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
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
Effect of maximum activity on the
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 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 Arazi 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 Arazi 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

<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±4</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: