Effect of Moderate Exercise Training on Diabetic Status and Pancreatic Insulin Content in Diabetic Rats

Supitchaya Traisaeng, Sompol Sanguanrungsirikul, Somboon Keelawat, Juraiporn Somboonwong

Abstract

Moderate-intensity exercise training appears to produce more positive impact for diabetic patients than low- or high-intensity exercise training. However, information regarding its effect on insulin content in pancreatic islets is still limited. The objective of this study was to examine the effect of moderate exercise training on pancreatic insulin content and diabetic status in streptozotocin-induced diabetic rats. Twenty four male Wistar rats were randomly divided into three groups with 8 animals per group as follows: 1) sedentary non-diabetic control group (CON), 2) sedentary diabetic group (DM), and 3) exercise-trained diabetic group (DME). The rats in DME group were engaged in moderate exercise training program that involved 30-min exercise on a treadmill at 18 m/min once daily, 5 days per week for 6 weeks. Body weight (BW) was recorded weekly. After 6-week experimental period, levels of fasting plasma glucose (FPG), plasma insulin, serum fructosamine, as well as islet size and density, and pancreatic insulin content were determined. The results showed that DME exhibited a significant decrease in FPG levels and an improvement in BW compared to DM. The levels of serum fructosamine and plasma insulin in DME were not different from those of DM. The density of pancreatic islets in DM and DME was reduced compared to that of CON. Islet diameter of DME tended to be greater than that of DM but was comparable to CON. Insulin content per islet and per beta cell in DME was higher than that of DM. Compared to CON, DME had a higher insulin content per beta cell despite a lower insulin content per islet. In conclusion, moderate exercise training improves diabetic status and increases pancreatic insulin content in type 1 diabetic rat model.

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Diabetes mellitus is mainly categorized into type 1 (T1D) and type 2 (T2D) diabetes. T1D results from failure of pancreatic beta cells to produce insulin while T2D is caused by a combination of the body's inability to produce enough insulin as well as insulin resistance. This leads to impaired glucose homeostasis with subsequent chronic hyperglycemia, which is associated with long-term damage and dysfunction of various organs, including the eyes, kidneys, nerves, heart, and blood vessels. Thus, diabetes covers a wide range of heterogeneous diseases.[1]

Exercise training is an adjunctive therapy for diabetes because it better assists in glycemic control, and also helps to reduce insulin dosage in patients with T1D.[2,3] Exercise training enhances glucose tolerance by increasing insulin sensitivity, and improves lipid profile, circulation, muscle mass, metabolic rate, and antioxidant capacity, thereby reducing the risk of diabetic complications.[4,5] In addition, voluntary running exercise has been reported to increase pancreatic insulin content and prevent beta-cell failure in diabetic animal models.[6,7]

Interestingly, exercise training at moderate intensity level can improve diabetic status, including insulin sensitivity, oxidative capacity, and blood glucose, to a greater extent than low- and high-intensity exercise training in T2D condition.[8,9] Moderate exercise training is reported to increase beta cell insulin content in Zucker genetically T2D fatty rats.[10] To our knowledge, reports focusing on the effect of intensity of exercise training on pancreatic beta cell in T1D are scanty. Therefore, the aim of this study was to demonstrate the effect of moderate exercise training on diabetic status and pancreatic insulin content and islet pathology in T1D animal model. In this study, T1D was induced in rats using a single high dose of streptozotocin (STZ), which causes DNA damage and beta cell death.[11,12]

Materials and Methods

Animals

A total of twenty-four male Wistar rats (8 weeks old; body weight 180-220 g) were used in this study. The animals were obtained from the National Laboratory Animal Center, Salaya Campus, Mahidol University, Nakhon Pathom, Thailand. All experimental proce-

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dures were conducted in accordance with the guidelines for experimental animals provided by the National Research Council of Thailand, and the protocols were approved by the Committee of Animal Care, Faculty of Medicine, Chulalongkorn University. Three rats were housed per cage in a room maintained temperature at 25ºC with 12:12 hour light-dark cycle. The rats were fed *ad libitum* with normal chow and water. Body weight (BW) was recorded at 9:00 am weekly.

Experimental protocol

All rats were randomly stratified into three groups with 8 animals per group as follows: 1) sedentary non-diabetic control group (CON), 2) sedentary diabetic group (DM), and 3) exercise-trained diabetic group (DME). Control animals were intraperitoneally administered with sodium citrate buffer (0.4 ml, pH 4.5). In DM and DME groups, diabetes was induced following an 8-hour fast by a single intraperitoneal injection of STZ (55 mg/kg body weight) (Sigma Chemical, St Louis, Missouri, USA), which was dissolved in acidified citrate buffer (0.1 M, pH 4.5). Two days later, after a 4-hour-fast, blood glucose levels from tail vein were measured using glucometer (Advantage Glucometer, Roche, Mannheim, Germany). Rats having glucose values of 200 mg/dl or greater were considered diabetic.

On the next day after diabetic confirmation, DME rats were allowed to practice running on treadmill for about one week prior to the commencement of moderate exercise training program which involved 30-min exercise on a treadmill at 18 m/min once daily, 5 days per week for 6 weeks.[13] The CON and DM groups were raised in normal condition for 7 weeks. At the end of the study, 24-28 hours after the last bout of exercise, all rats were killed using intraperitoneal injection of an overdose of thiopental sodium. The abdomen was opened and the whole pancreas was rapidly removed and fixed in 10% buffered formalin for further histomorphological examination of the islets and insulin immunohistochemistry. Blood samples were drawn from abdominal aorta and collected in three tubes containing sodium fluoride, EDTA, and no anticoagulant for measuring fasting plasma glucose (FPG), plasma insulin, and serum fructosamine, respectively.

Determination of blood levels of glucose, insulin, and fructosamine

Plasma glucose was determined by enzymatic method using COBAS INTEGRA Hexokinase (GLUC2) analyzer (Bangkok RIA Lab Co., Ltd.). Plasma insulin was analyzed by enzyme immunoassay (EIA) using insulin (mouse/rat) EIA kit (Bertin Pharma, *Montigny-le-Bretonneux*, France). Serum fructosamine was determined by colorimetric assay using Architech ci8200 (Bangkok RIA Lab Co., Ltd.).

Pancreatic sections

After being fixed in 10% buffered formalin, the pancreatic tissues were dehydrated in graded concentrations of ethanol, soaked in xylene, and embedded in paraffin wax at 55°C. The tissues were then sectioned at 8 μm thickness and mounted on a microscope.

Assessment of islet density and size

Tissue sections were stained with hematoxylin and eosin (H&E) for histomorphological examination of the islets. Islet density was defined as the number of islets per microscopic field, with two fields per section being randomly selected for measurement. Islet size was identified by measuring the diameter of the islet using the software Aperio ImageScope V.8.0.39.1065 (Aperio Technologies Inc., Vista, CA, USA). The sections of pancreatic tissues from all animals were assessed.

Immunohistochemical analysis of pancreatic insulin content

The paraffin-embedded sections were deparaffinized in xylene and ethanol for ten minutes. After water washing, sections were retrieved for tissue antigenicity lost during formalin fixation with target retrieval solution in microwave. Endogenous peroxidase activity was inhibited by incubating in 3% hydrogen peroxide for five minutes. The slides were then incubated with 3% normal horse serum for twenty minutes in order to block nonspecific binding. After that, primary antibody specific to insulin was applied to the sections which were then subjected to incubation with the secondary antibody, followed by 3,3-diaminobenzidine (DAB). Subsequently, the slides were counterstained with hematoxylin. Under light microscopy, the cells stained positive for insulin were indicated by dark brown staining in cytoplasm. The specificity of insulin immunoreactivity was confirmed by omitting the primary antibodies from some sections. Quantitative analysis of insulin immunoreactivity were done using Image J software (National Institutes of Health, Bethesda, Maryland, USA) by measuring the optical density value of staining per cell and per islet. In order to obtain net optical density, the background staining value was deduced from the value measured. Approximately 10-15 islets per section were randomly selected from each animal. The method of measuring optical density for immunoreactive insulin content was modified from those previously described by Huang *et al*. (2011) and Rawal *et al*. (2013).[7,10]

Statistical analysis

Data were presented as mean ± standard deviation (SD). For comparison among all groups of animals, one way analysis of variance (one-way ANOVA) and Tukey post-hoc comparisons were employed. Differences were considered statistically significant at $P < 0.05$.

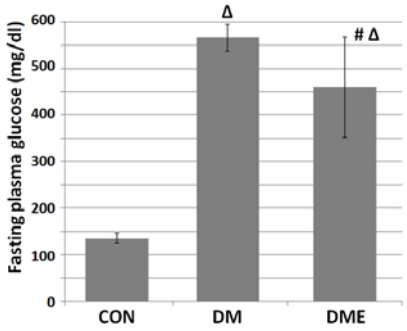

Figure 1 Effect of moderate exercise training on fasting plasma glucose levels in diabetic rats. CON, sedentary non-diabetic control group; DM, sedentary diabetic group; DME, exercise-trained diabetic group. Data are expressed as mean ± SD. **Δ**, Significantly different from CON ($P < 0.05$); #, significantly different from DM ($P < 0.05$).

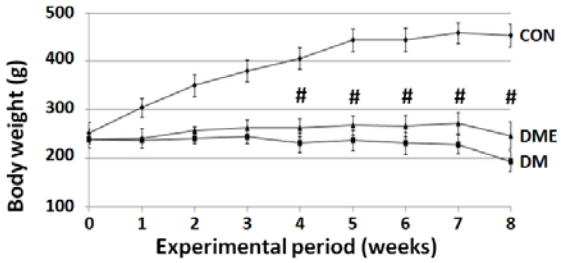

Figure 2 Effect of moderate exercise training on weekly body weight in diabetic rats. CON, sedentary non-diabetic control group; DM, sedentary diabetic group; DME, exercise-trained diabetic group. Data are expressed as mean ± SD. #, Significantly different from DM ($P < 0.05$).

Results

Body weight, fasting plasma glucose, plasma insulin and fructosamine

At the end of the experiment, the results showed a significant decrease in FPG levels in DME compared to DM (Figure 1). Exercise training significantly improved BW in DME compared to DM from the fourth week to the last week. The BW of both diabetic groups was significantly lower than that of CON from the second week to the last week (Figure 2). Plasma insulin levels were not significantly different between DME and DM (Figure 3). The serum levels of fructosamine did not differ among the three groups (Figure 4).

Islet density and size

There was a decrease in the islet density in both diabetic groups. The result showed a significantly lower islet density in DM (21.5 ± 10.60 islets/50 mm^2) and DME (18.50 ± 16.28 islets/50 mm^2) compared to that of CON (38.25 ± 26.99 islets/50 mm^2) (Figure 5A).

Islet diameters of CON, DM, and DME were 158.87 ± 9.64, 115.51 ± 6.20, and 145.21 ± 3.24 µm, respectively. Islet size of DM was decreased compared to that of CON. However, islet size of DME tended to be greater than that of DM but was comparable to CON (Figure 5B). Histopathology of

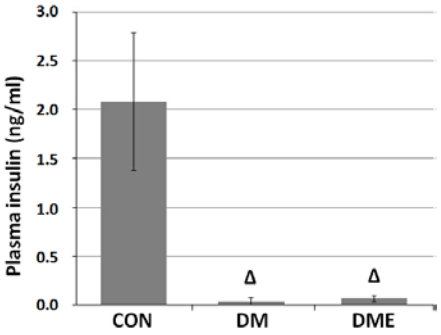

Figure 3 Effect of moderate exercise training on plasma insulin levels in diabetic rats. CON, sedentary non-diabetic control group; DM, sedentary diabetic group; DME, exercise-trained diabetic group. Data are expressed as mean ± SD. **Δ**, Significantly different from CON ($P < 0.05$).

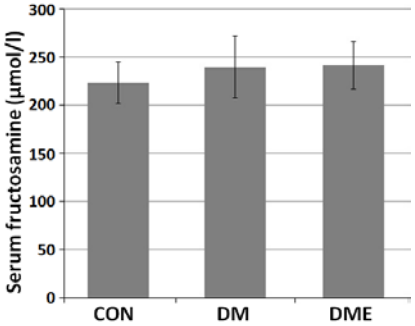

Figure 4 Effect of moderate exercise training on serum fructosamine levels in diabetic rats. CON, sedentary non-diabetic control group; DM, sedentary diabetic group; DME, exercise-trained diabetic group.

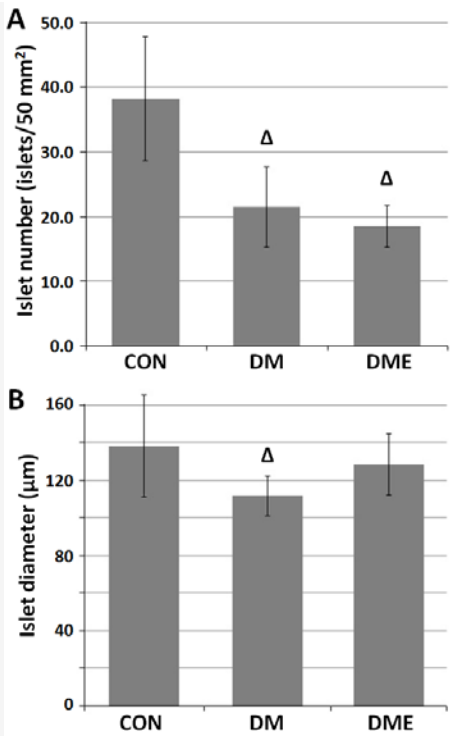

Figure 5 Effect of moderate exercise training on islet density (*A*) and islet size (*B*) in diabetic rats. CON, sedentary non-diabetic control group; DM, sedentary diabetic group; DME, exercise-trained diabetic group. Data are expressed as mean ± SD. **Δ**, Significantly different from CON ($P < 0.05$).

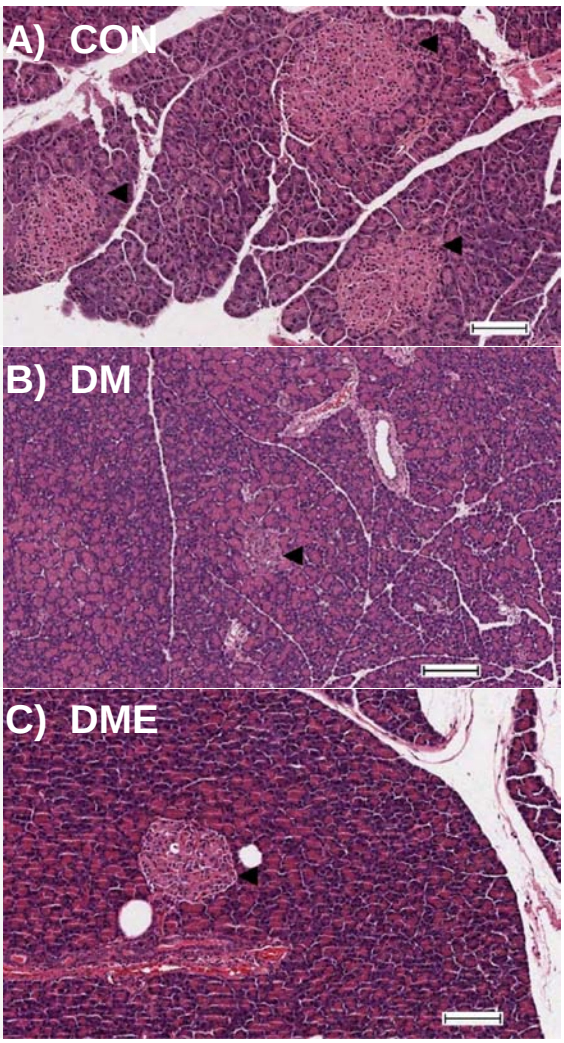

Figure 6 Histopathological features of pancreatic sections stained with hematoxylin and eosin in CON (*A*), sedentary non-diabetic control group; DM (*B*), sedentary diabetic group; and DME (*C*), exercise-trained diabetic group. Pancreatic islets are indicated by arrows. Scale bar length represents 100 μm.

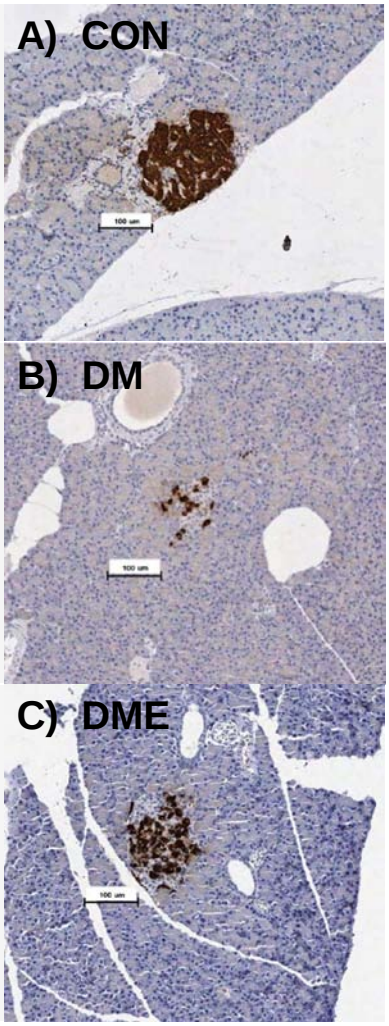

Figure 7 Immunohistochemical findings of pancreatic sections obtained from CON (*A*), sedentary non-diabetic control group; DM (*B*), sedentary diabetic group; and DME (*C*), exercise-trained diabetic group. Scale bar length represents 100 μm.

islets in pancreatic tissues of CON, DM, and DME is depicted in Figure 6A, 6B, and 6C, respectively.

Insulin content

Immunohistochemical features demonstrating insulin content in pancreatic islets of CON, DM, and DME are illustrated in Figure 7A, 7B, and 7C, respectively. Insulin content per islet of DME was significantly greater than that of DM (122.82 ± 15.95 *vs* 96.76 ± 10.27 optical density), but lower than that of CON (190.01 ± 6.86 optical density) (Figure 8A). Insulin content per beta cell of DME was significantly higher than those of CON and DM (214.27 ± 4.50 *vs* 201.29 ± 4.96 *vs* 190.63 ± 11.44 optical density, respectively), and DM values were significantly lower than that of CON (Figure 8B).

Discussion

The main findings of this study were that moderate exercise training significantly decreased FPG and improved BW of STZ-induced diabetic rats. Pancreatic analysis also revealed that such intensity of exercise training were able to significantly increase insulin content and tended to enlarge islet size.

Consistent with previous studies, diabetic rats engaged in a 6-week moderate exercise training program exhibited an improvement in diabetic status, including blood glucose and body weight.[6,14] These results may be in part explained by that exercise improves metabolic efficiency and stimulates endocrine response.[15] It has been shown that exercise increases muscle glucose uptake as a result of the stimulation of GLUT-4 receptor translocation to the cell membrane.[16] Another explanation for glucose-lowering effect of exercise is an enhancement in basal secretion of insulin.[6,7] Conversely, another study reported no effect of voluntary running exercise on glucose levels in STZ-induced diabetic mice.[6] A difference in exercise protocol and animal species may explain the discordant results. With regards to the effect of exercise on body weight, a study found

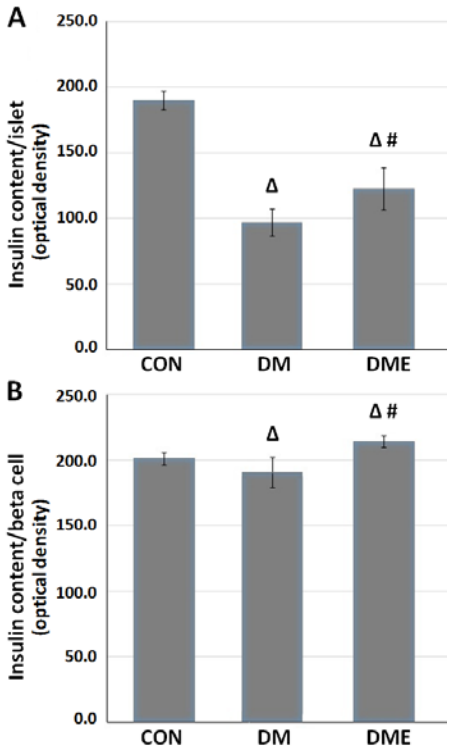

Figure 8 Effect of moderate exercise training on insulin content per islet (*A*) and insulin content per beta cell (*B*) in diabetic rats. CON, sedentary non-diabetic control group; DM, sedentary diabetic group; DME, exercise-trained diabetic group. Data are expressed as mean ± SD. **Δ**, Significantly different from CON ($P < 0.05$); #, significantly different from DM ($P < 0.05$).

that physical exercise improved body composition changes in the elderly by increasing skeletal muscle weights.[17]

However, serum fructosamine levels, an indicator of glycemic control, were unchanged despite a decrease of blood glucose in DME group. A longer period of time may be required to detect any exercise-induced changes in fructosamine levels.

Islet density was depleted following the injection of STZ, as demonstrated in the DM group. This is due to the effects of STZ which is toxic to islet beta cells, increases lipid peroxidation and reduces antioxidant enzyme activity. The present study showed that moderate exercise training was able to increase islet size, but not islet density, in diabetic rats. Additionally, exercise training tended to have a slight impact on ameliorating the impaired islet morphology as seen in sedentary diabetic group having irregular shaped islets with non-smooth edges (Figures 6A, 6B, and 6C). Nevertheless, some studies found that neither islet size nor islet density was affected by exercise training in diabetic animals.[7,10]

To the best of our knowledge, this is the first report that demonstrates the beneficial effect of moderate-intensity exercise training on improving islet morphology and insulin content in T1D rats. Apart from the enlargement of islets, there was an increment of insulin content per beta cell and per islet in trained diabetic rats compared to sedentary diabetic rats. Moreover, insulin content per beta cell in exercise-trained diabetics was greater than in non-diabetic condition. However, insulin content per islet in trained diabetic condition did not reach up to non-diabetic values, given that there was a significant loss of islets in STZ-induced diabetes. These findings support those of earlier studies reporting that voluntary exercise training increased insulin gene expression in T2D rats[6] and increased insulin content in pancreatic islet beta cells in T1D mice.[7] A previous study also revealed that aerobic exercise training could protect beta cells during the onset of DM in T2D animal model by promoting islet beta cell proliferation and beta cell mass compared with sedentary diabetic group.[18] Evidence has shown that exercise training, especially at moderate intensity, decreases lipid peroxidation and improves antioxidant enzyme activity, thus preserving islet beta cell integrity.[19,20] However, the mechanism whereby exercise training improves insulin content is still unclear. Several investigations have found that diabetic condition is related to zinc deficiency and relies on ZnT8, a kind of zinc transporter which is important for insulin synthesis.[21,22]

Although there was an increase in pancreatic insulin content after moderate exercise training, serum insulin levels of DME were not different from DM, which is in line with other research.[10] This may be due to timing of blood measurement that was during the fasting period when the levels of blood insulin were low.

Conclusion

This study indicates that moderate exercise training can improve diabetic status, and ameliorate islet size and pancreatic insulin content in T1D rats. As zinc and ZnT8 play an important role in insulin synthesis, therefore further studies are suggested in order to elucidate the underlying mechanism whether exercise training affects zinc content and ZnT8 expression in pancreatic islets.

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Conflict of interest

None to declare.

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