

## Original Research

# Moderate Intensity of Physical Exercise increased *B* (Beta) Cell and Size of Langerhans Islets in *Streptozotocin* Induced Diabetes Mellitus Rats

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## Abstract

**Background:** The death of  $\beta$  cells Langerhans islets in Diabetes Mellitus (DM) can cause loss of Langerhans islet function and worsen the progression of DM. Physical exercise plays a major part in DM treatment.

**Aim:** to observe the effect of moderate intensity exercise to  $\beta$  (beta) cell numbers and Langerhans islets area size in *Streptozotocin* (STZ) induced diabetes in rats.

**Methods:** Thirty adult male Wistar rats (*Rattus norvegicus*) divided into 3, Group 1 as the control, Group 2 received 35 mg/kg *streptozotocin* induction treatment, Group 3 received 35 mg/kg *streptozotocin* induction and physical exercise, swimming, with moderate intensity 70% from the swimming maximal ability, 9% of body weight load, 4 times a week for 4 weeks. Data collected were in the form of histopathology slide of pancreatic tissue after receiving treatment for 28 days.

**Results:** There are significant differences of  $\beta$ -cell pancreas number between group K1 and K2 ( $p < 0,001$ ), group K2 and to K3 ( $p < 0,001$ ). No significant difference between group K1 and K3 ( $p = 0,102$ ). The Langerhans islets area sizes of pancreas tissue between group K1, K2, and K3 are significantly different ( $p < 0,001$ ).

**Conclusion:** This study shows moderate-intensity physical exercise can increase the number of  $\beta$  cell and average area size of Langerhans islets. The effect of physical exercise depends on the intensity of exercise and the capacity of pancreatic function left of the diabetic.

**Keywords:** *diabetes mellitus, physical exercise,  $\beta$ -cell pancreas, Langerhans islet morphology*

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## Introduction

Diabetes Mellitus (DM) is a group of metabolic disease marked by blood hyperglycemia caused by insulin secretion disorder.<sup>1</sup> Diabetes Mellitus is a chronic disease with high prevalence in population all over the world. Glucose is the main physiologic regulator of  $\alpha$  and  $\beta$  cells Langerhans islets function which role are in

glucose metabolism regulation. Hyperglycemia caused histopathologic changes of Langerhans islet through direct glucotoxicity to  $\beta$  cells.<sup>2</sup> Oxidative stress may constitute a focal point for multiple therapeutic interventions and for therapeutic synergy. Hyperglycemia is thought to promote oxidative stress through both enzymatic and

non enzymatic mechanism. Oxidative stress plays an important role on the etiology of diabetes and diabetes complications.<sup>3</sup>

Physical exercise plays a major role on DM treatment. Mild and regular exercise might improve glucose, fatty acid, and ketone metabolism, decrease insulin necessity, and stimulate glycogen synthesis. Physical exercise is indicated for DM cases because of its imitation of insulin properties which increase the muscle capacity to intercept plasma glucose due to intramuscular fat storage degradation. The effect of moderate intensity exercise to  $\beta$  (beta) cell Langerhans islets in diabetic person is still not completely clear and human research has not yet been done due to ethical reason, therefore in this research we used *Rattusnorvegicus* induced by *Streptozocin* (STZ).<sup>4</sup> STZ, an antibiotic produced by *Streptomyces achromogenes*, is the most commonly agent used in experimental diabetes. STZ has been shown to damage pancreatic  $\beta$  cell membrane and induce DNA strand breaks and methylation in pancreatic islet cells.<sup>5</sup>

Pancreatic beta cells ( $\beta$ -cell) hold a central element in the maintenance of glucose homeostasis. To execute this function,  $\beta$ -cells are equipped with specialized transport mechanisms and complex metabolic pathways which couple glucose metabolism with depolarization events, culminating in calcium influx and insulin secretion. Several conditions such as overnutrition, malnutrition, and inflammatory processes may alter insulin sensitivity and  $\beta$ -cell functions, leading to impaired glucose-stimulated insulin secretion.<sup>6</sup>

Exercise is an important tool to reduce the incidence of diabetes and other metabolic diseases. However, studies investigating the direct effects of exercise on  $\beta$ -cell function and survival are scarce and still controversial. Considering the relevance of  $\beta$ -cells to diabetes onset and the benefits obtained from exercise, in this matter  $\beta$ -cell function and

survival, aim of this study is to evaluate the effects of moderate intensity exercise training on  $\beta$ -cell function in diabetes.<sup>7</sup>

## Material and Methods

This study used post test only randomized controlled group design. Inclusion criteria in this study were adult male Wistar rat, bodyweight 200-300 grams. Animals were housed in individual cage and received a standard diet. We monitored rats' body weight every week. All procedures were approved by Surabaya Faculty of Veterinary Medicine Airlangga University Health Research Ethical Committee. Thirty wistar rats (*Rattusnorvegicus*) met the inclusion criteria, divided to 3 groups. Group 1 was the healthy rats as a control group, Group 2 received 35 mg/kg of body weight *streptozotocin* induction treatment, Group 3 received 35 mg/kg of body weight *streptozotocin* induction treatment and physical exercise treatment. The physical exercise prescribed was moderate intensity swimming, 70% from the swimming maximal ability, with 9% of body weight load, 4 times a week for 4 weeks. Diabetes was induced by a single intraperitoneal injection of STZ 35 mg/kg of body weight, freshly dissolved in five mmol/liter citrate buffer, Ph 4,5.<sup>10</sup> Rats are natural swimmers, exercise protocols based on swimming are widely used, the load intensity of the exercise being determined by the weight of lead attached to the tail.<sup>11</sup>

### Exercise protocols

Each animal was weighted and a lead weight equivalent to 9% of body mass was attached to the tail of each rat, the exercise done at near moderate intensity with 70% of maximal capacity of swimming. The 9% lead weight consisted of three lead weight of equivalent mass, each suspended by a small

hook holder attached with rubber band to the animal's tail. The maximum intensity was measured with the time of the rats swimming to near exhaustion, exhaustion was defined as the point at which the animal could not remain at the water surface, then 70% of the measured time reached by each animals was prescribed to get the moderate intensity of swimming capacity. Swimming took place in a 50-cm-diameter plastic tank filled with water (70-cm deep) at 27-28°C.<sup>12</sup>

All data processed using SPSS 22.0. To determine the differences between groups, ANOVA test was used on normal data distribution, Kruskal Wallis test if the data were not normally distributed, significant difference if the value of  $p < 0.05$ . If there were significant differences, analysis continued to multiple comparison test.

### ***Histopathological Procedures***

Pancreatic tissues were harvested from the sacrificed animals, and the fragments from the tissues were fixed in 10% neutral formaline solution, embedded in paraffin and then stained with *Mallory Azan* (MA). Using ocular micrometer at 100X magnification. Calculated the number of Average  $\beta$  cell number in each of the three fields of view. Results are expressed in the number of cells per field of view.

### ***$\beta$ cell pancreas***

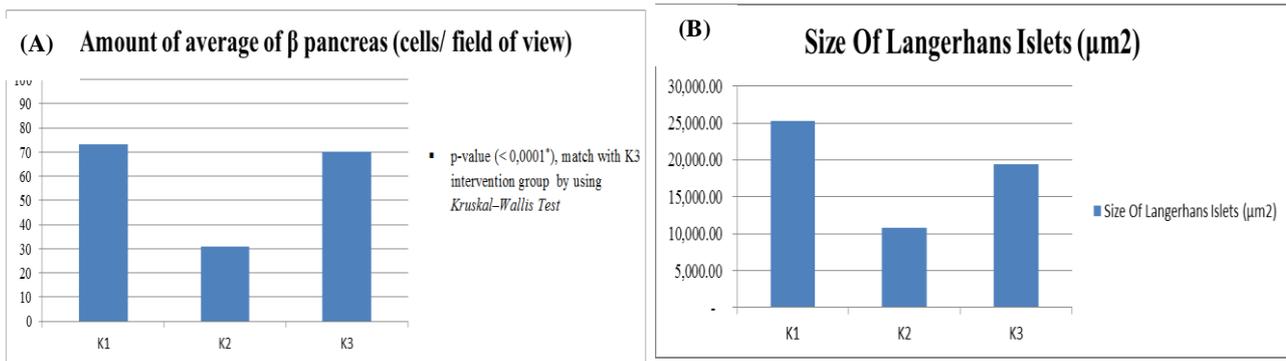
Average  $\beta$  cell number was defined as the average number  $\beta$  cells calculated from islets area of each pancreatic tissue examined. For each group, islets from pancreatic sections of the animals were analyzed independently for the cell composition. Islets were classified according to their morphology as either intact or disrupted.<sup>13</sup>

### ***Area size of Langerhans islet***

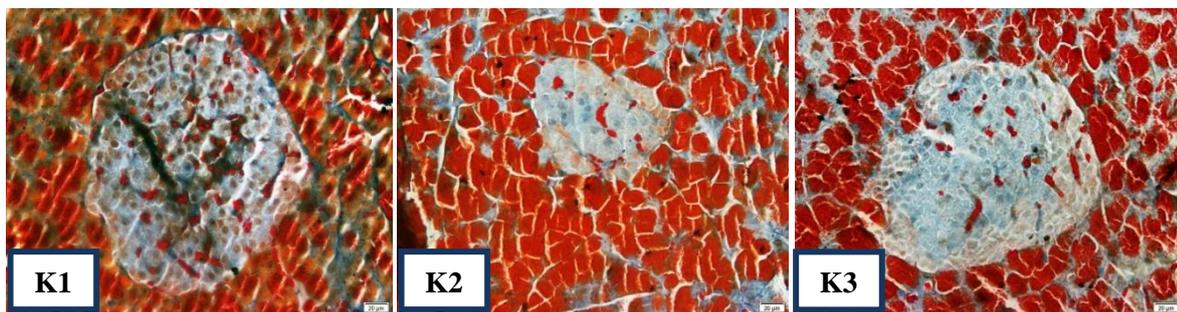
As diabetes resulted in further worsening changes in the appearance of the islets, determining the area size of the Langerhans islets from pancreas tissue was complicated. We measured the average islet core diameter, and excluded the extension area.<sup>14</sup>

### **Result**

The average fasting blood glucose levels of all of the diabetic rats was above 150 mg/dl<sup>21</sup> prior to the initiation of exercise, which was higher than the control group rats (K1) ( $p < 0.001$ ). There was no significant difference in the blood glucose levels at the end of the study between the two diabetic groups. Within the diabetics groups (K2 and K3) and control groups (K1), there was no difference in initial weight. There were significant difference of  $\beta$ -cell numbers between group K1 and K2, and between group K2 and K3, with  $p < 0,001$ , consecutively. However, there is no significant difference between group K1 compared to K3 ( $p = 0,102$ ), see Figure 1. The area size of Langerhans islets pancreas tissue showed significant difference between group K1, K2, and K3 ( $p < 0,001$ ). Different islet morphology and area size is shown at Figure 2.



**Figure 1.** (A). Averages of  $\beta$  pancreas (cells/ field of view) between group K1, K2 and K3; (B). Averages size of Langerhans islet (cells/ field of view) between group K1, K2 and K3



**Figure 2.** MA (Mallory Azan) stained histopathological of pancreas tissues between group: **K1.** Control group showing normal cells in the islets of Langerhans, intact  $\beta$  cells without missing sections or disrupted; **K2.** Cells in the islets of Langerhans shows  $\beta$  cells were not intact with missing sections and disrupted cells; **K3.** Cells in the islets of Langerhans shows  $\beta$  cells were more intact compared to group K2. There were regular borders on K1 group, K3 group had bigger islets than K2 group, and K2 group was irregular borders.

## Discussion

Due to the beneficial effects of physical exercise on glycemic homeostasis, we sought to investigate its effects on  $\beta$  cell pancreas and area size of the Langerhans islets from pancreas tissue.<sup>8,21</sup> In this study, rats with STZ induced diabetes underwent moderate intensity exercise for 4 weeks. The intensity of exercise resulted in significant difference on  $\beta$  cell pancreas number and area size of the Langerhans islets between the exercised intervention group (K3) and diabetes rats without exercised (K2) at the end of the study.

Maintenance of islet morphology with exercise in diabetic animals has been one of the most consistent findings across diabetes studies. Exercise prevented islet failure by

maintaining the insulin storing islet, thereby maintaining overall islet quality.<sup>15</sup>

Few human studies have examined the effect of exercise on islet morphology in diabetic populations. Columbo *et al.* showed that exercise improved  $\beta$ -cell health in ZDF rats without significant changes in islet cell gene expression profiles.<sup>16</sup> Our results were showing significant increase in the number of disrupted islets in STZ-induced diabetic rats.

Our study examined the effects of swimming exercise on  $\beta$ -cell pancreas number and area size of the Langerhans islets of STZ-induced diabetic rats. Free radical mechanism and other possible sources of oxidative stress in the pathogenesis of diabetes and diabetic complication have been extensively studied in animal models and in patients for years.<sup>17</sup> Regular physical exercise has shown  $\beta$  cell

pancreas protection effect from STZ induction toxicity by decreasing *superoxide dismutase* (SOD), *glutathion peroxidase* (GSHPx) and *catalase* (CAT).<sup>3</sup> Oxidative stress has recently been shown to be responsible at least in part for the  $\beta$  cell dysfunction caused by glucose toxicity. ROS are produced and causing tissue damage during this glucose toxicity.<sup>17</sup>

It is clear that exercise improves glucose homeostasis by enhancing glucose uptake in diabetic rodents and humans. Regular exercise also creates an anti-inflammatory environment that favors  $\beta$ -cell survival,<sup>18</sup> Protection for  $\beta$ -cells comes via insulin sensitization and reduced gluco-lipo toxicity, inflammation, and oxidative stress. Various alterations in response to exercise have been reported in diabetic patients and in rodent pancreatic islets.<sup>19</sup>

Huang *et al.* investigated the effects of exercise training on islet morphology, cell composition, and insulin secretion and content 3 days after the second STZ injection.<sup>20</sup> Compared with the sedentary diabetic group, the exercised diabetic mice displayed significantly lower glucose levels during the first 2 weeks of exercise. However, the difference was not statistically significant at later time. Cellular atrophy and extensive vacuoles were present in 80% of the islets from sedentary and exercised diabetic mice. Morphological analysis of islets from 2 weeks trained and sedentary diabetic groups did not reveal any differences.<sup>20</sup>

Oxidative stress is a condition where the body's free radical production exceeds the antioxidants of the cellular defense system causing damage to the cell membrane. This can lead to various health problems such as neurodegenerative, cancer, cardiovascular and premature aging. The resistance of pancreatic  $\beta$  cells is a balance between the formation and apoptosis of pancreatic  $\beta$  cells. On the physiological state,  $\beta$  cells experience apoptosis in a balanced rate with the

formation of new  $\beta$  cells, thus  $\beta$  cell function remains normal in producing insulin in regulation of blood sugar levels.<sup>21</sup>

Significant changes in the histological structure of the pancreatic Langerhans island are one of the typical pathological features and often found in patients and animal models with diabetes. The condition of hyperglycemia can cause hyperplasia and hypertrophy of the endocrine cells, especially in pancreatic  $\beta$  cell populations that make up most of the islands of Langerhans, as well as neogenesis of Langerhans from new cells. However, cells will experience apoptosis if blood glucose levels have crossed a certain critical threshold, giving in to a decrease in the area size and extent of the island of Langerhans.<sup>3</sup> In this study 9% weight was given to each K3 group sample, the load was tied to 2-3 cm from the base of the rat's tail, this was based on the assumption that the placement of the load closer to the base of the tail would affect the lever arm biomechanically, and will affect the magnitude of the intensity.<sup>17</sup>

Physical activity carried out regularly and measured carefully will have a positive effect on the body, but if done excessively it will have a negative impact on the body. This is in accordance with Halliwell and Gutteridge (1999) statement of low intensity exercises provide stimulation and high intensity exercises can cause toxic effects. High intensity exercise can cause excessive muscle activity which causes mechanical and metabolic stress, both are major causes of muscle cell damage.<sup>23</sup> Mechanical stress causes a strain on the sarcomere resulting in interference with contractile cells, cytoskeleton muscles, and proteins associated with sarcolemma which continues to damage cells.<sup>20</sup> While metabolic stress during exercise will increase ROS and cause oxidative stress. The results of this study show moderate intensity physical exercise can increase the number and

function of  $\beta$  cells, and the benefits of physical exercise depend on the intensity of exercise and the capacity of pancreatic function left of the diabetic.<sup>7</sup> Physical exercise has been proven to give an insulin-like effect. This is due to an increase in muscle capacity to capture plasma glucose due to a decrease in intramuscular fat reserves. Increased muscle capacity to oxidize fat in response to aerobic physical exercise is the main mechanism that increases insulin sensitivity in muscles.<sup>8</sup> This study has many limitations. The duration of the study is fairly short, which is 28 days, allowing for reversible biomolecular changes.

## Conclusion

This study showed moderate-intensity physical exercise can increase  $\beta$  cells number and average area size of Langerhans islets, where the benefits of physical exercise depend on the intensity of exercise and the capacity of pancreatic function left in the individual diabetes.

## Acknowledgement

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