

The Effect of High Intensity Intermittent Training (HIIT) on GLP-1R Expression in Pancreas Tissue and Serum Insulin of Male Wistar Rats with Type 2 Diabetes Mellitus

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Abstract

Objective: Insulin secretion and insulin resistance are associated with incidence and severity of type 2 diabetes mellitus (T2DM). This study aimed to assess the effect of high intensity intermittent training (HIIT) on GLP-1R expression in pancreas tissue and serum insulin of T2DM rats.

Materials and Methods: In this quasi-experimental study, T2DM induced by intraperitoneal injection of streptozotocin-Nicotinamide in 16 male wistar rats aged 10 weeks (220 – 20 g). The rats were divided into control (no-training, n= 8) and HIIT (5 times/week/12-week, n= 8) groups. GLP-1R expression in pancreas tissue, fasting glucose and serum insulin were measured 48 h after lasted exercise of exercise and control groups. Independent and paired T-test was used to compare variables between 2 groups.

Results: HIIT resulted in significant increase in GLP-1R expression compared to control subjects (*P*-value: 0.023). Fasting glucose decreased (*P*-value< 0.0001) and serum insulin increased (*P*-value< 0.0001) in response to HIIT when compared to control subjects.

Conclusion: HIIT can improve insulin secretion in male rats with T2DM and this improvement can be attributed to increased GLP-1R expression in pancreas tissue in response to training protocol.

Keywords: Exercise training, Elderly, Endothelial cells, microRNAs

Introduction

Type 2 diabetes mellitus (T2DM) often is related to dysfunction of β -cells of pancreas and lower level of insulin secretion. Reduced level of insulin secretion is often associated with increased insulin resistance, increased release of glucose-dependent glucagon from liver deposits, and a rapid increase in glucose uptake from nutrients

(1). Based on the presented laboratory evidence, destruction of pancreatic β -cells is a hallmark of T2DM, which is related to a reduction in the synthesis and secretion of insulin. The mentioned destruction is also connected to dysfunction of incretin hormones. Clinical studies revealed that the rate of apoptosis in β -cells is faster than the

proliferation of new cells rate (2). However, high concentrations of GLP-1 receptors or increased expression of the mentioned receptors in these cells can increase the cell mass, renewal and proliferation of β -cells (2). GLP-1 is a 31 amino acid produced in the intestinal epithelial endocrine L-cells and stimulates insulin secretion by direct impact on the pancreatic islets (3).

GLP-1 is secreted from the gut and detected by the proglucagon gene as an encoded hormone was named glucagon-like peptide (GLP-1) due to its 50% similarity with glucagon. Binding to its receptors in β -cells leads to an increase in insulin release. Moreover, GLP-1 reduces hepatic glucose releases (4). In young people, GLP-1 is responsible for 60% of insulin secretion after meals (5). Although GLP-1 is easily decomposed by dipeptidyl peptidase-4 (DPP-4) within a few minutes, its receptor agonists such as Exenatide and Liraglutide are used to prolong its effects. Some studies on animal species have referred to the pleiotropic effects of GLP-1 such as regeneration and proliferation on β -cells. In a study conducted by Perfetti et al, from the Endocrinology Center of California, it has been determined that continuous infusion of GLP-1 into both young and adult rats results in increased expression of pancreatic and duodenal homeobox 1 (PDX-1) in pancreatic tissue. The mentioned process plays a significant role in the differentiation and maturation of pancreatic cells and is associated with increased proliferation and renewal of cells (6).

Hence, in addition to increasing its secretion from intestinal cells, it also appears that an increase in the expression of its receptors in pancreatic cells is accompanied with an increase in the synthesis and secretion of insulin. Meanwhile, the response of serum GLP-1 and other effective genetic or hormonal factors in secretion of insulin to other external stimuli such as application of exercise training with various methods have also been reported (7,8,9). In a recent study, 12 weeks of low

intensity aerobic training led to a decreased glucose and serum leptin levels as well as a significant increase in GLP-1 in male patients with type 2 diabetes (10). However, although the effects of different training methods on protein or expression level of other effective genetic factors on the synthesis and secretion of insulin have been studied, the response of GLP-1R expression to the stimulants in the pancreatic tissue has been less studied. The reduction of Transcription factor 7-like 2 (TCF7L2) expressions in the pancreatic tissue as another stimulus for insulin secretion in response to long-term resistance training has been reported in type 2 diabetic rats (11). The mentioned finding supports the beneficial effects of exercise training on genetic factors affecting insulin secretion. Regarding the effective role of GLP-1R in the synthesis and secretion of insulin and the lack of evidence regarding the effect of exercise training on GLP-1R expression, the present study aimed at specifying the effect of 12 weeks of HIIT on GLP-1R and serum insulin levels in T2DM rats.

Materials and Methods

Experimental animals

Sixteen 10-weeks-old male wistar rats (220 ± 30 g), procured from the institutional animal house facility were used for all the experiments. Animals were provided with standard pellet diet and water ad libitum and they were maintained under standardized conditions (12-h light/dark cycle, 25 ± 2 °C & humidity 45-55 %). The rats were left for 1 week for acclimatization prior to the commencement of the experiment.

Induction of type 2 diabetes

After 1 week of acclimation, wistar rats were randomized and divided into two groups: exercise diabetes group (ED), control diabetes group (CD). T2DM induced by a single intraperitoneal (i.p.) injection of 60 mg/kg streptozotocin (dissolved in citrate buffer, pH 4.5), 15 min after the i.p. administration of 95 mg/kg of nicotinamide (dissolved in normal

saline) (12). Hyperglycemia was confirmed by elevated blood glucose levels on day 7 after injection and only animals with fasting blood glucose level between 150-400 mg/dl were selected for were served as T2D rats and used in the study (13).

Training protocol

HIIT lasted 12 weeks (5 time/ weekly). Each session lasted 30 min consist of 10 running repetitions on the treadmill and 2 minutes active rest (walking) between each repetition with aim to determine that effect on GLP-1R expression in pancreas tissue, serum insulin and fasting glucose to compare with control subjects (14). Details of the training program are summarized in table 1. The rat of control group remained no training during the study. All rats of 2 groups were described 48 hours after lasted exercise session.

Sample collection and biochemical assay

Finally, 48 hours after the lasted exercise session, the fasted rats in both groups (10-12 hours overnight fast) were anesthetized through intraperitoneal injection of 10% ketamine at a dose of 50 mg/kg along with 2% xylosine at a dose of 10 mg/kg, after which they were underwent dissection. After the rats were anesthetized, blood samples were collected through cardiac puncture. Then, pancreatic tissue was removed and immersed in RNA later until biochemical analysis was performed for determine GLP-1R expression. Blood samples were centrifuged for 10 minutes by 3000 rpm speed for serum separation to analysis serum insulin. Glucose was determined by the oxidase method (Pars

Azmoon kit, Tehran). Insulin was determined by ELISA method (Demeditec, Germany) and the intra- assay and inter-assay coefficient of variation of the method were 2.6% and 2.88 respectively. Glucose was determined by the oxidase method (Pars Azmoon kit, Tehran).

RNA extraction/ Real time- PCR

To purify RNA, 20 milligrams of tissue were ground using a mortar and pestle, and extraction was then performed employing the RNeasy Protect Mini Kit (manufactured by Qiagen Inc. in Germany) according to the manufacturer's protocol (15). In this stage, the One Step SYBR Prime Script RT-PCR Kit (manufactured by the Takara Bio Inc. in Japan) was employed according to the manufacturer's protocol to prepare the reaction product. The thermal cycle program used for the Rotor-Gene Q instrument was as follows: 42°C for 20 minutes, 95 °C for two minutes, and 40 cycles with 94°C for 10 seconds and 60°C for 40 seconds. Temperatures from 50 to 99°C were used for the melting curve after the PCR to study the characteristics of the primers. (Table 2)

Statistical analysis

All the data are expressed as mean (\pm SD). Data were analyzed by computer using the Statistical Package for Social Sciences (SPSS) for Windows, version 15.0. Student's T-tests for paired samples used to determine intra-group changes of weight in each group. Independent T-tests used to determine difference (inter-group) of variables between. Differences were considered to be statistically significant when P -value < 0.05.

Table 1. HIIT protocol based on the speed of running (m/min) on treadmill during training period

Weeks	1	2 - 3	4 - 5	6 - 7	8 - 9	10 - 12
Exercise stage (speed: m/min)	16	20	25	33	33	36
Active rest (Walking speed: m/min)	10	10	12	12	14	14

Table 2. Primer sequence

Genes	Primer sequence	Product size	T m	Gene bank
GLP-1R	For: GGGCTTTATGGTGGCTGTCTTG	159 bp	60	NM_001191052.1
	Rev: GTTTCATGCTGCTGTCCCTCTG			
RNA PolymraseII	For: ACTTTGATGACGTGGAGGAGGAC	164 bp	60	XM_008759265.1
	Rev: GTTGGCCTGCGGTCGTTG			

Ethical considerations

The study was approved by department of Physical Education and Sport Sciences of Qom University of Technology, Iran (Ethic code: IR.SSU.REC.1398.489) and carried out in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experiment on Animal) guidelines.

Results

The results of independent T-test showed that there was no significant difference in body weight between the two groups at baseline (P -value: 0.632).

In control group, although the body weight increased after study, it was not statistically significant (P -value: 0.148). On the other hand, the weight of rats was significantly increased when compared with baseline in the HIIT group (P -value: 0.012). However, there was no significant difference between the weight of rats in the post-exercise conditions between the two groups (P -value: 0.13).

Based on independent T-test, significant difference was found in serum insulin between 2 groups (P -value < 0.001). On the other hand,

HIIT resulted in significant increase in serum insulin compared to control rats (Figure 1). Fasting glucose was also significant lower in exercise than control rats (P -value < 0.001). In fact, fasting glucose decrease by HIIT when compare with control group (Table 3, Figure 2). In addition, statistical analysis showed significant increase in GLP-1R expression in pancreas tissue in response to HIIT (P -value: 0.023). On the other hand, GLP-1R expression in pancreas tissue increased 77% in the HIIT group compared to the control group (Figure 3).

Discussion

Increased expression of GLP-1R is the main finding of this study. In other words, 12 weeks of HIIT for five sessions per week resulted in a significant increase in the expression of GLP-1R in the pancreatic tissue of T2DM rats. Moreover, an increase in the serum insulin levels and a decrease in the glucose level in response to HIIT training are the other findings of the present study. The mentioned findings have been reported in some other studies, for example, some studies have

Table 3. Pre and post-training of studied variables

Group	Control diabetic	Exercise diabetic	P -value
Glucose (mg/dL)	293 (\pm 12)	230 (\pm 12)	< 0.0001
Insulin (μ IU/ml)	4.06 (\pm 0.25)	5.74 (\pm 0.57)	< 0.0001
GLP-1R expression	1	1.61 (\pm 0.68)	0.023

Data were compared by independent T-test

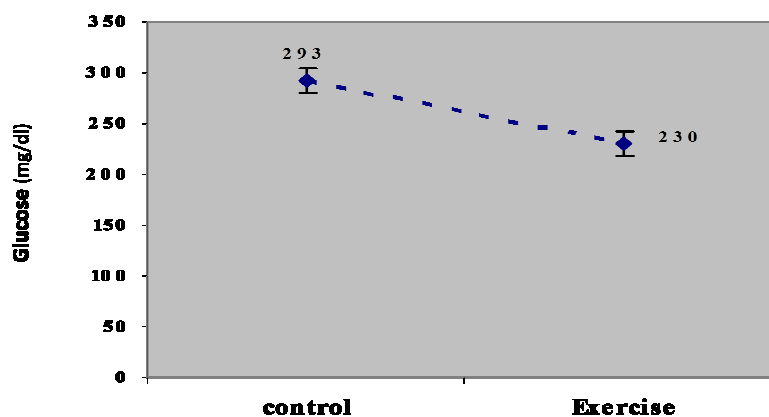


Figure 1. The changes in fasting glucose by HIIT in T2DM rats

reported the increased serum insulin levels along with decreased blood glucose levels in response to long-term training in type 2 diabetic rats (11). In this regard, Rashidi et al (2016) reported an increase in the serum insulin levels as well as an improved glucose uptake in type 2 diabetic rats following a 12-week aerobic training (16). Similarly, Eizadi et al (2017) reported an increase in the serum insulin level and a decrease in the blood glucose level in response to long-term HIIT training in type 2 diabetic rats (14). However, some studies have reported that insulin level does not change in response to continuous exercise training. In the study conducted by Marquis et al (2015), a significant decrease in insulin level was

reported following nine months of combined interval-resistance training in obese men (17). Increased serum insulin level may be attributed to the increased expression of GLP-1R in the pancreatic tissue in response to HIIT training. In this regard, although a study addressing the effects of HIIT training on the expression of GLP-1R in the pancreatic tissue of diabetic rats is not present, a change in the effective genes in insulin synthesis such as TCF7L2 and MTNR1B along with increased serum insulin levels have been reported in some other studies following HIIT and aerobic trainings (14,16). In another study, there was a significant improvement in both β -cells function and insulin resistance in response to eight weeks of HIIT training (18). Moreover,

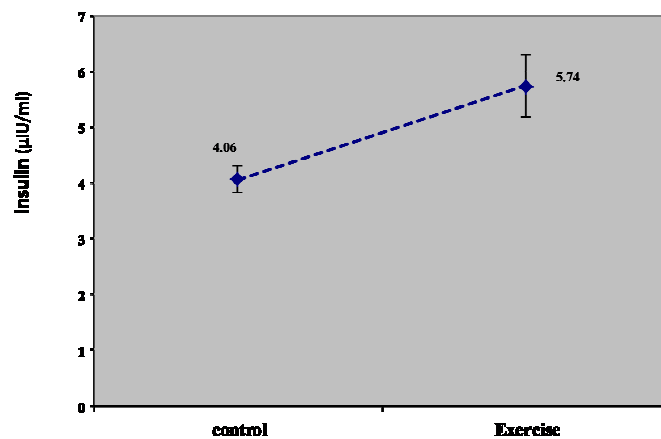


Figure 2. The changes in serum insulin by HIIT in T2DM rats.

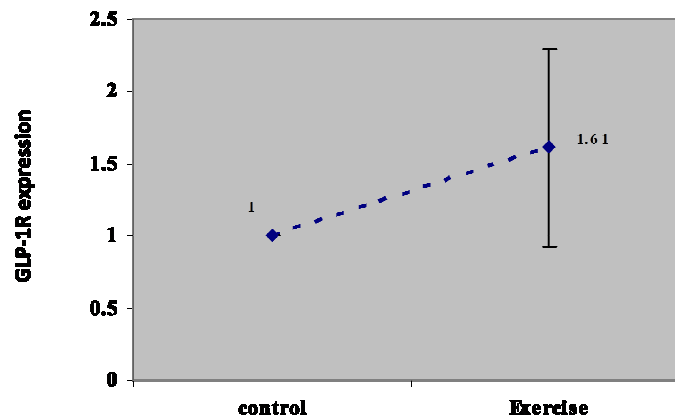


Figure 3. The changes in GLP-1R expression by HIIT in T2DM rats.

the findings of the clinical study conducted by Farilla et al. revealed that the removal of GLP-1 in isolated human pancreatic cells leads to an increase in cell death, while GLP-1 treatment results in decreased apoptosis, increased proliferation, and mass of β -cells (19). Another study revealed that GLP-1 increased the expression of some transcription factors that have pleiotropic effects such as DNA synthesis in pancreatic β -cells, metabolic enzyme expression, and insulin biosynthesis (20). Moreover, an increase in glucose-dependent insulin secretion by incretins has been reported in response to GLP-1 injection to healthy subjects (3). Therefore, based on the available evidence and the effective role of GLP-1R in the synthesis and secretion of insulin, the increased serum insulin levels in this study may be attributed to the increase of GLP-1R expression in pancreatic tissue in response to HIIT training. Precise mechanisms that can justify the role of GLP-1 binding to its receptors (GLP-1R) in improvement of insulin release from β -cells are still unidentified. However, it has been specified that the presence of GLP-1 is required to activate the cAMP messenger pathways and protein kinase A (PKA) that can be achieved by binding G-protein to GLP-1 receptors. The mentioned process can be achieved especially in the presence of increased GLP-1R expression in β -cells of pancreas. The second mechanism of the effect of GLP-1 on insulin secretion that is independent of PKA relates to glucose-dependent insulin secretion that depends on cAMP guanine nucleotide exchange factors (cAMP-GEFs) (21). Nevertheless, PKA activity seems to be necessary to optimize the effect of incretins on the stimulation of insulin vesicle exocytosis (21).

The insulinotropic effect of GLP-1 is demonstrated by its binding to its expressed receptors in β -cells. The receptors binding to incretins increase the intracellular cAMP levels (22), which in turn lead to increased activity of protein kinase A (PKA) (23) and nucleotide-2 cAMP guanine nucleotide exchange factor (GEF) (24). PKA and EPAC

are involved in extensive intracellular reactions including ion channel changes, increased levels of systolic calcium, and increased exocytosis of insulin granules, all of which contribute to glucose-dependent insulin secretion (25).

In this regard, a study on Japanese subjects revealed that only 5% of the pure GLP-1 reached the systemic circulation. Moreover, it was pointed out that the binding of GLP-1 to its receptors and the subsequent stimulation of insulin secretion are performed by activating vagus nerve (25). Based on the clinical observations, Yusta et al (2006) concluded that the signaling pathways of GLP-1 receptors lead to the adaptation and survival of pancreatic β -cells by their direct effect on the stress response in the endoplasmic reticulum (26).

In addition to increased expression level of GLP-1R in response to HIIT training in the present study, some studies have reported increased serum levels of GLP-1 following exercise trainings. In spite of reports about no changes in GLP-1 in some studies (27), one study revealed that a 12-week aerobic training program resulted in an increase of GLP-1 levels by 5 to 7 times in type 2 diabetic patients (28). Increases in the serum or plasma levels of GLP-1 following exercise trainings have been reported by some other studies, as well (10). Moreover, increased serum or expression levels of GLP-1R in other body tissues in response to exercise trainings have been reported. In a study, a 6-week resistance training program led to a significant increase in GLP-1 expression in the left ventricle of type 2 diabetic rats (29). Hence, considering the effective role of exercise trainings in increasing the serum levels of GLP-1 and also increasing the expression of its receptors in response to HIIT trainings in the present study, it may be concluded that changes in GLP-1 levels and its expression in pancreas are significantly involved in the synthesis and regulation of insulin secretion. The strength of this study is determined of GLP-1R expression in pancreas tissue in response to HIIT on

diabetes rats. However, the lack of measurement of other hormonal or genetic components affecting insulin synthesis from the pancreas such as GIP expression or indicators of oxidative stress and antioxidants in pancreatic tissue is a limitation of the present study.

Conclusions

Long-term HIIT training leads to a significant reduction in fasting glucose in type 2 diabetic rats. This improvement can be attributed to the increase in serum insulin and GLP-1R expression in pancreas tissue. However, understanding the mechanisms responsible for

lowering glucose in response to exercise training requires further study

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