**Effect of maximum activity on the immune system cells in diabetic rats**

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**Abstract**

**Introduction:**
Diabetes is a metabolic disorder that can be followed by a decrease in insulin secretion. Exercise is an important factor in the control of diabetes and is also effective on the immune system. The purpose of this study was to investigate the effect of exercise intensity on the number of immune cells in the peripheral blood of diabetic rats.

**Materials and Methods:**
In this experimental study, 28 white female Wistar rats with average weight of 260 ± 20 and age of 80 days were randomly divided into four groups with seven subjects: control, diabetic without exercise, diabetic with electric shock and diabetic with maximum exercise. After four weeks of running on a treadmill, the blood samples were collected and sent to the laboratory so as to determine the number of immune system cells in blood.

**Results:**
White blood cell counts in diabetic rats with maximum exercise showed that the lymphocyte count was increased significantly (P≤0.05) as compared with control and diabetic rats without exercise but the monocyte and neutrophil counts were decreased significantly (P≤0.05). Also, the eosinophil counts in diabetic rats with maximum exercise did not decrease significantly (P≤0.05) compared with diabetic rats without exercise.

**Conclusion:**
The results showed an increase in the lymphocytes count and a decrease in the monocytes and neutrophil counts of diabetic rats with maximum exercise compared with diabetic rats without exercise; the most and the least frequent cells were lymphocytes and eosinophil, respectively in diabetic rats with maximum exercise.

**Keywords:** Diabetes Mellitus, Exercise, Physical Activity, Rats

**Introduction**
Diabetes is one of the most common metabolic diseases in humans. If insulin is not adequately secreted in the body or cells develop insulin resistance, blood glucose rises and the person suffers diabetes. There are two types of diabetes; type I, and the more common type II (1, 2). According to the World Health Organization 2010 report, of the 221 million diabetics in the world, four million are in Iran. This number is forecast to rise to 300 million in
the world by 2025 (3). Diabetes is a disease that affects the immune system and depletes body’s defense system against infections. Regular exercise with metabolic control in people with diabetes can control blood sugar levels and amplify defensive responses of the body (4).

Exercise plays an essential role in control of diabetes, and in addition to strengthening muscles and maintaining health of the heart, blood vessels, and lungs, it reduces blood cholesterol and triglyceride levels (5). Through burning calories, exercise helps people with non-insulin dependent diabetes to achieve and maintain their ideal weights. Moreover, with increased insulin functioning, exercise helps control diabetes and it may even reduce diabetics need for medical therapy (6). Exercise affects the entire immune system, and change in immune parameters is often associated with the pressure experienced by the athlete during exercise (7). The immune system creates a powerful set of defense mechanisms to protect against the invading foreign agents. In the absence of this system, the invader will use the host body as a rich source of food. Generally, the immune system comprises five types of cells including basophils, lymphocytes, macrophages with monocytes, macrophages with neutrophils, and eosinophils (8). The principle function of the immune system is to eradicate the infectious agent to minimize any potential damage. In normal, healthy people, the immune system guarantees that infection is short-lived and damage is kept to a minimum (9).

The effect of diabetes on the immune system (4) and the effect of exercise on diabetes (10, 11) have been studied by many researchers. Although it is widely accepted that exercise has a positive effect on the immune system (9, 12-14), some studies have revealed different results (15). A few studies have attempted to explain the relationship between the intensity of exercise, diabetes, and the immune system. The role of exercise intensity in the diabetic rats’ immune systems has not been studied much, and the few studies focused on the effect of different exercise periods on the immune system, and rarely considered intense or light exercise program effects (16-19). A study examined the effect of intense exercise on the immune system in rats with a voluntary running exercise protocol, performed 3 days per week for 8 weeks. The results showed an improvement in the lymphocyte cell activity in rats (20). In another study, researchers showed that in athletes with overtraining symptoms, exercise had no effect on the number of lymphocytes. In the present study, in the early stages of intense exercise (2-4 weeks), the number of lymphocytes declined, but toward the end of intense exercise course (4-8 weeks) this number returned to normal state. During the long exercise periods, leukocytes were with the highest level of efficacy (21). In another study, it was shown that intense exercise or intense prolonged exercise can impair the defense system due to amplified glutamine burning caused by intense exercise. Glutamine affects performance of leucocytes, and if reduced, it increases athletes’ readiness to withstand infections (22). Also, in a study to investigate the effects of swimming on immune system in diabetic rats, it was found that physical exercise reduced diabetes, insulin, and glycogen supplies, increased glucose and neutrophil, and restored blood glucose, liver glycogen levels, and number of neutrophils and lymphocytes (4). Given the results of these studies and the positive effect of exercise on the immune system, the present study was conducted to evaluate the effect of exercise with maximal intensity on peripheral blood immune cells in diabetic rats.

Materials and Methods

In this experimental study, 28 female Wistar rats weighing 260±20 grams, aged 70-80 days were procured from Razi Vaccine and Serum Institute in Shiraz, and
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randomly divided into four groups of seven as follows: 1) control group, 2) diabetic-no exercise, 3) diabetic-electric shock, 4) diabetic-maximal exercise.

Exercise protocol: Before implementing the protocol, the exercise group members were trained to run on the treadmill on a daily basis for one month. For this group, the treadmill speed was set at 30 m/min. Exercise began with 10-minute run and 5-minute rest on the first day and gradually increased to 30 minutes without rest by the 25th day. The 30-minute exercise without rest remained constant in the last five days. Then the exercise group exercised on the treadmill for 4 weeks, 3 days per week (every other day), 30 minutes each day, at the speed of 30 m/min. The electric shock group received a shock 3 times per week for 4 weeks.

In this study, diabetes was induced in the diabetic group by use of the alloxan monohydrate (Sigma Company), which selectively destroys beta cells and is a suitable drug for inducing experimental diabetes. Alloxan is obtained from uric acid oxidation, and it is a pink powder that easily dissolves in water (23, 24). Given the limitation in alloxan dosage, and in accordance to the previous studies, a dose of 150 mg/kg body weight was selected as appropriate dose for the purposes of this study (25). For better efficacy, the rats were kept fasting 24 hours before injections, and also 3 to 4 hours after injections. After 4 days, to measure blood sugar levels, rats were kept fasting for 24 hours again, and on the fifth day using Glucometry device (Sigma Company), blood sugar levels were measured. To do this, first rats' tails were scratched by lancet, and a drop of blood was placed on the glucometer strip, and put in glucometer, and blood sugar level was read. Blood sugar levels between 200 and 300 mg/dl were considered diabetic (4).

At the end of the four weeks of exercise, blood samples were collected again. To observe and count white blood cells under the microscope, first, with the aid of methanol, blood smears were prepared and fixed, and then they were stained.

Data were analyzed by SPSS software using descriptive and inferential statistics including the one-way ANOVA and Duncan tests. All results were expressed in mean±standard deviation, and α=0.05 was considered the significant level.

Results

Given the results presented in table 1, of the immune system cells in the exercised diabetic rats compared to the non-exercised diabetic group, the highest frequency observed was related to lymphocytes and the lowest to eosinophils. With respect to lymphocytes, the non-exercise diabetic group compared to the electric shock and control groups, and the maximum intensity exercise group compared to other three groups showed a significant increase in lymphocytes (P≤0.05).

Also, according to table 1, despite the insignificant increase in monocytes in the diabetic groups without exercise and with electric shock compared to the control group, only the maximal exercise group showed a significant reduction compared to the other three groups (P≤0.05).

Although the difference in the number of eosinophil in the diabetic with electric shock group compared to the control group was insignificant. In the diabetic groups without exercise and with maximal exercise this difference was significant compared to the control and diabetic with electric shock groups (P≤0.05) (table 1).

Despite the lack of change in number of neutrophils in diabetic with electric shock group compared to the control group, there was a significant reduction in neutrophils in diabetic without exercise group compared to the shock and control groups (P≤0.05). Also, there was a significant reduction in the diabetic with maximal exercise group compared to the other three groups (P≤0.05) (table 1).

Discussion
Diabetes effect on the immune system, exercise effect on the immune system, and also, the relationship between exercise and diabetes have been the subject of many studies. But few have studied the relationship between diabetes, exercise, and the immune system (2, 4-6, 10-11, 18-20, 22, 26-28). In the present study, in the diabetic rats without exercise group, a significant increase in lymphocytes and a significant reduction in eosinophils and neutrophils were observed, but monocytes did not significantly increase.

The important change that occurred with exercise was the increase in lymphocytes in the exercise group compared to the diabetic group without exercise. This finding is consistent with results of studies by Browns Guard et al., Guying et al., Howell et al., Nelson et al., and Nyman et al. However, it differs from results obtained by Arazı et al., Shabkhiz et al., Crespilo et al., and Sharp that found no change or reduction in lymphocytes after exercise (4, 12-13, 15-16, 18, 26, 29, 30). It should be noted that in Sharp’s study, the subjects were not diabetics, and that they were malnourished due to strenuous exercise and immunodeficiency. In such conditions, large amounts of plasma glutamine are used during endurance exercise and hence its levels in plasma are reduced. Since glutamine is an important fuel source in rapid cell division of cells and lymphocytes, the deficiencies created restrict lymphocyte proliferation, or increases the number of immune cells. Perhaps, a main reason for this is high glucose levels in samples obtained from diabetic groups in the present study that reduces the body’s need for non-glucose sources like glutamines. In this study, monocytes reduced after exercise. This result concurs with those obtained by Arazı et al. and Howell et al. (15, 29). Nonetheless, it differs from results obtained by Nelson et al., Crespilo et al., and Guiney et al. that found an increase in the number of monocytes (4, 12, and 16). Rapid elimination of monocytes is probably due to redistribution and implantation of these cells in damaged tissues and also, their role in cytokine secretion like interleukin-6.

In this study, eosinophil levels reduced in exercise groups. This result is in line with those found by Crespilo et al and Howell et al (4, 15), but it disagrees with results of studies by Guiney et al and Shabkhiz et al (12, 13). It seems, eosinophils have a less important role in immune system at the time of exercise, and show phagocytic activity in parasite infections.

The number of neutrophils in this study, in the exercise group was less than in non-exercise group, and this was consistent with results found by Suzuki et al. and Blanin et al. (13, 27). However, it disagrees with results of studies by Nelson et al., Howell et al., Gilson et al., Shabkhiz et al., Crespilo et al., and Guiney et al. that showed an increase in neutrophils (4, 12-13, 15, 22). The reason for this reduction was probably the existing inflammation in the tissue that caused a response to such

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Table 1- Percentage of blood immune cells after four weeks exercise

<table>
<thead>
<tr>
<th>Groups</th>
<th>Index</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
<th>Eosinophils</th>
<th>Neutrophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>63±2</td>
<td>10±1</td>
<td>5±1</td>
<td>22±1</td>
<td></td>
</tr>
<tr>
<td>Diabetic with electric shock</td>
<td>63±1</td>
<td>11±1</td>
<td>5±1</td>
<td>21±1</td>
<td></td>
</tr>
<tr>
<td>Diabetic without exercise</td>
<td>68±2</td>
<td>11±1</td>
<td>2±1</td>
<td>19±1</td>
<td></td>
</tr>
<tr>
<td>Diabetic with maximal exercise</td>
<td>77±2</td>
<td>9±1</td>
<td>2±1</td>
<td>12±1</td>
<td></td>
</tr>
</tbody>
</table>

The difference in these groups is insignificant, if there is at least one shared letter (P≥0.05)
stimulants as infections and tissue injuries. Neutrophils are the very first cells that enter the tissue at early stages of inflammation. Hence, their number in blood is reduced to a minimum. Perhaps, the type of male/female rats used, keeping conditions, the induced exercise method, intensity and duration of exercise, physical readiness and time of blood sampling, all could have caused the results to differ from other studies results.

It is recommended that in future studies, the effect of exercise with different intensities on other immune system parameters also be investigated.

References:

25. Lenzen S, an
