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Impact of High-Intensity Interval Training on GLP-1R/ PKBα Axis in Pancreatic Tissue of Diabetic Rats Induced by High-Fat Diet and STZ

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Abstract

Objective: Apart from hormonal factors and oxidative stress, insulin synthesis is strongly dependent on transcription factors in the pancreas. The aim of the present study was to assess the impact of high-intensity interval training (HIIT) on genes affecting insulin synthesis in diabetic obese rats.

Materials and Methods: Type 2 diabetes (T2D) was induced by a 6-week high-fat diet (HFD) and intraperitoneal injection of streptozotocin (25 mg /kg) in 14 male Wistar rats (10 week old, 220±10 g). Rats with fasting glucose levels between 400 and 150 were considered T2D. The diabetic rats were randomly assigned to exercise (HIIT: 6 weeks/5 sessions weekly, n= 7) or control (n= 7) groups. Forty-eight hours after the intervention, fasting GLP-1R and PKBα gene expression in pancreatic tissue and plasma insulin and glucose levels were compared between the groups. Data were compared by independent t-test used to compare variables, version 22 between groups. A $P< 0.05$ was considered significant.

Results: HIIT led to significant increase in PKBα gene expression (*P:* 0.001) and insulin (*P:* 0.031) and decreases in glucose concentration (*P:* 0.001) compared with the control group. No change was observed in the GLP-1R gene expression response to HIIT (*P:* 0.093).

Conclusion: HIIT is associated with increased serum insulin levels in T2D obese rats. Despite no change in GLP-1R, this improvement is probably rooted in increased expression PKBα in pancreas in response to this type of exercise training.

Keywords: GLP-1R، PKBα ، High fat diet، High intensity interval training, Diabetes

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Introduction

Type 2 diabetes (T2D) is the most common metabolic disorder in the world today (1). The fact that obesity is the most important factor in the development of T2D has been recently supported by many previous studies (2-4). T2D occurs on the one hand in response to insulin resistance in body tissues such as adipose tissue or skeletal muscle and on the other hand due to the inability of beta cells to secrete enough insulin to compensate for insulin resistance (3,4). These deficiencies result from both genetic and environmental factors (5). Some recent studies have supported the direct role of genetic disorders in decreased beta cell function, sufficient insulin secretion, and subsequent onset or increase in the severity of T2D (6). Among them, the role of glucagon-like peptide-1 (GLP-1) as an incretin hormone and its receptor (GLP-1R) in insulin secretion from beta cells is prominent (7). Apart from its direct effects on insulin synthesis and secretion, it stimulates glucose uptake and transcription of insulin (8).

GLP-1 is an incretin hormone derived from intestinal L cells whose synthesis and release are increased in response to glucose availability and other nutrients, and by binding to its receptors in beta cells, it leads to increased insulin secretion. Increasing insulin synthesis and the release of insulin from pancreatic cells is one of its most important known features (9). In other words, decreased GLP-1 secretion and its receptor expression in pancreatic cells lead to decreased beta cell function and insulin secretion (9). Both serum GLP-1 level and its receptor expression in the beta cells of T2D are reduced compared with healthy individuals (10).

On the other hand, GLP-1 leads to an increase in protein kinase B (PKBα) in beta cells (11-13). PKBα, also called AKT1, is activated by phosphoinositide 3-kinases (PI3K). In mammals, three PKB isoforms are encoded as AKT1, AKT2, and AKT3 (13). Pancreatic beta cells contain large amounts of PKBα (14) and its overexpression leads to increased beta cell mass and function by affecting the number and size of cells (15,16).

Based on this evidence, it is hypothesized that increased protein levels or expression of these transcription factors in the pancreas leads to increased insulin synthesis and secretion and-cell function, particularly in diabetic patients. In this context, although no studies have been performed on the impact of exercise as an external stimulus on PKBα in pancreatic tissue, a relatively new study showed that long-term aerobic training increased GLP-1R expression in the pancreatic tissue of diabetic rats (17). In another study, resistance training reduced TCF7L2 expression as another insulin transcription factor in the pancreas of rats with diabetes (18). However, no study has measured the effect of high-intensity interval training (HIIT) on GLP-1R and PKBα expression in the pancreatic tissue of T2D rats. Based on this limitation, this study determined the effect of HIIT on GLP-1R and PKBα expression and insulin and glucose levels.

Material and methods

Experimental animals

Fourteen Wistar rats aged 10 weeks (220±10 g) were prepared at the Laboratory Animal Breeding Center of Baqiyatallah University of Medical Sciences, Tehran. Then, after the induction of T2D, they were randomly divided into exercise (n= 7) and control (n= 7) groups. Animals were provided with a high-fat diet (HFD) and were maintained (12-h light/dark cycle, 25± 2 °C & humidity 45 - 55 %).

Induction of obesity and type 2 diabetes

Type 2 diabetes induced by 6-week HFD (1% pure corn oil and 1% cholesterol powder) and injection (intraperitoneal) of 25 mg/kg STZ (sigma aldrich: S2130, CAS number: 18883-66-4) dissolved in citrate buffer(pH 4.5) (19). Obesity was diagnosed in the studied rats using the Lee index (20). Hyperglycemia

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was confirmed by elevated glucose levels using an ACCU-Glucometer (made in Chek) on day 7 after STZ injection, and rats with fasting glucose levels between 400 and 150 were considered T2D. (18,21). In this way, a drop of blood from the tail vein of the rat was taken, and the fasting glucose level was measured using a glucometer.

Training protocol

The rats of the exercise group participated in 6 weeks of HIIT (5 27-minute sessions/ weekly) in the form of running on the treadmill in the form of 10 repetitions of running for 40 s with active breaks of 2 min between running stages according to Table 1 (22).

Sample Collection and Biochemical Assay

All rats were anesthetized 48 h after the last training session by intraperitoneal injection of 10% ketamine along with 2% xylosine (50 mg/kg and 10 mg/kg respectively), then they were dissected (18). Blood samples were collected through cardiac puncture. The pancreatic tissue was removed and immersed in RNA later to determine GLP-1R and TCF7L2 gene expression. Insulin was assessed by ELISA (Demeditec; DEV 8811, Germany) and glucose was measured using the glucose oxidase method (Pars Azmoon).

RNA extraction/Real time-PCR

To purify RNA, 20 mg of pancreatic tissue was ground using a mortar and pestle, and extraction was performed using the RNeasy Protect Mini Kit according to the manufacturer's protocol (Qiagen Inc. Germany) (19). Therefore, the One Step SYBR Prime Script RT - PCR Kit (Takara, Japan) was employed to prepare the reaction product according to the manufacturer's protocol. The thermal cycle program used for the Rotor-Gene Q instrument was as follows 42 °C for 20 min, 95 °C for two minutes and 40 cycles with 94°C for 10 seconds and 60°C for 40 s. Temperatures from 50 °C to 99°C were used for the melting curve to study the characteristics of the primers. RNA polymerase was used as a control gene. Primer analysis software was used to design the primer based on PKBα and GLP-1R (Table 2).

Statistical analysis

According to the normal distribution of data, independent sample T-test was used to compare the variables between 2 groups. Data were analyzed using SPSS, 22.0 and are expressed as mean ± SD. The figures are drawn in Excel 2003.

Ethical considerations

This study was approved by the Ethics Committee of Islamshahr Azad University

Table 1. HIIT protocol based on running speed in the active and rest phases in the exercise group

Weeks	Exercise Running speed (m / min)	Active rest Walking speed (m / min)	Treadmill slope
1	25	10	5
2	25	10	10
3	28	10	10
4	32	10	10
5	35	10	10
6	35	10	10

*Running time in the exercise phase is 40 s and rest phase is 2 min

Table 2. Primer sequence

Genes	Primer sequence
PKBα	**For** : AGGAGGTCATCGTTGCCAAG **Rev** : GCTCACGAGACAGGTGGAAG
GLP-1R	**For** : GGGCTTTATGGTGGCTGTCTTG **Rev** : GTTTCATGCTGCTGTCCCTCTG
RNA Polymerase I I	**For** : ACTTTGATGACGTGGAGGAGGAC **Rev** : GTTGGCCTGCGGTCGTTC

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(Code: IR.IAU.PIAU.R.1400.011).

Results

Based on data by independent T-test according to the normal distribution of data, PKBα gene expression was significantly higher in the HIIT group than in the control group. In other words, 6 weeks of HIIT led to a significant increase in PKBα gene expression compared with control rats (*P:* 0.031).

In contrast, no significant difference was observed in GLP-1R expression between the control and HIIT groups. In other words, HIIT did not lead to a significant change in GLP-1R expression compared with the control group (*P:* 0.093).

A significant difference was also observed between the groups about insulin and beta cell function, in which HIIT led to a significant increase in insulin (*P:* 0.001) and beta cell function (*P:* 0.001) in the exercise group compared with the control group.

Discussion

Despite no change in GLP-1R expression, increased PKBα gene expression in pancreatic tissue was the main finding of this study. In other words, 6 weeks of HIIT with 5 sessions per week led to a significant increase in PKBα gene expression in the pancreatic tissue of obese T2D rats. This change was accompanied by decreased fasting glucose and increased insulin sensitivity and serum insulinlevels. Although the effect of exercise on GLP-1 receptor expression in T2D has not been studied, several studies aimed at the effect of exercise interventions on insulin, blood glucose, and insulin resistance and hormones or genetics affecting these variables have been performed in T2D and other healthy or diseased populations. There are contradictory results in this field, so some studies have reported decreased insulin secretion after exercise. For example, in Rawal et al. (2013), 12 weeks of aerobic training reduced insulin levels in healthy subjects (23). However, in a study in diabetic rats, prolonged exercise resulted in a 57% increase in plasma insulin compared with controls (24). In another study on diabetic rats, a significant increase in plasma insulin was observed after 5 weeks of exercise (25). However, in line with this study, all of these studies have reported significant reductions in blood glucose levels. Researchers have concluded that the response of insulin synthesis and beta cell function to exercise in humans or animal species differ depending on the presence or absence of diabetes, the severity of diabetes, and the age of onset of diabetes (23,26). It seems that the most beneficial effects of exercise on blood glucose levels in healthy non-diabetic individuals, such as obese people, are manifested by reduced insulin resistance of peripheral tissues, especially skeletal muscle (27).

During type 2 diabetes, the function of beta cells decreases, resulting in decreased insulin secretion from these cells. Laboratory studies have revealed that T2D results from both a decrease in-cell function and an increase in insulin resistance (28). The influence of genetic or hormonal factors affecting the synthesis, secretion, and function of insulin in target cells should not be ignored. In this context, it has been mentioned that GLP-1 and its receptors GLP-1R or some other genetic factors such as TCF7L2 play an important role in the concentration and secretion of insulin. Although glucagon secretion inhibition and reduction of gastric emptying are among the functions of GLP-1R, increasing insulin synthesis from beta cells is its most important known feature (7). In other words, clinical studies have shown that decreased secretion of GLP-1 as well as decreased expression of its receptors in pancreatic cells leads to decreased beta cell function and insulin secretion (9). On the other hand, it has been shown that both serum levels of GLP-1 and its receptor expression in the pancreatic cells of patients with T2D are reduced compared with healthy individuals (10).

In addition, PKBα plays a key role in the growth and survival of beta cells (29). Genetic studies have shown that PKBα increases

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GLP-1 anti-apoptotic function in beta cells (12). Lee et al.. (2005) revealed that the effect of GLP-1 inhibitors on cytokine-induced apoptosis and necrosis in beta cells is mediated by PKBα -dependent signaling pathways (30). Despite this evidence, in this study, PKBα gene expression in pancreatic tissue increased significantly in the absence of GLP-1R change. The lack of change in GLP-1R can probably be attributed to the low number of samples studied or scattering of data. Therefore, the increase in serum insulin in response to HIIT may be attributed to the increased expression of PKBα in the pancreas of diabetic rats.

Various studies have suggested that AKT /PKB-controlled messaging pathways not only play an important role in insulin function but also increase beta cell adaptation for greater insulin release. AKT/PKB activity is regulated by PI3K-dependent and non-PI3K-dependent mechanisms and requires multiple steps involved in their transfer to plasma membranes and phosphorylation (12). It is also possible that increased expression of PKBα in the downstream or upstream pathway is another genetic component involved in insulin transcription and synthesis, such as TCF7L2, associated with increased beta cell function or HIIT-induced insulin synthesis. Note that the lack of assessment of other transcription factors such as TCF7L2 and mTORc1 is a limitation of this study.

Conclusions

HIIT is associated with an increase in serum insulin levels in diabetic rats, and this improvement is somehow rooted in the increase in beta cell function in response to this training method. On the other hand, based on the effective role of PKBα in the transcription and synthesis of insulin and beta cell function, the improvement in serum insulin and beta cell function may be attributed to the increase in PKBα expression in the pancreas of diabetic rats in response to HIIT. Knowing other effective pathways in the synthesis and secretion of insulin in response to exercise training requires further studies in this area.

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Conflict of Interest

The authors declare no conflict of interest

Authors' contributions

All authors have accepted responsibility for the entire content of this manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved and approved the version to be published.

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