Articles

The effect of regular training on plasma cytokines response in healthy and diabetic rats

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ABSTRACT

الأهداف: تقييم أثر تمارين السباحة المنتظمة على مستويات البلازما ل (IL–1β)، (TNF–α) و (IL–6) لدى الجرذان السليمة والمصابة بالسكري.

الطريقة: في هذه الدراسة، التي أجريت من مارس 2008م إلى مارس 2009م –مركز أبحاث تطبيق الدواء بمدينة تابيز – إيران، تم تقسيم عدد 40 جرذ نوع ويستار (250–300g) عشوائياً إلى 4 مجموعات (n=10): مجموعة التحكم، مجموعة التمارين، مجموعة السكري، ومجموعة السكري والتمارين. أصبحت الحيوانات مصابة بالسكري بواسطة حقنة مفردة من عقار ستريبتوزوتوسين (50mg/kg). تكون برنامج التمارين من السباحة (ساعة واحدة/5 أيام/الأسبوع) لمدة 8 أسابيع. تم قياس حركة البلازما بواسطة استعمال طقم خاص وبطريقة إليسا.

النتائج: أظهرت النتائج لهذه الدراسة أن متوسط الفرق ل(IL–6) كان واضحاً بين المجموعات، لذلك قد تزيد السباحة المنتظمة من مستويات البلازما إلى 9 مرات لدى الجرذان السليمة و إلى 23 مرة لدى الجرذان المصابة بداء السكري (p=0.000 F (3.31) = 54.79). ولكن لم يكن هنالك فروق ملحوظة في مستويات IL-1ß، و (TNF–α) بين المجموعات.

خاتمة: وفقاً إلى نتائج لهذه الدراسة. فإن التمارين المنتظمة تسبب زيادة في مستوى البلازما ل (IL–6) وهذه التعزيز يكون أعلى في مجموعة السكري أكثر من الجرذان السليمين. لهذا، فإنه بزيادة سكر الجلوكوز المباشر في الدم عن طريق الهيكل العظمي العضلي، IL–6 يمكن أن يكون له دور مفيد في مواصلة الأنشطة لدى المرضى المصابين بالسكري.

Objectives: To evaluate the effect of regular swimming exercise on plasma levels of tumor necrosis factor (TNF)-α, interleukin (IL)-1ß and of IL-6 cytokines in healthy and diabetic rats.

Methods: In this study, carried out from March 2008 to March 2009 in the Drug Applied Research Center, Tabriz, Iran, 40 Wistar rats (250-300g) were randomly divided into 4 groups (n=10): control-sedentary, control-exercised, diabetic-sedentary, and diabetic-exercised. Diabetes was induced by a single injection of streptozotocin (50mg/kg, intraperitoneally). The exercise protocol consisted of swimming (one hour/day, and 5 days/week) for 8 weeks. The plasma cytokines were measured by using specific kits and the enzyme-linked immunosorbent assay method.

Results: The findings of this study showed that the mean difference of IL-6 was significant among the groups, and that regular swimming increased the plasma levels of IL-6 to 9-times in healthy rats and to 23-times in diabetic ones (p=0.000, F (3,31)=54.79). However, there were no significant differences in IL-1ß, and TNF-α levels among the groups.

Conclusion: According to findings of this study, regular exercise causes an increase in plasma levels of IL-6, and this enhancement is much higher in diabetics rather than healthy rats. Thus, by increasing direct absorption of blood glucose by skeletal muscle, IL-6 can have a beneficial role in continuing the activities of diabetic patients.

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The effects of regular aerobic exercise training (such as swimming and treadmill) on the body are different from those of non-regular, strenuous exercises. Regular exercises offer a host of benefits. The most prominent effect is its protective effects against cardiovascular diseases, obesity, and diabetes.[1] Pedersen & Saltin[2] reviewed the evidence for importance of exercise in the treatment of metabolic syndrome related disorders (insulin resistance, type 2 diabetes, dyslipidemia, hypertension, obesity), heart and pulmonary diseases, muscle and bone diseases, and type 1 diabetes. Therefore, they have prescribed exercise as a therapy in those chronic diseases.[2] It has been demonstrated that the production of pro- and anti-inflammatory cytokines is modulated by physical activity.[3,4] Interleukin-1ß (IL-1ß) and tissue necrosis factor-alpha (TNF-α) are usually referred to as inflammatory or pro-inflammatory cytokines, while IL-6 has primarily immune-modulatory effects.[5] The TNF-α is produced by adipose tissue and lipopolysaccharides of inducible pathogenic microorganisms and it can increase insulin resistance to insulin and atherosclerosis.[5] The production of IL-1ß, the other inflammatory cytokine, is augmented in tissue injuries, inflammation, and stress.[4] Infusion of IL-1ß and TNF-α in animals or humans is accompanied by an enhanced production of acute phase proteins by the liver.[6] The IL-6 directly inhibits the expression of IL-1ß and TNF-α and increases the expression of IL-1 receptor antagonist, as a biological inhibitor of inflammatory cytokines.[5,7] Acute, non aerobic, and exhausted physical activities elevate circulating levels of TNF-α, which has adverse effects on the body and immune system. However, regular exercises decrease TNF-α concentration and increase IL-6 level; resulting in initiation of adaptive mechanisms to counter the inflammation.[4] Besides, its anti-inflammatory properties, IL-6 is involved in the metabolism of carbohydrates, so that it enhances plasma glucose uptake by skeletal muscle.[5] In addition, IL-6 can reduce body weight by inducing the lipolysis in adipose tissues. Low-grade inflammatory responses in diabetes may increase resistance, and beta cell apoptosis.[1] The IL-6 can improve insulin resistance in tissues by enhancing the expression of IL-1 receptor antagonist and blocking the TNF-α receptor. Thus, IL-6 can be beneficial in both type-1 and type-2 diabetes and can reduce cardiovascular risk factors. It was reported that measuring the plasma levels of IL-6 and TNF-α may predict myocardial risk factors.[5,8] Therefore, when high plasma levels of IL-6 produced by regular exercise are accompanied with reduced TNF-α and IL-1ß, which results in acute phase proteins elevation, it can induce beneficial metabolic effects, and can minimize the adverse effects of diabetes including cardiovascular disturbances, retinopathy, neuropathy, and nephropathy.[8] It is revealed that acute activities increase the plasma levels of cytokines TNF-α and IL-1ß as well as IL-6 at the same time. However, the metabolic effects of nonacute and regular exercises are not well known and seem to be different from the acute and non-regular exercise influences. Therefore, the aim of present study was to evaluate the simultaneous effect of regular swimming exercise on plasma cytokines levels of TNF-α, IL-1ß, and IL-6 in rats with type-1 diabetes.

Methods. ***Animals.*** The study, carried out between March 2008 and March 2009 in the Drug Applied Research Center, Tabriz, Iran, was performed using male Wistar rats weighing 250-300g at the beginning of the experiments (aged 4 weeks). The animals were housed 5 per cage under controlled conditions of temperature (20-23˚C), humidity (40%) and light/dark cycle (12h/12h, lights on at 7 am) with ad libitum access to standard rat chow and water. The present study followed the principals and guidelines of the Iranian Council on Animal Care, and it received institutional ethical approval from the Committee for Animal Research, Tabriz University of Medical Sciences. Forty rats were divided into 4 groups of 10 animals: the control-sedentary group (CS), the control–exercised group (CE), the diabetic–sedentary group (DS), and the diabetic–exercised group (DE). In order to induce type-1 diabetes, streptozotocin (STZ; Sigma Chemical Co, St. Luis, MO, USA) was dissolved in saline and a single intra-peritoneal injection of STZ (50 mg/kg) was given to each animal. To confirm the induction of diabetes, 3 days after the STZ injection, the blood glucose levels were determined from blood samples obtained by tail prick using a strip-operated blood glucose monitoring system (Healthy Living, Samsung, Korea), and the animals with blood glucose levels higher than 300 mg/dl were selected.[4] The exercise protocol consisted of swimming daily for 60 minutes, and 5 days per week for 8 weeks, in a plastic tank filled with water at a temperature of 28 ± 2°C. Rats belonging to the sedentary group were placed daily in a novel cage. All groups of rats were weighed 3 times a week.

Blood sampling. Eight weeks before, and after 8 weeks of exercise, blood samples of all groups of rats were taken by tail pricking. Furthermore, to prevent acute exercise effects, at 24 hours after the last exercise session, rats were weighed and sacrificed under ether anesthesia; blood was collected from the abdominal aorta. All blood samples were collected in vials and centrifuged at 3000 rpm for 10 minutes to separate serum. One rat from the CS group, and 2 rats from both the DS and DE groups died and were excluded from the experiments.

Assay of cytokines. The concentrations of IL-1ß, IL-6, and TNF-α in serum were determined by

Table 1 - The circulating levels of cytokines (pg/ml) within sedentary and/or trained healthy groups before and after 8 weeks.

Cytokines	Control- sedentary group		Control-exercised group	
	Before	After	Before	After
IL-6	22.3 ± 17.86	32.8 ± 7.54	33.6 ± 5.14	303.7 ± 72.13*
IL-1ß	175.5 ± 57.85	136.8 ± 76.65	156.0 ± 61.65	173.1 ± 58.75
TNF-α	36.0 ± 21.19	28.7 ± 23.99	38.8 ± 20.69	43.2 ± 19.60

*p=0.000 significantly different as compared with baseline before levels within that group.
IL - interluken, TNF-α – tumor necrosis factor

Table 2 - The circulating levels of cytokines (pg/ml) within sedentary and or trained diabetic groups before and after 8 weeks.

Cytokines	Diabetic-sedentary group		Diabetic-exercised group	
	Before	After	Before	After
IL-6	24.9 ± 10.	36.30 ± 18.47	49.0 ± 3.67	1138.5 ± 174.38*
IL-1ß	133.1 ± 86.47	97.4 ± 47.41	165.13 ± 48.25	201.0 ± 58.75
TNF-α	25.9 ± 17.35	35.9 ± 27.93	19.75 ± 5.58	31.0 ± 17.14

*p=0.000 significantly different as compared with baseline before levels within that group.
IL - interluken, TNF-α – tumor necrosis factor

an enzyme-linked immunosorbent assay (ELISA) using commercially available rat sensitive assay kits (Bender Medsystems, Vienna, Austria, [BMS622MST, BMS625MST, and BMS623MST]). The assays were carried out according to the manufacturer's instructions. All cytokine assays were performed in duplicate and reported in picogram per milliliter serum.

Statistical analysis. All data are presented as means ± SEM. Analysis of cytokine level differences between groups was performed by 2 way ANOVA followed by Tukey as a post hoc test. Analysis of cytokine level differences within groups (before and after 8-weeks) was carried out by paired t-test using SPSS 13. A value of p<0.05 was accepted as significant.

Results. In the CE group, the level of IL-6 after 8 weeks training was significantly increased from the baseline level (p=0.000). The swimming exercise did not affect the circulating levels of IL-1ß and TNF-α in this group; TNF-α (p=0.69), IL-1ß (p=0.85) (**Table 1**). The levels of cytokines IL-6, IL-1ß, and TNF-α in plasma of untrained healthy rats (CS group) after 8 weeks were not significantly different from the levels of those measured before 8 weeks TNF-α (p=0.36), IL-1ß (p=0.43), IL-6 (p=0.28). In the third group, the untrained diabetic rats, there were no significant alterations in all 3 cytokines before and after 8 weeks, TNF-α (p=0.82), IL-1ß (p=0.42), IL-6 (p=0.44). However, swimming training in diabetic rats significantly increased the plasma level of IL-6 after 8 weeks training (p=0.000). There were no significant effects of exercise on the levels of IL-1ß,

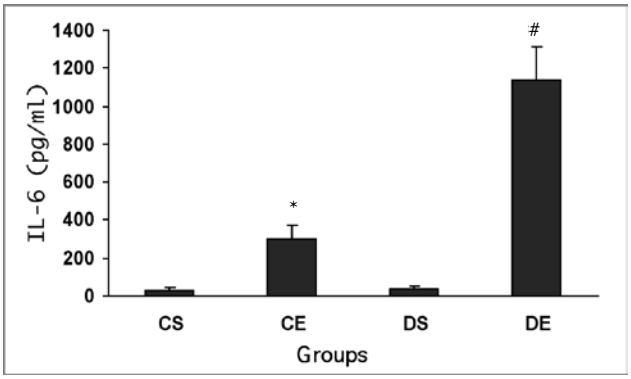

Figure 1 - The level of IL-6 (pg/ml) between all groups after 8 weeks. *p=0.000 as compared with group CS and #p=0.000 as compared with group DS, and ¥p=0.000 as compared with group CE. CS - control–sedentary group, CE - control–exercised group, DS - diabetic–sedentary group, DE - diabetic–exercised group. IL-6 - interleukin-6.

and TNF-α in this group as well TNF-α (p=0.15), IL-1ß (p=0.35) (**Table 2**). The comparison of circulating cytokine IL-6 levels after 8 weeks in sedentary and trained rats are shown in **Figure 1**. As seen, IL-6 was significantly increased in groups CE and DE as compared with those of groups CS and DS. The amount of this enhancement was 9-times in healthy trained rats (CE versus CS) but 23-times in diabetic trained rats (DE versus DS). Our results indicated that the swimming exercise led to much greater enhancement of IL-6 in diabetics rather than healthy control rats. However, the plasma levels of TNF-α and IL-1ß after 8 weeks were not statistically

significant among all groups, which reflects that neither swimming training nor 8-week diabetes could affect these inflammatory cytokines in this experiment.

Discussion. In this study, we investigated the cytokine response to regular swimming and its possible interaction with diabetic state in rats. We found that both control and diabetic rats that had swum for 8 weeks demonstrated a rise in IL-6 levels in plasma. There were no significant alterations in circulating levels of TNF-α and IL-1ß among non-exercised healthy and diabetic rats. Also, no significant effect of exercise was evidenced on TNF-α and IL-1ß concentrations in control and diabetic rats.

The body of evidence shows that production of IL-6 during exercise is primarily from adipose tissues that even extends over the recovery period.[9] However, the production of IL-6 from skeletal muscle in humans is transient; and abruptly returns to the basal levels after cessation of exercise.[10] In our study, the exercise-induced rise in levels of IL-6 was 9-fold in controls, and 31-fold in diabetic rats. This is likely because, lack of muscular glycogen in diabetic animals can induce a greater increase of IL-6 during exercise, which IL-6 in turn leads to further uptake of glucose by muscles. In this regard, previous studies have showed that low levels of muscular glycogen stimulate IL-6 gene expression in response to exercise.[9,11] Furthermore, dynamic knee extensor exercises for 5 hours at 25 watt in healthy males increased plasma concentration of IL-6 19-folds with comparison to rest,[12] which is in accordance with our finding in healthy rats. This indicates a very fast turnover of IL-6 during muscle exercise.

The elevated levels of IL-6 enhance the ability of contracting skeletal muscle cells to uptake greater amounts of glucose. In agreement with our results, Pedersen[5] found that the IL-6 level is increased 100-fold in marathon athletes, and is related to exercise duration. Exercised-induced release of IL-6 to the blood can have a hormone-like effect on liver and adipose tissue by which IL-6 acts to balance the glucose homeostasis and lipolysis stimulated by exercise. Furthermore, IL-6 can inhibit the production of TNF-α and IL-1ß by adipose tissue and inflammatory cells. These cytokines have important roles in the pathogenesis of insulin resistance and atherosclerosis.[4] Thus, by reducing the levels of TNF-α and IL-1ß, IL-6 can improve the insulin effect on tissues and minimize lipid plaque formation and atherosclerosis development in both type-1 and type-2 diabetes mellitus. Physical training, by rising IL-6 level up to 23-folds as in this study, can enhance insulin sensitivity, and muscular contraction-induced glucose uptake in the exercised muscle. In a Chinese study on the comparative effects of diet and exercise on 577 persons with impaired glucose tolerance,[2] it was demonstrated that the risk of diabetes was reduced 31% by the diet group and 46% by the exercise group.[2] Similar findings were also obtained in other studies,[12] which reflects the important effects of exercise.

The IL-6 directly increases uptake of glucose by muscle, and therefore regulates the blood glucose homeostasis.[13] Therefore, continuous regular physical activities would have many beneficial effects on diabetic subjects. The IL-6 released from skeletal muscle during regular exercise acts as an energy sensor to stimulate lipid oxidation and lipolysis.[14] This effect of IL-6 also may be favorable in diabetic patients by preventing obesity.

The TNF-α and IL-1ß are usually elevated in states of septic and aseptic inflammations. However, in our study, an 8 week diabetic state in rats could not significantly increase the IL-1ß and TNF-α level in both exercised and non-exercised diabetic groups. The reason may relate to the possibility that 8 weeks of diabetic condition could not induce any inflammatory responses in rats. This possibility is confirmed by no significant differences being evidenced on the levels of these pro-inflammatory cytokines among healthy swimming and diabetic non-swimming rats. Furthermore, IL-1ß and TNF-α stimulate the secretion of IL-6; whereas the increased levels of IL-6 inhibit the secretion or the elevation in secretion of both IL-1ß and TNF-α in diabetic states.[15]

As previously noted, physical training may be one of the therapies in chronic diseases such as diabetes and heart diseases. Furthermore, physical exercises may have profitable effects on the plasma lipoproteins profile, which seems to play a role in development of atherosclerosis in both type-1 and type-2 diabetic subjects.[16,17] We therefore, propose studying the effects of prolonged (more than 2 months) regular exercise training on immune function, blood glucose homeostasis, and lipid profiles of diabetic individuals as well as the protective effects of these types of exercises on cardiovascular risk factors and atherosclerosis in future research.

In conclusion, we have demonstrated that regular swimming exercise can induce IL-6 elevation in blood of both diabetic and nondiabetic rats with much greater response in diabetic ones. The increased IL-6 production may provide a link from contracting skeletal muscle to enhanced glucose uptake and fat metabolism in the diabetic state. Furthermore, regular exercise by increasing IL-6 can modulate the diabetes-induced inflammatory responses and plasma concentration of pro-inflammatory cytokines TNF-α and IL-1ß.

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