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Effect of Aerobic Training and Cinnamon on Expression of Resistin Gene in Adipose Tissue of Male Rats Fed with a High-Fat Diet

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Abstract

Background: The pathogenesis of obesity is very complex, and a number of factors are involved in its occurrence. To treat or control obesity and diabetes, various treatment methods, such as the use of natural and synthetic drugs or lifestyle modifications, are recommended for obese people. Exercise is an active lifestyle that can have beneficial effects on obese people. Due to people's concerns about the side effects of chemical drugs, including drug resistance, today in medicine, the use of herbal compounds with different properties, including cinnamon, has been considered by everyone.

Objectives: In the current work, simultaneous application of cinnamon extract (CE) and aerobic practices were studied on the expression of the resistin gene (RG) in adipose tissue of male rats on a high-fat diet.

Methods: The influence of 5 days a week of aerobic exercise for 6 weeks, along with a simultaneous high-fat diet (as an emulsion with the rate of 150 mL in excess of the routine diet) and consumption of CE (200 mg/kg), were studied. The expression of the RG in adipose tissue of male rats that received a high-fat diet in their daily food was assessed. Independent variables of the present study included performing 6 weeks of aerobic exercise, consumption of CE, and high-fat diet as the underlying variables. The mRNA expression of the proteins was evaluated using real-time PCR. The effect of variables on between-group and within-group changes was assessed using a one-way analysis of variance and the Bonferroni test.

Results: Different patterns, including high-fat diet, cinnamon, and combinations of high-fat diet/cinnamon, high-fat diet/aerobic exercise, and high-fat diet/aerobic exercise, were significant on resistin in adipose tissue.

Conclusions: The best results in terms of mitigating the adverse effects of diet on the expression of RG were observed for a combination of six weeks of exercise and CE. Therefore, the simultaneous utilization of aerobic exercise and CE is recommended.

Keywords: Resistin, Exercise, Cinnamon, Obesity, Aerobic Activity, High-Fat Diet

1. Background

Today, obesity and type 2 diabetes are epidemics worldwide. Obesity is a known risk factor for type 2 diabetes. Also, obesity complicates the control of type 2 diabetes by increasing insulin resistance and blood glucose concentration (1). The pathogenesis of obesity is very complex, and a series of factors are involved in its occurrence. The most important factors include sex, environmental factors, social-psychological factors, age, genetic predisposition, endocrine gland function disorders, food habits, environmental factors, sports, physical activities, and serum adipokines levels, among others (2-4).

An important way to control obesity, lose weight, and create a negative energy balance is to reduce energy intake, which is directly affected by a person's appetite and the amount and composition of food consumed (5). Notably, in this cycle, the imbalance of hormones and physiological factors can change the appetite and the processes related to energy intake. On the other hand, the extra energy intake caused by overeating can increase the fat mass, which results in a change in the pattern of the secretion of some hormones and physiological factors (5). The importance of adipose tissue is due to the secretion of a number of abnormal proteins such as leptin, adiponectin, and resistin and, therefore, the creation of insulin resistance. Resistin is a hormone secreted by adipocytes that belongs to a family of proteins with a carboxyl end rich in cysteine called resistin-like molecules or proteins found in inflammatory areas. Increased secretion of resistin disrupts insulin action and glucose metabolism and acts as an important link between insulin resistance and obesity (6-9). In order to treat or control obesity and diabetes, various treatment methods, such as the use of natural and synthetic drugs (kinetic) or lifestyle modification, are recommended for obese patients and people (1). Regular exercise activity as an active lifestyle increases glucose uptake in a constant concentration of insulin. On the other hand, due to people's concerns regarding the adverse effects of chemical drugs, including drug resistance, nowadays, in medical science, the use of herbal compounds with different properties is required. Studies show that cinnamon is more effective than other herbal products, such as green tea, olive oil, garlic seeds, and onions, in regulating glucose metabolism (1, 8). Among the complementary and alternative treatments in weight reduction and control is the use of medicinal plants or their effective substances.

Medicinal plants are affordable in terms of price and have fewer side effects compared to chemically synthesized drugs. In addition, they are easily available and widely used. These herbal medicines are consumed raw or with simple preparation methods that activate their effective compounds and cause useful therapeutic responses. Cinnamon can be mentioned among these plants. The compounds in cinnamon may control and reduce obesity by affecting the expression of various genes (10). Mohammad et al. (2012) investigated the protective effect of cinnamon on sugar indices, fat profile, and some adipokines. Their results indicate a positive influence on the mentioned indicators, and the levels of resistin decreased compared to the control group due to cinnamon (6). One of the active components derived from cinnamon is methylhydroxychalcone, which acts like insulin. Phosphorylation of insulin receptors decreases in obese people and type 2 diabetic patients. Cinnamon stimulates the autophosphorylation of the insulin receptor, and phosphotyrosine phosphatase is an enzyme active in dephosphorylating the insulin receptor, which is inhibited by cinnamon compounds, hence increasing insulin sensitivity. Also, this food additive increases glucose uptake by activating the insulin receptor, increases glycogen synthesis, improves body fat metabolism, and improves antioxidant status in obese people with diabetes, heart diseases, and metabolic syndrome (1).

Sports activity has beneficial effects on obesity and metabolic problems. Sports activity may also affect the status of adipocytes and thus change resistin levels (9, 11). Aerobic exercise increases the efficiency of aerobic energy production devices and increases cardio-respiratory endurance. Aerobic training refers to long-term, low-intensity activity that leads to muscle, cardiovascular, respiratory, energy, and metabolism adaptations. In this regard, Lee et al. (cited in Irandoost et al.) (1) demonstrated the positive effects of regular exercise on obesity and type 2 diabetes in Korean children. After 12 weeks of exercise, they observed improvements in blood sugar control, reduction in body weight, and decreased levels of adipokines (specifically resistin), as well as a decrease in cardiovascular risk factors. Ihalainen et al. (cited in Hejazi et al.) (8) showed that combined resistance and aerobic training decreased leptin and resistin in healthy subjects. However, Lopez Lopes et al. (cited in Irandoost et al.) (1) showed that 12 weeks of aerobic training without restricting caloric intake resulted in a significant change. However, it did not affect resistin and adiponectin (11).

Although the effect of aerobic activity on resistin and insulin resistance in diabetic and obese people has been investigated, there is little research on the effect of cinnamon on resistin, as well as the simultaneous effect of cinnamon consumption and aerobic exercise on resistin and sugar indicators (1). This issue raises the question of whether performing six weeks of aerobic training and consuming CE affects the expression of the resistin gene (RG) in the adipose tissue of male rats fed with a high-fat diet.

2. Methods

The present experimental research aimed to determine the effect of six weeks of aerobic exercise, consumption of CE, and high-fat food on leptin gene expression in the adipose tissue of male rats fed a high-fat diet using an animal model. The study involved 50 two-month-old male Wistar rats. It was a multi-group research project with a control group. The independent variables included six weeks of a high-fat diet as a background variable, CE consumption, and six weeks of aerobic exercise. Dependent variables included body weight, adipose tissue mass, and the amount of RG expression in adipose tissue.

The rats were familiarized with nutritional conditions, living environment, and training by keeping them in controlled conditions for two weeks. After that, weight matching was done in order to categorize the rats into 5 groups randomly. Each group consisted of ten mice. The control group, which did not participate in the program of aerobic exercise, the gavage of CE, and high-fat food, was sampled to determine the basic values of research variables. The high-fat diet group was to show the changes in the research variables after 6 weeks of high-fat food gavage. The CE and high-fat diet group was to show the changes in research variables after 6 weeks of CE gavage. The aerobic exercise and high-fat diet group were to show the changes in research variables after six weeks of aerobic exercise, and the aerobic exercise, CE, and high-fat diet group were to show the changes in research variables after six weeks of aerobic exercise and gavage of CE.

In addition to the daily and precise control of the amount of food and water consumed by the rats based on the existing standard guidelines, all groups receiving high-fat food were given daily high-fat food emulsion (1.5 mg/kg) for 6 weeks by gavage (Table 1).

Table 1. Composition of High-fat Food

Compounds	Amount (Grams)
Corn oil	400
Sucrose	150
Whole milk powder	80
Cholesterol	100
Multivitamin	2.5
Polysorbate	36.5
Propylene glycol	31
Salt	10
Distilled water (mL)	300

The exercise group participated in an aerobic exercise program on an intelligent animal treadmill 5 days a week for 6 weeks. The incline of the treadmill was 15% throughout the training period. The speed of the treadmill started from 25 m/min in the first week and reached 30 m/min in the sixth week. The duration of training started from 10 minutes a day in the first week and reached 50 minutes a day in the sixth week. At the beginning of the training session, each of the rats ran for 5 minutes at a speed of 10 - 15 m/min and a 0° inclination to warm up. Then, in order to reach the intensity of the desired exercise, the speed and incline of the treadmill were increased every 5 - 10 minutes. At the end of the training program, to cool down the subjects, the inclination of the device returned to 0°, and the speed slowly reached 10 - 15 m/min. The duration of the cooling phase lasted about 5 minutes in the first weeks and about 10 minutes in the final weeks (Table 2).

All rats were injected intraperitoneally with ketamine (90 mg/kg) and xylazine (10 mg/kg) 48 hours after the last training session and after 12 hours of fasting, according to a predetermined schedule in a standardized manner by trained experts in a desiccator. The weight of mice and the weight of fat tissue were measured and recorded using a digital scale according to the purpose of the present study. Fat tissue was removed and placed in 1.5 or 2 microliter microtubes containing RNAlater at -70°C. The mRNA expression of the desired proteins was analyzed with the real-time PCR method. Preparation of the PCR master mix for cDNA was conducted using the Takara Viragen kit. After performing the PCR, the threshold cycles obtained from the samples of the groups were collected in an Excel sheet, and by putting them in the formulas of $\Delta\Delta$Ct and $\Delta\Delta$Ct-2, the expression ratio of the target and reference genes was compared with each other. The characteristics of the designed primer, including length, type, and sequence for RG, are as follows:

TCATGCCCAGAACCGAGTTG (F) - 20 bp

GGCTTCATCCATGGGACACA (R) - 20 bp

The Shapiro-Wilk test was used to test the normality of the distribution of the variables, and the Lune test was used to test the homogeneity of the variance of the variables. The significant differences between the groups were investigated according to the comparison of the mean values of the variables among the groups, with the exception of the control group, using analysis of variance and Bonferroni tests. The mean values of each group's variables were compared with the control group to determine intra-group differences using the one-sample *t*-test. The significance level in all tests was $P \geq 0.05$.

3. Results

After conducting the Shapiro-Wilk test and Lunn's test, we observed the normality and convergence of the variance of different groups. Changes in RG expression in different groups are listed in Table 3. There was a significant difference in the resistance gene expression changes ($F = 48.1820$ and $P = 0.001$) between different groups according to the results of the analysis of variance. In Table 4, the results of the Bonferroni post hoc test showed that the RG expression levels in the training ($P = 0.001$), CE ($P = 0.001$), and cinnamon-exercise ($P = 0.001$) groups had a significant decrease compared to the control group. Also, Table 5 shows the results of the correlated *t*-test along with the mean and standard deviation of different groups compared to the control group and the significant change of resistance.

4. Discussion

In the present study, the effect of six weeks of aerobic exercise with a cinnamon supplement on the expression of RG in rats with a high-fat diet was investigated. This study showed that performing aerobic exercise and cinnamon supplementation, as well as the combination of aerobic exercise with cinnamon supplementation, reduced the expression of RG in mice with a high-fat diet. Physical inactivity increases the risk of obesity, and an inactive

Table 2. Aerobic Exercise Program

Aerobic Exercise Protocol	Weeks of Training					
	1	2	3	4	5	6
Training duration (minutes per day)	10	20	30	40	45	50
Treadmill speed (m/min)	25	26	27	28	29	30
Inclination of the treadmill (%)	15	15	15	15	15	15

Table 3. One-way Variance Analysis Test Results

Variables	Sum of Squares	Degrees of Freedom	Mean Square	F Value	Significance
RG in adipose tissue				1820.48	$P \leq 0.001$
Between groups	5.248	3	1.749		
Intergroup	0.035	36	0.001		
Total	5.282	39			

Table 4. Bonferroni Supplementary Test Results Comparing Resistin Gene (RG) in Adipose Tissue of Different Groups

Research Groups	High-Fat Diet	High-Fat Diet and Exercise	High-Fat Diet and Cinnamon
High-fat diet and exercise	$P \leq 0.001$		
High-fat diet and cinnamon	$P \leq 0.001$	P = 0.99	
High-fat diet, exercise, and cinnamon	$P \leq 0.001$	$P \leq 0.001$	$P \leq 0.001$

Table 5. The *t*-test Results of Samples Comparing the Resistance Gene of the Adipose Tissue in Different Groups with the Control Group

Groups	Mean ± SD	Difference of Means	t Value	Degrees of Freedom	P-Value
High-fat diet	1.84 ± 0.027	0.274	21.38	9	$P \leq 0.001$
High-fat diet and exercise	0.34 ± 0.023	-0.301	30.99	9	$P \leq 0.00$
High-fat diet and cinnamon	2.14 ± 0.030	-0.305	28.03	9	$P \leq 0.001$
High-fat diet, exercise, and cinnamon	1.35 ± 0.025	-0.776	125.50	9	$P \leq 0.001$

lifestyle is associated with a high risk of increasing insulin resistance (9, 12). Obesity leads to an increase in the expression of inflammatory adipokines and a decrease in the expression of anti-inflammatory adipokines, and as a result, enhances the chronic inflammatory condition. Pathogenesis of obesity-related complications can result from adipose tissue dysfunction, which in turn leads to irregular release of these adipokines (13). In addition, a high-calorie, high-fat diet can cause pro-inflammatory conditions in fat tissue. Such dietary changes are also effective in the secretion of adipokines, such as resistin, and the function of target tissues.

On the other hand, following a proper and balanced diet along with physical activities and proper and sufficient sports training has an important role in controlling and reducing body fat mass and improving the level of health and well-being (6, 7, 14). Studies have shown that the release of resistin as a result of exercise is influenced by the reduction of weight indicators, fat percentage, and pro-inflammatory cytokines such as IL-6, IL-1, and TNFα because these cytokines stimulate the expression of resistin in blood mononuclear cells. Studies have shown that physical activities and exercise play a role in controlling inflammation-related diseases such as diabetes and obesity by reducing the release of pro-inflammatory cytokines and creating an anti-inflammatory environment. Some of the anti-inflammatory properties of exercise may be associated with the modulation of adipokines produced by adipose tissue. In addition, long-term sports activities reduce the synthesis of atherogenic adipokines. Changes in the synthesis of these biomaterials as a result of exercise may play an important role in controlling cardiovascular diseases caused by obesity and insulin resistance (1). However, contrary to the current research findings, Tofighi et al. (cited in Irandoost et al.) (1) showed that 12 weeks of combined exercise did not have a significant effect on resistance levels and glycemic profile in postmenopausal women with type 2 diabetes. Haghighi et al. (cited in Irandoost et al.) also showed that aerobic exercise did not affect the serum resistance of obese men. The difference in the results of this research with the above findings may be due to the difference in the type of subjects and the type of exercises (1).

Additional studies related to resistin have shown that the increased expression of RG in the liver of mice causes insulin resistance, and in mice lacking this gene, fasting glucose decreases. This protein, through interference in the insulin signaling pathway, inhibits the action of insulin in the liver of mice, which is why it is an important molecular link between obesity and insulin resistance (1, 9, 11).

Another result of this present study was the reduction of RG expression in the CE group. Cinnamon, as an herbal anti-obesity agent, is effective in the control of obesity and the reduction of body fat mass (15). Cinnamon is one of the oldest medicinal plants in Iran's traditional medicine. It has a hot and dry nature and has been used in curing many health issues and diseases. Due to its polyphenols, cinnamon has useful insulin-like effects in controlling blood sugar and fats and can regulate appetite (16, 17). In addition, by increasing the body's metabolism, it can cause the breakdown of fats and excess energy consumption of fat tissue. Cinnamon, due to its high antioxidant activity, flavonoids, anticancer effects, antimicrobial and bacterial effects, and its composition of 65 to 80% cinnamic aldehyde and 5 to 10% eugenol, has anti-inflammatory effects and protects against myocardial ischemia and cardiovascular diseases. Additionally, it has inhibitory effects on nitric oxide production (10). Pharmacological and toxicological studies do not show any particular risk for the consumption of cinnamon in humans. However, there are reports of the swelling of hepatocytes, the increase in the thickness of the lining of the stomach, and the development of nephritis after long-term consumption of cinnamon, which is attributed to its essential oil (15, 17).

Diet is one of the factors affecting resistance changes. So, dietary restrictions cause a decrease in the expression of the resistin messenger gene. Studies have shown that CE can affect resistin by activating the PPARγ pathway and fat tissue metabolism. Increasing PPARγ activity improves inflammation and reduces resistin expression (1). On the other hand, studies have shown a positive relationship between resistin levels and atherogenic lipid profiles in people with metabolic syndrome. It seems that reducing the lipid profile as a result of cinnamon consumption can also reduce resistance. Nevertheless, Soleimani et al. (2013) showed that eating 200 mg of CE per kilogram of body weight caused a difference in the results. In line with the findings of the present research, Rashidlamir et al. (cited in Irandoost et al.) (1) showed that cinnamon, along with aerobic exercise, can be beneficial in improving glucose and blood lipids in diabetic patients. In general, more research is needed to understand better the effects of aerobic exercise and CE on adipokines, especially resistin (1). The results of the present study showed that aerobic exercise and consumption of cinnamon may reduce the expression of RG in mice fed with a high-fat diet. Improvement of insulin action and reduction of insulin resistance is mediated through the reduction of adipokines such as resistin. The combination of aerobic exercises and cinnamon consumption can be considered a preventive approach to improving insulin resistance and reducing resistin production.

Footnotes

Authors' Contribution: Study concept and design: N. N and T. B.; acquisition of data: M. M.; analysis and interpretation of data: M. M and N. N.; drafting of the manuscript: T. B.; critical revision of the manuscript for important intellectual content: N. N, T. B, and M. M.; statistical analysis: M. M.; administrative, technical, and material support: T. B.; study supervision: N. N.

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