ORGINAL ARTICLE

The Effects of Resistance Training on VCAM-1, ICAM-1 and CRP in Diabetic Rats

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Abstract

Objective: Many diabetic patients are susceptible to cardiovascular diseases which are known as one of the most important causes of mortality among diabetic patients. The aim of this study was to investigate the effects of eight weeks of resistance training on VCAM-1, ICAM-1 and CRP in diabetic rats.

Materials and Methods: In this experimental study, 24 male Sprague- Dawley diabetic rats were selected and divided into three groups; (1) diabetes victim first week, (2) diabetes victim last week, and (3) resistance training. In order to investigate the effects of induction of diabetes, 16 healthy male rats were divided into two groups of healthy victim first week and healthy victim last week. The resistance training group had progressively participated in eight weeks (3 weekly sessions) resistance training, which has included climbing up in a specified ladder for rats, based on the average weekly weight from 30% of body weight for the first week to 100% of the body weight for the last one. Kolmogorov-Smirnov test, one-way ANOVA and Tukey's post hoc test (P-value≤ 0.05) were used to analyze the results.

Results: The results showed that induction of diabetes significantly increases of VCAM-1 (P-value: 0.004), ICAM-1 (P-value: 0.001) and CRP (P-value: 0.02) in rats. The eight weeks of resistance training significantly decreases the serum levels of ICAM-1 (P-value: 0.005) and CRP (P-value: 0.001). However, the eight weeks of resistance training did not significantly reduce serum level of VCAM-1 (P-value: 0.51) in diabetic rats.

Conclusion: Resistance training has beneficial effects on the reduction of ICAM-1 and CRP levels, though an insignificant decrease in the levels of VCAM-1.

Keywords: ICAM-1, VCAM-1, CRP, Resistance training, Diabetes mellitus, Rats

Introduction

Diabetes is a type of metabolic disease that is addressed as high level of blood glucose and may access due to either insufficient insulin production by pancreatic cells or inappropriate response of cells to insulin (1). Diabetes may cause some problems such as Neuropathy, Retinopathy, Nephropathy, and cardiovascular diseases that affect both patients and society (2). Moreover, diabetes has been recognized as the fourth

major factor of mortality in most developed countries (3). Diabetic patients are susceptible to cardiovascular diseases, which are counted as the most paramount reason of mortality among those patients (4).

The insulin resistance and endothelial dysfunction would accelerate atherosclerosis process in patients with type 2 diabetes. Although the causes of atherosclerosis and cardiovascular diseases are complex in diabetic patients, but endothelial dysfunction is recognized as the major cause in progress and development of atherosclerosis and cardiovascular diseases (5).

The high levels of glucose have harmful effect on minor and major veins. Hence, appropriate control of diabetes may delay mortality (6). C-reactive protein (CRP) is the dominant protein of acute phase and releases from the liver in response to various injuries like surgery, tissue damage, inflammation and physical activity. CRP is the indicator of systematic inflammation affection. CRP is recognized as the most sensitive predictive index for risk of cardiovascular hazards independently (6). Inflammation is known as the origin of most cardiac diseases. Vascular stimulations and their resulting damages are because of either directly consumption of some substance or indirectly increase of shear stress on tissues. Those events cause the increase in the sensitivity of endothelial adherent molecules of vessels including vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1), selectins and integrin.

Increase of adhesion molecules would lead to invasion of monocytes to endothelial of the vessels and worsen permeability and activation of plackets. The sedimentation of fibrous tissue would be developed with migration of smooth muscle cells from walls of the vessels and atheromatous plaques would expand, consequently (7).

In contrast, exercise is a non-pharmaceutical strong strategy against diabetes and its side-effects. A regular exercise schedule may improve insulin resistance status, whereas physical inactivity will lead to decrease of insulin resistance. Body weight, level of blood glucose and insulin resistance would be reduced by aerobic exercises (7). The relative studies showed different results about the effects of exercise on serum levels on adhesion molecules and CRP in diabetes mellitus. For instance, endurance training with moderate and high intensities during eight weeks (3 weekly sessions about 60 min) would lead to decrease of ICAM-1, VCAM-1, and CRP serum levels in diabetic rats (6). Moreover, aerobic training with 60% to 70% of maximum heart rate (MHR) during 12 weeks would cause significant decrease of ICAM-1, VCAM-1, and CRP serum levels in middle-aged women with type-2 diabetes (8). Eight weeks (3 weekly sessions) endurance training with 50% to 80% of MHR would not make any significant effect on serum levels of ICAM-1 among obese men (9). Furthermore, eight weeks (3 weekly sessions) aerobic training with 50% to 70% of MHR intensity would lead to decreases in serum levels of ICAM-1 but no change in serum levels of VCAM-1, among middle-aged women (10). The present study has been done for the purpose of investigation of ICAM-1, VCAM-1, and CRP responses to resistance training in diabetic rats.

Materials and Methods

Forty male Sprague-Dawley rats were purchased for this experimental survey. The rats were undertaken a consistency period (8 days) in a room with controlled temperature (22±2°C) and light (12 hrs light/ 12 hrs darkness). The animals had free access to food and water, during the consistency period. After a fasting night and at the eighth day, the 40 rats had intraperitoneal injection of 60 (mg/kg) streptozotocin (STZ) (made in Sigma Co.) dissolved in citrate buffer (adjust pH to 4.7). In order to gauge levels of blood glucose, samplings were performed from tails of the animals, four days after the injections. Twenty-four rats with blood glucose levels greater than 300 (md/dl) were selected as the subject of the study.

The training schedule started one week after diabetes induction. The diabetic rats were divided into three groups of 8 sera (1) diabetes victim first week, (2) diabetes victim last week, and (3) resistance training. In order to investigate the effects of induction of diabetes on variations levels of ICAM-1, VCAM-1, and CRP, 16 healthy male rats were divided into two groups of healthy victim first week and healthy victim last week. At the beginning and after 16 hours fasting, the two groups of victim first week were sacrificed and blood sampling was done. Following the one-week consistency period, rats of the training group were educated to climb the ladder by trainer. They were located on the lowest step of the ladder and taught to climb the ladder, without connecting any weight to them and placing their hind legs on the steps. Whenever the rats stop at any step of the ladder, they were enforced to continue climbing up through touching their tails (conditional animal). The introduction climbing up schedule lasted one week (one-day session/ one-day rest). Each introduction session included three to four repetitions. The training protocol lasted eight weeks (3 weekly sessions) for the resistance training group, which included sessions consisting 1meter ladder climbing up. The distance between each two successive steps of the ladder was 4 cm and the slope of the ladder was 90° (vertical). At the beginning of each training session, the mice were warmed up through three to five repetitions of climbing up without any weight connection. Connection weight was assigned as 30% of rat body weight at the first week of the training period and increased to 100% of the body weight at the eighth weeks of the training period. The connection weights were attached to the beginning of the tails. The mice were trained two repetitions for each weight connection, and new weights were connected to their tails, after that. The exercise weights consisted of 50, 75, 90, and 100 percentage of maximum weight that rats could pull up. The maximum weight was assigned at the last training session of each week, following the session and rest.

The last lifting weight of the session was increased. At the end of each training week, blood samples were collected form healthy, diabetic and diabetic training mice to measure variables of the study. The animals were undertaken 16 hours fating before each blood sampling (11). Measurements of ICAM-1, VCAM-1 and CRP levels were performed via Zellbio kits (made in Germany) by using Eliza method. Animal experimental procedures were in accordance with institutional guidelines and approved by the ethical committee of laboratory animals Care at Marvdasht Islamic Azad University, Marvdasht, Iran; IR.REC.1396.162. The results were described as mean and standard deviation (SD). Kolmogorov-Smirnov test was applied to investigate data distribution normality. In addition, one-way ANOVA and Tukey's post hoc tests were used to analyze the results. Significance level was assigned as P-value< 0.05.

Results

The results of paired sample T-test showed that weight levels in the post-test of heath victim first week group were significantly increased compared to the pre-test (t= -3.14, P-value= 0.01), but there was no significant decreases in pre-test and post-test in diabetes victim last week group (t= 2.08, P-value= 0.07), and resistance training group (t= 0.84, P-value= 0.43). The results of Tukey's post hoc test in indicated that the levels of ICAM-1 (Figure 1), VCAM-1 (Figure 2), and CRP (Figure 3) in the group of diabetes victim first week were significantly higher than those of the group of healthy victim first week. Accordingly, diabetes induction would lead to significant accelerations of ICAM-1 (P-value: 0.001), VCAM-1 (P-value: 0.004), and CRP (P-value: 0.02) in rats. Serum levels of ICAM-1 and CRP in the training group were lower than those of the group of diabetes victim last week. Therefore, eight weeks resistance training did significant effect on increase of
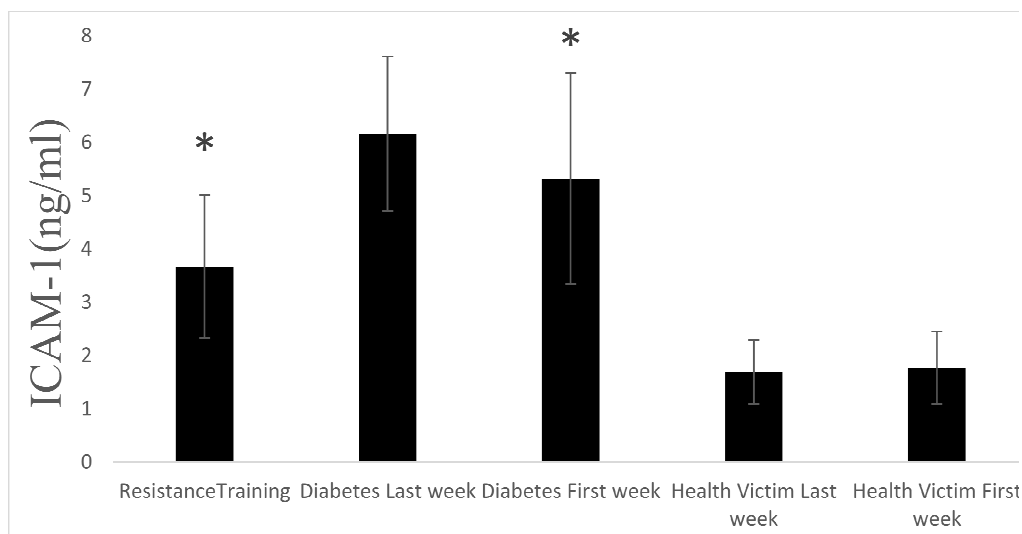
Figure 1. Levels of ICAM-1 in rats of the study groups.
* Serum levels of ICAM-1in group of diabetes victim first week were significantly higher than those of the group of healthy victim first week (P-value: 0.001).* Eight weeks' resistance training did significant effect on increase of ICAM-1 (P-value:0.005) of diabetic rats
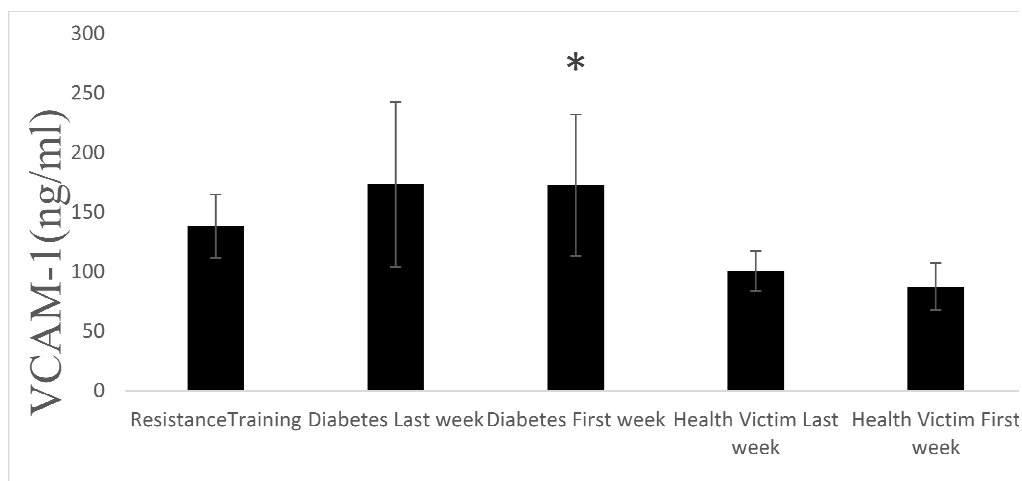

Figure 2. Levels of VCAM-1 in rats of the study groups.
* Serum levels of VCAM-1in group of diabetes victim first week were significantly higher than those of the group of healthy victim first week (P-value: 0.004).

ICAM-1 (P-value: 0.005) and CRP (P-value: 0.001) of diabetic rats. In the other hand, there was not observed any significant change in serum level of VCAM-1 in the training group than that of the diabetes victim last week one. Hence, eight weeks resistance trainings did not generate any significant effect for the sake of reduction of serum levels of VCAM-1 (P-value= 0.51) in diabetic rats.

Discussion

Almost the whole diabetic's cardiovascular risk factors including acceleration of blood pressure, obesity, disorder of blood lipids and insulin resistance. The accumulation of those mentioned factors is known as metabolic syndrome (4). Inflammation is recognized as an indirect hazard for arteriosclerosis, sudden death and diabetes (12). There are numerous

evidences that indicate cellular and vascular adhesion molecules have vital parts in the arteriosclerosis process. Attachment of blood cells to the arterial walls is one of the first incidents for recognition of arteriosclerosis process (13). The present results showed eight weeks resistance training would lead to decrease of serum levels of ICAM-1 in diabetic mice. ICAMs accelerate the formation of the foam cells through attaching to monocytes and moving them to the depth of the endothelium (13). In agreement with the present results, it has been reported ICAM-1 would increase with blood glucose in diabetics (6). Whereas diabetes induction might lead to increase of ICAM-1, VCAM-1, and CRP serum level in rats of the present study, though eight weeks resistance training would cause significant decreases of serum levels of ICAM-1 and CRP, in addition to an insignificant decrease of serum level of VCAM-1, in diabetic mice. Exercise may prevent the release of inflammatory mediators from lipid tissues via enhancement of anti-inflammatory cytokines. Indeed, exercise can reduce inflammatory indices though reinforcing antioxidant defense and reducing free radicals (6,7,13). The homeostasis response of the human body to exercise depends on intensity, duration and type of training schedule. There are few studies about effects of exercise on adhesion molecules level and their results were partly incongruous. Though, all of them mostly reported decreases in adhesion molecules following training schedules. Accordingly, Farsi et al (2016) reported a decrease in serum level of ICAM-1 in diabetic rats after eight weeks moderate and high intensities endurance training (6). Also, Kargarfard et al (2016) stated eight weeks endurance training and high intensity interval training would lead to ICAM-1 and VCAM-1decrease in obese men. While, only endurance training could cause a significant decrease of VCAM-1 in men with normal weights (14). In addition, Rosety et al (2016) reported significant decreases of levels of ICAM-1 and VCAM-1 following 12 weeks resistance training (3 weekly sessions) in elderly obese women (15). Abd El-Kader et al (2016) concluded three months treadmill endurance training would lead to significant decrease of ICAM-1, VCAM-1, and CRP in elderly obese women (16). Khademi et al (2016) reported a decrease of ICAM-1 gene expression within heart tissue of Wistar male rats, following 10 weeks (5 weekly sessions) high intensity interval training (90-95% of
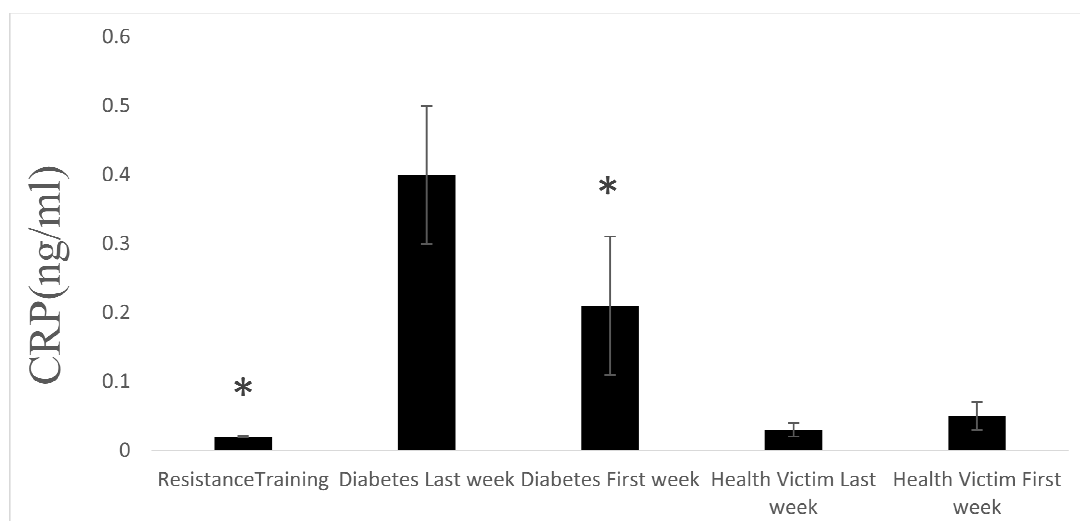

Figure 3. Levels of CRP in rats of the study groups.
* Serum levels of CRP in group of diabetes victim first week were significantly higher than those of the group of healthy victim first week (P-value: 0.02).* Eight weeks' resistance training did significant effect on increase of CRP (P-value: 0.001) of diabetic rats.

VO$_2$max) (17). In the other hand, Ryan et al (2014) stated that aerobic training accompanied by weight reduction would result in a significant decrease of CRP level and no significant changes of ICAM-1 and VCAM-1 in obese women (18). Moreover, Hejazi et al (2013) concluded that eight weeks (3 weekly sessions; 40 min each session) endurance training (75% of maximum HRR) would lead to significant decrease of ICAM-1 and E-SELECTIN in healthy middle-aged women. Though, changes in levels of VCAM-1 were not significant, among them (10). Exercise may adjust effective mechanisms of regulation of adhesion molecules, like renin–angiotensin system (RAS) (19), and decrease the release of chemical mediators and pro-inflammatory stimuli, such as the nuclear factor NF-κβ, for the sake of the decrement of vasculitis. The nuclear factor NF-κβ exists inactively within cytoplasm and instigates the beginning of endothelial activity via the mediators and ICAM-1 gene expression (20). Furthermore, angiotensin II increases the occurrence of ICAM-1 through stimulation of NADPH oxidase (19). Each of the above mentioned mechanisms can somewhat justify the variations of concentration of adhesion molecules in both training groups. In this regard, exercise may regulate endothelial activity and the body inflammation. Those regulations may promote through the improvement of antioxidant defense of endothelial, blood, and the restraint of nitric oxide degradation by active oxygen particles, on one hand, and the enhancement of production of nitric acid by endothelial cells, on the other hand. Consequently, Endothelium-derived relaxing factor EDRF, inhibition of platelet aggregation (IPA), control of adhesion of mediators to the vascular wall, endothelium activity, and inflammation of the body are regulated by exercise (21).

The present results indicated that the induction of diabetes would lead to a significant acceleration of CRP serum levels in rats. In contrast, the eight weeks resistance training would result in a significant decrease of CRP in diabetic rats. CRP is an acute phase reactant that is synthesized within the liver in response to IL-6 and may be effective in formation and progression of atherosclerotic plaque (22). Since the hs-CRP is an independent factor for the sake of prediction of the risk of cardiovascular diseases, it could be concluded the hs-CRP serum is solely counted as a risk indicator of cardiovascular incidents among diabetics, regardless of other risk factors (22). Accordingly, investigation of the effects of serum levels of CRP on prediction of diabetes has been noticed in various studies. For Instance, serum levels of CRP of 737 diabetics have been reported higher than those of 785 healthy people (23). Furthermore, a Futures study with five years follow up has been done and comparison of 127 diabetics to other participants (5245 people, overall) indicated that CRP is the predictor of diabetes in middle-aged men (24). Exercise may cause a decrease of the body fat mass. Hence, it has been thought that exercise may cause less production of inflammatory factors, in which produced by the fat tissue, through reducing fat mass and Less penetration of macrophages (25). In accordance with the present study, Rankovi et al (2009) showed six weeks home training (3 rehabilitation sessions and 3 controlled training weekly sessions) would lead to decrease of CRP serum levels in patients with coronary artery disease (26). Moreover, Safarzade et al (2012) showed four weeks (5 weekly sessions) resistance training would result in significant decreases of serum levels of CRP in diabetic rats (27). Ogawa et al (2010) stated twelve weeks (3 weekly sessions) training would lead to a significant decrease of CRP in middle-aged women (28). Hemmati Nafar et al (2014) showed six weeks high intensity interval training would cause accelerations of acute inflammatory factors (hs-CRP & fibrinogen) in inactive young men (29). Whereas, Abedi, et al (2012) did not report significant changes in level of CRP, following 12 weeks combination training (30). Incongruous results may be originated from

different training duration, diverse statistical society, and/or various methods of experimental measurements. Consequently, exercise may prevent aggravation of the disease though improvement of endothelial reconstruction capacity (via enhancing number and performance of the stem cells). After mobilizing the stem cells from bone marrow and migrating to the place of the damaged endothelium, the stem cells would be differentiate in circulating endothelial progenitor cells (EPCs) and aid in growth, repair of vessels and improvement of the endothelium function, as a result. IL-6 production, consequent CRP production (within the hepatic cells), and appearance of the adhesion molecules would be decreased simultaneously with raising the endothelium activity (31). Of course, intensity, duration, and type of exercise, musculoskeletal injuries, and relative stress and metabolic conditions have been considered as the reasons of those mentioned changes.

Conclusions

According to the results of the present study, eight weeks resistance training may lead to decrease of ICAM-1 and CRP in diabetic rats. Those exercises may decrease serum levels of VCAM-1 in diabetic rats. Though, the latest mentioned decrease was not significant.

Acknowledgments

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Conflict of Interest

The authors declare that they have no conflict of interest.

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