Arch Gynecol Obstet
DOI 10.1007/s00404-013-2742-y

REPRODUCTIVE MEDICINE

The effects of drospirenone-ethinyl estradiol and drospirenone-ethinyl estradiol + metformin on ovarian ultrasonographic markers, body fat mass index, leptin, and ghrelin

Y. Cakiroglu · B. Vural · S. Isgoren

Received: 25 October 2012 / Accepted: 22 January 2013

Abstract
Purpose Polycystic ovary syndrome (PCOS) is considered as the most common endocrinopathy among women of reproductive age. Oral contraceptives (OCs) and metformin are one of the main drug groups in the long-term treatment of PCOS. This study was undertaken to investigate the effects of drospirenone-ethinyl estradiol and drospirenone-ethinyl estradiol + metformin on ultrasonographic markers, body fat mass (BFM) index, leptin–ghrelin.
Methods This was a prospective clinical study conducted at Kocaeli University Department of Obstetrics and Gynecology on 42 PCOS patients. Patients were randomly allocated into two groups [Group I ($n = 22$): drospirenone-ethinyl estradiol (DEE); Group II ($n = 20$): drospirenone-ethinyl estradiol + metformin (M)] according to Body Mass Index (BMI) findings. Patients were evaluated in terms of leptin–ghrelin, ultrasound, and body fat distribution before and 6 months after therapy. Main outcome measures were to investigate the effects of drospirenone-ethinyl estradiol and drospirenone-ethinyl estradiol + metformin on ovarian ultrasonographic markers, BFM index, leptin, and ghrelin.
Results In patients with higher BMI, ovarian volume, numbers of follicles, stromal area, and echogenicity have been reported to be larger. In group II, a negative correlation between ghrelin and abdominal fat mass after treatment has been noted, whereas in group I a positive correlation between leptin and abdominal fat mass after treatment has been observed.
Conclusions Addition of metformin could have beneficial effects on abdominal fat mass. Stromal area measurement and assessment of fat mass with Dual X-ray Absorptiometry could be helpful as a quantitative way of measurement.

Keywords Polycystic ovary syndrome · Leptin · Ghrelin · Drospirenone-ethinyl estradiol · Metformin

Introduction

Polycystic ovary syndrome (PCOS)—defined as unexplained hyperandrogenic chronic anovulation—is considered as the most common endocrinopathy among women of reproductive age [1]. Although randomized controlled trials have not yet been studied on PCOS-related obesity, approximately 35–50 % of cases of PCOS are overweight [Body Mass Index (BMI) >25 kg/m^2) or obese (BMI >30 kg/m2) [2, 3].

Ultrasound criteria for PCOS were first described in 1986 and since then have undergone many changes. According to Rotterdam criteria, essential criteria for the diagnosis of polycystic ovarian syndrome have been described [4]. Increased ovarian volume has been the most agreed ultrasonographic parameter for PCOS [5].

Leptin is a 16-kDa protein hormone consisting of 167 amino acids [6]. It is mainly encoded by the obesity gene and is secreted from adipose tissue. The main function of leptin is to reduce the food intake by inhibiting the secretion of neuropeptide Y [7]. Neuropeptide Y is a strong appetite excitatory and lack of leptin in obese mice results in a desire for increased food intake. From this perspective, high neuropeptide Y levels in hypothalamus might be

Y. Cakiroglu (✉) · B. Vural
Department of Obstetrics and Gynecology, Faculty of Medicine, Kocaeli University, Umuttepe/Uctepeler, 41000 Kocaeli, Turkey
e-mail: dryigit1@yahoo.com

S. Isgoren
Department of Nuclear Medicine, Kocaeli University, Kocaeli, Turkey

Published online: 03 February 2013

responsible for obesity-related disorders [8]. Until today, the only hormone that was known to induce food intake by stimulating hunger was ghrelin [9]. Interestingly, an endogenous ligand for GHS receptor has been identified in the stomach and named as "ghrelin" [10].

In many randomized trials, the use of metformin has been demonstrated to improve ovulatory function in patients with PCOS [11, 12]. In clomiphene-resistant cases, when compared with trials of placebo, addition of metformin significantly increases pregnancy rates in PCOS patients [13]. Oral contraceptives (OCs) are one of the main drug groups in the long-term treatment of PCOS.

This objective of this study was to investigate the effects of drospirenone-ethinyl estradiol and drospirenone-ethinyl estradiol + metformin on ultrasonographic markers, body fat mass (BFM) index, leptin–ghrelin.

Materials and methods

Between 01 July 2007 and 01 September 2008 a total of 42 patients between the ages of 17–37years with the diagnosis of PCOS were recruited for the study. Rotterdam 2003 criteria were employed for the inclusion criteria [14]. Determination of at least two of three criteria were accepted for the diagnosis of PCOS: oligo-and/or anovulation (fewer than 6 menstrual cycles or amenorrhea for a period of longer than 6 months), clinical and/or biochemical signs of hyperandrogenism, polycystic ovaries (presence of at least 12 or more follicles ranging from 2 to 9 mm in each ovary, and/or increased ovarian volume ($\geq$10 mL; calculated by the formula 0.5 × length × width × thickness).

Patients with a history of alcohol–cigarette use, thyroid dysfunction, hyperprolactinemia, Cushing's syndrome, congenital adrenal hyperplasia, diabetes mellitus, infertility, glucocorticoid use, and OC use in the past 3 months were excluded from the study.

All patients were divided into two groups: group I ($n = 22$) and group II ($n = 20$). Patients in each group were separated again into two groups as overweight patients (BMI $\geq$25 kg/m^2) and normal weight patients (BMI <25 kg/m^2). In both groups, three patients were lost to follow-up and were removed from the study. The study protocol was approved by the local ethics committee of the board (CERD No.: 2/14). All patients included in the study filled out a consent form.

Study protocol is summarized in Fig. 1. After the establishment of a computer-mediated patients list, the patients were randomly assigned into two groups, including group 1 and group 2: for patients in group I, an OC (21 tablets, each tablet contains 3 mg drospirenone + 30 mcg ethinyl estradiol (DEE) (Yasmin®, Schering AG, Berlin, Germany) was initially given on the first day of the menstrual period and was continued for 6 months. For patients in group II an OC (21 tablets, each tablet contains 3 mg drospirenone + 30 mcg ethinyl estradiol (DEE) (Yasmin®, Schering AG, Berlin, Germany) was initially given on the first day of the menstrual period and was continued for 6 months with metformin (M) (Glifor® 850 mg, Bilim, Turkey). All patients' diet was regulated.

Detailed histories of all the patients were taken at the beginning of the study. Weights and heights were determined, and then BMI values were calculated [weight (kg)/height (m)^2]. Waist and hip circumferences were measured at the largest part of the gluteal region and at the narrowest part of the lumbar region in order to calculate the waist–hip ratio (WHR). Hirsutism was assessed with the modified Ferriman–Gallwey scale.

All laboratory tests and ultrasound examinations were performed on 2nd–4th day of spontaneous menstruation or 2nd–4th days of bleeding following progesterone withdrawal. All blood samples were obtained by vacuum in the morning following an overnight fasting period and were immediately centrifuged and stored at −20 °C. Levels of serum follicle stimulating hormone (FSH), lutheinising hormone (LH), total testosterone, sex hormone binding globuline (SHBG), free androgen index (FAI), 17 hydroxy progesterone (17-OH-P), fasting blood sugar, fasting blood glucose/insulin ratio, homeostasis model insulin resistance (HOMA-IR) were noted at the beginning of the study and re-evaluated 6 weeks and 6 months after the OC use. The same person recorded all the parameters (Y.C.). Free androgen index was calculated by the formula T/SHBG × 100. Insulin resistance was calculated with homeostasis model [HOMA-IR = (fasting plasma glucose × I/18)/22.5)]. HOMA-IR >2.5 and/or fasting glucose/fasting insulin <4.5 were considered as insulin resistance values.

At the beginning of the study, within the first 4 days in the follicular phase of menses, ultrasonography (3-D Voluson 730D Pro, version 4.03, General Electric, USA) was performed transvaginally by the same operator (Y.C.). Ovarian volume, number of follicles, ovarian stromal area, and ovarian stromal echogenicity were measured. Ovarian volume was calculated using the following formula: Volume = $D_1 \times D_2 \times D_3 \times 0.523$ (D_1 long diameter, D_2 anterior–posterior diameter, D_3 ovarian transverse diameter). While assessing the number of follicles, presence of at least 12 or more follicles ranging from 2 to 9 mm in each ovary was investigated. Ovarian stromal echogenicity was scored as 1 (normal), 2 (moderately increased echogenicity), 3 (marked increased echogenicity) [15]. Stromal area was calculated by circulating the caliper around the central echogenic area. Doppler flow measurement was performed on ovarian vessels. Each calculation was performed and recorded for each ovary separately.

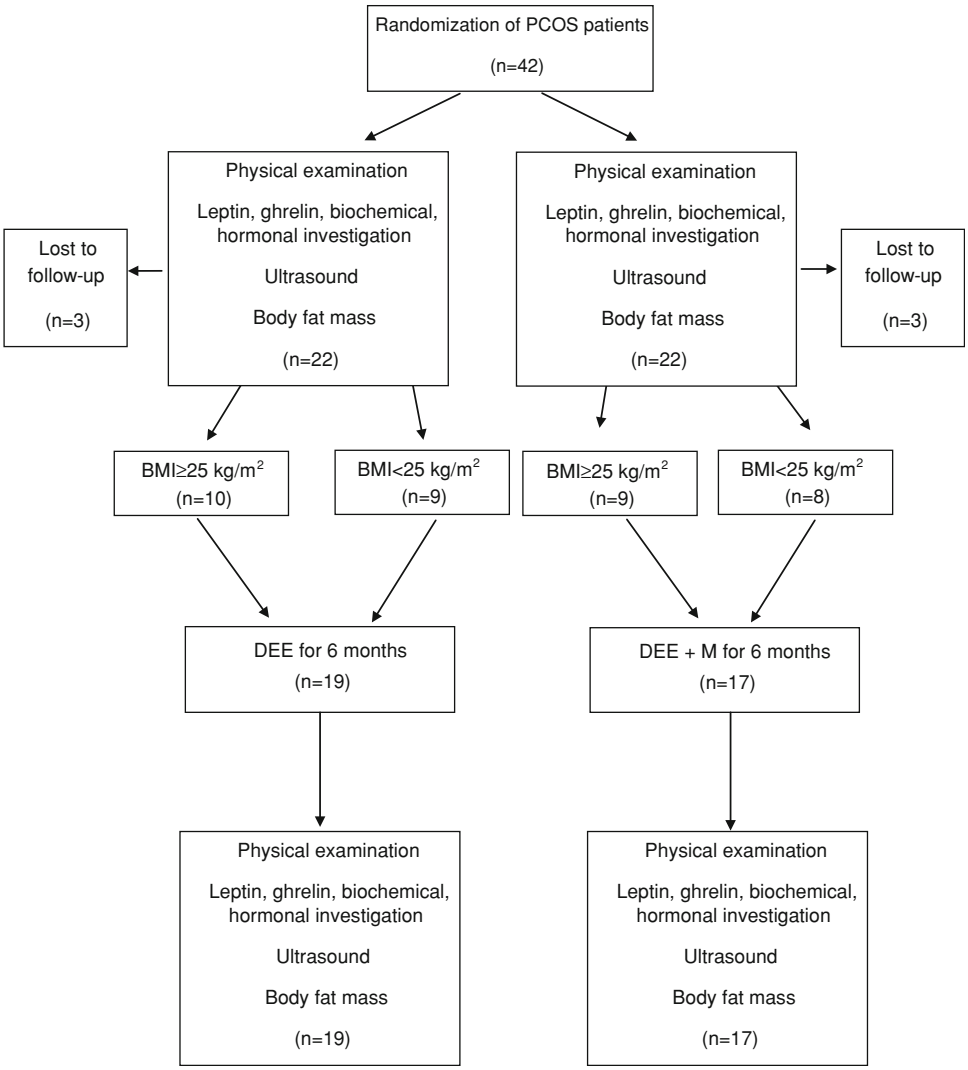

Fig. 1 Algorithm of the study

At the beginning of the study, the amount of BFM was determined in a total of six regions by Dual X-ray Absorptiometry (DEXA). These areas are (1) head, (2) trunk, (3) abdomen, (4) arms, (5) legs, and (6) whole body. The amount of fat mass was determined in each of the six regions. Measurement was performed with Norland XR-36 densitometer device (Norland Co., Fort Atkinson, WI, USA). The same investigator performed research on all patients (S.I.).

All data were analyzed using SPSS 11.5 (SPSS Inc., Chicago, IL, USA). Descriptive statistics are expressed as a mean ± standard deviation. Data distribution was tested with the Shapiro–Wilk test. As a result of an uneven distribution, the differences between groups were tested with the Mann–Whitney *U* test and differences between pre and post treatment values were compared using the Wilcoxon test. Spearman's correlation coefficient was used to calculate the correlation coefficients. *p* values less than or equal to 0.05 were considered statistically significant.

Results

Initial values were compared in between the groups on the basis of their BMI findings as normal weight (BMI <25) or overweight (BMI ≥25). All patients' sociodemographic characteristics are compared in Table 1.

Basal leptin, ghrelin, LH/FSH, total testosterone, FAI, SHBG, 17-OH-P, DHEAS, fasting blood glucose/insulin ratio, and HOMA-IR were compared in both groups between overweight and normal weight patients. In overweight patients, serum leptin levels were significantly higher and serum ghrelin levels were significantly lower (for both $p < 0.001$). Serum leptin levels were found to be increasing directly proportionally to the increase in BMI.

Table 1 Sociodemographic results of the patients

	Overweight group (BMI ≥25) ($n = 19$)	Normal weight group (BMI <25) ($n = 17$)	p
Age (years)		23.0 ± 4.8	0.1
Menarche age (years)	12.9 ± 1.4	12.6 ± 1.1	0.6
Body mass index (kg/m^2)	30.9 ± 6.6	20.9 ± 1.9	<0.001*
Waist–hip ratio	0.90 ± 0.11	0.68 ± 0.05	<0.001*
Ferriman–Gallwey score	8.6 ± 4.9	8.7 ± 3.3	0.9
Acne	0.3 ± 0.5	0.5 ± 0.7	0.4
Alopesia	0.2 ± 0.4	0.1 ± 0.4	0.9
Acanthozis nigricans	0.1 ± 0.2	0	0.8

* Statistical significance according to Mann–Whitney U test, $p < 0.05$

Similarly, the serum ghrelin levels were also found to be decreasing inversely proportionally to the increase in BMI.

Correlation analysis was performed between DEXA and serum leptin and ghrelin levels. The analysis has revealed a positive correlation between serum ghrelin levels and fat mass localized in the abdomen in the normal-weight group ($r = 0.549$, $p = 0.02$). However, except this positive correlation, no other correlation was found between serum leptin–ghrelin levels and abdomen, trunk, arms, legs, and total fat mass index.

Correlation analysis was performed for serum leptin and ghrelin levels and ovarian stromal area, echogenicity, and abdominal BFM (Table 2). As a result of the analysis, a strong positive correlation was noted between serum leptin levels, right and left ovarian stromal area, and the stromal echogenicity in patients diagnosed with PCOS.

Table 2 Correlation analysis of all patients for serum leptin and ghrelin levels and ovarian stromal area, echogenicity, and abdominal body fat mass (r)

	Leptin ($n = 36$)	Ghrelin ($n = 36$)
Right ovary		
Stromal area (cm^2)	0.625*	−0.485*
Stromal echogenicity	0.606**	−0.245
Left ovary		
Stromal area (cm^2)	0.610*	−0.413**
Stromal echogenicity	0.356*	−0.204
Body fat distribution (g)		
Abdomen	0.606*	−0.233
Insulin resistance		
HOMA-IR	0.178	0.155
FBG/insulin	0.47	−0.171

* $p < 0.01$

** $p < 0.05$

The examination of the right ovarian volume and the number of follicles showed no difference between before and after treatment for group I, while a significant reduction was observed in group II for both overweight and normal weight ($p = 0.01$, 0.02, 0.02, 0.02 for overweight and normal weight groups, respectively). A significant reduction in stromal echogenicity was observed in the overweight sub-group of group 2 ($p = 0.04$). Stromal area measurement was not different in the DEE group after treatment, but a statistically significant reduction was determined in the stromal area in the overweight sub-group after treatment (overweight group, $p = 0.02$). In the DEE + M group, both for overweight and for normal weight sub-groups, the stromal area measurements were statistically significantly decreased after treatment (p values 0.008 and 0.03, respectively). The left ovarian volume and the number of follicles were analyzed. In group II, for the overweight sub-group a significant reduction was observed both in the ovarian volume and in the number of follicles after treatment ($p = 0.007$ and 0.006, respectively). The comparison between stromal area measurements between groups I and II showed a reduction in the stromal area on the left ovary only in the overweight subgroup ($p = 0.007$ and 0.04, respectively).

Groups were compared in between according to BMI, WHR, and BFM. A mild reduction was observed in the mean BMI after treatment in DEE + M group, but it was not noted as statistically significant (29.7 ± 5.7, 28.6 ± 6.5; $p = 0.2$). Ferriman–Gallwey scores of patients were evaluated: for group I only the overweight subgroup and for group II, both the overweight and the normal weight sub-groups showed a statistically significant reduction in the scores (p values 0.006, 0.007, 0.03, respectively).

BFM was compared in between groups and a significant reduction in the abdominal region was noted for the normal weight sub-group in group 1, and for the overweight sub-group in group 2 after treatment ($p = 0.05$ and 0.03,

respectively). In addition, the distribution of fat in the arms was found to be decreased for group II after treatment ($p = 0.02$). Differences were only observed in the abdominal and arms regions for both groups.

Correlation analysis was performed between ovarian stromal area and echogenicity and abdominal and total BFM for before and after treatment. According to the analysis, in the DEE group, a strong correlation was observed between ovarian stromal area and echogenicity and abdominal and total BFM before and after treatment.

A correlation analysis was performed between ovarian stromal area and echogenicity with serum leptin and ghrelin levels before and after treatment (Table 3). According to the analysis, a positive correlation between serum leptin levels before and after treatment both in the DEE and in DEE + M group was observed. A significant negative correlation was determined between serum ghrelin levels and stromal area in the DEE + M group.

A correlation analysis was performed between body fat distribution and HOMA-IR and fasting blood glucose/insulin levels before and after treatment. In the analysis, while no correlation was found in DEE + M group before treatment, a statistically significant positive correlation was noted for fat mass in all regions especially in the abdomen with HOMA-IR and fasting blood glucose/insulin levels after treatment. The decrease in insulin resistance after the M treatment revealed the positive contribution of M treatment on abdominal fat distribution and other areas.

Discussion

Etiopathogenesis of PCOS is still unknown and many treatment modalities have been introduced. In this study, leptin–ghrelin levels are investigated and again in patients diagnosed with PCOS effects of drospirenone-ethinyl estradiol and Metformin on ovarian ultrasonographic markers, on the amount of BFM and on serum leptin–ghrelin levels were investigated.

The majority of studies on ghrelin have been investigations on the determination of circulating concentrations. In our study, ghrelin concentrations at the time of first diagnosis of the patients were compared on the basis of BMI, and similar to the literature, a decrease in plasma ghrelin levels were determined directly proportional to the increase in BMI. Looking at the literature, plasma ghrelin levels in patients with PCOS were found to be lower than the values observed in healthy people [16, 17]. Orio et al. [18] have compared ghrelin levels in patients with PCOS with weight-matched healthy obese patients' ghrelin levels. They have argued that ghrelin levels were inversely correlated with BMI in PCOS patients. In our study, we showed that ghrelin levels were positively correlated with BMI, but no correlation was determined with the overweight group. Schöfl et al. [19] have found serum ghrelin levels to be lower for PCOS patients than for healthy, obese and lean patients. How ghrelin levels after diet-related obesity show what kind of changes was investigated. For this purpose, Moran et al. [20] have recommended a diet for patients with PCOS and then re-evaluated the levels of ghrelin. In their study, it was shown that at least 7.5 % weight loss in overweight patients with PCOS leads to increased levels of ghrelin.

In our study, it was shown that basal serum leptin levels increase with obesity for patients with PCOS. Similarly, when patients were reanalyzed according to groups as normal-weight, overweight, and obese, serum leptin levels were also found to be increased. Erturk et al. [21] have investigated serum leptin concentrations for obese and lean patients with PCOS and showed in their study that the concentrations were associated with obesity. Panidis et al. [22] have compared leptin levels in weight-matched healthy patients with PCOS and pointed a close relationship

Table 3 Correlation analysis in between ovarian stromal area and echogenicity with serum leptin and ghrelin levels before and after treatment (r)

	Leptin				Ghrelin			
	Group I ($n = 19$)		Group II ($n = 17$)		Group I ($n = 19$)		Group II ($n = 17$)	
	Before	After	Before	After	Before	After	Before	After
Right ovary								
Stromal area	0.625**	0.311	0.643**	0.623**	−0.456*	−0.250	−0.637**	−0.604**
Echogenity	0.619**	0.662**	0.706**	0.295	−0.386	−0.220	−0.620**	−0.450
Left ovary								
Stromal area	0.668**	0.654**	0.489*	0.607**	−0.343	−0.326	−0.285	−0.488**
Echogenity	0.385	0.552**	0.496*	0.123	−0.469*	−0.418	−0.179	−0.281

* $p < 0.05$

** $p < 0.01$

between serum leptin levels and BMI and PCOS. When our study is evaluated in terms of the correlation analysis, no correlation was found between leptin levels and BMI.

Based on the probable role of serum leptin and ghrelin levels on etiopathogenesis, whether levels have changed after drospirenone-ethinyl estradiol + metformin treatment has been investigated. In our study, leptin levels were found to have decreased in all groups after treatment. Interestingly, in both patient groups, normal and overweight patients, an increase in ghrelin levels was observed after treatment; additionally, this certain increase was more apparent in the drospirenone-ethinyl estradiol group. Leptin and ghrelin levels have been investigated in the literature before, but as it is in our study, any study comparing levels before and after drospirenone-ethinyl estradiol + metformin treatment is not available. Romuald et al. [23] have examined changes in levels of leptin in obese PCOS patients after metformin therapy. In their study, improvement in insulin levels after administration of metformin has been determined. Sagsoz et al. [24] have compared leptin and ghrelin levels in PCOS patients treated with drospirenone-ethinyl estradiol before and after the treatment. They have reported a significant increase especially in ghrelin levels 3 months after treatment, but not in leptin levels. Contrary to this study, in the present study, although we have reported a decrease in leptin levels in the drospirenone-ethinyl estradiol group in patients with normal weight, similar to the findings of the Sagsoz et al.'s study, an increase in ghrelin levels has also been observed for both of the groups. According to research results, especially ghrelin levels vary after treatment, while leptin levels were found to be more stable.

Leptin's direct effect on the ovaries has also been proposed [25]. Karlsson et al. [26] have shown leptin receptor transcription in human granulosa and theca cells. In this study, it was shown that leptin inhibits estradiol production from granulosa cells mediated by LH. Similarly, after metformin treatment, reduced levels of insulin and IGFBP may cause an increase in response to gonadotropins [27]. Decreased leptin levels found in our study in the metformin group may contribute as a direct effect on ovaries for the response to gonadotropins.

Recently, particular attention has been focused on diagnostic criteria for ultrasound for PCOS patients. The incidence of polycystic ovary on ultrasonographic examination in normal population is about 20 %; for this reason, it is clear that ultrasonographic investigation alone would not be enough for the diagnosis. For this purpose, patients with the diagnosis of PCOS according to BMI were evaluated and found that ovarian volume and follicle number before treatment were statistically significantly higher in overweight patients. Jonard et al. [28] have evaluated the correlation between hormonal and metabolic properties and found a parallel relationship with the increasing number of follicles.

More sensitive additional ultrasonographic parameters have been introduced for patients with PCOS. For this purpose, ovarian stromal echogenicity and ovarian stromal area measurements were investigated. In our study, we found that BMI is correlated with stromal area and stromal echogenicity. According to our findings, increases in stromal echogenicity and stromal area were observed with the increase in pre-treatment BMI values. Belosi et al. [29] have recorded stromal area measurements and reported a positive correlation between the recordings and plasma androgen concentrations. Based on a visual interpretation of ultrasound practitioners to make a more objective evaluation of stromal echogenicity comments will eliminate the differences.

In literature, there are not enough studies comparing any changes in ultrasonographic markers after treatment with drospirenone-ethinyl estradiol + metformin. In this respect during our study, only in the drospirenone-ethinyl estradiol group changes were noted in ovarian volume and follicle counts. Furthermore, with the addition of metformin especially in the overweight group, significant reductions in ovarian volume and the number of follicles were detected. These findings may be interpreted as metformin being more effective on soft markers in the overweight group. Legro et al. [30] have investigated the effects of metformin and rosiglitazone treatment on ultrasonographic markers in PCOS patients. They have analyzed ovarian volume, the largest follicle diameter and endometrial thickness and, contrary to our findings, they have not noted significant differences for both 3 and 6 months after treatment. Other issues not yet discussed in the literature are changes in the stromal area and stromal echogenicity measurements. Both in group 1 and in group 2, stromal area was significantly decreased before and after treatment in overweight and normal weight patients. This decrease was more significant in the metformin group. Similarly, reduction in the stromal echogenity was more significant in the overweight subgroup. Especially these two parameters were the most varied with the treatment. According to our data, we have reached a positive correlation between the BFM and stromal area. In particular, ovarian stromal area follow-up would be a more ideal parameter for the evaluation of treatment in PCOS patients. In addition, this improvement may be more effective with the addition of metformin to drospirenone-ethinyl estradiol.

Association between obesity and BMI in PCOS is an issue that has been researched for a long time. In our study, before treatment, body fat distribution was analyzed in six different regions. Weight status was compared and no difference was detected ($p > 0.05$). Patients were analyzed whether parallel with increase in weight any concentration

in a specific region, no significant change was detected. Unlike our study, Yucel et al. [31] have compared fat distribution in PCOS patients with healthy controls and showed a significant increase in fat mass in trunk and arm regions. Toscani et al. [32] have researched body fat distribution in hirsute patients with and without diagnosis of PCOS by DEXA and calculated total fat mass without separating into sub-groups. In their study, they have shown that insulin resistance was associated with truncal obesity.

By DEXA examination, we also analyzed the changes in fat mass before and after treatment with drospirenone-ethinyl estradiol and drospirenone-ethinyl estradiol + metformin. According to our research of the relevant literature, no record of comparing changes with this treatment modality by DEXA was found. In our study, especially in the metformin group, for the overweight sub-group, a significant decrease was observed in the abdominal region after treatment. Reduction on fat mass for the overweight group with the addition of metformin suggests a relationship between insulin and abdominal obesity.

In conclusion, while serum leptin levels increase with the amount of body mass, ghrelin levels show the contrary. Now, whether this is the cause or the result of leptin and ghrelin is not yet clear. Addition of metformin could have beneficial effects on abdominal fat mass as in a decrease of ovarian stromal area irrespective from insulin resistance. Stromal area measurement could be an adjunctive method in PCOS patients and assessment of fat mass with DEXA could be helpful as a quantitative way of measurement.

Conflict of interest None.

References

1. ACOG Practice Bulletin No. 41 (2002) Polycystic ovary syndrome. Int J Gynecol Obstet 80(203):335–348
2. Kiddy DS, Sharp PS, White DM, Scanlon MF, Mason HD, Bray CS et al (1990) Differences in clinical and endocrine features between obese and non-obese subjects with polycystic ovary syndrome: an analysis of 263 consecutive cases. Clin Endocrinol (Oxford) 32:213–220
3. Gambineri A, Pelusi C, Vicennati V, Pagotto U, Pasquali R (2002) Obesity and the polycystic ovary syndrome. Int J Obes Relat Metab Disord 26:883–896
4. Balen AH, Laven JSE, Tan SL, Dewailly D (2003) Ultrasound of the polycystic ovary: international consensus definitions. Hum Reprod Updat 9(6):505–514
5. Van Santbrink EJP, Hop WC, Fauser BCJM (1997) Classification of normogonadotropic infertility: polycystic ovaries diagnosed by ultrasound versus endocrine characteristics of polycystic ovary syndrome. Fertil Steril 67:452–458
6. Goumenou AG, Matalliotakis IM, Koumantakis GE, Panidis DK (2003) The role of leptin in fertility. Eur J Obstet Gynecol Reprod Biol 106:118–124
7. Houseknecht KL, Baile CA, Matteri RL, Sporluck ME (1998) The biology of leptin: a review. J Anim Sci 76:405–420
8. Rohner-Jeanrenaud E, Jeanrenaud B (1997) Central nervous system and body weight regulation. Ann Endocrinol 58:137–142
9. Huda MSB, Wilding JPH, Pinkney JH, The International Association for the Study of Obesity (2006) Appetite regulatory peptides. Gut peptides and the regulation of appetite. Obes Rev 7:163–182
10. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K (1999) Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402:656–660
11. Pasquali R, Gambineri A, Biscotti D, Vicennati V, Gagliardi L, Colitta D et al (2000) Effect of long-term treatment with metformin added to hypocaloric diet on body composition, fat distribution, and androgen and insulin levels in abdominally obese women with and without the polycystic ovary syndrome. J Clin Endocrinol Metab 85:2767–2774
12. Moghetti P, Castello R, Negri C, Tosi F, Perrone F, Caputo M et al (2000) Metformin effects on clinical features, endocrine and metabolic profiles, and insulin sensitivity in polycystic ovary syndrome: a randomized, double-blind, placebo-controlled 6-month trial, followed by open, long-term clinical evaluation. J Clin Endocrinol Metab 85:139–146
13. Vandermolen DT, Ratts VS, Evans WS, Stovall DW, Kauma SW, Nestler JE (2001) Metformin increases the ovulatory rate and pregnancy rate from clomiphene citrate in patients with polycystic ovary syndrome who are resistant to clomiphene citrate alone. Fertil Steril 75:310–315
14. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group (2004) Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 19:41–47
15. Pache TD, Hop WC, Wladimiroff JW, Schipper J, Fauser BCJM (1991) Transvaginal sonography and abnormal ovarian appearance in menstrual cycle disturbances. Ultrasound Med Biol 17:589–593
16. Glintborg D, Andersen M, Hagen C, Frystyk J, Hulstrøm V, Flyvbjerg A et al (2006) Evaluation of metabolic risk markers in polycystic ovary syndrome (PCOS). Adiponectin, ghrelin, leptin and body composition in hirsute PCOS patients and controls. Eur J Endocrinol 155:337–345
17. Panidis D, Farmakiotis D, Koliakos G, Rousso D, Kourtis A, Katsikis I et al (2005) Comparative study of plasma ghrelin levels in women with polycystic ovary syndrome, in hyperandrogenic women and in normal controls. Hum Reprod 20(88):2127–2132
18. Orio F Jr, Lucidi P, Palomba S, Tauchmanova L, Cascella T, Russo T et al (1988) Circulating ghrelin concentrations in the polycystic ovary syndrome. J Clin Endocrinol Metabol 29:942–945
19. Schöfl C, Horn R, Schill T, Schlösser HW, Müller MJ, Brabant G (1987) Circulating ghrelin levels in patients with polycystic ovary syndrome. J Endocrinol Metabol 10:4607–4610
20. Moran LJ, Noakes M, Clifton PM, Wittert GA, Tomlinson L, Galletly C et al (1989) Ghrelin and measures of satiety are altered in polycystic ovary syndrome but not differentially affected by diet composition. J Endocrinol Metabol 7:3337–3344
21. Erturk E, Kuru N, Savci V, Tuncel E, Ersoy C, Imamoglu S (2004) Serum leptin levels correlate with obesity parameters but not with hyperinsulinism in women with polycystic ovary syndrome. Fertil Steril 82(5):1364–1368
22. Panidis D, Rousso D, Kourtis A, Tsimas V, Papathanasiou K, Makedos G (2003) Serum leptin levels in normal-weight and overweight women with polycystic ovary syndrome. Clin Exp Obstet Gynecol 30(4):207–210
23. Romualdi D, Campagna G, Selvaggi L Jr, Cento R, Proto C, Lanzone A et al (2008) Metformin treatment does not affect total leptin levels and free leptin index in obese patients with polycystic ovary syndrome. Fertil Steril 89(5):1273–1276

24. Sağsöz N, Orbak Z, Noyan V, Yücel A, Uçar B, Yıldız L (2008) The effects of oral contraceptives including low-dose estrogen and drospirenonee on the concentration of leptin and ghrelin in polycystic ovary syndrome. Fertil Steril (Epub ahead of print)
25. Jacobs HS, Conway GS (1999) Leptin, polycystic ovaries and polycystic ovary syndrome. Hum Reprod Updat 5(2):166–171
26. Karlsson C, Lindell K, Svensson E, Bergh C, Lind P, Billig H et al (1997) Expression of functional leptin receptors in the human ovary. J Clin Endocrinol Metab 82:4144–4148
27. Berker B, Emral R, Demirel C, Corapcioglu D, Unlu C, Kose K (2004) Increased insulin-like growth factor-I levels in women with polycystic ovary syndrome, and beneficial effects of metformin therapy. Gynecol Endocrinol 19:125–133
28. Jonard S, Robert Y, Cortet-Rudelli C, Pigny P, Decanter C, Dewailly D (2003) Ultrasound examination of polycystic ovaries: is it worth counting the follicles? Hum Reprod 18(3):598–603
29. Belosi C, Selvaggi L, Apa R, Guido M, Romualdi D, Fulghesu AM et al (2006) Is the PCOS diagnosis solved by ESHRE/ASRM 2003 consensus or could it include ultrasound examination of the ovarian stroma? Hum Reprod 21(12):3108–3115
30. Legro RS, Zaino RJ, Demers LM, Kunselman AR, Gnatuk CL, Williams NI et al (2007) The effects of metformin and rosiglitazone, alone and in combination, on the ovary and endometrium in polycystic ovary syndrome. AJOG 196(4):402.e1–402.e10
31. Yücel A, Noyan V, Sagsoz N (2006) The association of serum androgens and insulin resistance with fat distribution in polycystic ovary syndrome. Eur J Obsterics Gynecol Reprod Biol 126:81–86
32. Toscani M, Migliavacca R, Sisson de Castro JA, Spritzer PM (2007) Estimation of truncal adiposity using waist circumference or the sum of trunk skinfolds: a pilot study for insulin resistance screening in hirsute patients with or without polycystic ovary syndrome. Metab Clin Exp 56:992–997