# GHRELIN ANTAGONIST "(D-L YS$^3$) GHRP-6" AMELIORATES OVARIECTOMY-INDUCED OBESITY IN ADULT FEMALE ALBINO RATS.

By
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**ABSTRACT:**
The prevalence of obesity is increasing worldwide and is reaching epidemic proportions. The majority of the normal adults are becoming overweight and one of the subpopulation in which this is growing most rapidly is postmenopausal women.
In the present study we hypothesized that antagonizing the ghrelin action could be beneficial in treating the ovariectomy induced obesity and its associated metabolic disturbances. Twenty eight female albino adult rats of the local strain were used. They were left to acclimatize to lab condition for one week before the start of experiment. Rats were randomly divided into four groups (7 rats each): The control group (C) was sham operated and received no treatment. The ovariectomized (OVX) group in which rats were subjected to ovariectomy and received no treatment. The ovariectomized estradiol (OVX-E) group was treated with estradiol injection (30ug\kg s.c) for four weeks starting one week post-operative. The ovariectomized ghrelin antagonist (OVX-GA) group was treated with the ghrelin antagonist (D-Lys$^3$) GHRP-6 intraperitoneal in a dose of 0.5 mg\kg twice daily for one week starting four weeks post-operative. Data of the present study indicate that, the ghrelin antagonist significantly decreased food intake, body weight, body mass index (BMI), gastrocolic omentum fat weight, total cholesterol (TC), low density lipoprotein cholesterol (LDL-c), serum glucose (G), insulin (I), HOMA-IR (IR), and significantly increased high density lipoprotein cholesterol (HDL-c) and triglycerides (TGs) compared with the OVX group. Some parameters were normalized (food intake, body weight, BMI, gastrocolic omentum fat weight, G, I, IR, HDL-c). TC, TGs and LDL-c were improved but were still significantly different from the C group. It is concluded from this study that, ghrelin antagonist is benifial in ameliorating ovariectomy-induced obesity. So it may be used as a promising treatment in postmenopusal women.

**KEYWORDS:**
ghrelin antagonist (D-Lys$^3$) GHRP-6 Ovariectomy Obesity GHS-R- metabolic syndrome Postmenopusal obesity.

## INTRODUCTION:

The prevalence of obesity is increasing worldwide and is reaching epidemic proportions. The majority of the normal adults are becoming overweight and one of the subpopulation in which this is growing most rapidly is postmenopausal women (Sharma et al., 2008).

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, 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).

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 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).

Similar to humans, ovariectomized (OVX) rodents fed high energy diets develop obesity due to the lack of estrogen and the excess of consumed energy, and thus, these animals can serve as a model for commonly observed postmenopausal human obesity (Brown and Clegg, 2010).

The aim of this work is to study the potential effect of ghrelin antagonist (D-Lys$^3$) GHRP-6 on ameliorating the ovariectomy induced obesity and its associated metabolic effects in rats.

## MATERIALS AND METHODS:

### Animals Used

Twenty eight adult female albino rats from the local strain, of body weight ranging from 150-200 grams at the beginning of this study. They were housed at room temperature with natural light\dark cycles for one week for acclimatization. Rats were fed a standard diet of commercial rat chow and tap water ad libitum until the time of the experiment (Ahmadi and Oryan, 2009). During the acclimatization period, daily food intake was measured to know the mean daily food intake per rat.

Rats were randomly divided into four groups (7 rats each):

1. The control (C) group in which rats were sham operated sham operated.
2. **Ovariectomized (OVX) group:** in which the rats were subjected to ovariectomy and received no treatment.
3. **Ovariectomized estradiol treated (OVX-E) group:** in which the rats were subjected to ovariectomy, then after one week recovery, the rats started to receive daily subcutaneaous injection of estradiol benzoate (Folone from Misr Co. For Pharm. Ind. S.A.E.); 30 ug\kg body weight (Babaei et al., 2010) for 4 weeks (Rivera and Eckel, 2010).
4. **Ovariectomized ghrelin antagonist treated group (OVX-GA):** in which the rats were subjected to ovariectomy, then after four weeks, the rats started to receive daily intraperitoneal injection of 0.5mg\kg ghrelin antagonist (D-Lys$^3$ GHRP-6) (Sigma, St. Louis,USA) at 7:00 am and 7:00 pm for one week (Asakawa et al., 2003).

The following parameters were measured: body weight, food intake, body mass index (BMI, = body weight (g)/length$^2$ (cm$^2$) (Novelli et al., 2007),gastrocolic omentum fat weight (GOF) weight (Liang et al., 2002), Serum lipids (total cholesterol (TC) (Deeg and Ziegenhorn, 1983), triglycerides (TGs) (Cole et al., 1997), high density lipoprotein cholesterol (HDL-c) and low density lipoprotein cholesterol (LDL-c) (Schaefer and McNamara, 1997) were measured by quantitative enzymatic colorimetric methods, using kits supplied by Greiner Diagnostic GmbH- Germany.

Serum level of glucose (G) was determined by glucose oxidase enzymatic colorimetric technique, according to the method described by Tietz (1995), using kits supplied by Egyptian Company for Biotechnology (S.A.E), Egypt. Serum insulin (I) level was determined using insulin Enzyme-Linked Immunosorbent assay (ELISA) kit (Clark and Hales, 1991).

Results were statistically analyzed by one-way ANOVA for differences between means of different groups. And expressed as mean ± S.E.M. Significance of differences between means of controls and treated animals was evaluated by unpaired Student's t-test. A probability of 0.05 or less was considered as significant.

**RESULTS:**

**1- Effect of ghrelin antagonist on body weight, body mass index (BMI), gastrocolic omentum fat (GOF) weight.**

Data presented in Table (1), show the effect of ghrelin antagonist (D-Lys$^3$) GHRP-6 on the studied parameters. It is clear from the data that, ovariectomy caused a significant increase in body weight (BW) from the second week after OVX till the end of the study, GOF weight, and final body mass index (FBMI) compared with C group. Estradiol treatment normalized the above mentioned parameters to control level. ghrelin antagonist treatment restore the above parameters to control level.

**Table (1):** Effect of ghrelin antagonist on body weight, body mass index (BMI), gastrocolic omentum fat (GOF) weight

| Groups | IBW | One week BW | Two week BW | Three week BW | Four week BW | Five week BW | IBMI g/cm$^2$ | FBMI g/cm$^2$ | GOFg |
|---|---|---|---|---|---|---|---|---|---|
| **Control** | 182.57 ±5.51 | 191.36 ±6.94 | 194.89$^{bc}$ ±6.8 | 0.55 ± 0.02 | 0.53 ± 0.01 | 0.53 ± 0.02 | 0.55 ±0.02 | 0.56$^b$ ± 0.01 | 2.22$^a$ ±0.13 |
| **OVX** | 179.00 ±2.93 | 184.57 ± 3.17 | 212.36$^a$ ±2.39 | 229.43$^a$ ±4.01 | 242.43$^a$ ±3.43 | 254.71$^a$ ±2.51 | 0.53 ± 0.01 | 0.66$^a$ ± 0.02 | 3.76$^b$ ±0.21 |
| **OVX-E** | 177.00 ±4.9 | 179.89 ±5.98 | 187.86$^c$ ±6.19 | 196.57$^b$ ±6.55 | 202.71$^b$ ±7.02 | 214.43$^b$ ±6.57 | 0.53 ± 0.02 | 0.57$^b$ ± 0.02 | 2.47$^a$ ±0.21 |
| **OVX-GA** | 174.86 ±2.71 | 181.43 ±3.16 | 208.43$^{ab}$ ±4.14 | 226.19$^a$ ±3.6 | 245.24$^a$ ±4.24 | 230.2$^b$ ±3.17 | 0.51 ± 0.02 | 0.56$^b$ ± 0.01 | 2.26$^a$ ±0.17 |

Data are expressed as mean ± S.E.M. of 7 rats in each group. IBM: initial body weight, IBMI: initial body mass index, FBMI: final body mass index, OVX: Ovariectomized group, OVX-E: Ovariectomized estradiol treated group, and OVX-GA: Ovariectomized ghrelin antagonist treated group. Means in the same column with different superscripts (a, b, and c) are statistically significant ($P < 0.05$).

**2- Time course changes in food intake (g/day) in the different studied groups.**

Figure (1) showed that, the overall daily food intake of all ovariectomized groups dropped significantly during the first week following OVX. After that OVX group showed a significant increase in food intake till the end of the study. Injection of estradiol prevented this significant increase in food intake and kept it insignificantly different from the C group. Injection of ghrelin antagonist significantly reduced food intake to reach C group.

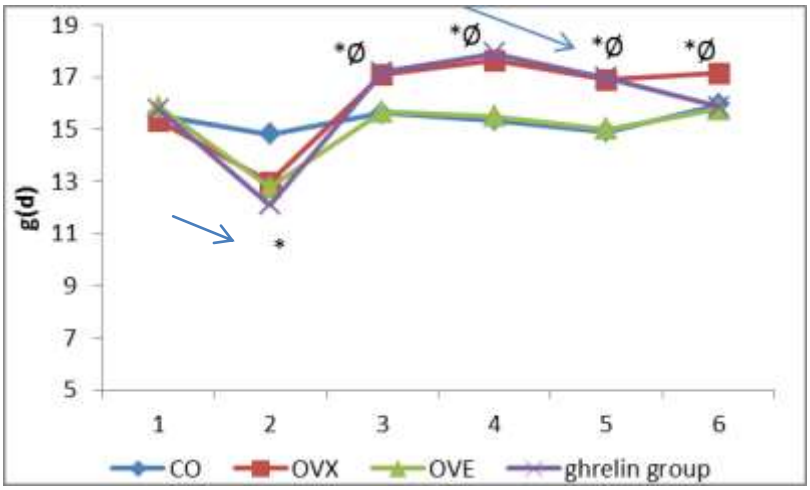


**Figure (1): Time course changes in food intake (g/day) of the different studied groups.** Data are expressed as means ± S. E. M. of 7 rats in each group.CO: Control, OVX: Ovariectomized, OVE:OVX+estradiol. *Significant from its overall daily food intake before ovariectomy. Ø Significant from the corresponding control. Start of ghrelin antagonist injection.

**3- Effect of ghrelin antagonist on lipid profile.**

Data presented in table (2) showed that, ovariectomy resulted in a significantly higher serum level of TC and LDL-c associated with a significantly lower serum level of TG and HDL-c compared with C group. Treatment with estradiol normalized the serum level of both TC , LDL-c and HDL-c but significantly increased serum level of TG compared with OVX and C groups. Ghrelin antagonist normalized HDL-c to C group. The other lipid profile parameters were improved (TC, LDL-c, TGs) but still significantly different from C group.

**Table (2):** Serum level of lipid profile in the different studied groups (mg/dl).

| Groups | TC | TGs | LDL-c | HDL-c |
|---|---|---|---|---|
| **Control** | $105.12^{c}$ ±1.85 | $88.45^{b}$ ±2.67 | $48.96^{c}$ ±2.42 | $39.72^{ab}$ ±1.56 |
| **OVX** | $167.56^{a}$ ±6.66 | $57.38^{c}$ ±1.59 | $124.37^{a}$ ±3.32 | $31.61^{c}$ ±1.46 |
| **OVX-E** | $112.03^{c}$ ±5.63 | $165.01^{a}$ ±4.62 | $44.13^{c}$ ±2.21 | $35.38^{bc}$ ±3.15 |
| **OVX-GA** | $142.44^{b}$ ±2.99 | $66.98^{c}$±6.61 | $84.63^{b}$ ±4.2 | $44.47^{a}$ ±3.25 |

Data are expressed as mean± S.E.M. of 7 rats in each group.TC: total cholesterol, TGs: triglycerides, LDL-c: low density lipoprotein cholesterol, and HDHL-c: high density lipoprotein cholesterol. Means in the same row with different superscripts (a, b, and c) were statistically significant.

**4- Effect of ghrelin antagonist on serum glucose, insulin levels and HOMA-IR.**
Figure (2) clearly demonstrated that, ovariectomy significantly increased serum level of glucose, insulin and HOMA-IR compared with C group. Estradiol treatment significantly lower serum levels of glucose, and HOMA-IR compared with OVX and C groups. Ghrelin antagonist treatment normalized these parameters to C group.

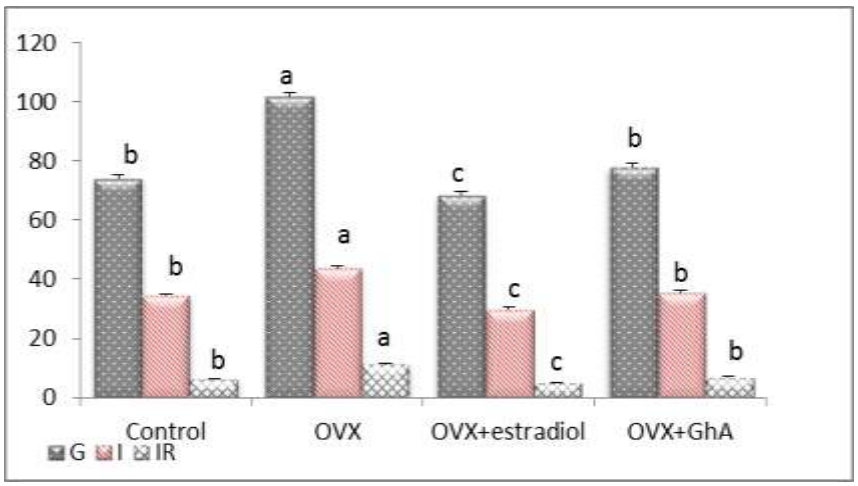


**Figure (2): Serum level of glucose (mg/dl), insulin (µU/ml), and HOMA-IR in different studied groups.**

Data are expressed as means ± S.E.M. of 7 rats in each group. OVX: Ovariectomized, GhA: ghrelin antagonist, G: Glucose, I: insulin, and IR: HOMA-IR. Means of columns of the same parameter carrying different superscripts (a,b, and c) are statistically different ($P< 0.05$).
HOMA -IR: Homeostasis model assessment of insulin resistance.HOMA- IR = Serum Glucose (mg/dl) X Serum insulin (µU/ml) / 405.

**DISCUSSION:**

The obesity epidemic calls for novel pharmacologic treatment methods. The inhibition of ghrelin action seems to represent a promising approach, as it attenuates food intake and subsequently improves other pathologies and metabolic parameters clustered within the so-called metabolic or insulin-resistance syndrome (Maletinska et al., 2011).

OVX rats were shown to have increased circulating levels of the orexigenic gut peptide ghrelin, which positively correlated with transient hyperphagia and resulted in permanent weight gain. This orexigenic action of ghrelin in OVX rats was decreased by estrogen replacement (Clegg et al., 2007). Based on these findings about ghrelin and ovariectomy, the ghrelin receptor antagonist (D-Lys$^3$) GHRP-6 was used in the present study to test its potential effect in the treatment of ovariectomy induced obesity and its associated metabolic effects.

The significant increase in body weight following OVX in the present study could be explained by a significant increase in food intake and increased lipogenisis which was reflected in the significant increase in BMI and GOF weight. This is consistent with liang et al. (2002) and Hamed et al. (2011).

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 (Yepuru et al., 2010). This was evidenced in the present study by the increase in lipid profile parameters (cardiovascular disease risk factors) and significant increases in serum levels of glucose, insulin, and HOMA-IR in OVX group. These data are in agreement with Gavrilla et al. (2003) in human and by Leite et al. (2009) in rats.

The mechanisms underlying these changes in lipid metabolism are not clear. However, Paquette et al. (2008) explained the ovariectomy induced dyslipidemia by a change in expression of key transcriptional factors related to hepatic lipid regulation, such as the peroxisome proliferator-activated receptor (PPAR)α, sterol regulatory element-binding protein-1c (SREBP-1c), and stearoyl- CoA desaturase-1 (SCD-1).

Obesity induced by ovariectomy may be involved in the induction of insulin resistance (IR). This is in agreement with Kaaja (2008) who found that reduced insulin sensitivity did not appear until postmenopausal women had accumulated levels of visceral adipose tissue that approximated levels seen in men, suggesting a possible threshold effect of abdominal fat and IR. Another mechanism of IR in estrogen deficient conditions is the impairment of lipid metabolism in the liver and adipocytes. The mechanism by which food intake and adiposity increased after OVX is the lack of estrogen hormone and lost repression of adipose tissue proliferation and adipokine synthesis as found by Yepuru et al. (2011). This is evidenced in the present work by reversal of almost all effects of ovariectomy by estradiol replacement.

The decreased body weight and BMI found in the present study with estradiol treatment is in agreement with El-Nasr et al. (2011) who explained that by a significant rise in plasma leptin, hence decreasing food intake and increasing energy expenditure and thereby decreasing body weight. However, studies evaluating the effect of estrogen replacement therapy on leptin levels were contradictory, with some authors supporting a stimulatory effect of estrogen whereas others suggested that estrogens do not have a stimulatory action on leptin in humans (Nar et al., 2009). Recently, the estrogen effects were found to be independent of leptin, and they were able to substitute for leptin anorexigenic effects in leptin resistant conditions (Matyškova et al., 2010).

In the present study, estradiol replacement to ovariectomized rats significantly decreased serum levels of TC and LDL- c and significantly increased serum level of HDL-c which are in agreement with Babaei et al. (2010) suggesting a preventive effect of estradiol against cardiovascular disease (CVD).

The reduction of LDL-c by estradiol is probably due to the ability of estradiol to stimulate the expression of LDL-receptor gene and increasing the number of LDL receptors. LDL-c internalizes into the cells through the process of LDL-receptor mediated endocytosis accelerating LDL catabolism (Gopalakrishnan and Chandra, 2006). Another protective mechanism offered by estradiol in lowering LDL-c and increasing HDL-c is through depression of hepatic lipase enzyme activity, thereby decreasing HDL-c catabolism. Estradiol fatty acyl esters

incorporate into HDL and enhance the atheroprotective properties of HDL by mediating the initial steps of reverse cholesterol transport (Badeau et al., 2009).

The increased TG level found with estradiol 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. The mechanism of increased TG by estradiol involves estradiol induced increase in hepatic TG secretion secondary to an increase in very low density lipoprotein (VLDL) triglyceride and apo B production (Gorodeski, 2002).

Improved glycemic control with estradiol treatment observed in the present study is in agreement with El-Nasr et al. (2011) who explained that by a significantly decreased renal gluconeogenesis after estradiol treatment, in addition to increase in the whole body as well as skeletal muscle glucose uptake through increasing the expression of glucose transporter -4 (GLUT-4) (Campello et al., 2011).

The ability of estradiol replacement in ameliorating the effects of ovariectomy found in the present study may be mediated partially by the reduction of ghrelin secretion which confirmed by the significant attenuation of ovariectomy induced effects in ovariectomized group treated with ghrelin antagonist.

The mechanisms underlying the body weight lowering effect of (D-Lys$^3$) GHRP-6 found may be explained by reduced food intake and increased lipolysis as evidenced by the significant reduction of food intake and decreasing the weight of GOF.

Which may be explained by increased mRNA expression of uncoupling protein-1 (UCP1) in brown adipose tissue (BAT) in the OVX groups, suggesting enhanced energy dissipation?. The BAT contains numerous mitochondria with UCP1 located on mitochondrial membrane that plays an important role in energy dissipation (Mano-Otagir et al., 2010).

In agreement with Asakawa et al. (2003), the present study showed that ghrelin antagonist was effective in reducing the CVD risk factors as evidenced by a significant decrease of serum total cholesterol and LDL-c and significantly increased HDL-c as compared to the OVX group with insignificant increase in TG level. The mechanisms by which ghrelin antagonist decrease the atherosclerotic risk factors may involve inhibition of lipogenesis and stimulation of lipolysis, actions which are opposite to ghrelin (Varela et al., 2011).

The reduction of food intake, body weight and adiposity with ghrelin antagonist treatment 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 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-Lys$^3$) GHRP-6-treated mice. FGF21 was shown to mediate insulin-independent glucose uptake into adipose tissue via increased expression of GLUT-1 (Dostalova et al., 2009). Also FGF21 treatment has been recently shown to reduce body weight, body fat, blood glucose, insulin, and lipid levels without affecting food

intake in ob/ob mice and mice with diet induced obesity (Xu et al., 2009**).**

**CONCLUSION:**

In conclusion, this study demonstrated that, ghrelin antagonist (D-Lys$^3$) GHRP-6 ameliorates OVX induced obesity and restored food intake, body weight, BMI, GOF weight, G, I, IR, HDL-c to normal values. TC, TGs, and LL-c were improved but still significantly different from control values. So ghrelin antagonist may be a promising treatment in postmenopusal obesity.

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## مضاد الجرلين (البيبتيد السداسي المحفز لافراز هرمون النمو ليسين$^3$) يخفف من السمنة المحدثة بإستئصال المبيضين في إناث الفئران البيضاء البالغة

**سليم محمود عبد الحكيم, مجدي قاسم عبد العال حسن, مريم يحي ابراهيم خليل, هناء محمد ابراهيم, ومني محمد ابراهيم.**
**من قسم الفسيولوجي بكلية الطب- جامعة المنيا.**

يهدف البحث الي التعرف علي تأثير مضاد الجريلين علي السمنة الناتجة عن استئصال المبيضين لدي اناث الفئران البيضاء البالغة, حيث اشتملت عينة البحث علي عدد 28 فأرة بالغة, تم تقسيمهن الي 7 مجموعات قوام كل مجموعة 7 فئران علي النحو التالي:-
1- المجموعة الضابطة 2- مجموعة اناث مستأصلة المبيضين 3- .مجموعة اناث مستأصلة المبيضين ومعالجة بهرمون الانوثة الاستراديول 4 مجموعة اناث مستأصلة المبيضين ومعالجة بمضاد الجريلين6-GHRP (D-Lys$^3$)

تم قياس كل من وزن الجسم وكمية الطعام المتناولة ووزن الدهن المعدي الثربي ومؤشر كتلة الجسم و تم فصل المصل لقياس كل من الكوليستيرول الكلي والكوليستيرل في البروتين الدهني منخفض الكثافة والكوليستيرول في البروتين الدهني مرتفع الكثافة والجليسريدات الثلاثية والجلوكوز والانسولين ومقاومة الجسم للانسولين.

وقد اثبتت الدراسة ان استئصال المبيضين ادي الي زيادة ذات دلالة احصائية في كل من وزن الجسم وكمية الطعام المتناولة ومؤشر كتلة الجسم ووزن الدهن المعدي الثربي والكوليستيرول الكلي والكوليستيرول في البروتين الدهني منخفض الكثافة والجلوكوز والانسولين ومقاومة الجسم للانسولين وانخفاض ذو دلالة احصائية في الجليسردات الثلاثية والكوليستيرول الكلي و البروتين الدهني مرتفع الكثافة في حين ادي العلاج التعويضي بالاستراديول الي انخفاض ذي دلالة احصائية في كل من وزن الجسم وكمية الطعام المتناولة ومؤشر كتلة الجسم ووزن الدهن المعدي الثربي والكوليستيرول الكلي والكوليستيرول في البروتين الدهني منخفض الكثافة والجلوكوز والانسولين ومقاومة الجسم للانسولين وزيادة ذات دلالة احصائية في كل من الكوليستيرول في البروتين الدهني مرتفع الكثافة والجليسريدات الثلاثية حتي اقتربت من المجموعة الضابطة.

ادي الحقن البريتوني لمضاد الجيرلين الي انخفاض ذي دلالة احصائية في كل من وزن الجسم الزائد وكمية الطعام المتناولة ومؤشر كتلة الجسم ووزن الدهن المعدي الثربي والجلوكوز والانسولين ومقاومة الجسم للانسولين حتي وصلت الي قيم المجموعة الضابطة, كما ادي الي انخفاض ذي دلالة احصائية في كل من الكوليستيرول الكلي والكوليستيرول في البروتين الدهني منخفض الكثافة مع عدم وصولهم الي المجموعة الضابطة في حين زاد الكوليستيرول في البروتين الدهني مرتفع الكثافة ووصل للمجموعة الضابطة. وزادات الجليسريدات الثلاثية زيادة ذات احصائية لكنها ظلت منخفضة معنويا عن المجموعة الضابطة.

الخلاصة:1- استئصال المبيضين يؤدي الي اختلال في بعض عمليات التمثيل الغذائي الخاص بالكربوهيدرات والدهون يقترب من اضطرابات متلازمة الأيض.2 - يمكن معالجة العديد من اضطرابات الأيض الناتجة عن استئصال المبيضين باستخدام العلاج التعويضي بالاسترادايول.

3- مضاد الجيرلين اعاد معظم القياسات الي مستواها الطبيعي وحسن كثيرا من بعض القياسات وبذلك يمكن ان يكون علاجا واعدا للسمنة المحدثة باستئصال المبيضين والسمنة المصاحبة لسن الياس.

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