Effects of ovariectomy and estrogen replacement therapy on visceral adipose tissue and serum adiponectin levels in rats

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Abstract

Background. Excess visceral adipose tissue accumulation after menopause is closely associated with decreased insulin sensitivity and adiponectin levels.

Objective. The purpose of this study was to determine the effect of ovariectomy and estrogen replacement on visceral fat and serum adiponectin levels in ovariectomized (OVX) rats.

Method. Forty 11-week-old female Wistar rats were divided into the four following groups (n = 10 rats per group): sham-operated control (SHAM); sedentary OVX (OVX-SED); OVX with estrogen replacement (OVX-ER); and OVX with sesame oil treatment (OVX-C). Rats in OVX-ER and OVX-C groups received 17β-estradiol valerate (30 μg/kg, subcutaneously) and sesame oil as vehicle, five days a week, respectively. All animals were sacrificed after eight weeks of intervention.

Results. Ovariectomy after eight weeks increased body weight and visceral fat (P, 0.05) in OVX-SED and OVX-C groups compared with SHAM rats with no change in plasma adiponectin levels. Estrogen replacement in OVX animals decreased body weight (13.4%, P, 0.05) and visceral fat (10.4%). Although they were not statistically significant, adiponectin, insulin sensitivity and lipid profile of OVX rats were ameliorated with estrogen treatment.

Conclusion. We conclude that ovarian hormone withdrawal leads to higher body weight and visceral adipose tissue in rats, but surprisingly does not change adiponectin levels. Although a substantial decrease in body weight was achieved by estrogen replacement therapy in OVX animals, the beneficial metabolic effects of weight loss seems to be only mechanical, having a tendency to improve insulin sensitivity without elevating adiponectin production.

Keywords: Ovariectomy, lipid profile, 17β-estradiol, HOMA-IR, adiponectin

Introduction

Estrogen deficiency is associated with an increase risk of cardiovascular diseases (CVD) in postmenopausal women.1–3 One important reason for this might be greater visceral fat and consequently insulin resistance.2–4 Cross-sectional studies have indicated that estrogen deficiency accelerates the selective deposition of abdominal fat and tends to accumulate visceral fat.5,6 In rodents, ovariectomy, which results in a dramatic reductions in circulating estrogen levels,5,7 is similar to naturally occurring menopause8,9 and provides an appropriate research tool to mimic the postmenopausal hormonal states in humans to study abdominal obesity,10 lipid metabolism11 and insulin resistance.12

Recently, it has become evident that adiponectin, an adipocyte-secreted hormone, has a protective role against atherosclerosis13 and is inversely correlated with accumulation of abdominal fat mass and insulin resistance.14–16 Based on this information, we hypothesized that adiponectin levels and lipid metabolism are modified in the absence of estrogens, which promotes visceral fat...
accumulation and other clusters of metabolic syndrome components.

There are different therapies for the prevention of obesity-related consequences in postmenopausal women such as exercise, diet and hormone replacement therapy.17,18 Our previous study indicated that aerobic exercise training failed to change adiponectin and insulin levels in ovariectomized (OVX) animals.19 Higher concentrations of adiponectin in women than in men suggest that estrogen might have a stimulatory impact on the production of adiponectin. Contradictory findings exist in the literature describing reduction20 as well as no change in plasma adiponectin concentrations by estrogens. Therefore, we investigated whether the loss and replacement of ovarian hormones would alter lipid profile, adiponectin levels and insulin sensitivity in ovariectomized rats.

Material and methods

Animal care

Female Wistar rats (n = 40) weighing 160–175 g were housed four per cage and fed standard pellet rat chow and had free access to tap water. The 12:12-h light cycle started at 07:00 hours and the room temperature was maintained at 21–23 °C. The experiments described in this report were conducted according to the policy of Ethics Committee of the Guilan University of Medical Sciences.

Groups and treatment protocol

Forty 11-week-old female Wistar rats were divided into the four following groups (n = 10 rats per group): sham-operated control (SHAM); sedentary (OVX-SED) group; OVX with estrogen replacement (OVX-ER); and OVX and sesame oil treatment or vehicle control (OVX-C). Rats were ovariectomized and sham-operated according to the technique described in our previous study.19 Rats in the OVX-ER group and OVX-C group received subcutaneous injections of 17β-estradiol valerate (30 μg/kg bw; Bayer Schering Pharma, Berlin, Germany) in 0.2 mL sesame oil as vehicle (sesame oil; Aburaihan Pharma Co, Tehran, Iran), five days a week, for eight weeks. Mesenteric fat pad consisted of adipose tissue surrounding the gastrointestinal tract from the gastro-esophageal sphincter to the end of the rectum with special care taken in distinguishing and removing pancreatic cells. Urogenital fat pad included adipose tissue surrounding the kidneys, ureters, and bladder as well as ovaries, oviducts and uterus. Retroperitoneal fat pad was taken as that distinct deposit behind each kidney along the lumbar muscles.

Analytical procedure

Rats were sacrificed between 09:00 and 12:00. The food was removed from the animal’s cage at least 12 hours before sacrifice. After complete anaesthesia, 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 concentrations were measured by rat adiponectin ELISA kit (AdipoGen, Seoul, Korea).

The serum glucose concentrations were determined by enzymatic (GOD-PAP, glucose oxidase-amino antipyrine) colorimetric method ( Pars Azmoun, Tehran, Iran) and serum insulin levels were measured by rat insulin ELISA kit (DRG, USA). The homeostasis model assessment (HOMA-IR: [fasting insulin (μU/mL) × fasting glucose (mmol/L)]/22.5) was used to estimate the insulin resistance. Serum total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) were determined by enzymatic (CHOD-PAP, Pars Azmoun, Tehran, Iran) colorimetric method. Serum triglyceride (TG) was determined by enzymatic (GPO-PAP, Pars Azmoun, Tehran, Iran) colorimetric method. The procedure of Friedewald et al.22 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 evaporative weight loss. Mesenteric fat pad consisted of adipose tissue surrounding the gastrointestinal tract from the gastro-esophageal sphincter to the end of the rectum with special care taken in distinguishing and removing pancreatic cells. Urogenital fat pad included adipose tissue surrounding the kidneys, ureters, and bladder as well as ovaries, oviducts and uterus. Retroperitoneal fat pad was taken as that distinct deposit behind each kidney along the lumbar muscles.

Statistical analysis

All data are presented as mean ± s.e. Before statistical analysis, normal distribution and homogeneity of the variances were tested. Parameters that did not fulfill these tests (HOMA-IR index, serum insulin, serum adiponectin) were log transformed. Statistical comparisons between groups were performed by one-way analysis of variance test, followed by Tukey’s post hoc test. Levels of statistical significance were set at P values <0.05.

Results

After eight-week of intervention, ovariectomy resulted in increased body weight and visceral fat (P < 0.05) as observed in OVX-SED and OVX-C groups compared with SHAM animals (Figures 1 and 2, respectively). Interestingly, 17β-estradiol replacement in OVX-ER rats decreased body weight and visceral fat gain induced by ovariectomy to the levels observed in intact rats (SHAM group). There were no significant changes in serum adiponectin concentrations after eight weeks of ovariectomy. Estrogen supplementation led to a reduction in serum adiponectin levels however; this reduction was not
significant (Figure 3). Food intake and serum glucose levels did not show any significant differences between groups (Table 1). Moreover, serum insulin concentrations, as well as HOMA-IR index, were higher in OVX-SED and OVX-C animals than in SHAM control group and were reduced in OVX-ER rats (Table 1) however, they were not statistically significant. Finally, despite showing a slight change in lipid profiles after estrogen supplementation, statistically they were not significant (Table 1).

Discussion

Similar to the results of previous studies,5,7,23,24 ovariectomy in the present work led to increased body weight and visceral fat. The weight gain in OVX rats in our study is not due to an increase in food intake since we did not observe any difference in terms of energy intake between OVX-SED and SHAM rats. This is in contrast with the results of Shinoda et al.,23 showing that OVX rats are hyperphagic. Estrogen is an important regulator of female adipose tissue deposition in OVX animals.25 It seems estrogen signalling produces a preferential increase in adipose tissue,26 probably through an effect on lipid metabolism rather than influencing the central mechanisms of food intake. Interestingly, replacement of estrogen in the present study reduced body weight of OVX rats to the levels found in our control animals. Lower weight in OVX-ER than those of OVX rats indicates that low estrogen levels in OVX rats are responsible for the body weight gain. It has been shown that estrogens exert important regulatory effects on adipocyte metabolism6 and are considered as an important regulator of female adipose tissue deposition and distribution in both human and rodents.7,25,26 For instance, while estrogens favour lipolysis in adipocytes, it appears that fat lipolysis is reduced in OVX animals.24,26 Estrogen therapy in our OVX animals caused only negligible decreases in visceral fat which contradicts the results of Shinoda et al. who found estradiol replacement reduces intra-abdominal fat depots in OVX rats. This discrepancy might be related to the type, dosage and duration of estrogen replacement as well as the method used to estimate abdominal adipose tissue.26,27 For instance, they used 17β-estradiol pellets (0.025 mg/day) as their estrogen replacement protocol that was efficient for 60 days.

We initially assumed that OVX rats might have lower adiponectin levels than in SHAM rats; however, the results showed no significant changes in plasma adiponectin levels. To our knowledge, only a few studies have examined the effect of hypoestradiolism on plasma adiponectin levels and they have reported conflicting results (increased,28 no change29 and decreased plasma adiponectin levels16,30). Our results are in agreement with the findings of Nishizawa and colleagues29 showing no change of plasma adiponectin concentrations in the absence of estrogen. One cannot exclude the possibility that ovariectomy might enhance the numbers of adipocytes,31 whereas enlargement of adipocyte size is necessary for the reduction in adiponectin production.32 In other words, adiponectin metabolism in the hypoestradiolism-induced obesity seems to be under complex hormonal control.

An interesting finding of the present study is that estrogen exerts a negative impact on serum adiponectin level. Our observation is in agreement with previous studies demonstrating the involvement of steroid hormones in the regulation of adiponectin metabolism.
Estrogen replacement in estrogen-deprived animals  
metabolism is different from other forms of obesity. 

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levels. In other words, in 
adipose tissue; but, unexpectedly, this does not decrease 
that ovariectomy increases body weight and visceral 
treatment.

Table 1 The metabolic variables, hormonal and morphometric measures after eight-week experimental period

<table>
<thead>
<tr>
<th></th>
<th>SHAM</th>
<th>O VX-SED</th>
<th>O VX-ER</th>
<th>O VX-C</th>
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</thead>
<tbody>
<tr>
<td>Food intake (g/day)</td>
<td>14.67 ± 0.69</td>
<td>15.39 ± 0.36</td>
<td>17.62 ± 0.5</td>
<td>15.44 ± 0.6</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>6.53 ± 0.68</td>
<td>5.41 ± 0.34</td>
<td>5.87 ± 0.4</td>
<td>6.77 ± 0.5</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>1.59 ± 0.17</td>
<td>1.85 ± 0.16</td>
<td>1.56 ± 0.12</td>
<td>1.76 ± 0.11</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.03 ± 0.16</td>
<td>1.22 ± 0.17</td>
<td>0.97 ± 0.12</td>
<td>1.22 ± 0.09</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>76.88 ± 7.77</td>
<td>87.4 ± 3.88</td>
<td>77.56 ± 2.9</td>
<td>80.22 ± 3</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>79.75 ± 5.99</td>
<td>60.91 ± 4.5</td>
<td>86.22 ± 8.3</td>
<td>63.9 ± 4.8</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>34.13 ± 5.5</td>
<td>35.6 ± 3.5</td>
<td>37.67 ± 4</td>
<td>35.33 ± 2.9</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>26.8 ± 2.63</td>
<td>39.62 ± 2.35</td>
<td>22.64 ± 5.6</td>
<td>32.11 ± 3.45</td>
</tr>
</tbody>
</table>

SHAM, sham operated; O VX-SED, sedentary ovariectomized; O VX-ER, ovariectomized with 17β-estradiol replacement; O VX-C, ovariectomized with sesame oil treatment (vehicle); HOMA-IR, homeostasis model assessment of insulin resistance.

Values are means ± SE, n = 10 rats per group. Insulin, HOMA IR and adiponectin concentrations were logarithmically transformed.

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synthesis.21,28,33,34 Combs et al.28 reported that estrogen implantation in ovariectomized mice reduces adiponectin levels. However, Siemiska et al.21 and Gaviria et al.16 reported similar levels of adiponectin in both estradiol receiving and control premenopausal women. On the other hand, Gui et al.35 reported elevated adiponectin messenger RNA levels in estrogen receiving mouse. Nevertheless, the lack of favourable effects of weight loss after estrogen therapy on the adiponectin levels in our OVX rats may be related to the lack of comparable reductions in visceral fat.

It has been well-established that hypoestrogenism-induced visceral fat accumulation is associated with insulin resistance.4,6,17,24 In the present study we did not observe significant changes in the serum levels of glucose and insulin along with HOMA-IR ratios following ovariectomy. Our findings are consistent with the findings of Toth et al.,36 who reported that early postmenopausal women have greater intra-abdominal fat than late premenopausal women, with no differences in insulin sensitivity. Nevertheless, OVX-SED and OVX-C animals tended to have the worsen insulin sensitivity status compared with SHAM and OVX-ER rats after eight weeks of treatment. The lack of significant changes in both insulin sensitivity levels and visceral fat depots with estrogen replacement might be attributed to short period of our intervention.

In women, menopause is associated with increased concentration of TG, TC, and LDL-C and decreased HDL-C.12 In animals, similar results as higher TG, TC, LDL-C with lower HDL-C12 and increased TC as well as HDL-C with no changes in TG levels23 with OVX have been reported. In the present study, the OVX-SED rats presented no differences in lipid profiles compared with SHAM animals. 17β-estradiol supplementation in our study had the tendency to ameliorate the lipid profile of OVX animals; however, it was not statistically significant, possibly due to the lack of substantial reduction in visceral fat and/or due to the short duration of employed treatment.

In summary, the results of the present study indicate that ovariectomy increases body weight and visceral adipose tissue; but, unexpectedly, this does not decrease adiponectin levels. In other words, in hypoestrogenism-induced obesity, the adiponectin metabolism is different from other forms of obesity. Estrogen replacement in estrogen-deprived animals prevented body weight gain and had the tendency to decrease the visceral fat along with improved insulin sensitivity and lipid profile.

From a clinical point of view, the present data confirm that menopause is associated with weight gain and obesity, which is not always associated with insulin resistance. In addition, adiponectin does not appear to be the link between beneficial effect of body weight loss and reducing insulin resistance in postmenopausal hormonal context.

Competing interests: None declared.

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