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The effects of 17β-estradiol plus drospirenone on anthropometric and biochemical measures of adiposity in menopausal women

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Abstract
Purpose To assess whether there are changes on anthropometric and biochemical measures of adiposity in pre- and postmenopausal women and in the latter before and after 6 months treatment with 17β-estradiol plus drospirenone.
Methods Twenty postmenopausal and 20 premenopausal women were enrolled in a prospective comparative study. Postmenopausal women received 1 mg 17β-estradiol plus 2 mg drospirenone daily for 6 months. Measurements of body mass index (BMI), waist/hip ratio and plasmatic levels of insulin, glucose, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride, leptin, adiponectin, orexin-A, glucagon-like peptide-1 (GLP-1) and ghrelin were performed in premenopausal (group 1) and postmenopausal women and in the latter before (group 2a) and after (group 2b) 6 months treatment with 17β-estradiol plus drospirenone.
Results No significant changes in BMIs, insulin and glucose were observed between group 1 and 2a; and group 2a and 2b. GLP-1 levels were significantly increased in group 1 compared to group 2a ($p = 0.035$). Leptin levels were significantly increased ($p = 0.001$) and GLP-1 levels were significantly decreased ($p = 0.021$) in group 2b compared to group 2a. HDL was significantly decreased while LDL and triglyceride levels were significantly increased in group 2a compared to group 1. ($p = 0.030$, $p = 0.001$, $p = 0.020$; respectively) LDL was significantly decreased ($p = 0.010$) in group 2b compared to group 2a. GLP-1 had a positive correlation with orexin-A ($p < 0.001$, $r = 0.520$) and negative correlation with leptin ($p = 0.008$, $r = -0.345$).
Conclusion Leptin was significantly higher and GLP-1 was significantly lower in women receiving 17β-estradiol plus drospirenone treatment. GLP-1 levels were significantly lower after the menopause compared to premenopausal levels. Orexin-A and GLP-1 were positively correlated.

Keywords Hormone replacement therapy · Leptin · Adiponectin · Ghrelin · Orexin-A · Glucagon-like peptide-1

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Introduction

Obesity is frequently observed with metabolic complications. Body fat distribution is critically important for obesity and cardiovascular disorders. Fat accumulation in the abdominal region is associated with an increased risk for cardiovascular diseases and mortality, whereas the gynecoid pattern of fat accumulation is a protective metabolic factor [1]. In most studies, the quantity of abdominal fat has been calculated as a waist/hip ratio and it has been argued that hormone replacement therapy (HRT) decreases

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abdominal fat [2, 3]. High levels of low-density lipoprotein (LDL) and triglycerides, and decreases in high-density lipoprotein (HDL) levels have been associated with ischemic heart disease [4].

One of the most controversial subjects is HRT and whether it influences weight gain. Body mass index (BMI) reaches its peak in women and men between the ages of 50–59 years after increasing continuously with age [5]. Waist circumference increased significantly in a 6-year study of 543 premenopausal women; these gains were caused by diminishing energy needs with age, increasing food intake, and falling metabolic activity [6]. Weight gain also causes changes in fat distribution as abdominal adiposity increases [7]. In many studies, abdominal obesity has been assessed using the waist/hip ratio [8]. Although estrogen deficiency resulting from menopausal changes was blamed initially, elevating androgen levels and decreasing sex hormone binding globulin (SHBG) levels also play important roles in increased abdominal obesity [9].

Adipose tissue is the largest endocrine organ and is influenced by leptin and adiponectin, which are autocrine secretions, ghrelin produced by the stomach and bowel, orexin-A released by central nervous system and the pancreas, and glucagon-like peptide-1 (GLP-1). These hormones all have important functions in energy intake and balance.

In this study, we determined whether HRT (Angeliq®: 1 mg 17β-estradiol [E_2] and 2 mg drospirenone daily for 6 months) has effects on fat tissue distribution and metabolism in postmenopausal women. First, we investigated the influences of the perimenopausal period on fat metabolism by comparing premenopausal women with postmenopausal women. Then, we focused on the effects of HRT on fat metabolism during menopause. We evaluated BMI, waist/hip ratio, adiponectin, ghrelin, orexin-A, and GLP-1 levels, as well as follicle stimulating hormone (FSH), luteinizing hormone (LH), LDL, HDL, triglycerides, fasting plasma glucose, and insulin parameters.

Methods and materials

Subjects

The study group comprised 20 premenopausal and 20 postmenopausal women (age 35–55 years) who admitted to the Istanbul University Cerrahpasa School of Medicine, Department of Obstetrics and Gynecology outpatient clinic between January 2008 and October 2009, and who had no other medical illnesses. Postmenopausal women were those who had not had a menstruation for the last year and premenopausal women were those who continued to have regular menstrual cycles. This study was approved by the Ethics Committee of Cerrahpasa Medical Faculty, and every patient gave informed consent. The manuscript was designed according to the STROBE guidelines.

All women agreed not to alter their diet and exercise regimen during the study period. The inclusion criteria were: (1) a naturally occurring menopause after 40 years of age; (2) menopause must have occurred at least 1 year prior to inclusion in the study; (3) a high FSH (>40 IU/l) and a low E_2 level (<10 pg/ml); (4) presence of climacteric symptoms such as hot flashes, palpitations, or night sweats. Exclusion criteria were significant systemic illnesses such as renal failure, liver impairment, cardiovascular disease, hypertension, acute or chronic infections, neoplastic diseases, gastrointestinal diseases, diabetes, or alcoholism. Women were followed up and evaluated monthly for compliance. All patients were periodically followed up, by the same endocrinologist.

Postmenopausal women received 1 mg E_2 plus 2 mg drospirenone (Angeliq®, Bayer Schering Pharmaceuticals, Berlin, Germany) for 6 months. All women were evaluated for BMI, waist/hip ratios, and levels of FSH, LH, HDL, LDL, triglycerides, glucose, insulin, and for hormones that act on fat tissue such as leptin, orexin-A, GLP-1, adiponectin, and ghrelin both before and after treatment. Furthermore, postmenopausal women were compared with premenopausal women for the parameters above. The height and weight were measured without shoes and with clothes using a calibrated stadiometer and balance beam scale, and BMI was calculated as weight (kg)/height $(m)^2$. Waist circumference, measured at the level of the umbilicus, was divided by the circumference of the hip, measured at its greatest gluteal protuberance, to obtain the waist to-hip ratio (WHR).

Blood samples

Blood was drawn after 12–14 h of fasting in the morning. Blood was drawn once from each patient into uncoated serum tubes (3 ml) and ethylenediaminetetraacetic acid (EDTA)-containing plasma tubes (3 ml), respectively. After a resting period of at least 30 min and a maximum of 60 min the tubes were centrifugated at 2,500 g for 10 min. and removal of the plasma. Serum and plasma samples were stored at −80 °C until assay. All icteric or haemolytic blood samples were discarded.

Biochemical assays

Measurement of plasma leptin levels

Plasma leptin levels were measured in duplicate aliquots, using a human enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (cat

no: EL2001-1, AssayMax Human Leptin ELISA kit; Assaypro, St. Charles, MO, USA). The coefficients of intra- and interassay variations for the leptin assay were 4.2 % ($n = 10$) and 9.1 % ($n = 10$), respectively.

Measurement of plasma adiponectin levels

Plasma adiponectin levels were measured in duplicate aliquots, using a human ELISA according to the manufacturer's instructions (cat no: EA2500-1, AssayMax Human Adiponectin ELISA kit; Assaypro). The coefficients of intra- and interassay variations for the adiponectin assay were 4.0 % ($n = 10$) and 9.0 % ($n = 10$), respectively.

Measurement of plasma ghrelin levels

Plasma ghrelin levels were measured in duplicate aliquots, using a human ELISA according to the manufacturer's instructions (cat no: Ek-031-30, Ghrelin Human EIA kit; Phoenix Pharmaceuticals, Burlingame, CA, USA). The coefficients of intra- and interassay variations for the ghrelin assay were 3.8 % ($n = 10$) and 8.7 % ($n = 10$), respectively.

Measurement of plasma orexin-A levels

Plasma orexin-A levels were measured in duplicate aliquots, using a human ELISA according to the manufacturer's instructions (cat no: Ek-031-30, Orexin Human EIA kit; Phoenix Pharmaceuticals). The coefficients of intra- and interassay variations for the orexin-A assay were 3.9 % ($n = 10$) and 9.1 % ($n = 10$), respectively.

Measurement of plasma GLP-1 levels

Plasma GLP-1 levels were measured in duplicate aliquots, using a human ELISA according to the manufacturer's instructions (cat no: Ek-031-30; GLP-1 Human EIA kit; Phoenix Pharmaceuticals). The coefficients of intra- and interassay variations for the GLP-1 assay were 3.6 % ($n = 10$) and 8.8 % ($n = 10$), respectively.

Serum FSH, LH, HDL, LDL, triglycerides, glucose levels were determined on the Olympus AU 800 analyzer by enzymatic methods using commercial kits (Roche Diagnostics, GmbH, Mannheim). Serum insulin was measured by radioimmunoassay using a commercial kit (DSL-1600, USA).

Statistical analysis

The values were expressed as medians. The comparisons of premenopausal with postmenopausal women were performed with the Mann–Whitney *U* test, whereas Wilcoxon's signed-rank test was used when pretreatment values of postmenopausal women were compared with posttreatment values. Correlations were evaluated by Spearman's correlation test. The data were evaluated using the Statistical Package for the Social Sciences version 16.0 for Windows (SPSS, Inc., Chicago, IL, USA). $p < 0.05$ was accepted as statistically significant.

Results

The effects of menopausal status and HRT on factors influencing fat metabolism are presented in Table 1. The median age was 39 years for premenopausal women and 50 years for postmenopausal women ($p < 0.001$). Premenopausal women had a median BMI of 25.5 kg/m^2, whereas postmenopausal women had a median BMI of 26.1 kg/m^2 ($p = 0.266$). The median waist/hip ratio was 0.83 in postmenopausal women and 0.81 in premenopausal women ($p = 0.855$).

No significant changes in BMIs (26.1 vs. 26.0 kg/m^2), waist/hip ratios (0.83 vs. 0.83) were observed before or after HRT in postmenopausal women.

Median leptin levels were similar in premenopausal and postmenopausal women (17 vs. 22 ng/ml, respectively, $p = 0.090$). The median adiponectin level in premenopausal women was 15.1 μg/ml, whereas it was 12.5 μg/ml in postmenopausal women ($p = 0.320$).

Median orexin-A levels were 0.86 pg/ml and 0.65 pg/ml in premenopausal and postmenopausal women, respectively ($p = 0.136$). Median GLP-1 levels were 27 pg/ml in premenopausal women and 19 pg/ml in postmenopausal women ($p = 0.035$). There was no significant difference in median ghrelin levels between postmenopausal and premenopausal women (13 vs. 17 pg/ml, respectively; $p = 0.438$).

After HRT, leptin levels were significantly elevated. (before HRT, 22 ng/ml vs. after HRT, 26 ng/ml; $p = 0.001$). Adiponectin slightly increased after HRT (before HRT, 12.5 μg/ml vs. after HRT, 14.8 μg/ml ($p = 0.086$). Orexin-A levels were 0.65 and 0.66 pg/ml before and after HRT, respectively ($p = 0.482$). The GLP-1 level before HRT was 19 versus 15 pg/ml after HRT ($p = 0.021$).

The ghrelin level was 13 pg/ml before HRT and after HRT ($p = 0.310$).

Glucose and insulin levels were not significantly different when compared to menopausal status. The median HDL was 54.5 mg/dl in premenopausal women and 48.5 mg/dl in menopausal women ($p = 0.030$). The median LDL was 86 versus 100.5 mg/dl in premenopausal and postmenopausal women, respectively ($p = 0.001$). Triglyceride levels were 165.5 mg/dl in premenopausal women and 186.5 mg/dl in postmenopausal women ($p = 0.020$). The median HDL level was 48.5 mg/dl before HRT and 50.5 mg/dl after HRT ($p = 0.198$). LDL levels were 100.5 and 92 mg/dl before and after HRT,

Table 1 Effects of menopausal status and hormone replacement therapy (HRT) on factors influencing adipose tissue and fat metabolism

	Premenopause		Before HRT (postmenopause)		HRT		*p*1*	*p*2**
	Median	25th–75th Percentiles	Median	25th–75th Percentiles	Median	25th–75th Percentiles		
Age	39	37.7–41.0	50	48.2–51.7	50	48.2–51.7	**<0.001**	1
BMI (kg/m^2)	25.5	24.3–27.3	26.1	24.9–28.2	26.0	24.9–28.1	0.266	0.914
Glucose (mg/dl)	93.5	78.5–101.2	93	81.5–98.5	91.5	81.2–102.5	0.849	0.828
Insulin (IU/ml)	8.15	6.55–10.8	9.45	6.72–14.5	9.3	6.17–14.6	0.372	0.989
LDL (mg/dl)	86	76.7–96.7	100.5	93.0–109.5	92.0	87.5–96.5	**0.001**	**0.010**
HDL (mg/dl)	54.5	47.0–58.2	48.5	41.5–53.5	50.5	47.0–56.2	**0.030**	0.198
Triglycerides (mg/dl)	165.5	143–191	186.5	174.5–203.5	179.5	156.7–190	**0.020**	0.083
FSH (mIU/ml)	8.2	5.96–10.0	70.6	54.5–86.5	52.4	44.0–51.5	**<0.001**	**<0.001**
LH (mIU/ml)	4.8	3.88–5.64	41.6	34.0–49.5	27.0	23.3–33.7	**<0.001**	**<0.001**
Leptin (ng/ml)	17	14.4–24	22	18–24	26	22–30	0.090	**0.001**
Adiponectin (μg/ml)	15.1	10.3–20.7	12.5	10.2–16.6	14.8	11.5–21.9	0.320	0.086
Ghrelin (pg/ml)	17	8–26	13	9–17	13	12–22	0.438	0.310
Orexin-A (pg/ml)	0.86	0.59–1.44	0.65	0.43–1.09	0.66	0.50–1.15	0.136	0.482
GLP-1 (pg/ml)	27	20–34	19	15–25	15	8–18	**0.035**	**0.021**

BMI body mass index, *GLP-1* glucagon-like peptide-1, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *FSH* follicle stimulating hormone, *LH* luteinizing hormone

Bold values indicate statistical significance ($p < 0.05$)

* Premenopause versus postmenopause

** Before HRT versus after HRT

respectively ($p = 0.010$). Triglyceride levels before HRT were 186.5 and 179.5 mg/dl after HRT ($p = 0.083$).

Leptin was positively correlated ($p = 0.007$, $r = 0.352$) and GLP-1 was negatively correlated ($p = 0.025$, $r = -0.293$) with age. Orexin-A was negatively correlated with BMI ($p = 0.003$, $r = -0.383$).

Since age and BMI may affect some of the parameters, a partial correlation test with control for age and BMI was performed in subgroups. Partial correlation showed that in the premenopausal group orexin-A ($p < 0.001$, $r = 0.943$) and adiponectin ($p = 0.046$, $r = 0.504$) had positive correlations with GLP-1; however, there was not any correlation between GLP1, leptin, adiponectin, ghrelin and orexin-A in the menopausal groups neither before nor after HRT (Table 2).

In our study group, there was no correlation between age and insulin, glucose, HDL, LDL and triglyceride. However, there were positive correlations between BMI and insulin ($p < 0.001$, $r = 0.465$), LDL ($p < 0.001$, $r = 0.516$) and triglyceride ($p < 0.001$, r = 0.660). There was a negative correlation between BMI and HDL ($p = 0.001$, $r = -0.415$).

Table 2 Correlations between GLP-1, leptin, adiponectin, ghrelin and orexin-A

	Premenopausal		Menopausal pre-HRT		Menopausal post-HRT	
	p	*r*	*p*	*r*	*p*	*r*
GLP-1 vs. leptin	0.883	0.040	0.688	0.102	0.466	−0.184
GLP-1 vs. adiponectin	**0.046**	0.504	0.511	−0.166	0.910	0.029
GLP-1 vs. ghrelin	0.494	−0.184	0.969	−0.010	0.434	0.197
GLP-1 vs. orexin-A	**<0.001**	0.943	0.084	0.418	0.226	0.301
Leptin vs. adiponectin	0.272	−0.292	0.802	−0.064	0.549	0.151
Leptin vs. ghrelin	0.161	0.368	0.553	−0.150	0.754	−0.079
Leptin vs. orexin-A	0.723	0.096	0.937	0.020	0.545	−0.153
Adiponectin vs. ghrelin	0.333	−0.259	0.096	−0.404	0.185	−0.327
Adiponectin vs. orexin-A	0.090	0.438	0.845	0.050	0.984	0.005
Ghrelin vs. orexin-A	0.922	−0.027	0.208	−0.312	0.185	−0.328

Bold values indicate statistical significance ($p < 0.05$)

GLP-1 glucagon-like-peptide-1

Discussion

In our study, no significant changes in BMIs, insulin and glucose were observed in premenopausal and postmenopausal women before or after 17β-estradiol plus drospirenone treatment. There were no significant differences in leptin, adiponectin, orexin-A and ghrelin levels between premenopausal and postmenopausal women. GLP-1 levels were significantly increased in premenopausal compared to postmenopausal women. There were no significant differences in adiponectin, orexin-A, ghrelin before and after 17β-estradiol plus drospirenone treatment. Leptin levels were significantly increased and GLP-1 levels were significantly decreased after 17β-estradiol plus drospirenone treatment. HDL was significantly decreased while LDL and triglyceride levels were significantly increased after menopause. LDL was significantly decreased after 17β-estradiol plus drospirenone whereas HDL and triglyceride did not show a significant change. Partial correlation showed that in the premenopausal group orexin-A and adiponectin had positive correlations with GLP-1; however, there was not any correlation between GLP1, leptin, adiponectin, ghrelin and orexin-A in the menopausal groups neither before nor after HRT.

Levels of leptin, also known as a satiety factor, are directly proportional to the quantity of adipose tissue in the body [10]. Consequently, it would be expected that leptin levels would be higher in women after menopause when the amount of adipose tissue increases. We found that leptin increased significantly after 17β-estradiol plus drospirenone treatment; however, there was no significant change after menopause compared to premenopausal levels. Lee et al. [11] and Bednarek-Tupikowska et al. [12] found no significant difference in leptin levels between pre and postmenopausal women, and leptin levels were reportedly related to BMI. Lambrinoudaki et al. [13] compared leptin levels in overweight and lean postmenopausal women (age, 43–60 years) before and after HRT, which included estrogen or estrogen plus MPA, and detected a trend toward an increase in both groups, but the difference was not significant. In general, studies indicate that HRT limits weight gain and increases leptin levels [13, 14].

Adiponectin, which is the most secreted hormone into the bloodstream by adipose tissue, was similar in pre- and postmenopausal in the present study. Milewicz et al. [15] compared two groups of postmenopausal women (BMIs were obese, 36.6 and non-obese, 22.8) with premenopausal women with a mean BMI of 23.4. The study groups consisted of patients between the ages 34–62 years. Adiponectin level tended to be lower in non-obese postmenopausal women, although the difference was not significant and adiponectin levels were inversely proportional to free testosterone in obese postmenopausal women. Sieminska et al. [16] detected no difference in adiponectin in pre and postmenopausal women.

Plasma adiponectin did not change significantly after 17β-estradiol plus drospirenone treatment in our study. This finding is supported by the study of Sieminska et al. [16]. However, Im et al. [17] showed that plasma adiponectin levels were significantly lower in the HRT (0.625 mg conjugated estrogens and 2 mg MPA for 5 years) group.

Orexin, which is an appetite enhancing hormone, was not significantly changed after menopause, as compared to premenopausal women in our study. Lou et al. [18] found that orexin-A decreases with increasing leptin level. Orexin-A levels are also inversely proportional to BMI [19]. We detected no significant decrease in orexin-A when compared before and after HRT. Exogenously administered leptin has a negative effect on orexin-A levels and orexin-A mRNA expression, suggesting that peripherally administered leptin decreases appetite by inhibiting the orexin system [20].

Another appetite and nutrient-intake enhancing hormone, ghrelin, was not significantly changed in postmenopausal women when compared to levels in premenopausal women. Whatmore et al. [21] found that ghrelin levels tended to decrease with advancing age. Iwamoto et al. [22] found that ghrelin is significantly lower in postmenopausal women, as compared to premenopausal women, and reported that increasing body weight was inversely related with ghrelin levels. In our study after 17β-estradiol plus drospirenone treatment, ghrelin remained unchanged. DiCarlo et al. [23] showed that the women that received the estradiol-nomegestrol therapy had higher ghrelin levels.

Insufficient data are available on the relationships between perimenopausal period, HRT, and GLP-1. In our study the changes in GLP-1 levels after menopause and 17β-estradiol plus drospirenone treatment were both significant. Ranganath et al. [24] compared GLP-1 levels in premenopausal women with those in postmenopausal women and found higher concentrations in the first group. Korosi et al. [25] reported that dipeptidyl peptidase, an enzyme that cleaves GLP-1, decreases with advancing age; thus, GLP-1 levels rise. We also found that GLP-1 levels decreased with increasing age. In the premenopausal group GLP-1 is positively correlated with orexin-A. Orexin-A are increased during fasting in human subjects and are lower in obese subjects compared with normal-weight subjects in the fasted state. GLP-1 is a major contributor to the ileal brake mechanism of the upper gastrointestinal tract, thereby modulating gastric emptying and acid secretion. These two molecules physiologically act in parallel ways. [26] However, in the postmenopausal group we can see this close correlation neither in pre- nor in post-HRT groups.

It's hard to explain this loss of correlation due to insufficiency of data in the literature on correlations between orexin-A, adiponectin and GLP-1 in the postmenopausal group.

The effects of HRT on the cardiovascular system are highly controversial. Although HRT has positive effects on serum lipid profiles, two comprehensive, prospective, randomized controlled studies revealed unexpected results. The first of these two studies (the Heart and Estrogen/Progestin Replacement study) found no significant positive influences of HRT on cardiovascular disorders [27].

GLP-1 was found to be cardioprotective in some studies. [28–30] Discordance on the hemodynamic consequences of GLP-1 pharmacotherapy in experimental animals and human patients has been reported in the literature. However, long-term pharmacological doses of GLP-1 have shown prolonged and beneficial actions on cardiovascular homeostasis in the adjuvant treatment of metabolic disease. [31] The effects of leptin on cardiovascular system remains controversial. Leptin was found to be pro-atherogenic in most of the studies [23, 32] and cardioprotective in few others. [33] In the study of Kantorova et al. [32] showed that significantly higher levels of leptin and lower levels of adiponectin and ghrelin were confirmed in the ischemic stroke group compared to the control group.

The Women's Health Initiative study was terminated in 2002, before completion, due to an increased risk for breast cancer and cardiovascular disease [34]. However, after patients who have been menopausal for 20 years or more were excluded from the study, HRT did not seem to increase the risk for cardiovascular disorders [35]. In the same study, young women, who used only estrogen, had a lower risk for cardiovascular disorders. Al-Azzawi et al. [36] reported that postmenopausal women receiving HRT had lower LDL levels and higher HDL and triglyceride levels. Saranyaratana et al. [37] detected higher HDL and lower LDL levels after HRT use. Dias et al. [38] also found that postmenopausal women receiving HRT had significantly higher levels of HDL and triglycerides and lower LDL than those who did not. In our study, HDL and triglyceride levels were significantly elevated after HRT, and LDL was significantly lower, in agreement with previous studies.

One of the weaknesses of our study was that we did not evaluate the premenopausal women till menopause since this would take more than 10 years. The metabolic features may not be the same although BMI, glucose and insulin levels were similar. Another one is that GLP-1 was negatively correlated with age. The significant decrease of GLP-1 after menopause could be due to the age as well as menopause itself. It is difficult to solve this riddle in a non-parametric evaluation. However, GLP-1 continued to decline after HRT in the same age (since group 2a and 2b both had same age median); which may give a clue that GLP-1 may have decreased due to metabolic changes as well.

Our search in pubmed with the keywords "leptin AND adiponectin AND ghrelin AND orexin AND GLP-1" in English language between years 1960 and 2012 did not show any study evaluating these parameters in the same patient group prospectively, therefore, our study is the first one.

In conclusion, leptin was significantly higher and GLP-1 was significantly lower in women receiving 17β-estradiol plus drospirenone treatment. GLP-1 levels were significantly lower after the menopause compared to premenopausal levels. Orexin-A and GLP-1 were positively correlated.

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Conflict of interest The authors declare that they have no conflict of interest related to the publication of this manuscript.

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