

Effect of Vigorous Aerobic Exercise on Serum Levels of SIRT₁, FGF₂₁ and Fetuin A in Women with Type II Diabetes

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ABSTRACT

Background and Objectives: Sirtuin (SIRT), Fibroblast Growth Factor₂₁ (FGF₂₁) and Fetuin A are proteins that cause a wide range of metabolic disorders such as type 2 diabetes mellitus (T2DM). On the other hand, regular physical activity is known to play a key role in prevention and management of T2DM. Thus, this study investigated the effect of vigorous aerobic exercise on serum levels of metabolic parameters including SIRT₁, FGF₂₁ and Fetuin A in women with T2DM.

Methods: The study was performed on 28 randomly selected women with T2DM who were divided into an exercise group and a control group. The training intervention consisted eight weeks of vigorous aerobic exercise (three times a week at 70-80% of maximum heart rate). The serum levels of SIRT₁, FGF₂₁ and Fetuin A were evaluated before the first session and 48 hours after the last session. Paired sample t-test and independent t-test were used to analyze within and between group differences, respectively. All statistical analyses were performed in SPSS (version 19) at significance of 0.05.

Results: The eight-week aerobic training caused a significant reduction in body weight, body mass index, insulin resistance, low-density lipoprotein, fasting blood sugar, triglycerides and Fetuin A of women with T2DM. In addition, it caused a significant increase in SIRT₁ and FGF₂₁ levels. There was no significant difference in the level of high-density lipoprotein and cholesterol between the two groups.

Conclusion: As a non-pharmacological therapy, regular aerobic exercise might improve the metabolic parameters, SIRT₁, FGF₂₁, and Fetuin A in women with T2DM.

Keywords: Diabetes Mellitus Type 2, SIRT₁, FGF₂₁, Fetuin A, Exercise.

INTRODUCTION

Reduced physical activity and sedentary lifestyle habits increase the incidence of chronic diseases, such as diabetes mellitus (DM). DM is a complex chronic disease associated with hyperglycemia due to deficiency in insulin secretion or action or both (1). According to statistical reports in 2011, the global prevalence of DM was 8% in adults and is estimated to reach 10% by 2030 (2). Proteins involved in the metabolic pathways such as sirtuin₁ (SIRT₁) and some hepatokines such as fibroblast growth factor₂₁ (FGF₂₁) and Fetuin A have been known to play a key role in type 2 diabetes. Diabetics have decreased level of SIRT₁ and FGF₂₁ and increased level of Fetuin A. SIRT₁ is a protein that acts as nicotinamide adenine dinucleotide (NAD)-dependent deacetylase (3) and an important regulator of nutritional homeostasis in several metabolic tissues. SIRT₁ positively regulates the secretion of insulin in beta-pancreatic cells and protects cells from oxidative stress and inflammation (1). It also plays a positive role in insulin signaling pathway in muscle and adipose tissue and its expression is associated with improved insulin sensitivity (4). SIRT₁ increases lipolysis, inhibits lipogenesis, and plays a role in the reverse cholesterol transport (5). FGF₂₁ is a protein involved in regulation of glucose and lipid metabolism. It is mainly expressed in metabolic tissues, such as the liver and muscles (6). Systemic induction of FGF₂₁ prevents obesity and decreases hyperglycemia and insulin resistance. It also leads to weight loss and decreases triglyceride (TG) and low-density lipoprotein (LDL) levels (7). Fetuin A is also a glycoprotein produced by the liver (6), which is associated with metabolic syndrome, type 2 diabetes and cardiovascular disease. It promotes insulin resistance by inhibiting phosphorylation insulin receptor (8) and is involved in development of atherosclerosis in diabetic patients (9). Exercise and regular physical activity have beneficial health effects, especially on chronic diseases such as diabetes. Various studies have been conducted on the effect of physical activity on SIRT₁. Sarga et al. reported an increase in SIRT₁ level of mice following endurance training (10). It has been shown that long-term exercise exerts cardio protective effects via activation of SIRT₁ (11). The results of studies on the effects of training on FGF₂₁

have been inconsistent. Some studies reported an increase in serum level of FGF₂₁ following physical activity (12,13), while in a study by Besse-Patin et al., eight weeks of endurance training in obese men did not affect serum level of FGF₂₁ (14). On the other hand, Yang et al. reported that serum level of FGF₂₁ decreases in obese women after three months of physical activity in obese women (15). In study of Malin et al., a reduction in serum level of Fetuin A was observed in obese adult men and women following a course of endurance training (16). Another study demonstrated that six weeks of aerobic exercise does not significantly change serum level of Fetuin A in obese women (17). Considering the lack of sufficient data regarding the effects of physical activity on serum level of SIRT₁ in humans and the increasing prevalence of diabetes, we conducted this study to investigate the effect of eight weeks of high intensity aerobic training on metabolic parameters and serum levels of SIRT₁, FGF₂₁, and Fetuin A in women with type 2 diabetes.

MATERIAL AND METHODS

This semi-experimental study (clinical trial registration number: IRCT20171104037225N1) was carried out on two groups (14 participants in each group) with a pretest and posttest design and a control group. The study population consisted of the females with type 2 diabetes who were referred to the Diabetes Center in Sari (Iran) for treatment.

Inclusion criteria included age range between 40-50 years old, fasting blood sugar of >126 mg/dl, history of type two diabetes for at least three years with A1C of more than 6.5%, no history of insulin injection, no history of regular physical activity in the past six months, and no history of hepatic and chronic heart disease requiring treatment. Consent was obtained from all participants before taking part in the study. They were asked not to perform any other type of activities. Exclusion criteria included fasting blood sugar of >300 mg/dl, any cardiovascular, liver or kidney diseases, intermittent attendance to the training sessions, and unwillingness to continue participating in the study. Exercise intervention was based on the American Diabetes Association

recommendations and included three sessions of high intensity exercise per week at 70-80% of maximum heart rate (18). The initial training duration was 25 minutes, which was increased by one minute after each training session, and reached 43 minutes at the end of week six. The training intensity started at 65% of the maximum heart rate and increased by 5% every week until reaching 80% of maximum heart rate. The exercise program was performed for eight weeks. Height and weight of the participants were measured when they wearing light clothing and without shoes. Body mass index (BMI) was calculated by dividing body weight (Kg) by height squared (m²). Insulin resistance was evaluated using the following formula:

$$\text{HOMA-IR} = \left[\frac{\text{fasting insulin } (\mu\text{U/ml}) \times \text{fasting glucose (mmol/l)}}{22.5} \right]$$

Metabolic parameters and serum levels of SIRT₁, FGF₂₁, and Fetuin A were evaluated before the first session and 48 hours after the last session. Venous blood samples were taken between 8:00-10:00 AM following 12 hours of overnight fasting. Serum levels of metabolic parameters were measured by an auto analyzer and serum levels of SIRT₁ (Bio Vision, USA), FGF₂₁, and Fetuin A (Bio Vender, USA) were

measured with enzyme-linked immunosorbent assay (ELISA). Descriptive statistics including mean ± standard deviation (SD) were used to describe the data. Kolmogorov Smirnov test and Levene's test were applied to determine normality of data distribution and homogeneity of variances. Paired sample t-test and independent t-test were performed in order to analyze differences within and between groups, respectively. All statistical analyses were performed in SPSS (version 19) at significant of 0.05.

RESULTS

Before the intervention, there was no significant difference between the control and the exercise groups. The eight weeks of high-intensity aerobic exercise caused a significant decrease in body weight, BMI, A1C level, insulin resistance, low-density lipoprotein (LDL) level, fasting glucose level, TG, and Fetuin A levels. However, a non-significant decrease was also noted in the cholesterol level of the participants in the exercise group. Moreover, there was a statistically significant increase in serum levels of SIRT₁ and FGF₂₁ (P=0.001) and a non-significant increase in level of high density lipoprotein (HDL) (Table1).

Table1- Statistical analysis of metabolic parameters, SIRT1, FGF21, and Fetuin A before and after the exercise intervention

Variable	Groups	Pre-test	Post-test	P-value within groups	P-value between groups
Weight (Kg)	Control	70.61 ± 2.11	71.02 ± 2.26	0.220	0.043*
	Exercise	71.17 ± 2.30	69.24 ± 2.15	0.001	
BMI (Kg/m ²)	Control	28.53 ± 1.62	28.68 ± 1.54	0.237	0.025*
	Exercise	28.18 ± 1.32	27.41 ± 1.28	0.001	
FBS (mg/dl)	Control	165.14 ± 11.82	164.79 ± 12.21	0.662	0.001*
	Exercise	164.79 ± 9.9	143.93 ± 8.31	0.001	
A1C (%)	Control	7.94 ± 0.52	7.93 ± 0.55	1.000	0.01*
	Exercise	8.04 ± 0.50	7.18 ± 0.41	0.001	
IR	Control	4.97 ± 0.64	4.95 ± 0.62	0.52	0.02*
	Exercise	4.97 ± 0.61	4.38 ± 0.60	0.001	
TG (mg/dl)	Control	169.57 ± 12.36	166.64 ± 2.49	0.195	0.001*
	Exercise	168.85 ± 12.14	149.92 ± 12.33	0.001	
LDL (mg/dl)	Control	118.64 ± 7.72	119.00 ± 6.21	0.583	0.01*
	Exercise	119.57 ± 8.21	110.92 ± 8.29	0.001	
HDL (mg/dl)	Control	40.85 ± 5.98	40.78 ± 5.07	0.444	0.17
	Exercise	41.07 ± 5.18	43.21 ± 4.82	0.001	
Cholesterol (mg/dl)	Control	180.75 ± 14.08	179.42 ± 13.94	0.126	0.09
	Exercise	179.21 ± 13.18	170.71 ± 12.64	0.001	
SIRT ₁ (ng/ml)	Control	8.51 ± 1.18	8.34 ± 1.14	0.353	0.001*
	Exercise	9.32 ± 1.14	9.99 ± 1.56	0.001	
FGF ₂₁ (pg/ml)	Control	209.71 ± 12.02	210.42 ± 10.42	0.373	0.001*
	Exercise	210.92 ± 8.7	228.07 ± 7.76	0.001	
FetuinA (ng/ml)	Control	25.64 ± 3.44	26.55 ± 3.42	0.19	0.001*
	Exercise	25.48 ± 3.35	20.94 ± 2.98	0.001	

* Significant difference compared to the control group after exercise

DISCUSSION

In this study, we examined the effects of eight weeks of high-intensity aerobic exercise on metabolic parameters and serum levels of SIRT₁, FGF₂₁, and Fetuin A in women with type 2 diabetes. The exercise intervention caused a significant decrease in the level of fasting blood glucose, A1C, insulin resistance, triglyceride, LDL, and Fetuin A compared to the control group. We also noted a significant increase in serum levels of SIRT₁ and FGF₂₁ in the exercise group. Dyslipidemia and cardiovascular disease are common among patients with DM. Our results showed that regular physical activity can improve cardiovascular risk factors, which was supported by other studies (1,19).

The expression and activity of the SIRT₁ are decreased in DM (1). In this study, the eight-week intense aerobic training increased the serum levels of SIRT₁ and FGF₂₁ in diabetic women. Previous studies have not extensively investigated the effect of physical activity on serum level of SIRT₁. Saremi et al. reported a significant increase in serum level of SIRT₁ after two months of exercise (1). In another study, Chi Huang et al. found that swimming increased SIRT₁ level in gastrocnemius and soleus muscle of mice (20). Similarly, Causu et al. reported that six weeks of training increased SIRT₁ levels in healthy mice (21). However, a study by Marton et al. reported that physical activity had no effect on serum levels of SIRT₁ (22). In our study, the eight-week high-intensity aerobic exercise increased serum levels of FGF₂₁, which was in consistent with the results of Yang et al. (15), Farzanegi (23), and Abbasi et al. (24). Farzanegi stated that eight weeks of regular physical activity in women with type 2 diabetes brought about a significant increase in serum levels of FGF₂₁. In addition, the study of Abbasi et al. on obese men demonstrated an increase in serum levels of FGF₂₁ in response to aerobic training. Yang et al. also observed an increase in serum FGF₂₁ levels after 12 weeks of combined exercise training in obese women. Contradictory to the results of these studies, a study claimed that plasma level of FGF₂₁ was reduced following three weeks of intertropical training (25).

It seems that the positive effects of regular physical exercise on the metabolic parameters of diabetics may partially be exerted through proteins associated with mitochondrial biogenesis and glucose regulation such as

SIRT₁ and FGF₂₁. Physical activity increases glucagon and free fatty acids release, which simultaneously increase the secretion of FGF₂₁ from hepatocytes (26). FGF₂₁ increases glucose uptake in adipose tissue independent of insulin (7), which acts through the expression of glucose transport and regulation of lipolysis (27). FGF₂₁ increases the mitochondrial oxidative capacity in adipose tissue, which is manifested by increasing oxygen consumption and induction of key metabolic genes (28). In other tissues, such as the liver and pancreas, FGF₂₁ also helps maintain homeostasis (6).

AMP-activated protein kinase (AMPK) is activated by reduction of ATP/AMP ratio, phosphocreatine (CP), and glycogen levels. On the other hand, the CP and glycogen depletion is influenced by exercise intensity (29). The activation of AMPK (e.g., by exercise) triggers an increase in the NAD⁺/NADH ratio, which activates SIRT₁ (30). Increased level of AMPK increases NAD⁺ intracellular levels, which in turn activates SIRT₁ (31). SIRT₁ also increases fat oxidation and glucose uptake and mitochondrial biogenesis (32). In addition to lipolysis, FGF₂₁ mediated activation of the AMPK-SIRT₁ is accompanied by decreased expression of lipogenic genes involved in fatty acid synthesis (5, 28).

In the present study, eight weeks of aerobic training significantly reduced the serum levels of Fetuin A. This is in agreement with the results of Lee et al. (33), Salama et al. (34), and Malin et al. (16). Lee et al. reported 11% reduction in Fetuin A level after 12 weeks of training in middle-aged men. In the study of Salama et al., regular exercise in diabetic rats caused a significant decrease in Fetuin A level compared to the control group. In the study of Malin et al., 12 weeks of training in adult males and females decreased Fetuin A levels by 8%. On the other hand, Blumenthal et al. reported an increase in serum levels of Fetuin A after six months of aerobic exercise in middle-aged men (35). Another study on non-diabetic obese women did not find any significant changes in Fetuin A levels after six weeks of training (17). These inconsistencies could be attributed to the differences in the type of exercise protocol, duration, and intensity of exercise. Fetuin A is linked to the insulin receptor thereby inhibiting the autophosphorylation of tyrosine kinase and

reducing insulin signaling in skeletal muscle and adipose tissue (8). Fetuin A also stimulates release of cytokines and reduces adiponectin release, which in turn leads to insulin resistance (36). Stimulation of lipogenesis is another consequence of increased Fetuin A level (37). It seems that regular exercise can increase FGF₂₁ and SIRT₁ and decrease Fetuin A levels.

CONCLUSION

As a non- pharmacological therapy, physical activity may exert some beneficial

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effects on metabolic parameters in the body through activation of the AMPK-SIRT₁ pathway and improvement of insulin sensitivity and associated pathways.

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CONFLICT OF INTEREST

All authors declare no conflicts of interest.

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