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 adlibitum 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 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 RNasey 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 (± 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.

| 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 |

| Genes | Primer sequence | Product size | T m | Gene bank |
|-------|-----------------|--------------|-----|-----------|
| GLP-1R | For: GGGCTTTATGTTGCTGTTG | 159 bp | 60 | NM_001191052.1 |
| | Rev: GTTTCAATGCTGTTGCCCTTCTG | | | |
| RNA PolymraseII | For: ACTTGTATGAGCTGGAAGGAGGAC | 164 bp | 60 | XM_008759265.1 |
| | Rev: GTGGCCTGCGGTGTCGTC | | | |
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 (± 12)       | 230 (± 12)        | < 0.0001|
| Insulin (µIU/ml) | 4.06 (± 0.25)    | 5.74 (± 0.57)     | < 0.0001|
| GLP-1R expression | 1                | 1.61 (± 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,
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 incretines 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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References
1. DeFronzo RA. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. Clinical Diabetology. 2009;10(3):101-28.
2. Garber AJ. Incretin effects on β-cell function, replication, and mass: the human perspective. Diabetes care. 2011;34(Supplement 2):S258-63.
3. Kreymann B, Ghatei MA, Williams G, Bloom SR. Glucagon-like peptide-1 7-36: a physiological incretin in man. The Lancet. 1987;330(8571):1300-4.
4. Garber AJ. The Role of GLP-1 and GLP-1 Agonist in Type 2 Diabetes. Living Medical eTextbook, Point in Knowlege. Little Falls, New Jersey. 2012.
5. Salehi M, Aulinger B, Prigeon RL, D’Alessio DA. Effect of endogenous GLP-1 on insulin secretion in type 2 diabetes. Diabetes. 2010;59(6):1330-7.
6. Perfetti R, Zhou JI, Doyle ME, Egan JM. Glucagon-like peptide-1 induces cell proliferation and pancreatic-duodenum homeobox-1 expression and increases endocrine cell mass in the pancreas of old, glucose-intolerant rats. Endocrinology. 2000;141(12):4600-5.
7. Abedi B, Abbasi-Bakhtiari R. The effect of a 12-week combined training program on serum leptin, C-reactive protein and the insulin resistance index in overweight men. KAUMS Journal (FEYZ). 2015;19(4):293-301. (in Persian)
8. Malekyian-Fini E, Kaviani-Nia A, Mahmoudi F. The interactive effect of aerobic training and resveratrol supplementation on C-reactive protein and metabolic profiles in women with type 2 diabetes. Feyz Journal of Kashan University of Medical Sciences. 2015; 19(5): 372-81. (in Persian)
9. Eizadi M, Behboudi L, Zahedmanesh F, Afsharmand Z. Effect of Acute and Chronic Exercise on Beta-Cell Function in Diabetic Patients.2012; 6(4): 15-19. (in Persian)
10. Lee SS, Yoo JH, So YS. Effect of the low-versus high-intensity exercise training on endoplasmic reticulum stress and GLP-1 in adolescents with type 2 diabetes mellitus. Journal of physical therapy science. 2015;27(10):3063-8.
11. Eizadi M, Ravasi AA, Soory R, Baesi K, Choobineh S. The effect of three months of resistance training on TCF7L2 expression in pancreas tissues of type 2 diabetic rats. Avicenna Journal of Medical Biochemistry. 2016;4(1):12-34014.
12. Eizadi M, Ravasi AA, Soori R, Baesi K, Choobineh S. Effect of three months aerobic training on TCF7L2 expression in pancreatic tissue in type 2 diabetes rats induced by streptozotocin-nicotinamide. KAUMS Journal (FEYZ). 2017;21(1):1-8. (in Persian).
13. Garrow JS. Obesity: Definition, etiology and assessment. In: Allen LH, Prentice A, Caballero B, Editors. Encyclopedia of human nutrition. 3rd ed. New York: Academic press; 2013; (3): 1430-34.
14. Eizadi M, Soori R, Ravasi AA, Baesi K, Choobineh S. Relationship between TCF7L2 Expression in Pancreas Tissue with Changes in Insulin by High Intensity Interval Training (HIIT) in Type 2 Diabetes Rats. Journal of Shahid Sadoughi University of Medical Sciences 2017; 24(12): 981-93. (in Persian)
15. Coughlin CC, Finck BN, Eagon JC, Halpin VJ, Magkos F, Mohammed BS, et al. Effect of marked weight loss on adiponectin gene expression and plasma concentrations. Obesity. 2007;15(3):640-5.
16. Rashidi M, Soori R, Choobineh S, Ravasi AA, Baesi K. The Effect of an Aerobic Exercise on MTNR1B Gene Expression, Insulin and Glucose
Levels in Pancreas of Induced Diabetic Rat with Streptozotocin-Nicotinamide. Journal of Knowledge & Health 2016; 11(3): 40-48. (in Persian)

17. Marquis-Gravel G, Hayami D, Juneau M, Nigam A, Guilbeault V, Latour É, et al. Intensive lifestyle intervention including high-intensity interval training program improves insulin resistance and fasting plasma glucose in obese patients. Preventive medicine reports. 2015;2:314-8.

18. Madsen SM, Thorup AC, Overgaard K, Jeppesen PB. High intensity interval training improves glycaemic control and pancreatic β cell function of type 2 diabetes patients. PloS one. 2015;10(8):e0133286.

19. Farilla L, Bulotta A, Hirshberg B, Li Calzi S, Khoury N, Noumehr H, et al. Glucagon-like peptide 1 inhibits cell apoptosis and improves glucose responsiveness of freshly isolated human islets. Endocrinology. 2003;144(12):5149-58.

20. Buteau J, Roduit R, Susini S, Prentki M. Glucagon-like peptide-1 promotes DNA synthesis, activates phosphatidylinositol 3-kinase and increases transcription factor pancreatic and duodenal homeobox gene 1 (PDX-1) DNA binding activity in beta (INS-1)-cells. Diabetologia. 1999;42(7):856-64.

21. McIntyre N, Holdsworth CD, Turner DF. New interpretation of oral glucose tolerance. The Lancet. 1964;284(7349):20-1.

22. Szecowka J, Grill V, Sandberg E, Efendić S. Effect of GIP on the secretion of insulin and somatostatin and the accumulation of cyclic AMP in vitro in the rat. European Journal of Endocrinology. 1982;99(3):416-21.

23. Fehmann HC, Göke R, Göke B. Cell and molecular biology of the incretin hormones glucagon-like peptide-I and glucose-dependent insulin releasing polypeptide. Endocrine reviews. 1995;16(3):390-410.

24. Holz GG. Epac: a new cAMP-binding protein in support of glucagon-like peptide-1 receptor-mediated signal transduction in the pancreatic β-cell. Diabetes. 2004;53(1):5-13.

25. Holz GG. Epac: a new cAMP-binding protein in support of glucagon-like peptide-1 receptor-mediated signal transduction in the pancreatic β-cell. Diabetes. 2004;53(1):5-13.

26. Yusta B, Baggio LL, Estall JL, Koehler JA, Holland DP, Li H, et al. GLP-1 receptor activation improves β cell function and survival following induction of endoplasmic reticulum stress. Cell metabolism. 2006;4(5):391-406.

27. Ueda SY, Miyamoto T, Nakahara H, Shishido T, Usui T, Katsura Y, et al. Effects of exercise training on gut hormone levels after a single bout of exercise in middle-aged Japanese women. Springerplus. 2013;2(1):83.

28. Dela F, Stallknecht B. Effect of physical training on insulin secretion and action in skeletal muscle and adipose tissue of first-degree relatives of type 2 diabetic patients. Am J Physiol Endocrinology and Metabolism. 2010; 299(1):E80-91.

29. Kalhor H, Peeri M, Matin Homaee H, Izadi M. The Effect of 6 Weeks Resistance Training and HITT on GLP-1 Gene Expression of Diabetic Rats. Iranian Journal of Diabetes and Obesity. 2018;10(1):42-9.