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The effect of endurance training and extract of fenugreek seed on serum visfatin and vaspin levels in diabetic rats

Peeri. Maghsoud[1], Yazdanshenasan Shahraki. Saeedeh[1*], Azarbayjani. Mohamad Ali[1] and Arshadi. Sajad[2]

[1]Physical Education Faculty, Exercise Physiology Department, Islamic Azad University Central Tehran Branch-Tehran-Iran
[2]Physical Education Faculty, Exercise Physiology Department, Islamic Azad University South Tehran Branch-Tehran-Iran

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ABSTRACT

The goal of the present study was to determine effect of endurance training and to supplement aqueous extract of fenugreek seed on visfatin and vaspin levels in diabetic rats with streptozotocin. Material and Methods: Diabetes was induced by intraperitoneal injection of streptozotocin to 96 male Wistar rats (weight of 200-250 g and age of 12 weeks old). The rats were randomly divided into 8 groups: endurance training and fenugreek with dose of 1.74 g/kg, swimming endurance training, endurance training and fenugreek with dose of 0.87 g/kg, swimming endurance training and glibenclamide, diabetic control group, fenugreek group with dose of 0.87 g/kg, fenugreek group with dose of 1.74 g/kg and glibenclamide group. Swimming endurance training was performed for 6 weeks, 5 days a week and 1 h a day, in a water tank. In each dose of glibenclamide medicine, fenugreek extract and saline solution were orally fed to the subjects using gastric gavage. Results: The results showed that combination of endurance training and aqueous extract of fenugreek seed with dose of 1.74 g/kg significantly decreased plasma visfatin levels but combination of endurance training and aqueous extract of fenugreek with dose of 1.74 g/kg did not have any significant effect on Vaspin plasma levels. Conclusion: Considering the presented results in this research which showed that swimming endurance training and extract of fenugreek seed were effective on decrease of visfatin, it can be said that endurance training and fenugreek plant could be probably regarded a preventive or treatment method for diabetes.

Key words: Diabetes mellitus, Endurance training, Fenugreek, Visfatin, Vaspin

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INTRODUCTION

Diabetes mellitus is a heterogeneous metabolic disease characterized by hyperglycaemia and resulting in defective insulin secretion, resistance to insulin action or both [1]. If diabetes is not controlled, it can lead to blindness, cardiac disease, kidney disorders, amputation and nerve dysfunction [2, 3, 4]. One of the suitable methods for controlling blood sugar is to perform regular exercise programs [5]. Endurance training as the most suitable form of exercise is assossiated with many positive metabolic effects, such as improvements in lipid profile, decrease of body fat, and decrease of blood glucose levels. Endurance training is also effective in improving insulin resistance in patients with type 2 diabetes mellitus and in obese subjects without diabetes [6]. One of the simple and safe methods for treatment of diabetes is to use herbs which have low side effects. Fenugreek is a plant with traditional medicinal use in diabetes [7] and it is effective for lowering postprandial (after meal) blood sugar. The applicable part of fenugreek is its seed with active constituents include trigonelline, 4-hydroxyisoleucine and sotolon. About 80% of the total content of free amino acids in the seeds is present as 4-hydroxyisoleucine, which appears to directly stimulate

insulin. This effect is glucose dependent and only occurs in the presence of moderate to high glucose concentrations [8]. On the other hand, it has been specified that visfatin is an insulin mimicking–dipokine .Visfatin is elevated in obesity and type2 diabetes [9, 10]. Like insulin, it increases glucose transport and lipogenesis by adipocyte and myocyte and decreases glucose production by hepatocyte [11]. Vaspin hormone has been known as a new dipokine relative to obesity and insulin sensitivity [12, 13]. It has been reported that mRNA concentration of serum vaspin in human samples is associated with blood glucose, insulin sensitivity, body mass index (BMI) or percent body fat [14]. Some comprehensive studies have been done on the effect of endurance training and effect of fenugreek on lipid profile and diabetes has been limitedly investigated; however, few studies have been done on the effect of combination of such trainings and consumption of aqueous extract of fenugreek seed on levels of visfatin and vaspin in diabetic rats with STZ and Based on previous studies, researches in this field are quite limited which clarifies the need for more researches in this field.

MATERIALS AND METHODS

Subjects:
In the present research, 96 Wistar rats were used with the mean weight of 200-250 g and age of 12 weeks old. The animals were purchased from Laboratory Animal Rearing House in Ahwaz and were kept in special shelves under controlled environmental conditions with mean temperature of 24±2ºC and light-dark period of 12:12 with free access to water and special rat food. The animals were randomly divided to 8 groups of 1- combination of swimming endurance training- aqueous extract of fenugreek seed with dose of 1.74 g/kg , 2- swimming endurance training group, 3- combination of swimming endurance training - aqueous extract of fenugreek seed with dose of 0.87 g/kg, 4- combination of swimming endurance training - glibenclamide, 5- diabetic control group (C), 6- aqueous extract of fenugreek seed group with dose of 0.87 g/kg, 7- aqueous extract of fenugreek seed with dose of 1.74 g/kg and 8- glibenclamide.

Induction of diabetes
Diabetes induction was done with one time of intraperitoneal injection of streptozotocin solution diluted in a citrate buffer of 0.1 molars with the amount of 60 mg per kg of body weight. 72 h after injection and 12 h of fasting, concentration of blood glucose was measured using blood samples collected from tail of animals. Diabetes criterion was concentration of blood glucose of above 300 mg /deciliters while resting [15].

Preparing Aqueous Extract of Fenugreek Seed
First, 1 kg of powdered Trigonella foenum-graecum (Fenugreek) seed were boiled in 10000 ml distills water for 30 mins. Then, the decoction was cooled for 30 mins at room temperature. Next,the cooled decoction was filtered through a coarse sieve twice. Finally, the filtrate was concentrated by flash evaporation at 358 °c to a thick paste [16].

Endurance Training
Swimming endurance training was done for 6 weeks, 5 days a week and 1 h a day, in a water tank with dimensions of 150cm×90cm×70cm with water temperature of 30°C [17]. The subjects were familiar with swimming conditions in water tank and swimming training protocol was such that period of swimming was 10 min on the first day and 10 min was added to the swimming time on the days after each session so that swimming period of rats reached 6 min after one week and this period remained 60 min until the end of the sixth week [18].

Prescription of Glibenclamide Medicine
At any meal, 0.5 mg/kg was given orally to the rats for 6 weeks. Fenugreek extract, glibenclamide medicine and saline solution were orally fed to the target groups for 6 weeks using gastric gavage .

Blood sampling
After 6 weeks of training protocol and 12 h of fasting, the rats were anesthetized with ether and their blood was taken directly from the heart. Then, the blood samples were centrifuged with speed of 3000 rpm for 10 min and, after plasma separation; they were frozen at -20°c in order to measure visfatin and vaspin at proper time. Concentration of plasma visfatin and vaspin in the subjects was measured using ELISA method and using visfatin and vaspin kits(Glory science co,USA). Visfatin and vaspin were tested with the wavelength of 450 nanometers. It is necessary to note that all ethical and legal aspects of this research were studied and confirmed considering Helsinki Protocol (October 2000) by Committee of Ethics at Ilam University of Medical Education.

Statistical Methods
ANOVA was used for statistical analysis of the data and comparison of the difference between the groups, and Tukey Post Hoc test was used in the case of significance of variance oanalysis in order to determine difference

between the groups. Statistical calculation was done in SPSS statistical software, version 17, and significance level of all the tests was considered P<0.05.

RESULTS

The results of weight showed that no significant difference was found in groups in the first week before starting the protocol (P>0.05) which indicated equal weight conditions of the subjects. This research was done under equal weight conditions. At the end of the sixth week, there was significant weight reduction in all the groups (P<0.05), which was higher in diabetic control group and lower in the combination of endurance training and aqueous extract of fenugreek seed with dose of 1.74 g/kg of body weight (15.90%), endurance training group(10.03%) and combination of endurance training and aqueous extract of fenugreek seed with dose of 0.87 g/kg of body weight (14.51%) and indicated that endurance training by itself and the combination of training and aqueous extract of fenugreek seed could prevent losing weight in diabetic rats (Fig. 1).

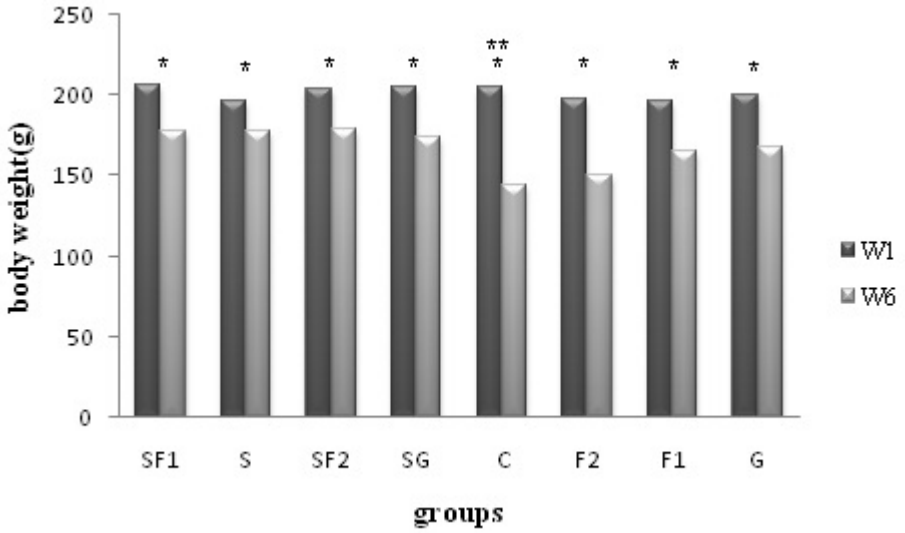

Figure 1: the effect of 6 weeks treatment by swimming endurance training, fenugreek and glibenclamide on serum body weight (g)
*SF1: swimming training + fenugreek seed extract 1.74gr/kg. S: swimming training. SF2: swimming training + fenugreek seed extract 0.87g/kg. SG: swimming training + glibenclamide. C: diabetic control. F2: fenugreek seed extract 0.87g/kg. F1: fenugreek seed extract 1.74g/kg. G: glibenclamide. * Significant difference in the first week than the sixth week. ** The weight loss maximum*

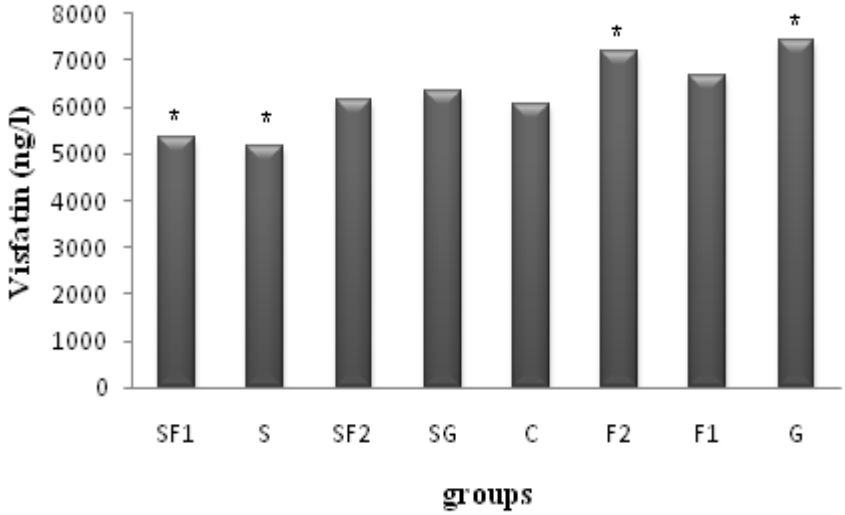

Figure2: the effect of treatment by swimming endurance training, fenugreek and glibenclamide on serum visfatin (ng/l)
*SF1: swimming training + fenugreek seed extract 1.74g/kg. S: swimming training. SF2: swimming training + fenugreek seed extract 0.87g/kg. SG: swimming training + glibenclamide. C: diabetic control. F2: fenugreek seed extract 0.87g/kg. F1: fenugreek seed extract 1.74g/kg. G: glibenclamide. * Significant difference between groups*

The results also demonstrated that variable of visfatin had significant reduction between the group which combined endurance training and aqueous extract of fenugreek seed with dose of 1.74 g/kg of body weight and the group of

aqueous extract of fenugreek seed with dose of 0.87 g/kg (p=0.048) and there was significant reduction between combination of endurance training and aqueous extract of fenugreek seed with dose of 1.74 g/kg and glibenclamide (p=0.019). There was also significant reduction between combination of endurance training and aqueous extract of fenugreek seed with dose of 0.87 g/kg (p=0.020). Moreover, significant decrease was observed between combination of endurance training and glibenclamide (p=0.007). But, there wasn't significant difference between other groups (P>0.05) (Fig. 2).

The results of the present research on variable of vaspin showed no significant difference between all the groups and the control group. Then, it can be concluded that swimming endurance training, combination of endurance training and aqueous extract of fenugreek seed, glibenclamide and combination of endurance training and glibenclamide did not have any effect on vaspin levels in the diabetic samples (p>0.05) but more reduction of vaspin levels resulting from the treatment by combining endurance training and glibenclamide was observed (Fig.3).

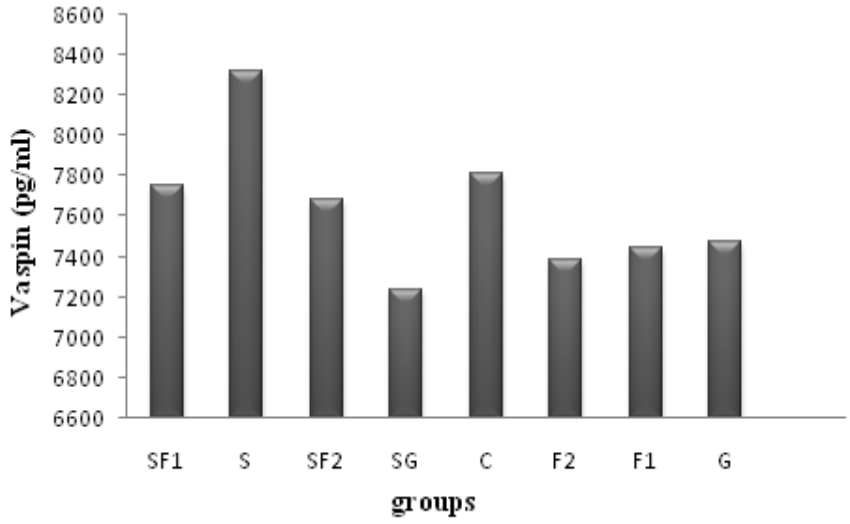

Figure3: the effect of treatment by swimming endurance training, fenugreek and glibenclamide on serum vaspin (pg/ml)
SF1: swimming training + fenugreek seed extract 1.74g/kg. S: swimming training. SF2: swimming training + fenugreek seed extract 0.87g/kg. SG: swimming training + glibenclamide. C: diabetic control. F2: fenugreek seed extract 0.87g/kg. F1: fenugreek seed extract 1.74g/kg. G: glibenclamide

DISCUSSION

The results of the present research showed that 6 weeks of endurance training in diabetic rats caused significant reduction of plasma visfatin levels compared with other groups and combination of endurance training and aqueous extract of fenugreek seed with dose of 1.74g/kg after 6 weeks caused less reduction of plasma visfatin levels than that of endurance training. Therefore, 6 weeks endurance training caused significant reduction of plasma visfatin levels in diabetic rats (P=0.002) with streptozotocin. In this field, research of Chi et al (2007) showed that plasma visfatin levels were reduced following the loss of weight after 12 weeks of aerobic and strength training program and these results demonstrated that changes in visfatin levels may be accompanied by useful effects of exercise. Subjects of the mentioned research included 48 healthy, overweight, or obese Korean women with mean age of 35-50 years. Participants were encouraged to train five times per week. In each session after a brief warm-up, they performed aerobic training with intensity of 60-70% of maximum heart rate (300 Kcal/day) for 45 min and muscular strength training for 20 min (Kcal/day100) and followed by a brief cool-down. Aerobic exercise training included treadmill walking /running and cycling [19]. That research was different from the present work in terms of type and gender of the subjects, training protocol and period of training protocol but its results were similar to those of the present research to some extent. In the research by Amin Mohammadi et al., (2010) plasma visfatin levels significantly decreased after 8 weeks of endurance training program and caused the researchers to interpret the obtained results and mention that changes in visfatin levels might be accompanied by useful effects of exercise. The subjects of the above research included 19 middle-aged healthy men with mean age of 37.5±4.8 years. The subjects performed endurance training 3 days a week with intensity of 65-80% of maximum heart rate for 20-34 min [20]. This research was also different from the present research in terms of type and gender of the subjects, training protocol and period of training protocol but its results were similar to those of the present research to some extent. Therefore, according to the above studies and the present research, it can be concluded that changes in visfatin levels may be accompanied by useful effects of exercise. Thus, exercise can be probably one of the effective factors on visfatin levels. In the studies on metabolic and endocrinological effects of physical exercise, it has been specified

that exercise promotes the use of blood glucose and free fatty acid in muscles and reduces blood glucose levels in diabetic patients who are well-controlled [21]. Advantages of regular exercise for diabetes include: low A1C levels in children and adults with type 1diabetes, decrease in risk of cardiovascular diseases, hypertension, colon cancer, obesity and osteoporosis, increase of life expectancy, increase of cardiovascular endurance, muscle fitness and flexibility and whole body insulin sensitivity [22]. It seems that physical activity can be useful for controlling type2 diabetes. Structured aerobic exercise (walking, jogging, or cycling) or resistance training(weightlifting) reduces absolute hemoglobin A_{1C} value by about 0.6%. The hemoglobin A_{1C} value reflects the mean plasma glucose concentration over the previous 2 to 3 months. 1% absolute decrease in the hemoglobin A_{1C} value is associated with a 15% to 20% decrease in major cardiovascular events and a 37% reduction in microvascular complications [23].

It seems that accessible energy and, to be more specific, rate of plasma glucose are the factors which can affect changes in visfatin levels because, in a study on the effect of an acute training session on changes of gene expression of visceral adipose tissue in diabetic rats, improvement of in the expression of visfatin mRNA levels of visceral adipose tissue following physical training was proved, and the results of this research showed that increase in visfatin mRNA levels of visceral adipose tissue can probably play an important role in glucose metabolism and act as a paracrine messenger [24]. Therefore, decrease in visfatin levels following endurance training can be attributed to many positive metabolic effects of endurance training such as improvement of lipid profile, decrease of body fat and decrease of blood glucose levels. Endurance training seems to be effective on the improvement of insulin resistance in type 2 diabetic patients and non-diabetic fat people [6].

On the other hand, research results in this field showed that fenugreek seeds can reduce blood glucose and cholesterol in type 1&2 diabetic people and experimental diabetic animals [16]. In the research by Ravi Kumar and Anuradha (1999), improvement of glucose metabolism, normalization of creatine kinase activity in heart, skeletal muscle and liver of the diabetic rats as well as decrease in hepatic and renal glucose 6-phosphatase and fructose-1, **6**-biphosphatase activity were observed following the use of fenugreek seeds [25]. In humans, fenugreek seeds exert the hypoglycemic effects by stimulating glucose-dependant insulin secretion from pancreatic β-cells and by controlling activities of alpha-amylase and sucrase, two intestinal enzymes involved in carbohydrate metabolism [26].

Fenugreek seeds contain a high amount of fiber (30% soluble fiber and 20% insoluble fiber). The role of fiber in reducing blood glucose of diabetic patients has been extensively studied and established. Therefore, one of the other mechanisms by which fenugreek seeds decrease blood glucose level is caused by its high content of soluble fiber which would postpone gastric emptying and interfere with glucose absorption [27]. Fenugreek seeds also reduce serum triglycerides, total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C). These effects may be due to sapogenins, which increase biliary cholesterol excretion in order to lead to lowered serum cholesterol levels. The lipid-lowering effect of fenugreek might be also attributed to its estrogenic part, which indirectly increases thyroid hormone T4 [26].

In the research by Tu et al., (2007) 6 weeks of oral use of fenugreek extract for the diabetes caused by streptozotocin led to decrease in blood glucose and glycated hemoglobin. This effect of fenugreek was also observed when natural rats were applied. A potential mechanism for anti-diabetic effects of fenugreek may be due to its bioactive constituents which exist in the fenugreek seed extract, i.e. 4-hydroxyisoleucine which is a newly discovered amino acid and helps insulin secretion. In addition, the soluble fibers existing in fenugreek seed extract could inhibit absorption of glucose in the gastrointestinal tract [16]. According to the research by Tu et al.,(2007) which was similar to the present research to some extent, it is likely that factors such as 4-hydroxyisoleucine as well as soluble fibers existing in fenugreek seed extract may be able to affect reduction of plasma visfatin levels of the rats studied in the present research. Therefore, some factors such as sapogenins and estrogenic part of the fenugreek may affect changes in visfatin levels of the rats studied in the present research.

Other results of the research showed that plasma visfatin levels were affected by combination of endurance training and aqueous extract of fenugreek seed with dose of 1.74 g/kg and were not affected by combination of endurance training and aqueous extract of fenugreek seed with dose of 0.87 g/kg. In the research by Alireza Safarzadeh et al.,(2011) 4 weeks of endurance training in non-diabetic rats significantly reduced vaspin level in serum while vaspin level of diabetic training group was significantly higher than that of diabetic control group. Increase in serum concentration of vaspin after 4 weeks of endurance training in diabetic rats may indicate transient compatibility mechanism.

Subjects of this research were 36 Wistar rats. Their training program included two preliminary and main stages and the period of each stage was 2 weeks. In the preliminary stage, training period (frequencies and weight) gradually increased every day. Trainings were done 3 days a week on every other day [28]. The results of this research were

not congruent with the present research results due to the difference in training program type. Mechanism of sugar metabolism and particularly diabetes can be effective on vaspin changes. On the other hand, the above-mentioned researchers attributed one of the potential mechanisms of vaspin level increase after 4 weeks of training to antioxidant supplements and, especially to vitamins C and E. Of course, these researchers reported that the people who had higher physical readiness had also higher vaspin levels [28]. According to the results of the present research on vaspin, endurance training had probably no effect on vaspin levels and, according to the research by Alireza Safarzadeh et al. which showed increase in serum concentration of vaspin after 4 weeks of endurance training in diabetic rats, it is likely that this dipokine could be more affected by endurance training.

There are few studies on the effect of combination of endurance training and aqueous extract of fenugreek seed on plasma visfatin and vaspin levels of diabetic rats which are not accessible. They are not also in line with the results of the present research. Thus, the present work could be considered a pioneer in this regard.

CONCLUSION

The goal of the present study was to determine effect of endurance training (for 6 weeks, 5sessions per week , and each session for 60 min) on supplementation of aqueous extract of fenugreek seed with two doses of 0.87 g/kg and 1.74 g/kg on visfatin and vaspin levels in diabetic rats with streptozotocin. The results of the present research in both doses showed that swimming endurance training and aqueous extract of fenugreek seed significantly reduced visfatin and vaspin levels. Therefore, one can say that combination of endurance training and use of aqueous extract of fenugreek seed can be regarded as a treatment or preventive method for diabetes. More studies are definitely needed since there are limited informations and few studies in this field.

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