Clinical Endocrinology (2013) **78**, 379–384 doi: 10.1111/j.1365-2265.2012.04415.x

ORIGINAL ARTICLE

Ethinyl estradiol–drospirenone *vs* ethinyl estradiol–drospirenone plus metformin in the treatment of lean women with polycystic ovary syndrome

Nese Cinar, Ayla Harmanci, Miyase Bayraktar and Bulent O. Yildiz

Endocrinology and Metabolism Unit, Department of Internal Medicine, Hacettepe University School of Medicine, Ankara, Turkey

Summary

Objective Oral contraceptive use might be associated with cardiometabolic risk in PCOS. We aimed to compare the effects of ethinyl estradiol–drospirenone (EE/DRSP) alone *vs* EE/DRSP plus metformin on clinical and cardiometabolic parameters in PCOS.

Design Prospective observational study.

Patients Forty-five lean patients with PCOS who received EE/DRSP (30 μg/3 mg) ($n = 25$) or EE/DRSP plus metformin (1700 mg/day) ($n = 20$) and 45 BMI-matched healthy controls.

Measurement BMI, waist-to-hip ratio (WHR), hirsutism scores, androgens, lipids, glucose and insulin levels during an OGTT were measured before and after 6 months of treatment in patients and compared to controls.

Results At baseline, patients with PCOS showed similar glucose, insulin and lipids but increased 2 h glucose values compared to controls. Hirsutism scores and free androgen index decreased in both treatment groups. BMI and WHR did not show any change in the EE/DRSP group, while metformin addition resulted in a decrease in BMI. Lipid levels increased in both groups. Glucose and insulin parameters did not change in any group, but metformin addition compared to EE/DRSP alone significantly decreased waist circumference, fasting insulin and HOMA-IR. After-treatment values for both EE/DRSP alone and in combination with metformin compared to the control group showed increased 2 h glucose and increased lipids in patients with PCOS.

Conclusion EE/DRSP alone or in combination with metformin improves clinical and biochemical hyperandrogenism in lean PCOS. Both treatments similarly alter lipid profile. EE/DRSP alone does not affect insulin sensitivity, whereas combining EE/DRSP with metformin might improve it.

(Received 6 February 2012; returned for revision 27 February 2012; finally revised 11 April 2012; accepted 12 April 2012)

Correspondence: Bulent Okan Yildiz, Endocrinology and Metabolism Unit, Department of Internal Medicine, Hacettepe University School of Medicine, Hacettepe, 06100 Ankara, Turkey. Tel.: +90 312 3051707; Fax: +90 312 3116768; E-mail: yildizbo@yahoo.com

Introduction

Polycystic ovary syndrome (PCOS) is a common and complex disorder characterized by hyperandrogenism, ovulatory dysfunction and polycystic ovaries (PCO). Women with PCOS have an increased risk for development of diabetes, dyslipidaemia, hypertension and the metabolic syndrome.[1] Even though these patients have increased metabolic risk factors, there is still a lack of information regarding the long-term cardiovascular outcome in PCOS.[2,3]

Oral contraceptive pills (OCPs) are first-line medical therapy in women with PCOS who are not seeking pregnancy. However, potential adverse cardiometabolic effects of OCPs represent a major concern. There is still controversy about the effects of OCP use on insulin sensitivity in PCOS. The results of studies are inconsistent in that decreased, unchanged and increased insulin sensitivity measurements have been reported.[4,5] The combination of ethinyl estradiol and drospirenone (EE/DRSP) is a new oral contraceptive used in the treatment for PCOS. DRSP is a nonsteroidal progestin, derived from 17-α- spirolactone, with antimineralocorticoid and antiandrogenic properties.[6] It is suggested that metabolic effects might be less severe compared to other OCPs.[4] There are few studies in the literature evaluating the effect of this treatment on clinical and cardiometabolic parameters in PCOS, especially in nonobese patients.[7–10]

The strong association between PCOS and insulin resistance and increased risk of diabetes has resulted in common use of insulin sensitizers in the treatment for PCOS. Metformin is the most widely used insulin sensitizer. Several studies have reported that metformin may improve menstrual pattern, increase ovulation, reduce serum androgen levels[11–16] and improve hirsutism in PCOS.[17] The effect of metformin in PCOS shows variability depending on body weight: in some studies, nonobese women respond better to treatment with metformin than obese women,[18] while metformin was found to be more effective in obese patients in others.[19] To date, only three studies of up to 4–6 months are available comparing OCP alone and the combination of OCP plus metformin in PCOS.[20–22] In two of these studies, norgestimate[20] and cyproterone acetate (CA)[21] were the proges-

tins used in the OCP, whereas one study in normal and overweight PCOS women compared the DRSP containing OCP alone *vs* in combination with metformin or cyproterone acetate.[22] In this study, we aimed to compare the effects of EE/DRSP alone or in combination with metformin on clinical and cardiometabolic parameters in lean women with PCOS (BMI $\leq$ 27 kg/m^2).

Materials and methods

Subjects

Forty-five lean, normal glucose-tolerant patients with PCOS (BMI $\leq$ 27 kg/m^2) of 83 patients, who attended to the Outpatient Clinic of Endocrinology and Metabolism of Hacettepe University between January 1 and December 31, 2008 were enrolled in this prospective observational study. The patients were not taking any medication including oral contraceptives and insulin sensitizers. All patients fulfilled the diagnostic criteria for PCOS according to revised 2003 Rotterdam criteria.[23] Hyperandrogenism and chronic oligo-anovulation were defined as previously described.[24] Hirsutism was defined as modified FG score (mFG) $\geq$7. PCO was defined as the presence of $\geq$12 follicles in each ovary each measuring 2–9 mm in diameter and/or increased ovarian volume (>10 ml; Rotterdam 2004). Women with a secondary endocrine disorder, such as hyperprolactinaemia, nonclassical form of congenital adrenal hyperplasia, thyroid function disorder, androgen secreting tumours and women with contraindications to OCP use were excluded, as suggested.[23] Forty-five BMI-matched healthy women without features of hyperandrogenism, oligo-amenorrhoea or PCO formed the control group. The control group was included to determine whether baseline and after-treatment cardiometabolic features of lean patients with PCOS differ from healthy women from the background population. The study was approved by the Local Ethics Committee of the Hacettepe University School of Medicine, and informed consent was obtained from all subjects.

Study design

All participants were evaluated by means of a standardized form that included a medical history and physical examination. Weight, height, waist and hip circumferences (waist: midway between the lower rib margin and the iliac crest, hip: widest circumference over the great trochanters) were measured. The body mass index [BMI; weight (kilograms)/height (metres)^2] and waist-to-hip ratio (WHR) were calculated. Hirsutism was evaluated by modified Ferriman–Gallwey scoring system.

An OGTT was performed at 08·00–10·00 AM after a 3 day, 300 g carbohydrate diet and an overnight fast of 10–14 h. A 75 g oral glucose load was administered, and blood samples for glucose and insulin determinations were collected through an intravenous cannula at 0 and 120 min. Additional baseline blood samples were obtained for hormonal and metabolic parameters. Hormonal evaluation included total testosterone, androstenedione, dehydroepiandrosterone sulphate (DHEAS) and sex hormone-binding globulin (SHBG), whereas metabolic evaluation included total cholesterol, triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C). All subjects were studied at baseline, during the follicular phase of the menstrual cycle (2–5 days after onset of last spontaneous or progestin-induced menstrual bleeding).

Patients received EE/DRSP (30 μg/3 mg) alone for 21 days on and 7 days off or EE/DRSP in combination with metformin (2 × 850 mg/day) for a duration of 6 months. All laboratory tests were repeated after treatment. None of the patients took any concurrent medication during the study.

Free androgen index (FAI = [testosterone (nM)/SHBG (nM)] × 100), homoeostatic model assessment of insulin resistance (HOMA-IR = [fasting insulin (mU/l) × fasting plasma glucose (FPG) (mM)]/22·5) and low-density lipoprotein cholesterol (LDL-C) (LDL = total cholesterol [HDL + (TG/5)]) were calculated as previously described.[24]

Assays

Blood samples were taken through venepuncture and centrifuged within 2 h after withdrawal. Serum was stored at −80 °C until analysis. Testosterone and insulin concentrations were measured by chemiluminiscent immunoassay kits (Roche Diagnostics GmbH, Mannheim, Germany). The average intra- and inter-assay coefficients of variation (CV) for insulin were $\leq$4·3% and $\leq$3·4%, respectively. The concentration of DHEAS and androstenedione was determined by chemiluminiscent immunoassay (Immulite® 2000, Los Angeles, USA). SHBG was measured by immunoradiometric assay (ZenTech, Angleur, Belgium). Total cholesterol, TG and HDL-C concentrations were measured using enzymatic colourimetric kits with intra- and interassay CV of <10% (Roche Diagnostics GmbH, Mannheim, Germany). Plasma glucose concentration was determined by the glucose oxidase method (Olympus AU 2700, Beckmann Coulter Inc., MA, USA).

Statistical analysis

Differences in baseline characteristics and laboratory data among the groups (each PCOS treatment group and the control group) were analysed by one-way analysis of variance (ANOVA) and post hoc Tukey's test for pairwise comparisons for normally distributed parameters, Kruskal–Wallis one-way ANOVA for parameters with skewed distribution and Mann–Whitney test for pairwise comparisons. The treatment effect was evaluated by comparing the differences before and after treatment by independent samples Student's *t*-test. For the primary outcome parameter, the mFG score, assuming an average change of 3 units from baseline with treatment and 3 units of standard deviation, our study has 80% power to detect a clinically significant difference of 2·5 units between groups as statistically significant with 5% type-I error level. SPSS Release 15.0 (Statistical Package for Social Sciences for Windows, SPSS Inc., Chicago, IL, USA) was used for these analyses. Values were described as mean ± SD. $P < 0{\cdot}05$ was considered statistically significant.

Results

A total of 90 women, 45 lean patients (BMI $\leq$ 27 kg/m²) and 45 BMI-matched controls were enrolled in the study. The participants were 17–33 years of age (mean ± SD of age for control; OCP and OCP plus metformin group, respectively; 24·0 ± 3·6, 21·8 ± 4·1, 21·5 ± 4·3; $P < 0{\cdot}05$). Table 1 shows the comparison of the clinical, hormonal and metabolic characteristics of each patient group before and after treatment and comparison with the control group. All patients had regular menses during the study.

Twenty-five patients received OCP alone, whereas 20 patients received OCP in combination with metformin. At baseline, mFG score, serum androgen levels and 2 h plasma glucose levels were significantly higher in patients with PCOS than controls ($P < 0{\cdot}05$ for all; Table 1). All the clinical and hormonal parameters were similar between the two treatment groups (NS; Table 1) except testosterone and androstenedione levels ($P < 0{\cdot}05$ for both).

After treatment, while there was no change in BMI, waist circumference or WHR in the OCP group (NS; Table 1), there was a significant decrease in waist circumference ($P < 0{\cdot}05$; Table 1) and BMI at a P level of 0·05 in the combination group (Table 1). A significant decrease in FAI and mFG scores and a significant increase in SHBG levels were observed in both groups ($P < 0{\cdot}05$; Table 1). Serum levels of total cholesterol, TG, HDL-C and LDL-C increased significantly in both groups ($P < 0{\cdot}05$; Table 1). FPG and 2 h plasma glucose levels, fasting insulin and HOMA-IR values did not change compared with baseline values in the OCP group (NS; Table 1). On the other hand, OCP plus metformin treatment caused a nonsignificant decrease in the levels of FPG, fasting insulin and HOMA-IR values (NS; Table 1). One patient in each group developed IGT, and 2 h plasma glucose levels were analysed after exclusion of these patients. No significant change occurred in 2 h plasma glucose levels (NS; Table 1).

Comparing the effect of treatments between the groups, the changes in fasting insulin, HOMA-IR and waist circumference in the OCP plus metformin group were found to be statistically significant (P-values < 0·05; Table 1).

After-treatment values for both OCP alone and in combination with metformin compared to the control group showed increased 2 h glucose and increased lipids in patients with PCOS ($P < 0{\cdot}05$ for all; Table 1).

One patient complained of oedema, and four patients in the OCP plus metformin group reported mild nausea and diarrhoea during the treatment period, but all patients completed the study.

Discussion

In this prospective observational study, we compared the effects of treatment with EE/DRSP vs EE/DRSP plus metformin on the hormonal, metabolic and cardiovascular risk profiles of lean patients with PCOS. Our data showed that (i) both treatment regimes significantly improved hyperandrogenaemia (FAI) and hirsutism scores and metformin treatment did not cause an additional benefit in terms of clinical and biochemical hyperan-

Table 1. Comparison of the clinical and hormonal characteristics of OCP and OCP + metformin groups before and after treatment and the healthy control group

Parameters	Control (n = 45)	OCP (n = 25)			OCP + metformin (n = 20)			Effect P treatment P between groups
		Baseline	After	P	Baseline	After	P	
BMI (kg/m^2)	22·1 ± 2·2	22·1 ± 2·9	22·1 ± 3·1	NS	22·8 ± 3·2	22·1 ± 2·3	0·05	NS
Waist circumference (cm)	73·5 ± 8·1	73·6 ± 8·1	74·4 ± 8·6	NS	74·9 ± 10·1	71·0 ± 6·6	<0·05	<0·05
WHR	0·77 ± 0·07	0·76 ± 0·05	0·78 ± 0·05	NS	0·78 ± 0·06	0·76 ± 0·06	NS	NS
mFG score	0·4 ± 0·7	8·9 ± 5·1[a]	5·3 ± 3·5[a]	<0·001	11·1 ± 4·8[a]	7·6 ± 4·6[a]	<0·05	NS
Total testosterone (nM)	1·2 ± 0·4	2·8 ± 0·9[a]	2·2 ± 1·0[a]	<0·01	2·1 ± 0·9[a]	1·8 ± 1·0	NS	NS
SHBG (nM)	56·3 ± 37·2	35·9 ± 24·6[a]	159·4 ± 63·3[a]	<0·001	31·9 ± 29·4[a]	151·4 ± 82·3[a]	<0·001	NS
Androstenedione (nM)	8·0 ± 3·8	12·9 ± 4·5[a]	11·2 ± 3·1[a]	NS	9·1 ± 3·1	9·4 ± 4·9	NS	NS
DHEAS (mM)	5·9 ± 3·2	7·5 ± 3·0[b]	5·9 ± 3·4	<0·01	7·6 ± 3·3[b]	6·3 ± 2·5	<0·05	NS
FAI	2·8 ± 1·7	10·9 ± 7·6[a]	1·9 ± 1·8[b]	<0·001	11·2 ± 11·2[a]	1·9 ± 1·9[b]	<0·01	NS
FPG (mM)	4·6 ± 0·5	4·6 ± 0·5	4·6 ± 0·4	NS	4·8 ± 0·4	4·6 ± 0·7	NS	NS
2 h plasma glucose (mM)	4·9 ± 0·9	5·7 ± 0·9[b]	5·8 ± 0·9[b]*	NS	5·6 ± 1·1[b]	6·1 ± 0·9[b]*	NS	NS
Fasting insulin (pM)	65·3 ± 26·4	69·4 ± 24·3	79·9 ± 40·3	NS	78·5 ± 31·9	68·1 ± 31·9	NS	<0·05
HOMA-IR	1·9 ± 0·9	2·1 ± 0·8	2·4 ± 1·3	NS	2·5 ± 1·1	2·1 ± 1·0	NS	<0·05
T.cholesterol (mM)	4·2 ± 0·7	4·2 ± 0·9	5·4 ± 1·0[a]	<0·001	3·9 ± 0·6	4·8 ± 0·9[b]	<0·001	NS
HDL-C (mM)	1·6 ± 0·3	1·6 ± 0·4	2·1 ± 0·5[a]	<0·001	1·5 ± 0·4	1·9 ± 0·5[b]	<0·01	NS
LDL-C (mM)	2·3 ± 0·6	2·3 ± 0·6	2·8 ± 0·8[b]	<0·05	2·1 ± 0·5	2·6 ± 0·6	<0·05	NS
TG (mM)	1·7 ± 0·6	2·3 ± 0·9[b]	3·6 ± 1·4[a]	<0·05	1·8 ± 0·6	3·2 ± 1·4[a]	<0·01	NS

Results are expressed as mean ± SD. NS, Not significant. [a]$P < 0{\cdot}001$ vs. control group. [b]$P < 0{\cdot}05$ vs. control group. * 2 h plasma glucose levels were calculated after patients with IGT (one in each treatment group) were excluded.

drogenism; (ii) EE/DRSP alone did not cause significant weight gain or any change in anthropometric measures, whereas adding metformin caused a decrease in these parameters; (iii) EE/DRSP alone did not change insulin resistance, but adding metformin to the OCP resulted in an improvement in insulin sensitivity; (iv) lipid levels increased significantly in both groups reaching higher levels compared to controls and metformin addition had no beneficial effect on lipids.

The effects of EE/DRSP on hyperandrogenaemia and body weight in our study are consistent with previous studies.[7,8,22,25,26] Regarding the effect of DRSP on lipid profile, the data are still inconsistent in that some studies report decreased LDL levels and increased HDL and TG levels,[10,27,28] whereas others show no significant change in lipid profile.[22] We observed a significant increase in all components of lipid profile.

Considering insulin sensitivity parameters, studies comparing EE/DRSP with another OCP or rosiglitazone shown that EE/DRSP does not affect insulin sensitivity or glucose tolerance,[7,8,10,26] or even improves these parameters.[22] Our results are in line with previous data and suggest no change in insulin sensitivity with EE/DRSP use in lean normal glucose tolerant PCOS unless it is combined with metformin.

The effect of metformin on hirsutism and hyperandrogenaemia in PCOS shows variability in different studies. In some, no change was observed in hirsutism score in patients with PCOS with metformin monotherapy,[29] whereas significant improvement in hirsutism was reported in others.[30] Metformin administration induced a reduction in FAI and serum testosterone levels in some studies.[13,15,16] Metformin reduces fasting and glucose-stimulated insulin levels and decreases ovarian cytochrome P450c17α activity inducing a reduction in the serum-free testosterone concentration in lean patients with PCOS.[13] Moreover, the inhibitory effect of insulin on the synthesis of SHBG is reduced by decreasing hyperinsulinaemia with metformin treatment for PCOS.[13] On the other hand, a Cochrane review including four randomized controlled trials comparing the effect of metformin *vs* OCPs in PCOS reported that metformin monotherapy was less effective than OCPs in reducing serum total testosterone levels.[31] In our study, combining metformin with EE/DRSP did not show any additional impact on decreasing hirsutism or hyperandrogenaemia.

Metformin either decreases or causes no change in BMI and WHR [32–36] and either improves or shows no effect on insulin sensitivity [15,16,32–35,37] when used alone in patients with PCOS. The available data on the effect of meformin in nonobese patients are inconclusive.[13,18,19] Trolle *et al.*[19] reported that nonobese PCOS women did not benefit from metformin. On the other hand, fasting insulin, the area under the insulin curve and serum androgens decreased significantly in nonobese patients with PCOS with metformin monotherapy in other studies.[15,16,18] In our study, we found that adding metformin to OCP might have beneficial effects on insulin sensitivity in lean patients with PCOS. Metformin monotherapy either improves lipid profile or causes no change in patients with PCOS.[32,35,38] In a meta-analysis by Costello,[31] it was documented that metformin monotherapy significantly decreased TG levels. However, in our study, metformin, when added to an OCP, did not show a similar beneficial effect on lipids.

Three studies have been published to date comparing OCP and OCP plus metformin combination in patients with PCOS. These studies did not include a healthy control group for comparison at baseline, and EE/DRSP was used in only one of them. The first study by Elter *et al.*[21] evaluated OCP (EE/CA) (n = 20) *vs* EE/CA plus metformin (n = 20) for 4 months in nonobese patients with PCOS. Combination therapy compared to OCP alone in this study resulted in a significant decrease in BMI and WHR, and similar improvement in testosterone and free testosterone levels. While combination therapy resulted in an improvement in insulin sensitivity, OCP alone did not show an effect in this study. There was no change in lipid profile except for an increase in total cholesterol in the OCP alone group. The second study, by Cibula *et al.*,[20] compared EE/norgestimate (n = 15) with EE/norgestimate plus metformin (n = 13) for 6 months in lean and overweight PCOS. In this study, there was no significant change in BMI, WHR, insulin sensitivity measured by euglycaemic clamp or lipids in either group. When comparing the effects of treatments between the groups, only a more pronounced decrease in FAI was found in combination group. In the third study, Fruzzetti *et al.*[22] compared EE/DRSP alone (n = 16) *vs* in combination with metformin (n = 15) or CA (n = 16) for 6 months in both lean and overweight patients with PCOS. The dose of EE was 20 μg in this study. They reported similar improvement in hyperandrogenism in all groups, no change in BMI or WHR in either group, and improvement in insulin sensitivity in OCP alone and OCP plus metformin groups compared to OCP plus CA group. EE/DRSP did not change the lipid profile, whereas EE/DRSP plus metformin significantly increased HDL-C, and EE/DRSP plus CA significantly increased total cholesterol and TG.

Our results are in line with all three studies in that OCP alone and OCP plus metformin have similar effects on hyperandrogenism. Similar to the results of Elter *et al.*[21] and different from Cibula *et al.*[20] and Fruzzetti *et al.*,[22] our data suggest that metformin addition results in a significant decrease in BMI and waist circumference. Our finding of increased lipids in both groups might in part be related to the 30-μg dose of EE in the OCP we used considering that Cibula *et al.*[20] showed similar changes in lipids with an OCP containing 35 μg EE, whereas Fruzzetti *et al.*[22] failed to show this alteration with an OCP containing 20 μg EE.

An interesting finding in our study is that one patient in each group developed IGT regardless of the use of OCP alone or in combination with metformin. Previous studies in obese PCOS showed an increased conversion rate from normoglycaemia to IGT and diabetes.[39,40] Age, BMI at baseline, family history and gestational diabetes are among the independent risk factors for adverse changes in glycaemia.[39,40] The patients developing IGT in our study were young, had normal BMI and did not have personal history of gestational diabetes or family history of diabetes. Even though a duration of 6 months of any treatment in a small group of patients may not be sufficient to fully evaluate effects on glucose homoeostasis, our results suggest that adding

metformin to an OCP may not retard or prevent the development of diabetes in PCOS. Longer-term studies with larger sample size including both lean and obese patients are needed to resolve this intriguing question.

A limitation of our study was the lack of randomization. Secondly, our sample size was relatively small although it was similar to previously published studies. Nevertheless, post hoc power analysis showed that our study had 80% power with 5% type 1 error level to detect a clinically significant difference in the primary outcome of hirsutism score. Thirdly, we included only lean patients with PCOS in the study, and our results might not be extrapolated to obese patients with PCOS. Lastly, the duration of our study was relatively short even though it was sufficient to observe improvement in hyperandrogenism and alterations in cardiometabolic risk profile.

In conclusion, our results suggest that the use of EE/DRSP alone or in combination with metformin improves hyperandrogenism in lean PCOS and metformin addition might decrease BMI even in lean subjects. Both EE/DRSP alone and EE/DRSP plus metformin therapies for PCOS are associated with an increase in all components of the lipid profile. Use of EE/DRSP alone in lean patients with PCOS with normal glucose tolerance does not affect insulin sensitivity, while addition of metformin might improve insulin sensitivity parameters.

Disclosure statement

The authors have nothing to disclose.

References

1 Ehrmann, D.A. (2005) Polycystic ovary syndrome. *New England Journal of Medicine*, **352**, 1223–1236.

2 Legro, R.S. (2003) Polycystic ovary syndrome and cardiovascular disease: a premature association? *Endocrine Reviews*, **24**, 302–312.

3 Fauser, B.C., Tarlatzis, B.C., Rebar, R.W. *et al.* (2012) Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. *Fertility and Sterility*, **97**, 28–38. e25.

4 Yildiz, B.O. (2008) Oral contraceptives in polycystic ovary syndrome: risk-benefit assessment. *Seminars in Reproductive Medicine*, **26**, 111–120.

5 Vrbikova, J. & Cibula, D. (2005) Combined oral contraceptives in the treatment of polycystic ovary syndrome. *Human Reproduction Update*, **11**, 277–291.

6 Shulman, L.P. (2002) Safety and efficacy of a new oral contraceptive containing drospirenone. *Journal of Reproductive Medicine*, **47**, 981–984.

7 Guido, M., Romualdi, D., Giuliani, M. *et al.* (2004) Drospirenone for the treatment of hirsute women with polycystic ovary syndrome: a clinical, endocrinological, metabolic pilot study. *Journal of Clinical Endocrinology and Metabolism*, **89**, 2817–2823.

8 Pehlivanov, B. & Mitkov, M. (2007) Efficacy of an oral contraceptive containing drospirenone in the treatment of women with polycystic ovary syndrome. *European Journal of Contraception and Reproductive Health Care*, **12**, 30–35.

9 Mancini, F., Cianciosi, A., Persico, N. *et al.* (2010) Drospirenone and cardiovascular risk in lean and obese polycystic ovary syndrome patients: a pilot study. *American Journal of Obstetrics and Gynecology*, **202**, 169. e1-8.

10 Kriplani, A., Periyasamy, A.J., Agarwal, N. *et al.* (2010) Effect of oral contraceptive containing ethinyl estradiol combined with drospirenone vs. desogestrel on clinical and biochemical parameters in patients with polycystic ovary syndrome. *Contraception*, **82**, 139–146.

11 Lord, J.M., Flight, I.H. & Norman, R.J. (2003) Metformin in polycystic ovary syndrome: systematic review and meta-analysis. *British Medical Journal*, **327**, 951–953.

12 Baillargeon, J.P., Jakubowicz, D.J., Iuorno, M.J. *et al.* (2004) Effects of metformin and rosiglitazone, alone and in combination, in nonobese women with polycystic ovary syndrome and normal indices of insulin sensitivity. *Fertility and Sterility*, **82**, 893–902.

13 Palomba, S., Falbo, A., Zullo, F. *et al.* (2009) Evidence-based and potential benefits of metformin in the polycystic ovary syndrome: a comprehensive review. *Endocrine Reviews*, **30**, 1–50.

14 Palomba, S., Falbo, A., Giallauria, F. *et al.* (2010) Effects of metformin with or without supplementation with folate on homocysteine levels and vascular endothelium of women with polycystic ovary syndrome. *Diabetes Care*, **33**, 246–251.

15 Orio, F., Palomba, S., Cascella, T. *et al.* (2005) Improvement in endothelial structure and function after metformin treatment in young normal-weight women with polycystic ovary syndrome: results of a 6-month study. *Journal of Clinical Endocrinology and Metabolism*, **90**, 6072–6076.

16 Orio, F., Manguso, F., Di Biase, S. *et al.* (2007) Metformin administration improves leukocyte count in women with polycystic ovary syndrome: a 6-month prospective study. *European Journal of Endocrinology*, **157**, 69–73.

17 Harborne, L., Fleming, R., Lyall, H. *et al.* (2003) Metformin or antiandrogen in the treatment of hirsutism in polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism*, **88**, 4116–4123.

18 Maciel, G.A., Soares Junior, J.M., Alves da Motta, E.L. *et al.* (2004) Nonobese women with polycystic ovary syndrome respond better than obese women to treatment with metformin. *Fertility and Sterility*, **81**, 355–360.

19 Trolle, B., Flyvbjerg, A., Kesmodel, U. *et al.* (2007) Efficacy of metformin in obese and non-obese women with polycystic ovary syndrome: a randomized, double-blinded, placebo-controlled cross-over trial. *Human Reproduction*, **22**, 2967–2973.

20 Cibula, D., Fanta, M., Vrbikova, J. *et al.* (2005) The effect of combination therapy with metformin and combined oral contraceptives (COC) versus COC alone on insulin sensitivity, hyperandrogenaemia, SHBG and lipids in PCOS patients. *Human Reproduction*, **20**, 180–184.

21 Elter, K., Imir, G. & Durmusoglu, F. (2002) Clinical, endocrine and metabolic effects of metformin added to ethinyl estradiol-cyproterone acetate in non-obese women with polycystic ovarian syndrome: a randomized controlled study. *Human Reproduction*, **17**, 1729–1737.

22 Fruzzetti, F., Perini, D., Lazzarini, V. *et al.* (2010) Comparison of effects of 3 mg drospirenone plus 20 mug ethinyl estradiol alone or combined with metformin or cyproterone acetate on classic metabolic cardiovascular risk factors in nonobese women with polycystic ovary syndrome. *Fertility and Sterility*, **94**, 1793–1798.

23 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. *Fertility and Sterility*, **81**, 19–25.

24 Yildiz, B.O., Bozdag, G., Harmanci, A. *et al.* (2010) Increased circulating soluble P-selectin in polycystic ovary syndrome. *Fertility and Sterility*, **93**, 2311–2315.

25 Batukan, C. & Muderris, I.I. (2006) Efficacy of a new oral contraceptive containing drospirenone and ethinyl estradiol in the long-term treatment of hirsutism. *Fertility and Sterility*, **85**, 436–440.

26 Tfayli, H., Ulnach, J.W., Lee, S. *et al.* (2011) Drospirenone/ethinyl estradiol versus rosiglitazone treatment in overweight adolescents with polycystic ovary syndrome: comparison of metabolic, hormonal, and cardiovascular risk factors. *Journal of Clinical Endocrinology and Metabolism*, **96**, 1311–1319.

27 Oelkers, W., Foidart, J.M., Dombrovicz, N. *et al.* (1995) Effects of a new oral contraceptive containing an antimineralocorticoid progestogen, drospirenone, on the renin-aldosterone system, body weight, blood pressure, glucose tolerance, and lipid metabolism. *Journal of Clinical Endocrinology and Metabolism*, **80**, 1816–1821.

28 Parsey, K.S. & Pong, A. (2000) An open-label, multicenter study to evaluate Yasmin, a low-dose combination oral contraceptive containing drospirenone, a new progestogen. *Contraception*, **61**, 105–111.

29 Krstevska, B., Dimitrovski, C., Pemovska, G. *et al.* (2006) Metformin improves menstrual patterns, endocrine and metabolic profile in obese hyperinsulinemic women with a polycystic ovary syndrome. *Prilozi*, **27**, 57–66.

30 Oppelt, P.G., Mueller, A., Jentsch, K. *et al.* (2010) The effect of metformin treatment for 2 years without caloric restriction on endocrine and metabolic parameters in women with polycystic ovary syndrome. *Experimental and Clinical Endocrinology & Diabetes*, **118**, 633–637.

31 Costello, M.F., Shrestha, B., Eden, J. *et al.* (2007) Metformin versus oral contraceptive pill in polycystic ovary syndrome: a Cochrane review. *Human Reproduction*, **22**, 1200–1209.

32 Velazquez, E.M., Mendoza, S., Hamer, T. *et al.* (1994) Metformin therapy in polycystic ovary syndrome reduces hyperinsulinemia, insulin resistance, hyperandrogenemia, and systolic blood pressure, while facilitating normal menses and pregnancy. *Metabolism*, **43**, 647–654.

33 Acbay, O. & Gundogdu, S. (1996) Can metformin reduce insulin resistance in polycystic ovary syndrome? *Fertility and Sterility*, **65**, 946–949.

34 Ehrmann, D.A., Cavaghan, M.K., Imperial, J. *et al.* (1997) Effects of metformin on insulin secretion, insulin action, and ovarian steroidogenesis in women with polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism*, **82**, 524–530.

35 Morin-Papunen, L.C., Koivunen, R.M., Ruokonen, A. *et al.* (1998) Metformin therapy improves the menstrual pattern with minimal endocrine and metabolic effects in women with polycystic ovary syndrome. *Fertility and Sterility*, **69**, 691–696.

36 Kolodziejczyk, B., Duleba, A.J., Spaczynski, R.Z. *et al.* (2000) Metformin therapy decreases hyperandrogenism and hyperinsulinemia in women with polycystic ovary syndrome. *Fertility and Sterility*, **73**, 1149–1154.

37 Diamanti-Kandarakis, E., Kouli, C., Tsianateli, T. *et al.* (1998) Therapeutic effects of metformin on insulin resistance and hyperandrogenism in polycystic ovary syndrome. *European Journal of Endocrinology*, **138**, 269–274.

38 Morin-Papunen, L.C., Vauhkonen, I., Koivunen, R.M. *et al.* (2000) Endocrine and metabolic effects of metformin versus ethinyl estradiol-cyproterone acetate in obese women with polycystic ovary syndrome: a randomized study. *Journal of Clinical Endocrinology and Metabolism*, **85**, 3161–3168.

39 Ehrmann, D.A., Barnes, R.B., Rosenfield, R.L. *et al.* (1999) Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. *Diabetes Care*, **22**, 141–146.

40 Norman, R.J., Masters, L., Milner, C.R. *et al.* (2001) Relative risk of conversion from normoglycaemia to impaired glucose tolerance or non-insulin dependent diabetes mellitus in polycystic ovarian syndrome. *Human Reproduction*, **16**, 1995–1998.