The Effect of 12 Weeks Resistance Training on FOXO1 Expression in Hepatocytes, Glucose and Insulin in Diabetic Rats- A Brief-Report

Masoomeh Yarmohammadi[1], Laleh Behboudi[*1], Mojtaba Eizadi[2]

1. Department of Exercise Physiology, Islamshahr Branch, Islamic Azad University, Islamshahr, Iran.
2. Department of Exercise Physiology, Saveh Branch, Islamic Azad University, Saveh, Iran.

***Correspondence:**
Laleh Behboudi**,** Department of Exercise Physiology, Islamshahr Branch, Islamic Azad University, Islamshahr, Iran.
Tel: (98) 912 260 0327
Email: behboudil2018@yahoo.com

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Abstract

Objective: In diabetic patients, hyperglycemia is associated with impaired FOXO signaling pathways in liver cells. This study aimed to determine the effect of resistance training on FOXO1 expression in liver hepatocytes and fasting glucose levels in type 2 diabetic rats.

Materials and Methods: In this experimental study, type 2 diabetes induced by intraperitoneal injection of nicotinamide-STZ in 16 male wistar rats (220±20 g) and were randomly divided into exercise (n=8) and control (n=8) groups. Exercise group were participated in resistance training program (12 weeks, 5 days/weekly). Fasting glucose and insulin as well as FOXO1 expression in liver hepatocytes were measured lasted exercise session of 2 groups and compared by independent T-test.

Results: Compared to control group, resistance training resulted in significant decrease in fasting glucose (*P*-value< 0.0001) and decrease in serum insulin (*P*-value< 0.0001). However, no significant difference was found in FOXO1 expression in liver cells between exercise and control groups (*P*-value: 0.725).

Conclusion: Based on this data, improvement of glycemic profile in response to resistance training in diabetic rats cannot be attributed to FOXO1 expression in liver cells. This improvement may be attributed to an increase in serum insulin or other hormonal or genetic changes in response to resistance exercise, which will require further studies in this area.

Keywords: Resistance training, FOXO1 expression, Fasting glucose, Type 2 diabetes

Introduction

In spite of training research findings about the effect of training on FOXO1 expression in non-diabetic ones, the study on the effect of training on FOXO1 expression in liver cells of patients with type 2 diabetes is not apparent (1-3). On the other hand, considering that increased glucose release has a significant role in hyperglycemia in type 2 diabetic patients (4,5). The aim of this study was to determine the effect of resistance training on FOXO1 expression in liver cells as one of the most important transcription factors affecting the release of glucose and also fasting glucose levels in type 2 diabetic rats.

Materials and Methods

Statistical population of the experimental study (IR code: IR.SSRI.REC.1397.383) consists of male wistar rats of the Pasteur Institute of Iran, among which sixteen 10 weeks old male rats weighing 220 ± 220 grams were purchased and after type 2 diabetes induction divided into training group (resistance training, 8 weeks for 5 sessions per week) and control (non training) (each group consists of 8 rats).

Resistance training protocol

The resistance training program in the rats of the training group started from the 13th week and lasted for 12 weeks. The distribution pattern of the intensity of training with a gradual increase in the strength by adding weights to the tails of rats equivalent to different percentages of their body weight, which was performed in 5 sessions per week in the form of 3 courses with 6 repetitions per courses. Rest intervals between the courses was 3 minutes and the intervals between repetitions was 45 seconds. At the end of the program, the control group was also autopsied at the same time as the training group.

Blood samples were used to measure fasting glucose by colorimetric enzymatic method using glucose oxidase technology by Pars Test Tehran Co. kit. Variation coefficient of Intra and extra-test of glucose were 1.74 and 1.19%, respectively, and the sensitivity of measurement was 5 mg / dL. Measurement of insulin by ELISA was performed using a laboratory kit (Demeditec insulin ELIZA DE2935, Germany), respectively.

Liver tissue was extracted and after washing was immediately immersed in physiological serum in 1.8 milliliters microtubes in nitrogen and transferred to the Pasteur Institute of Tehran for analysis of gene expression. RNA extraction was performed by the QIAGEN RNeasy commercial kit (6). The determination of FOXO1 mRNA by RT-Real time PCR was performed by the 6000 Rotrogen system using the One Step SYBR TAKARA kit according to the kit instructions. To study the properties of primers for a melting curve, temperatures from 50 to 99 ° C was used. The sequence pattern of primers is reported in Table 1. A comparative ΔΔCT method was used to quantify expression of FOXO1 mRNA. RNA Polymerase II was used as control gene. The mean CT for the control gene was 19 and for FOXO1 was different depending on the sample concentration.

Kolmogorov-Smirnov test was used to assess the normal distribution of data among the groups. Also, for comparing the means, independent T-test was used at the significance level of *P*-value< 0.05. Statistical analysis was performed in SPSS software version 16.

Ethical considerations

This study was approved by committee of Ethics Standard in Research of the Institute of Physical Education and Sport Science, Ministry of Science, Research and Technology, Tehran, Iran, with number of IR.SSRI.REC.1397.383.

Results

Statistical analysis by independent T-test indicates a significant difference in blood glucose levels between training and control groups. On the other word, training resulted in a significant decrease in fasting glucose in the training group compared to the control group (Table 2). On the other hand, the implementation of resistance training led to a significant increase in serum insulin levels compared to the control group (Table 2).

Table 1. The primer sequences of the variables under study

Genes	Primer sequence	Product size	T m	Gene Bank
FOXO1	**For**: GCAGTTGGAGTTGGAGAACCTG **Rev**: CGTGCTCTGGGCTGAGTG	159 bp	60	NM_001191052.1
RNA Polymerase II	**For**: ACTTTGATGACGTGGAGGAGGAC **Rev**: GTTGGCCTGCGGTCGTTC	164 bp	60	XM_008759265.1

Table 2. Fasting glucose levels and relative expression of FOXO1 in exercise compared to control group

Variable	Exercise group	Control group	*p*-value
Serum insulin (μIU / ml)	6.11 (± 0.84)	4.89 (± 0.68)	<0.0001
Fasting Glucose (mg / dL)	247 (± 13)	292 (± 11)	<0.0001
FOXO1 expression	1.08 (± 0.53)	1	0.725

In spite of significant difference in fasting glucose and insulin levels in the training group compared to the control group, there was no significant difference in expression of FOXO1 in rat's liver cells in the training and control group. In other words, resistance training did not lead to a significant change in expression of FOXO1 in the liver cells of the training group compared to the control group (Table 2).

Conclusions

12 weeks of resistance training reduces fasting glucose levels in the absence of FOXO1 expression in hepatocytes of type 2 diabetic rats. On the other hand, improving glucose levels may be attributed to increased insulin levels in response to resistance trainings. Glucose improvement also probably stems from other metabolic, hormonal or genetic changes that point to the need for more molecular-cell studies.

In the end, it is noted that the improvement of fasting glucose in the present study is likely to be caused by the alteration of other metabolic, hormonal or genetic variables in response to resistance trainings, which not measuring them is one of the main constraints of the present study and further studies are needed to understand the mechanisms behind the glycemic profiles in response to various training.

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Conflict of Interest

The authors report no conflicts of interest.

References

1. Lin HV, Accili D. Hormonal regulation of hepatic glucose production in health and disease. Cell metabolism. 2011;14(1):9-19.
2. Haeusler RA, Hartil K, Vaitheesvaran B, Arrieta-Cruz I, Knight CM, Cook JR, Kammoun HL, Febbraio MA, Gutierrez-Juarez R, Kurland IJ, Accili D. Integrated control of hepatic lipogenesis versus glucose production requires FoxO transcription factors. Nature communications. 2014;5(1):1-8.
3. Xiong X, Tao R, DePinho RA, Dong XC. Deletion of hepatic FoxO1/3/4 genes in mice significantly impacts on glucose metabolism through downregulation of gluconeogenesis and upregulation of glycolysis. PloS one. 2013;8(8).
4. Li M, Li W, Yoon JH, Jeon BH, Lee SK. Resistance exercise training increase activation of AKT-eNOS and Ref-1 expression by FOXO-1 activation in aorta of F344 rats. Journal of exercise nutrition & biochemistry. 2015;19(3):165.
5. Slopack D, Roudier E, Liu ST, Nwadozi E, Birot O, Haas TL. Forkhead BoxO transcription factors restrain exercise-induced angiogenesis. The Journal of physiology. 2014;592(18):4069-82.
6. Coughlin CC, Finck BN, Eagon JC, Halpin VJ, Magkos F, Mohammed BS, Klein S. Effect of marked weight loss on adiponectin gene expression and plasma concentrations. Obesity. 2007;15(3):640-5.