*Research Article*

# Role of Sympathetic Nervous System in Ovariectomy Induced Obesity in Adult Female Albino Rats

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## Abstract

**Aim**: To investigate the potential role of the sympathetic nervous system in the development of ovariectomy (OVX)-induced obesity and its metabolic complications in adult female albino rats.
**Methods:** ٤٢ adult female albino rats were randomly divided into the following groups (٧ rats each): Control (C), OVX non-treated, and OVX-treated groups with estradiol (E), atenolol (A); (a selective ß١-adrenergic receptor blocker), reserpine (R); (an inhibitor of catecholamine reuptake at the sympathetic nerve terminals) and tyramine (T); (a stimulant of catecholamine release). Daily food intake, initial and final body mass index (BMI) was recorded for all rats. Blood samples were taken from jugular vein for determination of lipid profile, insulin (I), glucose (G), homeostasis model assessment of insulin resistance **(**HOMA-IR) and catecholamines (epinephrine; EP, norepinephrine; NE and dopamine; DA). The brains were also removed and the hypothalamic content of catecholamines was determined. Finally, peritoneal omental fat was removed as the whole gastrocolic omental fat (GCOF) and weighed. **Results:** OVX produced a significant increase in food intake and weight gain starting from the ٢nd wk after OVX till the end of the study along with significant increases in BMI, GCOF and disturbed lipid profile and insulin resistance. This was accompanied with increased sympathetic activity as evidenced by the higher serum levels and hypothalamic content of catecholamines than the control group. These effects were almost completely prevented by estradiol supplementation except for serum triglycerides (TGs) level which was significantly higher than the control group. Either blocking β١adrenergic receptors by atenolol or chemical sympathectomy by reserpine partially prevented the OVX-induced obesity and its metabolic effects. Reserpine was better than atenolol in terms of glycemic control and correction of dyslipidemia. Meanwhile, increased sympathetic activity by tyramine, failed to produce any significant change in all tested parameters except for serum TGs and insulin levels which were significantly lower than those of OVX non-treated group. **In conclusion,** OVX can cause detrimental metabolic alterations including obesity, insulin resistance, and disturbed lipid profile. Theses effects were almost completely prevented by estrogen supplementation probably due to its inhibitory effect on food intake and subsequent weight gain. Increased sympathetic activity secondary to estrogen lack could be a contributing factor in such condition possibly via stimulation of β١-adrenergic receptors present on the ghrelin secreting cells of the stomach resulting in ghrelin release.
**Keywords:** Ovariectomy, Obesity, Estradiol, Sympathetic nervous system and Ghrelin

## Introduction

The prevalence of obesity is increasing worldwide and is reaching epidemic proportions(١). Currently, there is no effective treatment for obesity, despite there are several measures used including caloric restriction, pharmacological, behavioral and surgical treatments(٢). The problem is that conventional treatment of obesity by caloric restriction reduces fat in adipocytes through increased lipolysis and hepatic oxidation of fatty acids to ketones. However, the adipocytes retain their complement of lipogenic enzymes capable of resynthesis of fat, which probably explains the high rate of relapse after such treatment(٣).

Postmenopausal women are one of the subpopulation in which obesity is growing most rapidly(٤). Withdrawal of estrogen together with physical inactivity, are probably the major

causes of post menopausal obesity[٥]. Other contributing factors include ethnicity, reduced lean mass, and treatment with certain drugs, e.g. steroids, insulin, glitazones[٦].

Estrogen withdrawal during menopause has a detrimental effect on metabolism and results in changes in body fat distribution from a gynoid to an android pattern, reduced glucose tolerance, abnormal plasma lipids, increased blood pressure, endothelial dysfunction and vascular inflammation. As a result, there are increased rates of hypertension, diabetes mellitus, coronary artery disease and mortality[٧].

The exact mechanisms of post menopausal complications including weight gain are not clear. In animal models of postmenopausal obesity, ovariectomized rats were shown to have increased levels of the orexigenic gut peptide ghrelin, which positively correlated with transient hyperphagia and permanent weight gain[٨]. These data suggest that estrogen inhibits the orexigenic action of ghrelin in females, and that the OVX-induced weight gain could be ghrelin mediated.

Previous studies have shown that ghrelin secretion increases when sympathetic nerves are stimulated artificially[٩] or when adrenergic hormones are infused locally into the stomach lining[١٠]. Furthermore, $\beta_1$-adrenergic receptors were found on the ghrelin secreting cells in the stomach suggesting a regulatory role for the sympathetic nervous system in ghrelin release and induction of obesity after menopause[١١,١٢].

Therefore, the present study was designed to investigate the effect and potential mechanisms of modulation of sympathetic activity on the development of OVX-induced obesity and its associated metabolic effects in a rat model of postmenopausal obesity.

## Materials and methods

### I. Animals:

Adult female albino rats from the local strain, weighing ١٥٠-٢٠٠ grams at the beginning of this study were used. Animals were housed at room temperature with natural light/dark cycles for one week acclimatization to lab conditions. Rats were fed a standard diet of commercial rat chow and tap water ad libitum until the time of the experiment[١٣]. During the acclimatization period, daily food intake was measured to know the mean daily food intake per rat. All the experimental procedures were approved by the local animal care committee in Minia University, Egypt.

### II. Ovariectomy induced obesity:

All rats (except for control group) were bilaterally ovariectomized under light ether anesthesia. In a glass hood saturated with ether vapor, rats were placed to inhale ether until the surgical stage of anesthesia was reached (judged by loss of withdrawal reflexes), rats were then removed and placed on the operating board for ovariectomy as previously described[١٤].

Briefly, under clean aseptic conditions and after shaving the hair of the lower abdomen, a single longitudinal skin incision was made in the midline of abdominal wall above symphysis pubis. The skin was retracted laterally and the abdominal wall and the peritoneum were incised. The ovarian fat pad was pulled out to locate the ovary and oviduct. A hemostat was placed on the oviduct just proximal to the ovary, then a ligature with absorbable cut gut was done and the ovary was excised and the procedure was repeated on the other side. Finally, the incision was closed in two layers with absorbable sutures and each rat was observed until the recovery from anesthesia. Histological sections were used to assess ovariectomy. The same surgical procedures were done in the sham operated control group except for removal of the ovaries[١٣].

After ovariectomy, the animals were left for ٥ weeks during which daily food intake was measured for all groups by subtracting the remaining part from the initial amount put to each group and body weight was measured every week.

### III. Experimental Groups

The rats were divided randomly into the following groups (٧ rats each):

١. Control sham operated group (C); in which rats were left freely wandering in their cages and received no medication throughout the period of the experiment.
٢. Ovariectomized group (OVX); in which the rats were subjected to bilateral ovariectomy.

٣. OVX-estrogen treated group (OVX-E): in which the rats were subjected to ovariectomy. One week later, each rat started to receive daily subcutaneous injection of estradiol benzoate (Folone from Misr Co. For Pharm. Ind. S.A.E.), ٣٠µg/kg for ٤ weeks[١٥].

٤. OVX-atenolol treated group (OVX-At): in which the rats were subjected to ovariectomy, and after one week recovery, each rat started to receive the selective ß١-adrenergic receptor blocker, atenolol (Sigma, St. Louis, USA), ٢٥ mg/kg/day orally for ٤ weeks[١٦]. This receptor was found to be present on the gastric cells which secrete ghrelin and may affect its release[١١].

٥. OVX-reserpine treated group (OVX-R): in which the rats were subjected to ovariectomy, and one week later, each rat started to receive reserpine (Sigma, St. Louis, USA), ٥٠ µg/kg/day i.p. for ٤ weeks[١٧]. It acts by inhibiting the active reuptake of catecholamines into the vesicles of nerve terminals causing their oxidation by monoamine oxidase (MAO) and subsequent depletion.

٦. OVX-tyramine treated group (OVX-T): in which the rats were subjected to ovariectomy, and after one week recovery, each rat started to receive tyramine hydrochloride (Sigma, St. Louis, USA), ٢٠ mg/kg/day i.p. for ٤ weeks [١٨], which causes release of catecholamines.

## IV. Body mass index (BMI):

Body length (nose-to-anus length) was determined in all rats under light ether anesthesia using a measuring tape to determine the distance between the bottoms of the lower incisors to the anus from the ventral surface. Rats were also weighed using electronic balance (FY ٢٠٠٠) and BMI was calculated weekly according to the following formula[١٩]:

Body mass index (BMI) =

$$\text{body weight (g)/length}^{٢}\ (\text{cm}^{٢})$$

## V. Sample collection:

At the end of the experimental period and after an overnight fasting, all rats were sacrificed by decapitation and blood samples were collected from the jugular vein. Sera were separated and stored in aliquots at -٨٠°C till used for estimation of the following parameters;

١. Total cholesterol (TC), Triglycerides (TGs), Low density lipoprotein cholesterol (LDL-c), High density lipoprotein cholesterol (HDL-c) and glucose by enzymatic colorimetric methods using commercial kits (Biodiagnostic, Egypt)

٢. Insulin using ELISA kits.

٣. HOMA-IR[٢٠] was calculated from the following formula:

HOMA-IR= Serum fasting glucose (mg/dl) X Serum fasting insulin (µU/ml) / ٤٠٥

٤. Catecholamines including; (norepinephrine, epinephreine and dopamine) were also measured spectrophotoflurometrically as previously described by[٢١] using spectroflurometer (Shimaduz RF-٥٠٠٠, Japan).

Finally, the heads were dissected, then the brain was removed and the hypothalamus was isolated for determination of its catecholamines content. Peritoneal omental fat was also removed as the whole gastrocolic omentum and was weighed [٢٢].

## VI. Serum Parameters:

### ١. Total Cholesterol (TC) level:

Serum TC level was determined using enzymatic colorimetric method depending on the following reactions[٢٣];

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{Cholesterol Esterase}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{٤-Cholestenone-٣-one} + H_2O_2$$

$$٢H_2O_2 + \text{٤-Aminoantipyrine} + \text{phenol} \xrightarrow{\text{Peroxidase}} \text{Red quinone} + ٤H_2O$$

The intensity of the red quinone dye can be measured calorimetrically at ٥٠٠-٥٥٠ nm which is directly proportionate to the TC concentration in the sample.

**٢. Triglycerides (TGs) level:**

Serum TGs level was determined using enzymatic colorimetric method depending on the following reactions[٢٤];

$$\text{Triglycerides} \xrightarrow{\text{Lipoprotein lipase}} \text{Glycerol} + \text{Fatty acids}$$

$$\text{Glycerol} + ATP \xrightarrow{\text{Glycerol Kinase}} \text{Glycerol-٣-phosphate} + ADP$$

$$\text{Glycerol-٣-phosphate} + O_2 \xrightarrow{\text{Glycerol ٣-phosphatoxidase}} \text{Dihydroxyacetonephosphate} + H_2O_2$$

$$٢\,H_2O_2 + \text{Aminoantipyrine} + \text{٤-Chlorphenol} \xrightarrow{\text{Peroxidase}} \text{Quinonimine} + HCl + ٤\,H_2O$$

The intensity of the red Quinonimine dye can be measured calorimetrically at ٥٠٠ nm which is directly proportionate to the TGs concentration.

**٣. Low-Density Lipoprotein cholesterol (LDL-c) level:**

Serum level of LDL-c was determined using precipitation method and based on the precipitation of (LDL-c) by heparin/sodium citrate. After centrifugation, high Density Lipoproteins (HDL) and Very Low Density Lipoproteins (VLDL) remain in the supernatant. They are measured by common enzymatic cholesterol determination. Then this value is subtracted from the total cholesterol and yields the final result of LDL- Cholesterol [٢٥].

**٤. High Density Lipoprotein cholesterol (HDL-c) level:**

Low- and Very low-density lipoproteins (LDL, VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstate and magnesium ions. After centrifugation, the cholesterol concentration in the HDL (high density lipoprotein) fraction, which remains in the supernatant, is determined [٢٦].

**٥. Serum glucose (G) level:**

Serum fasting glucose level was determined using enzymatic colorimetric method depending on the enzymatic oxidation of glucose in the presence of glucose oxidase (GOD). The formed hydrogen peroxide reacts under catalysis of peroxidase with phenol and ٤-aminoantipyrine to form a red violet quinoneimine dye, the absorbance of which can be measured spectrophotometrically at ٥٤٦ nm[٢٧].

$$\text{Glucose} + ٢H_2O + O_2 \xrightarrow{\text{GOD}} \text{gluconic acid} + H_2O_2$$

$$٢H_2O_2 + \text{Phenol} + \text{٤-aminoantipyrine} \xrightarrow{\text{Peroxidase}} ٤H_2O + \text{quinoneimine}$$

**٦. Serum insulin Level (ELISA):**

United biotech ink (UBI) MAGIWELTM Insulin Enzyme-Linked Immunosorbent assay (ELISA) kit was used for determination of serum fasting insulin level [٢٨]. It is a solid phase ELISA kit in which the wells are coated with monoclonal antibody with higher affinity for insulin. When the samples, and controls are incubated in the wells with enzyme conjugate, which is another antibody linked to horseradish peroxidase to form a sandwich complex bound to the well. Unbound conjugate are then washed off with wash buffer. The amount of bound peroxidase is proportional to the concentration of the insulin present in the sample. Upon addition of tetramethoxypropane (TMB) substrate, the intensity of the color developed is proportionate to insulin concentration present in the samples.

**٧. Homeostasis Model Assessment of Insulin Resistance (HOMA-IR):**

HOMA-IR was calculated according to the following formula [٢٠].

HOMA- IR= Serum fasting glucose (mg/dl) X Serum fasting insulin (µU/ml) / ٤٠٥

**٨. Serum Catecholamines (EP, NE, DA) levels:**

Serum catecholamines were determined spectro-photoflurometrically as previously described by Ciarlone (١٩٧٨)[٢١] using spectroflurometer (Shimaduz RF- ٥٠٠٠), Japan).

**VII. Determination of hypothalamic content of catecholamines:**

The hypothalamus of each rat was weighed and placed in a conical glass homogenizer tube containing ٥ ml of an acidified solution of n-butanol and submerged in ice. The mixture was homogenized and placed on a vortex mixer for ٣٠ seconds. The crude homogenate was centrifuged at ١٠٠٠ r.p.m. in a cooling centrifuge (Hettich centrifuge) for ٥ minutes. ٢.٥ ml of the supernatant was transferred to a glass tube containing ١.٦ ml of ٠.٢ N acetic acid and ٥ ml n-heptane. The content of the tube was thoroughly mixed for ٣٠ seconds using vortex mixer (Vortex-Genie Scientific Industries Inc., USA) and then centrifuged in a cooling centrifuge (Hettich centrifuge) at ٢٠٠٠ r.p.m for exactly ٥ minutes. The supernatant organic layer was discarded and one ml of the remaining fluid was transferred to a tube for determination of epinephrine (EP), norepinephrine (NE), and dopamine (DA) spectrophotoflurometrically as previously described [٢١].

## Statistical analysis

All data were expressed as means ± standard errors (mean ± SEM). Data were analyzed using one-way analysis of variance (ANOVA) with repeated measurements. All the statistical analyses were performed using general linear model procedure (SAS Institute Inc., NC, USA, ٢٠٠٣). Significant differences among groups were detected using Duncan's multiple rang test (١٩٥٥) with a value of $P \leq ٠.٠٥$ considered statistically significant.

## Results

**١. Time course changes in body weight in different experimental groups;**

Table (١) show the effect of OVX with or without treatments on body weight during the first ٥ weeks after OVX, and the percentage change in body weight of each group in each week from its corresponding body weight in the preceding one. The mean of the initial body weights (IBW) and first week body weights were not significantly different among all groups, after that the following was found:

OVX caused a significant and progressive increase in body weight starting from the second week after OVX till the end of the study. This effect was completely prevented by estradiol replacement therapy (OVX+E group) and chemical sympathectomy with reserpine (OVX+ R group) during the ٥th week of the experiment. On the other hand, β١-adrenergic blocker, atenolol treatment partially prevented the effect of OVX; although the body weight significantly decreased yet, it was still significantly higher than the control starting from the second week after OVX till the end of the experiment. Under the effect of tyramine treatment, a significant increase in body weight was observed in OVX+T group as compared to the control group without any significant difference from the OVX non-treated group (table ١).

**Table (١): Time course changes in body weight in different experimental groups:**

| GPs / BW (g) | C | OVX | OVX+E | OVX+A | OVX+R | OVX+T | Sig |
|---|---|---|---|---|---|---|---|
| **initial** | ١٨٢.٦±٥.٥ | ١٧٩±٢.٩ | ١٧٧±٤.٩ | ١٧٤±٥.١ | ١٧٥±٤.٦ | ١٧٥.٤±٦.٣ | NS |
| **١ week after OVX** | ١٩١.٤±٦.٩ | ١٨٤.٥٧±٣.٢ | ١٧٩.٨٩±٥.٩٨ | ١٨٠.١±٥.٤ | ١٨٢.٩±٦.١ | ١٨٠.٥±٦.٥ | NS |
| **% ch. from initial** | ٤.٦±٠.٨ | ٣.١±١.٢ | ١.٦±٠.٧ | ٣.٥ ±٠.٨١ | ٤.٤±٠.٩ | ٢.٩±١.١ | |
| **٢ weeks after OVX** | ١٩٤.٩±٦.٨$^{bc}$ | ٢١٢.٣٦±٢.٤$^{a}$ | ١٨٧.٧±٦.٢$^{c}$ | ١٩٠.٣±٥.٤$^{c}$ | ٢٠٢.٩±٤.٦$^{abc}$ | ٢١٤.٤±٦.٤$^{a}$ | ** |
| **% change from ١week** | ١.٧±٠.٣ | ١٥.٢±٢.٢ | ٤.٥±٠.٦ | ٥.٨ ±١.٦ | ١١.٢ ±١.٨ | ١٩±١.١٦ | |
| **٣ weeks after OVX** | ١٩٧.٩±٥.٩$^{bc}$ | ٢٢٩.٤±٤.٠١$^{a}$ | ١٩٦.٦ ±٦.٦$^{c}$ | ٢١٩.٦±٦.٩$^{a}$ | ٢١٤.٥±٣.٦$^{ab}$ | ٢٣٠.٥±٧.٥$^{a}$ | *** |
| **% change from ٢ weeks** | ١.٧±٠.٩٨ | ٧.٨±١.٤ | ٣.٩±٠.٩ | ١٥.٤±١.٥ | ٥.٦±١.٧ | ٧.٥ ±٠.٨ | |
| **٤ weeks after OVX (start of GA injection)** | ٢٠٤.٨± ٦.٨$^{c}$ | ٢٤٢.٤±٣.٤$^{a}$ | ٢٠٢.٧±٧.٠٢$^{c}$ | ٢٢٦.٣ ±٧.٩٩$^{b}$ | ٢٢٠.٩ ±٤.٣$^{bc}$ | ٢٤٦.٤ ±٣.٤$^{a}$ | *** |
| **% change from ٣ weeks** | ٥.٢±١.٢ | ٥.٨±١.٩ | ٣.١±٠.٤ | ٢.٩٧±٠.٩ | ٢.٩±٠.٧ | ٦.٦±٠.٨ | |
| **٥ weeks after OVX** | ٢١٥.٢±٦.١$^{c}$ | ٢٥٤.٧±٢.٥$^{a}$ | ٢١٤.٤ ±٦.٦$^{c}$ | ٢٣٥.٤ ±٧.٧$^{b}$ | ٢٣١.٥ ±٣.٠$^{bc}$ | ٢٦٣.٨ ±٦.٧$^{a}$ | *** |
| **% change from ٤ weeks** | ٥.٢±٠.٩٥ | ٥.١±١.٣ | ٥.٩±٠.٧ | ٤.١ ±٠.٩ | ٤.٩±٠.٩ | ٧.١ ±١.١ | |
| **% change from initial** | ١٧.٩±١.٤ | ٤٢.٥±٢.٢ | ٢١.٢±١.٧ | ٣٥.٣±١.٦ | ٣٢.٧ ±٢.٧ | ٥٠.٧±٣.٣ | |

Means in the same horizontal row sharing similar superscripts ($^{a,b \text{ and } c}$) are statistically insignificant (P≤٠.٠٥)NS: not significant. *p<٠.٠٥ ** P< ٠.٠١ ***P < ٠.٠٠١
Data are expressed as mean ± SEM of ٧ rats in each group.
C: control; E: estrogen; A: atenolol; R: reserpin; T: tyramin

**٢. Time course changes in food intake (g/day) in different studied groups;**

In table (٢), the overall daily food intake of all OVX groups dropped significantly during the first week following OVX. After that, only OVX non-treated and OVX+tyramine-treated groups showed a highly significant increase in their food intake till the end of the study. Meanwhile, administration of estradiol, atenolol or reserpine prevented this significant increase in food intake and kept it insignificantly different from the control group.

**Table (٢): Time course changes in overall daily food intake (g\day) in different experimental groups**

| Groups / Food intake (g\d) | C | OVX | OVX+E | OVX+A | OVX+R | OVX+T | Sig |
|---|---|---|---|---|---|---|---|
| One week before OVX | ١٥.٥±٠.٢ | ١٥.٣±٠.٣ | ١٥.٩ ± ٠.٢ | ١٥.٤±٠.٣ | ٥.٥±٠.٣ | ١٥.٧±٠.٣ | NS |
| ١st week after OVX | ١٤.٨±٠.٣ | ١٣.٤±١.١ | ١٢.٨ ± ٠.٧ | ١٢.٤±١.١ | ٢.٨±١.٢ | ١١.٣±١.٤ | NS |
| ٢nd week after OVX | ١٥.٦±٠.٢[a] | ١٧.١±٠.٦[b] | ١٥.٧ ± ٠.٣[a] | ١٤.٦±٠.٢[a] | ٥.٢±٠.٤ [a] | ١٧.٤±٠.٦[b] | *** |
| ٣rd week after OVX | ١٥.٣±٠.٤[a] | ١٧.٦±٠.٥[b] | ١٥.٥ ± ٠.٤[a] | ١٥.١±٠.٤[a] | ٥.٩±٠.٣[a] | ١٧.٤±٠.٤[b] | *** |
| ٤th week after OVX (start of GA injection) | ١٤.٩±٠.٦[a] | ١٦.٩±٠.٣[b] | ١٤.٩٩ ± ٠.٥[a] | ١٤.٠٤±٠.٨[a] | ٥.٥±٠.٤[a] | ١٧.١±٠.٤[b] | ** |
| ٥th week after OVX | ١٥.٩±٠.٥[a] | ١٧.١±٠.٢[b] | ١٥.٨ ± ٠.٣[a] | ١٥.٨±٠.٥[a] | ٥.٣±٠.٤[a] | ١٧.٥±٠.٥[b] | ** |

Means in the same horizontal row sharing similar superscripts ([a, b and c]) are statistically insignificant (P≤٠.٠٥)
NS: not significant. *p<٠.٠٥ **P<٠.٠١ ***P<٠.٠٠١
Data are expressed as mean ± SEM of ٧ rats in each group.
C: control; E: estrogen; A: atenolol; R: reserpin; T: tyramin

**٣. Effect of OVX with and without treatment on body mass index (g/cm٢);**

As shown in table (٣), the initial body mass index (IBMI) was insignificantly different among all groups. However the final body mass index (FBMI) was significantly higher (p<٠.٠٠٠١) in the OVX group than in the control group. Only, the OVX group treated with tyramine showed a significantly higher FBMI than the control group but not significantly different from OVX group. On the other hand, all other treatments including estradiol, atenolol or reserpine prevented this significant increase in the FBMI and kept it insignificantly different from the control group.

**Table (٣): Effect of OVX with and without treatment on body mass index (BMI) (g\cm٢)**

| Groups / BMI (g\cm٢) | C | OVX | OVX+E | OVX+A | OVX+R | OVX+T | Sig |
|---|---|---|---|---|---|---|---|
| Initial BMI | ٠.٥٥±٠.٠٢ | ٠.٥٣±٠.٠١ | ٠.٥٣ ± ٠.٠٢ | ٠.٥٢±٠.٠١ | ٠.٥٢±٠.٠٣ | ٠.٥٣±٠.٠١ | NS |
| Final BMI | ٠.٥٦±٠.٠١[b] | ٠.٦٦±٠.٠٢[a] | ٠.٥٧ ± ٠.٠٢[b] | ٠.٦±٠.٠٤[b] | ٠.٥٨±٠.٠١[b] | ٠.٦٦±٠.٠٢[a] | *** |

Means in the same horizontal row with different superscripts ([a and b]) are significantly different (P≤٠.٠٥)
NS: not significant. ***P < ٠.٠٠٠١
C: control; E: estrogen; A: atenolol; R: reserpin; T: tyramin
Data are expressed as mean ± SEM of ٧ rats in each group.

**٤. Effect of OVX with and without treatment on the weight of gastrocolic omentum (g);**

As shown in table (٤) the weight of the gastrocolic omentum was significantly higher in the OVX group than the control group. Apart from OVX group treated with tyramine that showed a significantly higher weight of gastrocolic omentum than the control, all other treatments including estradiol, atenolol or reserpine prevented this significant increase in the gastrocolic omental weight and kept it insignificantly different from the control group with the lowest weight value found in the OVX+estrogen supplemented group.

**Table (٤): Effect of OVX with and without treatment on the weight of the gastrocolic omentum (g)**

| Groups / parameter | C | OVX | OVX+E | OVX+A | OVX+R | OVX+T | Sig |
|---|---|---|---|---|---|---|---|
| wt. of gastrocolic omentum (g) | ٢.٢٢±٠.١٣[a] | ٣.٧٦±٠.٢١[b] | ٢.٤٧±٠.٢١[a] | ٢.٦٦±٠.٣٣[a] | ٢.٦٤±٠.١٨[a] | ٣.٨٣±٠.٤٨[b] | *** |
| % difference from control | …….. | ٦٩.٣٧٪ | ١١.٢٦٪ | ١٩.٨٢٪ | ١٨.٩٢٪ | ٧٢.٥٪ | |
| % difference from OVX | …….. | …….. | -٣٤.٣١٪ | -٢٩.٢٦٪ | -٢٩.٧٩٪ | ١.٨٦٪ | |

Means in the same horizontal row with different superscripts ([a and b]) are significantly different ($P \leq ٠.٠٥$) ***$P < ٠.٠٠٠١$

C: control; E: estrogen; A: atenolol; R: reserpin; T: tyramin

Data are expressed as mean ± SEM of ٧ rats in each group.

**٥. Effect of OVX with and without treatment on serum lipid profile;**

Table (٥) shows that; in comparison to the control group, OVX resulted in significantly higher TC and LDL-c along with significantly lower TGs and HDL-c serum levels.

Estradiol supplementation restored the normal control levels of both TC and LDL-c without any significant change in serum HDL-c level. While, TGs level showed a more significant increase than both control and OVX groups.

Atenolol treatment to OVX rats caused a significant reduction in TGs level as compared to both control and OVX rats, normalization of serum TC level, partial reduction in LDL-c level without any significant change in HDL-c level as compared to the OVX non-treated rats.

Reserpine treatment to OVX rats restored completely the OVX-induced decrease in HDL-c level and returned it back to the control level. It also caused a partial reduction in serum levels of both TC and LDL-c along with partial increase in TGs levels as compared to the OVX non-treated group but not to the control level.

Finally, tyramine treatment to OVX rats failed to produce any significant change in serum TC, LDL-c or HDL-c levels as compared to the OVX non-treated group which remained significantly higher than the control group except for TGs level which was significantly lower than both the OVX and control groups.

**Table (٥): Serum changes in lipid profile in the different studied groups (mg/dl)**

| Groups / parameter | C | OVX | OVX+E | OVX+A | OVX+R | OVX+T | Sig |
|---|---|---|---|---|---|---|---|
| **TC (mg/dl)** | ١٠٥.١٢± ١.٨٥$^{d}$ | ١٦٧.٥٦± ٦.٦٦$^{a}$ | ١١٢.٠٣± ٥.٦٣$^{d}$ | ١٢١.٩٧± ٧.٥٣$^{cd}$ | ١٢٩.٧٦± ٧.٨$^{bc}$ | ١٦٢.٦٧± ٧.٢٨$^{a}$ | *** |
| **%ch. from control** | ..... | ٥٩.٣٩٪ | ٦.٥٧٪ | ١٦.٠٣٪ | ٢٣.٤٤٪ | ٥٤.٧٥٪ | |
| **% ch. from OVX** | ..... | ..... | -٣٣.١٤٪ | -٢٧.٢١٪ | -٢٢.٥٦٪ | -٢.٩٢٪ | |
| **TGs (mg/dl)** | ٨٨.٤٥± ٢.٦٧$^{b}$ | ٥٧.٣٨± ١.٥٩$^{d}$ | ١٦٥.٠١±٤. ٦٢$^{a}$ | ٣٤.٩٤± ٠.٦٨$^{e}$ | ٧٣.٨٥± ٢.٩٦ $^{c}$ | ٣٤.٣٦± ١.٩٧$^{e}$ | *** |
| **%ch. from control** | ..... | ..... | ٨٦.٥٦٪ | -٦٠.٤٩٪ | -١٦.٥١٪ | -٦١.١٥٪ | |
| **% ch. from OVX** | ..... | ..... | ١٨٧.٥٧٪ | -٣٩.١١٪ | ٢٨.٧٪ | -٤٠.١٢٪ | |
| **HDL-c (mg/dl)** | ٣٩.٧٢ ±١.٥٦$^{ab}$ | ٣١.٦١± ١.٤٦$^{c}$ | ٣٥.٣٨±٣.١ ٥$^{bc}$ | ٣١.٣٩± ٢.٥٩$^{c}$ | ٤٣.٨± ٢.١١$^{a}$ | ٣٤.٨٥± ٢.٩٣$^{bc}$ | ** |
| **% ch. from control** | ..... | -٢١.٧٪ | -١٠.٩٣٪ | -٢٠.٩٧٪ | ١٠.٢٧٪ | -١٢.٢٦٪ | |
| **% ch. from OVX** | ..... | ..... | ١١.٩٣٪ | -٠.٧٪ | ٣٨.٥٦٪ | ١٠.٢٥٪ | |
| **LDL-c (mg/dl)** | ٤٨.٩٦± ٢.٤٢$^{d}$ | ١٢٤.٣٧± ٣.٣٢$^{a}$ | ٤٤.١٣± ٢.٢١$^{d}$ | ٨٣.٥٩± ٦.٩$^{bc}$ | ٧١.١٢± ٥.٠٧$^{c}$ | ١٢٠.٥٩± ٥.٢٩$^{a}$ | *** |
| **%ch. from control** | ..... | ١٥٤.٠٢٪ | -٩.٨٧٪ | ٧٠.٧٣٪ | ٤٥.٢٦٪ | ١٤٦.٣٪ | |
| **%ch. from OVX** | ..... | ..... | -٦٤.٥٢٪ | -٣٢.٧٩٪ | -٤٢.٨٢٪ | -٣.٠٤٪ | |

Means in the same row with different superscripts ($^{a,b,c,d \text{ and } e}$) were statistically significant. ** P<٠.٠١ *** P <٠.٠٠٠١

C: control; E: estrogen; A: atenolol; R: reserpin; T: tyramin

Data are expressed as mean ± SEM of ٧ rats in each group.

**٦. Effect of OVX with and without treatment on serum fasting glucose, insulin and HOMA-IR levels.**

As shown in table (٦), OVX group showed a significantly higher serum levels of glucose, insulin and HOMA-IR than the control group. Estradiol supplementation to OVX rats reversed the whole condition and effieciently decreased the serum glucose, insulin and HOMA-IR levels even below those of the control group.

Reserpine treatment to OVX rats effeciently restored the normal control levels of glucose, insulin and HOMA-IR. On the other hand, atenolol treatment to OVX rats produced a significant reduction in serum insulin and HOMA-IR levels without any significant change in serum glucose level when compared to the OVX non-treated group. Although serum insulin level was not significantly different from that of control group, serum glucose and HOMA-IR levels were still significantly higher. Finally, tyramine treatment to OVX rats did not significantly change the elevated serum glucose and HOMA-IR levels induced by OVX. On the other hand, the OVX-induced increase in serum insulin level was significantly decreased but remained significantly higher than the control levels.

**Table (٦): Effect of OVX with and without treatment on serum glucose level (mg/dl), serum insulin level (µIU/ml) and HOMA-IR**

| Groups / parameter | C | OVX | OVX+E | OVX+A | OVX+R | OVX+T | Sig |
|---|---|---|---|---|---|---|---|
| **Serum glucose (mg/dl)** | ٧٤.٢٢±١.١٢$^{b}$ | ١٠١.٧±١.٣$^{a}$ | ٦٨.٣٠ ± ١.٤$^{c}$ | ١٠٤.٢٢ ± ٢.٧$^{a}$ | ٧٨.٧٩ ± ١.٧$^{b}$ | ٩٩.٦٤ ± ١.٧$^{a}$ | *** |
| **% ch. from control** | ..... | ٣٧.٠٣٪ | -٧.٩٨٪ | ٤٠.٤٢٪ | ٦.١٦٪ | ٣٤.٢٥٪ | |
| **% ch. from OVX** | -٢٧.٠٢٪ | ..... | -٣٢.٩٪ | ٢.٤٨٪ | -٢٢.٥٣٪ | -٢.٠٣٪ | |
| **Serum insulin (µIU/ml)** | ٣٤.٥±٠.٧١$^{c}$ | ٣.٧١±٠.٦٨$^{a}$ | ٢٩.٦٦ ± ٠.٨٧$^{d}$ | ٣٥.٨٤ ± ١.٠٨$^{c}$ | ٣٦.٩٢ ± ١.٠٤$^{c}$ | ٤٠.٣٢ ± ١.٠٤$^{b}$ | *** |
| **% ch. from control** | ..... | ٢٦.٧٪ | -١٤.٠٢٪ | ٣.٩٪ | ١٥.٣٪ | ٥.٣٪ | |
| **% ch. from OVX** | -٢١.١٪ | ..... | -٣٢.١٤٪ | -١٨٪ | -٨.٩٩٪ | -١٦.٩٪ | |
| **HOMA-IR** | ٦.٣٢±٠.٢٣$^{c}$ | ١٠.٩٨±٠.٤٠$^{a}$ | ٥.٠±٠.٢٢$^{d}$ | ٩.٢٢±٠.٤٩$^{b}$ | ٧.١٨±٠.٢١$^{c}$ | ٩.٩٢±٠.٧٦$^{ab}$ | *** |

Means in the same horizontal row with different superscripts ($^{a,b,c \text{ and } d}$) are statistically different ($P \leq ٠.٠٥$) ***$P < ٠.٠٠٠١$.
C: control; E: estrogen; A: atenolol; R: reserpin; T: tyramin
HOMA -IR: Homeostasis Model Assessment of Insulin Resistance.
Data are expressed as means ± SEM of ٧ rats in each group.

**٧. Effect of OVX with and without treatment on serum levels of epinephrine (EP), norepinephrine (NE) and dopamine (DA) (ng/ml);**

Table (٧) shows that, in comparison to the control group, OVX rats showed significantly higher serum levels of EP, NE and DA. These changes were completely prevented by reserpine treatment but not by atenolol or tyramine administration to OVX rats. On the other hand, estradiol supplementation prevented the OVX-induced increase in EP and NE but not DA which remained significantly higher than the control level.

**Table (٧):** Serum levels of epinephrine (EP), norepinephrine (NE) and dopamine (DA) in the different studied groups (ng/ml).

| Groups / parameter | C | OVX | OVX+E | OVX+A | OVX+R | OVX+T | Sig |
|---|---|---|---|---|---|---|---|
| **Serum EP (ng/ml)** | ٢٨.٢٩±.٨٩$^{c}$ | ٣٧.٤٣ ±٢.٤١$^{ab}$ | ٢٩.٥٧±٢.٢٦$^{c}$ | ٤١.٤٣±١.٨$^{a}$ | ٢٤.١٤±١.٣٢$^{c}$ | ٤٢.٥٧±٢.٣٣$^{a}$ | *** |
| **Serum NE (ng/ml)** | ٥٥.٤٣± ٤.٣$^{b}$ | ١٠٩.٩٧±٣.٦$^{a}$ | ٧٥.١٤±٢.٩ $^{b}$ | ١١٥.٠٠ ±١٤.٢$^{a}$ | ٦١.٤٣±٣.٤٣$^{b}$ | ١٢٤.٥±١٣.٥٣$^{a}$ | *** |
| **Serum DA (ng/ml)** | ١٨.٥٧±٤.٠٤$^{b}$ | ١٧١.٤٣ ±٤.٠٤$^{a}$ | ١٧٦.١٤±٥.٤$^{a}$ | ١٧٧.١٤±٤.٧$^{a}$ | ١١٣.٨٦±٥.٢$^{b}$ | ١٨٧.١٤±٨.٤$^{a}$ | *** |

Means in the same horizontal row with different superscripts ($^{a \text{ and } b}$) are statistically different ($P \leq ٠.٠٥$) *** $P < ٠.٠٠٠١$.
C: control; E: estrogen; A: atenolol; R: reserpin; T: tyramin
Data are expressed as mean ± SEM of ٧ rats in each group.

**٨. Effect of OVX with and without treatment on hypothalamic content of epinephrine (EP), norepinephrine (NE) and dopamine (DA) (ng/mg tissue);**

As shown in table (٨), the hypothalamic EP did not significantly change among the different experimental groups throughout the study.

Regarding hypothalamic NE and DA, OVX rats showed a significantly higher hypothalamic NE and DA levels than the control.

Estradiol supplementation to OVX rats prevented the OVX-induced increase in hypothalamic NE level but not DA which showed a significantly higher level than the control group.

Chemical sympathectomy with reserpine prevented completely the increase in hypothalamic NE and DA levels induced by OVX. The levels were not significantly different from the control group. On the other hand, atenolol treatment prevented completely the OVX-induced increase in hypothalamic DA level but not NE which showed a significantly higher level than the control group. Finally, hypothalamic NE and DA levels in OVX rats treated with tyramine were not significantly different from the levels observed in OVX non-treated group.

**Table (٨):** The hypothalamic content of epinephrine (EP), norepinephrine (NE), and dopamine (DA) in the different studied groups in (ng/mg)

| Groups / parameter | C | OVX | OVX+E | OVX+A | OVX+R | OVX+T | Sig |
|---|---|---|---|---|---|---|---|
| **Hypothalamic EP (ng/mg)** | ٥.١٢±٠.٦٣ | ٥.١٦±٠.٧١ | ٦.٩٣±٠.٨٢ | ٥.٧١±٠.٣٩ | ٥.١١±٠.٦٢ | ٥.٨٣±٠.٤٢ | NS |
| **Hypothalamic NE (ng/mg)** | ٢٤.٥٤±١.٣$^{cd}$ | ٣٢.٦٢±١.٣$^{a}$$_{b}$ | ٢٣.٩٤±٢.٥$^{cd}$ | ٣١.٣١±١.٧ | ٢٠.٦٦±٢.٣$^{d}$ | ٣٧.١٦±١.٠٠$^{a}$ | *** |
| **Hypothalamic DA (ng/mg)** | ١٨.٩٢±١.٧$^{b}$ | ٢٦.٩٨±١.١$^{a}$ | ٢٩.٢٣±٢.٥$^{a}$ | ١٩.٨٤±١.٨ | ١٧.٣٨±١.٤$^{b}$ | ٢٧.٧١±.٢.٠٥$_{a}$ | *** |

EP: Epinephrine, NE: Norepinephrine, DA: Dopamine.
Means in the same horizontal row with different superscripts ($^{a, b and c}$) are statistically different.
($P\leq٠.٠٥$) NS: Not significant. *** $P<٠.٠٠٠١$.
C: control; E: estrogen; A: atenolol; R: reserpin; T: tyramin
Data are expressed as mean ± SEM of ٧ rats in each group.

## Discussion

The present study provides an evidence for the possible role of adrenergic overactivity in the development of postmenopausal obesity and its metabolic syndrome. Proposed mechanisms may involve sympathetic-induced increase in food intake, body weight, and adiposity mostly via stimulation of β١-adrenergic receptors present on the ghrelin secreting cells in the stomach resulting in ghrelin release.

Similar to humans, ovariectomized rodents develop obesity due to the lack of estrogen, and thus, these animals can serve as a model for commonly observed postmenopausal human obesity[٢٩]. So, OVX was chosen in this study as an experimental model for induction of postmenopausal obesity.

The results of the present study revealed that, OVX rats showed a significantly higher body weight than sham-operated group starting from the second week after OVX till the end of the study. This was positively correlated with significantly high levels of food intake, BMI, GCOF weight as well as serum TC, LDL-c, glucose, insulin, and HOMA-IR levels, along with a significant reduction in serum HDL-c and TGs levels. Sympathetic activity also showed a significant increase in OVX rats as evidenced by significantly high serum levels of EP, NE and DA and hypothalamic contents of EP and DA; findings which are in agreement with Menozzi et al. (٢٠٠٠) and Liu et al. (٢٠٠٤)[٣٠, ٣١].

It seems that the major attributer to OVX-induced adiposity and its associated metabolic

disturbances is estrogen lack and lost repression on adipose tissue proliferation and adipokine synthesis[٢٩, ٣١] as evidenced by the reversal of almost all the effects of OVX by estradiol (E) supplementation.

In the present study, a positive correlation between increased body weight and food intake was observed in OVX rats which is consistent with Liang et al. (٢٠٠٢), Clegg et al. (٢٠٠٧) and Jiang et al. (٢٠٠٨) who explained the increase in body weight as a consequence of increased food intake[٢٢, ٨, ٣٢]. OVX-induced weight gain could also be attributed to increased lipogenesis and decreased lipolysis as evidenced by the significant increase in BMI and GCOF weight in OVX group. Toth et al. (٢٠٠١) reported that OVX-induced estrogen lack stimulates adipose tissue lipoprotein lipase, a regulatory enzyme responsible for the hydrolysis of circulating triglycerides and their uptake and storage into adipocytes, thereby promoting the growth of fat mass and its mobilization and deposition into the viscera (ectopic fat syndrome) [٣٣].

The OVX-induced metabolic effects were completely prevented by estradiol supplementation to OVX rats as evidenced by the significant reduction in food intake and subsequent prevention of OVX-induced weight gain. Findings, which are consistent with Liang et al. (٢٠٠٢), who found that the anorectic action plays an important role in the anti-obesity effects of estrogen[٢٢]. This effect could be mediated via leptin release from adipose tissue. Leptin is well known for its inhibitory effect on food intake and increasing energy expenditure and thereby decreasing body weight[٣٤]. However, other studies have shown that estrogen-mediated anorectic effects are independent of leptin and estrogen per se could substitute for leptin anorexigenic effects in leptin resistant conditions[٣٥].

In humans, the increase in BMI that often accompany menopause is usually consistent with normal aging[٣٦]. However, even in absence of weight gain, changes in body fat distribution (visceral adiposity) occurring across menopause could partially explain the higher risk of cardiovascular disease (CVD), type ٢ diabetes and hyperlipidemia in postmenopausal women[٣٧,٣٨,٣٩]. The mechanisms involved may include, increased food intake[٣٢], imbalance between pathways of uptake, synthesis and oxidation or hepatic secretion of lipids[٤٠], decreased liver fatty acid oxidation[٤١] and finally, the relative increase in androgen to estrogen ratio after OVX, which could promote visceral fat accumulation by inhibiting adenosine monophosphate kinase (AMPK) activation[٤٢].

In the present study, OVX rats showed a significant increase in CVD risk factors including higher TC and LDL-c and lower HDL-c and TGs levels as well as significant increases in serum glucose, insulin, and HOMA-IR levels. These results are in agreement with Yepuru et al. (٢٠١٠) who found that, the deposition of fat mass and particularly central fat mass in postmenopausal obesity due to lack of estrogens, is responsible for an increase in circulating adipocytokines, which have implications for insulin resistance and cardiovascular diseases[٤٣].

The mechanisms underlying the disturbed lipid profile after OVX are not clear. However, Paquette et al. (٢٠٠٨) explained the OVX-induced dyslipidemia by a change in expression of key transcriptional factors related to hepatic lipid regulation secondary to estrogen lack, such as the peroxisome proliferator-activated receptor (PPAR)α, sterol regulatory element-binding protein-١c (SREBP-١c), and stearoyl-CoA desaturase-١ (SCD-١). They demonstrated a decrease in lipid oxidation systems associated with down regulation of PPARα mRNA expression, along with an increase in the expression of lipogenesis transcriptional factors (SREBP-١c and SCD-١) in OVX rats[٤٤].

In the present study, the OVX-induced dyslipidemia was almost completely prevented by estradiol supplementation as evidenced by the significant decrease in serum TC and LDL-c levels along with a significant increase in serum HDL-c levels; findings which are consistent with Liu et al. (٢٠٠٤) and Babaei et al. (٢٠١٠) and suggesting a preventive effect of estradiol against CVD[٣١,٢٥].

The LDL-c lowering effect of estradiol is probably due to its ability to stimulate the expression of LDL-receptor gene and increasing the number of LDL receptors[٤٥]. LDL-c

internalizes into the cells through the process of LDL-receptor mediated endocytosis accelerating LDL catabolism. The expression of LDL-receptor on the cell surface is a function of various hormone-regulated transcription factors of the receptor gene; and β-estradiol is considered the prime hormonal regulator of LDL-receptor expression[٤٦]. Another protective mechanism offered by estradiol is through the depression of hepatic lipase enzyme activity[٣٣], thereby decreasing HDL-c catabolism which was confirmed by the significant increase in HDL-c levels in OVX+E treated group. Similarly, Walsh et al. (١٩٩٤) found HDL elevation following oral estradiol treatment in postmenopausal women that was dose dependent[٤٧]. In turn, estradiol fatty acyl esters incorporate into HDL and enhance the atheroprotective properties of HDL by mediating the initial steps of reverse cholesterol transport[٤٨].

Unfortunately, the significant increase in TGs level in OVX+E treated group does not support the cardiovascular protection of estrogen. In contrast, it supports clinical as well as experimental findings that estrogen replacement therapy has harmful cardiovascular effects[٤٩, ٥٠]. The mechanisms involved may include estradiol-induced increase in hepatic TGs secretion secondary to an increase in very low density lipoprotein (VLDL) triglyceride and apo B production[٥١, ٥٢].

Obesity induced by OVX may be involved in the induction of insulin resistance (IR) as evidenced, in the present study, by the significant increase in fasting serum levels of glucose, insulin and HOMA- IR in OVX rats. These findings are consistent with Tsunekawa et al. (٢٠٠٥) and Tamakoshi et al. (٢٠٠٧) who found that postmenopausal women had significantly higher insulin resistance (IR) than premenopausal women[٥٣,٥٤]. Kaaja (٢٠٠٨) found that reduced insulin sensitivity did not appear until postmenopausal women had accumulated levels of visceral adipose tissue that approxi-mated the levels seen in men, suggesting a possible threshold effect of abdominal fat on the development of IR[٥٥]. Another possible mechanism of IR in estrogen deficient condi-tions could be due to estrogen lack-induced impairment of lipid metabolism in the liver and adipocytes. Bryzgalova et al. (٢٠٠٨) found that estrogen therapy decreased the expression of lipogenic genes such as fatty acid synthase (FAS), and SCD١ in adipocytes which were parallel with an improvement in insulin sensitivity[٥٦]; findings which were confirmed by the significant improvement in glycemic control in OVX+E treated rats. These effects could be attributed to the inhibitory effect of estrogen on renal gluconeogenesis[٥٧], in addition to an estrogen-induced increase in the whole body as well as skeletal muscle glucose uptake by up regulating the expression of glucose transporter-٤ (GLUT-٤)[٥٨]. Blood glucose level therefore was significantly reduced. Final mechanism could be attributed to the roles played by estradiol at both β-cells of the pancreas as well as the peripheral insulin-sensitive tissues. It was found that ١٧β-estradiol at physiological concentrations protects pancreatic β-cells against lipotoxicity, oxidative stress, and apoptosis[٥٩].

Increased sympathetic activity secondary to estrogen lack seems to be a major contributing factor in OVX-induced obesity. This was evident in the present study by the significant increase in serum levels of EP, NE and DA as well as hypothalamic contents of NE and DA in OVX rats along with significantly increased food intake, body weight, and adiposity. Similar findings were reported by Gomes et al. (٢٠١٢)[٦٠] and could be due to an increase in the activity of dopamine-beta-hydroxlase (DBH) enzyme which converts the monoamine; DA to NE in the sympathetic as well as the central nervous system[٦١]. This increase of NE is indicative to the increase of NPY which is co-localized and released with norepinephrine (NE) from both central and peripheral adrenergic neurons and thus could explain the increased food intake observed after OVX[٦٢].

Estradiol supplementation to OVX rats reversed the OVX-induced increase in both serum and hypothalamic NE levels, which may explain the anorexigenic effect of estradiol as previously reported by Serova et al. (٢٠١٠) and Sabban et al. (٢٠١٠) adding more evidence for the role of neuronal NE in the pathogenesis of OVX-induced obesity and its metabolic effects[٦٣, ٦٤]. Only, the hypothalamic content of DA was significantly increased with estradiol supplementation which is consistent with Alfinito et al. (٢٠٠٩)[٦٥]. One possible explanation for the estrogen-induced increase in hypothalamic DA

content may be attributed to the stimulatory effect of estrogen on the hypothalamic tyrosine hydroxylase enzyme activity[٦٦].

Previous studies have shown that estrogen administration to OVX rats caused significant reductions in the number of ghrelin-producing cells, ghrelin mRNA in the stomach, and plasma total ghrelin levels [٨]. In addition, ghrelin and estrogen receptors (ER)-α are co-localized in the stomach[٦٧], suggesting a direct inhibitory effect of estrogen on ghrelin expression and the estrogen lack-induced weight gain could be ghrelin mediated.

In the present study, we hypothesized that the sympathetic-induced weight gain following OVX could be mediated via stimulation of $\beta_1$-adrenergic receptors present on the ghrelin secreting cells in the stomach resulting in ghrelin release. To confirm this hypothesis, we tested the effect of atenolol, a selective $\beta_1$ adrenergic blocker, reserpine, which causes depletion of NE in the sympathetic nerve terminals and finally, tyramine, which stimulates release of catecholamines, on the changes in body weight and associated metabolic disturbances occurring following OVX.

The results of the present study proved that, the stimulatory effect of increased sympathetic activity on food intake and weight gain could be mediated via stimulation of $\beta_1$ adrenergic receptors and subsequent ghrelin release; finding which are in accordance with Zhao et al. (٢٠١٠)[١٢]. This was evidenced by the effectiveness of the selective $\beta_1$-adrenergic blocker, atenolol in keeping the food intake insignificantly different from the control group throughout the study period along with significant decrease in body weight, TC, and LDL-c as compared with OVX non-treated group.

Although atenolol efficiently reduced the food intake and decreased accumulation of omental fat, it worsened the glycemic state, as it significantly lowered the serum insulin level and kept the high serum glucose level unchanged as compared to the OVX non-treated group; findings which are in agreement with Goyal et al. (٢٠١١)[٦٨]. $\beta_1$ adrenergic blockers have been implicated in altering glucose homeostasis, primarily through inhibition of pancreatic insulin secretion and promoting insulin resistance[٦٩]. So, β-receptor selectivity appears to play an important role in the degree of downstream metabolic effects, which include not only glucose increases but also weight gain and dyslipidemia.

The results of the present study showed that chemical sympathectomy by reserpine in OVX+R group resulted in a significant decrease in food intake, body weight, BMI, GCOF weight, serum levels of glucose, insulin, and HOMA-IR, TC, LDL-c, serum and hypothalamic EP, NE, and DA as compared with OVX group. This was accompanied with a significant increase of HDL-c. These results are in agreement with Shafi et al. (٢٠٠٢) and indicating an anti-atherosclerotic effect of reserpine[٧٠]. The later effect may be attributed to upregulation of hepatic LDL-c receptors which uptake LDL-c from the plasma and/or increased TC, LDL-c clearance from the plasma[٧١]. Finally, the effectiveness of reserpine in glycemic control may be explained by the associated lowered body weight, GCOF weight and enhanced insulin sensitivity observed in OVX+R treated group. On the other hand, tyramine administration to OVX rats failed to produce any significant change in food intake or body weight and adiposity as compared with the OVX non-treated rats. Probably, it could be stated that the sympathetic stimulation response mechanism with OVX has reached its maximal limit and no further added response could be mediated by tyramine under such condition.

**In conclusion**, Ovariectomy and subsequent estrogen lack can cause detrimental metabolic alterations that mimic features of the metabolic syndrome including, obesity, IR, and disturbed lipid profile. Although estrogen supplementation successfully prevented almost all OVX-induced disturbances, it had a potential cardiovascular risk due to the significant increase in serum TGs level.

Increased sympathetic activity secondary to estrogen lack could be a contributing factor in OVX-induced obesity possibly via increased food intake, body weight, and adiposity. One suggested mechanism may involve stimulation of $\beta_1$-adrenergic receptors found on ghrelin secreting cells in the stomach resulting in ghrelin release. Therefore, blocking sympathetic

activity with either reserpine or atenolol could have beneficial effects in treatment of postmenopausal obesity. However, reserpine may have more therapeutic advantage over atenolol in terms of glycemic control and correction of dyslipidemia.

## References


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