Exercise and dexamethasone oppositely modulate β-cell function and survival via independent pathways in 90% pancreatectomized rats

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Abstract

Long-term dexamethasone (DEX) treatment is well known for its ability to increase insulin resistance in liver and adipose tissues leading to hyperinsulinemia. On the other hand, exercise enhances peripheral insulin sensitivity. However, it is not clear whether DEX and/or exercise affect β-cell mass and function in diabetic rats, and whether their effects can be associated with the modulation of the insulin/IGF-I signaling cascade in pancreatic β-cells. After an 8-week study, whole body glucose disposal rates in 90% pancreatectomized (Px) and sham-operated male rats decreased with a high dose treatment of DEX (0.1mg DEX/kg body weight/day)(HDEX) treatment, while disposal rates increased with exercise. First-phase insulin secretion was decreased and delayed by DEX via the impairment of the glucose-sensing mechanism in β-cells, while exercise reversed the impairment of first-phase insulin secretion caused by DEX, suggesting ameliorated β-cell functions. However, exercise and DEX did not alter second-phase insulin secretion except for the fact that HDEX decreased insulin secretion at 120 min during hyperglycemic clamp in Px rats. Unlike β-cell functions, DEX and exercise exhibited increased pancreatic β-cell mass in two different pathways. Only exercise, through increased proliferation and decreased apoptosis, increased β-cell mass via hyperplasia, which resulted from an enhanced insulin/IGF-I signaling cascade by insulin receptor substrate 2 induction. By contrast, DEX expanded β-cell mass via hypertrophy and neogenesis from precursor cells, rather than increasing proliferation and decreasing apoptosis. In conclusion, the improvement of β-cell function and survival via the activation of an insulin/IGF-I signaling cascade due to exercise has a crucial role in preventing the development and progression of type 2 diabetes.


Introduction

Normal levels of glucocorticoids are important for glucose metabolism. In a fasting state, normal concentrations of cortisol stimulate several processes, which collectively serve to maintain normoglycemia. By contrast, chronically excessive numbers of glucocorticoids are known to induce insulin resistance, which is possibly involved in impairing the insulin signaling in liver, muscle, and adipose tissues (Giorgino et al. 1992, Haber & Weinstein 1992, Buren et al. 2002). Insulin resistance induces hyperinsulinemia to normalize blood glucose levels in humans and experimental animals (Barbera et al. 2001, Ahren & Pacini 2005). Genetically modified animal studies have revealed that the decompensation for insulin resistance is associated with not only β-cell function, but also its mass, through alteration of the insulin/insulin-like growth factor (IGF)-I signaling cascade (Withers et al. 1998, White 1999, Rhodes & White 2002). The enhancement of insulin receptor-substrate 2 (IRS2)–PI3 kinase-phosphorylated Akt by IRS2 induction in β-cells promotes sufficient and sustained compensatory insulin secretion via increased pancreatic β-cell mass (Hennige et al. 2003, Park et al. 2006).

The failure of this compensatory mechanism in β-cells is believed to play a crucial role in the pathogenesis of type 2 diabetes (Taylor 1999, Mathis et al. 2001). However, when insulin resistance is aggravated, the mechanism and the role of factors involved in compensatory insulin secretion are only partly understood. Furthermore, it has not been verified whether the modulation in β-cell function and mass via dexamethasone (DEX) results from an impairment of the insulin/IGF-I signaling cascade in β-cells.

In contrast, exercise is well known to lessen hyperglycemia and insulin resistance by improving glucose utilization in muscles and decreasing fat deposits in the body (Sato et al. 2003, Hawley 2004). Few studies have been performed to verify whether exercise affects β-cell function and mass in diabetic rats (Shima et al. 1997). The effect of exercise and DEX on β-cell function and mass and their mechanism have not been revealed, even though both are involved in glucocorticoid metabolism. Therefore, we studied whether (1) DEX and exercise affect islet function and mass along with insulin resistance in pancreatectomized (Px) and sham-operated rats, and (2) whether the insulin/IGF-I signaling cascade in β-cells is associated with their modulation.
Materials and Methods

Animals

Male Sprague–Dawley rats weighing 248 ± 14 g had 90% of their pancreas removed using the Hosokawa technique (Hosokawa et al. 1996) or received a sham pancreatectomy operation. After a 90% pancreatectomy (Px), the pancreas which remained was within 2 mm of the common bile duct and extended from the duct to the first part of the duodenum. Px rats with random fed serum glucose levels less than 9·4 mM were excluded after 2 weeks from surgery, and the Px rats included in the experiments showed characteristics of mild diabetes mellitus. A sham pancreatectomy was performed by disengaging the pancreas from the mesentery and gently rubbing it between the fingers. The sham-operated rats did not have any symptoms of diabetes. All experimental animals were allowed free access to standard laboratory food (Sam Yang Co., Kangwon-Do, Korea) and water. They were housed individually in stainless-steel cages in a controlled environment (23 °C and a 12 h light:12 h darkness cycle). All surgical and experimental procedures were performed according to the guidelines of the Animal Care and Use Review Committee at Hoseo University, Korea. Overnight fasted serum-glucose levels, food intake, and body weight were measured weekly every Tuesday at 1000 h.

Px and sham rats were divided into two groups; half of the rats ran on an uphill treadmill at 20 m/min for 30 min four times a week during the experimental periods, and were designated as the exercise group, while the rest did not exercise. Exercised and unexercised rats ran on an uphill treadmill at 20 m/min for 30 min four times a week during the experimental periods, and were designated as such. Exercised and unexercised rats were further divided into three groups respectively; each group received a daily oral administration of 0·1 mg cellulose/kg body weight (bw) (control), 0·1 mg DEX (Yuhan Co., Seoul, Korea)/kg bw (HDEX) or 0·01 mg DEX/kg bw (a low dosage treatment of DEX (LDEX)) for 8 weeks.

Insulin secretion and insulin resistance

After 7 weeks of treatment, catheters were surgically implanted into the right carotid artery and left jugular vein of rats anesthetized with i.p. injections of ketamine and xylazine (100 mg and 10 mg/kg bw respectively). After 5–6 days of implantation, a hyperglycemic clamp was performed in conscious and overnight fasted rats to determine insulin–secretion capacity (Dobbins et al. 2002). An i.v. bolus of 25% glucose over 5 min was given to instantaneously raise blood glucose to 12 mM. Subsequently, a continuous and variable glucose infusion was applied to hold the rate at a constant 12 mM during the time from 60 to 120 min. Serum glucose and insulin levels from artery blood were measured at 0, 2, 5, 10, 60, 90, and 120 min.

Two days after use of the hyperglycemic clamp, a euglycemic hyperinsulinemic clamp (Choi & Park 2002) was used under the same conditions as the hyperglycemic clamp to determine insulin resistance. Insulin-stimulated whole body glucose flux was estimated using a prime continuous infusion of [3-3H]glucose (10 μCi bolus, 0·1 μCi/min, NEN Life Science, Boston, MA, USA) throughout the clamps. Regular human insulin (Humulin, Eli Lilly and Co.) was continuously infused at a rate of 20 pmol/kg per min in order to raise the plasma insulin concentration to approximately 1100 pm. Blood samples from arteries were collected at 10-min intervals for glucose estimation and 25% glucose was infused as needed at variable rates to clamp glucose levels at approximately 6 mM. To determine plasma [3-3H]glucose concentrations, the plasma was deproteinized with ZnSO4 and Ba(OH)2, dried to remove 3H2O, resuspended in water and disintegrations per minute (d.p.m.) of 3H were recorded. The plasma concentration of 3H2O was determined by the difference between 3H counts with and without drying. Rates of whole body glucose uptake and basal glucose turnover were determined as the ratio of the [3H]glucose infusion rate to the specific activity of plasma glucose (d.p.m./μmol) during the final 30 min of the respective experiments. Hepatic glucose production during clamps was determined by subtracting the glucose infusion rate from the whole body glucose uptake. The rats were anesthetized with sodium pentobarbital (35 mg/kg bw) (Nembutal, Abbott Laboratories) and were killed by decapitation at the end of the clamp. Tissues were rapidly dissected, weighed and frozen in liquid nitrogen, and stored at −70 °C until further analysis could be performed.

Serum–glucose levels were analyzed with a Glucose Analyzer II (Beckman, Palo Alto, CA, USA). Serum–insulin levels were measured by RIA (Linco Research, St Charles, MO, USA). Advanced glycated endproducts (AGE) of s.c. tissues were measured using fluorescence methods (Oddetti et al. 1990). Briefly, s.c. tissues were homogenized in PBS and the lysates centrifuged at 10 000 × g for 30 min at 4 °C. The pellet was defatted with chloroform and methanol (2:1, v:v) and digested with collagenase type 7 and proteinase K in PBS for 48 h, followed by overnight incubation with an equal amount of 0·2 M NaOH at 4 °C. After centrifugation, half of the supernatant was used for fluorescence determination at excitation 370 nm, emission 440 nm to measure general AGE–associated fluorescence. The rest was used for assaying the content levels of hydroxyproline, which was determined by colorimetric measurement.

In order to determine the glycogen content in the liver, its lysates were centrifuged at 100 g for 10 min and the supernatants deproteinized with 1·5 M perchloric acid. The glycogen content was calculated from glucose concentrations derived from glycogen hydrolyzed by α-amylglucosidase in an acid buffer (Frontoni et al. 1991). Insulin content in the pancreas was measured by acid–ethanol methods (Hennige et al. 2003). The pancreas was homogenized with acid–alcohol, stored overnight at 4 °C and followed by centrifugation at 700 g for 30 min. The supernatant was removed and stored at −20 °C. pending measurement of insulin content by RIA kit (Linco Research).
Five to six rats from each group were treated with 5-bromo-2-deoxyuridine (BrdU; Roche Molecular Biochemicals; 100 μg/kg bw) at the end of the 8-week experimental period. Six hours postinjection, pancreas samples were prepared and analyzed as described above (Hennige et al. 2003). The pancreas was dissected, fixed in a 4% paraformaldehyde solution (pH 7.2) overnight at room temperature and embedded in paraffin blocks. Serial 5 μm paraffin-embedded tissue sections were mounted on slides. To prevent the selection of sections with similar areas, after rehydration, every sixth or seventh section was selected to determine β-cell area, BrdU incorporation, and apoptosis. The randomly chosen sections were immunostained as described above (Hennige et al. 2003).

Endocrine β-cells were identified by applying a guinea pig anti-insulin antibody in paraffin-embedded pancreatic sections. β-Cell proliferation was examined by the incorporation of BrdU in β-cells from rats injected with BrdU. This incorporation was determined by performing a double-label immunohistochemistry with anti-insulin (Zymed Laboratories, South San Francisco, CA, USA) and anti-BrdU antibodies (Roche Molecular Biochemicals) on rehydrated paraffin sections. Apoptosis of β-cells was measured by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling kit (Roche) in paraffin sections of the pancreas containing insulin-positive cells, measured by IP Lab software (Scanalytics, Inc., Fairfax, VA, USA). Pancreatic β-cell area was measured by acquiring random (selecting every other islet in the window), non-overlapping images from two sets of eight to ten distal images of insulin-stained pancreatic sections at a magnification of 10× with a Zeiss Axiovert microscope (Carl Zeiss Microimaging, Thornwood, NY, USA). Results of β-cell quantification are expressed as the percentage of the total surveyed area containing insulin-positive cells, measured by IP Lab Spectrum software (Scanalytics, Inc., Fairfax, VA, USA). Pancreatic β-cell mass was calculated by multiplying the percentage of insulin-positive area by the weight of the corresponding pancreatic portion. The individual β-cell size was determined as the insulin-positive area divided by the number of nuclei counted in the corresponding insulin-positive structures in immunofluorescence staining, which were chosen at random and corresponded to 125–150 nuclei per sample. Bigger size of individual β-cell indicates the induction of β-cell hypertrophy. The number of small β-cell clusters was determined as the number of measurements in an arbitrarily set area of <250 μm² (islets containing less than five nuclei were excluded) and expressed as the percentage of the total number of measurements in the section (Rooman et al. 2002).

To immunostain duct cells, mouse monoclonal anti-cytokeratin-19 antibodies from Zymed were used. β-Cell proliferation was expressed in the number of BrdU+ β-cells per square millimeter pancreas and was calculated as the total BrdU+ nuclei in β-cell nuclei per pancreas section, two sections per animal and five to six animals per group. Apoptosis of β-cells was determined by counting the total number of apoptotic bodies in a β-cell nucleus, and was calculated in the same manner as that of β-cell proliferation.

Pancratic islets from nine to ten rats of each group were isolated by collagenase digestion at the end of an 8-week treatment of DEX and/or exercise (Hennige et al. 2003). Through the pancreatic duct, 3 ml 1·0 g/ml collagenase (Sigma) in Dulbecco’s modified Eagle’s medium (DMEM)—high glucose were injected into the pancreas of rats anesthetized with sodium pentobarbital. The pancreas was immediately removed and incubated at 37 °C for 15 min. The digested pancreas was washed with DMEM—high glucose four times on ice and islets were isolated with a separation medium consisting of Ficoll reagent (Sigma). The islets washed with cold DMEM—high glucose were pooled from two to three rats from each group. Prior to lysing islets, they were administered with 10 nM IGF-I for 10 min to determine insulin/IGF-I signaling cascade.

Islets isolated from rats treated with DEX and/or exercise, as mentioned above, were lysed with a 20 mM Tris buffer (pH 7.4) containing 2 mM EDTA, 137 mM NaCl, 1% NP40, 10% glycerol and 12 mM α-glycerol phosphate and protease inhibitors. After 30 min on ice, the lysates were centrifuged for 10 min at 11 000 g at 4 °C. Lysates with equivalent amounts of protein (400 μg) were immunoprecipitated with specific antibodies (anti-IRS1 and IRS2 antibodies) or resolved directly by SDS-PAGE. Lysates with equal amounts of protein (30 μg) were used for immunoblotting with specific antibodies against IRS1 (UBI, Waltham, MA, USA), IRS2 (UBI), protein kinase B (PKB or Akt, Cell Signaling Technology, Beverly, MA, USA), phosphorylated PKB<sup>Thr473</sup> (cell signaling), pancreatic homeodomain protein (PDX-1; Santa Cruz Biotechnology, Santa Cruz, CA, USA), and β-actin (Santa Cruz Biotechnology) as described above (Giraud et al. 2004). The intensity of protein expression was determined using Imagequant TL (Amersham Biosciences). These experiments were repeated four times for each group.

All results are expressed as means±S.D. Statistical analysis was performed using the SAS statistical analysis program (Committee of SAS Institute 1985). One-way ANOVA was carried out to determine DEX effect in cell culture studies. In animal studies, the two main effects of DEX and exercise were determined by two-way ANOVA, since there was no significant interaction between exercise and DEX. Significant differences in the main effects among groups were identified.
Results

Exercise delays the progression of diabetes in Px rats as opposed to DEX treatment

Px rats treated with HDEX, not LDEX, exhibited hyperglycemia and hyperinsulinemia during an overnight fast and increased AGE levels at the end of the experimental periods. Exercise improved glucose homeostasis in HDEX-treated Px rats, which led them to exhibit near normoglycemia and decreased AGE levels similar to the control group (Table 1). DEX increased serum-insulin levels in a dose-dependent manner in Px rats, compared to the control, and exercise did not reverse this effect. Sham rats maintained normoglycemia despite DEX treatments. Even sham rats with HDEX treatment displayed normoglycemia with hyperinsulinemia, while exercise corrected the hyperinsulinemia induced by HDEX without changing serum-glucose levels (no data shown).

Pancreatectomy decreased whole body glucose disposal rates by 21–51% during a euglycemic hyperinsulinemic clamp, compared with sham rats, indicating increased peripheral insulin resistance due to the procedure. DEX and exercise exhibited a similar effect on the rates in both Px and sham rats, but the intensity of the effects was lower in sham rats than in Px rats (Fig. 1A). Px rats, administered with HDEX, displayed decreased glucose disposal rates in comparison with the control rats, while exercise improved the rates in DEX-treated diabetic rats (P<0.05, Fig. 1A). However, LDEX did not decrease glucose disposal rates comparable with the control (Fig. 1A).

Table 1: Overnight fasted serum glucose and insulin levels in 90% pancreatectomized rats at the end of experimental periods. Means ± s.e.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum glucose (mmol/l)</th>
<th>Serum insulin (pmol/l)</th>
<th>AGE (AU/mg collagen)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDEX (n=10)</td>
<td>7.7±0.8ab</td>
<td>580±65b</td>
<td>7.1±0.5b</td>
</tr>
<tr>
<td>HDEX-EX (n=11)</td>
<td>6.9±0.7ab</td>
<td>430±52b</td>
<td>6.3±0.5b</td>
</tr>
<tr>
<td>LDEX (n=11)</td>
<td>7.2±0.6b</td>
<td>315±38b</td>
<td>6.2±0.6b</td>
</tr>
<tr>
<td>LDEX-EX (n=11)</td>
<td>6.2±0.6b</td>
<td>255±39c†</td>
<td>5.4±0.7c</td>
</tr>
<tr>
<td>CON (n=10)</td>
<td>7.0±0.7ab</td>
<td>299±35c</td>
<td>6.1±0.7b</td>
</tr>
<tr>
<td>EX (n=11)</td>
<td>6.0±0.5c‡</td>
<td>195±26d‡</td>
<td>5.5±0.6c**</td>
</tr>
</tbody>
</table>

Significant dexamethasone (DEX) effect in two-way ANOVA at *P<0.05 and †P<0.01. Significant exercise (EX) effect in two-way ANOVA at †P<0.05 and §P<0.01. Values in the same column, with different superscript letters (a–c, b–d) were significantly different at P<0.05. HDEX, high dosage DEX treatment; LEDX, low dosage DEX treatment; AGE, advanced glycation endproducts in s.c. tissues (arbitrary unit (AU)/mg collagen); CON, control.
Figure 1 Whole body glucose disposal rate, hepatic glucose output, and liver glycogen. (A) At the end of experimental periods, after administration of saline (CON) or dexamethasone (DEX) and/or exercise (EX), a euglycemic hyperinsulinemic clamp was performed in overnight fasted 90% pancreatectomized (Px) diabetic rats. Whole body glucose disposal rates during a euglycemic hyperinsulinemic clamp in Px rats and sham rats. (B) Hepatic glucose output at basal and clamped states during a euglycemic hyperinsulinemic clamp in Px rats. (C) Liver glycogen deposits were measured after a euglycemic hyperinsulinemic clamp in Px rats. (D) Hepatic glucose output at basal and clamped states during a euglycemic hyperinsulinemic clamp in sham rats. Sample size of each group was the same as in Table 1. *Significant DEX effect in two-way ANOVA at P<0.05. †Significant EX effect in two-way ANOVA at P<0.05. Columns with different superscript letters (a, b, c) were significantly different in Tukey’s test at P<0.05. HDEX, high dosage DEX treatment; LDEX, low dosage DEX treatment.
The expression of glucose transporter 2 (GLUT2) and glucokinase was analyzed under different treatment paradigms to assess their roles in the improvement of insulin secretion observed in Fig. 2. GLUT2 expression remained unchanged under all conditions (Fig. 3). Glucokinase expression in islets was increased in exercised rats, while its expression was lowered by DEX when compared with the control (Fig. 3).

**Exercise and DEX increased β-cell mass expansion in two different pathways**

After 90% pancreatectomy, the pancreas, including islets in Px rats, was regenerated up to 50–60% of the size of sham rats for 8 weeks, which was the same among all groups of Px rats (no data shown). Regeneration of the pancreas was evident due to an increase in pancreas weight. However, exercise and DEX increased absolute β-cell mass, which was calculated by multiplying pancreas weight by β-cell area (percentage of total pancreas area), per section in both Px and sham rats. Although the percentage of the β-cell area in the pancreas was greater in Px rats than in sham rats, absolute β-cell mass was lower in Px rats due to a smaller pancreas (Tables 2 and 3). HDEX treatment enlarged β-cell area and mass in sham rats in higher proportions than in Px rats, suggesting that Px rats expand β-cell mass at insufficient rates.

DEX enlarged β-cell mass via an increase in individual β-cell size and in the number of small β-cell clusters in both Px and sham rats (Tables 2 and 3). Even with HDEX treatment, the number of small β-cell clusters was significantly lower in sham rats when compared with Px rats. Figure 4 exhibits an example of small β-cell clusters in Px rats. Small β-cell clusters were checked at random to ensure they were not peripheral parts of larger islets in the series of sections. Most small β-cell clusters were located close to the ductal complexes, and some of them were within the complex (Fig. 4). An increase in the number of small β-cell clusters was an indication of increased neogenesis in DEX-treated Px rats, yet exercise did not affect their production in Px and sham rats.

BrdU incorporation into β-cells, representing the degree of proliferation, was decreased in DEX-treated rats in a dose-dependent manner in both Px and sham rats, while the number of apoptotic bodies was increased (Tables 2 and 3). HDEX had a greater impact on β-cell proliferation and apoptosis in Px rats than in sham rats. Exercise increased β-cell mass by increasing the number of β-cells via elevating β-cell proliferation and decreasing apoptosis. By contrast, HDEX decreased β-cell proliferation and increased apoptosis. However, DEX expanded the mass by β-cell hypertrophy, determined by individual β-cell size in sham and Px rats, possibly due to increased insulin resistance (Tables 2 and 3). The individual size of a β-cell is represented by the cellular area with one nucleus. Exercise suppressed β-cell hypertrophy, which had been induced by DEX. LDEX plus exercise exhibited the biggest additive increase in β-cell mass via increased neogenesis and proliferation and decreased apoptosis in Px and sham rats (Tables 2 and 3).

**Exercise and DEX modulate insulin/IGF-I signaling cascade in islets of Px rats via the induction of IRS2 expression**

Islets isolated from DEX-administered rats exhibited decreased IRS2 expression (Fig. 5). The effect of DEX on IRS2 expression was reversed by exercise; IRS2 content was elevated in the islets from exercised rats and tyrosine phosphorylation was stimulated in parallel with IRS2 expression levels when isolated islets were administered with 10 nM IGF-I for 10 min. According to the tyrosine phosphorylation of IRS2, the insulin/IGF-I signaling cascade in the islets was positively modulated. Along with the potentiation of the insulin/IGF-I signaling cascade, PDX-1 expression was elevated in islets from exercised rats and lowered in those from HDEX-treated rats. Exercise overcame the attenuation of the insulin/IGF-I signaling cascade resulting from DEX administration in the combination of treatments.

**Discussion**

In this study, we observed that DEX, a potent synthetic corticosteroid, increased insulin resistance in peripheral tissues and impaired β-cell function in Px and sham rats, while exercise reversed these effects. Unlike β-cell function, β-cell mass was enlarged in both DEX and exercise in Px and sham rats. However, their mechanism of action and intensity were different in diabetic and normal rats. HDEX sufficiently increased β-cell mass to compensate for induced insulin resistance in sham rats, but not in Px rats. Exercise restored β-cell function, not only by reducing insulin resistance, but...
Thus, in an insulin-resistant state, superscript letters (a, b, c, d) were significantly different at Apoptosis (% apoptotic bodies of islets). Also by increasing the number of β-cells, through potentiating insulin/IGF-I signaling.

Progression into diabetes can be viewed as having definable stages, characterized by changes in serum-glucose levels and β-cell function. In addition, each stage is marked by important changes in β-cell mass. Until β-cell mass increases to compensate for insulin resistance, blood glucose levels remain within a normal range (Weir & Bonner-Weir 2004). Thus, in an insulin-resistant state, β-cell mass needs to expand abruptly. β-cell mass is normally tightly regulated through a balance of β-cell birth (β-cell replication and islet neogenesis from precursor cells) and β-cell death through apoptosis (Taylor 1999). Since insulin resistance disrupts this regulation and impairs β-cell growth and survival, hypertrophy (an enlargement of individual islet size) plays a predominant role in increasing β-cell mass during insulin-resistant states. This expansion due to hypertrophy could not be sustained for long periods and fell into decompensation.

Table 2 Effect of dexamethasone (DEX) and exercise (EX) on the modulation of islet morphology in 90% pancreatectomized rats. Means ± S.D.

<table>
<thead>
<tr>
<th></th>
<th>HDEX (n = 6)</th>
<th>HDEX+EX (n = 6)</th>
<th>LDEX (n = 7)</th>
<th>LDEX+EX (n = 6)</th>
<th>CON (n = 7)</th>
<th>EX (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Cell area per section (% of pancreas)</td>
<td>2.9 ± 0.5&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3.6 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.1 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.9 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.4 ± 0.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.2 ± 0.5&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;+++&lt;/sup&gt;</td>
</tr>
<tr>
<td>Absolute β-cell mass (mg)</td>
<td>6.9 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.7 ± 1.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.3 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.9 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.7 ± 0.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.5 ± 1.0&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;+++&lt;/sup&gt;</td>
</tr>
<tr>
<td>Individual β-cell size (μm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>239 ± 63 ± 5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>201 ± 67 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>197 ± 52 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>175 ± 34 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>177 ± 48 ± 8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>168 ± 52 ± 6&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;+++&lt;/sup&gt;</td>
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<tr>
<td>Small β-cell cluster (%)</td>
<td>20.4 ± 2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.2 ± 2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.2 ± 2.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.4 ± 2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.2 ± 1.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.9 ± 1.6&lt;sup&gt;aa&lt;/sup&gt;</td>
</tr>
<tr>
<td>BrdU&lt;sup&gt;+&lt;/sup&gt; cells (% of BrdU&lt;sup&gt;+&lt;/sup&gt; β-cells of islets)</td>
<td>0.56 ± 0.16&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.04 ± 0.18&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.79 ± 0.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.22 ± 0.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.98 ± 0.21&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.55 ± 0.11&lt;sup&gt;aa&lt;/sup&gt;</td>
</tr>
<tr>
<td>Apoptosis (% apoptotic β-cells of islets)</td>
<td>0.58 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.47 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.46 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.32 ± 0.08&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.38 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.24 ± 0.08&lt;sup&gt;+++&lt;/sup&gt;</td>
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Significant DEX effect in two-way ANOVA at *P<0.05. †Significant EX effect in two-way ANOVA at *P<0.05. Values in the same column with different superscript letters (a, b, c, d) were significantly different at P<0.05. HDEX, high dosage DEX treatment; LDEX, low dosage DEX treatment; CON, control.
yet it temporarily prevented a sudden progression of diabetes during an insulin-resistant state. In contrast, exercise blocked any hypertrophy in HDEX-treated rats and expanded β-cell mass by hyperplasia of β-cells, resulting in longer compensation. Thus, consistent with Taylor’s results, our data revealed that DEX and exercise expanded β-cell function and mass, even though a contrasting, HDEX treatment decreased weight gain despite increased food consumption compared with the control. In our study, LDEX−treated rats exhibited higher body weight with increased food intake per 100 g bw compared with the control, possibly due to elevated energy expenditure through peripheral catabolism. We did not measure serum NEFA levels, but we expected that HDEX increased serum NEFA levels, which would participate in elevated insulin resistance and impaired β-cell function.

However, long-term treatment with NEFA inhibited glucose-induced insulin secretion. Thus, elevated NEFA in an insulin-resistant state contributed to the impairment of β-cell function (Grill & Qvigstad 2000). Human and animal studies have shown that DEX elevates serum NEFA via increased lipolysis by impairing the anti-lipolytic action of insulin (Mokuda & Sakamoto 1999, Willi et al. 2002). In our study, LDEX−treated rats exhibited higher body weight with increased food consumption compared with the control. In contrast, HDEX treatment decreased weight gain despite increased food intake per 100 g bw compared with the control, possibly due to elevated energy expenditure through peripheral catabolism. We did not measure serum NEFA levels, but we expected that HDEX increased serum NEFA levels, which would participate in elevated insulin resistance and impaired β-cell function.

Table 3 Effect of dexamethasone (DEX) and exercise (EX) on the modulation of islet morphometry in sham-operated rats. Means ± S.D.

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<thead>
<tr>
<th></th>
<th>HDEX (n=5)</th>
<th>HDEX-EX (n=6)</th>
<th>LDEX (n=6)</th>
<th>LDEX-EX (n=6)</th>
<th>CON (n=6)</th>
<th>EX (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Cell area per section (%) of pancreas</td>
<td>2.8 ± 0.5ab</td>
<td>3.1 ± 0.5a</td>
<td>2.6 ± 0.4b</td>
<td>2.9 ± 0.4a</td>
<td>1.8 ± 0.4c</td>
<td>2.3 ± 0.4b*†</td>
</tr>
<tr>
<td>Absolute β-cell mass (mg)</td>
<td>15.8 ± 1.7a</td>
<td>17.4 ± 1.8a</td>
<td>14.5 ± 1.6b</td>
<td>16.4 ± 1.8a</td>
<td>10.2 ± 1.4c</td>
<td>12.9 ± 1.5b*†</td>
</tr>
<tr>
<td>Individual β-cell size (μm²)</td>
<td>228.4 ± 22.1b</td>
<td>187.3 ± 20.4b</td>
<td>184.6 ± 20.1b</td>
<td>151.4 ± 19.9c</td>
<td>150.5 ± 18.9c</td>
<td>147.6 ± 17.3b*†</td>
</tr>
<tr>
<td>Small β-cell cluster (%)</td>
<td>9.7 ± 1.2a</td>
<td>9.3 ± 1.0a</td>
<td>7.8 ± 0.9b</td>
<td>7.6 ± 0.9b</td>
<td>5.4 ± 0.8c</td>
<td>5.8 ± 0.7c*</td>
</tr>
<tr>
<td>BrdU+ cells (% BrdU+ cells of islets)</td>
<td>0.71 ± 0.09c</td>
<td>0.93 ± 0.14b</td>
<td>0.91 ± 0.11b</td>
<td>1.16 ± 0.18ab</td>
<td>0.9 ± 0.11b</td>
<td>1.37 ± 0.16b*†</td>
</tr>
<tr>
<td>Apoptosis (% apoptotic bodies of islets)</td>
<td>0.49 ± 0.07a</td>
<td>0.41 ± 0.06ab</td>
<td>0.39 ± 0.07ab</td>
<td>0.27 ± 0.05c</td>
<td>0.35 ± 0.05b</td>
<td>0.25 ± 0.04b*†</td>
</tr>
</tbody>
</table>

Significant DEX effect in two-way ANOVA at *P<0.05. †Significant EX effect in two-way ANOVA at P<0.05. Values in the same column with different superscript letters (a, b, c, d) were significantly different at P<0.05. HDEX, high dosage DEX treatment; LEDX, low dosage DEX treatment; CON, control.

Figure 4 Immunohistochemical staining for the transdifferentiated ductal complexes and β-cells of 90% Px rat pancreas. (A) The pancreas of rats treated with high dosage DEX treatment and exercise for 8 weeks. (B) The pancreas of rats exercised for 8 weeks. Brown represents β-cells stained with anti-insulin antibody. Single and double arrows indicate small β-cell cluster and duct respectively. Islets of A contained similar number of nuclei as those of B, but the size of islets was bigger than B, indicating hypertrophy of β-cells. Most small β-cell clusters were located close to the ductal complexes, and a few of them were within the complex.
Figure 5 The modulation of insulin/IGF-I signaling in islets isolated from 90% pancreatectomized (Px) rats treated with dexamethasone (DEX) and exercise (EX) for 8 weeks. Islets isolated from the rats of each group were administered with 10 nM IGF-I and immediately lysed with a lysis buffer and the phosphorylation and expression levels of the specific proteins were determined by immunoblotting with specific antibodies. The intensity of protein expression was determined using Imagequant TL (Amersham Biosciences). These experiments were repeated four times for islets, and the values are means ± S.D. *Significant DEX effect in two-way ANOVA at \( P < 0.05 \). †Significant EX effect in two-way ANOVA at \( P < 0.01 \). Columns with different superscript letters \( ^{a, b, c, d} \) were significantly different at \( P < 0.05 \). HDEX, high dosage DEX treatment; LDEX, low dosage DEX treatment; IRS, insulin receptor substrate; IB, immunoblotting; IP, immunoprecipitation; AU, absolute unit.
Another possible mechanism to reduce β-cell mass is decreased expression of transcription factors, which are important for islet development, such as PDX-1, Neuro D and hepatic nuclear factor-1α (HNF-1α) (Laybutt et al. 2003). Several studies have demonstrated that mRNA levels of these transcription factors were reduced by 50–60% in Px rats with 120 mg/dl glucose levels after 4 weeks from pancreatectomy (Jonsson et al. 1994, Stoffers et al. 1997). The mRNA expression levels of GLUT2 and glucokinase associated with glucose sensing in β-cells were downregulated in Px rats with hyperglycemia. During insulin-resistant states, PDX-1 expression in precursor cells, such as ductal and acinar cells, may be elevated during differentiation into β-cells, and the expression may be reduced when differentiation is completed. Thus, an increase in small clusters transiently prevents induction of hyperglycemia in order to compensate for insulin resistance.

In our study, exercise induced IRS2 expression in islets leading to an enhanced insulin/IGF-I signaling cascade, which, in turn, possibly led to β-cell mass expansion and improved function. Several studies have demonstrated that IRS2 induction in β-cells contributed to increasing their function and mass via enhancing insulin/IGF-I signaling (Hennige et al. 2003, Park et al. 2006). However, due to an attenuated insulin/IGF-I signaling cascade, DEX eventually exacerbated β-cell function, even though hypertrophy could be temporarily sustained to compensate for peripheral insulin resistance. By contrast, exercise plays a crucial role in diabetic treatment, not only by lessening insulin resistance, but also through potentiating β-cell function and mass via enhancement of the insulin/IGF-I signaling cascade. Enhanced signaling promoted the expansion of β-cell mass by increasing hyperplasia via increased β-cell proliferation and decreased apoptosis (Hennige et al. 2003, Hashimoto et al. 2005). Thus, hyperplasia is necessary in order to strengthen β-cell function to compensate for insulin resistance.

In addition to proliferation, β-cell neogenesis from precursor cells, such as ductal cells and acinar cells is known to contribute to β-cell regeneration after 90% partial pancreatectomy and administration of a high dosage of streptozotocin (Rooman et al. 2002, Li et al. 2003, Pospisilik et al. 2003). The present study revealed that DEX increased β-cell mass not through β-cell proliferation, but via neogenesis in parallel with elevated insulin resistance. β-Cell neogenesis was represented by an increased frequency of small β-cell clusters, and it appeared to be crucial for β-cell mass expansion during times of increased insulin resistance in which β-cell proliferation was decreased and apoptosis was increased. Insulin resistance elevated PDX-1 expression in precursor cells of β-cells during differentiation and in early stages of islet development (Li et al. 2003, Kulkarni et al. 2004, Jetton et al. 2005). However, the elevation of PDX-1 expression can only be maintained in β-cells if the insulin/IGF-I signaling cascade is activated. Small β-cell clusters generated from neogenesis required an enhanced insulin/IGF-I signaling cascade for growth and survival. However, DEX attenuated insulin/IGF-I signaling in β-cells causing small β-cell clusters to slowly grow into bigger islets. In contrast, the number of small clusters was fewer in exercised rats than in rats treated with DEX in this study, which can be explained as follows: exercise did not stimulate neogenesis as much as DEX, and/or it made newly created small β-cell clusters grow into bigger islets.

In conclusion, DEX reduces β-cell numbers but still increases β-cell mass via hypertrophy of individual β-cells and neogenesis, which play an important role in temporarily preventing diabetes development and progression when peripheral insulin resistance is induced. However, hypertrophy is insufficient to compensate for glucose dysregulation in Px rats due to inherent low insulin storage and abnormal β-cell function. In contrast, exercise ameliorates glucose homeostasis by improving β-cell function and mass as well as reducing insulin resistance. As with DEX treatment, exercise expands β-cell mass but through a different pathway. Exercise induces hyperplasia by stimulating β-cell proliferation and suppressing apoptosis via activation of the insulin/IGF-I signaling cascade. Thus, exercise plays an important role in preventing diabetic development and progression during insulin-resistant states.

Acknowledgements

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References


Choi SB & Park S 2002 Does fluoxetine administration influence insulin resistance in 90% pancreatectomized rats? Metabolism 51 38–43.


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Withers DJ, Gutierrez JS, Towery H, Burks DJ, Ren JM, Previs S, Zhang Y, Bernal D, Pons S, Shulman GI et al. 1998 Disruption of IRS-2 causes type 2 diabetes in mice. *Nature* **391** 900–904.

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