Effect of Exercise on Pancreatic Islets in Zucker Diabetic Fatty Rats

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Abstract

Exercise and physical activity improve the glycemic control in people with type 2 diabetes (T2D). It is known that activity improves muscle utilization of glucose, and that exercise can spare islets if initiated prior to the onset of diabetes. However, any effect of exercise on pancreatic islet function after the diagnosis of overt diabetes is unknown. The aim of the study was to investigate the effects of exercise training on pancreatic islets in a rodent model of overt T2D. 12-week old male Zucker Diabetic Fatty (ZDF) rats and control lean rats were divided into 4 groups: sedentary control, exercised control, sedentary diabetic and exercised diabetic. Exercised rats were trained with moderate intensity running on a treadmill for 7 weeks. Assessment of plasma insulin levels, islet cell composition (relative proportion of α, β and δ cells), islet density, insulin content and islet core diameter was conducted at the end of the study. Diabetes in ZDF rats lead to high Hba1c and BGLs, which was not reversed by exercise. Diabetes caused destruction of the islet structure and significant loss of β-cells, with an increased proportion of α- and δ-cells. Exercise improved islets morphology in the diabetic group, while islet density and islet cell composition were not affected by exercise. Increased insulin immunostaining of the pancreatic islets was identified in the diabetic animals after exercise. Although exercise did not affect the diabetes-induced decrease in the proportion of islet β cells, there appeared to be an improvement in the islet architecture and in β-cell insulin content.

Keywords: Insulin; Glucagon; Somatostatin; Islet; Type 2 diabetes; Obesity; ZDF rat; β-cell; Islet morphology

Introduction

Diabetes affects more than 300 million people in the world [1]. The recent staggering increase in the number of people with type 2 diabetes (T2D) can be attributed partially to changing lifestyle. Increasing obesity and physical inactivity lead to a metabolic imbalance between energy intake and expenditure [2]. Insulin is at the heart of metabolic control, and when the insulin secretion or utilization pathways are impaired, diabetes ensues.

A loss of insulin production and secretion by the pancreatic β-cells is typically associated with type 1 diabetes, while ineffective action of insulin at the peripheral tissue, such as skeletal muscle is associated with T2D [3]. Yet, it has long been known that dysfunctional β-cells exist in people with T2D [4]. Nearly 30 years ago, it was determined that the total β-cell mass of the pancreas from people with T2D was decreased compared to age- and weight-matched non-diabetics [5]. Since then, research has confirmed that people with T2D have reduced islet numbers along with β-cell reductions, and inadequate insulin secretion [6,7].

Early exercise training improves the metabolic status and insulin sensitivity of people, thereby reducing their risk of developing T2D later in life [8-10]. The majority of animal studies focused on the effects of exercise have concentrated on the early stage of T2D, initiating exercise before the animals are overtly diabetes [11-16]. In this situation, exercise can delay or even inhibit the development of the disease.

However, most people diagnosed with the disease are inactive, and may be motivated to start exercising until after the diagnosis of T2D. Increased physical activity and changes in diet and medication are the essence of management for people with T2D [17]. Numerous reports have shown that increased daily activity and/or exercise can improve glucose control and reduce medication requirements in those with T2D [18,19]. While lifestyle modifications such as exercise can improve the insulin utilization of the peripheral tissues [20], an effect of exercise on pancreatic β-cell function in diabetics remains unclear. In humans, moderate-intensity exercise for 3-8 months improved insulin sensitivity and insulin secretion in response to a glucose challenge in those with overt T2D [3]. However, the method for measuring β-cell function in vivo in humans is indirect [3]. The purpose of this study was to examine the direct effect of exercise on pancreatic islets in the presence of overt T2D.

Material and Methods

Animals

The animal protocol was approved by the Institutional Animal Care and Use Committee. The standard animal model of T2D is the leptin receptor deficient (fa/fa) Zucker diabetic fatty (ZDF) rat. The disease develops spontaneously in males when fed the appropriate diet (Purina #5008), and is associated with many of the clinical symptoms of the disease [21]. Male ZDF (fa/fa) rats and control Zucker Lean (fa/+), rats were obtained from Charles River Laboratories and assigned to 4 groups: (1) sedentary (non-exercised) diabetic (n = 10), (2) exercised diabetic (n = 12), (3) sedentary control (n = 12), and (4) exercised control (n = 10). Animals were placed on the 12:12 light-dark cycle with food and water ad libitum. In order to induce the development of type 2 disease process the animals were fed with Purina 5008 diet.

Exercise training

Aerobic exercise training was started at 12 weeks of age, immediately...
samples were incubated with secondary antibody conjugated with Abcam, ab53165) at 4°C overnight in a wet chamber. Subsequently, anti-glucagon (1:200, Abcam, ab10988), and anti-somatostatin (1:200, Cambridge, Mass, USA, ab7842), for 30 min. Incubation with the following primary antibodies was completed: anti-insulin (1:100, Abcam, Cambridge, Mass, USA, ab7842), anti-pancreatic serum albumin, and 0.03% Triton X-100 diluted in 0.1 M PBS, pH 7.4 was conducted according to manufactures instructions. Slides were read at 450 nm with a reference wavelength of 620 nm.

**Pancreatic tissue sections**

Sample preparation and staining was completed according to the protocol previously published [24]. In short, harvested pancreatic tissue was fixed in normal buffered formalin. Pancreatic tissue was embedded in paraffin wax and cut and mounted on Superfrost/Plus microscope slides (Fisher Scientific, Pittsburgh, Pa, USA, no. 12-550 15). The sections were dried overnight at 40°C, and stored at 4°C until processing. Later, while processing, serial tissue was fixed in normal buffered formalin. Pancreatic tissue was completed the exercise training protocol, with occasional breaks in the running, if the animals appeared fatigued. Blood glucose and body weight measurements were taken every week. Blood glucose levels were measured with pricks from the rat tail using Accu-Check Active meter (Roche Diagnostics, Indianapolis, IN). Glycated hemoglobin (HbA1c) levels were measured at the Termination of the study using a A1CNow meter (Metrika, Sunnyvale, CA). For statistical purposes, the highest detectable value of blood glucose and HbA1c (600 mmol/L or 13%, respectively) was used when rats had blood glucose or HbA1c levels higher than detectable. Animals were sacrificed within 36 hours of the final exercise training episode.

**Plasma Insulin levels**

Plasma insulin levels were determined using the insulin rat (high range) sandwich ELISA kit from ALPCO, as we have previously published [23]. Briefly, 5 μl samples were loaded to microplates following the manufacturer’s instruction. After washing, incubation with horseradish peroxide enzyme-labeled monoclonal antibody, subsequent washing and exposure to the stop solution, the plates were read at 450 nm with a reference wavelength of 620 nm.

**Immunohistochemistry (IHC)**

Insulin staining was developed on the pancreatic sections using anti-insulin (1:100, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, # sc-9168) primary antibody and Histostain-Plus Broad Spectrum (AEC) Kit (Invitrogen, Frederick, MD, # 859943). The IHC procedure was conducted according to manufactures instructions. Slides were counterstained with hematoxylin to identify cell nuclei.

**Immunofluorescence staining**

Sections were blocked with 10% normal donkey serum, 1.0% bovine serum albumin, and 0.03% Triton X-100 diluted in 0.1 M PBS, pH 7.4 for 30 min. Incubation with the following primary antibodies was completed: anti-insulin (1:200, Abcam, Cambridge, Mass, USA, ab7842), anti-glucagon (1:200, Abcam, ab10988), and anti-somatostatin (1:200, Abcam, ab53165) at 4°C overnight in a wet chamber. Subsequently, samples were incubated with secondary antibody conjugated with DyLight 488 (1:400, Jackson ImmunoResearch Laboratories Inc, West Grove, PA, 706-485-148), Alexa 555 (1:400, Molecular Probes, Eugene Ore, A31570), or Alexa 647 (1:400, Molecular Probes, A31573). Images were collected on a Nikon C1Si confocal microscope (Nikon Instruments Inc. Melville, NY).

**Islet density, morphology and cell composition**

Islet density was defined as the average number of islets per area of pancreatic tissue using previously published procedures [25]. All islets contained on 9 sections of pancreatic tissues from 3 animals per group were analyzed. Cell composition was determined by measuring the relative proportion of all three types of endocrine cells (α-, β-, or δ-cells) present in each islet. For each group, 65 to 81 islets from pancreatic sections of 3 animals were analyzed independently for the cell composition. Islets were classified according to their morphology as either intact or disrupted. Intact islets had a distinct circular or ellipsoidal perimeter. Disrupted islets contained an identifiable border, but were missing sections of insulin-, glucagon-, or somatostatin-stained cells. For an islet to be classified as disrupted, 25% or more of the islet area lacked endocrine cell staining.

**Islet diameter**

As diabetes resulted in changes in the appearance of the islets giving the “spider-like shape”, which has been described previously [26]. Because of the lost islet cells and remaining extensions, determining the diameter of the islets was complicated. We measured the average islet core diameter, and excluded the extension area.

**Insulin content**

To determine the insulin content, insulin immunohistochemistry (IHC) was performed on the pancreatic sections according to previously published procedures [27]. Images were analyzed with Ps Adobe Photoshop CZ4 extended software. Insulin intensity was determined from each cell and then the background staining was subtracted from each of the values and then the average was calculated. The insulin content analysis was conducted by a blinded reviewer who did not participate in the animal portion of the studies.

**Statistics**

One-way ANOVA on ranks (Kruskal-Wallis) followed by Dunn’s pairwise comparisons was used. T- test and Mann Whitney Rank sum test was used to compare the difference between groups. Results were expressed as averages of each group or cell population ± SEM. P < 0.05 was defined as significant.

**Results**

Daily random blood glucose measurements for each animal were averaged per week and plotted for the course of the study. At the initiation of the exercise study there was already a statistical increase in the starting weight of the fatty diabetic rats (ZDF) compared to the lean controls (Figure 1A). Within the diabetics and control groups there was no initial statistical difference (sedentary vs. exercise) in initial weight. Over the course of the study, there was a significant difference in the amount of weight gained by the control animals. Sedentary control animals gained an average of 18.2% of their baseline weight while exercise control animals gained 26.2% of their baseline weight (Figure 1A). Sedentary diabetic animals gained an average of 5.6% of their weight while exercise diabetic animals lost 0.5% of their initial weight. Although the non-diabetic animals gained a greater percentage of their weight during the 7 week study, still the sedentary diabetic rats
weighed more than the control animals at the termination of the study (p<0.05).

The average blood glucose levels of all of the ZDF rats were classified as diabetic with levels above 300 mg/dl prior to the initiation of exercise, which was statistically higher than the control lean rats (Figure 1B, p<0.001). There was no significant difference in the blood glucose levels at the end of the study between the two diabetic groups (exercise diabetic vs. sedentary diabetic) or between the two control groups (exercise control vs. sedentary control). Likewise, there was no difference in the average HbA1c levels (sedentary control = 4.2% ± 1.3%, exercise control = 4.7% ± 1.3%, sedentary diabetic = 12.2% ± 3.5%, exercise diabetic = 12.1% ± 3.8%). There was no significant effect of exercise on blood ketone levels in the lean animals (sedentary control = 0.36 ± 0.05 mmol/l, exercise control = 0.27 ± 0.02 mmol/l). Likewise, there was no effect of exercise on blood ketone levels in the diabetic groups at the termination of the study (sedentary diabetic 0.70 ± 0.13 mmol/l, exercise diabetic = 0.70 ± 0.11 mmol/l). However, there was a significant difference between the levels in the lean animals versus the diabetic with levels significantly higher in the diabetic rats (p < 0.01).

**Islet morphology**

Islets from lean control rats had β-cells located in the core of the islet with α- and δ-cells on the outer mantle, as described previously (Figure 2A) [27]. Diabetes resulted in a loss of endocrine cells resulting in disrupted islets (Figure 2B). These islets had a “spider”-like shape with entire sections of cells missing. Exercise training had no effect on the morphology of the islets from lean rats (Figure 2C), but appeared to improve islet morphology in the diabetic groups (Figure 2D). Islets were classified according to intact, with no missing sections or spider-like extensions, or disrupted. Figure 2E illustrates the high level of intact islets in the lean control rats. Diabetes significantly increased the percentage of disrupted islets (p<0.001). Exercise maintained a higher level of intact islets in diabetic rats compared to the sedentary diabetic rats (p < 0.001). The results suggest that exercise helps the diabetic pancreatic islets maintain more of their normal morphology.

**Islet diameter**

The diameter of each islet was measured. In non-diabetic lean control rats exercise had no effect on the core islet diameter (Figure 3A). For islets from diabetic animals, measurements of islet diameter were difficult, because a large percentage of the islets were disrupted with unclear borders as noted in Figure 2. Thus, the core of the remaining islet cells was measured whenever the islet had disrupted regions. There was no effect of exercise on the core diameter in the diabetic animals.

**Islet density**

With loss of endocrine cells within islets apparent in the diabetic rats, we hypothesized that the total number of islets, or islet density, would be affected by diabetes and potentially by exercise. Overt diabetes in the ZDF rat did not result in a decrease in islet density (Figure 3B). Further, exercise had no significant effect on islet density in the control or diabetic populations.

**Islet cell composition**

In the immunofluorescence images of the α-, β-, and δ-cells, there was clearly a rearrangement of the islet morphology in diabetic rats. However, it was unclear from images whether the percentage of each cell type changed. Cell counts of immunofluorescently stained sections indicated that islets from the control groups (sedentary and diabetic)
were comprised predominantly of β-cells (Figure 4). However, in the islets from the diabetic animals, there was a statistically lower percentage of β-cells compared to the lean rats (p < 0.05). The percentage of glucagon-positive α-cells was significantly higher in islets from diabetic rats compared to the lean controls (p < 0.05). Finally, the percentage of somatostatin-positive δ-cells was also significantly higher in islets from diabetic rats compared to the controls (p < 0.05). There was no significant difference between the relative proportions of β-, α-, δ-cells between the sedentary diabetic and exercise diabetic groups suggesting that exercise did not affect the endocrine cell composition.

**Insulin content**

Immunohistochemistry staining for insulin provided estimates of insulin content (Figure 5, red). In lean control rats (sedentary or exercised) there was an abundance of insulin (Figure 5A and 5C). In sedentary diabetic animals there was far less insulin within each islet (Figure 5B), and exercise appeared to improve the insulin amount (Figure 5D). Quantification of the over 2400 β-cells from more than 100 islets is summarized in Figure 5E. Exercise was associated with a significant decrease in insulin staining/islet in the healthy, non-diabetic rats (p < 0.001). Diabetes caused a significant decrease in the insulin content of sedentary animals (sedentary control vs. sedentary diabetic; p < 0.001). In contrast to the non-diabetics, in diabetic animals, exercise actually increased insulin content (p < 0.001). The only comparison in Figure 5E that was not statistically different was between the exercise control rats and the sedentary diabetics (NS).

**Plasma insulin levels**

Plasma insulin levels did not correlate with the islet insulin content. Not surprisingly, non-diabetic control animals had less plasma insulin than the diabetic animals (Figure 6). Exercise reduced the insulin level in the lean controls significantly (p < 0.05). Exercise did not significantly alter the plasma insulin levels of the diabetic groups. However, there was a large variation in the plasma insulin levels in the diabetic groups that was not present in the controls.

**Discussion**

Exercise and diet have been the cornerstones of lifestyle interventions as a way to prevent diabetes. In healthy individuals and animals, physical activity alone can delay or even prevent the development of T2D later in life. In non-diabetics, exercise improves islet function within the pancreas, specifically the β-cells [28-31]. However, far less is understood about the effects of exercise on the pancreatic islets in people who already have established diabetes.
Certainly exercise, begun after the diagnosis of overt diabetes can reverse or minimize the peripheral tissue insulin resistance [20,32,33]. However, studies demonstrating exercise-induced improvement in β-cell function in those with overt diabetes have been scant.

In this study, ZDF rats with mature diabetes underwent moderate intensity exercise for 7 weeks. The intensity of exercise resulted in a significant difference in weight at the termination of the study between the exercised and sedentary ZDF rats. Exercise failed to alter plasma insulin levels or random glucose values in the diabetic rats. Further, exercise did not change the islet density or core diameter, or islet cellular composition. However, exercise was associated with an increase in islet insulin content and maintenance of more normal islet morphology in the diabetic animals.

Disrupted islet morphology and loss of islet function has been well established with progressing diabetes [12,26,34]. Maintenance of islet morphology with exercise in diabetic animals has been one of the most consistent findings across diabetes studies [35]. Exercise prevented islet failure by maintaining the islet insulin stores, thereby maintaining overall islet quality [13]. Columbo et al. showed that exercise improved β-cell health in ZDF rats without significant changes in islet cell gene expression profiles [36]. Our results were in agreement with these three studies, showing a significant increase in the number of disrupted islets in the diabetic rats, which was mitigated by exercise. Of note, all of the previously mentioned studies focused on the ability of diabetes to maintain islet health prior to the onset of T2D. Only our study initiated exercise after overt diabetes.

When discussing changes in islet morphology with diabetes, one of the main factors is loss of β-cells. Previously only Kiraly et al. measured changes in islet endocrine cell composition in animal models of T2D. Similar to our findings, they showed that ZDF rats had decreased β-cell mass compared to the non-diabetic controls [35]. They also found a significant decrease in α-cell mass and an increase in δ-cells [35]. In their study, swimming caused an increase in β-cell mass with a decrease in α-cell and δ-cell mass [35], which is different than the results reported here showing no exercise effect in the diabetic animals. The exercise effect in Kiraly et al. [35] is likely because the ZDF rats in their study were only 5 weeks old when exercise was initiated, and thus were still in the prediabetic stage. ZDF rats used in this study were 12 weeks of age, which correlates with mature overt diabetes model [37], thus the disease was well established prior to the intervention.

Although exercise had no effect on the percentage of remaining β-cells in diabetic rats, the results of this study illustrate clearly the attempt of individual β-cells to overcome the peripheral insulin resistance with increased insulin production in the remaining cells. Exercise caused a significant increase in insulin content per β-cell in the pancreata of the exercised diabetic rats. Few human studies have examined the exercise effect on islet insulin secretion in diabetic populations. However, 3 months of aerobic exercise training in people with T2D increased the islet insulin secretion to both oral glucose and arginine challenges [38]. In ZDF rats exercised for 11 weeks, in vivo and in vitro insulin release from isolated islets was higher than sedentary rats [39]. In another study with the ZDF rats also showed that the islet insulin store was greater in exercised compared to sedentary ZDF rats [13]. This finding has been consistent across diabetic animal models. In partially-pancreatectomized rats, 8 weeks of exercise improved insulin secretion [40]. In a toxin-induced diabetic animal model using streptozotocin, exercise increased staining for insulin and preservation of β-cells [23,41]. Importantly the current study confirms that the same positive benefits can be achieved in an overtly T2D animal model.

An important marker of islet function is plasma insulin levels, yet we found no change in plasma insulin levels in the exercised diabetic rats. It is not surprising that there is a disconnect between β-cell function and plasma insulin levels. When insulin resistance is present so that insulin cannot be utilized in the periphery, β-cells attempt to overcome the resistance with more insulin secretion [37]. Thus, plasma insulin levels can actually rise with a corresponding loss in β-cell mass, as demonstrated previously [37]. Over time, the rising blood glucose levels are a result of an inability of the β-cells to replicate at a rate fast enough to overcome the T2D-associated β-cell death [37]. Tokuyama et al. measured a 7 fold increase in plasma insulin levels in fully diabetic ZDF rats compared to non-diabetic controls [26]. In contrast, we measured a nearly 3 fold increase with diabetes.

Exercise in healthy individuals and animals consistently cause a decline in insulin levels. A significant decrease in plasma insulin levels with long-term (12 week) exercise training in healthy individuals was demonstrated by deLemos et al. [42]. The authors concluded that most of the improvement in plasma insulin levels was due to improved peripheral insulin resistance rather than changes in the β-cells. Not as much insulin was necessary in the exercised groups because contraction of the muscle during exercise increased glucose transport into the muscle without insulin and increased GLUT4 to the plasma membrane to increase the effectiveness of insulin [43]. These findings are in agreement with our results in the non-diabetic group.

In diabetic animals, exercise was associated with a 57% increase in plasma insulin levels in the exercised ZDF rats compared to the sedentary ZDFs [12]. Another study found an increase with 5 weeks of exercise in Zucker rats [36]. Likewise, 5 weeks of regular swimming caused a significant increase in plasma insulin levels in ZDF rats [35]. In contrast, a different group of researchers found no change in plasma insulin levels in Zucker rats with treadmill exercise, consistent with our results [15]. It is again important to note that all of these studies, including the latter, were conducted on prediabetic ZDF rats. In contrast, our study used animals that were already diabetic based on their age and blood glucose [26]. The timing of the initiation of exercise is vitally important as β-cell loss continues to occur as the disease progresses. In an interesting study on people with overt T2D, Dela
et al. aerobically trained people for 3 months [44]. They found that people who already had a low level of insulin secretion (more β-cell loss) did not have a positive response to exercise with improved β-cell function. In contrast, if individuals started with a moderate level of insulin secretion before the onset of exercise, then the aerobic activity improved β-cell function. This is consistent with our results showing no effect of exercise on plasma insulin levels in the diabetic populations.

Conclusion

The study shows that exercise training, even in mature overt T2D, can improve the insulin content and morphology of islets. These improvements did not transfer to improved glycemic control or plasma insulin levels. However, that may be because the disease had progressed too far by the time exercise was initiated. It will be important to time the exercise intervention according to the onset of the disease and determine, in humans, the window of opportunity for benefitting the islets.

Acknowledgements

The authors wish to acknowledge the assistance of Drs. Rairprasad Loganathan and Muhammad Al-Jarrah and Punvi Patel in exercise training the animals. NIH funding through the INBRE program of the National Center for Research Resources to IVS provided resources to conduct the study. The National Institute of Child Health and Human Development supported core facility services.

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