

The Effect of Resistance Training with Genistein on Interleukin-6 and C-Reactive Protein in Induced Diabetic Rats

Saeed Ghodsbin¹, Sirous Farsi^{1*}, Seyed Ali Hosseini²

1. Department of Sport Physiology, Islamic Azad University– Larestan Branch, Larestan, Iran.

2. Department of Sport Physiology, Islamic Azad University– Marvdasht Branch, Marvdasht, Iran.

*Correspondence:

Sirous Farsi, Department of Sport Physiology, Islamic Azad University– Larestan Branch, Larestan, Iran.

Tel: (98) 917 924 3431

Email: sirous.farsi@gmail.com

Received: 11 January 2019

Accepted: 21 June 2019

Published in August 2019

Abstract

Objective: The aim of this study was to investigate the effect of Resistance Training (RT) with Genistein on Interleukin-6 (IL-6) and C-reactive protein (CRP) in diabetic rats.

Materials and Methods: 64 induced diabetic rats into 8 groups were investigated with experimental study: first week control, last week control, RT, 15 mg/kg Genistein consumption, resistance exercise with 15 mg/kg Genistein consumption, 30 mg/kg Genistein consumption, resistance exercise with 30 mg/kg Genistein consumption and placebo (dimethyl sulfoxide). Rats in groups 3, 5 and 7 performed 8 weeks of RT with 30 to 100% of body weight in 3 weekly sessions, and groups 4, 5, 6, and 7 received Genistein peritoneally with the mentioned doses. Also, to investigate the effects of diabetes induction on the study variables, 16 healthy rats were selected and divided into two groups: healthy first week control and healthy last week control. The data were analyzed using independent sample T-tests, two-way and one-way ANOVA with Bonferroni's post-hoc test in SPSS software (P -value \leq 0.05).

Results: RT had a significant effect on CRP reduction in induced diabetic rats (P -value \leq 0.05). Genistein consumption decreased IL-6 and CRP in diabetic rats (P -value \leq 0.05). Moreover, the interaction between RT and Genistein consumption had statistically significant effects on the reduction of IL-6 and CRP in diabetic rats (P -value \leq 0.05); 15 mg/kg Genistein consumption had a greater effect on the reduction of CRP than 30 mg/kg Genistein consumption in induced diabetic rats (P -value \leq 0.05).

Conclusion: RT alone could not significantly reduce the levels of IL-6 in induced diabetic rats; however, it seems that Genistein consumption with RT have interactive effects on reducing inflammatory factors in induced diabetic rats.

Keywords: Training, Genistein, Interleukin- 6, C- reactive protein, Diabetes

Introduction

Diabetes is a metabolic disorder characterized by symptoms such as chronic hyperglycemia and impaired metabolism of glucose, fat, and protein. These

disorders are caused by impaired function or insulin secretion. In 2013, 347 million people in the world were involved with the disease, and it is expected to be one of the main causes

of death in 2030 (1). Diabetes mellitus is permanently associated with decreased strength in skeletal muscle and increased levels of blood lipids and inflammatory markers. The adipose tissue acts as an active endocrine gland, and its increase in diabetic patients can destroy adipokines, adiponectin, leptin, and retinol bound protein- 4 (RBP-4); in addition, the disturbance in glucose homeostasis, causes inflammatory responses (2). The process of inflammation in diabetic patients is associated with elevated levels of acute phase proteins including CRP, fibrinogen, and pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α) (3). Increased levels of these inflammatory factors are associated with the progression of diabetes and atherosclerosis, which increases insulin resistance and ineffectiveness of endothelial cells (4,5). On the other hand, sports activities have been introduced as a non-pharmacologic method for treating diabetes mellitus (5,6). Increased glucose consumption as well as hypertrophy due to muscle contractions has been shown to be effective in treating a number of chronic diseases such as diabetes (7). Moderating effects of regular physical activity, and in particular resistance training (RT) on the immune system, may have positive results on the innate immune system, and thus, in addition to improving the strength and functional abilities of diabetic patients, it may have other benefits as well (8). However, the response of systemic inflammation to CRP and IL-6 following exercise in diabetic patients is contradictory (4,5). In this regard, a study showed that three weekly sessions of aerobic and combined (aerobic and resistance) training for eight weeks had a significant effect on reducing insulin and glucose resistance, but did not change IL-6 in patients with type 2 diabetes (4); four weeks, three sessions per week, of RT on rodents' ladder had a significant effect on the reduction of serum CRP levels in diabetic rats, but had no effect on the reduction of IL- 6 levels in them (9); on the other hand, three weekly 60-

minute-sessions of moderate and high intensity exercise for eight weeks led to a significant decrease in serum CRP levels in diabetic rats. Also, there was no significant difference between the two exercise intensities in reducing CRP in this study (5); six 60-minute-weekly sessions of yoga for three months had a significant effect on IL- 6 and CRP reduction in men (10); 12 months of low- intensity aerobic training resulted in IL- 6 and CRP reduction in elderly men and women (11). Researchers suggest that using proper diet along with exercise can help reduce glucose fluctuations in short term, and it can reduce the long-term complications of type 1 diabetes by controlling the amount of glucose and lipid in blood (12). Literature on dietary supplements and medicinal plants used in traditional medicine suggests that, in addition to lowering blood lipids, their existing compounds including nutrient fibers, vitamins, Flavonoids, sterols and other antioxidant compounds, can play a role in the inhibition of oxidation and free oxygen radicals removal, and hence enhance complications of diabetes mellitus by affecting the immune system and improving metabolic disorders in the body (13). Recent studies have examined Genistein and its effects on diabetes. Genistein is a major isoflavone in soybean which its protective role in blood vessels and heart and its effectiveness on the function and secretion of insulin have been shown (14). In this regard, researchers have shown that the use of Genistein for four weeks with 5, 10, and 20 mg/kg body weight of rats in a day led to a reduction in glucose and insulin levels in diabetic rats (15). The use of Genistein has a significant effect on the reduction of inflammatory factors such as IL-6, TNF- α and nuclear factor kappa light chain enhancer of activated B cells (Nf- κ B) in cell culture (16); consumption of Genistein for 8 weeks with 1 mg/kg of body weight resulted in decreased expression of Nf- κ B and IL-1 β in diabetic rats (17). The use of Genistein improves the inflammatory activity and regulates the expression of IL- 6, TNF- α in human stem cells. Genistein significantly

increases nitric oxide and decreases interleukin- 6 (19); consuming 30 mg/kg of body weight Genistein for eight weeks in rats had a significant effect on the improvement of lipid profiles of diabetic rats (20). Considering the above points and the lack of information on concurrent effects of using Genistein and RT on inflammatory factors in diabetic patients, the necessity of conducting the present study in order to find suitable nutrients and herbal products with the most anti-inflammatory effects on diabetic patients was strongly felt. Therefore, the present study was carried out to investigate the effect of RT and Genistein injection on IL- 6 and CRP in diabetic rats.

Materials and Methods

In this experimental study, 80 adult male Sprague Dawley rats from animal breeding center in the house of animals at Islamic Azad University–Marvdasht Branch were purchased and transferred to the animals retention room of the sport physiology laboratory. During the study, rats underwent a seven-day adaptation period under standard conditions at the ambient temperature of $22 \pm 2^\circ\text{C}$ and controlled light (12-hour light and dark cycle), with free access to water and food and relative humidity of 55- 60 %. On day 8, after a fasting night, 64 rats were intraperitoneally injected with a single dose of 60 mg/ kg streptozotocin (manufactured by Sigma Company) dissolved in citrate buffer. Four days later, using punching method, blood samples were taken from the tails to measure the glucose levels by glucometer (21). The rats with blood glucose greater than 300 mg/ dl were included in the study. The onset of the training program and the use of Genistein took place a week after induction of diabetes and maintenance of the rats. Blood glucose-induced diabetic rats were randomly assigned to 8 groups of 8, including (1) first week control, (2) last week control, (3) RT, (4) 15mg/ kg Genistein, (5) RT with 15 mg/ kg Genistein, (6) 30mg/ kg Genistein, (7) RT with 30mg/ kg Genistein and (8) placebo (dimethyl sulfoxide). Note that the

Genistein used in this study was produced by Chinese Hangzhou Dingyan Cem Co., Ltd. with Batch No 20151105.

In order to investigate the effects of diabetes induction on the research variables, 16 healthy rats were selected and divided into two groups: healthy first week control group and healthy last week control group. At first, first week healthy control and diabetic control groups were sacrificed by euthanasia method and blood samples were taken. Then, the rats in groups 3, 5 and 7 performed 8 weeks of RT at intensity of 30 to 100% of body weight during 3 weekly sessions (12). Groups 4 and 5 received daily 15 mg/kg peritoneal Genistein for 8 weeks and groups 6 and 7 received daily 30 mg/ kg peritoneal Genistein for eight weeks (22). After this period, blood samples were taken from the rest of the healthy and diabetic rats to measure the studied variables. To measure IL- 6 serum levels, the German-made zellbio was used; also, measurement of CRP was done using DIACOLON commercial kit manufactured in France.

To familiarize the rats with RT and how to climb the ladder, each rat was placed on the lowest ladder staircase and trained to climb the ladder without connecting the weights and by placing their hind legs on the stairs. To force the rats to move on the ladder while standing on a stair and preventing a pause, they were conditioned by touching their tails and making a sound, simultaneously. The introduction program proceeded by climbing the ladder every day for one week and in each session, three to four repetitions were performed without connecting weight. The RT protocol consisted of eight weeks of climbing a ladder to the height of one meter, with four centimeters between each staircase and a vertical slope. Before beginning of the training program in each session and in order to warm up the rats, they were forced to climb the ladder three times without connecting weight or rest between repetitions. The weight selected at the first phase of training was 30% of rats' body weight which was increased up to 100% by the last week. Based on the

training protocol, weights were connected to the tip of rats' tails by using a leucoplast adhesive (before training the vulnerability of the rats' tails to this type of adhesive was examined). The rats performed two repetitions with each attached weight. Then a new weight was added to their tails. The training loads, including 50, 75, 90 and 100 percent, were the highest weights that the rats managed to raise from the ladder. In the last session of weekly training, after training program of the relevant session and rats' resting, the maximum weight that rats were able to raise was determined. For this purpose, weights were added to the last repetition weight, and it continued until the rats were not able to lift the weight anymore (12). After eight weeks of training and supplementation, rats were anesthetized with ketamine and xylazin to measure research variables and then blood samples were directly taken from the left ventricle of them. All the ethical and legal aspects of this research were reviewed and approved by the Islamic Azad University of Marvdasht Branch (IR.MIAU.REC.1396.123). The findings of this study were analyzed using paired sample T- test, one-way ANOVA test, two-way ANOVA test along with Bonferroni's post hoc test in SPSS version 19 (P -value \leq 0.05).

Results

In Table 1 paired sample T-test showed that IL-6 ($t = -17.58$; P -value= 0.001) and CRP ($t = -6.29$; P -value= 0.001) in the diabetic first week control group were significantly higher than healthy first week control group. The results of one-way analysis of variance showed a significant difference in rats' levels

of IL-6 in 15 mg/kg Genistein consumption, 30 mg/kg Genistein consumption, control and sham groups ($F = 86.99$; P -value= 0.001). In addition, results of Tukey's post hoc test showed that IL-6 levels did not differ significantly in last week control group and sham group ($M = -15.58$; P -value= 0.32). Levels of IL-6 in the sham group were significantly lower than 15 mg/kg Genistein consumption ($M = -64.94$; P -value= 0.001) and 30 mg/kg Genistein consumption ($M = -71.06$; P -value= 0.001) groups. Also, there was no significant difference in the levels of IL-6 in 15 mg/kg Genistein consumption and 30 mg/kg Genistein consumption groups ($M = 6.12$; P -value= 0.86).

In Table 2, results of two-way ANOVA showed that RT ($F = 1.04$; P -value= 0.31; and effect size= 0.02) had no significant effect on decreasing IL-6 levels of diabetic rats, but Genistein consumption ($F = 18.61$; P -value= 0.001; and effect size= 0.47) resulted in significant decrease of IL-6 in diabetic rats. RT and Genistein consumption have interactive effects in reducing IL-6 in diabetic rats ($F = 8.38$; P -value= 0.001; and effect size = 0.28). In Table 3, based on the results of Bonferroni's post hoc test, using 30 mg/kg Genistein (P -value= 0.001) and 15 mg/kg Genistein (P -value= 0.001) significantly decreased IL-6 compared to the groups which did not receive Genistein. Nevertheless, consuming 15 and 30 mg/kg doses of Genistein had the same effect on the reduction of IL-6 levels in diabetic rats (P -value= 0.44). The results of one-way analysis of variance in 15 mg/kg Genistein, 30 mg/kg Genistein, control and sham groups showed a significant

Table 1. Mean and standard deviation of variables in research groups

Group	IL-6 (ng/L)	CRP (mg/L)
Control (first week)	72.11± 76.33	0.31± 0.13
Control (last week)	19.69± 11.77	0.0± 05.35
Diabetic (first week)	9.163± 11.16	0.0± 13.73
Diabetic (last week)	25.145± 96.56	0.0± 12.76
RT	24.110± 29.50	0.0± 09.39
Resistance with 30 mg/ kg Genistein consumption	11.94± 80.46	0.0± 08.34
30 mg/kg Genistein consumption	11.88± 53.07	0.0± 07.38
sham	12.159± 79.14	0.0± 13.64
Resistance with 15 mg/ kg Genistein consumption	16.107± 76.00	0.0± 09.24
15 mg/ kg Genistein consumption	9.94± 35.20	0.0± 12.30

difference between CRP levels in rats ($F=31.96$; P -value= 0.001). Moreover, the results of Tukey's post hoc test showed that CRP levels did not differ significantly in last week control group and sham group ($M= 0.15$; P -value= 0.60). CRP levels of sham group were significantly lower than 15 mg/ kg Genistein (P -value= 0.001) and 30 mg/ kg Genistein (P -value= 0.001) groups; there was no significant difference between levels of CRP in 15 mg/ kg Genistein and 30 mg/ kg Genistein (P -value= 0.47) groups.

The results of two-way ANOVA indicated that eight weeks of RT ($F= 29.15$; P -value= 0.001; effect size= 0.41) and Genistein supplementation ($F= 37.62$; P -value= 0.001; effect size= 0.64) had a significant effect on CRP reduction in diabetic rats. RT and Genistein consumption have interactive effects on reducing CRP in diabetic rats ($F= 13.94$; P -value= 0.001; effect size= 0.39). In Table 4 results of Bonferroni's test showed that consumption of 30 mg/ kg Genistein (P -value= 0.001) and 15 mg/ kg Genistein (P -value= 0.001) had a significant effect on the reduction of CRP in diabetic rats; in addition,

compared to 30 mg/ kg Genistein, the consumption of 15 mg/ kg Genistein had a greater effect on reducing levels of CRP in diabetic rats (P -value= 0.04).

Discussion

Increased blood glucose is a common disorder that affects a significant percentage of people in our country. This disorder may be due to decreased levels of insulin or cellular receptor deficiency. Based on the results, induction of diabetes increased serum levels of IL-6 and CRP in rats. CRP serum levels were reported above normal healthy subjects in diabetic patients (23,24). Considering the limitation of research on human specimens, it seems necessary to use animal models to better investigate this disease. One of the diabetic chemicals used in laboratory studies on animal models, such as rats, is streptozotocin (STZ). STZ degrades the membrane of pancreatic cells, splits DNA and reacts with enzymes such as glucokinase, which can increase blood glucose levels in animals. STZ increases the expression of glucose- 6 hepatic phosphatase enzyme mRNA and, thereby, increases blood

Table 2. Results of two-way ANOVA test to examine the levels of research variables

Factor	Variable	Sum of Square	df	F	P-value	Effect Size
IL-6	Genistein Consumption	11867.71	2	18.61	0.001	0.47
	RT	334.23	1	1.04	0.31	0.02
	Genistein Consumption with RT	5345.81	2	8.38	0.001	0.28
CRP	Genistein Consumption	0.77	2	37.62	0.001	0.64
	RT	0.30	1	29.15	0.001	0.41
	Genistein Consumption with RT	0.28	2	13.94	0.001	0.39

Table 3. Results of Bonferroni's post-hoc test to compare the effect of Genistein consumption with different doses on IL- 6 serum levels of streptozotocin-induced diabetic rats

Factor	No Genistein Consumption	15 mg/kg Genistein Consumption
30 mg/kg Genistein Consumption	$M= -36.76^*$, P -value= 0.001	$M= -9.32$, P -value= 0.44
15 mg/kg Genistein Consumption	$M= -27.43^*$, P -value= 0.001	

* results of Bonferroni's post hoc test, using 30 mg/kg Genistein (P -value= 0.001) and 15 mg/ kg Genistein (P -value= 0.001) significantly decreased IL- 6 compared to the groups which did not receive Genistein.

Table 4. Results of Bonferroni's post-hoc test to compare the effect of different doses of Genistein on serum CRP levels of streptozotocin-induced diabetic rats

Factor	No Genistein Consumption	15 mg/kg Genistein Consumption
30 mg/kg Genistein Consumption	$M= -0.21^*$, P -value= 0.001	$M= 0.09$, P -value= 0.04
15 mg/kg Genistein Consumption	$M= -0.30^*$, P -value= 0.001	

* results of Bonferroni's test showed that consumption of 30 mg/ kg Genistein (P -value= 0.001) and 15 mg/ kg Genistein (P -value= 0.001) had a significant effect on the reduction of CRP in diabetic rats.

* compared to 30 mg/kg Genistein, the consumption of 15 mg/ kg Genistein had a greater effect on reducing levels of CRP in diabetic rats (P -value= 0.04).

glucose (25). Studies have shown that increased STZ-induced hyperglycemia causes an increase in reactive oxygen species (ROS). Following ROS increase in cells, the disturbed balance between antioxidants and ROS contributes to progression of diabetes. Increasing oxidative stress activates the signal cascade of inflammation and increases IL-6 and TNF- α (26). In line with results of the present study, injection of 55 mg/kg STZ had a significant effect on increased levels of TNF- α , CRP and blood glucose in rats (9); induction of diabetes with a dose of 60 mg/kg of STZ increased inflammatory factors in rats (27,28). Reasons for the alignment of these studies with the present study can be attributed to using the same dosages of STZ. However, inconsistent with the present study, induction of diabetes by 55 mg/kg of STZ had no significant effect on elevated levels of IL-6 in rats (9); possible reasons for this inconsistency can be attributed to levels of measurement and different measurement methods of the two studies.

The results showed that RT had a significant effect on reduced levels of serum CRP in diabetic rats, but the effect of RT on IL-6 reduction in diabetic rats was not significant. Along with the present study, three weekly sessions of high intensity interval training for six weeks did not significantly decrease IL-6 in overweight women with type 2 diabetes (29). Three weekly sessions of aerobic training and combined training for eight weeks with 60-80% intensity of maximum heart rate had no significant effect on the reduction of IL-6 in type 2 diabetic patients (4). Reasons for the similarity of these studies with the present study can be attributed to diabetic subjects in both studies. Researcher stated that RT with different intensities reduced the levels of CRP and hs-CRP in young men (30), elderly men (31) and diabetic and non-diabetic rats (9). Moreover, combined trainings (aerobic and resistance) had a significant effect on the reduction of CRP and no change in levels of IL-6 in inactive men (32) and healthy elderly women (33). Eight weeks, three sessions a

week, of endurance training had a significant effect on reducing CRP levels in diabetic rats (5); similarity of the statistical population and the induction of diabetes by the same methods can be explained as the reasons for the compatibility of these studies with the present study.

Eight weeks, three sessions per week, of endurance training with 60-75% intensity of maximal heart rate significantly reduced the CRP of middle-aged women with diabetes (34); reasons for the consistency of this study with the present study can be the initial levels of variables in both studies.

On the other hand, in contrast with the present study, researchers have stated that 18 months of walking exercise combined with weight lifting had no significant effect on the CRP of elderly obese male patients (35). The reason for the inconsistency of this study with this study is the difference between type and intensity of exercises and statistical population. The beneficial effects of long-term RT on health and improvement of metabolism in diabetic patients are well known. RT improves the complications of diabetes by increasing levels of insulin sensitivity to facilitate glucose uptake by muscle cells. It is worth mentioning that the difference in results may be due to the type of training, the difference in the statistical population and the primary levels of CRP. Since reduction in CRP levels can be due to the potential effect of exercising on modulation of inflammation from diabetes, hormonal changes occur in response to physical activity and exercise that increase the concentration of several hormones, including cortisol, growth and epinephrine. It appears that IL-6 released from muscles is responsible for increasing cortisol secretion in sports activities. IL-6 stimulates cortisol secretion with an effect on the hypothalamus and adrenal glands. Cortisol and epinephrine suppress the production of inflammatory cytokines. RTs lead to decreased amount of stored body fat by increasing the stimulation of protein synthesis and muscle mass. Following that, the extinction of

inflammatory cytokines gene in muscle tissue and reduction of leukocyte adhesion molecules serum levels by inhibiting the reaction of monocytes and endothelial cells, ultimately lead to less inflammation (31). In addition, exercise activity can reduce CRP levels in the blood circulation by directly reducing the production of cytokines in the adipose tissue, muscle and mononuclear cells, and by indirectly increasing insulin sensitivity and improving endothelial function. However, the mechanism for the role of RT on reducing inflammation is not well defined. Studies have shown that increased caloric intake can lead to lower levels of CRP in several ways, including weight loss (4). Researchers also believe that lack of significant changes in IL-6 levels following RT can be explained as exercise training is likely to increase pro-inflammatory cytokines such as vaspin, which play an inhibitory role in reactive oxygen species (ROS) and inflammatory agents in smooth muscle and vascular cells; this mechanism is associated with the prevention of phosphorylation of Nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and the protein C θ kinase. Increasing blood glucose during exercise results in oxidative stress, which in turn results in activation of NF- κ B and thus an increase in levels of pro-inflammatory cytokines in blood circulation (36).

Genistein consumption has a significant effect on the reduction of IL-6 and CRP in diabetic rats. Cell damage in diabetes and development of insulin resistance are closely related to the presence of oxidative stress in the cell. Oxidative stress is likely to directly increase the risk of diabetes by reducing insulin sensitivity and destruction of insulin-producing cells and degradation of pancreatic cells. Increasingly active oxidative stress can also contribute to systemic inflammation and increased levels of pro-inflammatory cytokines such as IL-6, TNF- α and IL-1 β . Inhibition of insulin receptor downstream signaling cascade is a primary mechanism through which inflammation produces insulin

resistance (37). Most researchers believe that elevated cholesterol, neutrophil accumulation and hypoxia in the cell will result in production of inactive IL-1 β form, and inactive IL-1 β with interfering caspase-1 will change into an active form of IL-1 β , resulting in the production of IL-6 as well as CRP (38). Nevertheless, Genistein isoflavone via cAMP/PKA increases the production of NOS and NO from non-genomic pathways. cAMP is the central molecule in many messaging pathways and plays an important role in maintaining the vascular function. cAMP/PKA pathway activity results in endothelial nitric oxide phosphorylation, resulting in its activity, and consequently in NO production. In addition, the activation of the cAMP/PKA pathway also inhibits vascular inflammation by suppressing the adhesion of leukocytes to endothelial cells (39). Genistein also increases the mass of pancreatic beta cells and serum insulin levels in diabetic patients through protein diet-induced weight loss, and subsequently facilitates the transfer of glucose into the cell. Researchers also believe that following weight loss and decreased fat mass, reduction of pro-inflammatory factors from signaling pathway inhibition of NF- κ B and IL-1 β takes place. Genistein leads to a decrease in the production of TNF- α , IL-6 and CRP by inhibiting the inhibitory protein of I κ B α , increasing phosphorylation of IKK (an enzyme which inhibits the I κ B protein and phosphorylation of IL-1 β receptors (9,17,37,40).

In line with decreasing the inflammatory factors following the use of Genistein, researchers reported that taking one mg of Genistein per kg of body weight for eight weeks had a significant effect on the reduction of Nf- κ B and IL-1 β and a significant increase in SIRT1 in ovariectomized rats and ovariectomized and high fatty foods and STZ-induced diabetic rats (17). Daily injection of 30 mg per kg of body weight Genistein for eight weeks had a significant effect on the improvement of fat profile in STZ induced diabetic rats, thus reducing low density lipoproteins and low density lipoprotein and

cholesterol can lead to decreased inflammation (20). On the other hand, inconsistent with the results of the present study, consumption of 14 grams of soy protein per day for eight weeks did not have a significant effect on serum TNF- α changes in peritoneal dialysis patients (41). Possible reasons for this inconsistency can be seen in the manner of application and dose of Genistein and different statistical population. Also, the use of Genistein at 50 mg/kg of body weight per day for two weeks did not have a significant effect on TNF- α reduction in doxorubin-induced nephropathy rats (42). Possible reasons for the inconsistency of this study with the current study could be the different doses of two studies and different time periods, which was eight weeks in the current study but two-weeks in the referred study. Six months of using daily doses of Genistein at a rate of 30-54 mg/ dl had no significant effect on reducing CRP levels in women with menopause (43); among the reasons for inconsistency with results of this study, we can point to different statistical populations as well as CRP basal levels.

The results of present study showed that RT with Genistein have interactive effects in reducing serum IL-6 and CRP levels in rats. Most researchers have examined the effects of Genistein and exercise on inflammatory factors separately. In this regard, no study was found to investigate the effect of simultaneous application of Genistein and RT on IL-6 and CRP in diabetic patients. The results of studies have shown that both Genistein and exercise lead to reducing inflammation in diabetic patients by decreasing mass and consequently reducing pro-inflammatory factors from the signal pathway of I κ B α protein inhibition, increasing IKK phosphorylation and decreasing the expression of Nf-kB, as well as phosphorylation of IL-1 β receptors, (9,17,37,44,45). In confirmation of the findings of this study, Osali et al. (2016) concluded that endurance training with moderate intensity and 30 mg/ kg injection of Genistein had an interactive effect on improving fat profile in STZ induced diabetic

rats (20). Eight weeks, three sessions per week, of endurance training with moderate intensity and daily consumption of 100 grams of soy nuts had an interactive effect on reducing the mean systolic blood pressure and systolic blood pressure in obese menopausal women (46). On the other hand, inconsistent with the results of the present study, 30 sessions of combined training (aerobic and resistance) and 100 mg of isoflavone per day had no interactive effects on the reduction of inflammatory factors (IL-6 and IL-8) in postmenopausal women (47). The reasons for the inconsistency of this study may be the difference in the statistical population and the initial levels of inflammatory factors measurement. One of the limitations of the present study was the lack of control over conflicts among rats and their damage during non-training hours that could affect the changes in inflammatory factors in diabetic rats. Considering the lack of control of the calorie intake among the research groups and the importance of this point during exercise, it is suggested that in future studies, the effects of endurance exercise be studied along with controlling the accurate amount of food consumed to control the amount of calories received and consumed in diabetic rats. In addition, regarding the effect of fat mass and its relation with cytokines, the lack of fat mass measurement and its relation with levels of research variables were among other limitations of this research. Therefore, it is suggested that in the future studies different intensities of RT and their effects on fat mass as well as their correlation with inflammatory factors in diabetic rats be investigated and the results be compared with this study.

Conclusions

Regarding the findings of this study, it can be generally concluded that although RT alone could not significantly reduce levels of IL-6 in diabetic rats, RT with Genistein consumption had interactive effects on reducing serum levels of IL-6 and CRP in rats.

Acknowledgments

Considering the fact that this study is the result of the doctoral dissertation of Mr. Saeed Ghodsbin, approved by Islamic Azad University, Larestan Branch, we thank and

appreciate the spiritual assistance of the research deputy of this university unit.

Funding

The study costs have been provided by the authors and there was no external funding.

References

- Costa Melo L, Dativo-Medeiros J, Menezes-Silva CE, Barbosa FT, de Sousa-Rodrigues CF, Luiza A. Physical Exercise on Inflammatory Markers in Type 2 Diabetes Patients: A Systematic Review of Randomized Controlled Trials. *Oxidative Medicine and Cellular Longevity*. 2017;10.
- Annibalini G, Lucertini F, Agostini D, Vallorani L, Gioacchini A, Barbieri E, et al. Concurrent Aerobic and RT Has Anti-Inflammatory Effects and Increases Both Plasma and Leukocyte Levels of IGF-1 in Late Middle-Aged Type 2 Diabetic Patients. *Oxidative Medicine and Cellular Longevity*. 2017;3937842.
- Koloverou E, Panagiotakos DB, Georgousopoulou EN, Chrysohoou C, Tousoulis D, Stefanadis C, et al. ATTICA Study Group. Single and combined effects of inflammatory markers on 10 year diabetes incidence: The mediating role of adiposity-Results from the ATTICA cohort study. *Diabetes Metab Res Rev*. 2018;34(1):25.
- Yousefipoor P, Tadibi V, Behpoor N, Parnow A, Delbari E, Rashidi S. The Effect of 8-week Aerobic and Concurrent (aerobic- resistance) Exercise Training on Serum IL-6 Levels and Insulin Resistance in Type 2 Diabetic Patients. *SSU_Journals*. 2013;21(5):619-31.
- Farsi S, Azarbayjani MA, Hosseini S, Pirei P. Response of Serum Levels of ICAM-1 VCAM-1 and CRP to High and Moderate Endurance Trainings in Sprague Dawley Male Diabetic Rats. *Armaghane danesh*. 2016;21(8):757-71.
- Salehi OR, Hoseini A. The Effects of Endurance Trainings on Serum BDNF and Insulin Levels in Streptozotocin-Induced Diabetic Rats. *Shefaye Khatam*. 2017;5(2):52-61.
- Irvine C, Taylor NF. Progressive resistance exercise improves glycaemic control in people with type 2 diabetes mellitus: a systematic review. *Australian Journal of Physiotherapy*. 2009;55:237-46.
- Li ZZ, Liu JB, Li L, Jiao L, Chen L. Intensive therapy for diabetes through influence on innate immune system. *Medical Hypotheses*. 2009;72:675-6.
- Safarzade AR, Gharakhanlou R, Hedayati M, Talebi-Garakani E. The Effect of 4 Weeks RT on Serum Vaspin, IL-6, CRP and TNF-A Concentrations in Diabetic Rats. *Iranian Journal of Endocrinology and Metabolism*. 2012;14(1):68-74.
- Shete SU, Verma A, Kulkarni DD, Bhogal RS. Effect of yoga training on inflammatory cytokines and C-reactive protein in employees of small-scale industries. *Journal of education and health promotion*. 2017;6:76
- Nicklas BJ, Hsu FC, Brinkley TJ, Church T, Goodpaster BH, Kritchevsky SB, et al. Exercise training and plasma C-reactive protein and interleukin-6 in elderly people. *Journal of the American Geriatrics Society*. 2008;56(11):2045-52.
- Hosseini S, Nikbakht H, Azarbayjani M. The effect of aqua extract of saffron with RT on glycemic indexes of streptozotocin induced diabetic rats. *Armaghane danesh*. 2013;18(4):284-94.
- Fallah Huseini H, Alavian S, Toliati T, Jamshidi A, Heshmat R, Naghdi Badi H, et al. The efficacy of herbal medicine Khar Maryam (*Silybum marianum* (L.) Gaertn.) on liver cirrhosis in chronic hepatitis B patients. *Journal of Medicinal Plants*. 2005;1(1):1-6
- Elizabeth RG, dongmin I. Anti-diabetic functions of soy isoflavone genistein: mechanisms underlying effects on pancreatic β -cell function. *Food & function*. 2013; 4(2):200-12.
- El-Kordya, et al. Effect of genistein, a natural soy isoflavone, on pancreatic β -cells of streptozotocin-induced diabetic rats: Histological and immunohistochemical study. *Journal of Microscopy and Ultrastructure*, 2015;3:108-19.
- Ji G, Zhang Y, Yang Q, Cheng S, Hao J, Zhao X, et al. Genistein suppresses LPS-induced inflammatory response through inhibiting NF- κ B following AMP kinase activation in RAW 264.7 macrophages. *PLoS One*. 2012;7(12):53101.
- Yousefi H, Alihemmati A, Karimi P, Alipour MR, Habibi P, Ahmadiasl N. Effect of genistein on expression of pancreatic SIRT1, inflammatory cytokines and histological changes in ovariectomized diabetic rat. *Iranian journal of basic medical sciences*. 2017;20(4):423.
- Choi JH, Chung MJ, Jeong DY, Oh DH. Immunostimulatory activity of isoflavone-glycosides and ethanol extract from a fermented soybean product in human primary immune cells. *Journal of medicinal food*. 2014;17(10):1113-21.

19. Choi EY, Bae SH, Ha MH, Choe SH, Hyeon JY, Choi JI, et al. Genistein suppresses Prevotella intermedia lipopolysaccharide-induced inflammatory response in macrophages and attenuates alveolar bone loss in ligature-induced periodontitis. *Archives of oral biology*. 2016;62:70-9.
20. Hashemi HS, Hosseini SA. The effect of moderate intensity endurance training and lipid lowering genistein in Streptozotocin induced diabetic rats. *Journal of Shahrekord Uuniversity of Medical Sciences*. 2017;19(1):10-23. (in Persian)
21. Zar A, Hoseini A, Ahmadi F, Rezaei M. Effects of ginger together with swimming training on blood fat profiles in adult diabetic rats with streptozotocin. *Iranian Journal of Nutrition Sciences & Food Technology*. 2016;11(2):65-74.
22. Sosić-Jurjević B, Filipović B, Ajdžanović V, Brkić D, Ristić N, Stojanoski MM, et al. Subcutaneously administrated genistein and daidzein decrease serum cholesterol and increase triglyceride levels in male middle-aged rats. *Experimental biology and medicine* (Maywood, NJ). 2007;232(9):1222-7.
23. Hu FB, Meigs JB, Li TY, Rifai N, Manson JE. Inflammatory markers and risk of developing type 2 diabetes in women. *Diabetes Journal*. 2004;53:693-700.
24. Freeman DJ, Norrie J, Caslake MJ, Gaw A, Ford I, Lowe GD. West of Scotland C-reactive Protein is an Independent Predictor of Risk for the Development of Diabetes in the West of Scotland Coronary Prevention Study. *Diabetes Journal*. 2002;51:1596-600.
25. Amini A, Parto P, Yousofvand N. The effect of induced diabetes and its treatment with zinc sulfate & vanadium on reproductive system in rat. *The Journal of Urmia University of Medical Sciences*. 2016;27(6):476-85.
26. S Samarghandian S, Borji A, Farkhondeh T. Attenuation of oxidative stress and inflammation by *Portulaca oleracea* in streptozotocin-induced diabetic rats. *Journal of evidence-based complementary & alternative medicine*. 2017;22(4):562-6.
27. Kazemi F, Zahedi Asl S. Atni-inflammation effect of 8-week aerobic training on apelin plasma concentration in diabetic male rats. *Iranian Journal of Diabetes and Metabolism*. 2017;16(2):85-93. (in Persian)
28. Kim JS, Lee YH, Kim JC, Ko YH, Yoon CS, Yi HK. Effect of exercise training of different intensities on anti-inflammatory reaction in streptozotocin-induced diabetic rats. *Biology of sport*. 2014;31(1):73.
29. Nemati J, Ghodrat L, Hojjati F. Inflammatory effect of severe periodic exercises in overweight women and type 2 diabetes. *J Physiol Exe Phys Act*. 2017;10:79-88. (in Persian)
30. Sheikholeslami VD, Ahmadi S, Mojtahedi H, Marandi M, Ahmadi DK, Faraji H, et al. Influence of different intensities of resistance exercise on inflammatory markers in young healthy men. *Iranian Journal of Endocrinology And Metabolism*. 2011;12(6):618-25.
31. Taghian F, Ghatreh Samani K. Dose 12 week RT Influence IL-18 and CRP levels in Elderly men?. *Razi Journal of Medical Sciences*. 2018;24(165):85-92.
32. Abedi B. The effects of 12-wk combined aerobic/RT on C-reactive protein (CRP) serum and interleukin-6 (IL-6) plasma in sedentary men. 2012;14 (4):95-106.
33. Mardanpour Shahrekordi Z, Banitalebi E, Faramarzi M. The Effect of RT on levels of interleukine-6 and high-sensitivity C - reactive protein in older-aged women. *Elderly Health Journal*. 2017;3(1):35-41.
34. Kakhka H, Nasrabadi R, Nuraeinjar M, Effect of Twelve Weeks Aerobic Training on Selected Molecules 1, TNF- α , CRP, ICAM-1, VCAM-1 Type 2 Diabetes in Middle-aged Women. *Journal of Scientific Research and Development*. 2015;2(1):154-7.
35. Nicklas BJ, Ambrosius W, Messier SP, Miller GD, Pennix B, Losser RF. Exercise and chronic inflammation in older, obese adults: a randomized controlled clinical trial. *The American Journal of Clinical Nutrition*. 2004;79:554-1.
36. Hedayati M, Safarzade A, Gharakhanlou R, Talebi-Garakani E, The effect of 4 weeks RT on serum vaspin, Il-6, CRP and TNF-A concentrations in diabetic rats. *Iranian Journal of Endocrinology and Metabolism*. 2012;14(1):68-74.
37. Mohammad Sadeghi H, Mansourabadi A, Rezvani M, Esmaeeli Dahaj M, Shams A. A Review of the Impact of Flavonoids on Diabetes. *Journal of Diabetes Nursing*. 2015;2 (4):45-59.
38. Ridker PM. From C- reactive protein to interleukin-6 to interleukin-1. *Circulation Research*. 2016;118:145-56.
39. Sahafian M, Farzanegi P, Abbaszadeh H, Vizvari E. Effect of caffeine and genistein supplement on cardiac levels of nitric oxide and vascular endothelial growth factor in wistar rats, following an acute swimming exercise. *Pathobiology Research*. 2018;21(1):23-8.
40. Azamian Jazi A, Maghsoudi A, Emadi S. Effect of four weeks of endurance exercise training on TNF- α and IL-10 genes expression following experimental MI in male rats. 2018;13(26):237-48.
41. Imani H, Tabibi H, Atabak S, Rahmani L, Hedayati M, Neyestani T et al. Effects of soy consumption on serum lipid and apoprotein levels in peritoneal

- dialysis patients. *Iranian Journal of Nutrition Sciences & Food Technology*. 2007;2(3):33-44.
42. Yousefinejad A, Siassi F, Javanbakht MH, Mohammadi H, Ghaedi E, Zarei M, et al. Effect of genistein and L-carnitine and their combination on lipid profile and inflammatory cytokines in experimental nephrotic syndrome. *Reports of biochemistry & molecular biology*. 2018;7(1):1-8.
43. D'Anna R, Baviera G, Corrado F, Cancellieri F, Crisafulli A, Squadrito F. The effect of the phytoestrogen genistein and hormone replacement therapy on homocysteine and C-reactive protein level in postmenopausal women. *Acta Obstet Gynecol Scand*. 2005;84(5):474-7.
44. Osali A, Choobineh S, Soori R, Ravasi AA, Mostafavi H. The effect of three-month aerobic exercise with moderate intensity on IL1 β , IL-6, and brain volume in 50-65 years old women with metabolic syndrome. *yafte*. 2017;19(2):60-71.
45. Salimi Avansar M. The effects of eight weeks interval training and curcumin consumption on TNF- α and BDNF levels in men with metabolic syndrome. *Journal of Ardabil University of Medical Sciences*. 2017;17(3):299-310. (in Persian)
46. Zarneshan A. Synergic effect of aerobic exercise training (moderate intensity) and soya intake on blood pressure & rest heart rate in obese postmenopausal women. *Hormozgan Medical Journal*. 2014;18(1):52-60. (in Persian)
47. Giolo J, Costa J, da Cunha-Junior J, Pajuaba A, Taketomi E, de Souza A, et al. The effects of isoflavone supplementation plus combined exercise on lipid levels, and inflammatory and oxidative stress markers in postmenopausal women. *Nutrients*. 2018;10(4):424.