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# Evaluation of Some Lipid and Hematological Parameters of Blood Plasma in Ovariectomized Rats after Eight Weeks of Aerobic Exercise

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**Article Type**

**Research Paper**

**ABSTRACT**

**Background and Objective:** Due to the physiological changes caused by estrogen deficiency in menopause and due to the importance of aerobic exercise as one of the non-pharmacological strategies to improve these changes, the present study was performed to evaluate the effect of moderate-intensity aerobic exercise on lipid profile and the number of some plasma blood cells after ovariectomy.

**Methods:** This experimental study was performed on 40 female rats that were divided into two equal groups and one group underwent ovariectomy. After two weeks of recovery, the rats were randomly divided into four groups: control-healthy, control-ovariectomy, exercise-healthy and exercise-ovariectomy. The rats exercised for 25 weeks with 25 meter per minute intensity and blood samples were taken 48 hours after the last training session.

**Findings:** Ovary removal in the control-ovariectomy group increased body weight (240.20±11.37) compared to the healthy-control group (198.36±6.79) (p≤0.01), increased triglyceride (82.33±12.38) compared to control-healthy (36.83±3.55) (p≤0.01), increased total cholesterol (130.66±3.82) compared to healthy-control (95.5±1.33) (p≤0.0001) and increased monocytes (3.73±0.58) compared to the control-healthy (0.233±0.06) (p≤0.0001). Ovariectomy caused an increase in white blood cells in the control-ovariectomy group (6.33±1.39) compared to the healthy-exercise group (3.68±0.74) (p≤0.05). Total cholesterol levels decreased in exercise-ovariectomy (108±3.53) compared to control-ovariectomy (130.66±3.82) (p≤0.001). Eight weeks of aerobic exercise reduced monocytes in the exercise-ovariectomy group (0.268±0.03) compared to the control-ovariectomy group (3.73±0.58) (p≤0.001).

**Conclusion:** The results of the study showed that ovariectomy increased inflammation due to an increase in white blood cells, monocytes and platelets, and aerobic exercise was able to partially compensate for these changes in lipid and hematological parameters resulting from ovary removal. It seems that aerobic exercise can improve the effects of ovariectomy on white blood cells and platelets after adaptation, which has a significant positive effect on body weight and lipid profile.

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## Introduction

The onset of menopause in women significantly increases the risk of metabolic diseases such as obesity, cardiovascular disease and diabetes (1). The protective effect of estrogen disappears after menopause (2, 3). Numerous reports indicate that estrogen deficiency increases total cholesterol, increases low-density lipoprotein (LDL) cholesterol levels, activates white blood cells, and increases platelet production (4, 5). Weight gain during menopause appears to cause white blood cells to move towards inflammation due to increased production of pro-inflammatory cytokines. Increased coagulation, which is directly related to gender and menopause, also increases after a decrease in estrogen (6). The total number of white blood cells and its subsets increases with age, menopause and body mass index, and all subsets of white blood cells are positively correlated with triglyceride levels and negatively correlated with high density lipoprotein (HDL) cholesterol (7). In menopause, platelets are more responsive to fusion, indicating an increased incidence of thrombosis in postmenopausal women, and aerobic exercise appears to reduce platelet fusion in postmenopausal women (5). Studies have shown that exercise can be effective as an anti-inflammatory therapeutic agent after adaptation (8) and reduce platelet aggregation, reduce the risk of cardiovascular disease and ultimately, reduce mortality (9).

Decreased adipose tissue following exercise improves the balance of adipokines; As a result, it promotes the flow of leukocytes, the production of blood from the bone marrow, and the transfer of white blood cells (10). Ovary removal or ovariectomy in animals, similar to menopause in women, is associated with decreased lipid oxidation and decreased estrogen production from the ovaries (11), and moderate-intensity exercise can have the greatest effect on lipid oxidation, inflammation, and subsequently, reduction in cardiovascular disease (12). Aging, menopause, and increased inflammation reduce bone and muscle tissue and are directly related to decreased immune function, and subsets of white blood cells such as neutrophils and lymphocytes are also directly associated with cardiovascular disease during menopause (13, 14).

Physical activity has been shown to improve muscle and bone tissue density and boost the immune system (14). In addition, moderate-intensity aerobic exercise is one of the appropriate exercises for postmenopausal period due to its higher safety as well as movement limitations in menopause. However, very little research has been done on the effect of moderate-intensity aerobic exercise on the coagulation system and white blood cells. In a study performed on postmenopausal women, the effect of resistance training on liver enzymes and platelets was investigated and it was shown that resistance training in postmenopausal women has no significant effect on platelets but reduces liver enzymes and as a result, inflammation is reduced (15). A study also showed that aerobic exercise reduced the total number of white blood cells in postmenopausal women (7).

Considering the importance of menopause and finding various factors that can improve the effects of estrogen deficiency during this period, and since very few studies have been conducted about the changes in white blood cells and its subsets, blood platelets and their association with changes in lipid profile and body weight after exercise during estrogen depletion, this study was performed to evaluate some lipid and hematological parameters of blood plasma after eight weeks of aerobic exercise during estrogen deficiency in ovariectomized rats, which is a condition similar to menopause.

## Methods

After approval by the Ethics Committee of Mazandaran University with the code IR.UMZ.REC.1400.005, this experimental study was performed on 40 11-week-old Wistar female rats with an average weight of 200±10. All animals at all stages of the experiment were under close control in terms

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of ambient temperature (23±2 °C) and light/dark cycle (12h:12h). In all stages of the research, the animals had access to sufficient water and food, and all steps were performed in accordance with the "Guide for the Care and Use of Laboratory Animals" approved by the University of Mazandaran.

**Surgery:** To perform ovariectomy, after anesthesia with ketamine (70 mg/kg) and xylazine (3-5 mg/kg) by intraperitoneal injection and by making an incision to the skin and muscle in the lower abdomen, the ovaries were removed.

**Exercise:** After undergoing a two-week recovery period after ovariectomy, all rats were randomly divided into four groups: control-healthy, control-ovariectomy, exercise-healthy, and exercise-ovariectomy. In the first week, the animals were familiarized with the treadmill in a way that the rats ran on the treadmill for 15 minutes a day at a speed of 12 meters per minute without slope. After that, the rats exercised on a treadmill for eight weeks at a speed of 25 meters per minute for 60 minutes a day. In order to match the conditions for the experimental and control groups, the control group also walked on a treadmill for 10 minutes a day at a speed of 10 meters per minute (16).

**Sample collection:** 48 hours after the last training session and after 12 hours of fasting, the animals were anesthetized with ketamine-xylazine injection (70 mg/kg ketamine and 3-5 mg/kg xylazine) and blood sample was collected from the left ventricle. After centrifugation (3000 rpm in 15 minutes), the study variables including white blood cells and its subset, platelets and lipid profile were measured by bionic kit (Tehran, Iran).

**Statistical analysis:** Before performing statistical tests, Kolmogorov-Smirnov and Levene's tests were used to evaluate the normality of data distribution and homogeneity of variance. Then, to evaluate intragroup changes, two-way ANOVA test was used to evaluate the interaction effect of exercise and ovariectomy, while Tukey test was used to evaluate the intergroup changes. For data analysis, GraphPad Prism software version 6.07 and Excel 2016 software were used and $p<0.05$ was considered significant.

## Results

The results showed that ovariectomy resulted in a significant increase in the mean final body weight (g) in the control-ovariectomy group (240.20±11.37) compared to the healthy-control group (198.36±6.79) ($p\leq0.01$). In addition, aerobic exercise caused a significant increase in final body weight in the exercise-ovariectomy group (252.90±8.01) compared to the control-ovariectomy group (240.20±11.37) and an increase in the exercise-healthy group (216.00±4.18) compared to the control-healthy group (198.36±6.79), which was not significant.

The results of lipid profile analysis showed that triglyceride levels in the control-ovariectomy group (82.33±12.38) increased significantly compared to the control-healthy (36.83±3.55) and exercise-healthy groups (39.83±2.60) ($p\leq0.01$, Table 1). In addition, triglyceride levels in the exercise-ovariectomy group (71.33±9.29) showed a significant increase compared to the control-healthy group (36.83±3.55) ($p\leq0.05$) (Table 1). Total cholesterol levels in the control-ovariectomy group (130.66±3.82) increased significantly compared with the control-healthy (95.5±1.33) and exercise-healthy (95.83±3.67) groups ($p\leq0.0001$, Table 1). The results also showed that the level of total cholesterol in the exercise-ovariectomy group (108±3.53) decreased significantly compared to the control-ovariectomy group (130.66±3.82) ($p\leq0.001$) (Table 1).

The results of two-way ANOVA test showed that the effect of ovariectomy ($p=0.0001$) and the effect of exercise ($p=0.03$) on white blood cells count were significant. The results showed that the interaction effect of aerobic exercise and ovariectomy on white blood cell count was not significant. The results of Tukey post hoc test showed that ovariectomy caused a significant increase in white blood cell count in the control-



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ovariectomy group (6.33±1.39) compared to the healthy-exercise group (3.68±0.74) (p≤0.05). In addition, white blood cell count in the exercise-ovariectomy group (6.043±0.72) showed a significant increase compared to the healthy-exercise group (3.68±0.74) (p≤0.001) (Table 2).

**Table 1. Mean and standard deviation of lipid profile levels in experimental groups (n=6)**

| Groups / Variable | Exercise-ovariectomy Mean±SD | Control-ovariectomy Mean±SD | Exercise-healthy Mean±SD | Control-healthy Mean±SD |
|---|---|---|---|---|
| Triglycerides (mg/dL) | 71.33±9.29[bc] | 82.33±12.38[b] | 39.83±2.60[ac] | 36.83±3.55[a] |
| Total cholesterol (mg/dL) | 108±3.53[a] | 130.66±3.82[b] | 95.83±3.67[a] | 95.5±1.33[a] |
| Low density cholesterol (mg/dL) | 68.12±3.94[a] | 68.035±2.34[a] | 64.95±3.51[a] | 57.35±1.62[a] |
| High density cholesterol (mg/dL) | 34.33±1.34[a] | 34.66±2.5[a] | 37.66±2.09[a] | 32.33±1.5[a] |

**Non-similar letters in each row indicate significant difference.**

**Table 2. The mean levels of white blood cells, neutrophils, lymphocytes, monocytes and platelets in experimental groups (n=6)**

| Groups / Variable | Exercise-ovariectomy Mean±SD | Control-ovariectomy Mean±SD | Exercise-healthy Mean±SD | Control-healthy Mean±SD |
|---|---|---|---|---|
| White blood cells (×1000/µl) | 6.043±0.72[ac] | 6.33±1.39[ac] | 3.68±0.74[ab] | 5.091±0.56[abc] |
| Platelets (×1000/µl) | 627.16±28.74[a] | 684.16±29.89[a] | 592.3±16.10[a] | 602.33±24.25[a] |
| Neutrophils (×1000/µl) | 1.122±0.038[a] | 1.67±0.46[a] | 0.696±0.11[a] | 0.771±0.15[a] |
| Lymphocytes (×1000/µl) | 3.9±0.27[a] | 2.91±0.44[a] | 2.6±0.22[a] | 2.68±0.37[a] |
| Monocyte (×1000/µl) | 0.268±0.03[a] | 3.73±0.58[b] | 0.146±0.04[a] | 0.233±0.06[a] |

**Non-similar letters in each row indicate significant difference.**

The results of two-way ANOVA test showed that the effect of ovariectomy (p≤0.0001), the effect of exercise (p≤0.0001) and the interaction effect of exercise and ovariectomy (p≤0.0001) on monocyte count were significant. The results of Tukey post hoc test showed that ovariectomy caused a significant increase in the number of monocytes in the control-ovariectomy group (3.73±0.58) compared to the healthy-control group (0.233±0.06) (p≤0.0001) and exercise-healthy group (0.146±0.04) (p≤0.0001). In addition, monocyte count in the control-ovariectomy group (3.73±0.58) showed a significant increase compared to the exercise-ovariectomy group (0.268±0.03) (p≤0.0001).

The results of two-way ANOVA test of platelet count showed that the effect of ovariectomy on platelet count was significant (p=0.03). The results showed no significant effect of aerobic exercise factor (p=0.2) and the interaction effect of exercise and ovariectomy (p=0.3). Although platelet count in the ovariectomy group increased compared to other groups, no significant difference was observed with other experimental groups in this study. Platelet count in the exercise-ovariectomy group (627.16±28.74) decreased compared to the control-ovariectomy group (684.16±29.89), which was also not statistically significant (p=0.4). No significant result was observed in other factors. The results of two-way ANOVA test showed that only the effect of ovariectomy on neutrophil count (p≤0.016) and lymphocyte count (p≤0.037) was significant. Neutrophil count decreased in the exercise-ovariectomy group (1.122±0.038) compared to the control-ovariectomy group (1.67±0.46), which was also not statistically significant (p=0.43). Lymphocyte count in

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the exercise-ovariectomy group (3.9±0.27) increased compared to the control-ovariectomy group (2.91±0.44), which was also not statistically significant (p=0.43).

## Discussion

The results of the present study showed that ovariectomy caused weight gain, and increased triglycerides and total cholesterol, but the interaction effect of exercise and ovariectomy failed to improve the disorders caused by weight gain and triglycerides and also failed to improve the effects of ovariectomy on white blood cells and platelets. Eight weeks of aerobic exercise in this study could not improve weight gain due to ovariectomy, which is similar to the results of studies by Pighon et al. and Farahnak et al. (17, 18). These results are not consistent with the studies by Hao et al., which showed that eight weeks of moderate-intensity aerobic exercise can improve weight gain due to ovariectomy (19). On the other hand, as ovariectomy in animals leads to weight gain and obesity, several studies have shown that menopause in women increases BMI and obesity (1). However, some studies have reported no association between ovary removal and BMI (20).

In the present study, body weight increased in ovariectomized rats after exercise, but since visceral fat and bone density were not measured, it is difficult to determine which part of the body had been more influenced by exercise. On the other hand, it was observed that exercise increased body weight in healthy rats; however, this weight gain was not significant. Since estrogen is directly related to muscle building and bone formation (21), exercise has a greater effect on body weight in healthy rats with higher estrogen levels and this compensatory weight gain may occur in muscle and bone, and this might have been the cause of increase in body weight after exercise in exercise-ovariectomy group. In this study, ovariectomy increased plasma levels of triglycerides and total cholesterol, and exercise could only reduce total cholesterol in ovariectomized rats, but had no significant effect on HDL, LDL, and TG levels, which is consistent with studies by Saengsirisuwan et al. and Kazeminasab et al. (22, 23). These studies have shown that in ovariectomized animals and postmenopausal women, exercise lowers total cholesterol but does not cause any significant change in triglyceride. Mawi et al. showed that 12 weeks of moderate-intensity aerobic exercise could lower total cholesterol and triglyceride levels in a positive way compared to control group (24).

Considering the effect of ovariectomy and overweight on white blood cells, the present results are in accordance with the research of Kim et al. who reported that the total number of white blood cells and its subset increases with weight (25). In this regard, it was observed in a study that overweight men with low level of fitness experience higher levels of white blood cells, neutrophils, lymphocytes and basophils compared to men with higher level of fitness (26). In the present study, exercise could not have a significant lowering effect on white blood cells in ovariectomized rats. However, in a study by Johannsen et al., it was demonstrated that after adaptation to aerobic exercise, white blood cell count and neutrophil count decrease in obese postmenopausal women and when exercise improves abdominal obesity, it reduces inflammatory cytokines such as interleukin 6 and adiponectin and may have the greatest effect on correcting total white blood cell count (7). Another study, inconsistent with the present study, showed that six months of aerobic exercise was able to reduce total white blood cell count and neutrophil count in sedentary, overweight and postmenopausal women (27). As mentioned earlier, there is a significant relationship between white blood cell count and predictors of inflammation that occur after lipid profile disorder and overweight (28). Physical activity seems to help reduce systemic inflammation and aerobic exercise reduces white blood cell count and associated inflammatory markers (29, 30). In this study, no such effect of exercise on white blood cells was observed, which may be due to lack of change in body weight. In the present study, ovariectomy also


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increased the number of monists, and exercise in the ovariectomy group was able to reduce it greatly. Disruption of lipid profile has been shown to lead to monocytosis, and hyperlipidemia alters the production of monocyte precursors and adult monocytes from the bone marrow, causing lipid droplets to accumulate in monocyte subsets and pro-inflammatory monocytes, and increased tissue damage during atherogenesis. Considering the effect of exercise on monocytosis, the present results are in line with the study of Timmerman et al. who showed that strength endurance training reduces the number of monocytes in a passive demographic model (31). Since exercise had no effect on platelet activation in the present study, the current results are incontinent with the study of Heber et al. who reported that exercise reduces platelet aggregation in postmenopausal women (32). Lundberg et al. also reported that platelet activity levels increased after ovariectomy (33). Platelets contain estrogen receptor beta, and this receptor prevents the accumulation of platelets in the presence of estrogen, but when this effect disappears during menopause, the platelets are more likely to coalesce and cause coagulation disorders (34).

Overall, total white blood cells and platelets, which can be potential indicators for the effectiveness of exercise and the reduction of mild inflammatory diseases, were not affected by intensity and duration of exercise, and it seems that changes in total white blood cell count and platelet count may be related to changes in physical and physiological mechanisms associated with increased level of fitness because it has been shown that aerobic exercise during menopause, even up to the recommended intensity, may not directly affect platelets, adiponectin, monocytes, lymphocytes or basophils (7). But even if exercise is not able to show positive effects on some of our target factors, it may alter the course of subsets of white blood cells and platelets between the secondary lymphatic organs and the blood. Since the amount of visceral fat was not measured in this article, by measuring visceral fat in animal samples and measuring body composition in human samples with the help of body composition analysis, we can have a better comparison between the effectiveness of the intensity and type of exercise and body composition on white blood cells and platelets in future studies.

The results of the present study showed that removal of the ovaries causes weight gain, increases triglycerides, total cholesterol, white blood cells, platelets and thus increases inflammation, and aerobic exercise can partially improve some of these changes in lipid and hematological parameters caused by ovariectomy. It seems that aerobic exercise can improve the effects of ovariectomy on white blood cells and platelets after adaptation, which has a significant positive effect on body weight and some lipid profile parameters.

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