ORGINAL ARTICLE

The Effect of 12 Weeks Aerobic Training on PDX-1 and GLUT2 Gene Expression in the Pancreatic Tissue of Type 2 Diabetic Rats

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Abstract

Objective: The aim of this study was to investigate the effect of 12 weeks aerobic training on PDX-1 and GLUT2 gene expression in the pancreatic tissue of type 2 diabetic rats.

Materials and Methods: 21 wistar male rats were placed in 3 groups (healthy, diabetic, aerobic diabetic). Diabetes was induced by peritoneum injection of nicotine amid. Training program lasted 12 weeks, five sessions per week by gradual increase of speed (18 to 26 meters on minutes) and time (10 to 55 minutes) in the form of running on the treadmill. After 12 week aerobic training, PDX-1 and GLUT-2 genes were measured. Data was analyzed using ANOVA with 5 % significance level.

Results: Aerobic training caused a significant increase in the expression of GLUT2 (*P*-value: 0.043) and PDX-1 genes (*P*-value: 0.007) in the pancreatic tissue of rats with type 2 diabetes (p value: 0.05). Also aerobic training had a significant effect on serum glucose (*P*-value: 0.001) and insulin levels (*P*-value: 0.001).

Conclusion: It is concluded that aerobic training has significant effects on diabetic control by increasing the expression of PDX1 and GLUT-2 which lowers serum glucose.

Keywords: Aerobic training, PDX-1, GLUT-2, Type 2 diabetes.

Introduction

Endurance training improves insulin sensitivity in humans (1). About one week of endurance training is appropriate to improve glucose tolerance in type 2 diabetes (T2DM) patients (2). The progressive degradation or progressive disorder of β-cell function leads to the inability of secretes sufficient insulin to compensate or dominate insulin resistance (3). Of the effective factors in this disorder, we can point to the role of insulin receptors in regulating β-cells' functions (4) and β-cell mass, and the complex interactions between genetic and environmental factors affecting pancreatic cells (5).

GLP-1 increases glucose-depleted insulin, inhibits glucagon secretion, stimulates insulin biosynthesis, and increases the mass of cells and increase the β-cell phenotype through path dependent on PDX-1 (6).

Six weeks of swimming can prevent hyperglycemia in fat diabetic rats, improve the morphology of the Langerhans's lands, increase β-cells mass and accelerate the increase of these cells (7,8). Extreme walking exercise also leads to the improvement of β-cell function, in addition to improving insulin function (9). Muller Madsen et al. (2015) investigated the extreme interval training's effects on blood glucose control and pancreatic β-cell function in patients with T2DM in adults over 56 years. Training program included ergometer bike running3 times in week (10 × 60 minutes, HIIT) for 8 weeks. Another notable point is that no research has ever addressed the role of aerobic exercise on PDX-1 gene expression, which has a crucial role in the differentiation of β-cells and their maintenance and function in patients with T2DM. Therefore, the purpose of this study was to investigate the effect of aerobic training on PDX-1 and GLUT2 gene expression of pancreatic tissue in T2DM rats.

Materials and Methods

This is experimental study on 21 wistar male rats (age, 10 weeks; weight, 20 ± 220 g) placed in 3 groups of seven members. The sample size was based on previous studies in this field and then based on the estimation of G * power software. The first group (control), the second group (diabetic) did not participate in training program. Third group (aerobic diabetic) participated in 12 weeks training program 5 sessions per week. During Research stages, the experiment's animals cases were kept in transparent polycarbonate cages under light controlled conditions (12 hours Lighting and 12 hours darkness, light beginning at 6 p.m and darkness beginning at 6 am) with temperature of 22 ± 3 cm, and 30 to 60

moisture average. They had enough access to water and food. All rats were kept in animal's laboratory for two weeks to get familiar with maintenance environment.

Induction of T2DM: Peritoneum nicotine amide 110 mg / kg on every kg of rat's weigh and STZ with dose of 60 mg / kg on rat's weigh (10) were induced to rats of diabetic group. After one week from T2DM induction, fasting blood glucose was measured and blood sugar more than 150 millimeter on deciliters was considered as a criterion to ensure the rats getting T2DM (11).

Training protocol: Training program lasted 12 weeks, 5 sessions per week by gradual increase of speed (18 to 26 meters on minutes) and time (10 to 55 minutes) in the form of running on the treadmill (Table 1).

Blood and tissue sampling: 48 hours after the last training session and 10-12 hours fasting, rats were anaesthetized using ketamine 10 % with a dose of 50 mg / kg and xylosin 2 % with a dose of 10 mg / kg. Then the animal's chests were dissected, and blood samples were taken directly from the animal's heart to ensure minimal animal harm. Blood samples were centrifuged at 1000 g G for 20 min to separate the serum and stored at -80 C to measure serum glucose and insulin. The pancreatic tissue of the rats was sampled and immersed in 1.8% RNA later TM fluid after rinsing in physiological serum in 1.8 micro-tubes and transferred to the laboratory for genetic testing (12).

Chemical Reagent and Materials: The concentration of glucose was measured by enzymatic staining method with glucose oxidase technology and using Pars Azmoon-Tehran glucose kit. The coefficients of changes in the test and out-of-test glucose were 1.74 and 1.19 percent, respectively, and

Table 1. Aerobic exercise pattern for aerobic rats group on treadmill

Week	Running time (min)	Running speed (m / min)
First	10	18
Second and third	20	20
Fourth and fifth	30	22
Sixth and seventh	40	22
Eighth and ninth	50	24
Tenth, eleventh and twelfth	55	26

the sensitivity of the measurement was 5 mg / dL. Serum insulin was measured by ELISA and in accordance with the standards of the German Kit (Demeditec Diagnostic insulin ELIZA). The coefficients of changes within the test and outside the insulin test were 2.6 and 2.88 percent, respectively, and the sensitivity of the measurement was 1.76 (Table 2).

The RNA was extracted from the pancreatic tissue by the Rneasy protect mini (QIAGEN) kit according to the company's instructions. 20 mg of tissue was cut using a scalpel and inserted into the micro-tube.

The determination of PDX mRNA and GLUT2 mRNA by RT-Real time PCR was performed by Rotorgen 6000 system using One Step SYBR TAKARA single-stage kit from Takara Company according to the company's instructions. The melting curve analysis was performed at the end of the PCR cycle to determine the validity of the expected PCR product. The thermal cycle protocol used by the Rotogen device in Real time-PCR included: 42 for 20 minutes, 95 for 2 minutes, and 40 cycles with 94 for 10 seconds and 60 for 40 seconds. After the PCR stage, to study the characteristics of the primers, temperatures of 50 to 99 were used to prepare the melting curve. RNA Polymrase II was used as a control gene to determine the expression of the genes under study. CTs related to the reactions were extracted and recorded by Real time-PCR software. A comparative DDCT method was used to quantify TCF mRNA expression.

Statistical analysis

Data was analyzed using SPSS Statistical software version 25. After normalizing variables, paired T-test and one way variance analysis (ANOVA), tukey's post hoc test were conducted. The mean variables were statically studied in groups. The results of this study were reported in the form of standard deviation ± mean and statistical difference was considered at the significance level of 5 %.

Ethical considerations

The study was approved by the ethics committee of Sport Sciences Research Institute of Iran, Ministry of Science, Research and Technology, Tehran, Iran (IR.SSRI.REC. 1397.377).

Results

The results of rats weight in Table 3 show that there was a significant increase in rat weight compared to pre-test (*P*-value: 0.001), but this increase was less in the diabetic group than in the control group. Biochemical factors were measured and presented in the form of mean in Table 4.

According to the results, the results of one-way analysis of variance showed changes in the measured indices between groups. The use of post hoc tests in control and diabetic groups showed that induction of diabetes leads to a decrease in insulin level and increased serum glucose levels (*P*-value: 0.001).

Table 2. Primer pattern

Genes	Primer sequence	Product size	T m	Gene Bank
PDX-	**For**: ATACGCAGCAGAACCGGAG **Rev**: ACTTCATGCGACGGTTTTGGA	159 bp	60	NM_001191052.1
GLUT2	**For**: GCATGTCTGTTACCCCAGGATAG **Rev**: AGAGGAGTAACAAGCTCAAGGTG	159 bp	60	NM_001191052.1
RNA Poly maraes II	**For**: ACTTTGATGACGTGGAGGAGGAC **Rev**: GTTGGCCTGCGGTCGTTC	164 bp	60	XM_008759265.1

Table 3: Pre and post-training of body weight of 2 groups (Mean ± SD)

Group	Pre-training	Post-training	*P*-value (Paired T-test)
Control	221.28 (± 2.28)	290.2 (± 6.7)	0.0001*
Diabetes	219.28(± 3.25)	254.5 (± 8.8)	0.001*
Aerobic diabetes	225.55 (± 2.99)	241.4 (± 2.2)	0.001*

*compared to pre-test

Table 4. Fasting glucose and serum insulin and gene expression of studied groups (Mean ± SD)

Variable	Control	Diabetes	Aerobic diabetes	P-value (ANOVA)	P-value (Tukey's post hoc test)	
Fasting glucose (mg/dl)	92.8 (± 4.6)	294.5 (± 10.8)	242.2 (± 13.2)	0.001*	Diabetes – control	0.001*
					Diabetes – Aerobic Diabetes	0.001*
					control – Aerobic Diabetes	0.001*
Insulin (m unit/ml)	8.42(± 0.87)	4.14 (± 0.28)	5.05 (± 0.24)	0.001*	Diabetes – control	0.001*
					Diabetes – Aerobic Diabetes	0.001*
					control – Aerobic Diabetes	0.001*
GLUT2 expression	1	0.76 (± 0.47)	2.14 (± 1.55)	0.001*	Diabetes – control	0.018*
					Diabetes – Aerobic Diabetes	0.043*
					control – Aerobic Diabetes	0.001*
PDX1 expression	1	0.48 (± 0.42)	1.84 (± 1.02)	0.009*	Diabetes – control	0.001*
					Diabetes – Aerobic Diabetes	0.007*
					control – Aerobic Diabetes	0.001*

*-ANOVA and Tukey's post hoc test

Also, induction of diabetes in the diabetic group reduced the expression of PDX and GLUT2 (*P*-value: 0.01). On the other hand, aerobic exercise increased insulin levels and decreased serum glucose in diabetic rats (*P*-value: 0.01)**,** In addition, these training in the diabetic group increased the expression of PDX (*P*-value: 0.007) and GLUT2 (*P*-value: 0.043).

Discussion

In our study, the results showed that aerobic training has had significant increase on PDX-1 and GLUT 2 gene expression. The recent findings show that one of the mechanisms by which Pdx-1 increases insulin gene transcription is the changes and conversions of Histone in Insulin promoter.

It has been proved that PDX-1 uses HAT (histone acetyltransferase) P300 for the insulin promoter only in high amount of glucose (10-30 mM) that increases histone acetylation (13-16). It has also been proved that histone methyl transferaseSet9 is used for insulin via PDX-1 that leads to histone demethylationH3 Lys ^ (17). These events histone acetylation and methylation changes the chromatin structures, which in turn increase the transcription of insulin gene (17-20). PDX-1 connection to insulin promoter for POLII (RNA polymerase II) is required to get isoform extension for active transcription. It seems that in the absence of PDX-1, Pol II is unable to change from onset isoform (pSer^5) to extension isoform (pSer ^ 2) that is necessary for the extension of active transcription (17,20). The study pointed out that PDX-1 increases insulin gene transcription by increasing the chromatin structure of active transcription which risen the extension via Pol II.

Conclusions

There are also evidences that PDX-1 participates in the suppression of gene expression and is stops glucagon, sitocratin K19and c- myc transcription (13,22). However, it is not clear that Pdx-1 ability to stop transcription is related to the changes of chromatin structure or not. It is interesting that Pdx-1 uses HDAC (histone deacethylation) -1 and -2 for insulin promoter in response to low glucose values(1-3 mM) to down regulate insulin gene transcription (23). Therefore, it is likely that PDX-1 stops gene expression using common repressive. It has also been reported that PDX-1 interacts with PCIF-1 (interacting factor ofc-terminusPDX-1 1), TRAF (factor related to the receptive factor of necroztomori) and also nuclear factor containing domain POZ that inhibits the activation ability of PDX-1 independent of histone common repression and stops using HDAC (24,25). However, the precise mechanism by which PCIF-1 stops activation by PDX-1has not been discovered yet. Bridge-1 (PSMD9) is another protein that interacts with PDX-1 and induces transcription activity.Bridge-1common regulator is a domain protein like PDZ and it seems its ability for activating transcription depends on

its interaction with HAT P300 (26,25). In short, it seems that PDX-1 regulates gene transcription by increasing multi protein complexes synthesis in insulin promoter.

Acknowledgements

Conflict of Interest

The authors have not any conflict of interest.

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