Effects of aerobic exercise training on visceral fat and serum adiponectin concentration in ovariectomized rats

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ABSTRACT

 Objective The aim of this experimental study was to investigate the effects of exercise training on visceral fat and adiponectin levels in ovariectomized (OVX) rats.

 Method Female Wistar rats were divided into OVX (n = 20) and sham-operated control (SHAM; n = 8) groups. OVX rats were subdivided into a sedentary (OVX-SED; n = 10) and an exercise (OVX-EX; n = 10) group. The exercise consisted of 8 weeks of aerobic exercise (26 m/min, 5 days/week, 60 min/day, 10% slope).

 Results In OVX rats, body weight was 21% greater (255.2 ± 9.31 vs. 211.63 ± 3.23; p < 0.01) and visceral fat was 29% greater (10.87 ± 0.66 vs. 8.43 ± 0.45; p < 0.05) than in SHAM rats. After training, visceral fat was 20% lower in OVX-EX rats than in OVX-SED rats (8.72 ± 0.46 vs. 10.87 ± 0.66; p < 0.05). After 8 weeks of running on the treadmill, levels of serum glucose, insulin and serum adiponectin, and the homeostasis model assessment of insulin resistance were not changed significantly in the OVX-EX group.

 Conclusion These results suggest that 8-week exercise training induces a decrease in visceral fat, and this reduction without weight loss does not change serum adiponectin levels and insulin sensitivity in ovariectomized rats.

INTRODUCTION

Obesity is a common problem after menopause and is a major risk factor for the development of cardiovascular diseases. Estrogen has great importance in the determination of body fat distribution1–3 and deficiency of this hormone tends to lead to an accumulation of visceral fat4–6. Excess visceral adipose tissue accumulation after menopause is highly associated with decreases in insulin sensitivity, a predisposing factor for cardiovascular disease7–9. Consistent with these studies, rodent models have revealed that removal of visceral fat leads to a marked improvement in insulin resistance10.

Recently, it has become evident that adipose tissue is a source of metabolically active cytokines such as adiponectin and its levels decrease in obese humans11 and postmenopausal women12. A decrease in adiponectin levels is postulated not only

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to promote visceral fat accumulation, but also to have a role in the pathogenesis of insulin resistance\textsuperscript{11} and cardiovascular diseases\textsuperscript{13}. It has been shown that the use of hormone replacement therapy to prevent obesity-related diseases in women has some risks\textsuperscript{14}; other alternative therapies such as exercise and diet have been recently considered\textsuperscript{15}. In contrast to diet therapy, weight loss followed by exercise training has no negative effects on muscles\textsuperscript{16,17}.

To our knowledge, the effects of exercise on visceral fat and adiponectin levels in the absence of estrogen have not been fully understood\textsuperscript{18–23}. Therefore, we evaluated the effect of exercise training on visceral fat, insulin sensitivity and adiponectin concentration in ovariectomized rats. We also examined the relationship between body weight, visceral fat, insulin sensitivity and adiponectin concentration in trained and control groups. Since aging is associated with an increase in visceral fat and insulin resistance\textsuperscript{24–26}, we conducted our experiment on ovariectomized, young adult rats, similar to Ainslie and colleagues\textsuperscript{4} and Bellino\textsuperscript{27}.

**MATERIAL AND METHODS**

**Animal care**

Female Wistar rats ($n=32$; Razi Institute, Tehran, Iran) weighing 170–185 g were housed four per cage and fed standard-pellet rat chow and tap water \textit{ad libitum}. The 12-h:12-h light cycle started at 07.00 and the room temperature was maintained at 21–23°C. The experiments described in this study were conducted according to the policy of the Iranian Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes, and the protocol was approved by the Ethics Committee of Guilan University of Medical Sciences, Rasht, Iran.

**Groups and surgery**

Two weeks after acclimatization, 11-week-old rats were randomly divided into two groups: the sham-operated control group (SHAM) and the ovariectomized (OVX) group. At the onset of the experiment, OVX rats were randomly divided into two subgroups: OVX + sedentary (OVX-SED) and OVX + exercise (OVX-EX). Rats were ovariectomized and sham-operated under general anesthesia with an intraperitoneal injection of ketamine (50 mg/ml; Rotex Medica, Germany) and xylazine (20 mg/ml; Alfasan, the Netherlands) in a ratio of 4:1, according to the technique described by Shinoda and colleagues\textsuperscript{28}.

**Protocol for exercise training**

Rats in the OVX-EX group were trained to run on a motor-driven treadmill (Iranian Model, eight lanes, designed by Sports Sciences Research Center, Tehran, Iran) from the 3rd week after ovariectomy (14 weeks old). During the first week of training, rats began to practice walking at the speed of 6 m/min, 10 min/day, without any inclination of the treadmill and then were trained to run at the experimental protocol. Exercise training consisted of running on the rodent treadmill for 5 days/week for 8 weeks. Rats progressively ran from 15 min/day at 15 m/min, 0% slope, up to 60 min/day at 26 m/min, 10% slope for the last 4 weeks\textsuperscript{28}. The regular endurance exercise used in this study is equivalent to 70–85% VO$_{2\text{max}}$\textsuperscript{29}. It has been established that this protocol induces a reduction in visceral fat and improvement of insulin sensitivity in intact and OVX rats\textsuperscript{8,30,31}.

**Body weight, food intake and body mass index**

Body weight, food intake and visceral fat were measured by using a scale (Sartorius, Germany) accurate to 0.1 g. All rats were weighed twice weekly, between 09.00 and 11.00. The average of these two weights was calculated to yield a weekly weight for each animal.

To measure the food intake, an equal amount of food (20 g/day/rat) was given to each rat in each cage in each identical group, and food consumption was measured by subtracting the weight of the remaining uneaten food. The difference between the weights was divided by the number of rats in the cage. After 8 weeks of exercise, rats were anesthetized as described for the measurement of body length (nose-to-anus). The body weight and body length were used to determine the body mass index (BMI) according to the equation: BMI (g/cm$^2$) = body weight (g)/length (cm)$^2$.

**Blood analysis and visceral adipose tissue**

At the end of the treatment period, all animals were killed between 09.00 and 23.00. All trained rats were restrained from training 40 h before...
sacrifice, and food was removed from the animal’s cage at least 12 h before sacrifice. After complete anesthesia, the abdominal cavity was rapidly opened and blood samples were drawn from the inferior vena cava. The serum was separated by centrifugation (3000 rpm for 15 min) and stored at −80°C for later biochemical and hormonal measurements. Serum adiponectin concentration was measured in duplicate by a rat adiponectin ELISA kit (AdipoGen, Seoul, Korea).

The serum glucose concentration was determined by enzymatic (GOD-PAP, glucose oxidase-amino antipyrine) colorimetric method (Pars Azmoun, Tehran, Iran) and the serum insulin concentration was measured by a rat insulin ELISA kit (DRG, USA). HOMA-IR (fasting insulin (µU/ml) × fasting glucose (mmol/l)/22.5) was used to estimate the insulin resistance. Serum total cholesterol and high density lipoprotein cholesterol (HDL-C) were determined by the enzymatic (CHOD-PAP, Pars Azmoun, Tehran, Iran) colorimetric method. Serum triglyceride was determined by the enzymatic (GPO-PAP, Pars Azmoun, Tehran, Iran) colorimetric method. The procedure of Friedewald and colleagues 32 was used to estimate low density lipoprotein cholesterol (LDL-C). All measurements were performed in duplicate.

After collecting the blood samples, all intra-abdominal fat depots including mesenteric, urogenital and retroperitoneal, were dissected out by one experimenter and weighed immediately after dissection to avoid weight loss by evaporation. The mesenteric fat pad consisted of the adipose tissue surrounding the gastrointestinal tract from the gastroesophageal sphincter to the end of the rectum, with special care taken in distinguishing and removing pancreatic cells. The urogenital fat pad included the adipose tissue surrounding the kidneys, ureters and bladder as well as the ovaries, oviducts and uterus. The retroperitoneal fat pad was taken as the distinct deposit behind each kidney along the lumbar muscles.

### Statistical analysis

All data are presented as mean ± standard error. Before statistical analysis, the normal distribution and homogeneity of the variances were tested. Parameters that did not fulfill these tests (HOMA index, serum insulin, serum adiponectin) were log-transformed. Statistical comparisons between groups were performed by the one-way ANOVA test, followed by Tukey’s post-hoc test. Correlation between body weight, visceral fat and the HOMA index with serum adiponectin concentrations were analyzed with the Pearson’s correlation method. Levels of statistical significance were set at $p < 0.05$.

### RESULTS

Twenty-eight animals successfully completed the intervention protocols. At the beginning of the study, there was no difference in body weight between the groups (Figure 1a). Figure 1a also illustrates the increased rate of weight gain that occurred after ovariectomy. Figure 1b shows that the average weekly food intake in trained rats was significantly ($p < 0.05$) higher than in other groups.

At the end of the experiment, body weight was 21% ($p < 0.01$) and BMI was 17% ($p < 0.01$) greater in the OVX-SED group compared to the SHAM rats (Table 1). The OVX-SED group had a 29% ($p < 0.05$) greater visceral fat level than the SHAM rats. Serum glucose levels decreased by 17% and insulin levels increased by 14% after ovariectomy. OVX-SED rats had a 16% greater HOMA-IR index and 9% greater serum adiponectin concentration compared to the SHAM group; however, these increases were not significant. No significant differences in lipid profiles were observed between OVX-SED and SHAM rats (Figure 2).

Exercise reduced visceral fat significantly by 20% ($p < 0.05$) in the OVX-EX group compared to the OVX-SED rats, whereas body weight and BMI remained unchanged (Table 1). Exercise increased serum glucose levels by 15% and decreased serum insulin levels by 25% in the OVX-EX group. HOMA-IR index and serum adiponectin levels decreased by 31% and 3.5%, respectively in the OVX-EX group compared to the OVX-SED rats. However, these changes were not significant (Table 1). In addition, lipid profiles did not show any significant changes after an 8-week exercise intervention (Figure 2). No significant correlation was found between serum adiponectin levels and body weight, visceral fat and HOMA-IR index.

### DISCUSSION

It is well documented that ovariectomy results in a significant reduction in circulating 17β-estradiol levels4,33–35 and consequently leads to weight gain and an increase in adipose tissue1,2,4,28,33,35. Therefore, estrogen deficiency and weight gain are indices of the effectiveness of ovariectomy1,36,37. In the...
present study, ovariectomy resulted in a significant increase in body weight and also an increase in visceral fat. Since there were no significant differences in food intake between the OVX-SED and SHAM rats, the weight gain in OVX-SED rats could not be due to an increase in food intake. This

Figure 1  (a) Average body weight during the intervention period and (b) average food intake per rat per day. SHAM, sham-operated group; OVX-SED, sedentary ovariectomized group; OVX-EX, ovariectomized-exercise group. *, p < 0.05 vs. exercise (EX) group. Data are means ± standard error

Figure 2  (a) Total cholesterol (TC), (b) triglyceride (TG), (c) high density lipoprotein cholesterol (HDL-C), (d) low density lipoprotein cholesterol (LDL-C) in sham-operated (SHAM) group, sedentary ovariectomized (OVX-SED) group and ovariectomized-exercise (OVX-EX) group. Data are means ± standard error
Table 1  The metabolic variables and hormonal and morphometric measurements after the 8-week experimental period. Data are means ± standard error

<table>
<thead>
<tr>
<th></th>
<th>SHAM (n = 8)</th>
<th>OVX-SED (n = 10)</th>
<th>OVX-EX (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>211.63 ± 3.23</td>
<td>255.2 ± 9.31**</td>
<td>261.8 ± 5.34**</td>
</tr>
<tr>
<td>Body mass index (g/cm²)</td>
<td>0.47 ± 0.01</td>
<td>0.55 ± 0.02**</td>
<td>0.51 ± 0.01</td>
</tr>
<tr>
<td>Visceral fat (g)</td>
<td>8.43 ± 0.45</td>
<td>10.87 ± 0.66*</td>
<td>8.72 ± 0.46↑</td>
</tr>
<tr>
<td>Insulin (mU/ml)</td>
<td>1.59 ± 0.17</td>
<td>1.85 ± 0.16</td>
<td>1.39 ± 0.14</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>6.53 ± 0.68</td>
<td>5.41 ± 0.34</td>
<td>6.39 ± 0.32</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.03 ± 0.16</td>
<td>1.22 ± 0.17</td>
<td>0.84 ± 0.14</td>
</tr>
<tr>
<td>Adiponectin (ng/ml)</td>
<td>1.30 ± 0.05</td>
<td>1.43 ± 0.03</td>
<td>1.38 ± 0.03</td>
</tr>
</tbody>
</table>

SHAM, sham operated group; OVX-SED, ovariectomized sedentary group; OVX-EX, ovariectomized-exercise group; HOMA-IR, homeostasis model assessment of insulin resistance; insulin, HOMA-IR and adiponectin concentrations were log-transformed

*, p < 0.05 vs. SHAM group; **, p < 0.01 vs. SHAM group; †, p < 0.05 vs. OVX-SED group

indicates that the lack of estrogen following ovariectomy influences body weight, partially by increasing visceral fat1–4. Consistent with this result, a study on mice deficient in estrogen receptor α showed a marked increase in adipose tissue mass38. There is extensive evidence that estrogen is an important regulator of female adipose deposition2–4,5 and may exert an important regulatory role in adipocyte metabolism6. Some part of the redistribution of fat to the intra-abdominal depot in the absence of estrogen relates to the change in lipoprotein lipase activity or decreased lipolysis1.

In the present study, there were no significant changes in glucose and insulin levels and the HOMA index, whereas visceral fat increased in ovariectomized rats. These results indicate that accumulation of visceral fat following ovariectomy is only one of several factors determining insulin sensitivity. Our finding is in agreement with the findings of Toth and colleagues39 who reported that early postmenopausal women with greater intra-abdominal fat mass compared to late premenopausal women had no differences in insulin sensitivity. However, there are some inconsistent studies indicating that excess visceral fat accumulation following estrogen deficiency is associated with insulin resistance1,6,8,40.

One of the findings of this study was that the 8-week exercise training program increased body weight and food intake, but remarkably decreased the visceral fat. The reduction in visceral fat with no significant changes in body weight probably reflected an increase in another component of the body (e.g. muscle weight) after exercise training28.

The lack of significant reduction in insulin sensitivity by exercise in the present study probably relates to the lack of dietary restriction. As mentioned earlier, the average food consumption was significantly higher in the exercise group compared to the control group. Exercise with energy restriction is postulated to result in a decreased demand for insulin and thus attenuate beta cell exhaustion31. It is thought that much of the effects of exercise on insulin action occur by energy restriction-induced weight loss42.

Moreover, there were no significant changes in lipid profiles after ovariectomy and 2 months of exercise. Many studies have reported that lipid profiles undergo changes during the menopause and ovariectomy. In women, menopause is associated with increased concentrations of triglycerides, total cholesterol and LDL cholesterol and decreased concentrations of HDL cholesterol43,44. Our findings are consistent with the results of the study by Antunes and colleagues36 which reported no significant differences in levels of triglycerides, total cholesterol and LDL cholesterol in ovariectomized rats. However, the findings of Meli and colleagues37, showing an increase in the number of lipid profile components, are not in agreement with our study. We also showed that 2 months of exercise did not affect significantly the serum lipid levels in OVX rats. Similar findings have been previously reported by Shinoda and colleagues38 and Klebanoff and colleagues45.

There are only a few studies that have examined the effect of hypoestrogenism on plasma adiponectin concentrations in humans and they have discrepant results36,47. To our knowledge, we have studied the level of adiponectin concentration in ovariectomized rats for the first time and we found no significant changes in serum adiponectin levels 2 weeks after ovariectomy. This is in contrast to
some studies in which plasma adiponectin levels correlate inversely with adiposity. Surprisingly, in spite of significant weight gain in the ovariectomized rats, the serum adiponectin concentration not only did not reduce but also slightly increased. However, other studies have reported a reduction in adiponectin levels in obese and postmenopausal women. In addition to adipose tissue, it seems that estrogen may be involved in the determination of serum adiponectin levels.

In this study, moderate-intensity exercise did not induce a significant change in serum adiponectin levels despite a significant decrease in visceral fat in ovariectomized rats. This finding is consistent with the findings of Boudou and colleagues who found a similar level of adiponectin in trained and control groups of diabetics. Consistent with this, O’Leary and colleagues and Hulver and colleagues reported unchanged adiponectin levels following aerobic exercise alone, despite the decrease in visceral fat. The lack of a significant change in adiponectin concentrations is in agreement with the finding of Kraemer and colleagues and Boudou and colleagues, indicating that the loss of body weight is necessary for increases in adiponectin levels. In our study, the weight loss was not achieved in trained rats, probably because of the increase in food consumption which has been reported in previous studies too. Calorie restriction is an important factor that increases insulin sensitivity and adiponectin levels in rodent models. Hara and colleagues also observed a decrease in fat mass without a significant increase in plasma adiponectin levels after aerobic exercise training in young obese men.

The response of adiponectin to exercise training is not yet well known and the literature shows unchanged, increased or decreased adiponectin levels. One of the reasons for the discrepancy in the literature could be due to the volume of exercise training. Kraemer and colleagues, in a review study, suggested that training for longer than 2 months that utilizes enough exercise volume to decrease body weight and increase insulin sensitivity will increase plasma adiponectin concentration. It seems that the 2 months of exercise used in our study was not long and intensive enough to induce significant changes in serum adiponectin levels.

Finally, we did not observe any significant correlation between adiponectin concentration and body weight, visceral fat and insulin resistance. The increase in visceral fat following ovariectomy with no changes in adiponectin levels might explain why adiponectin levels are not only determined by visceral fat in ovariectomized rats.

CONCLUSIONS
The results from the present study suggest that 8 weeks of exercise training successfully targets and decreases intra-abdominal fat, but does not influence serum adiponectin levels in ovariectomized rats. Exercise training, as an effective treatment program in preventing metabolic syndrome, acts partially by decreasing visceral fat; adiponectin is not necessarily a mediator of metabolic syndrome in ovariectomized rats. Additional investigation is necessary to evaluate the mechanisms underlying adiponectin levels in the estrogen deficiency status. Therefore, from a clinical point of view, we conclude that aerobic exercise training is a positive strategy to control visceral fat accumulation in postmenopausal women.

Conflict of interest Nil.

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