

Original Article



## Effects of different intensities of endurance training on serum fibroblast growth factor level, glucose, and insulin resistance in streptozotocin-diabetic male rats

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### Abstract

**Background and aims:** Fibroblast growth factor 21 (FGF21) is a myokine which is produced and secreted by skeletal muscle. Given the inconsistent results on the relationship between the intensity of training and the improvement of blood indices in diabetic patients, the current research investigated the effect of endurance training at different intensities on serum FGF21 level, glucose, and insulin resistance in streptozotocin (STZ)-diabetic male rats.

**Methods:** To this end, 50 rats (with a mean weight of 23.28±25.05 g) were randomly divided into healthy (non-diabetic) control, diabetic control, as well as low, moderate, and high-intensity endurance training groups. Diabetes was induced in all rats by the injection of STZ. Three days after STZ injection, the blood samples were taken from the cut tip of the tails, and those rats with blood glucose levels above 300 mg/dL were considered diabetic and included in the study. The program included 8-week aerobic training at different intensities. The blood samples (5 cc) were directly collected from the hearts 48 hours after the last training session, followed by measuring the serum FGF21 level, glucose, and insulin. Finally, the analysis of variance and Bonferroni post-hoc test were used for inter-group comparison and the significance level was considered <0.05.

**Results:** Based on the results, the serum glucose level, insulin resistance, and FGF21 reduced after eight weeks of endurance training. The reduction of FGF21 in higher intensity endurance training group was greater compared to the other groups ( $P < 0.05$ ) and the reduction of glucose in moderate intensity group was more significant in comparison to that of the other groups ( $P < 0.001$ ). However, the insulin level increased, which was more pronounced in the moderate intensity training group compared to the other groups ( $P = 0.002$ ).

**Conclusion:** Overall, both moderate and high-intensity endurance training led to a comparatively more effective reduction in blood glucose and insulin resistance. Therefore, these two parameters may have a protective effect on the complications associated with diabetes in the rats.

**Keywords:** Endurance training; Fibroblast growth factor; Insulin resistance; Diabetes

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### Introduction

Diabetes is regarded as a heterogeneous and metabolic disease that leads to increased blood glucose level and metabolic disorders due to the lack of or a reduction in insulin function and its complications affect both the patient and the society in the long term (1). Over 90%-95% of diabetic people suffer from type 2 diabetes (2). Statistics published by the International Federation of Diabetes indicate that more than 418 million adult people all over the world have diabetes, which reaches 600 million by the next 25 years. According to the estimations, a double increase is observed in the number of persons with diabetes in the Middle East and North Africa (3). The prevalence of diabetes is also growing in Iran such that it has affected about 5.5% of the population, making it a health care and social issue (4). Insulin resistance is

considered as the most important comorbidity of type 2 diabetes, which occurs due to defective insulin signaling, changes in the expression of insulin target genes, other metabolic impairments, and involvement with other hormones (1-5). Performing sports activities is one of the most important approaches to control and treat diabetes that leads to a reduction in the incidence of diabetes and cardiovascular complications (5).

Several studies demonstrated the effects of training on the improvement of blood indices in diabetic patients (4-7) or were conducted to investigate the impact of effective factors on the reduction of insulin resistance (8).

Various factors and molecular mechanisms play a role in insulin resistance; therefore, changes in these factors and mechanisms can be useful for improving diabetes (9). Myokines, cytokines, and other expression peptides are

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produced, released, and expressed through muscle fibers that exert autocrine and paracrine effects. In addition, as a part of the extracellular signaling pathway, these peptides are released in response to certain causes such as training and have a role in producing nutrients and causing angiogenesis and myogenesis (10). Fibroblast growth factor 21 (FGF21), as a member of the FGF family, is a protein with 181 amino acids that is expressed in the liver, skeletal muscle tissue, pancreas, and to a lesser extent, in thymus (11). Further, FGF21 is an important intrinsic regulator of blood glucose and lipid metabolism and has anti-diabetic effects. Increased FGF21 in diabetic patients causes a decline in insulin resistance and effectively improves diabetes. According to previous studies, FGF21 regulates lipolysis in adipocytes (12) and affects weight loss and adipose tissue reduction. Additionally, it has been found that the blood serum level of FGF21 increases in obese people (13). Nevertheless, there is little information about the physiological and regulatory effects of FGF21 on lipid metabolism, and thus the physiological role of FGF21 in controlling the adipose tissue remains a controversial issue (14).

Moreover, FGF21 increases glucose consumption in an insulin-dependent manner while it reduces lipolysis in human and mouse adipocytes *in vitro*. In most patients with type 2 diabetes, the purpose of physical activity is to increase the energy consumption that is directly related to the amount of muscle mass which is used during training. Therefore, an activity that involves a larger amount of muscle mass leads to better results for patients with type 2 diabetes (15).

In a study, the plasma level of FGF21 was evaluated before and after speed training at three-week intervals and the results showed that training reduced the plasma FGF21 levels (16). In contrast to these results, another study demonstrated that two-week intensive aerobic training caused an increase in the level of FGF21. Given the inconsistency in the results, further investigation is required regarding determining the amount of training and its effect on the serum levels of FGF21 (17,18).

In addition, although regular training is accepted as a strategy for improving the patients' conditions, it is unclear which training program and how its intensity produce more favorable effects. It is therefore essential to determine an appropriate training protocol. In light of the above-mentioned discussion, the present study aimed to evaluate the effect of endurance training with different intensities on the serum FGF21 level and insulin resistance.

## Materials and Methods

### Animals and Study Design

To conduct this experimental *in vitro* study, 50 eight-week male Wistar rats were obtained from the Pasteur Institute Animal Care Center in Karaj, Iran. The rats were transported to the laboratory and randomly divided into 5

groups, including healthy control, diabetic control, as well as low, moderate, and high intensity endurance training. Throughout the study, the animals were maintained under routine conditions (12-h light/12-h darkness cycles at  $23\pm 3^{\circ}\text{C}$ ), given a standard laboratory diet and enough water and housed under the same conditions (4). After a few days, first, the animals were weighed and then anesthetized with ether and received a single intraperitoneal injection of streptozotocin (STZ) at 60 mg/kg body weight. To prepare the solution, 9.5 mg citrate buffer with a pH of 4.5 (sterile) was added to one g STZ such that the color of the mixture became yellow after stirring. To determine the dose of STZ, a drop of blood was taken from the tip of the tail of the animal and then fasting blood sugar level was measured and injection was performed using a blood glucose test strip and a blood glucose meter. Three days after injection, blood was taken by the tail-tip amputation method, followed by measuring blood sugar level. Animals with blood glucose levels higher than 300 mg/dL were considered as diabetic cases. The rats were allowed to acclimatize to the laboratory environment for one week and then randomly assigned to three training groups and one control group. First, the rats became familiar with running on a treadmill at a speed of 3 m/min for 15-20 minutes within one week. The control group (n=10) underwent no training, but in the experimental groups, the training protocol was performed during 8 weeks 4 days per week for 30 minutes as follows.

- Low-intensity training group including 10 rats (speed 5-8 m/min, equivalent to 50-60%  $\text{Vo}_{2\text{max}}$ );
- Moderate intensity training group containing 10 rats (speed 14-16 m/min, equal to 65-70%  $\text{Vo}_{2\text{max}}$ );
- High-intensity training group encompassing 10 rats (speed 22-25 m/min, equivalent to 80%  $\text{Vo}_{2\text{max}}$ ).

The data related to the training protocol used for all groups are provided in Table 1.

Following the completion of the training protocol, all rats were weighed 48 hours after the last training session. Then, the rats were anesthetized by intraperitoneal injections of ketamine (90 mg/kg) and xylazine (10 mg/kg) and blood samples (5 mL) were directly drawn from the hearts and poured into sterile tubes. After the blood sample was left under room temperature, the serum was isolated using centrifugation at 2500 rpm for 10 minutes and then was frozen and stored in nitrogen at  $-180^{\circ}\text{C}$ .

### Blood parameters

In the post-test step, all the blood samples were taken out from the refrigerator within one day and were tested according to the respective protocols. In addition, plasma glucose assays were performed by the glucose oxidase method using Auto Analyzer Blood Test Machine (Hitachi 902, Beringer Mannheim, Germany) and a special Kit (Pars Azmoon Company, Iran) with a coefficient of variation of 5%. Insulin was measured by ELISA (Pars

Table 1. Training protocol in different groups

Group	N	Period (wk)	Number of Sessions (day/wk)	Intensity		Time (min)
				Speed (m/min)	Gradient (%)	
Control	10	8	0	0	0	0
Low	10	8	4	5-8	0	30
Medium	10	8	4	14-16	0	30
High	10	8	4	22-25	0	30

Azmoon Company, Iran) according to the manufacturer's instructions as well.

Further, insulin resistance (HOMA-IR) and insulin sensitivity indices were used to investigate insulin resistance. If HOMA-IR values were higher than 2.5 and the fasting insulin levels were above 60 pmol, samples would be regarded as insulin resistance cases (12) and their data would be included in the final analysis. Next, the collected data were entered into the SPSS software, version 19. Finally, the Kolmogorov-Smirnov test was used to determine whether the data were distributed normally.

### Statistical tests

The analysis of variance (ANOVA) and Bonferroni post-hoc test were utilized to examine the difference in the serum levels of the variables between the groups. Furthermore, data were analyzed by GraphPad Prism, version 5.01 (GraphPad, USA) and  $P \leq 0.05$  was considered statistically significant.

### Results

Given the reported significance level, the Kolmogorov-Smirnov test results showed that all data related to biochemical variables (e.g., glucose, insulin, insulin resistance, and FGF21) were normally distributed ( $P \geq 0.05$ ). Therefore, parametric tests were used to analyze the data. Table 2 demonstrates information about the mean, standard deviation, as well as the minimum and maximum values of the studied variables in different groups.

Based on the results of data analysis, the effect of the independent variable on FGF21 was different among the low, moderate and high-intensity endurance training groups. Besides, the results of one-way ANOVA and Bonferroni post-hoc test showed different levels of FGF21 in the moderate and high-intensity endurance training groups compared to the healthy control group, indicating a statistically significant difference ( $P < 0.05$ ). However, no significant difference was observed between healthy control and low-intensity endurance training groups, as well as diabetic control and low-intensity endurance training groups ( $P > 0.05$ ). Therefore, these results represent that the independent variable in the moderate and high-intensity endurance training groups affected plasma FGF21 level, but had no significant effect on FGF21 in the low-intensity endurance training group (Figure 1).

Table 2. Descriptive statistics of the studied groups

Groups	Variables	Mean $\pm$ SD
Healthy control	Weight (g)	229.66 $\pm$ 23.09
	Glucose (mg/dL)	27.2 $\pm$ 15.3
	Insulin (mU/L)	320.03 $\pm$ 0.01
	Resistance to insulin	3.40 $\pm$ 0.52
	FGF21 (pg/mL)	0.83 $\pm$ 0.24
Diabetic control	Weight (g)	176.5 $\pm$ 18.09
	Glucose (mg/dL)	557.75 $\pm$ 158.84
	Insulin (mU/L)	0.05 $\pm$ 0.04
	Resistance to insulin	4.83 $\pm$ 1.22
Low-intensity endurance training	Weight	183.62 $\pm$ 18.42
	Glucose (mg/dL)	497.00 $\pm$ 25.43
	Insulin (mU/L)	0.34 $\pm$ 0.04
	Resistance to insulin	0.168 $\pm$ 0.04
	FGF21 (pg/mL)	0.704 $\pm$ 0.117
Moderate intensity endurance training	Weight	207.12 $\pm$ 19.80
	Glucose (mg/dL)	322.5 $\pm$ 175.8
	Insulin (mU/L)	0.48 $\pm$ 0.05
	Resistance to insulin	0.150 $\pm$ 0.02
	FGF21 (pg/mL)	0.558 $\pm$ 0.95
High intensity endurance training	Weight (g)	199.50 $\pm$ 14.78
	Glucose	341.7 $\pm$ 108.7
	Insulin	0.45 $\pm$ 0.03
	Resistance to insulin	0.145 $\pm$ 0.04
	FGF21	0.5118 $\pm$ 0.168

Abbreviation: FGF21, fibroblast growth factor 21.

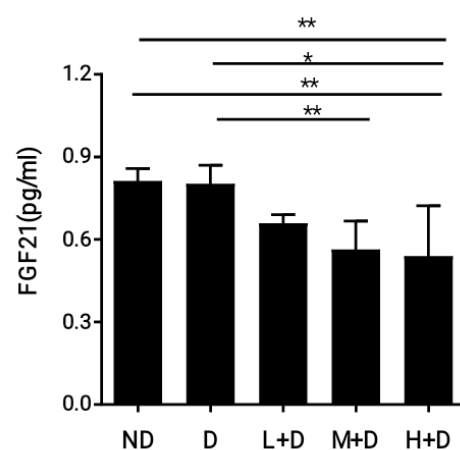


Figure 1. The effect of different intensities of endurance training on serum fibroblast growth factor 21 level

Note. ND: Nondiabetic group; D: Diabetic group; L+D: Undergoing low endurance training and having diabetes; M+D: Undergoing moderate endurance training and having diabetes; H+D: Undergoing high-intensity endurance training and having diabetes.

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According to the results of one-way ANOVA and Bonferroni post-hoc test, the glucose level in low-intensity endurance training group significantly differed compared to that of the healthy control group ( $P < 0.05$ ), but the difference between the diabetic control and low-intensity endurance training groups was not statistically significant. In addition, this value was greater than the significance level in moderate and high-intensity endurance training groups compared to the healthy control group, but the difference was not statistically significant ( $P \geq 0.05$ , Table 3). Further, the  $P$  value for glucose in low-intensity endurance training group was not statistically significant ( $P \geq 0.05$ ) while it was statistically significant in moderate and high-intensity endurance training groups ( $P < 0.05$ ) compared to the diabetic control group. In other words, the independent variable had a significant effect on blood glucose reduction in groups 4 and 5 (Figure 2).

The results of one-way ANOVA and Bonferroni post-hoc test demonstrated various effects of the independent variable on insulin in low, moderate, and high-intensity endurance training groups. The  $P$  value for insulin was  $< 0.05$  and  $< 0.01$  in moderate and high-intensity endurance training groups, respectively, which were statistically significant. In addition, the  $P$  value was significant in moderate and high-intensity endurance training groups compared to the diabetic control group ( $P < 0.01$ ). Besides, no significant difference was observed between the healthy control and low-intensity endurance training groups, as well as between diabetic control

and low-intensity endurance training groups ( $P \geq 0.05$ ). These results showed that the independent variable in the moderate and high endurance training groups had an effect on the plasma level of insulin, but it failed to have any significant effect on insulin level in low-intensity endurance training (Figure 2).

One-way ANOVA was performed to investigate the effect of low-intensity endurance training on healthy control and diabetic control groups, and the difference between low-intensity endurance training, healthy control, and diabetic control groups was found to be significant ( $P < 0.05$ ). Moreover, the difference between moderate and high-intensity endurance training groups was significant compared to healthy control and diabetic control groups. In other words, the independent variable had a significant effect on reducing the blood insulin resistance in all groups (Table 4).

Discussion

Diabetes is clinically one of the most common endocrine diseases (19). Regular physical activity improves blood glucose control and can prevent or delay the development of type 2 diabetes. Evidence shows that high levels of physical activity are associated with a reduction in the risk of developing type 2 diabetes (8). The results of the current study indicated that following eight-week endurance training, serum FGF21 level decreased significantly compared to the control group. In addition, different intensities of endurance training had different effects on the level of FGF21 such that the serum FGF21 level decreased significantly by increasing the endurance training intensity, indicating the positive effect of endurance training on FGF21 reduction. The results of our study are inconsistent with those reported by Besse-Patin et al (20), Yang et al (21), and Kim et al (22). Besse-Patin et al, in their study on non-diabetic obese men, found that eight-week endurance training had no significant effect on the level of FGF21 expression in muscles, but it increased the sensitivity to insulin by increasing apelin expression (20). These results show that endurance training can be effective in increasing the sensitivity to insulin and treating diabetes by affecting the other myokines. The results of Besse-Patin et al contradict those of the current study. This inconsistency can be attributed to different training types

Table 3. The results of one-way ANOVA and Bonferroni post hoc test

Groups	Intensity	Difference in Mean Values	T	P
Healthy control	Diabetic	-410.5	4.867	0.0017
	Low	-79.42	0.8421	0.0028
	Moderate	-58.33	0.5647	0.22
	High	69.83	0.5647	0.15
Diabetic control	Low	69.83	0.7405	0.27
	Moderate	333.1	3.511	0.0026
	High	3.409	352.2	0.0029

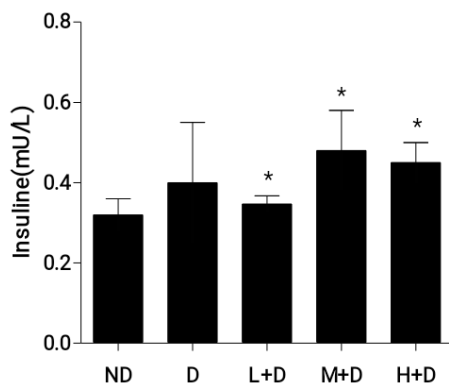


Figure 2. The effect of different intensities of endurance training on insulin serum level

Table 4. The results of one-way ANOVA and Bonferroni post hoc test

Groups	Intensity	Difference in Mean Values	T	P
Healthy control	Diabetic	0.1125	3.020	0.034
	Low	0.1623	3.042	0.03
	Moderate	0.2578	3.910	0.012
	High	0.2411	3.122	0.024
Diabetic control	Low	0.2652	3.452	0.017
	Moderate	0.2325	3.0633	0.03
	High	0.2358	3.852	0.012

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Moreover, Yang et al investigated the combination effect of aerobic training and endurance training on the level of fetuin-A and FGF21 and reported that training had no significant effect on increasing the levels of these two parameters. Increasing the duration of the training and the performance of either of the two training types may lead to different results.

Additionally, the results of the study conducted by Tanimura et al revealed that acute physical activity caused an increase in the serum FGF21 level, as well as in FGF21 secretion in skeletal muscles and the liver of mice, which is not in agreement with our study results. Based on the results of the present study, the expression level of FGF21 increased in the liver following acute physical activity. This difference is likely due to the investigation of variables at different times (23). Besides, different studies reported that the secreted FGF21 from the muscles regulates the metabolism of the whole body by increasing the glucose uptake, the oxidation of fatty acids, and glycolysis in the skeletal muscle. As regards hunger, acute physical activity is found to increase FGF21 in the liver. In other words, FGF21 binds to peroxisome proliferator-activated receptor (PPAR) and causes an increase in the blood FGF21 level.

In a study conducted by Taniguchi et al on Japanese elderly men, the 5-week endurance training program led to a reduction in the liver fat content and the serum FGF21 level (24), which is in line with the results of our study.

Similarly, Cuevas-Ramos et al showed that 2-week endurance training in 60 sedentary young healthy women led to a significant increase in the serum FGF21 level. Thus, they concluded it is likely that more training sessions might change the FGF21 (25). In addition, Taniguchi et al conducted a study on 28 women and found that the serum FGF21 level decreased 24 hours after a period of endurance training (26). Moreover, Yang et al investigated the effect of three-month combination training on FGF21 in obese women. Aerobic training was performed 45 minutes at the intensity of 60-75% of the maximum heart rate and resistance training was performed 20 minutes five times a week. All training sessions were supervised by a professional training physiologist. Based on the results, a significant reduction was observed in FGF21 after training evidenced by a significant decline in the waist circumference to the body mass index ratio, which is in agreement with the results of the current study (21).

In a recent study, Taniguchi et al observed that although five-week endurance training failed to change the elderly men's weight, it led to a significant reduction in the serum FGF21 level (26). In addition, the results obtained by Pernille showed that FGF21 is secreted in the skeletal muscle of humans in response to insulin stimulation, indicating that FGF21 is one of the most important insulin-regulated myokines (27). Serum FGF21 concentration increases in human under the conditions of

insulin resistance such as glucose intolerance and type 2 diabetes. Recent evidence demonstrates that FGF21 is the important intrinsic regulator of blood glucose and lipid metabolism. Further, it increases glucose consumption in an insulin-dependent manner while reducing lipolysis in human and mouse adipocytes. The therapeutic use of FGF21 in diabetic mice or monkeys improves lipid profile and plasma glucose until they reach natural levels and obesity is improved (28). Acute endurance training causes an increase in the serum FGF21 level. This increase may be associated with increased free fatty acids (FFAs). FFAs induce the expression of PPAR that is one of the most important regulators of FGF21 expression (29). Therefore, a sharp increase in the serum FGF21 level is regulated by FFA which is released from the fat-free adipose tissue. Besides, regular aerobic training leads to the reduction of FFA release from adipose tissue and reduces the amount of FGF21 expression whereas regular endurance training fails to change the FFA level and thus FGF21 (24). Like resistance training, Akt1 signaling pathway stimuli in skeletal muscle weaken metabolic diseases associated with obesity through producing and secreting metabolic regulators such as FGF21. The results of the current study obviously indicated that the serum FGF21 level significantly decreased following endurance training. There was also a negative correlation between the intensity of endurance training and the serum FGF21 level. These results further suggest that other regulatory factors and unknown molecular mechanisms play an important role in regulating the serum FGF21 level. Therefore, further research is required for discovering the molecular mechanisms of FGF21 regulation in treating metabolic disorders such as diabetes and cardiovascular diseases. In this research, plasma glucose level, and subsequently resistance to insulin significantly reduced in diabetic subjects after endurance training compared to the control group ( $P=0.0006$ ).

Similar to the current study, Souri et al investigated the effect of aerobic training on serum RBP4 and insulin resistance index in patients with type 2 diabetes and observed that serum RBP4 and insulin levels significantly decreased and insulin resistance index improved in the experimental group after eight weeks of aerobic training, including periodical running (30). Furthermore, Ravasi et al evaluated the effect of endurance training on inflammatory cytokines and resistance to insulin in obese men. The subjects performed endurance training, including continuous running at 75%-80% of the maximum heart rate for 13 minutes. The results revealed that endurance training caused a significant reduction in baseline tumor necrosis factor alpha, interleukin 6, and serum insulin levels, and improved insulin resistance index in obese men compared to the lean ones (31). Likewise, Mohammadi et al studied the effect of 12-week endurance training on the levels of blood glucose, insulin, and the

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heart structure of the mice with type 2 diabetes. They observed that the average blood glucose level was higher in the diabetic control group compared to the endurance training group after 12 sessions of endurance training. In addition, the average insulin index was reported to be higher in the endurance training group compared to the diabetic control group while no significant difference was observed in insulin resistance index between diabetic control and diabetic endurance training groups in this regard (32). Daneshyar et al investigated the effect of endurance training on changes in blood lactate and the plasma levels of calcitonin gene-related peptide in rats with type 2 diabetes. In their study, 50 rats were divided into four healthy control, healthy training, non-training diabetic, and training diabetic groups. Then, the training groups performed the endurance training program on a treadmill. Research findings showed that the blood glucose and the blood lactate levels at rest were higher in diabetic subjects compared to the non-diabetic healthy control group while blood glucose and lactate levels were lower in training diabetic subjects compared to the non-training diabetic control group (33).

Insulin resistance and variation in glucose metabolism are often gradual processes and begin with excess weight gain and obesity. Moreover, insulin resistance is considered as the central base of metabolic syndrome and research studies indicated that aerobic training improves glucose homeostasis and increases insulin sensitivity (34,35). Aerobic training also improves insulin sensitivity in subjects with insulin resistance. In spite of the effects of the physical method on insulin sensitivity and although even one session will improve insulin sensitivity, the effects of several weeks of regular aerobic training can be considerable (36), which supports our study results.

### Conclusion

The results of this study showed that different intensities of endurance training caused a reduction in glucose level, but an increase in the serum insulin and positively affected the insulin resistance-related indices. In addition, the 8-week endurance training protocol was observed to reduce the serum FGF21 levels. In other words, there was a negative correlation between the intensity of endurance training and serum FGF21 level. These results suggest that there may be other regulatory factors and unknown molecular mechanisms that play a role in the regulation of serum FGF21 level. Therefore, further research helps discover the molecular mechanisms of FGF21 regulation in the treatment of metabolic diseases such as diabetes and cardiovascular diseases.

### Conflict of interests

None.

### Ethical considerations

This study was approved by the Ethics Committee of Biomedical

Research of Islamic Azad University, Najafabad Branch (IR.IAU.KHUISF.REC.1396.26).

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