doi:10.4149/endo_2014_03_126

The effect of ghrelin antagonist (D-Lys^3) GHRP-6 on ovariectomy-induced obesity in adult female albino rats

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Objective. We aimed to investigate the effect of ghrelin antagonist (D-Lys3) GHRP-6 on the treatment of ovariectomy-induced obesity as compared to hormone replacement therapy with estradiol.

Methods. Twenty eight rats were divided into four groups: control sham operated (C), ovariectomized non-treated (OVX), ovariectomized+estradiol-treated (OVX+E) groups, and ovariectomized+ghrelin antagonist-treated group (OVX+GA). Rats were allowed free water and commercial standard diet ad libitum for 5 weeks after surgery. Body mass index (BMI) was determined at the beginning and the end of the experiment. Rats were sacrificed by decapitation and blood samples were collected for measurements of serum lipid profile, insulin, and glucose levels. Gastrocolic omental fat (GCOF) was removed and weighed.

Results. Ovariectomy was accompanied with a significantly higher body weight, food intake, BMI, GCOF, serum total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), glucose, insulin, and homeostatic model assessment – insulin resistance (HOMA-IR), with a significant decrease in high density lipoprotein-cholesterol (HDL-C) and triglycerides (TGs) in comparison with C group. Estradiol reversed the ovariectomy-induced changes except that of TGs. Administration of ghrelin antagonist was effective in treating the ovariectomy-induced obesity as evidenced by normalization of body weight, food intake, BMI, and GCOF weight, serum levels of insulin, glucose, HOMA-IR, and HDL-C. The serum levels of TC, LDL-C and TGs were improved but did not reach the control values.

Conclusion. Although estradiol succeeded in the prevention of almost all ovariectomy-induced disturbances, it had a potential cardiovascular risk due to a marked increase in serum TGs. Ghrelin antagonist was effective in ameliorating ovariectomy-induced obesity, so it may be used as a promising treatment for postmenopausal obesity, irrespective of hormonal replacement.

Key words: ovariectomy, obesity, ghrelin, rat

Obesity is a complex disease that has created an increasing demand for drugs that reduce body weight and also treat conditions associated with obesity such as diabetes, insulin resistance, osteoporosis, inflammation, muscle weakness, and others. Most of the drugs in development to treat obesity target the G protein coupled receptor (GPCR) class and are associated with side effects ranging from nausea to depression (Yepuru et al. 2010).

Postmenopausal women are one of the subpopulation in which obesity is growing most rapidly. Estrogen replacement therapy is one of the most controversial issues in the field of reproductive medicine. Complications of this therapy include endometrial cancer, breast cancer, hypertension, hyperlipidemia, and gall bladder disease; the last three complications presumably result from hepatic actions of estrogen replacement therapy

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(Lukes 2008). The presence of all these serious complications increases the demand for other solutions for postmenopausal symptoms.

Ghrelin is a 28-amino acid peptide produced predominantly by the stomach and is the endogenous ligand for the growth hormone secretagogue receptor (GHSR). In addition to potently stimulating growth hormone (GH) secretion from the pituitary, ghrelin administration stimulates food intake, carbohydrate utilization, and increases adiposity in rodents, suggesting a role for this hormone in the regulation of energy balance. These findings indicate that the gastric peptide ghrelin and GHSR may be involved in the pathophysiology of obesity and associated complications (Esler et al. 2007).

In animal models, obesity can be induced by ovariectomy which leads to a marked increase in energy stores of the rat (Rogers et al. 2009). Ovariectomized rats have been shown to have increased levels of orexigenic gut peptide ghrelin, which positively correlated with transient hyperphagia and resulted in permanent weight gain (Clegg et al. 2007). The aim of this work is to study the potential effect of ghrelin antagonist (D-Lys3) GHRP-6 on the treatment of ovariectomy-induced obesity and its associated metabolic effects in ovariectomized rats as compared to hormonal replacement therapy with estradiol.

Materials and Methods

Animals. Twenty-eight adult female albino rats from the local strain weighing 150-200 g were purchased from national research centre (Giza, Egypt). Rats were freely allowed tap water and standard diet of commercial rat chow ad libitum and were left to accommodate for one week under natural light/dark regular cycles in partially humid and well-aerated room. From the beginning of the experiment, rats were housed individually and the daily food intake was measured. The Protocol was approved by the local animal care committee at Minia University, Egypt.

Chemicals. Estradiol benzoate (folone) was purchased from Misr Co. for Pharm. Ind. S.A.E. Ghrelin antagonist (D-Lys3) GHRP-6 was purchased from Sigma, St. Louis, USA. All other chemicals were of analytical grade and were obtained from commercial sources.

Experimental protocols. Rats were randomly divided into 4 equal groups (7 rats each) as follows: 1) Control sham operated group (C); 2) Ovariectomized non-treated group (OVX): in which the rats were subjected to ovariectomy according to Zhang et al. (2007) and received no treatment; 3) Ovariectomized estradiol-treated group (OVX-E): in which the rats were subjected to ovariectomy, then after one week recovery, each rat started to receive daily subcutaneous injection of estradiol benzoate 30 μg/kg body weight (Babaei et al. 2010) for 4 weeks (Rivera and Eckel 2010); and 4) Ovariectomized ghrelin antagonist-treated group (OVX-GA): in which the rats were subjected to ovariectomy, then after four weeks, each rat started to receive daily intraperitoneal injection of 0.5 mg/kg ghrelin antagonist (D-Lys3) GHRP-6 at 7:00 a.m. and 7:00 p.m. for one week (Asakawa et al. 2003).

Body mass index (BMI). Body length (nose-to-anus length) was determined in all rats at the beginning and end of the experiment. The measurements were made in anaesthetized rats with light ether. The body weight and body length were used to determine BMI according to the following formula: BMI = body weight (g)/length^2 (cm^2) (Novelli et al. 2007).

Biochemical analysis. At the end of the experiment blood samples were obtained from carotid vessels during decapitation of the rats. After collection, the blood samples were left to clot at room temperature, and then centrifuged at 3000 rpm for 15 min in a cooling centrifuge (Hettich centrifuge). The serum layer was stored at -20°C till the time of assay of:

1. TC, TGs, LDL-C were determined by an enzymatic colorimetric methods described by Deeg and Ziegenhorn (1983), Cole et al. (1997), Schaefer and McNamara (1997), respectively, using kits purchased from Greiner Diagnostic GmbH-Germany.

2. Serum HDL-C by precipitation method (National Cholesterol Education Program, 1995).

3. Serum glucose by an enzymatic colorimetric methods described by Tietz (1995), using (Spectrum, Egyptian Company for Biotechnology Egypt' kit).

4. Serum insulin by enzyme-linked immunosorbent assay described by Clark and Hales (1991) (united biotech ink; UBI; MAGIWEL Insulin Enzyme-Linked Immunosorbent assay; ELISA).

HOMA-IR was calculated according to the following formula (Yada et al. 2008): HOMA-IR= serum glucose (mg/dl) × serum insulin (μU/ml)/405.

Peritoneal omental fat was removed as the whole gastrocolic omentum and weighed (Liang et al. 2002).

Statistical analysis. Data were expressed as mean ± standard error of the mean (S.E.M.) of 7 observations. Means of the different groups were analyzed using paired ANOVA and repeated measure ANOVA tests by

introducing the data into the statistical analysis system (SAS) 9.1.3. Program. Post hoc test used is Fisher's least significant difference (LSD). Levels of $p \leq 0.05$ were accepted as statistically significant.

Results

Time course changes in body weight in the different studied groups. Ovariectomy caused a significant increase in the body weight from the second week after ovariectomy till the end of the study. Injection of estradiol prevented the marked increase in body weight and kept it insignificantly different from the control throughout the study period as shown in Table 1. Injection of ghrelin antagonist after induction of obesity significantly decreased the body weight to an insignificant level from the control as shown in Table 1.

Time course changes in food intake (g/day) in the different studied groups. The overall daily food intake of all OVX groups dropped significantly during the first week following ovariectomy. After that, OVX group showed a highly significant increase in their food intake till the end of the study. However, injection of estradiol prevented this significant increase in food intake and kept it insignificantly different from the control group. Also injection of ghrelin antagonist resulted in a significant reduction of the increased food intake that was insignificantly different from the control group as shown in Table 2.

Effect of ovariectomy with and without treatment on BMI (g/cm^2). The initial BMI (IBMI) was insignificantly different among all groups. However, the final BMI (FBMI) was significantly higher ($p<0.0001$) in the OVX group than in the control group. On the other hand, estradiol and ghrelin antagonist treatment prevented this significant increase in the FBMI and kept it insignificantly different from the control group as shown in Table 3.

Effect of ovariectomy with and without treatment on the weight of gastrocolic omentum (GCO) (g). The weight of the GCO was significantly higher in the OVX group than the control group. OVX-E and OVX-GA groups showed an insignificantly different GCO weight from the control group with the lowest weight of GCO found in the OVX-GA group as shown in Table 4.

Effect of ovariectomy with and without treatment on serum level of TC, TGs, HDL-C and LDL-C (mg/dl). Data presented in Table 5 showed that in comparison to the control group, ovariectomy resulted in a significantly higher serum level of TC and LDL-C associated with a significantly lower serum level of TGs and HDL-C. Treatment with estradiol showed significantly lower serum levels of both TC and LDL-C than OVX group and insignificant from the control group. This was associated with highly significant higher serum levels of TGs than both OVX and control groups with insignificantly different serum HDL-C from both OVX and control groups. OVX-GA group showed significantly lower serum levels of both TC and LDL-C than the OVX group. The levels were still significantly higher than in the control group. It did not significantly change serum TGs level as compared with the OVX group, but the level was significantly lower than the control group. As regard HDL-C, it was significantly higher than the OVX group but was not significantly different from the control group (Table 5).

Effect of ovariectomy with and without treatment on serum glucose and insulin levels and HOMA-IR. As shown in Fig. 1, 2, and 3, OVX group showed significantly higher serum levels of glucose, insulin and HOMA-IR than the control group. OVX-E group showed a significantly lower serum level of glucose, insulin and HOMA-IR than the OVX group. The levels of glucose and insulin were also significantly lower than the control group without change in HOMA-IR. OVX-GA group showed significantly lower serum levels of glucose, insulin and HOMA-IR than the OVX group. The levels were insignificantly different from the control group.

Discussion

The prevalence of obesity is increasing worldwide and more adults are becoming overweight and one of the subpopulation in which this is growing most rapidly is postmenopausal women (Sharma et al. 2008). The obesity epidemic calls for novel pharmacologic treatment methods.

Estrogen replacement therapy is shown to inhibit the increase in body weight and fat accumulation in post-menopausal women. Animal studies have also demonstrated that ovariectomy increases body weight and estrogen replacement reverses this effect. These studies suggest that estrogen deficiency plays a role in the development of obesity but the anti-obesity effect of estrogen and its underlying mechanism is still unclear (Liang et al. 2002).

Recently, OVX rats were shown to have increased circulating levels of the orexigenic gut peptide ghrelin, which positively correlated with transient hyperphagia

Table 1
Time course changes in body weight (g)

Body weight (g)	Control	OVX	OVX + estradiol	OVX + GA	P
Initial	182.57±5.51	179.00±2.93	177.00±4.9	174.86±2.71	NS
1 week after OVX	191.36±6.94	184.57±3.17	179.89±5.98	181.43±3.16	NS
% change of initial	4.57±0.84	3.13±1.15	1.55±0.70	2.43±0.71	
2 weeks after OVX	194.89±6.8^{bc}	212.36±2.39^{a}	187.86±6.19^{c}	208.43±4.14^{ab}	**
% change of 1 week	1.69±0.25	15.24±2.15	4.50±0.59	14.92±1.55	
3 weeks after OVX	197.93±5.94^{bc}	229.43±4.01^{a}	196.57±6.55^{c}	226.19±3.6^{a}	***
% change of 2 weeks	1.67±0.98	7.84±1.4	3.92±0.94	8.58±0.85	
4 weeks after OVX (start of GA)	204.80±6.75^{c}	242.43±3.43^{a}	202.71±7.02^{c}	245.24±4.24^{a}	***
% change of 3 weeks	5.20±1.15	5.76±1.85	3.10±0.35	8.47±1.44	
5 weeks after OVX	215.21±6.06^{c}	254.71±2.51^{a}	214.43±6.57	230.2±3.17^{bc}	
% change of 4 weeks	5.20±0.95	5.14±1.28	5.87±0.68	6.04±0.76	***
% change of initial	17.94±1.36	42.47±2.16	21.15±1.70	31.78±1.88	

GA-ghrelin antagonist. Data are expressed as mean ± S.E.M. of 7 rats in each group. Means in the same horizontal row with different superscripts ($^{a, b, c}$) are significantly different (p<0.05), while means with similar superscripts are insignificantly different. P-significance; NS-not significant; **p<0.01; ***p<0.0001

Table 2
Effect of ovariectomy with or without treatment on overall daily food intake (g\day) during 5 weeks following ovariectomy

Food intake (g\d)	Control	OVX	OVX + estradiol	OVX + GA	P
1 week before OVX	15.5±0.22	15.3±0.33	15.86±0.15	15.77±0.34	NS
1st week after OVX	14.8±0.32^{a}	13.4±1.14^{b}	12.76±0.71^{b}	12.10±1.46^{b}	***
2nd week after OVX	15.6±0.19^{a}	17.1±0.63^{b}	15.67±0.34^{a}	17.18±0.36^{b}	***
3rd week after OVX	15.3±0.44^{a}	17.6±0.51^{b}	15.49±0.39^{a}	17.89±0.78^{b}	***
4th week after OVX (start of GA)	14.9±0.63^{a}	16.9±0.34^{b}	14.99±0.51^{a}	16.96±0.34^{b}	**
5th week after OVX	15.9±0.45^{a}	17.1±0.18^{b}	15.77±0.33^{a}	15.85±0.30^{a}	**

GA-ghrelin antagonist. Data are expressed as mean ± S.E.M. of 7 rats in each group. Means in the same horizontal row with different superscripts ($^{a, b}$) are significantly different (p<0.05), while means with similar superscripts are insignificantly different. P-significance; NS-not significant; **p<0.01; ***p<0.0001

Table 3
Effect of ovariectomy with and without treatment on body mass index (BMI) (g\cm^2)

BMI (g\cm2)	Control	OVX	OVX + estradiol	OVX + GA	P
Initial BMI	0.55±0.02	0.53±0.01	0.53±0.02	0.51±0.02	NS
Final BMI	0.56±0.01^{b}	0.66±0.02^{a}	0.57±0.02^{b}	0.56±0.01^{b}	***

GA-ghrelin antagonist. Data are expressed as mean ± S.E.M. of 7 rats in each group. Means in the same horizontal row with different superscripts ($^{a, b}$) are significantly different (p<0.05). P-significance; NS-not significant; ***p<0.0001

Table 4
Effect of ovariectomy with and without treatment on the weight of the gastrocolic omentum (g)

Parameter	Control	OVX	OVX + estradiol	OVX + GA	P
Weight of gastrocolic omentum (g)	2.22±0.13^{a}	3.76±0.21^{b}	2.47±0.21^{a}	2.26±0.17^{a}	***
% difference of control	-	69.37%	11.26%	1.8%	-
% difference of OVX	-40.96%	-	-34.31%	-39.89%	-

GA-ghrelin antagonist. Data are expressed as mean ± S.E.M. of 7 rats in each group. Means in the same horizontal row with different superscripts ($^{a, b}$) are significantly different (p<0.05) while means with similar superscripts are insignificantly different. P-significance, ***p<0.0001

Table 5

Serum level of lipid profile in the different studied groups (mg/dl)

Parameter	Control	OVX	OVX + estradiol	OVX + GA	P
TC (mg/dl)	105.12±1.85^{d}	167.56±6.66^{a}	112.03±5.63^{d}	142.44±2.99^{b}	
% change of control	-	59.39%	6.57%	35.5%	***
% change of OVX	-37.26%	-	-33.14%	-14.99%	
TGs (mg/dl)	88.45±2.67^{b}	57.38±1.59^{d}	165.01±4.62^{a}	66.98^{cd}±6.61	
% change of control	-	-35.13%	86.56%	-24.27%	***
% change of OVX	54.15%	-	187.57%	16.73%	
HDL-C (mg/dl)	39.72^{ab}±1.56	31.61±1.46^{c}	35.38^{bc}±3.15	44.47±3.25^{a}	
% change of control	-	-21.7%	-10.93%	11.96%	**
% change of OVX	25.66%	-	11.93%	40.68%	
LDL-C (mg/dl)	48.96^{d}±2.42	124.37±3.32^{a}	44.13±2.21^{d}	84.63±4.2^{b}	
% change of control	-	154.02%	-9.87%	72.86%	***
% change of OVX	-60.63%	-	-64.52%	-31.96%	

GA-ghrelin antagonist. Data are expressed as mean ± S.E.M. of 7 rats in each group. Means in the same row with different superscripts ($^{a, b, c, d, e}$) were significantly different, while means with similar superscripts are insignificantly different. P-significance; **p<0.01; *** p<0.0001

and resulted in permanent weight gain. This orexigenic action of ghrelin in OVX rats was decreased by estrogen replacement. The pivotal role of ghrelin in OVX-related weight gain was further supported by the finding that ghrelin receptor (GHS-R, growth hormone secretagogue receptor)-null mice were resistant to ovariectomy-induced increases in food intake and body weight (Clegg et al. 2007).

Based on these findings about ghrelin and ovariectomy-induced obesity, the ghrelin receptor antagonist (D-Lys3) GHRP-6 was used in the present study to test its potential effect in the treatment of ovariectomy-induced obesity as compared with estradiol replacement therapy.

The results of the present study revealed that, ovariectomy was accompanied with a significantly higher body weight than sham-operated rats. This was correlated with a significantly higher food intake, BMI, weight of GCOF, increased cardiovascular disease (CVD) risks as evidenced by significant increase in serum levels of TC, LDL-C with a significant decrease in HDL-C and TGs, as well as disturbed carbohydrate metabolism evidenced by significant increase in serum levels of glucose, insulin, and HOMA-IR.

The correlated increase in body weight and food intake found in the present study following ovariectomy is in agreement with Jiang et al. (2008) who have explained the increase in body weight by the increase in food intake. This was contradictory to Muller and Hsiao (1981) who have found no change in food intake following ovariectomy.

Hamed et al. (2010) have attributed the ovariectomy-induced obesity to metabolic changes as a result of ovarian hormone deficiency, which leads to an increased fat synthesis and deposition in the adipocyte and when adipocytes reach their capacity of fat storage, fat becomes mobilized to be deposited in the viscera as the skeletal muscles, heart and liver (ectopic fat syndrome).

The results of the present study confirmed that the increase in body weight following ovariectomy occurred secondary to increased food intake and lipogenesis as evidenced by increase in GCOF weight and BMI.

The mechanism by which food intake and adiposity increased after ovariectomy is the lack of estrogen hormone and lost repression of adipose tissue proliferation and adipokine synthesis as found by Yepuru et al. (2010). Estrogen lack resulted in increasing ghrelin level and releasing of ghrelin orexigenic activity from the inhibitory effect exerted by estrogen as found by Clegg et al. (2007). This is confirmed in our study by the effectiveness of ghrelin antagonist in reducing food intake, adiposity, and body weight.

The increased BMI found in the present study is in agreement with Hamed et al. (2010) who have explained that by the increased lipogenesis or decreased lipolysis or both. This is evidenced in the present study by the significant increase in weight of GCOF.

The mechanisms underlying the intra-abdominal fat accumulation in ovariectomized rats include 1) increased food intake as found by Paquette et al. (2008); 2) imbalance between pathways of uptake, synthesis

and oxidation or hepatic secretion of lipids as found by Barsalani et al. (2008); and 3) decreased liver fatty acid oxidation as found by Paquette et al. (2009).

Significant accumulation of visceral fat is known to play more important roles than subcutaneous fat in diseases associated with obesity as type 2 diabetes, hyperlipidemia, and hypertension (Liang et al. 2002). This was evidenced in the present study by an increase in CVD risk factors and increases in serum levels of glucose, insulin, and HOMA-IR. These results are in agreement with Yepuru et al. (2010) who have found that the deposition of fat mass and particularly central fat mass in postmenopausal obesity due to lack of estrogens is also responsible for an increase in circulating adipocytokines, which have implications for IR and CVD.

The increased CVD risk factors found in the present study following ovariectomy was evidenced by a significant increase of serum TC and LDL-C and reduction of HDL-C and TGs. Similar results have been reported by Leite et al. (2009) which confirmed the fact that ovariectomy negatively affects the lipid profile. In turn this alteration in lipid profile is strongly associated with an increased risk of CVD and atherogenic profile in postmenopausal women (Schneider et al. 2006).

In the present work, the increased body weight and GCOF weight were accompanied with IR as evidenced by a significant increase of fasting serum levels of glucose, insulin and HOMA-IR. These findings are consistent with Tsunekawa et al. (2005), who have found an alteration in carbohydrate metabolism in female rats.

AMP-activated protein kinase (AMPK) is an enzyme activated during low cellular energy charge. In peripheral tissues, the activation of AMPK influences various metabolic pathways, including glucose uptake, glycolysis, and fatty acid oxidation, all of which help to re-establish a normal cellular energy balance. AMPK is also present in the neurons of the hypothalamus, a critical center in the regulation of energy homeostasis (Kim and Lee 2005). Ghrelin has similar effects on AMPK activity in various tissues: it stimulates hypothalamic and heart AMPK activity, while inhibit adipose tissue and liver AMPK activity (Kola et al. 2008).

The present study showed that estradiol treatment of the ovariectomized rats prevented the significant increase of food intake, body weight, BMI, and GCOF weight and kept them insignificantly different from the control group. This was accompanied with improvement of the CVD risks as evidenced by keeping the serum level of TC, LDL-C, and HDL-C insignificantly different from the control group, while estradiol increased

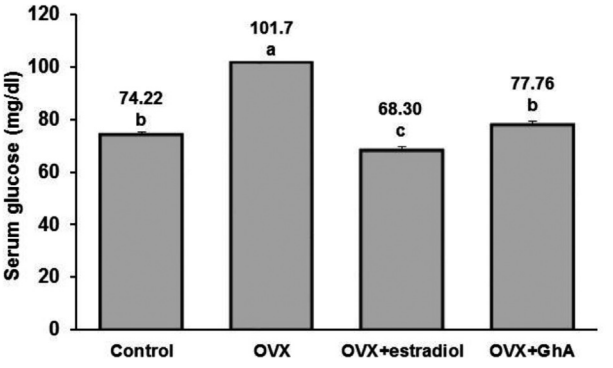

Fig. 1. Serum glucose level (mg/dl) in the different studied groups. OVX: ovariectomized, GhA: ghrelin antagonist. a, b, c, d means that columns with only different superscripts are significantly different (p<0.0001 in all significantly different groups). Data are expressed as means ± S.E.M. of 7 rats in each group.

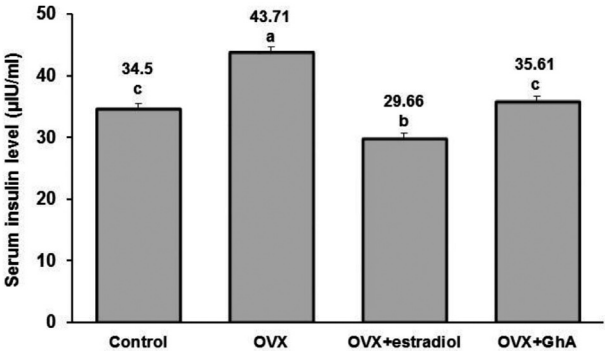

Fig. 2. Serum insulin level (µIU/ml) in the different studied groups. OVX: ovariectomized, GhA: ghrelin antagonist. a, b, c means that columns with only different superscripts are significantly different (p<0.0001 in all significantly different groups). Data are expressed as means ± S.E.M. of 7 rats in each group.

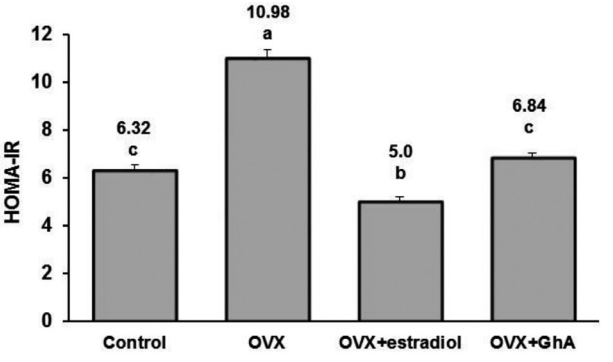

Fig. 3. Homeostasis model assessment of insulin resistance (HOMA-IR) in the different studied groups. OVX: ovariectomized. GhA: ghrelin antagonist. a, b, c means in that columns with only different superscripts are significantly different (p<0.0001 in all significantly different groups). Data are expressed as means ± S.E.M. of 7 rats in each group.

significantly one of the CVD risks which is serum TGs. This improvement of lipid profile was accompanied with better glycaemia control as evidenced by significantly lower serum level of insulin and glucose and HOMA-IR than the control group.

Estrogen has been found to correlate negatively with ghrelin expression and secretion in the stomach (Kellokoski et al. 2005). The present study showed that ghrelin antagonist (D-Lys3) GHRP-6 was effective in treating the ovariectomy-induced obesity as evidenced by a significant reduction of body weight, food intake, BMI, and GCOF weight near to the control levels. The mechanisms underlying the body weight lowering effect of (D-Lys3) GHRP-6 may be explained by reduced food intake as evidenced in the present study and increased lipolysis as proved by the decreased weight of GCOF and is consistent with Asakawa et al. (2003) and Maletinska et al. (2011).

The present study also showed that ghrelin antagonist was effective in reducing the CVD risk factors as evidenced by a significant decrease of serum TC and LDL-C and significantly increased HDL-C as compared to the OVX group with insignificant increase in TGs levels. Asakawa et al. (2003) have reported that treatment of ob/ob obese mice with D-Lys3 GHRP-6 resulted in insignificant decrease of TC and TGs levels as compared to the control saline treated ob/ob obese mice. The mechanisms by which ghrelin antagonist decreases the atherosclerotic risk factors may involve inhibition of lipogenesis and stimulation of lipolysis, actions which are opposite to ghrelin (Varela et al. 2011). In addition, Andrews et al. (2010) have found that central ghrelin administration may induce expression of various fat storage promoting enzymes as lipoprotein lipase (LPL), acetyl-CoA carboxylase (ACCα), fatty acid synthase (FAS), and stearoyl-CoA desaturase (SCD1) in white adipose tissue (WAT); while the rate limiting step in fat oxidation, carnitine palmitolyltransferase 1 (CPT1), was decreased. These effects could be reversed by the ghrelin antagonist as proved by the changed lipid profile observed in the present study.

The reduction of food intake, body weight, and adiposity was accompanied with return of fasting serum level of glucose, insulin, and HOMA-IR to normal. This is consistent with Maletinska et al. (2011) who have explained the lower glucose level by the enhanced fibroblast growth factor 21 (FGF21) production that could directly contribute to an increase in GLUT-1 expression in abdominal fat of (D-Lys3) GHRP-6 -treated mice. FGF21 has been shown to mediate insulin-independent glucose uptake into adipose tissue via increased expression of GLUT-1 (Dostalova et al. 2009).

Contrary to the results of the present study, Esler et al. (2007) have found that ghrelin dose-dependently suppressed insulin secretion from dispersed rat islets. This effect was fully blocked by a GHS-R1a antagonist demonstrating that GHS-R1a antagonists have a potential to improve the diabetic condition by promoting glucose-dependent insulin secretion and promoting weight loss. Theoretically, the decrease in insulin secretion with ghrelin administration could be an adaptation to an increase in the peripheral insulin sensitivity. Previous studies in humans and animals have suggested that ghrelin consistently reduces, rather than improves, peripheral insulin sensitivity (Vestergaard et al. 2008). So, reduced insulin sensitivity and increased insulin level after ovariectomy could be mediated by increased ghrelin secretion and adiposity; an effect which was antagonized by ghrelin antagonist.

In conclusion, ovariectomy can cause various metabolic alterations that mimic features of the metabolic syndromes. Although estradiol replacement therapy succeeded in the prevention of almost all ovariectomy-induced disturbances, it had a potential cardiovascular risk due to a marked increase in serum TGs level and it produces many other serious complications. Ghrelin antagonist (D-Lys3) GHRP-6 was effective in ameliorating the ovariectomy-induced obesity without hormonal replacement and it was more effective to correct the cardiovascular risky lipid profile. So, it may be used as a promising treatment in postmenopausal women. Conducting further researches on postmenopausal obesity in women and the use of ghrelin antagonist as compared with hormone replacement therapy is currently performed.

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