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The Effects of Eight Weeks Swimming Training on Interleukin- 17, Interleukin- 18 and Tumor Necrosis Factor-α in Streptozotocin Induced Diabetic Rats

Mahboobe Karbalayi* [1], Mehdi Noora [2], Mohsen Noura [1]
1. Department of Sport Physiology, Zabol Branch, Islamic Azad University, Zabol, Iran
2. Department of Sport Physiology, Shiraz Branch, Islamic Azad University, Shiraz, Iran

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***Corresponding author:**
Mahboobe Karbalayi. Department of Sport Physiology, Zabol Branch, Islamic Azad University, Zabol, Iran

Phone: +989128031332
Fax: +985432662776

Email: ma_karbalaie@yahoo.com
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Abstract
Introduction: Inflammation is a feature of the diabetes disease. The aim this study was to determine the effects of eight weeks swimming training on Interleukin- 17 (IL-17), Interleukin- 18 (IL-18) and tumor necrosis factor- α (TNF-α) in streptozotocin-induced diabetic rats.
Methods: In this experimental study, 30 diabetic rats were accidentally divided into two equal groups of 15: swimming training and control. The rats in the swimming training group swam for eight weeks, three sessions per week; in the first two weeks for 10 minutes and six weeks later for 30 minutes. The control group rats had no sports activity. 24 hours after the last training session, the rats were sacrificed to measure the parameters in the study. The data were analyzed using SPSS version 19 and paired sample t-test ($p \leq 0.05$).
Results: eight weeks of swimming training had no significant effect on reduction of IL-17 ($p = 0.30$), IL-18 ($p = 0.41$) and TNF-α ($p = 0.81$) in diabetic rats.
Conclusion: it can be concluded that the present training has no significant effect on inflammatory factors in diabetic rats.
Keywords: Diabetes, IL- 17, IL- 18, TNF- α, Training

Introduction
Diabetes is a kind of metabolic syndrome that causes problems such as neuropathy, retinopathy, nephropathy and cardiovascular problems in long term that affect both afflicted person and society (1). This illness occurs because of excessive glucose increase caused by insulin secretion disorder or insulin resistance (2). The pathogenesis of inflammation is important in the pathology of various diseases, including diabetes, in which the increase in blood glucose has been reported as a result of an increase in inflammatory and pro-inflammatory factors (2). Studies have shown that Interleukin- 17 (IL-17) and Interleukin- 18 (IL-18) are inflammatory cytokines and are connected with a lot of symptoms of chronic low-grade inflammation in fat people (3). IL-17 may contribute to pathogenesis of cardiovascular diseases through the induction of C reactive protein (CRP) production. In addition, it has been reported that IL-17 causes factors such as IL-8 and chemokine type one ligand to produce through effecting on endothelial cells and finally these factors cause the neutrophil accumulation within inflamed tissues (4). Also, IL-18 and inflammation cause increasing blood glucose and eventually lead to the activation of Th1 cells and production of interferon gamma both in the fat tissue and in the affiliate page of arteriosclerosis (5). Tumor necrosis factor alpha (TNF-α) plays an important role in the occurrence of metabolic syndrome. The diabetics are of gene expression and high amount of TNF-α protein in the skeletal and plasma muscle and it is likely that the fat tissue is the major source that produces TNF-α (6). TNF-α causes a reduction (negative regulation) of GLUT4 and

inhibits the signaling and activity of the insulin receptor (7). It also contributes to insulin resistance with increased non-estrogenic fatty acids in blood, resulting in a TNF-α causing reduced insulin action and hence insulin resistance in obese people (8). Many researches have been done about diabetes and the methods of its prevention and treatment. Among different researches we can mention the examination of the effect of different sports activities on controlling or curing this disease (9). Studies regarding the effect of sport on the immune system and some cytokines indicate that the expression of cytokines due to exercise is to a high degree dependent on the type, intensity, duration, size and training course (10). For example, combined trainings led to the reduction of IL-17 in women with Multiple sclerosis (MS) and increased their power and balance (11). Also, one session of high intensity training has been shown to increase IL-17 in training rats, and this cytokine may be involved in inflammatory processes of skeletal muscle (12). Twelve weeks aerobic trainings with intensity of 40 to 75 percent reserved heartbeat caused significant reduction of IL-18 levels in elderly men (13). A circular resistance training session with 60% of one maximal repetition (1RM) resulted in a significant decrease in IL-18 in obese subjects (14). Six weeks, five sessions per week of moderate endurance training resulted in a significant decrease in serum TNF-α levels and tumor volume growth rate in rats bearing breast tumor (15); also, six weeks, five sessions a week swimming training with arbutin consumption reduced TNF-α in the heart tissue of diabetic male rats (16). Considering the controversy of the studies as well as the lack of adequate information on the type, intensity and duration of exercise in reducing inflammatory factors to reduce the complications of inflammation in these patients and also the effects of different exercises on inflammatory cytokines, the present study was aimed to investigate the effects of eight weeks of swimming training on serum levels of IL-17, IL-18 and TNF-α in streptozotocin-induced diabetic rats.

Methods

In this experimental study, 30 male adult rats of Sprague Dowley were purchased from animal breeding center located at the Animal House of Physical Education and Sport Sciences at Islamic Azad University, Fars Science and Research Branch and were transferred to the keeping place at the Laboratory of Sport Physiology (ambient temperature 22 ± 2 degrees centigrade, controlled light, 12 hour cycle light and darkness) and went through 7 day compromise period. During the course, the animals' access to water and food was free. In the eighth day after a fasting night, the rats were anesthetized with chloroform, and by intraperitoneal injection 60 mg/kg streptozotocin (manufactured by Sigma Company) were dissolved in citrate buffer. Four days after injection, the animals' tails, by punching method were used to measure blood glucose. A glucometer was used to collect blood samples. Rats with blood glucose greater than 300 mg / dl were included in the study. The beginning of the training program was one week after induction of diabetes and keeping the rats. The diabetic rats were accidentally split into two equal groups of 15, including (1) control, (2) swimming training. The rats in the swimming training group received swimming trainings for 8 weeks, 3 sessions per week according to the training protocol of Nayanatara *et al.* (2005) (17), so that the rats swam in the swimming tank for 10 minutes in the first two weeks; then they swam for 30 minutes in following six weeks. After this period, blood samples were taken from rats after anesthesia using a combination of ketamine (70 mg / kg) and xylosin (3-5 mg / kg) to measure the variables studied. Before blood collection, animals were kept fasting for 16 hours. The variables were measured by ELISA method and the IL-17 commercial kit (manufactured by Cusabio, China), IL-18 kit

(manufactured by Boster), and TNF-α kit (Alfa Diaclone Besancon, France). Kolmogorov-Smirnov statistical tests and paired sample t-test were used for inferential statistics. Data were analyzed using SPSS software version 19 and significant level was considered ($p \leq 0.05$).

Results

Serum levels of research variables along with weight of rats are presented in Table 1. Results of paired sample t-test showed that there is no significant difference in IL-17 ($t = 1.01$, $p=0.30$), IL-18 ($t=-0.69$, $p=0.41$) and TNF-α ($t=-0.16$, $p = 0.81$) levels in swimming training and control group rats.

Table 1. The research variables along with the weight of the swimming training and control rats

Variable Group	IL-17 (ng/l)	IL-18 (ng/l)	TNF-α (ng/ml)	Weight Pretest	 posttest
Swimming training	62.71±13.94	54.46±3.22	83.33±14.72	226.54±15.31	279.58±17.67
Control	69.09±17.28	49.54±12.28	81.45±24.29	246±20.58	294.55±12.23

Table 2. The results of the independent t-test for the study variables in the swimming training and control groups

Variable	Group	t	df	p
IL-17	Swimming training Control	1.01	22	0.30
IL-18	Swimming training Control	-0.69	22	0.41
TNF-α	Swimming training Control	-0.16	22	0.81

Discussion

The results showed that there was no significant difference in the changes of IL-17 in diabetic rats in swimming and control groups. IL-17 is an inflammatory cytokine and is associated with a lot of symptoms of chronic low-grade inflammation in obese people. Increased levels of acute and intermediate inflammatory stage protein in the serum of obese people compared to lean individuals are indicative of this claim. Of course, in human specimens, other roles such as the role of IL-17 in osteoporosis have been proposed (18). It has also been shown that IL-17 increases angiogenesis in cancer cells and has high correlation with the number of blood vessels in ovarian cancer in humans. In contrast, researchers have shown that IL-17 inhibits the growth of fatty cells. The mechanism of the inhibitory effect of IL-17 on the growth of lipid cells is distinguished by prostaglandin E2 (PGE2) secretion to produce cyclooxygenase (COX2) in fat cells (3). Research has shown that few studies have examined the effects of sports activities on IL-17, reporting contradictory results. For example, an exercise session in cold and natural weather does not change the IL-17 (19), but six week endurance training resulted in a significant decrease in IL-17 (15). Eight weeks of aerobic and anaerobic treadmill trainings with Omega 3 resulted in a significant increase in IL-17 and CRP in male rats; these results are not consistent with the results of the present study. (20). Twelve weeks of interval aerobic training has a significant effect on the reduction of serum IL-17 levels, body mass index, insulin and insulin resistance in obese men (3); also, 12 weeks of high intensity exercise training has been shown to increase IL-17 (21). Among

the reasons for incompatibility of the results of these studies with the present study we can mention the type of exercise, the intensity of exercise, the difference in the statistical population, the type of disease and the duration of the training period. Perhaps the mechanism involved is that intense exercise causes the release of pro-inflammatory cytokines, and these cytokines produce anti-inflammatory cytokines such as interleukin-2 (IL-2), interleukin-6 (IL-6) and interleukin-10 (IL-10). The sequential production of pro-inflammatory and anti-inflammatory cytokines seems to be the reason for the production of IL-17 through peripheral blood leukocytes and skeletal muscle (21, 22). According to the results of various studies, it can be said that the intensity or duration of exercise is an important factor in increasing or decreasing the production of IL-17 (20). The results showed that there was no significant difference in IL-18 changes in swimming and control groups, which means that although swimming training led to an increase in IL-18, this increase was not significant. Several studies have shown that obesity has been associated with high levels of IL-6, IL-18, CRP and insulin serum, as well as insulin resistance index. Also, greater increase in serum IL-18 by rising insulin resistance increases insulin production in obese people (23). In this line, 12 weeks, three sessions per week and 60 minutes each session of weight training resulted in a significant decrease in serum IL-18 and hs CRP levels in obese men (24); on the other hand, eight weeks of rope skipping has a significant effect on the reduction of IL-18 and CRP in overweight and obese adolescents (25); Eight weeks of aerobic training results in a significant decrease in IL-18 of elderly men; this study is not coherent with the present study, and its possible reasons are the difference in the statistical population, the duration of the training period, and the type of training. Non-athletes, for the effects of exercise on IL-18 levels, it has been shown that IL-6 exerts its anti-inflammatory effects by stimulating and increasing the expression of IL-10 and IL-1α and blocking of TNF-α; on the other hand, the positive correlation between TNF-α and IL-18 has been shown (14). The researchers also argued that the anti-inflammatory effects of exercise in IL-18 production were attributed to the production of IL-6, IL-Ira, and IL-10 anti-inflammatory cytokines, which results in a reduction in pro-inflammatory cytokines such as IL-6 and IL-18 from adipose tissue. In addition, reducing the concentration of these cytokines can be attributed to the reduction in the percentage of body fat in which they are made (23). However, the most significant lack of change in serum IL-18 levels may be the significant lack of change in TNF-α levels following swimming training in the current study. The results showed that there was no significant difference in TNF-α changes in swimming and control group rats. The relationship between TNF-α and energy metabolism is very complex, so that increasing TNF-α concentration can increase the metabolism of the rest and ultimately lead to weight loss. Diabetes also increases the appearance of angiogenesis growth factors in some tissues (16). Studies on the effect of exercise on TNF-α have reported different results. For example, in line with the present study, eight weeks aerobic activity had no significant difference on visfatin, IL-6 and TNF-α in the rats' research groups in TNF-α serum levels (26). 10 weeks of resistance training and aerobic training did not show change on TNF-α (27); eight weeks of strength training did not show any significant changes in TNF-α in active adolescent girls (28). Six months of aerobic training did not have a significant effect on plasma levels of IL-6 and TNF-α (29); on the other hand, six months of weight loss and exercise (aerobic and resistance) reduced TNF-α receptor in postmenopausal obese women (30). The reason for inconsistency is the number of exercise sessions, the type of exercise, the intensity of exercise, as well as the amount of fat in the subjects and the levels

of measurement of variables. Eight weeks and three sessions of high interval intensity training per week can lead to significant reduction in visfatin and TNF-α. The researchers stated that the decrease in TNF-α was related to intensity and duration of activity, hence reducing this factor could reduce metabolic diseases (26). Exercise plays an important role by reducing fat resources, changes in the secretion of adipose tissue cells, and improving the lipophilic hypoxia (related to obesity and overweight) (31). Also, sports training reduces the production of IL-6 which inhibits TNF-α and activates the cholinergic anti-inflammatory pathway (32). The present study has many limitations such as the failure to check the results of diabetes induction on the levels of research variables, the effect of injection of streptozotocin poison on the inflammatory factors, and not checking the relationship between the serum levels of research variables together. Also, regarding the results of the present study, it is suggested that considering the effects of type, intensity and duration of exercises on the inflammatory factors in the future studies, it is necessary to do some manipulations in these interventions to achieve better results about the effects of exercise on the inflammatory factors in diabetic patients.

Conclusion

According to the results of the present study, swimming training has no significant effect on the serum levels of IL-17, IL-18 and TNF-α in in streptozotocin-induced diabetic rats.

Ethical issues

No applicable.

Authors' contributions

All authors equally contributed to the writing and revision of this paper.

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