

Original Article

Improvement of Fasting Glucose Following Resistance Training with Emphasis on The Some Gluconeogenic Genes Expression in Type 2 Diabetes Rat Model

Ali Ahmadian¹, Heshmatolah Parsian^{2*}, Seyed Ali Hosseini³, Shahram Soheili⁴, Rouhallah Ershadi⁵

1. Department of Exercise Physiology, Ki.C., Islamic Azad University, Kish, Iran.
2. Department of Physical Education and Sport Science, ShQ.C., Islamic Azad University, Shahr-e-Qods, Iran.
3. Department of Sport Physiology, Marv.C., Islamic Azad University, Marvdasht, Iran.
4. Department of Physical Education and Sport Science, ShQ.C., Islamic Azad University, Shahr-e-Qods, Iran.
5. Department of Physical Education and Sport Science, Bu.C., Islamic Azad University, Bushehr, Iran.

Abstract

Background and Aim: Laboratory studies have pointed to the key role of hepatic glucose release in fasting glucose levels, especially in patients with diabetes. This study was conducted with the aim of determining the effect of resistance training on hepatocyte PEPCK and FTO expression in the rats with type 2 diabetes (T2D).

Methods: For this purpose, 28 male Wistar rats (220 ± 10 grams) were fed a high-fat diet (HFD) in order to induce obesity. Among them, T2D induced in 14 rats by intraperitoneal injection of Streptozotocin (STZ) (30 ml/kg). Finally, the studied rats were divided into 4 groups with equal number: 1) control healthy, 2) resistance healthy, 3) control diabetic, and 4) resistance diabetic. The rat in resistance healthy and diabetic groups was completed 8 weeks resistance training (5 times/weekly) and control rats remained non-training. 48 hours after exercise intervention, fasting blood glucose, and hepatocyte PEPCK and FTO expression were compared by two-way analysis of variance and Tukey's post hoc test. Level of significance was set at $P < 0.05$.

Results: Resistance training in resistance diabetes group resulted in significant decrease in fasting glucose ($P = 0.001$), FTO expression ($P = 0.024$) and PEPCK expression ($P = 0.002$) compared to control diabetes group. No significant differences were observed in mentioned variables between control and resistance healthy groups ($P > 0.05$).

Conclusion: Improvement of blood glucose following resistance training is likely to be rooted in decrease the hepatic glucose release induced by reducing the gluconeogenic genes expression following this training method.

Keywords: Type 2 diabetes; Hepatic Gene expression; Resistance Training; Gluconeogenesis; Type 2 Diabetes Rat Model; Streptozotocin (STZ) Induced Diabetes Mellitus.

***Corresponding Author:** Heshmatolah Parsian; **Email:** h.parsian@iau.ac.ir; parsian52heshmat@gmail.com;; **ORCID ID:** [0009-0009-5088-4696](https://orcid.org/0009-0009-5088-4696)

Please cite this article as: Ahmadian A, Parsian H, Hosseini SA, Soheili S, Ershadi R. Improvement of Fasting Glucose Following Resistance Training with Emphasis on The Some Gluconeogenic Genes Expression in Type 2 Diabetes Rat Model. Arch Med Lab Sci. 2026;12:1-7 (e1). <https://doi.org/10.22037/aml.v12.47527>

Introduction

In the pathogenesis of type 2 diabetes (T2D), progressive destruction or dysfunction of beta cells leads to the inability to secrete insulin to compensate for and overcome insulin resistance (1).

Based on laboratory findings, both insulin resistance and beta cell dysfunction lead to the prevalence of T2D and its increased severity (2). Accordingly, most studies have sought to understand how hormonal or metabolic factors affect insulin function and its synthesis or release from beta cells, but the processes of glucose production by some body tissues such as

the liver, which leads to hyperglycemia, especially in patients with diabetes, have received less attention.

The liver is one of the key organs in maintaining and balancing systemic glucose homeostasis in mammals. It is capable of producing glucose by some pathways such as glycogen breakdown (glycogenolysis) and glucose synthesis from non-carbohydrate precursors such as pyruvate, glycerol, lactate, and alanine (gluconeogenesis) (3).

The rate of gluconeogenesis is controlled and regulated by the activity of some enzymes such as phosphoenolpyruvate carboxykinase (PCPK),

fructose 1,6-diphosphatase, and glucose-6-phosphatase (G6Pase) (4).

These studies have revealed that FTO variants have an important role in both body weight and glucose metabolism (7,8). Hepatic FTO plays an important role in regulating metabolism and its expression in liver cells changes in response to changes in other metabolic factors.

FTO expression increases during fasting without any change in its protein levels (9). On the other hand, similar to FTO, the expression of the gluconeogenic PEPCK gene changes in response to metabolic changes. Genetic studies have revealed that FTO protein levels and expression are directly related to glucose levels and PEPCK expression in liver cells (9).

In this context, the findings of one study revealed that increased insulin levels in laboratory mice lead to a decrease in FTO and PEPCK expression in liver cells (10). Although some studies have reported no change in hepatic FTO protein levels and expression in mice fed a high-fat diet for 14 to 17 weeks, increased hepatic FTO protein levels and expression have been reported in other studies following 6 to 12 weeks of high-fat diet in laboratory mice (10,11). On the other hand, FTO protein levels and expression in liver cells of patients with nonalcoholic fatty liver who have hyperinsulinemia and hyperglycemia are increased compared to healthy individuals (12).

In summary, evidence suggests that hepatic FTO plays an important role in whole-body homeostasis, either directly or indirectly, through regulation of gluconeogenic gene expression. It has been shown that increased FTO expression in mouse hepatocytes leads to increased hepatic PEPCK expression by reducing leptin activity and STAT3 nuclear translocation. These changes are associated with increased blood glucose levels and glucose intolerance (12).

On the other hand, clinical evidence indicates that their expression is increased in the presence of obesity and type 2 diabetes. Based on this evidence, it is hypothesized that if hepatic FTO expression is reduced by internal or external stimuli such as drug therapy or exercise, it will lead to a decrease in PEPCK expression, which will result in inhibition of hepatic gluconeogenesis and subsequent reduction in hepatic glucose release into the bloodstream, especially in T2D.

However, there is no study to date that reports the effect of exercise training on the expression of both FTO and PEPCK in hepatocytes. Based on this limitation, the present study aimed to determine the effect of resistance training, as one of the common training methods, on the hepatic expression of FTO and PEPCK as well as fasting glucose levels in T2D rats.

Methods

In the present quasi-experimental study, which was approved by the institute's ethics committee, 28 male Wistar rats (10-week-old, 220 ± 10 grams) were fed a high-fat diet (HFD) in order to induce obesity.

To prepare the HFD, 1% cholesterol powder and 1% pure corn oil were added to the standard diet (13, 14). Among them, T2D induced by intraperitoneal injection of Streptozotocin (STZ) (30 ml/kg) in 14 obese rats. Fasting blood sugar between 150 and 400 mg/dL was considered a criterion for ensuring that rats developed T2D (15). Finally, the studied rats were divided into the following groups: 1) control healthy, 2) resistance healthy, 3) control diabetic, and 4) resistance diabetic. Each group contains 7 rats.

The studied rats were kept under controlled light conditions (12 hours of light and 12 hours of darkness, lighting starts at 6 in the evening and turns off at 6 in the morning) with temperature ($22 \pm 3^{\circ}\text{C}$) and humidity in the range of 30 to 60. For this purpose, plexiglass cages with a mesh door and dimensions of $25 \times 27 \times 43$ cm were prepared so that the mice could have free access to water and HFD.

Resistance training protocol: Rats in the resistance diabetic and resistance non-diabetic groups participated in an 8-week resistance training, 5 sessions per week. The resistance group participated in a resistance training for 8 weeks (5 sessions/weekly). Each exercise session was performed in the form of 3 sets with 6 repetitions in each set (table 1).

The rest time between sets is 3 minutes and the rest time between repetitions in each set are 45 seconds. Applying resistance in the form of tying weights to the rats' tails is equivalent to different percentages of body weight during the training program (15).

Rats in the control diabetic and control non-diabetic groups did not participate in exercise training during this period. At the end, 48 hours after the last training session, the rats were killed and dissected to extract the hepatocytes to measure FTO and PEPCK expression.

Table 1. Resistance training based on applying resistance as a percentage of body weight.

Exercise Session (Weeks)	Applying Resistance (% of Body Weight)
1	40
2	50
3-4	60
5-6	80
7-8	100

Tissue extraction: 48 hours after the last training session, the overnight fasted studied rats in both groups were injected intraperitoneally with a mixture of 10% ketamine at a dose of 50 mg/kg and 2%

xylosin at a dose of 10 mg/kg were anesthetized. Next, the liver tissue was extracted and after washing in physiological serum, it was immersed in 1.8 microtubes containing 20% RNeasy liquid to perform genetic tests. RNA extraction was performed using the commercial kit RNeasy mini kit of QIAGEN company. mRNA gene determination was done by RT-Real time PCR by Rotor-Gene 6000 cyclor using One Step SYBR TAKARA kit from Takara company according to the company's instructions. RNA Polymerase II was used as the control gene. The sequence pattern of the primers is shown in table 2.

Table 2. The sequence pattern of the primers.

Genes	Primer Sequence	Product Size(bp)	Tm	Gene Bank
PEPCK	For: TGCCCCAGGAAGTGAGGAAG	159	60	NM_001191052.1
	Rev: CAGTGAGAGCCAGCCAACAG			
FTO	For: TACACAGAGGCCGAGATTGC	159	60	NM_001191052.1
	Rev: AAGGTCCACTTCATCATCGCAG			
RNA Polymerase II	For: ACTTTGATGACGTGGAGGAGGAC	164	60	XM_008759265.1
	Rev: GTTGGCCTGCGGTCGTTC			

Statistical Analysis

The Shapiro-Wilk test was used to ensure the normal distribution of the data. Descriptive statistics were used to describe the data and draw graphs, and two-way analysis of variance was used to compare groups in the variables under study. Tukey's post hoc test was also used to compare groups in each variable. The significant level will also be considered $\alpha = 5\%$.

All statistical analyses were performed using SPSS/Win version 22 software.

Results

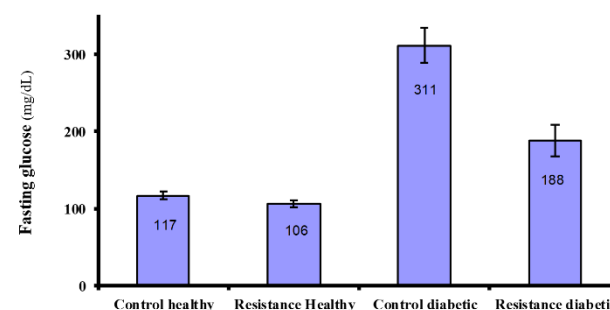
The mean and standard deviation of fasting glucose and PEPCK and FTO gene expression in liver hepatocytes in response to diabetes induction and resistance training are summarized in Table 3.

Table 3. Mean and standard deviation of fasting glucose and gene expression in studied groups.

Variable	Control Healthy	Resistance Healthy	Control Diabetic	Resistance Diabetic
Fasting glucose (mg/dL)	117 ± 5	106 ± 5	311 ± 23	188 ± 20
PEPCK Expression	1	0.82 ± 0.20	1.66 ± 0.40	0.87 ± 0.58
FTO Expression	1	0.83 ± 0.31	1.66 ± 0.40	1.08 ± 0.48

Based on the results of the two-way analysis of variance test, the main effects of diabetes ($P = 0.001$) and resistance training ($P = 0.001$) on fasting glucose were significant, and the interaction effect of diabetes and resistance training was also significant on fasting glucose ($P = 0.001$) (Fig. 1). On the other hand, based on Tukey's post hoc test, T2D induction resulted in a significant increase in glucose compared to the control healthy group ($P = 0.001$), and exercise training by the resistance diabetic group resulted in a significant decrease compared to the control diabetic group ($P = 0.001$). However, fasting glucose in the resistance

diabetic group were still significantly higher than those in the control healthy group ($P = 0.001$).

**Figure 1. Fasting glucose after exercise training in resistance compared with control groups.**

On the other hand, the main effects of diabetes ($P = 0.017$) and resistance training ($P = 0.002$) on PEPCK expression were significant, and the interaction effect of diabetes and resistance training was also significant on PEPCK expression ($P = 0.035$) (Fig. 2).

In addition, T2D induction resulted in a significant increase in hepatocytes PEPCK expression compared to the control healthy group ($P = 0.012$), and exercise training in the resistance diabetic group resulted in a decrease in its expression compared to the control diabetic group ($P = 0.002$).

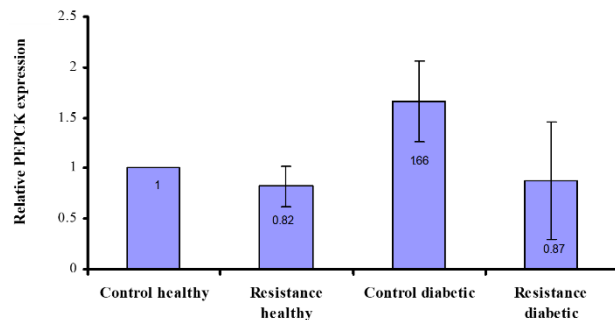


Figure 2. Relative PEPCK expression after exercise training in resistance compared with control groups.

In addition, the main effects of diabetes ($P = 0.002$) and resistance training ($P = 0.009$) on FTO expression were significant, but the interaction effect of diabetes and resistance training on FTO expression was also non-significant ($P = 0.135$) (Fig. 3). T2D induction resulted in a significant increase in hepatocytes FTO expression compared to the control healthy group ($P = 0.008$), and exercise training in the resistance diabetic group resulted in a significant decrease in its expression compared to the control diabetic group ($P = 0.024$).

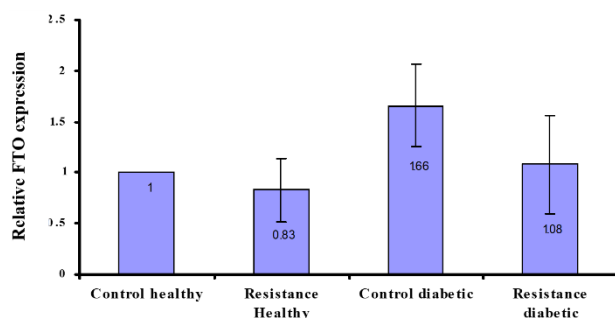


Figure 3. Relative FTO expression after exercise training in resistance compared with control groups.

Discussion

Based on the analysis and comparison of the response of variables dependent on the exercise intervention between the study groups, the main findings of the present study were a decrease in hepatic expression of PEPCK and FTO in response to resistance training in diabetic rats compared to the control group. It should be noted that, in confirmation of the research background on genetic transformation in the presence of diabetes, the induction of T2D was associated with an increase in hepatic expression of FTO and PEPCK compared to healthy rats. Changes in dependent variables in response to resistance training in the diabetic group are reported while none of the variables were observed in response to this type of training by the healthy resistance group compared to the healthy control group. The potential effect of resistance training on the expression of the aforementioned genes is reported while the implementation of the exercises was also associated with a significant improvement in glucose and insulin resistance. Therefore, the decrease in circulating glucose can be attributed in some way to the reduction in hepatic glucose release related to the inhibition of the gluconeogenesis process.

In a study conducted by Chuna et al (2015) the effect of eight weeks of exercise at different intensities on GLUT4 protein and blood glucose control in obese diabetic and non-diabetic obese rats was investigated. The results showed that both low and high intensity exercise were effective, but the high-intensity exercise program significantly improved blood glucose levels compared to inactive controls (16). Also, Soori et al (2017) reported a significant reduction in blood glucose levels after twelve weeks of resistance training in rats with T2D (17). A significant reduction in glucose in diabetic or non-diabetic rats after interval training has also been observed by some other studies (18).

Laboratory evidence supports the increased rate of hepatic gluconeogenesis in patients with diabetes, and the role of hepatic glucose release dependent on the gluconeogenesis pathway in increasing glucose levels in these patients is well established. In a study conducted by Di Moura et al (2013) the effect of a long period of swimming exercise on the levels of proteins and expression of some genes involved in hepatic glucose release in elderly obese mice was investigated. The experimental mice swam for 1.5

hours in two sessions with an interval of 45 minutes. The results showed that the levels of PEPCK proteins in the liver tissue of this group decreased significantly 16 hours after exercise (19).

Based on this evidence, the improvement in fasting glucose in response to resistance training in the present study may be attributed to changes in the expression of PEPCK or FTO in hepatic hepatocytes. It should be noted that resistance training in the diabetic group resulted in a decrease in hepatic PEPCK or FTO expression compared to the control group, but was not affected in the healthy group. In this regard, although studies are limited, Chang et al.'s study in 2006 showed that regular endurance training for 8 weeks, 7 sessions per week, each session consisting of one hour of treadmill running at a speed of 20 m/min and without incline had a significant effect on the activity of the PEPCK enzyme, one of the limiting enzymes of gluconeogenesis, in obese Zucker rats. The results of this study showed that regular endurance training improved insulin sensitivity and reduced PEPCK expression in insulin-resistant rats compared to the obese group without exercise (20).

Marinho et al (2012) reported that long-term endurance exercise improves insulin signalling mechanisms in liver tissue, even in the absence of weight loss. These researchers noted that this exercise is associated with a decrease in the expression of genes that express PEPCK and G6Pase, which are important for the rate of gluconeogenesis (21). Some studies have pointed to the key role of FTO in gluconeogenesis-dependent hepatic glucose release. These researchers attributed the role of FTO on hepatic glucose release to its effect on the activity or expression of the hepatic enzymes G6Pase and PEPCK. It should be noted that in the present study, although FTO expression in healthy mice was not affected in response to resistance training, this training regimen resulted in a significant decrease in FTO expression in diabetic mice.

In normal individuals, increased glucose or insulin in healthy rats leads to inhibition of FTO expression in hepatic hepatocytes. On the other hand, decreased hepatic FTO expression in turn leads to decreased G6Pase and PEPCK, which results in inhibition of gluconeogenesis or, in other words, decreased hepatic glucose release into the bloodstream. In other words, in healthy individuals, increased glucose itself results in a compensatory mechanism of decreased activity or

expression of FTO-dependent G6Pase and PEPCK. In contrast, increased blood glucose in type 2 diabetics leads to increased FTO expression, which results in increased hepatic G6Pase and PEPCK expression. Apart from diabetes, hepatic protein and FTO levels in humans with non-alcoholic fatty liver disease, who also have hyperglycemia and hyperinsulinemia, are higher than in healthy individuals (12).

Based on these findings and what was mentioned earlier, the decrease in PEPCK expression in response to resistance training in the present study may be attributed to the decrease in hepatic FTO expression in response to this training regimen. FTO also positively regulates another transcription factor, ATF4. In other words, increased FTO expression leads to accelerated hepatic glucose production by stimulating increased ATF4. ATF4, in turn, acts as a positive regulator of G6Pase and PEPCK gene expression, which leads to increased hepatic glucose production (22). ATF4 deficiency is associated with improved glucose tolerance, increased insulin sensitivity, and reduced hepatic glucose release in laboratory mice (23,24). On the other hand, hepatic gene expression is reduced in ATF4-deficient mice (22,24). This evidence supports the idea that hepatic ATF4 affects the effect of FTO on glucose metabolism through hepatic gluconeogenesis. Despite the aforementioned evidence, the effect of obesity and diabetes on FTO expression remains controversial.

Conclusion

Resistance training is associated with improved glucose in T2D rats. Based on the findings of the present study, this improvement was attributed to reduced glucose production by the liver, which is caused by changes in the expression of genes related to gluconeogenesis. As the reduction of FTO expression by resistance training leads to a decrease in the activity and expression of hepatic PEPCK, which leads to the inhibition of gluconeogenesis and hepatic glucose release. On the other hand, the improvement in the glycemic profile caused by resistance training is not limited to metabolic and genetic changes in the liver and requires further studies in this field.

Acknowledgments

The authors wish to thank the Dr. Kazem Baesi for analysis of gene expression.

Conflict of Interest

The authors declare no conflict of interest.

Funding/Support

Funding of this study was provided by its researchers.

Ethics

This study was approved by Azad Marvdasht University (ethical code: IR.IAU.M.REC.1403.306).

Authors' contributions

All authors were involved in conception and design of the study as well as in data generation, data analysis, and drafting the manuscript. All authors approved the final draft.

Using AI Declaration

Artificial intelligence was not used in the writing of this article.

References

1. Bergman RN, Finegood DT, Kahn SE. The evolution of β -cell dysfunction and insulin resistance in type 2 diabetes. *Eur J Clin Invest*. 2002; 32(3):35–45.
2. Weyer C, Bogardus C, Mott DM, Pratley RE. The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. *J Clin Invest*. 1999; 104(6): 787–794.
3. Liao J, Barthel A, Nakatani K, Roth RA. Activation of protein kinase B/Akt is sufficient to repress the glucocorticoid and cAMP induction of phosphoenolpyruvate carboxykinase gene. *J Biol Chem*. 1998 Oct 16; 273(42):27320–4.
4. Schmoll D, Walker KS, Alessi DR, Grempler R, Burchell A, Guo S, et al. Regulation of glucose-6-phosphatase gene expression by protein kinase Balpha and the forkhead transcription factor FKHR. Evidence for insulin response unit-dependent and -independent effects of insulin on promoter activity. *J Biol Chem*. 2000 Nov 17; 275(46):36324–33.
5. Dina C, Meyre D, Gallina S, Durand E, Körner A, Jacobson P, et al. Variation in FTO contributes to childhood obesity and severe adult obesity. *Nat Genet*. 2007 Jun; 39(6):724–6.
6. Scuteri A, Sanna S, Chen WM, Uda M, Albai G, Strait J, et al. Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits. *PLoS Genet*. 2007 Jul; 3(7):e115.
7. Li H, Kilpeläinen TO, Liu C, Zhu J, Liu Y, Hu C, et al. Association of genetic variation in FTO with risk of obesity and type 2 diabetes with data from 96,551 East and South Asians. *Diabetologia*. 2012 Apr; 55(4):981–95.
8. Yang Y, Liu B, Xia W, Yan J, Liu HY, Hu L, Liu SM. FTO Genotype and Type 2 Diabetes Mellitus: Spatial Analysis and Meta-Analysis of 62 Case-Control Studies from Different Regions. *Genes (Basel)*. 2017 Feb 11; 8(2):70.
9. Poritsanos NJ, Lew PS, Mizuno TM. Relationship between blood glucose levels and hepatic Fto mRNA expression in mice. *Biochem. Biophys. Res. Commun*. 2010; 400(4): 713–717.
10. Mizuno TM, Lew PS, Luo Y, Leckstrom A. Negative regulation of hepatic fat mass and obesity associated (Fto) gene expression by insulin. *Life Sci*. 2017 Feb 1; 170:50–55.
11. Chen J, Zhou X, Wu W, Wang X, Wang Y. FTO-dependent function of N6-methyladenosine is involved in the hepatoprotective effects of betaine on adolescent mice. *J Physiol Biochem*. 2015 Sep; 71(3):405–13.
12. Guo J, Ren W, Li X, Xi G, Li Y, Gao L, et al. Altering of FTO in the serum and livers of NAFLD patients: A correlation analysis. *Int J Clin Exp Med* 2018; 11(6):6046–6053.
13. Sun YP, Lu NC, Parmley WW, Hollenbeck CB. Effects of cholesterol diets on vascular function and atherogenesis in rabbits. *Proc Soc Exp Biol Med*. 2000 Jul; 224(3): 166–71.
14. Karimi, M. and M. Eizadi, The effect of interval training on FOXO1 expression in pancreas tissue of diabetes rats with high fat diet and STZ. *Razi Journal of Medical Sciences*, 2019. 26(6): p. 95–104.
15. 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 J Med Biochem*. 2016 June; 4(1): e34014.
16. Cunha VN, de Paula Lima M, Motta-Santos D, Pesquero JL, de Andrade RV, de Almeida JA, et al. Role of exercise intensity on GLUT4 content, aerobic fitness and fasting plasma glucose in type 2 diabetic mice. *Cell Biochem Funct*. 2015 Oct;33(7):435–42.
17. Soori, R., Rashidi M, Choobineh S, Ravasi AA, Baesi K, Rashidy-Pour A. Effects of 12 weeks resistant training on MTNR1B gene expression in the pancreas and glucose and insulin levels in type 2 diabetic rats. *Koomesh*. 2017; 19(1); 46–55.

18. Eizadi M, Soory R, Ravasi A, Baesy K, Choobineh S. Relationship between TCF7L2 Relative Expression in Pancreas Tissue with Changes in Insulin by High Intensity Interval Training (HIIT) in Type 2 Diabetes Rats. *JSSU*. 2017; 24 (12): 981-993.
19. de Moura LP, Souza Pauli LS, Cintra DE, de Souza CT, da Silva ASR, Marinho R, et al. Acute exercise decreases PTP-1B protein level and improves insulin signaling in the liver of old rats. *Immun Ageing*. 2013 Feb 25; 10(1):8.
20. Chang SP, Chen YH, Chang WC, Liu IM, Cheng JT. Merit of physical exercise to reverse the higher gene expression of hepatic phosphoenolpyruvate carboxykinase in obese Zucker rats. *Life Sci*. 2006 Jun 13; 79(3): 240-6.
21. Marinho R, Ropelle ER, Cintra DE, De Souza CT, Da Silva AS, Bertoli FC, et al. Endurance exercise training increases APPL1 expression and improves insulin signaling in the hepatic tissue of diet-induced obese mice, independently of weight loss. *J Cell Physiol*. 2012 Jul; 227(7):2917-26.
22. Li K, Zhang J, Yu J, Liu B, Guo Y, Deng J, et al. MicroRNA-214 suppresses gluconeogenesis by targeting activating transcriptional factor 4. *J Biol Chem*. 2015 Mar 27; 290(13):8185-95.
23. Seo J, Fortuno ES, Suh JM, Stenesen D, Tang W, Parks EJ, et al. Atf4 regulates obesity, glucose homeostasis, and energy expenditure. *Diabetes*. 2009 Nov;58(11):2565-73.
24. Yoshizawa T, Hinoi E, Jung DY, Kajimura D, Ferron M, Seo J, et al. The transcription factor ATF4 regulates glucose metabolism in mice through its expression in osteoblasts. *J Clin Invest*. 2009 Sep; 119(9):2807-17.