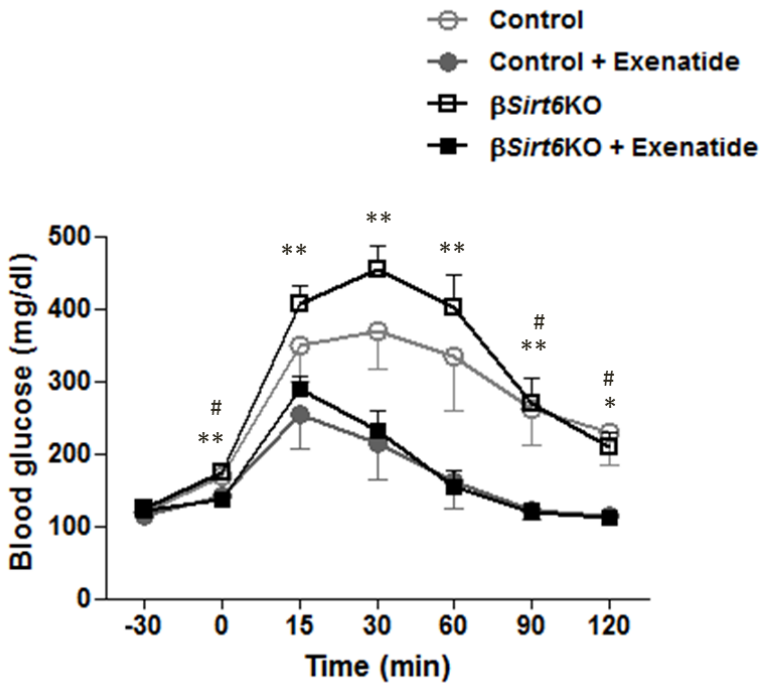

Figure 14. Exenatide treatment improved impaired glucose tolerance in β*Sirt6*KO mice. Intraperitoneal glucose tolerance test following exenatide treatment of 12-week-old male mice. After 18 hours of fasting, mice were intraperitoneally injected with 10 nM of exenatide 30 minutes before intraperitoneal glucose injection (control, n = 4; control + exenatide, n = 4; β*Sirt6*KO, n = 5; β*Sirt6*KO + exenatide, n = 5). #$p < 0.05$; *$p < 0.05$ and **$p < 0.01$ (#, comparison between control and control + exenatide; *, comparison between β*Sirt6*KO and β*Sirt6*KO + exenatide).

6. Exenatide treatment partially restored impaired *Insulin-1* mRNA synthesis in response to glucose in β*Sirt6*KO mice

We further investigated the effects of exenatide treatment on insulin synthesis in response to glucose stimulation. Figure 15 shows that *Insulin-1* mRNA expression levels in control islets increased 3.7-fold after 60 minutes of glucose stimulation in a static incubation experiment and also, exenatide treatment enhanced this effect, producing a 7.1-fold increase in response to glucose stimulation. Although islets from β*Sirt6*KO mice did not show increased *Insulin-1* mRNA expressions under glucose stimulation, exenatide treatment significantly increased these levels by 4.5-fold after glucose stimulation of β*Sirt6*KO islets. *Munc18c* mRNA expression levels after exenatide administration did not increase under glucose stimulation in β*Sirt6*KO islets, whereas in control islets exenatide treatment significantly increased these levels after glucose stimulation (Figure 16). These findings suggest that exenatide treatment strongly affects synthesis of *Insulin-1* transcripts in β*Sirt6*KO islets in response to glucose stimulation within 60 minutes, and suggest further that this could be attributed to improved insulin tolerance in β*Sirt6*KO mice treated with exenatide.

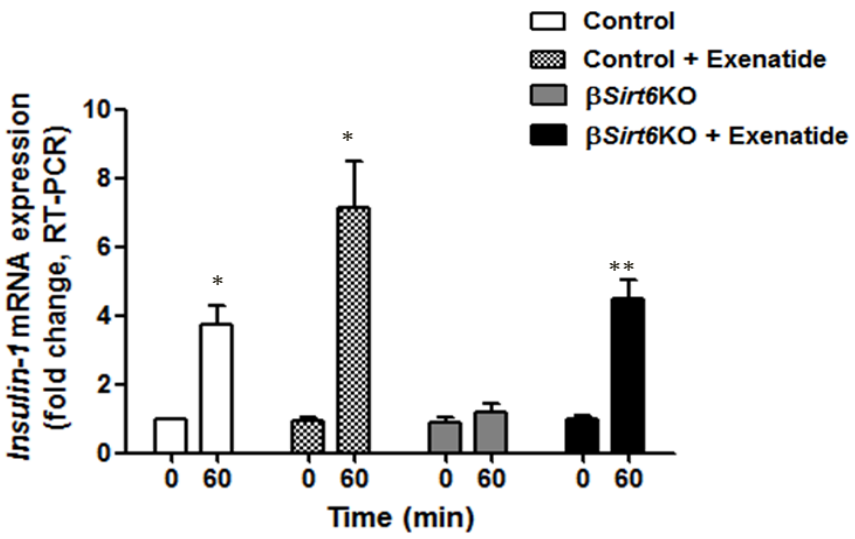

Figure 15. Exenatide treatment increased *Insulin-1* gene mRNA expression levels in response to glucose stimulation in β*Sirt6*KO islets. Isolated islets from 12-week-old control and β*Sirt6*KO mice treated with 15 mM glucose for 60 minutes. Exenatide (100 nM) was treated 30 minutes before the glucose administration. Expression levels of *Insulin-1* mRNA from islets of control and β*Sirt6*KO mice were quantitated by RT-PCR (n = 3-5 each). Recordings were made from each group of 50 islets of each mouse. All mRNA levels were normalized to *Gapdh* mRNA. *$p < 0.05$ and **$p < 0.01$ (comparison between 0 and 60 minutes after glucose administration).

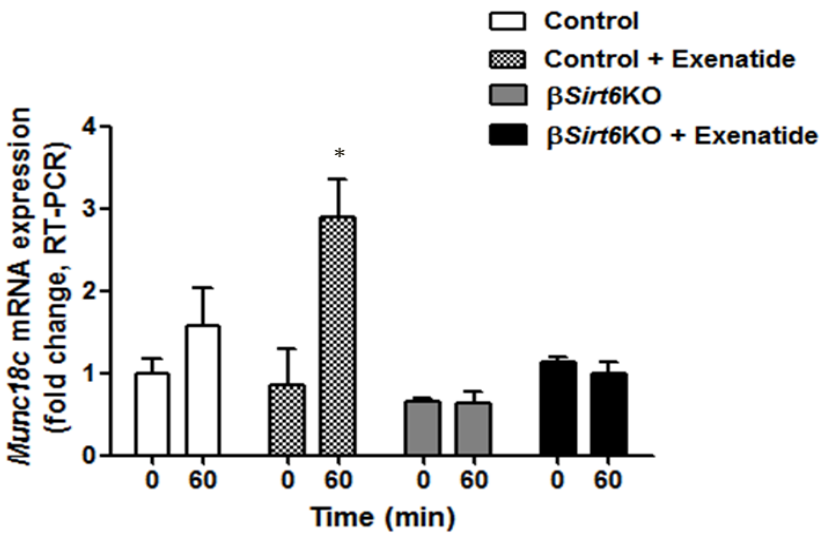

Figure 16. Effect of exenatide treatment on *Munc18c* gene mRNA expression levels in response to glucose stimulation in β*Sirt6*KO islets. Isolated islets from 12-week-old control and β*Sirt6*KO mice were treated with 15 mM glucose for 60 minutes. Exenatide (100 nM) was administered 30 minutes before glucose administration. Expression levels of *Munc18c* mRNA from islets of control and β*Sirt6*KO mice were quantified by RT-PCR (n = 3-5 each). Recordings were made from each group of 50 islets of each mouse. All mRNA levels were normalized to *Gapdh* mRNA. *$p < 0.05$ (comparison between 0 and 60 minutes after glucose administration).

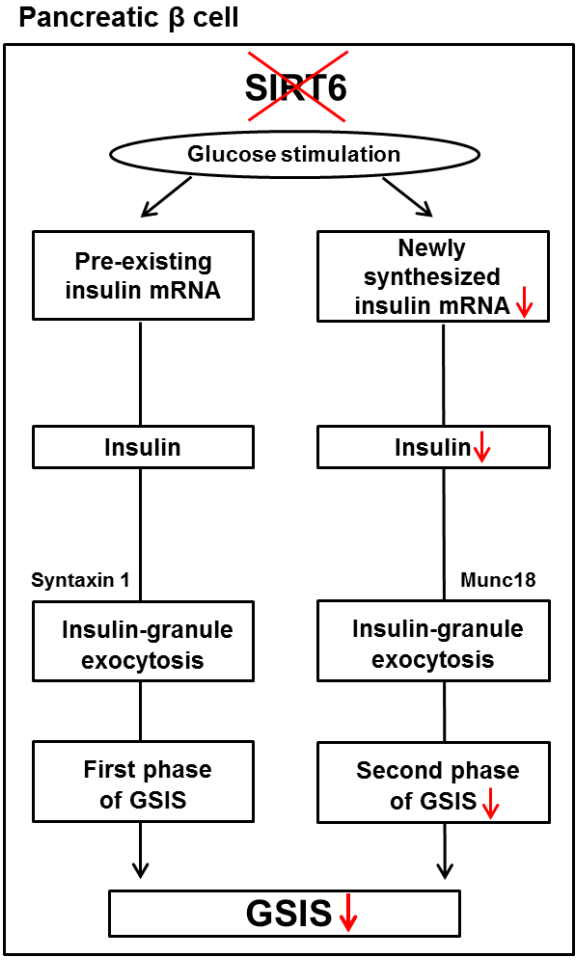

Figure 17. A schematic illustration showing the plausible mechanism by which SIRT6 relates to insulin synthesis and glucose-stimulated insulin secretion.

IV. DISCUSSION

In the present study, we investigated whether deletion of SIRT6 in pancreatic beta cells alters insulin synthesis and secretion. To this end, we generated beta cell-specific SIRT6 knockout mice by crossmatching *Sirt6* floxed mice with RIP-Cre mice. Depleting SIRT6 in pancreatic beta cells resulted in impaired glucose tolerance with defects in GSIS *in vivo*, although islets morphometry was not affected. Additional analyses revealed that GSIS *ex vivo* was significantly impaired, and synthesis of *Insulin-1* transcripts was decreased under glucose stimulation in isolated islets from β*Sirt6*KO mice. Furthermore, exenatide treatment partially restored decreased *Insulin-1* transcripts in response to glucose in β*Sirt6*KO islets and improved impaired glucose tolerance in β*Sirt6*KO mice. The results suggest that beta cell-specific SIRT6 is a key mediator of *Insulin-1* transcription within a short period of 60 minutes in response to glucose stimulation and further, that it mediates the second phase of GSIS and glucose tolerance (Figure 17).

Type 2 diabetes mellitus is characterized by insulin resistance and progressive beta cell failure, involving a decrease in beta cell mass and deterioration of beta cell functions such as GSIS.[19-21] Moreover, defective insulin secretion in response to glucose is known to be an important contributing factor to the beta cell dysfunction that occurs during diabetes progression.[22,23] Defects in insulin secretion are present at a relatively early stage in the evolution of diabetes, as seen in subjects who show impaired glucose tolerance (IGT) even before the onset of overt hyperglycemia.[24] Also, studies in the Zucker Diabetic Fatty (ZDF) rats have demonstrated that significant abnormalities of insulin secretion and profound alterations of multiple genes in the islet, involving aspects of insulin biosynthesis and secretion are present in the prediabetic stage.[25,26] In addition, in the overt diabetic condition, controlling postprandial glucose levels can help to achieve optimal glycemic control and may be more crucial for the prevention of cardiovascular complications than controlling fasting plasma glucose levels.[27,28] However, controlling postprandial glucose levels

is usually undercontrolled and does not reach the desired goal as readily. The exact mechanisms underlying this progressive beta cell dysfunction including the disruption of GSIS have not been revealed. Therefore, investigation of beta cell function, including insulin synthesis and secretion, in response to glucose stimulation could potentially provide insight into the pathophysiology as well as treatment and prevention of the beta cell dysfunction seen in both prediabetes and diabetes.

Insulin secretion from the pancreatic beta cell in response to stimulation by glucose exhibits biphasic insulin release, beginning with a rapid and immediate (within 10 minutes) first phase of exocytosis from readily releasable insulin granule pools, which is then followed by a slow and sustained second phase of granule translocation, docking, priming, and refilling of readily releasable insulin granule pools.[29,30] Still, there have been controversies as to whether glucose-induced inulin biosynthesis is dependent on either transcriptional or translational levels, and whether insulin secretory capacity of beta cell is mainly attributed to either glucose-stimulated insulin biosynthesis or insulin granule exocytosis. In particular, an imbalance between insulin biosynthesis and secretion is likely linked to insulin secretary dysfunction, but this has not been fully investigated.[31,32] In the current study, GSIS in β*Sirt6*KO islets seemed to primarily exhibit defects in the second phase of insulin secretion mostly after 15 minutes of glucose stimulation, and diminished glucose-stimulated *Insulin-1* transcription within 60 minutes. In a previous study, up-regulation at the transcriptional level of insulin by glucose stimulation occurred in the short term, and it directly contributed to acute insulin biosynthesis within 15 minutes.[33] These results may suggest that internal stores of insulin granule pools are depleted by continuous insulin secretion in the first phase of GSIS and the second phase of GSIS is impaired in β*Sirt6*KO islets because the second phase of GSIS would be attributable to newly synthesized insulin granules within an unexpectedly short time frame of 60 minutes. Therefore, attenuation of

new insulin synthesis at the transcription level and depletion of insulin storage by SIRT6 deficiency in pancreatic beta cell might be connected to impairments in GSIS, primarily in its second phase, and to insulin intolerance.

In addition, exenatide, a GLP-1 agonist, appeared to augment glucose-stimulated *Insulin-1* mRNA levels in control islets. Although *Insulin-1* mRNA levels was not changed by glucose stimulation in β*Sirt6*KO islets, exenatide partially potentiated regulation of *Insulin-1* mRNA in β*Sirt6*KO islets at the transcription level within 60 minutes in response to glucose stimulation, and consequently improved impaired glucose tolerance in β*Sirt6*KO mice. This was in marked contrast to glibenclamide, which did not sustain the immediate effect of potentiating insulin release. Glibenclamide, a sulfonylurea, promotes insulin secretion but not insulin synthesis, in contrast to exenatide.[34] Previous studies have revealed that GLP-1 enhances GSIS and that its action involves the stimulation of adenylate cyclase, leading to an increase in levels of cytoplasmic AMP and activation of protein kinase A.[35] Moreover, exendin-4, a GLP-1 agonist, has been reported to enhance proinsulin gene expression at the levels of gene transcription and the translational biosynthesis of proinsulin.[35-37] These finding suggest that the distinguishing effect of exenatide (as opposed to glibenclamide) on β*Sirt6*KO mice might be made possible by its augmentation of glucose-stimulated proinsulin biosynthesis at the transcriptional level. Further study is needed to determine the pathways by which exenatide affects *Insulin-1* transcription in the absence of SIRT6.

Multifactorial mechanisms contribute to the process of biosynthesis of insulin and insulin secretion including transcription factors such as pancreatic and duodenal homeobox-1 (PDX-1), v-maf musculoaponeurotic fibrosarcoma oncogene homologue A (MafA), and neurogenic differentiation factor 1 (NeuroD1).[38] PDX-1 is associated with the proliferation of pancreatic cells during development and also with glucose intolerance in adults.[39-41] MafA acts synergistically with PDX-1 and NeuroD1 to activate the insulin gene promoter and has an effect on GSIS involving

insulin granule release.[42-44] Therefore, further investigation is required to determine whether SIRT6 and key transcriptional factors work directly or indirectly in concert to control insulin synthesis under glucose stimulation.

V. CONCLUSION

Pancreatic beta cell-specific SIRT6 plays a critical role in the homeostasis of glucose, particularly in response to glucose stimulation by regulating insulin synthesis at the transcription level. The second phase of GSIS is impaired in beta cells lacking SIRT6, which may contribute to the decreased *Insulin-1* mRNA expression levels in the short term. Exenatide, a GLP-1 receptor agonist, partially restores impaired glucose tolerance and decreased *Insulin-1* mRNA expression. Collectively, research into pancreatic beta cell-specific SIRT6 has therapeutic potential for defective GSIS, beta cell dysfunction, and diabetes.

REFERENCE

1. Blander G, Guarente L. The Sir2 family of protein deacetylases. Annu Rev Biochem 2004;73:417-35.
2. Guarente L, Kenyon C. Genetic pathways that regulate ageing in model organisms. Nature 2000;408:255-62.
3. Saunders LR, Verdin E. Sirtuins: critical regulators at the crossroads between cancer and aging. Oncogene 2007;26:5489-504.
4. Frye RA. Characterization of five human cDNAs with homology to the yeast SIR2 gene: Sir2-like proteins (sirtuins) metabolize NAD and may have protein ADP-ribosyltransferase activity. Biochem Biophys Res Commun 1999;260:273-9.
5. Frye RA. Phylogenetic classification of prokaryotic and eukaryotic Sir2-like proteins. Biochem Biophys Res Commun 2000;273:793-8.
6. Finkel T, Deng CX, Mostoslavsky R. Recent progress in the biology and physiology of sirtuins. Nature 2009;460:587-91.
7. Michishita E, Park JY, Burneskis JM, Barrett JC, Horikawa I. Evolutionarily conserved and nonconserved cellular localizations and functions of human SIRT proteins. Mol Biol Cell 2005;16:4623-35.
8. Michishita E, McCord RA, Berber E, Kioi M, Padilla-Nash H, Damian M, et al. SIRT6 is a histone H3 lysine 9 deacetylase that modulates telomeric chromatin. Nature 2008;452:492-6.
9. Michishita E, McCord RA, Boxer LD, Barber MF, Hong T, Gozani O, et al. Cell cycle-dependent deacetylation of telomeric histone H3 lysine K56 by human SIRT6. Cell Cycle 2009;8:2664-6.
10. Yang B, Zwaans BM, Eckersdorff M, Lombard DB. The sirtuin SIRT6 deacetylates H3 K56Ac in vivo to promote genomic stability. Cell Cycle 2009;8:2662-3.
11. Kugel S, Mostoslavsky R. Chromatin and beyond: the multitasking roles for

SIRT6. Trends Biochem Sci 2014;39:72-81.

12. Mostoslavsky R, Chua KF, Lombard DB, Pang WW, Fischer MR, Gellon L, et al. Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. Cell 2006;124:315-29.

13. Dominy JE, Jr., Lee Y, Jedrychowski MP, Chim H, Jurczak MJ, Camporez JP, et al. The deacetylase Sirt6 activates the acetyltransferase GCN5 and suppresses hepatic gluconeogenesis. Mol Cell 2012;48:900-13.

14. Zhang P, Tu B, Wang H, Cao Z, Tang M, Zhang C, et al. Tumor suppressor p53 cooperates with SIRT6 to regulate gluconeogenesis by promoting FoxO1 nuclear exclusion. Proc Natl Acad Sci U S A 2014;111:10684-9.

15. Zhong L, D'Urso A, Toiber D, Sebastian C, Henry RE, Vadysirisack DD, et al. The histone deacetylase Sirt6 regulates glucose homeostasis via Hif1alpha. Cell 2010;140:280-93.

16. Kim HS, Xiao C, Wang RH, Lahusen T, Xu X, Vassilopoulos A, et al. Hepatic-specific disruption of SIRT6 in mice results in fatty liver formation due to enhanced glycolysis and triglyceride synthesis. Cell Metab 2010;12:224-36.

17. Xiong X, Wang G, Tao R, Wu P, Kono T, Li K, et al. Sirtuin 6 regulates glucose-stimulated insulin secretion in mouse pancreatic beta cells. Diabetologia 2016;59:151-60.

18. Ramsey KM, Mills KF, Satoh A, Imai S. Age-associated loss of Sirt1-mediated enhancement of glucose-stimulated insulin secretion in beta cell-specific Sirt1-overexpressing (BESTO) mice. Aging Cell 2008;7:78-88.

19. DeFronzo RA. Lilly lecture 1987. The triumvirate: beta-cell, muscle, liver. A collusion responsible for NIDDM. Diabetes 1988;37:667-87.

20. Kahn SE. The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes. Diabetologia 2003;46:3-19.

21. Ohn JH, Kwak SH, Cho YM, Lim S, Jang HC, Park KS, et al. 10-year

trajectory of beta-cell function and insulin sensitivity in the development of type 2 diabetes: a community-based prospective cohort study. Lancet Diabetes Endocrinol 2016;4:27-34.

22. Weir GC, Bonner-Weir S. Five stages of evolving beta-cell dysfunction during progression to diabetes. Diabetes 2004;53 Suppl 3:S16-21.
23. Muoio DM, Newgard CB. Mechanisms of disease:Molecular and metabolic mechanisms of insulin resistance and beta-cell failure in type 2 diabetes. Nat Rev Mol Cell Biol 2008;9:193-205.
24. Polonsky KS. Lilly Lecture 1994. The beta-cell in diabetes: from molecular genetics to clinical research. Diabetes 1995;44:705-17.
25. Sturis J, Pugh WL, Tang J, Ostrega DM, Polonsky JS, Polonsky KS. Alterations in pulsatile insulin secretion in the Zucker diabetic fatty rat. Am J Physiol 1994;267:E250-9.
26. Johnson JH, Ogawa A, Chen L, Orci L, Newgard CB, Alam T, et al. Underexpression of beta cell high Km glucose transporters in noninsulin-dependent diabetes. Science 1990;250:546-9.
27. Ceriello A, Hanefeld M, Leiter L, Monnier L, Moses A, Owens D, et al. Postprandial glucose regulation and diabetic complications. Arch Intern Med 2004;164:2090-5.
28. Fava S. Role of postprandial hyperglycemia in cardiovascular disease. Expert Rev Cardiovasc Ther 2008;6:859-72.
29. Rorsman P, Renstrom E. Insulin granule dynamics in pancreatic beta cells. Diabetologia 2003;46:1029-45.
30. Rorsman P, Eliasson L, Renstrom E, Gromada J, Barg S, Gopel S. The Cell Physiology of Biphasic Insulin Secretion. News Physiol Sci 2000;15:72-7.
31. Fu Z, Gilbert ER, Liu D. Regulation of insulin synthesis and secretion and pancreatic Beta-cell dysfunction in diabetes. Curr Diabetes Rev 2013;9:25-53.
32. Uchizono Y, Alarcon C, Wicksteed BL, Marsh BJ, Rhodes CJ. The balance

between proinsulin biosynthesis and insulin secretion: where can imbalance lead? Diabetes Obes Metab 2007;9 Suppl 2:56-66.

33. Leibiger B, Wahlander K, Berggren PO, Leibiger IB. Glucose-stimulated insulin biosynthesis depends on insulin-stimulated insulin gene transcription. J Biol Chem 2000;275:30153-6.

34. Alarcon C, Wicksteed B, Rhodes CJ. Exendin 4 controls insulin production in rat islet beta cells predominantly by potentiation of glucose-stimulated proinsulin biosynthesis at the translational level. Diabetologia 2006;49:2920-9.

35. Renstrom E, Eliasson L, Rorsman P. Protein kinase A-dependent and -independent stimulation of exocytosis by cAMP in mouse pancreatic B-cells. J Physiol 1997;502 (Pt 1):105-18.

36. Fehmann HC, Habener JF. Insulinotropic hormone glucagon-like peptide-I(7-37) stimulation of proinsulin gene expression and proinsulin biosynthesis in insulinoma beta TC-1 cells. Endocrinology 1992;130:159-66.

37. Bai L, Meredith G, Tuch BE. Glucagon-like peptide-1 enhances production of insulin in insulin-producing cells derived from mouse embryonic stem cells. J Endocrinol 2005;186:343-52.

38. Shao S, Fang Z, Yu X, Zhang M. Transcription factors involved in glucose-stimulated insulin secretion of pancreatic beta cells. Biochem Biophys Res Commun 2009;384:401-4.

39. Jonsson J, Carlsson L, Edlund T, Edlund H. Insulin-promoter-factor 1 is required for pancreas development in mice. Nature 1994;371:606-9.

40. Serup P, Jensen J, Andersen FG, Jorgensen MC, Blume N, Holst JJ, et al. Induction of insulin and islet amyloid polypeptide production in pancreatic islet glucagonoma cells by insulin promoter factor 1. Proc Natl Acad Sci U S A 1996;93:9015-20.

41. Reimer MK, Ahren B. Altered beta-cell distribution of pdx-1 and GLUT-2

after a short-term challenge with a high-fat diet in C57BL/6J mice. Diabetes 2002;51 Suppl 1:S138-43.

42. Hang Y, Yamamoto T, Benninger RK, Brissova M, Guo M, Bush W, et al. The MafA transcription factor becomes essential to islet beta-cells soon after birth. Diabetes 2014;63:1994-2005.

43. Zhang C, Moriguchi T, Kajihara M, Esaki R, Harada A, Shimohata H, et al. MafA is a key regulator of glucose-stimulated insulin secretion. Mol Cell Biol 2005;25:4969-76.

44. Aramata S, Han SI, Kataoka K. Roles and regulation of transcription factor MafA in islet beta-cells. Endocr J 2007;54:659-66.

`ABSTRACT (IN KOREAN)

췌장의 베타 세포에서 SIRT6 유전자 결손이 인슐린 분비에 미치는 영향

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김 규 리

Sirtuin은 NAD+ 의존적인 탈아세틸화효소 (deacetylase)로 작용한다고 알려져 있으며, 포유류에서는 7종류의 Sirtuin homolog (SIRT1-7)가 존재하고 있다. SIRT6는 chromatin과 연관되어 histone H3 lysine 9 (H3K9)를 탈아세틸화시키거나 FoxO1, GCN5 등과 같은 non-histone 기질을 탈아세틸화시킨다. 기존 연구에서 SIRT6가 포도당 및 지방 대사 조절과 밀접한 관계가 있음이 보고되었다. 또한 췌장 베타 세포 특이적 SIRT6 결핍 마우스에서 포도당 자극 시 인슐린 분비의 저하가 일어나는데 이 때 미토콘드리아의 기능 부전, 세포질 내 칼슘 이온 농도의 이상 변화가 동반된다고 보고된 바 있다. 하지만 아직까지 세부적인 기전이 밝혀지지 않은 바, 본 연구에서는 췌장 베타 세포 특이적 SIRT6 결핍 마우스를 생산하여 췌장 베타 세포의 SIRT6의 인슐린 합성 및 분비에 미치는 영향에 대해서 확인하고자 하였다.

Sirt6 floxed 마우스 (*Sirt6* flox/flox)와 RIP-Cre라고 불리는 B6.Cg-Tg(Ins2-Cre)25Mgn/J transgenic 마우스를 교배하여 후손으로부터 췌장

베타 세포 SIRT6 결핍 마우스 동종군 (homozygous)인 β*Sirt6*KO 마우스 모델을 생산하였다. 생산한 췌장 베타 세포 SIRT6 결핍 마우스 모델을 16주령까지 일반식이를 섭취하도록 한 후 18시간 금식 이후 공복혈당을 확인한 결과 실험군 간의 유의한 차이는 없었다. 경구 당부하 검사는 생후 4주, 8주, 12주, 16주령 때 시행하였으며, 대조군에 비해 췌장 베타 세포 SIRT6 결핍 마우스 동종군에서 포도당 섭취 후 30분 이후부터 혈당이 유의하게 증가하여 내당능장애가 있음을 나타냈다. 15주령에 시행한 인슐린 내성 검사와 pyruvate 내성 검사는 대조군과 췌장 베타 세포 SIRT6 결핍 마우스 동종군 사이 유의한 차이는 보이지 않아 전신적 인슐린 저항성 및 간 내 포도당 합성에는 차이를 보이지 않았다. 포도당 투여 후 혈중 인슐린 농도는 대조군에 비해서 8주, 12주, 16주령의 췌장 베타 세포 SIRT6 결핍 마우스 동종군에서 포도당 섭취 후 15분, 30분에서 낮게 측정되었다. 반면에, 췌장의 췌도 면적과 췌도의 수는 췌장 베타 세포 SIRT6 결핍 마우스 동종군과 대조군에서 유의한 차이가 없어, SIRT6 결핍 마우스 동종군에서 혈당 자극 시 인슐린 분비능의 저하가 췌장 췌도의 형태와는 무관함을 확인하였다. 마우스 췌도 세포 분리를 시행하여 포도당 자극 인슐린 분비능 측정 시 대조군에서는 포도당 자극 후 5분 이후부터 60분까지 분비된 인슐린 농도가 점차적으로 증가하였으나, SIRT6 결핍 췌도 세포에서는 5분 이후 인슐린 분비가 더 이상 증가되지 않음을 확인하였다. 또한 *Insulin-1*의 mRNA 발현이 대조군의 췌도 세포에서는 포도당 자극 60분 후 3.7배 증가하였지만, 췌장 베타 세포 SIRT6 결핍 마우스 동종군의 췌도 세포에서는 증가하지 않았다. 인슐린의 분비에 관여하는 설폰요소제인 Glibenclamide 투여 시에는 효과가 뚜렷하지 않았지만, 인슐린의 합성 및 분비에 관여하는 글루카곤 유사 펩타이드-1 수용체 길항제인

exenatide를 투여 후 시행한 복강 내 당부하 검사에서 췌장 베타 세포 SIRT6 결핍 마우스의 내당능장애가 대조군과 유사하게 호전되었다. SIRT6 결핍 췌도 세포에서 포도당 자극에 변화가 없던 *Insulin-1* mRNA의 발현이, exenatide 투여 후 포도당 자극 시 뚜렷하게 증가하는 것을 확인하였다. 반면에 인슐린 분비 2차 단계에서 인슐린 과립의 세포외배출 (exocytosis)에 관여하는 *Munc18c*의 mRNA 발현은 대조군에서 포도당 자극시 증가하나 SIRT6 결핍 췌도 세포에서는 포도당 자극 또는 exenatide를 투여 후 포도당 자극에도 변화를 보이지 않았다.

결론적으로, 본 연구에서는 췌장 베타 세포 SIRT6 결핍 마우스 모델을 생성하여, SIRT6의 췌장 베타 세포에서의 결손이 포도당 자극 시 전사 단계의 인슐린 합성의 저하, 2차 단계의 포도당 자극 시 인슐린 분비 저하 및 내당능장애와 연관됨을 확인함으로써 추후 SIRT6를 통한 새로운 당뇨병 치료 기전의 가능성이 있음을 확인하였다.

핵심되는 말: sirtuin6, 당뇨병, 베타 세포, 인슐린, 포도당 대사