The Effects of High Intensity Interval Training on HNF-4 α Gene Expression in Liver Tissue of Type 2 Diabetic Male Wistar Rats

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Received: 10 December 2018

Accepted: 24 February 2018

Published in May 2019

Abstract

Objective: This study examined the effect of high intensity interval training (HIIT) on HNF-4α gene expression, glucose and insulin in liver tissue of type 2 diabetic male Wistar rats.

Materials and Methods: In this study, 20 male Wistar rats weighing 220 (±20) grams were selected. After type 2 diabetes (T2D) induction, the samples were divided into two groups of HIIT and control. The training program was 12 weeks and 5 times a week. Fasting glucose, serum insulin and HNF-4α gene expression in the liver tissue were measured in both groups after the last exercise session by using independent T-test.

Results: The results showed that HIIT decreased significantly the fasting glucose (*P*-value: 0.001) and increased serum insulin (*P*-value: 0.001). Also, the expression of HNF-4α after HIIT significantly decreased in comparison to the control group (*P*-value: 0.003).

Conclusion: Based on the findings, HIIT resulted in a significant decrease in fasting glucose and a significant increase in insulin that is likely to reduce the liver HNF-4α gene expression of T2D male Wistar rats by increasing serum insulin.

Keywords: High intensity intermittent exercise, HNF-4α gene expression, Type 2 diabetic, Wistar rat

Introduction

Disruption of insulin function or insufficient insulin secretion leads to type 2 diabetes (T2D) (1). Increased production of glucose in the liver is responsible for increasing fasting blood glucose (FBG) and an important part of glucose uptake after eating a meal in diabetic people (2).

Insulin controls the production of glucose in the liver by controlling the expression of two glucose-6-phosphatase enzymes and phosphonalcarboxy kinase pyruvate, involved in the gluconeogenesis process (2). These enzymes are regulated by some genes including Foxo1 (Forkhead box O1), HNF-4α (hepatocyte nuclear factor-4), PGC-1α (peroxisome proliferator- activated receptor gamma coactivator- 1α), Sirt 1 (sirtuin 1) and STAT3 (signal transducer and activator of transcription 3) (3-5). The study of genes

expression in various diseases, especially T2D, is a new method in recent years. Therefore, in this study, the gene expression of HNF-4α is investigated.

HNF-4α belongs to the steroid hormone receptor, and is also a transcription factor that was first recognized by the interaction of specific gene promoters for the regulatory system of hepatic cysts (6). HNF-4α is a transcription factor expressed in the liver, intestine, pancreas and kidneys, also plays an important role in regulating the transcription of the liver and pancreas (6). One study found that one of the mechanisms that inhibits the effect of insulin on gluconeogenesis depends on the ability to reduce the expression of HNF-4a and Foxo1 in the liver (7). Indeed, in insulin resistant models, both Foxo1 and HNF-4α protein levels increased in steady state and after insulin stimulation (8). It has been suggested that HNF-4α modulates glucose metabolism in the liver by controlling the expression of glucose 6-phosphate and phosphonalcarboxy kinase pyruvate genes (7).

On the other hand, exercise is generally recommended because of the beneficial effects on glucose control in the treatment of T2D (9). Exercise improves insulin function and has a significant effect on insulin signaling pathways (10). It also improves glucose hemostasis and insulin sensitivity. After acute exercise, insulin sensitivity increases in tissues such as skeletal muscle, fat, liver and hypothalamus (11). One study investigated the effect of acute exercise on reducing liver glucose production through HNF-4a pathway in insulin resistant mice, which resulted in a reduction in HNF-4a pathway and, as a result of acute exercise, improved glucose hemostasis (12). Despite limited studies on the effect of exercise on HNF-4a expression, as well as the lack of a study that directly measures the effect of exercise training on HNF-4a expression in the liver tissue and its interaction with insulin and glucose, The present study aimed to determine the effect of HIIT on HNF-4a expression in liver, insulin and FBG in T2D rats.

Materials and Methods

The present research was done on male Wistar rats (produced at Pasteur Institute of Iran). The sample consisted of 20 rats in a 10-week-old age range of 220 (± 20) grams, selected randomly. All procedures of this study were approved by the Ethic Committee of south Tehran Branch Azad University (14121407951010). The rats were kept in a laboratory environment at 22 (± 3) ºC, humidity in the range of 30-60, 12 hours of light and 12 hours of darkness. Every two rats were kept in a rat cage for free access to standard water and food. (13). After a fasting night, for induction of T2D, a nicotine amide solution at a dose of 110 mg per kg of rat weight was injected in peritoneal. After 15 minutes, the freshly prepared STZ solution was injected intraperitoneally with a dose of 65 mg / kg in citrate buffer (PH = 4.5) (14). A week after injection, to ensure diabetes induction in rats, blood drops were collected from the venous vein and the glucose blood level was measured by the glucometer (15). In the next step, diabetic rats were randomly divided into two groups of exercise (12 weeks HIIT) and 10 controls. The HIIT program was conducted for 12 weeks each week, 5 sessions per running treadmill with 30 minute time in accordance with Table 1 (13).

After 12 hours of fasting and 48 hours after the last training session, intraperitoneal injection of 10% ketamine (50 mg / kg) and xylazine 2% (10 mg / kg) were used to anesthetizing the rats. The blood samples were taken directly from the animal's heart. The rat liver tissue was also sampled and washed in a physiologic serum in 1.8 μm microelements containing RNA later fluid with a 20% ratio for genetic testing. FBG were measured using glucose oxidase enzyme method and with glucose Kit (Pars test compani). The coefficient of internal and external test changes was 1.74 and 1.19%, respectively, and the sensitivity was 5 mg/dL. The serum insulin was measured by ELISA and in accordance with the instructions for the commercial kit (Demeditec Diagnostic Insulin made in Germany). The coefficient of internal

and external test changes was 2.6 and 2.88, respectively, and the sensitivity was 1.76. RNA extraction was performed using the RNeasy mini commercial kits (QIAGEN Company). Determination of TCF mRNA by RT-Real time PCR using the Rotator 6000 system using the One Step Single Step SYBR TAKARA kit from Takara Company in accordance with the company's instructions. RNA polymerase II was used as control gene to determine the expression of HNF-4a. The sequence pattern of primers is given in Table 2. Reactions CTs were extracted and recorded using Real Time PCR software. A CTΔΔ comparison was used to quantitatively express HNFMRNA.

After confirming the normal distribution of data with Kolmogorov-Smirnov test and homogeneity of data with Levene's test, independent T-test was used to examine the effect of training on dependent variables. The whole operation was performed by SPSS software (Version 20) and the significance level of the tests was considered to be 0.05.

Results

Body weight was measured in both groups before and after the training period. The values of rat weight in both HIIT and control groups are presented in Table 3. There was no significant difference in the weight of rats between the two groups in the pre-exercise program (*P*-value: 0.523).

The results of independent t-test showed that FBG level decreased significantly between the two groups (*P*-value: 0.001). The findings also showed a significant increase in serum insulin levels (*P*-value: 0.001). Also, the relative expression of HNF-4a gene in the liver tissue in the HIIT group significantly decreased (*P*-value: 0.003) (Table 4).

Discussion

In this study, the effect of 12 weeks of HIIT on HNF-4α in liver, glucose and insulin tissues in T2D rats was studied. The results of this study showed that HIIT led to a significant decrease in HNF-4α and glucose and a significant increase in serum insulin level in T2D rats.

Despite the fact that genetic studies refer to the interconnection of insulin signaling pathways with HNF-4α expression in T2D, excessive production of glucose is one of the main mechanisms for increasing FBG, which is due to increased glycogenolysis, reduced glycogen synthesis and gluconeogenesis. Insulin resistance with insulin deficiency in inhibiting phosphoanol-pyruvate carboxy kinase and glucose 6-phosphatase enzymes play an important role in this process (16). Possibly, there are many factors affecting the ability of insulin on the expression of Phosphoenolpyruvate carboxylase kinase and glucose 6-phosphatase enzymes, one of which is HNF-4α (3,7). Therefore, inhibition of gluconeogenesis and production of glucose in the liver is an attractive treatment in diabetic patients, so that metformin may act by inhibiting liver glucose production in hepatic patients (17).

Table 1. HIIT Exercise Program

Activity time (week)	One-minute repetitions (Speed: m/min)	Active rest (Speed: m/min)
1	16	10
2-3	20	10
4-5	25	12
6-7	30	12
8-9	33	14
10-12	36	14

Table 2. Primers pattern used in research

Genes	Primer sequence	Product size	Tm	Gene Bank
HNF4	**For**: GCAGAGATGAGCCGTGTGTC **Rev**: TTGATCTTGCCTGGGTCACTC	159bp	60	NM_001191052.1
RNA Polymerase II	**For**: ACTTTGATGACGTGGAGGAGGAC **Rev**: GTTGGCCTGCGGTCGTTC	159bp	60	NM_001191052.1

Table 3. Body weight changes (grams) before and after training in the HIIT and control groups

Group	Before HIIT Mean (±SD)	After HIIT Mean (±SD)	*P*-value
HIIT	218.42±1.13	271.42±6.65	0.001
Control	219.28±3.25	253.14±5.61	0.001

SD: standard deviation

Table 4. Glucose and insulin levels after exercise in the HIIT and control group

Variable	Control group Mean(±SD)	HIIT group Mean(±SD)	*P*-value
FBG (mg/dl)	294.57(±10.84)	229.00(±9.91)	0.001
Insulin (µIU/mL)	4.14(±0.28)	5.87(±0.49)	0.001
HNF-4α	1.00(±0.0001)	0.62(±0.20)	0.003

SD: standard deviation

Sport activity is one of the effective methods known to control blood glucose levels in diabetics by various mechanisms such as reduction in liver glucose production as an effective factor in hemostasis in glucose (18,19). A study investigated the effect of acute exercise on reducing liver glucose production through FOXO1 and HNF-4α pathways in insulin resistant mice, which resulted in a decrease in FOXO1 and HNF-4α pathways and therefore acute exercise would improve glucose hemostasis (12). Also, the results of the study showed that the association between HNF-4α polymorphisms with glucose and insulin is moderated by physical activity. Therefore, the results of this study showed that the effect of HNF-4α polymorphisms on the risk of T2D is affected by physical activity (20). The results of these studies are consistent with the results of the present study, which reported a reduction in HNF-4α expression.

Also, in several studies, the effect of exercise activity on insulin and glucose in diabetic animal samples has been investigated, some of which are consistent with the present study and some others are inconsistent. For example, in an 8 weeks study, moderate and high endurance training did not affect insulin levels in diabetic rats (21). Other studies also found no effect of exercise on insulin levels in diabetic rats (22-25). In contrast, in a 7 weeks treadmill study, moderate treadmill increased the insulin level in diabetic rats (26), which is in line with the results of this study. The reasons of contradiction between the findings could be due to study situation.

On the other hand, the results of this study indicate a decrease in blood glucose levels, which has been shown to significantly reduce blood glucose following long-term training (27,28). Aerobic exercise seems to control liver glucose production by several mechanisms. One of these mechanisms is the protein 1C binding to the sterol responsive element, which is a physiological inhibitor of liver glucose production (29), which done the expression of phosphoanol pyruvate carboxykinase by controlling the expression of HNF-4α (30). Other studies also showed that HNF-4α activity depends on the transmission of insulin signal via the Foxo1 insulin pathway (3). In the absence of insulin, HNF-4α, along with Foxo1, is likely to activate the glucose 6-phosphatase gene and phosphoanol pyruvate carboxy kinase via PGC-1α, which is associated with activation of gluconeogenesis, but in the presence of phosphorylated Foxo1 insulin it is removed from the nucleus, resulting in HNF-4α being isolated, which, with its separation, activates HNF-4α and the glucokinase gene and inhibits the genes involved in gluconeogenesis. (3,31).

In this study, there was a relationship between the increase in insulin levels and the reduction of HNF-4α expression, which is probably due to the reduction of the expression of phosphoanol pyruvate carboxy kinase and glucose 6-phosphate, and ultimately the reduction of gluconeogenesis and liver glucose production. It has also been shown that HNF-4α is involved in expression glucose metabolism and insulin secretion genes in

pancreatic beta cells (32,33). This increase in insulin in the present study may also be associated with expression of HNF-4α in Beta cells.

Conclusions

The findings of this study showed a decrease in the expression of HNF-4α, glucose uptake and increased serum insulin levels in T2D rats in response to HIIT. The increased liver glucose production and gluconeogenesis as an important pathologic factor in diabetic patients, as well as the role of HNF-4α on liver gluconeogenesis, HIIT activity may lead to increased levels of insulin and phosphorylation of the Foxo1 / HNF-4α pathway. Reducing the expression of phosphoanol pyruvate carboxy kinase and glucose 6-phosphatase and ultimately reducing gluconeogenesis and glucose production. However, more studies are needed in this area.

Acknowledgments

This research is based on a PhD thesis at the Faculty of Physical Education of south Tehran Branch Azad University.

Conflict of Interest

We declare that there was no conflict of interest.

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