

## Effects of Aerobic Training on mTORC1 Gene Expression in Male Wistar Rats with Type 2 Diabetes

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### Abstract

**Objective:** Although type 2 diabetes is a multifactorial illness, one of the major risk factors is the prevalence of obesity. In this context, recent genetic studies on diabetics or pre-diabetics, have shown that some of the newly-known genes make the conditions for type 2 diabetes even in the absence of obesity. One of these genes is called mTORC1, which plays an important role in the synthesis of beta cells. This study examined the effect of 12 weeks aerobic training on the mTORC1 expression, glucose, serum insulin and beta-cell function on diabetic male Wistar rats

**Materials and Methods:** To evaluate the effect of exercise activities on expression of this gene, 30 male Wistar rats were divided into three groups: healthy control, diabetic control and diabetic training group. The two groups of diabetic control and exercise were diabetic with nicotinamide and streptozotocin injecting, and the training group participated in aerobic training on a treadmill for 12 weeks.

**Results:** The results of the study showed that expression of mTORC1 gene increased significantly after 12 weeks of aerobic training, which resulted in a significant decrease in blood glucose concentration and increased beta cell function.

**Conclusion:** Regarding the results of this research and previous studies, the participation in sport exercises, and especially aerobic exercises, increased the expression of mTORC1 gene, increased the synthesis of beta cells, and ultimately control and treatment of type 2 diabetes.

**Keywords:** mTORC1 gene expression, Type 2 diabetes, Aerobic exercise

### Introduction

Type 2 diabetes (T2D) is one of the most common metabolic disorders of the present century (1). Obesity is one of the most important factors in the development of type 2 diabetes recently or by many previous studies (2). On the other hand, the question is also why all obese people are not

diabetic, or why some type 2 diabetic patients have normal weight.

Hence, it seems that apart from obesity, other important factors also play a crucial role in the development of this disease, which has recently been at the center of many researchers' attention. In this regard, recent

genetic studies on pre-diabetics or diabetic individuals, especially since 2007, have shown that some of the most recently recognized genes (mTORC1, AKT, CDKAL1, CDKN2A / B, IGF2BP2, HHEX, TCF7L2, SLC30A8, WFS1, ADAMTS9, CDC123, TSPAN8 and LGR5) increase the risk of developing type 2 diabetes even in the absence of obesity (3). What's interesting is that some of these genes do not affect body weight or obesity, but the function of beta cells and insulin secretion varies greatly (4,5). One of these genes is mTORC1 that controls the growth, size, number and metabolism of beta cells by regulating the protein 4E-BP1,2,3 and the ribosomal protein S6 kinase (6). Research has shown that mTORC1 plays a role in regulating mass and beta cell cycle by adapting these cells to insulin resistance (7,8). So, the role of exercise in controlling and treating type 2 diabetes and obesity has been reported many times (9,10). Therefore, considering the effect of exercise on the genome and the relationship between mTORC1 gene expression and type 2 diabetes, this study examined the effect of 12 weeks aerobic training on the mTORC1 expression, glucose, serum insulin and beta-cell function on diabetic male Wistar rats.

## Materials and Methods

Among male Wistar rats in the animal house of the Pasteur Institute, 30 rats 10 weeks old between  $220 \pm 20$  gr were randomly selected to study. Wistar rats were randomly divided into 3 groups including 2 diabetic and 1 healthy control groups. The rats were kept at the animal house of Parand Azad University in a 5 to 10 meter room under controlled light conditions (12 hours of light and 12 hours of darkness), temperature ( $22 \pm 3^\circ\text{C}$ ), and humidity in the range of 30 to 60. At first, the rats became acquainted with the environment with the living conditions in the animal house for 2 weeks and how to run on the treadmill. Then, after a fasting night (12 hours), nicotinamide and streptozotocin were injected to induce type 2 diabetes. Initially, a solution of nicotinamide at a dose of 110 mg

per kg of rat mice was injected in to peritoneal and after 15 minutes, the freshly prepared STZ solution in the citrate buffer with PH : 4.5 was also administered at a dose of 60 mg per kilogram were injected. The healthy control group received only the same volume citrate buffer (11). One week after diabetes induction, fasting blood glucose (FBS) was measured and glucose levels above 150 mg/dL were considered as an indicator to ensure that rats had type 2 diabetes (12). A training program for 12 weeks of aerobic training and 5 sessions per week with gradual increase in speed (18-26 m/min) and time (10 to 55 minutes) in the form of running on treadmill with the aim of determining its effect on FBS, insulin Serum, beta cell function and relative expression of mTORC1 in pancreatic tissue were compared to the control group that did not participate in the training program. 48 hours after the last training session (10 to 12 hours fasting), all rats were anesthetized by injecting 10% ketamine (50 mg/kg) and xylosin 2% (10 mg/kg). After assuring anesthesia, the animal's chest was splited by a surgical blade and blood samples were taken directly from the animal's heart. Blood samples were centrifuged for 20 minutes for serum separation and maintained at  $80^\circ\text{C}$  for glucose and insulin measurement. Rat pancreatic tissue in physiological serum transferred to the laboratory for genetic tests. Also, the function of beta cells was calculated using fasting insulin and glucose level in the software HOMA2-Calculator. All statistical analyzes were performed using SPSS version 16. The Kolmogorov-Smirnov test was used to ensure that the distribution of data is normal. Data analysis was performed using independent T-test. Changes less than 5% were reported significant.

## Results

Based on the independent T-test, aerobic training resulted in a significant decrease in fasting glucose and a significant increase in serum insulin and beta-cell function in the aerobic diabetic group compared to the diabetic control group. (Table 1)

Also, aerobic exercise led to a significant increase in the relative expression of mTORC1 in the pancreatic tissue of the aerobic diabetic group compared to the diabetic control group. (Table 2)

## Discussion

The results of this study showed a significant increasing in expression of mTORC1 and serum insulin and a significant decrease in fasting glucose. For decades, exercises along with diet and drug use have been proven to be effective ways of managing diabetes. Some researchers have suggested that exercise for 150 minutes per week and a weight reduction of 5 to 7 percent lead to a 60% reduction in the risk of progression or prevalence of glucose tolerance in type 2 diabetes (13,14). The importance of exercise and physical activity in diabetes management is clear. Some studies suggested that exercise can lead to a significant reduction in blood glucose in diabetic patients even in the absence of weight loss or body mass index (15). Hence, the beneficial effects of exercise activity on improving glycemia do not necessarily correlate with changes in body weight. (16,17,18). So high blood glucose in the absence of weight change may be attributed to genetic modification. Increasing of mTORC1 expression in this study after 12 weeks of aerobic exercise indicates the effect of aerobic training on mTORC1 expression. However, it is difficult to determine the exact effect of exercise protocols on these variables in animal models. In one study, the improvement in insulin secretion capacity from isolated islet pancreas was reported after 8 weeks of swimming training (19) which was associated

with a decrease in blood glucose levels in type 1 diabetic rats (20).

Findings from another study showed that after 8 weeks of training on treadmill, insulin secretion increased in pancreatic islets of diabetic Wistar rats compared with control group (21). Studies on beta cells in the pancreas in mice and humans have shown that glucose activates mTORC1. The activation of mTORC1 stimulates cell proliferation (22). Researchers have argued that the role of glucose and amine acids in activating mTORC1 is exerted through an increase in mitochondrial metabolism. In other words glucose and amino acids such as lucien and glutamine increase the production of ATP by inhibiting AMPK activity. As a result, mTORC1 activity increases (23). Also one of the steps in translating mRNA into a protein is where the eIF4E cytoplasmic connects to eIF4G. eIF4E activity is regulated by mTOR phosphorylation. In other words, mTOR increases the eIF4E phosphorylation, so increasing the expression of eIF4E leads to increased protein synthesis in mammalian cells (24). On the other hand, endurance exercises increase the myofibrillar and mitochondrial protein synthesis, both due to the increased expression of the mTORC1 gene. Studies have also shown that treatment with rapamycin is effective in improving insulin secretion (25,26). The role of mTORC1 in beta-cell proliferation has been reported in several studies, which includes the following results:

- Rapamycin treat abnormalities in the development and growth of beta cells (27).
- Stop proliferation of beta cells is as a result of changes in levels of cyclinD2 and cyclinD3 and CDK4 activity.

**Table 1. Fasting Glucose, Insulin and Beta Cell Function in Aerobic and Controlled Diabetic Groups**

Variable	Diabetic control group	Aerobic diabetic group	P-value
Glucose (mg/dL)	294 ± 11	240 ± 14	0.001
Insulin (μIU/ml)	4.06 ± 0.21	5.11 ± 0.25	0.001
Function of beta cell (HOMA-BF)	6.32 ± 0.31	10.42 ± 0.81	0.001

**Table 2. Relative expression of mTORC1 in aerobic and control groups**

Variable	Diabetic control group	Aerobic diabetic group	P-value
Relative expression of mTORC1	1	2.004± 0.99	0.021

- Rapamycin regulate cyclinD2 synthesis.
- Rapamycin enhances the expression of beta cells as an insulin resistance model, in which mTORC1 coordinates beta-cell compatibility with increased blood glucose and type 2 diabetes (28).

## Conclusions

Regarding the results of this research and previous studies, the participation in sport exercises, and especially aerobic exercises, increased the expression of mTORC1 gene, increased the synthesis of beta cells, and ultimately control and treatment of type 2 diabetes.

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