ORIGINAL ARTICLE

The Effects of Endurance Training with Genistein on ICAM-1, VCAM-1, and CRP of Diabetic Rats

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Abstract

Objective: The purpose of this study was to investigate the effects of endurance training accompany with taking genistein on Intercellular Adhesion Molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and C-reactive protein (CRP) levels in diabetic rats.

Materials and Methods: Sixty-four rats were divided into eight groups (1; healthy &sacrificed at the first week, 2; healthy &sacrificed at the last week, 3; diabetic &sacrificed at the first week, 4; diabetic &sacrificed at the last week, 5; endurance training, 6; taking genistein, 7; endurance training with taking genistein, and 8; placebo). The training groups were participated in three week sessionsof running on animal treadmill with span of 60 min and intensity range from 17 to 28 (m/s), during eight weeks. In addition, the genistein groups took daily amounts of 30 (mg/kg), through Intraperitoneal injection. In order to analyze data, one-way ANOVA and Toki post-hoc test were implemented ($P \leq 0.05$).

Results: The results showed that induction of diabetes would lead to increases in ICAM-1, VACAm-1, and CRP levels, among the rats. Though, all the three of endurance training, taking genistein and endurance training with taking genistein would cause significant reductions in the increased levels of ICAM-1 and CRP ($P<0.05$). Nevertheless, those mentioned three could not significantly decrease the increased level of VCAM-1, in the statistical aspect ($P>0.05$). Moreover, there was no significant difference between the effects of the three endurance training, taking genistein, and endurance training with taking genistein ($P>0.05$).

Conclusion: Eight weeks endurance training and also taking genistein do own improving influences on Inflammatory and cardiovascular risk factors among diabetic rats. Though, their combination does not have any synergistic effect.

Keywords: Endurance training, Genistein, ICAM-1, VCAM-1, CRP, Diabetes.

Introduction

Diabetes is one of the most prevalent chronic and non-communicable diseases in developing and developed countries. Due to the inappropriate diet and lifestyle diabetes prevalence is rising around the world. The evolution of diet and life style from traditional to industrial led to increase the prevalence of diabetes (1). One hundred and

forty-seven millions diabetic patients were estimated around the world in 2001 and will reach to three hundred and twenty millions people in 2035 (2).The increased level of glucose has toxic effects on veins and capillaries through time (3). C-reactive protein (CRP) is the acute phase protein which is released from the liver in response to various injuries such as surgery, tissue damage, inflammation and exercise. CRP shows the systematic inflammation (4). CRP is recognized as the most sensitive inflammatory index of cardiovascular diseases (CVD) risk prediction. Increase of CRP level would lead to increase in CVD risk two to five times (5). In addition, CRP was mentioned as an effective index to predict type-2 diabetes (T2DM) (6). CRP variation levels may affect insulin sensitivity and glucose metabolism, either directly or indirectly. CRP levels of the people with high fat density would rise and has a reverse relation to insulin sensitivity (7). In the other hand, the inter-cellular adhesive molecules 1 (ICAM-1) and vascular cell adhesive molecules 1 (VCAM-1) are the most sensitive cellular indicators for recognition of the process of atherosclerotic plaque formation in the vascular endothelial wall. There are growing evidences that indicate ICAM-1lead to vascular endothelial dysfunction.ICAM-1 could be used for prediction of CVD (8). Serum levels of ICAM-1 would rise with increase of body fat (9). In addition, a significant positive correlation between blood glucose level and ICAM-1 was reported in diabetic patients (10). ICAM-1 blood level rise in diabetic patients with nephropathy, retinopathy, neuropathy and microalbuminuria (11-14). There are different findings about aerobic trainings effects on serum levels of adhesive molecules and CRP (as authentic indices for evaluation of general inflammation). As, some studies showed exercise would lead to either considerable decrease (15-20) or invariance (21,22) serum levels of adhesive molecules and CRP. Hence, the researchers cannot achieve to any certain conclusion, about variations of those inflammatory indices-induced exercise, in order to predict cardiovascular diseases, among diabetic patients, yet.

In the other hand, various herbs were used to reduce blood glucose level and correct the diabetes effects, in Persian traditional medicine and those of other countries. In the recent years, herbal medications were prescribed all around the world, due to their minor adverse side effects, convenient availability, comparative inexpensive price, and effectiveness (23).

Genistein is a major is of lavone in soybeans. The cardiovascular protection role of genistein was approved. It can reduce low density lipoprotein (LDL) induced VCAM-1 in cells and vessels of the vascular endothelial of human (24). Affirmative effects of genistein against cardiovascular disorders are appreciated through improvement in glucose homeostasis, decrease in oxidative stress and reduce in diabetes-induced endothelial dysfunction among Zucker Diabetic Fatty (ZDF) Rats. Therefore, genistein can be booked as a virtual option for prevention of CVD (24). However, its mechanism was not investigated. In addition, genistein stimulates and activates insulin excretion in beta pancreatic cells through a cAMP-dependent protein kinase pathway, severely. There are incongruous results about effects of soybeans genistein on diabetic persons in the recent years. The present study was accomplished to investigate the co-effects of endurance trainings and genistein consumption on ICAM-1, VCAM-1 and CRP among diabetic rats.

Materials and Methods

Seventy male Sprague–Dawley rats were purchased from animal breeding center of the animal house of Islamic Azad University (Marvdasht branch). All of the mice were transferred to animal maintaining chamber of exercise physiology laboratory (environment temperature of 22±2 °C and controlled illumination of 12 hrs light/12 hrs dark), and underwent the eight days adaptation period.

The mice had free access to food and water during the adaptation period.

In the eighth day and after one night fasting, 54 rats were chloroformed and undergone an intraperitoneal injection of 60 (mg/kg) Streptozotocin (STZ; made in Sigma Co.) dissolved in citrate buffer. In order to gauge blood glucose by glucometer device, blood samples were taken from tails of the rats, through the punching method. Forty-eight rats which has glucose levels of higher than 300 (ml/dl) were qualified to enter the study.

In addition, 16 healthy rats were selected and entered the research. Hence, six rats were eliminated from the study. Based on the blood glucose levels, those selected 48 diabetic rats and other 16 healthy ones (64 mice totally) were divided to eight groups: (A) healthy/ sacrificed at first week, (B) healthy/ sacrificed at 8th week, (C) diabetic/ sacrificed at first week, (D) diabetic/ sacrificed at 8th week, (E) endurance training, (F) genistein, (G) endurance training with genistein, and (H) placebo. The sixteen healthy rats were separately selected and divided to A and B groups, to investigate induction effects of diabetes on variations amounts of ICAM-1, VCAM-1 and CRP. At first, mice of the two groups of A and C were euthanized to collect blood samples after 16 hours fasting. Then, mice of the two groups of E and G were participated in eight weeks endurance trainings schedule. In addition, the two groups of F and G received 30 (mg/kg) daily consumption of genistein, through intra-peritoneal injection. Thereafter, in order to measure values of the under study variables, blood samples were taken from both healthy and diabetic rats. The animals were maintained in 16 hours fasting situation before blood sampling. All ethical and legal aspects the present study were checked and approved by Islamic Azad University (Marvdasht branch).

Initially, the mice were put on the treadmill with speed of 8 (m/min), slope of 0°,and span of 10 min to make them familiar with the protocol of endurance trainings. Those running speed has no influence on aerobic power enhancement and physiologic parameters of the mice. A very weak electrical shock was embedded at the path ending of the animal treadmill to force the mice to continue the running. In order to avoid probable damages from electrical shock the rats were conditionally trained to run by either knocking the treadmill or hearing a low sound or touching their tails. The training protocol of the present study consisted of eight weeks increasing running sessions (Three weekly sessions) on the animal treadmill with no slope (0°), speed of 17 to 28 (m/min), and session span of 60 min (23). At the beginning of each session, the mice were put on the treadmill and made to run with speed of 8 (m/min) and span of 10 min for warming up purpose. Then, the training schedule was executed. At the end of each session training schedule speed of the device was gradually and reversely reduced to zero, to perform cooling down. The cooling down of the sessions had lasted 5 to 7 min. In order study the effects of the independent variables on probable changes of dependent ones, the whole selected mice were properly euthanized, in regard to a designated program.

Anesthetizing was carried by ketamine and Tylosin and blood sampling was directly from the left ventricular (LV).Five cc blood samples were directly taken form the LV by using syringe. In order to measure the levels of ICAM-1, VCAm-1 and CRP the ELISA kits (Zell Bio GmbH of Germany) were implemented.

The collected data were analyzed by using statistical tests of Kolmogorov-Smirnov, two-way ANOVA and Toki-post hoc, in significance level of 0.05, utilizing SPSS.

Results

The relevant results of rats weight of the eight groups were presented in table 1. Descriptive statistics were presented in tables 2.

There were significant differences between values of VCAM-1, ICAm-1 and CRP-1 of the eight groups ($P \leq 0.05$). Toki Post-hoc test was implemented to observe fashion of those significant differences. The results indicated

that diabetes would cause a significant increase in level of VCAM-1 (P=0.001). Although the endurance trainings, genistein consumption and their composition would lead to decreases in VCAM-1, but those reductions were not significant (P was 0.08, 0.053, and 0.12, respectively). The results showed that diabetes would lead to significant increase in level of ICAM-1 (P=0.001). Though, endurance training, genistein consumption and their composition would lead to significant decreases in levels of ICAM-1 (P was 0.03, 0.001, and 0.001, respectively). In addition, the results of the present study have shown diabetes would cause a significant increase in CRP level (P<0.05). However, endurance trainings, genistein consumption and their composition would lead to significant decreases in CRP (P was 0.001, for the three posture). Also, there was not observed any significant difference between the values of VCAM-1, ICAM-1, and CRP of the three groups of endurance trainings, genistein consumption, and endurance trainings/genistein (P>0.05).

Discussion

The results of the present study indicated that diabetes induction would cause significant increases in ICAM-1 and VCAM-1in rats. In agreement with the present results, it was reported ICAM-1 would rise with blood glucose level increase in diabetic patients (28). In addition, of adhesive molecules levels would rise, in patients with diabetic glomerular hyperfiltration (11), diabetic retinopathy (12), diabetic neuropathy (13), and diabetic microalbuminuria (14). Although diabetes would cause significant increases in levels of ICAM-1 and VCAM-1 in rats, but the eight weeks endurance trainings would lead to decreases in levels of ICAM-1 (a significant decrease) and VCAM-1 of diabetic rats. Perhaps, further intervention might also cause a significant decrease in VCAM-1. That declaration requires more future studies, to be approved. It appears regular exercise conduces to reduction in sympathetic stimulation and enhancement of anti-inflammatory cytokines and consequently leads to restrain inflammatory mediators from lipid tissue. Therefore, that restraint would lead to decrease concentrations of ICAM-1 and VCAM-1 (29). The antioxidative effect of aerobic training may be another mechanism in reduction of ICAM-1 and VCAM-1 inflammatory indices. Oxygen free radicals would cause increases in the appearance of inflammatory mediators and ICAM-1 (30). However, most of studies denoted reductive effects of exercise on such molecules. For instance, it was stated six week high intensity interval training (three weekly

Table 1. Rats' weights of the eight groups

Group	Pre-test weight (gr)	Post-weight weight
Healthy/sacrificed at 1st week	176.12±19.63	Killed at 1st week
Healthy/sacrificed at 8th week	215.00±34.64	223±37.61
Diabetic/sacrificed at 1st week	187.25±32.53	Killed at 1st week
Diabetic/sacrificed at 8th week	204.12±52.62	187.21±12.14
Endurance training	187.21±12.14	194.12±18.13
Genistein	166.37±27.08	159.37±45.34
Endurance training with genistein	176.06±11.65	181.63±18.15
Placebo	192.75±24.77	201.62±44.61

Table 2. Mean and standard deviation of principal the variables

Group	VCAM-1	ICAM-1	CRP
Healthy/sacrificed at 1st week	87.32±18.98	1.77±0.68	0.053±0.045
Healthy/sacrificed at 8th week	100.51±16.92	1.69±0.60	0.039±0.006
Diabetic/sacrificed at 1st week	172.72±59.28	5.32±1.98	0.21±0.07
Diabetic/sacrificed at 8th week	184.46±40.75	6.16±1.45	0.41±0.19
Endurance training	130.15±28.21	4.18±1.31	0.033±0.015
Genistein	126.85±27.34	3.12±0.52	0.024±0.011
Endurance training with genistein	133.31±33.88	3.24±0.90	0.019±0.006
Placebo	176.47±48.62	4.49±1.27	0.313±0.101

sessions, in the fashion of 4 to 6 repetition and maximum speed of greater than 20 m, and then 20 to 30 sec active rest) would lead to a significant decrease in ICAM-1in overweight young girls (31). It has been declared by another study that twelve weeks endurance training (three weekly sessions, with intensity of 50 to 75% HRR Max) would cause significant reductions of ICAM-1 in 50 to 70 year old elderly men (32). Another research (similar to the present study) declared twelve weeks endurance training (three weekly 60 min sessions, with intensity of 55 to 85% VO_2 Max) would lead to a significant reduction of ICAM-1 in rats (33). One other research that has studied diabetic patients, has stated eight weeks aerobic training (three weekly 45 to 60 min sessions, with intensity of 60% HRR Max) would lead to significant decreases in levels of adhesive molecules in patients with type 2 diabetes (20). In addition, it was stated in a research that eight weeks aerobic training (three 15 to 50 min weekly sessions, with intensity of 55 to 85% VO_2 Max) would cause significant reductions in levels of adhesive molecules. Nevertheless, those levels returned to the basic ones, following four weeks without training (34). Also, six weeks physical training (in the fashion of three week rehabilitation and then three weeks at home controlled training) would lead to significant decreases in levels of ICAM-1 and VCAM-1, among patients with coronary artery disease (35). And, twelve weeks aerobic training (three weekly sessions, with intensity of 60 to75% HR Max) would cause significant reductions in levels of ICAM-1 and VCAM-1, among elderly women with diabetes (15). Moreover, four weeks high intensity trainings with maximal oxygen uptake would lead to significant decreases in plasma ICAM-1 and VCAM-1in men with T2DM (17). Despite the previous mentioned researches, in some other studies that exercise does not influence on ICAM-1 and VCAM-1or exercise affects levels of ICAM-1, further than those of VCAM-1, which has been confirmed by the results of the present study. In this regard, although eight weeks aerobic trainings (three weekly sessions, with intensity of 80% HRR Max) would cause a significant decrease in ICAM-1, but it did not lead to any significant reduction of VCAM-1 (36). In addition, although six month moderate intensity endurance training on treadmill (three weekly sessions) did own a significant reduction in ICAM-1, but those trainings did not make any significant decrease in VCAM-1, among 40 to 65 year old men with diabetes (16). Hejazi et al (2013) have reported a similar statement (37). In the other hand, it has been reported that eight weeks aerobic training (three weekly sessions, with intensity of 65 to 70% VO_2 Max) did not have any significant influence on adhesive molecules, among middle-aged patients with type 2 diabetes (21). Also, another research reported eight weeks elementary swimming training (four 50 min weekly sessions, with intensity of 60 to 75% VO_2 Max) did not own any significant effect on ICAM-1, in postmenopausal women with high blood pressure (38). In addition, eight weeks aerobic training (five weekly running on treadmill sessions, with spans of 40 to 50 min) did not have any significant effect on VCAM-1 levels of overweight middle-aged women (22). Nonbeing reduction of adhesive molecules, which was stated in the recent mentioned studies, is in contrary to the present results, which has denoted the reduction in blood adhesion, due to exercise, and its influence on decrease in levels of ICAM-1. Reduction of shear stress would lead to decrease in the level of ICAM-1 and consequently cause its release from the endothelial cells lining blood vessels, according to the evidences (39). Therefore, low intensity trainings of those studies have not caused any reduction in serum levels of ICAM-1, through hematocrit changes. In regard of incongruous reports, the means of exercise effects on adhesive molecules is far from certainty. However, it can be stated that OxLDL would increase TNF-α (producer of VCAM-1), and glycated LDL would increase TNF-α (producer of VCAM-1) by 35

percentage. Those founds indicate that oxidation of fatty acid and oxidized phospholipid components of OxLDL would increase the ability of endothelial cell for occurrence of ICAM-1 and VCAM-1, through the cytokines. Regular endurance trainings accompany with decrease in level of blood fat and glucose would reduce cardiovascular diseases risk. Moreover, endurance training accompany with weight reduction, sympathetic stimulation, and enhancement of anti-inflammatory cytokines would lead to release the pre-inflammatory cytokines (TNF-α, & IL-1β) from the lipid tissue, and consequently decreases in levels of adhesive molecules (20).

In addition, results of the present study have indicated diabetes induction would led to significant increments in serum levels of CRP, among rats. Nevertheless, he eight weeks endurance training of the present work would cause significant decreases in serum levels of CRP, among rats. Various studies have confirm the statement of the present research about the importance of CRP values examination, to predict diabetes risk. For example, it has been reported in a research that serum levels of 737 diabetic patient were higher than those of 785 healthy persons (40). Also, a prospective study with five year follow-up and 5245 participants compared 127 diabetic patients to the other subjects and has indicated CRP of middle-aged men is the prediction parameter of diabetes (7). It has been shown exercise could lead to reduce in rest levels of CRP. However, long and intense exercise might cause a remarkable rise of CRP (greater than 20 times) (41). There are different results in the literature. For instance, twelve weeks endurance trainings (three weekly sessions, with intensity of 60 to 75% HR Max) would lead to significant decreases in levels of CRP, among middle-aged women with diabetes (15). And, six weeks physical trainings (three week rehabilitation & three weeks controlled training at home) would cause significant reductions of CRP levels, in patients with coronary artery diseases (35). Also, four weeks resistance training (five weekly sessions) would conduce to a significant decrease in serum level of CRP, in diabetic rats (18). In addition, twelve weeks physical trainings (three weekly sessions) would cause significant decreases in CRP levels, among middle-aged women (42). Effects of aerobic trainings have been compared to those of flexibility-resistance trainings, and it has been declared three weekly aerobic training session (during 10 months & each session with span of 45 min) could reduce CRP, significantly. Though, those flexibility-resistance trainings would not reach any significant result (43). In contrast, eighteen months compound trainings of walking accompany with weight training did not effect on CRP of elderly obese man, significantly (44). The appeared differences between the mentioned results could be originated from varieties in either types of subjects, initial levels of CRP, training protocols, or the amount of consuming calories. Nevertheless, reduces in levels of CRP could come from the potential influence of exercise on moderation of diabetes-induced inflammation. But, exercise can make decreases in CRP levels of the systemic circulation, both directly through reduction of cytokine production within lipid tissue, muscle, and mononuclear cells, or indirectly through enhancement of insulin sensitivity and improvement in endothelial performance. However, influence mechanism of endurance training on inflammation reduction has not been recognized, well. Literature have indicated that enhancement in consuming calories, via various ways such as weight cutting, could lead to decrease in CRP levels. Perhaps, endurance training of the present study might lead to a significant decrease in CRP level of the rats, via the weight cutting mechanism, accordingly.

In the other hand, the present results have indicated that the eight weeks genistein consumption did not have any significant influence on reduction of VCAM-1, among diabetic mice. Though that consumption would

cause decreases in ICAM-1 and CRP, in diabetic rats. Since the reduction magnitude of VCAM-1 (P=0.053) was near to the significant level (0.05), if either the dose of genistein or the duration of the schedule was more, the magnitude of VCAM-1 reduction would become significant. However, due to the uncertainty about effect of genistein on VCAM-1 level, that issue must be paid, in future studies. Genistein is the second primary ingredient of soybeans, after diadzein (45). Genistein is recognized as a formidable material for the purpose of diabetes treatment and prevention from diabetes, to aid patients with insulin resistance and diabetes (45). Several studies, which have carried out on animal models, shown genistein do possess anti-diabetic influences. It has been reported, 600 (ml/kg rat weight) daily dose of genistein nutritional supplement for diabetes-induced rats (via streptozotocin (STZ)) and in duration of three weeks, would lead to remarkable reduction in fasting blood glucose level and increments in insulin plasma levels (45). Heng-Song Tian et al (2015) investigated probable cardioprotective effects of genistein and its potential herbal mechanism, and have concluded twelve weeks genistein consumption would decrease diabetes-induced heart disorder and pathologic changes. Actually, those reductions would accomplish through improvements in glucose tolerance and insulin resistance, facilitation of (ATK) activity and glucose consumption amount, and decrease in intensity of oxidative stress. Also, they would be achieved through the related MAP kinase pathways and NF-κB signaling pathway. Moreover, it has been stated that genistein can reduce mRNA transcription of ICAM-1 and VCAM-1, in ZDF mice, significantly (24). In fact, it has been reported genistein might probably cause decreases in levels of ICAM-1 and VCAm-1, through activation of Nrf2/HO-1 pathway (46). Generally, few researches have adverted to the composition of endurance training and genistein. Maybe, the present study is the first one that has carried out that issue. Therefore, further future studies are required, to achieve more reliable results.

Conclusion

Diabetes increases the serum levels of ICAM-1, VCAM-1 and CRP in rats. Though, eight weeks endurance training, genistein consumption and their composition would lead to reduce in the increased levels of ICAM-1, VCAM-1 and CRP. The decreases in levels of ICAM-1 and CRP were significant. Perhaps, the insignificant reduction of VCAM-1 might be originated from insufficient duration of the intervention. However, there were no significant differences between correspondent results of endurance training, genistein consumption and their composition. Therefore, it can be concluded that eight weeks endurance trainings as well as genistein consumption are effective on the improvement of inflammatory and cardiovascular risk factors, in diabetic rats. But, their composition does not possess any synergistic effect.

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Conflict of Interest

There is no conflict of interest.

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