Effects of Ovariectomy on Insulin Resistance and β-Cell Function and Mass

Soo Bong Choi, Chun Hee Park[1], Dong Wha Jun[1], Jin Sun Jang[1] and Sunmin Park[1†]

Department of Internal Medicine, Konkuk University, Chungbuk 380-062, Korea
[1]Department of Food Science and Nutrition, Hoseo University, Chungnam 336-795, Korea

Abstract

The prevalence of type-2 diabetes increases remarkably in post-menopausal women, possibly because insulin secretion fails to compensate for the insulin resistance induced in various tissues by estrogen insufficiency. However, this has not been fully defined. Therefore, the present study investigated whether an ovariectomy (OVX) would increase insulin resistance and decrease the β-cell function and mass in female rats with and without a 90% pancreatectomy (Px). Female rats aged 15 weeks were divided into four groups: 1) OVX + Px, 2) SOVX (sham operation of OVX) + Px, 3) OVX + SPx (sham operation of Px), and 4) SOVX + SPx, and given a 30% fat diet for 8 weeks. At the end of the experimental period, the islet function and insulin resistance were determined using a hyperglycemic clamp and a euglycemic hyperinsulinemic clamp, respectively. The OVX only increased the body weight in the SPx rats, which was partially related to the food intake. Yet, the OVX did increase the peripheral insulin resistance, while the Px increased this resistance further. The OVX and Px both exacerbated the islet function, as measured by the insulin secretion pattern, while delaying and decreasing the first-phase insulin secretion. The OVX only decreased the proliferation of β-cells in the Px rats, while increasing apoptosis in both the Px and SPx rats. As a result, the OVX decreased the β-cell mass in the Px rats, but increased the mass in the SPx rats. In conclusion, an OVX was found to accelerate the development and progression of diabetes by increasing the insulin resistance and decreasing the β-cell mass. Therefore, menopause can be a risk factor for type-2 diabetes, mainly due to a deceased proliferation of β-cells.

Key words: pancreatectomy, estrogen, diabetes, insulin, β-cell, proliferation, apoptosis

INTRODUCTION

Insulin resistance is a characteristic feature of type-2 diabetes. However, even in an insulin resistant state, normoglycemia is maintained by compensatory hyperinsulinemia until the pancreatic β-cells become unable to meet the increased demand for insulin, at which point type-2 diabetes begins (1). The mechanism by which β-cells become unable to meet the rising insulin demand has never been elucidated, primarily because of the unavailability of human pancreatic islets for appropriate study. Yet, post-mortem studies in patients with type-2 diabetes have indicated that the β-cell mass is reduced (1).

The dysregulation of insulin secretion is an important factor for developing diabetes, and possibly insulin resistance. When increased insulin secretion is able to compensate for insulin resistance, type-2 diabetes does not develop. Insulin resistance is known to be accompanied by hyperinsulinemia and islet hyperplasia, and some studies (2-4) have shown that a sufficient β-cell mass is essential for insulin secretion to compensate for insulin resistance. Insulin resistance has also been associated with a biphasic effect, initially enhancing insulin output by stimulating hyperplasia (5,6), then subsequently reversing these compensatory changes when the insulin resistance becomes extremely high (5,6). However, there is still no explanation for the initial development of hyperplasia and its eventual failure.

When insulin resistance is induced by a high fat diet and insulin receptor substrate (IRS)-1 deletion, islet hyperplasia and hyperinsulinemia are both exhibited, yet not diabetes (2,3). In contrast, in IRS2 knockout mice insulin secretion cannot compensate for insulin resistance because of a decreased β-cell cell mass, in which case they developed severe diabetes, unlike IRS1 knockout mice (4). Therefore, it is important to increase the islet mass in a high insulin resistant state to prevent progression to diabetes. An increased β-cell mass may result from an increased insulin signaling cascade in β-cells. Pancreatic β-cells predominantly contain IRS2, not IRS1, and the insulin signaling cascade is mediated via the

---

increased phosphorylation of IRS2.

The development of insulin resistance and diabetes is linked to both genetic and environmental factors (7-9). Various environmental factors, such as aging and diet, are involved in their development and progression. Aging is an important risk factor for insulin resistance in both men and women (7). However, the prevalence and progression of diabetes increases markedly in post-menopausal women, possibly due to an estrogen insufficiency leading to an increased insulin resistance and/or decreased compensatory insulin secretion. The administration of estradiol alone would seem to have potentially beneficial effects for the improvement of glucose tolerance or insulin sensitivity or both in post-menopausal women (10,11). Accordingly, this study examined whether an ovariectomy (OVX) would modulate insulin resistance, insulin secretion, and pancreatic β-cell mass in 90% pancreatectomized (Px) and sham-operated (SPx) rats, since an OVX effectively induces estrogen insufficiency. The compensatory insulin secretion was found to be lower in the OVX-Px rats, yet only associated with a decreased β-cell mass in the Px rats. In contrast, in the SPx rats, the insulin secretion compensated for the insulin resistance and was accompanied by an increased β-cell mass. The decreased β-cell mass in the Px rats resulted from a decreased proliferation and increased apoptosis. Thus, estrogen insufficiency was found to impair the β-cell compensatory response in mild type-2 diabetes.

MATERIALS AND METHODS

Experimental animals

Female Sprague Dawley rats weighing 277±22 g were randomly divided into two groups, then 90% pancreatectomies were performed on one group and sham-operations (SPx) on the other. Using the technique of Hosokawa et al. (12), 90% of the pancreas was removed, leaving less than 2 mm of the common bile duct, extending from the duct to the first part of the duodenum. The Px rats showed characteristics of mild type-2 diabetes, and those with a random fed serum glucose level of less than 9.4 mmol/L two weeks after surgery were eliminated. The remaining Px rats and the SPx rats were randomly subdivided into two groups each (13) and either overectomized or sham-operated (SOVX). The result was four groups as follows: 1) OVX+Px, 2) SOVX (sham operation of OVX)+Px, 3) OVX+SPx (sham operation of Px), and 4) SOVX+SPx. After the OVX or SOVX surgeries, all the rats in the four groups freely consumed a 30% fat diet for 8 weeks. The animals were individually housed in stainless steel cages in a controlled (23°C;

12 hour light and dark cycle) environment. All surgical and experimental procedures were performed according to the guidelines of the Animal Care and Use Review Committee at Konkuk University, Korea. The serum glucose levels, food intake, and body weight were measured weekly on Tuesday at 10 AM.

Insulin secretion and insulin resistance

After seven weeks of treatment, catheters were surgically implanted into the right carotid artery and left jugular vein of the rats anesthetized with ketamine and xylazine (100 mg and 10 mg/kg body weight, respectively) via intraperitoneal access. After 5~6 days of implantation, a hyperglycemic clamp was performed in conscious and fasting rats to measure the insulin secretion capacity (14). Bolus glucose (375 mg glucose/kg body weight) was infused through the cannula for the first 5 minutes of the clamp, followed by 25% glucose to keep the blood glucose levels at 6 mM above the fasting level. Blood was collected from the carotid artery at 0, 5, 10, 40, 50, and 60 min and the glucose and insulin levels measured.

Two days after the use of the hyperglycemic clamp, a euglycemic hyperinsulinemic clamp (15) was used under the same conditions as the hyperglycemic clamp to determine insulin resistance. The basal and insulin-stimulated whole-body glucose flux was estimated using a primed continuous infusion of [3-^{3}H] glucose (10 μCi bolus, 0.1 μCi/min, NEN, Boston, MA). After basal artery blood sampling, a primed continuous infusion of regular human insulin (Humulin, Eli Lilly and Co., Indianapolis, IN) was infused at a rate of 20 pmol/kg/min to raise the plasma insulin concentration to approximately 1100 pM. Blood samples were collected from the arteries at 10-min intervals for glucose estimation, then 25% glucose was infused at variable rates as needed to clamp the glucose levels at approximately 6 mM. The glucose disposal rate was considered as the total glucose infused, which was calculated and expressed in terms of mg of glucose per kg body weight per minute. The glucose disposable rate is an index of the whole-body response to exogenous insulin. The serum glucose levels were analyzed using a Glucose Analyzer II (Beckman, Palo Alto, CA), while the serum insulin, leptin, and 17β-estradiol levels were measured by a radioimmunoassay (Linco Research, St. Charles, MO). The advanced glycated endproducts of the subcutaneous tissue were measured using fluorescence methods (16).

The rats were killed by decapitation at the end of the clamp. The liver and muscles were rapidly dissected, weighed, and frozen in liquid nitrogen, all tissues were stored at -70°C until further analyses were performed.

Growth, maintenance, and survival of β-cells

The β-cell area was measured by acquiring microscopic images from two sets of eight to ten distal, random non-overlapping images at 10x of insulin stained pancreatic sections. The results of the β-cell quantification were expressed as the percentage of the total surveyed area containing insulin-positive cells. The β-cell mass was then determined based on the β-cell area multiplied by the pancreas weight.

Five rats from each group were treated with 5-bromo-2-deoxyuridine (BrdU; Roche Molecular Biochemicals, Indianapolis, Indiana, USA; 100 mg/kg body weight) six hours before death (17). The β-cell proliferation was then examined in the pancreas from the rats injected with BrdU by performing double-label immunohistochemistry with anti-insulin (Zymed Laboratories, South San Francisco, CA) and BrdU antibodies (Roche Molecular Biochemicals) on rehydrated paraffin-embedded sections. The BrdU-positive β-cell ratios were calculated as the BrdU-positive β-cells over the total β-cell nuclei per section, two sections per animal, five animals per group.

Statistical analysis

All results were expressed as the mean ± standard deviation (SD). The statistical analyses were performed using SAS software (18). Two-way analyses of variance (ANOVA) were carried out to determine the main effects of an OVX and Px. Since most factors did not have any interaction, the significant differences of two main effects were determined. When necessary, an interaction term was considered. Since the number of groups in each main effect was 2, multiple comparisons were not used. Differences with $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Body weight, food intake, and serum 17β-estradiol levels

The OVX increased body weights more than did the SOVX in the SPx rats throughout the experimental period (Fig. 1A, Table 1). However, the OVX did not affect the body weight in the Px rats. The body weight gain
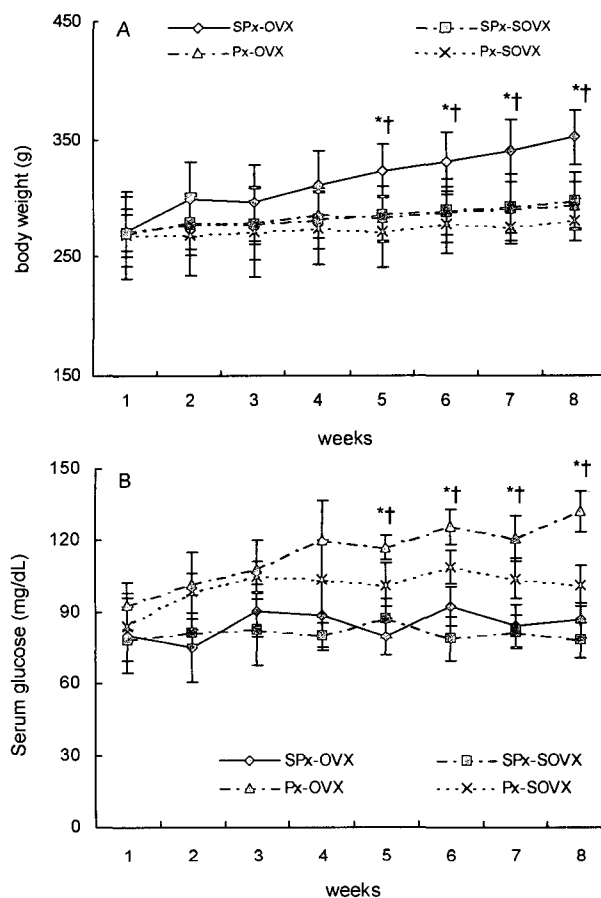

Fig. 1. Changes of body weight (A) and serum glucose levels (B) during the experimental periods. Serum glucose levels and body weight were weekly measured on Tuesday at 10 AM after overnight fasting.
OVX, ovariectomy; SOVX, sham operation of OVX; Px, 90% pancreatectomy; SPx, sham operation of Px.
$^*p < 0.05$ in OVX effect. $^\dagger p < 0.05$ in Px effect.

was only associated with an increased food intake in the SPx rats. The Px rats gained less weight than the SPx rats, even though the Px rats consumed more calories than the SPx rats. However, the serum leptin levels did not differ among the groups. A previous study by the current authors (19) and several other studies (20,21) have also found similar results, where OVX rats without diabetes gain more weight than SOVX rats, regardless of the diet composition. In the present study, the weight

Table 1. Body weight, energy intake, and serum leptin and 17β-estradiol levels after 8 week experimental period

	SPx[1]-OVX	SPx-SOVX	Px-OVX	Px-SOVX
Body weight (g)	351.6 ± 22.6[2]	297.6 ± 24.3	292.2 ± 19.6	281.4 ± 18.4*[†]
Energy intake (kcal/day)	28.6 ± 2.1	24.7 ± 2.3	32.7 ± 3.1	28.8 ± 2.8*[†]
Serum leptin (ng/mL)	3.3 ± 0.9	3.0 ± 0.8	3.5 ± 1.0	3.2 ± 0.9
Serum 17β-estradiol (pg/mL)	2.6 ± 0.6	6.2 ± 1.0	2.5 ± 0.8	6.3 ± 0.9***

[1]OVX, ovariectomy; SOVX, sham operation of OVX; Px, 90% pancreatectomy; SPx, sham operation of Px.
[2]Values are mean ± SD, n=8～9.
$^*p < 0.05$ and $^{***}p < 0.001$ in OVX effect.
$^\dagger p < 0.05$ in Px effect.

Table 2. Serum glucose and insulin levels and advanced glycated endproducts (AGE) in subcutaneous tissues after 8 week experimental period

	SPx[1]-OVX	SPx-SOVX	Px-OVX	Px-SOVX
Serum glucose (mg/dL)	$84.4 \pm 8.4^{2)}$	78.4 ± 7.2	132.2 ± 11.6	$101.5 \pm 12.3^{*\dagger}$
AGE (arbitrary unit/mg collagen)	5.9 ± 0.6	5.4 ± 0.7	7.2 ± 0.8	$6.1 \pm 0.7^{*\dagger}$
Serum insulin (pmol/L)	570.3 ± 61.5	452.7 ± 52.4	309.2 ± 34.6	$358.7 \pm 42.2^{*\dagger}$

[1]See the legend of Table 1.
[2]Values are mean $\pm$ SD, n=8~9.
$^{*}p < 0.05$ in OVX effect. $^{\dagger}p < 0.05$ in Px effect.

gain was partially due to a higher caloric intake by the OVX rats than by the sham rats. The serum 17β-estradiol levels in the OVX rats decreased to one third of those in the SOVX rats, regardless of the Px (Table 1). However, estrogen insufficiency was the major factor disrupting the energy and glucose metabolism in the OVX rats. Several studies (20,21) have already demonstrated the reversal of an impaired energy and glucose metabolism with estrogen replacement therapy. Kimura et al. (20) showed that an OVX increased body weight by decreasing the leptin signaling in the brain, even though the serum leptin levels were unchanged due to a decreased expression of leptin receptors in the brain. However, Burguera et al. (21) revealed that leptin treatment was only partially effective in modulating appetite and limiting weight gain in OVX rats. Thus, the body weight increase in the OVX rats would appear to be related to more than altered leptin signaling.

Serum glucose and insulin levels

The OVX was found to increase the overnight fasting serum glucose levels in the Px and SPx rats during the experimental period (Fig. 1B, Table 2), with a greater increase in the Px rats than in the SPx rats. The AGE, representing long-term glucose regulation, was also increased by the OVX and Px (Table 2), and was higher in the Px rats than in the SPx rats. The AGE reflected the changes in the serum glucose levels during the experimental period. Meanwhile, the OVX exacerbated the glucose metabolism under diabetic conditions.

The fasting serum insulin levels were increased with the OVX in the SPx rats (Table 2), possibly due to an increased insulin resistance. However, unlike the SPx rats, these levels were lower in the OVX-Px rats than in the SOVX-Px rats. As such, the hyperglycemia in the OVX-Px rats was apparently related to lower serum insulin levels than in the SOVX-Px rats, possibly due to an impaired compensatory insulin secretion and impaired β-cell function. Previous human and animal studies (22, 23) have shown higher fasting serum insulin levels in non-diabetic post-menopausal women and ovariectomized rats with an estrogen insufficiency than in the same subjects with an estrogen sufficiency; indicating that the

serum insulin levels were affected by an increased insulin resistance via the estrogen insufficiency, although a sufficient level of insulin was found in the pancreatic β-cells until diabetes developed.

Insulin resistance

In both the Px and SPx rats, the OVX was found to have decreased the whole-body glucose disposal rates when applying the euglycemic hyperinsulinemic clamp, revealing an insulin resistance in the body (p < 0.05, Fig. 2A). The Px also decreased the glucose disposal rates, indicating an increased insulin resistance (Fig. 2A). The liver is an important organ for maintaining normoglycemia, as such, the hepatic glucose output is important in preventing hypoglycemia and maintaining normoglycemia in a fasting state. The hepatic glucose output is regulated by the PEPCK, which in turn is regulated by the blood glucagon and insulin levels. Thus, the hepatic glucose output reflects any impairment of insulin action as exhibited by high levels of serum insulin. The hepatic glucose output in the basal (fasting) state was higher in the Px-OVX rats than in the other groups (Fig. 2B), as reflected by the higher glucose levels in the Px-OVX rats. In a clamped state, the hepatic glucose output should be suppressed with high serum insulin levels, but not suppressed in an insulin resistant state. As such, the hepatic glucose output in the clamped state was increased by the OVX and Px, and the Px-OVX rats exhibited the highest hepatic glucose output (Fig. 2B), with a simultaneous decrease in liver the glycogen storage (Fig. 2C). Many studies have shown that an OVX increases insulin resistance accompanied by obesity, and the increased insulin resistance is related to increased serum free fatty acids as a result of the OVX (24,25). Higher serum free fatty acids are a common symptom of insulin resistance. Yet, the OVX itself does not induce diabetes as long as insulin secretion can compensate for insulin resistance.

Insulin secretion

To characterize the effects on β-cell function by OVX and Px, the glucose-stimulated insulin secretion was measured using a hyperglycemic clamp. The intensity
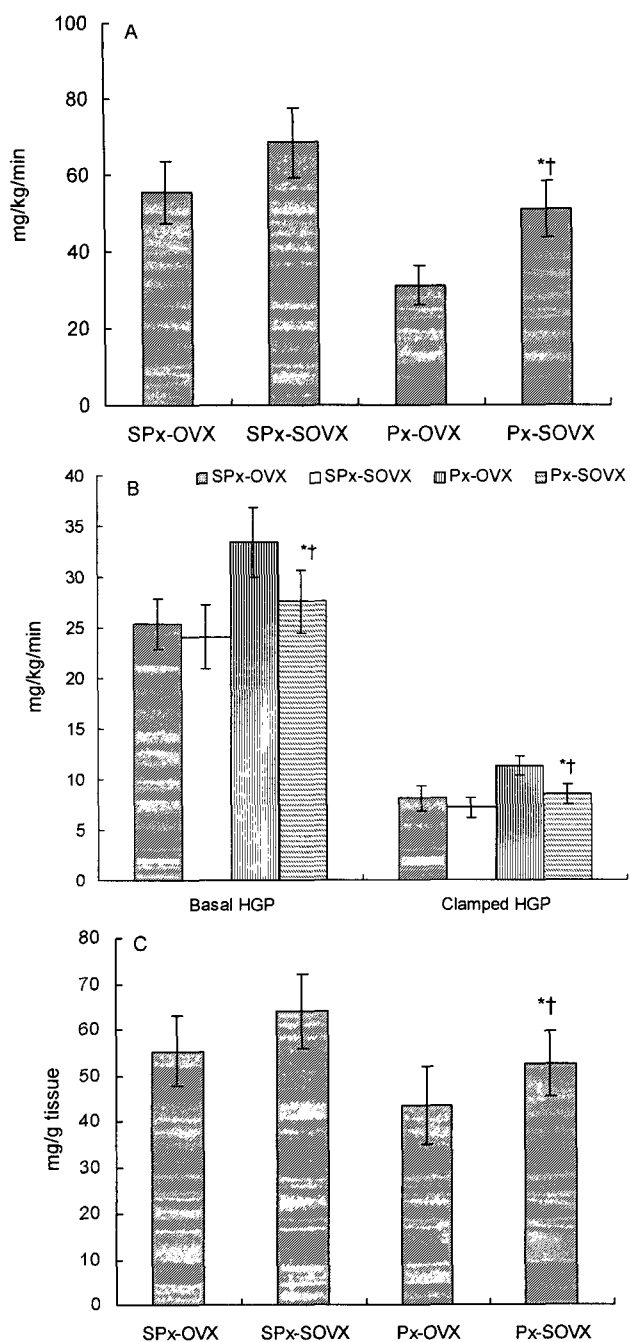
Fig. 2. Insulin resistance: glucose disposal rates (A), hepatic glucose output (B) and liver glycogen contents (C). At the end of experimental periods, euglycemic hyperinsulinemic clamp was performed in overnight fasted mice to determine whole body glucose disposal rates and hepatic glucose output in basal and clamped states (n=8~9).

OVX, ovariectomy; SOVX, sham operation of OVX; Px, 90% pancreatectomy; SPx, sham operation of Px.; HGP, hepatic glucose output.

*p<0.05 in OVX effect. †p<0.05 in Px effcet.

of the acute-phase insulin secretion was delayed and lower in the OVX and Px rats. In contrast, the second-phase insulin secretion was not changed by the OVX in the SPx rats, but decreased by the OVX in the Px

rats. Thus, the Px decreased the insulin secretion capacity and function, while the OVX impaired them further (Fig. 3). Not many studies have focused on insulin secretion capacity and/or islet function in post-menopausal women and OVX animals. Hirose et al. (23) showed that high serum free fatty acids, an OVX symptom, impaired the compensatory insulin secretion in obese Zucker rats, but not in Wister rats, which was associated with a decreased pancreatic β-cell mass.

Growth, maintenance, and survival of β-cells

The β-cell area was only lowered by the OVX in the Px rats, whereas it was increased by the OVX in the SPx rats (Table 2). The pancreas weight was not changed as a result of the OVX in the SPx rats, but was lowered in the Px rats. The β-cell mass, as determined by the pancreas weight multiplied by the β-cell area, was used to indicate the proliferation and apoptosis of β-cells. As such, the β-cell mass was increased in the OVX-SPx rats, yet not in the OVX-Px rats. Furthermore, the β-cell mass was increased when insulin resistance was exhibited with hyperinsulinemia or sufficient insulin. However, the β-cell mass was not increased in an insulin-resistant state with low insulin.

The current data showed that the above relationships were associated with the proliferation of β-cells more than apoptosis, as the proliferation of β-cells exhibited the same results as the β-cell mass. Apoptosis was increased by the OVX in both the Px and SPx rats. An estrogen insufficiency increased apoptosis, regardless of the Px. The insufficiency of estrogen decreased β-cell proliferation in Px rats, while it increased β-cell proliferation
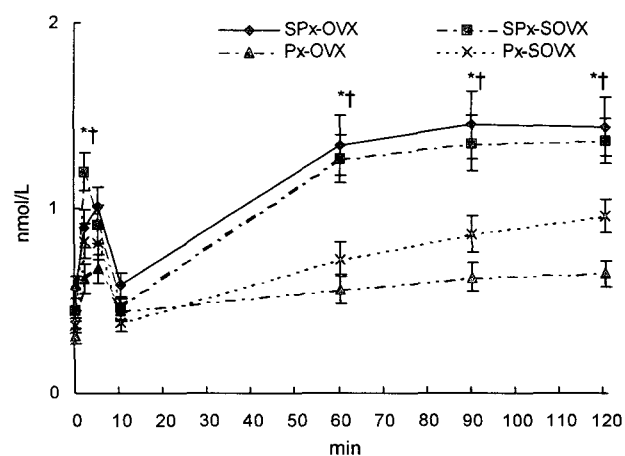

Fig. 3. Insulin secretion at hyperglycemic clamp. At the end of experimental periods, hyperglycemic clamp was performed in overnight fasted mice to determine insulin secretion pattern and amounts (n=8~9).

OVX, ovariectomy; SOVX, sham operation of OVX; Px, 90% pancreatectomy; SPx, sham operation of Px.

*p<0.05 in OVX effect. †p<0.05 in Px effcet.

Table 3. Area, mass, proliferation, and apoptosis of β-cells

	SPx[1]-OVX	SPx-SOVX	Px-OVX	Px-SOVX
β-cell area (%)	35.3±3.6[2]	28.1±4.2	20.3±2.9	32.4±4.8
β-cell mass (mg)	105.2±10.1	84.4±16.3	39.6±6.1	62.8±8.8
β-cell proliferation (# of BrdU⁺ cells/mm² pancreas)	2.1±0.3	1.6±0.4	2.2±0.4	2.8±0.5
Apoptotic bodies (# of brown dots/mm² pancreas)	0.1±0.02	0.06±0.01	0.18±0.04	0.08±0.02*

[1]See the legend of Table 1.
[2]Values are mean±SD, n=5.
*$p<0.05$ in OVX effect.

in SPx rats. Yet, proliferation was the main regulatory factor for the islet mass in the Px-OVX rats. When proliferation overcame apoptosis, the islet mass was increased in SPx-OVX rats. Various studies (2,3,5,6) with different animal models, such as IRS1 knockout mice and diet-induced insulin resistant rodents, have shown that insulin resistance can be compensated for by hyperinsulinemia, in which case diabetes does not develop. As such, insulin-resistant animals have an increased β-cell mass with increased proliferation via increased pancreatic homeodomain protein (PDX-1) expression levels, the transcription factor involved in β-cell proliferation. Additionally, Zucker diabetic rats do not develop type-2 diabetes while the islet mass is sufficient to secrete insulin to normalize the blood glucose levels (5,6). However, IRS2 knockout mice do develop type-2 diabetes, since the proliferation of β-cells decreases, leading to an extremely low β-cell mass, so the insulin secretion cannot compensate for the insulin resistance (4).

In summary, the current study found that an estrogen insufficiency increased insulin resistance and impaired the β-cell function, as represented by the insulin secretion patterns and amounts. The rats without diabetes overcame insulin resistance with compensatory insulin secretion. However, the rats with mild diabetes exhibited a uncompensated insulin resistance, possibly due to an insufficient increase in the β-cell mass. Thus, estrogen insufficiency was only found to impair the β-cell compensatory response in the case of mild type-2 diabetes. However, the development of diabetes can also be increased in the case of extreme insulin resistance due to environmental factors, such as stress, a high-fat diet, lack of physical activities, etc. In conclusion, after menopause, a diabetic condition can be rapidly aggravated, and the prevalence of diabetes remarkably increased. Consequently, postmenopausal women need to improve their insulin sensitivity by increasing physical activity and maintaining a normal weight to prevent and delay β-cell failure.

ACKNOWLEDGEMENT

REFERENCES

1. Rahier J, Goebbels RM, Henquin JC. 1983. Cellular composition of the human diabetic pancreas. *Diabetologia* 24: 366-371.
2. White MF. 1998. The IRS-signalling system: a network of docking proteins that mediate insulin action. *Mol Cell Biochem* 182: 3-11.
3. Kulkarni RN, Jhala US, Winnay JN, Krajewski S, Montminy M, Kahn CR. 2004. PDX-1 haploinsufficiency limits the compensatory islet hyperplasia that occurs in response to insulin resistance. *J Clin Invest* 114: 828-836.
4. Withers DJ, Gutierrez JS, Towery H, Burks DJ, Ren JM, Previs S, Zhang Y, Bernal D, Pons S, Shulman GI, Bonner-Weir S, White MF. 1998. Disruption of IRS-2 causes type 2 diabetes in mice. *Nature* 391: 900-904.
5. Hirose H, Lee YH, Inman LR, Nagasawa Y, Johnson JH, Unger RH. 1996. Defective fatty acid-mediated beta-cell compensation in Zucker diabetic fatty rats. Pathogenic implications for obesity-dependent diabetes. *J Biol Chem* 271: 5633-5637.
6. Milburn JL Jr, Hirose H, Lee YH, Nagasawa Y, Ogawa A, Ohneda M, BeltrandelRio H, Newgard CB, Johnson JH, Unger RH. 1995. Pancreatic beta-cells in obesity. Evidence for induction of functional, morphologic, and metabolic abnormalities by increased long chain fatty acids. *J Biol Chem* 270: 1295-1299.
7. Lee CC, Kasa-Vubu JZ, Supiano MA. 2004. Androgenicity and obesity are independently associated with insulin sensitivity in postmenopausal women. *Metabolism* 53: 507-512.
8. Shoelson SE, Lee J, Yuan M. 2003. Inflammation and the IKK beta/I kappa B/NF-kappa B axis in obesity- and diet-induced insulin resistance. *Int J Obes Relat Metab Disord* 27: S49-52.
9. Axen KV, Dikeakos A, Sclafani A. 2003. High dietary fat promotes syndrome X in nonobese rats. *J Nutr* 133: 2244-2449.
10. Friday KE, Dong C, Fontenot RU. 2001. Conjugated equine estrogen improves glycemic control and blood lipoproteins in postmenopausal women with type 2 diabetes. *J Clin Endocrinol Metab* 86: 48-52.
11. Andersson B, Mattsson L, Hahn L, Marin P, Lapidus L, Holm G, Bengtsson BA, Bjorntorp P. 1997. Estrogen replacement therapy decreases hyperandrogenicity and improves glucose homeostasis and plasma lipids in postmenopausal women with non-insulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 82: 638-643.
12. Hosokawa YA, Hosokawa H, Chen C, Leahy JL. 1996. Mechanism of impaired glucose-potentiated insulin-secretion in diabetic 90 percent pancreatectomy rats-Study using glucagon-like peptide-1 (7-37). *J Clin Invest* 97: 180-186.
13. Picard F, Deshaies Y, Lalonde J, Samson P, Labrie C,

Belanger A, Labrie F, Richard D. 2000. Effects of the estrogen antagonist EM-652. HCI on energy balance and lipid metabolism in ovariectomized rats. *Int J Obes Relat Metab Disord* 24: 830-840.

14. Dobbins RL, Szczepaniak LS, Myhill J, Tamura Y, Uchino H, Giacca A, McGarry JD. 2002. The composition of dietary fat directly influences glucose-stimulated insulin secretion in rats. *Diabetes* 51: 1825-1833.

15. Choi SB, Park S. 2002. Does fluoxetine administration influence insulin resistance in 90% pancreatectomized rats? *Metabolism* 51: 38-43.

16. Oddeti PR, Borgrglio A, Pascal AD, Rolandi R, Adezati L. 1990. Prevention of diabetes-increased aging effect on rat collagen linked fluorescence by aminoguanine and rutin. *Diabetes* 39: 796-802.

17. Hennige AM, Burks DJ, Ozcan U, Kulkarni RN, Ye J, Park S, Schubert M, Fisher TL, Dow MA, Leshan R, Zakaria M, Mossa-Basha M, White MF. 2003. Upregulation of insulin receptor substrate-2 in pancreatic beta cells prevents diabetes. *J Clin Invest* 112: 1521-1532.

18. Committee of SAS Institute. 1985. Guide for personal computers. SAS Institutes Inc, Cary, NC. p 257-260.

19. Park S, Park CH, Jun DW, Choi SB. 2004. A high carbohydrate diet induces insulin resistance through decreased glucose utilization in ovariectomized rats. *Kr J Internal Med* 19: 87-92.

20. Kimura M, Irahara M, Yasui T, Saito S, Tezuka M, Yamano S, Kamada M, Aono T. 2002. The obesity in bilateral ovariectomized rats is related to a decrease in the expression of leptin receptors in the brain. *Biochem Biophys Res Comm* 290: 1349-1353.

21. Burguera B, Hofbauer LC, Thomas T, Gori F, Evans GL, Khosla S, Riggs BL, Turner RT. 2001. Leptin reduces ovariectomy-induced bone loss in rats. *Endocrinology* 142: 3546-3553.

22. Friday KE, Dong C, Fontenot RU. 2001. Conjugated equine estrogen improves glycemic control and blood lipoproteins in postmenopausal women with type 2 diabetes. *J Clin Endocrin Metab* 86: 48-52.

23. Hirose H, Lee YH, Inman LR, Nagasawa Y, Johnson YH, Unger RH. 1996. Defective fatty acid-mediated β-cell compensation in Zucker diabetic fatty rats. pathogenic implications for obesity-dependent diabetes. *J Biol Chem* 271: 5633-5637.

24. Pansini F, Bonaccorsi G, Genovesi F, Folegatti MR, Bagni B, Bergamini CM, Mollica G. 1990. Influence of estrogens on serum free fatty acid levels in women. *J Clin Endocrinol Metab* 71: 1387-1389.

25. Shimabukuro M, Zhou YT, Levi M, Unger RH. 1998. Fatty acid-induced β cell apoptosis: A link between obesity and diabetes. *Proc Natl Acad Sci USA* 95: 2498-2502.