

# 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

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**BACKGROUND:** Oral contraceptive pills (OC) are usually the first choice of treatment for polycystic ovarian syndrome (PCOS), when fertility is not desired. However, they do not improve, or may even further induce impairment of insulin sensitivity, which is already impaired in women with PCOS. In this prospective, randomized study, we analysed the additional benefits of adding metformin to the OC treatment in non-obese women with PCOS. **METHODS:** After a baseline work-up including body mass index (BMI), waist:hip ratio (WHR), Ferriman–Gallwey score, ovarian volume, serum gonadotrophin, androgen and sex hormone-binding globulin (SHBG) levels, and fasting lipid, glucose and insulin levels, 40 non-obese women with PCOS were assigned either to the OC or to the OC + metformin treatment by computer-assisted randomization. At the end of the 4 month follow-up period, subjects were re-evaluated. **RESULTS:** The two groups were similar at baseline. After treatment, women in the OC + metformin group had significant decreases in BMI and WHR, and a significant increase in insulin sensitivity, in contrast to those in the OC group, who had insignificant changes in these parameters. Adding metformin also caused significant improvements in serum androstenedione and SHBG levels compared with the OC treatment alone. **CONCLUSIONS:** Adding metformin to the OC treatment may improve the insulin sensitivity, and may further suppress the hyperandrogenaemia in non-obese women with PCOS.

*Key words:* cyproterone acetate/insulin resistance/metformin/oral contraceptive/polycystic ovarian syndrome

## Introduction

Polycystic ovarian syndrome (PCOS) is the most common cause of infertility and hirsutism, affecting 5–10% of women of reproductive age (Knochenhauer *et al.*, 1998). It is characterized by chronic anovulation and hyperandrogenism. In addition to these major features, insulin resistance and compensatory hyperinsulinaemia are intrinsic features of the disorder (Hacihanefioglu *et al.*, 2000). Approximately 50% of women with PCOS are obese, exacerbating insulin resistance (Nestler and Jakubowicz, 1997). However, several investigators have demonstrated that even lean women with PCOS exhibit a form of insulin resistance out of proportion to body mass index (BMI) (Chang *et al.*, 1983; Dunaif *et al.*, 1987, 1989; Morales *et al.*, 1996), underscoring the universality of insulin resistance in women with PCOS and the important role hyperinsulinaemia has in the pathophysiology of this syndrome.

Metformin is an oral biguanide antihyperglycaemic drug used for decades for the treatment of type 2 diabetes mellitus. Recent studies have indicated that this drug improves metabolic

abnormalities, results in decreased androgen levels, and improves menstrual pattern and ovulatory function in women with PCOS (Velazquez *et al.*, 1994, 1997; Nestler and Jakubowicz, 1996, 1997; Diamanti-Kandarakis *et al.*, 1998; Morin-Papunen *et al.*, 1998; Nestler *et al.*, 1998; Moghetti *et al.*, 2000). Furthermore, it has been suggested that metformin improves the dyslipidaemia which is commonly associated with PCOS (Wild *et al.*, 1985; Talbott *et al.*, 1995; Moghetti *et al.*, 2000). There is not enough evidence to suggest the insulin-reducing medications as first-line therapy for women with PCOS. First, not all women with PCOS are insulin resistant, and it is not clear that insulin resistance is the primary defect of the heterogeneous disorder of PCOS (Taylor, 2000). More important, none of the drugs has been administered for long enough periods of time or to a sufficient number of patients to prove superiority over other established therapies (Taylor, 2000).

In hyperandrogenic women, who do not require fertility, oral contraceptive pills (OC) are usually the first choice of

treatment. They typically suppress ovarian and adrenal androgen production, increase sex hormone-binding globulin (SHBG) levels, and improve clinical hirsutism scores in ~80% of patients (Erenus *et al.*, 1996; Taylor, 2000). However, suppression of ovarian and/or adrenal androgenism does not diminish insulin resistance (Geffner *et al.*, 1986; Singer *et al.*, 1989) and, in addition, OC may induce impairment of glucose tolerance, and elevate insulin levels in women with PCOS as well as in healthy women (Wynn *et al.*, 1979; Godsland *et al.*, 1990; Korytkowski *et al.*, 1995). They also do not improve, or may even worsen, the dyslipidaemia associated with PCOS (Prelevic *et al.*, 1990; Korytkowski *et al.*, 1995). Therefore, addition of an insulin-lowering drug to the OC treatment may have metabolic benefits in women with PCOS. In addition, it may further improve hyperandrogenaemia since it has been shown that insulin, in synergy with LH, has a direct ovarian effect to modulate ovarian androgen secretion (Taylor, 2000; Attia *et al.*, 2001).

Therefore, we designed the present study to compare the clinical, endocrine and metabolic effects of the two treatment modalities: (i) the commonly used ethinyl estradiol (EE)–cyproterone acetate (CA) OC; and (ii) metformin added to this OC, in non-obese women with PCOS.

## Materials and methods

### Patient selection

Forty women with PCOS, aged 16–36 years, were enrolled into the study. All women were either of normal weight or thin (BMI,  $\leq 26$  kg/m<sup>2</sup>). PCOS was defined as the presence of: (i) bilateral polycystic ovaries on ultrasound examination; (ii) chronic oligomenorrhoea ( $< 6$  menstrual periods in the previous year) or amenorrhoea; and (iii) manifestations of hyperandrogenism and/or hyperandrogenaemia, such as a hirsutism score of  $> 8$  (Ferriman and Gallwey, 1961); acne; elevated serum testosterone and/or androstenedione and/or free testosterone levels.

All the women were euthyroid (serum TSH level, 0.35–5.5 mU/l) and had normal prolactin levels (serum prolactin level, 3.4–24  $\mu$ g/l). Their serum testosterone levels were  $< 7$  nmol/l, and dehydroepiandrosterone sulphate (DHEA-S) levels were  $< 19$   $\mu$ mol/l. If the basal serum 17OH-progesterone level was  $> 6$  nmol/l, an adrenocorticotrophic hormone (ACTH) stimulation test was done to exclude patients with late-onset congenital adrenal hyperplasia (Speroff *et al.*, 1994). Serum 17OH-progesterone levels were determined before and 30 min after an i.v. injection of 0.25 mg of synthetic ACTH (Synacthen; Ciba, Basel, Switzerland). Women who had an increase of  $> 10$  nmol/l in the serum 17OH-progesterone level after ACTH stimulation were excluded from the study (Elter *et al.*, 1999). A serum cortisol level of  $> 140$  nmol/l after an overnight dexamethasone suppression test was also an exclusion criterion (Elter *et al.*, 1999). Women were excluded from the study if an adnexal mass was noted on pelvic sonography.

After 3 days on a high carbohydrate diet (300 g/day) and an overnight fast of 10–12 h, all subjects underwent an oral glucose tolerance test (OGTT; a load of 75 g glucose in 300 ml water). Venous blood samples were drawn at 0, 30, 60 and 120 min for plasma glucose determination. Women who were found to have diabetes according to published criteria (American Diabetes Association, 1997) were excluded from the study. Women who had any other known endocrinological disease, and those taking drugs known

to affect carbohydrate or lipid metabolism and OGTT results during the 6 months preceding the study, were also excluded.

Medical histories were taken, and all subjects underwent gynaecological examination and pelvic ultrasonography. Weight and height were obtained and BMI [weight (kg)/height (m)<sup>2</sup>] was calculated. Waist and hip circumferences were measured to the nearest centimeter with a soft tape at the narrowest part of the torso and at the widest part of the gluteal region, and waist:hip ratio (WHR) was calculated.

All laboratory investigations and ultrasound examinations were performed in the early follicular phase (days 3–5) of spontaneous bleeding or withdrawal bleeding induced with medroxyprogesterone acetate at baseline or with the OC after the follow-up period. All ultrasound examinations were performed by one operator (G.I.), either transabdominally or transvaginally (3.5 and 5 MHz sector probes respectively; GE Logiq 200 Pro, GE Medical Systems, Milwaukee, WI, USA). Polycystic-appearing ovaries were defined sonographically as the presence of multiple ( $> 10$ ), small (2–8 mm in diameter) follicles in the periphery (in one plane) and increased stromal echogenicity as described (Adams *et al.*, 1986). Ovarian volumes were also measured, and volume determinations were carried out using the formula for the volume of an ellipsoid:  $0.523 \times \text{length (cm)} \times \text{width (cm)} \times \text{thickness (cm)}$  (Robert *et al.*, 1995). The study was approved by the Institutional Review Board at Marmara University, and written informed consent was obtained from each subject.

### Protocol

After baseline clinical (BMI, WHR, Ferriman–Gallwey score and ovarian volume), endocrinological (FSH, LH, testosterone, free testosterone, androstenedione, 17OH-progesterone, DHEA-S and SHBG) and metabolic [fasting glucose and insulin, total cholesterol, triglycerides, high density lipoprotein (HDL)-cholesterol and low density lipoprotein (LDL)-cholesterol] work-ups, the subjects were randomized to either the OC group or to the OC + metformin group. Women in the OC group were prescribed EE, 35  $\mu$ g, and CA, 2 mg (Diane 35; Schering AG, Berlin, Germany) for 21 days per month followed by a 7 day pill-free period. Women in the OC + metformin group were prescribed metformin 500 mg three times per day orally (for the first 15 days, 500 mg twice daily for adequate compliance (Glucophage; Ilsan and Iltas Pharmaceuticals, Istanbul, Turkey) in addition to the above-mentioned pill. The women were prospectively followed for 4 months, and at the end of this follow-up period, the baseline evaluation was repeated. Glucose:insulin ratio [GIR; glucose (mmol/l)/insulin (pmol/l)] was calculated for each subject at baseline and after treatment.

### Assignment

Randomization was produced from a computer-generated random list, where even and odd numbers were allocated OC and OC + metformin treatments respectively.

### Masking

Clinical parameters (BMI, WHR, Ferriman–Gallwey score and ovarian volume) of the subjects were evaluated by the same person, who was blind to the type of treatment. No attempt was made to mask the treatments from the subjects, and placebo was not used.

### Assays

Blood samples were obtained through venepuncture, and centrifuged within 2 h after withdrawal. Serum was stored at  $-20^{\circ}\text{C}$ ; it was assayed for FSH, LH, testosterone, SHBG and DHEA-S with chemiluminescent immunoassay kits (FSH, LH and testosterone kits were provided by Roche Diagnostics Corporation, Indianapolis, IN, USA;

SHBG and DHEA-S kits were provided by Diagnostic Products Corporation, Los Angeles, CA, USA), and for free testosterone, androstenedione and 17OH-progesterone with commercially available radioimmunoassay kits (17OH-progesterone kit was provided by Diagnostic Systems Laboratories, Inc., Webster, TX, USA; free testosterone and androstenedione kits were provided by Diagnostic Products Corporation).

The average intra-assay coefficients of variation (CV) were 1.5% for FSH, 0.7% for LH, 1.4% for testosterone, 9% for free testosterone, 6.1% for SHBG, 9.5% for DHEA-S, 6.5% for androstenedione, and 4.1% for 17OH-progesterone. The average total CV were 3.8% for FSH and 1.6% for LH. The average inter-assay CV were 2.2% for testosterone, 8.5% for free testosterone, 8% for SHBG, 13% for DHEA-S, 10% for androstenedione, and 6.8% for 17OH-progesterone.

Plasma glucose concentrations were measured with the glucose oxidase technique using an auto-analyser (BM/Hitachi 917; Boehringer Mannheim GmbH, Mannheim, Germany). Serum insulin concentrations were measured by chemiluminescent enzyme immunoassay (Diagnostic Products Corporation). Intra-assay and total CV for different values of insulin were between 3.8 and 4.8%, and 4.2 and 7.6% respectively. Fasting serum triglyceride, total cholesterol, HDL- and LDL-cholesterol concentrations were determined by using the BM/Hitachi 917 auto-analyser utilizing enzymatic calorimetric assays with intra- and inter-assay CV of <10% (Roche Diagnostics Corporation).

**Sample size calculation and statistical analysis**

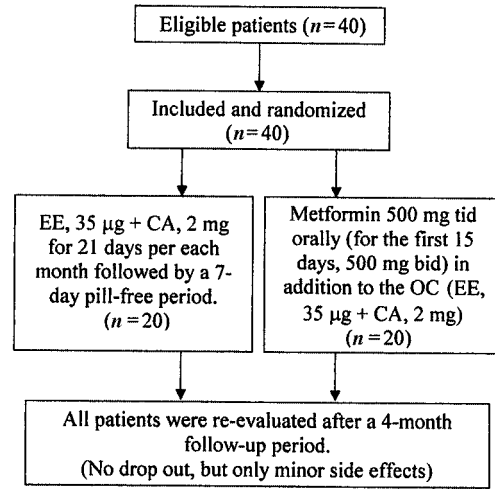
A power calculation showed that a sample size of 20 in each group has a power of 80% to detect a 0.56 kg/m<sup>2</sup> difference in BMI change during treatment, between groups at the 5% level of significance. The corresponding values were 0.03 for the WHR and 0.04 mmol/pmol for the GIR. Randomization and power analysis were made by using StatMate version 1.01 (GraphPad Software, San Diego, CA, USA).

Differences in baseline characteristics between the two groups were analysed by Student's *t*-test. Clinical, endocrine and metabolic features were analysed by repeated measures analysis of variance (ANOVA). In this repeated measures model, simple contrasts were used to test differences between baseline and after-treatment values. While evaluating the effect of treatment on hirsutism, analysing hirsute and non-hirsute subjects together may not be conclusive. Therefore, in addition to analysis of the Ferriman–Gallwey scores in all subjects, we also analysed only hirsute subjects in each group.

To analyse the role of weight loss in metformin's effect on insulin sensitivity, we calculated percentage changes in BMI, WHR and GIR during treatment, for each subject. After the median value for the percentage change in BMI was determined, subjects in the OC + metformin group were divided into two groups by using this median as the cut-off value. GIR in each group was compared by using repeated measures ANOVA. Correlations between changes in BMI, WHR and GIR were also analysed in the OC + metformin group by using the Pearson's correlation test. SPSS Release 10.0 (SPSS, Inc., Chicago, IL, USA) was used for these analyses. Values are expressed as mean ± SD, and *P* < 0.05 was considered statistically significant.

**Results**

Forty women, 20 in each group and aged 16–36 years, were eligible for the study. All of them agreed to participate in the trial, and were randomized (Figure 1). Tables I and II show the clinical, hormonal and metabolic characteristics of women in both groups, at baseline and after treatment. At baseline,



**Figure 1.** Flow chart of subjects. OC = oral contraceptive pill; EE = ethinyl estradiol; CA = cyproterone acetate.

all the clinical, hormonal and metabolic parameters were similar between the two groups (*P* > 0.05).

Four patients in OC + metformin group reported mild nausea and gastrointestinal problems that did not necessitate discontinuation of the treatment. After treatment, Ferriman–Gallwey score, ovarian volume, serum testosterone, free testosterone and androstenedione levels decreased, and SHBG levels increased significantly in both groups (Table I). These changes were comparable between groups, except the androstenedione and SHBG levels. Subjects who received OC + metformin had significantly greater reductions in serum androstenedione and increases in serum SHBG than those who received OC alone (*P* = 0.04 and *P* = 0.02 between groups for androstenedione and SHBG respectively; Table 1).

After treatment, women in the OC group had a slight, but insignificant, increase in BMI compared with those in the OC + metformin group, who had a significant decrease (*P* = 0.003; Table I; Figure 2). WHR also decreased significantly in the OC + metformin group (*P* = 0.001), while there was no change in the OC group (Table I; Figure 3). Fasting GIR improved significantly, mainly due to the decreased insulin levels, in the OC + metformin group (*P* = 0.006 and 0.001 for GIR and fasting insulin respectively), while OC treatment alone did not cause any significant change in the other group (Table II; Figure 4). Treatment caused no change in serum lipids in the two groups, except total cholesterol levels in the OC group. Total cholesterol levels increased significantly in the OC group (*P* = 0.002), but insignificantly in the OC + metformin group (Table II). However, these differences between groups did not reach significance (Tables I and II).

When only hirsute subjects (*n* = 14, in each group) were analysed, the Ferriman–Gallwey score (mean ± SD) decreased from 13.92 ± 3.84 [95% confidence interval (CI), 11.60–16.24] to 12.15 ± 3.46 (10.06–14.24) in the OC group (*P* < 0.001) and from 12.00 ± 2.60 (10.00–14.00) to 10.33 ± 2.12 (8.70–11.96) in the OC + metformin group (*P* < 0.001). The effects of both treatments were comparable (*P* = NS).

When percentage changes during treatment were analysed

**Table I.** Clinical and hormonal characteristics of women in both groups

	OC group (n = 20)				OC + metformin group (n = 20)				Effect of treatment P between groups
	Baseline		After		Baseline <sup>a</sup>		After		
	Mean ± SD	P within group	Mean ± SD	P within group	Mean ± SD	P within group	Mean ± SD	P within group	
Age (years)	23.45 ± 6.07 (20.61–26.29)		22.08 ± 1.90 (21.03–23.13)	NS	24.90 ± 6.62 (21.80–28.00)		22.20 ± 2.80 (20.51–23.89)	0.003	NS
BMI (kg/m <sup>2</sup> )	21.83 ± 1.40 (21.06–22.61)		0.796 ± 0.04 (0.78–0.82)	NS	22.74 ± 2.66 (21.13–24.35)		0.791 ± 0.06 (0.76–0.82)	0.001	NS
Waist:hip ratio	0.804 ± 0.04 (0.78–0.83)		10.47 ± 4.80 (8.00–12.94)	< 0.001	0.827 ± 0.06 (0.80–0.86)		7.65 ± 3.66 (5.77–9.53)	0.004	NS
FG score	12.06 ± 5.25 (9.36–14.76)		5.95 ± 2.09 (4.84–7.06)	0.003	9.47 ± 5.48 (6.65–12.29)		5.96 ± 2.41 (4.68–7.24)	0.001	NS
Ovarian volume (cm <sup>3</sup> )	7.33 ± 2.77 (5.86–8.81)		1.22 ± 0.56 (0.92–1.51)	< 0.001	7.78 ± 1.75 (6.84–8.71)		1.54 ± 0.66 (1.18–1.89)	NS	NS
LH (IU/l)	1.94 ± 0.61 (1.62–2.27)		1.63 ± 1.40 (0.86–2.41)	0.011	1.67 ± 0.40 (1.46–1.88)		1.59 ± 1.62 (0.70–2.49)	0.032	NS
FSH (IU/l) ratio	2.76 ± 1.61 (1.87–3.65)		8.89 ± 4.06 (6.64–11.13)	0.010	2.72 ± 1.21 (2.05–3.39)		8.11 ± 3.07 (6.41–9.81)	0.001	NS
Testosterone (nmol/l)	13.00 ± 4.62 (10.44–15.56)		6.06 ± 2.86 (4.41–7.71)	0.001	13.10 ± 3.11 (11.38–14.83)		3.34 ± 1.21 (2.53–4.15)	< 0.001	0.04
Free testosterone (pg/ml)	10.87 ± 2.53 (9.40–12.33)		2.36 ± 0.86 (1.84–2.88)	0.009	10.65 ± 3.53 (8.26–13.01)		2.51 ± 2.61 (1.12–3.90)	NS	NS
Androstenedione (nmol/l)	3.57 ± 1.54 (2.64–4.50)		7.80 ± 2.90 (5.95–9.64)	NS	2.74 ± 1.56 (1.91–3.58)		7.15 ± 2.74 (5.41–8.89)	NS	NS
17OH-Progesterone (nmol/l)	7.69 ± 3.21 (5.66–9.73)		84.80 ± 23.67 (67.87–101.7)	0.003	6.31 ± 2.30 (4.86–7.77)		114.7 ± 32.70 (94.93–134.5)	0.001	0.02
DHEA-S (µmol/l)	52.97 ± 19.08 (39.32–66.62)				54.99 ± 22.27 (41.53–68.45)				
SHBG (nmol/l)									

Results are expressed as mean ± SD (95% confidence interval).

<sup>a</sup>No significant differences between groups in baseline values.

OC = oral contraceptive pill; BMI = body mass index; FG = Ferriman–Gallwey; DHEA-S = dehydroepiandrosterone; SHBG = sex hormone-binding globulin; NS = not significant.

**Table II.** Metabolic characteristics of women in both groups

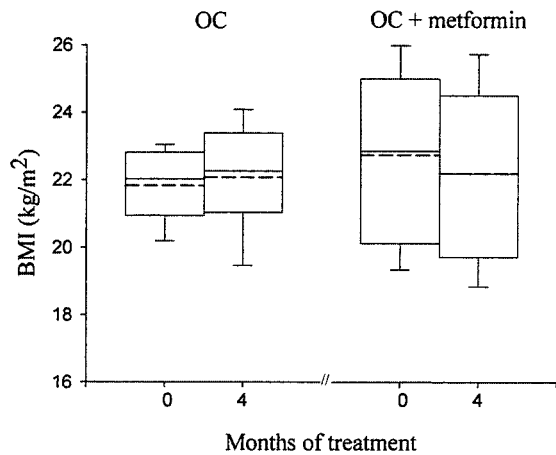
	OC group (n = 20)				OC + metformin group (n = 20)				Effect of treatment P between groups	
	Baseline		After		Baseline <sup>a</sup>		After			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Fasting glucose (mmol/l)	5.09 ± 0.68	(4.66–5.52)	4.68 ± 0.70	(4.24–5.13)	4.82 ± 0.89	(4.34–5.29)	4.34 ± 0.62	(4.02–4.67)	0.012	NS
Fasting insulin (pmol/l)	159.7 ± 108.1	(91.00–228.3)	118.6 ± 60.16	(80.40–156.9)	122.1 ± 58.65	(90.81–153.3)	81.84 ± 36.79	(62.24–101.4)	0.001	NS
Glucose (mmol/l): insulin (pmol/l) ratio	0.049 ± 0.043	(0.021–0.076)	0.052 ± 0.033	(0.031–0.072)	0.048 ± 0.022	(0.036–0.060)	0.063 ± 0.027	(0.049–0.078)	0.006	NS
Total cholesterol (mmol/l)	4.77 ± 1.07	(4.21–5.34)	5.36 ± 1.07	(4.79–5.93)	5.07 ± 0.91	(4.55–5.60)	5.46 ± 0.70	(5.05–5.86)	NS	NS
Triglycerides (mmol/l)	1.12 ± 0.46	(0.86–1.37)	0.97 ± 0.42	(0.74–1.20)	1.08 ± 0.40	(0.87–1.29)	1.10 ± 0.34	(0.92–1.28)	NS	NS
HDL-cholesterol (mmol/l)	1.32 ± 0.48	(1.04–1.60)	1.35 ± 0.41	(1.12–1.59)	1.22 ± 0.27	(1.07–1.36)	1.26 ± 0.29	(1.10–1.42)	NS	NS
LDL-cholesterol (mmol/l)	2.87 ± 0.72	(2.47–3.27)	2.97 ± 0.89	(2.47–3.46)	3.42 ± 0.83	(2.96–3.88)	3.31 ± 0.78	(2.88–3.74)	NS	NS

Results are expressed as mean ± SD (95% confidence interval).

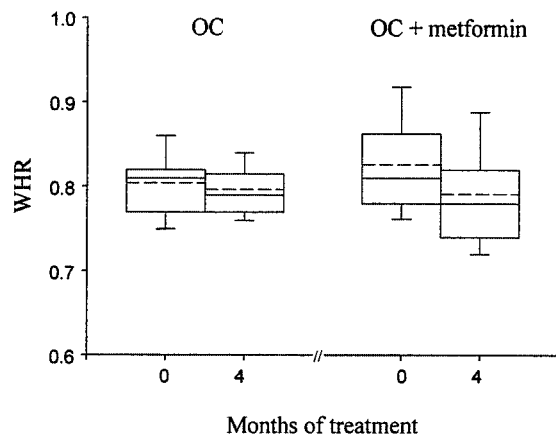
<sup>a</sup>No significant differences between groups in baseline values.

OC = oral contraceptive; HDL = high density lipoprotein; LDL = low density lipoprotein; NS = not significant.





**Figure 2.** Baseline and after-treatment body mass index (BMI) values in the two groups. Data are presented as box-plots. The solid and dashed lines within the box represent the median and mean respectively. The lower and upper boundaries of the box indicate the 25th and 75th percentiles respectively. The 10th and 90th percentiles are indicated by error bars. OC = oral contraceptive pill.

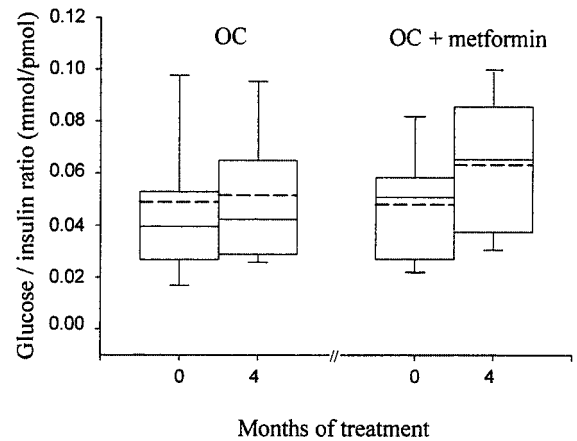


**Figure 3.** Baseline and after-treatment waist:hip ratios (WHR) in the two groups. Data are presented as box-plots. The solid and dashed lines within the box represent the median and mean respectively. The lower and upper boundaries of the box indicate the 25th and 75th percentiles respectively. The 10th and 90th percentiles are indicated by error bars. OC = oral contraceptive pill.

in the OC + metformin group, there was no correlation between BMI, WHR and GIR ( $P > 0.05$ ). The median value for the percentage change in BMI in the OC + metformin group was  $-2.5\%$ . Mean ( $\pm$  SD) percentage increase in the GIR for the subjects, who lost more weight than  $2.5\%$ , was  $47.20 \pm 24.25\%$  (95% CI, 24.77–69.62). The corresponding value for the subjects who lost less weight than  $2.5\%$  was  $14.79 \pm 45.26\%$  ( $-41.41$  to  $70.99$ ). The change in the GIR was comparable between these groups ( $P = NS$ ).

**Discussion**

To our knowledge, this is the first study on the effect of adding metformin to the OC treatment on clinical, hormonal and metabolic findings in non-obese women with PCOS. Our data



**Figure 4.** Baseline and after-treatment fasting glucose:insulin ratios in the two groups. Data are presented as box-plots. The solid and dashed lines within the box represent the median and mean respectively. The lower and upper boundaries of the box indicate the 25th and 75th percentiles respectively. The 10th and 90th percentiles are indicated by error bars. OC = oral contraceptive pill.

showed that adding metformin results in improvement of the glucose:insulin ratio in non-obese PCOS subjects. Combined metformin and OC treatment also caused significant decreases in BMI and WHR. The hormonal changes improved by the addition of metformin to the OC treatment were higher reductions in serum androstenedione and increases in serum SHBG. Indeed, most of the previous studies have shown significant improvement in insulin sensitivity during metformin treatment of PCOS subjects (Velazquez *et al.*, 1994; Nestler and Jakubowicz, 1996; Diamanti-Kandarakis *et al.*, 1998; Moghetti *et al.*, 2000), although contradictory results have also been reported (Acbay and Gundogdu, 1996; Ehrmann *et al.*, 1997). However, metformin has not been administered previously in combination with an OC to women with PCOS.

There is considerable evidence that OC can cause deterioration of glucose tolerance in normal women (Wynn *et al.*, 1979; Godsland *et al.*, 1990; Watanabe *et al.*, 1994) as well as in women with PCOS (Korytkowski *et al.*, 1995; Nader *et al.*, 1997). However, it has been recently reported that insulin sensitivity did not decrease during OC treatment in non-obese women with PCOS (Cibula *et al.*, 2002). The results of studies involving CA have been controversial. One study has shown a significant decrease in insulin sensitivity, as measured by using the euglycaemic clamp in women with PCOS before and after 6 months of EE-CA OC treatment (Dahlgren *et al.*, 1998). However, in other studies, either no effects on insulin sensitivity (Pasquali *et al.*, 1986; Morin-Papunen *et al.*, 2000a) or impairment of carbohydrate metabolism (Prelevic *et al.*, 1990) have been observed. In the present study, fasting GIR, which is a reliable indicator of insulin sensitivity in women with PCOS (Legro *et al.*, 1998), did not change significantly in the OC group. However, it increased significantly in the OC + metformin group. Therefore, EE-CA OC treatment does not improve, or may even adversely affect insulin sensitivity in women with PCOS (Dahlgren *et al.*, 1998), and glucose tolerance should be monitored during the OC treatment.

Adding metformin to the OC treatment may have beneficial effects in terms of carbohydrate metabolism, and may be considered especially in those subjects with a familial predisposition to type 2 diabetes mellitus.

Metformin either decreases or causes no change in BMI and WHR, when used alone in women with PCOS (Velazquez *et al.*, 1994, 1997; Nestler and Jakubowicz, 1996; Acbay and Gundogdu, 1996; Ehrmann *et al.*, 1997; Morin-Papunen *et al.*, 1998; Kolodziejczyk *et al.*, 2000; la Marca *et al.*, 2000). However, in most of these studies, metformin has not been administered specifically to non-obese women with PCOS, or non-obese subjects have not been analysed separately. To our knowledge, the present study is the second in which metformin has been administered to non-obese women with PCOS. Only one study (Nestler and Jakubowicz, 1997) has given metformin to normal weight or thin women in a randomized, placebo-controlled study, and reported no change in BMI but a decrease in the WHR after metformin treatment. In the present study, metformin, in combination with the OC, resulted in significant decreases in BMI and WHR among non-obese women. The EE-CA OC treatment alone did not cause any change in BMI and WHR, as it has been reported previously (Morin-Papunen *et al.*, 2000).

It has been suggested that insulin resistance in PCOS women is, at least partly, related to obesity and fat distribution and not entirely to PCOS itself (Morin-Papunen *et al.*, 2000b). Whether metformin improves insulin sensitivity independently of weight loss remains to be determined (Ehrmann, 1999). One way to address this question would be the separate analysis of the subjects who maintained, and those who lost, their weight during the treatment. To our knowledge, no investigators have analysed the study subjects in this manner. In most of the studies, metformin administration either improved or had no effect on insulin sensitivity, in parallel with changes in BMI (Velazquez *et al.*, 1994; Acbay and Gundogdu, 1996; Ehrmann *et al.*, 1997; Pasquali *et al.*, 2000). This may support the idea that metformin improves insulin sensitivity through weight loss. However, correlations between changes in BMI, WHR and insulin sensitivity have not been analysed in these studies. In addition, there are studies in which improvement of insulin sensitivity has been observed without a significant change in BMI (Diamanti-Kandarakis *et al.*, 1998; Morin-Papunen *et al.*, 1998). In the present study, we divided the subjects in the OC + metformin group into two groups by using the median percentage change in BMI as the cut-off value. 'Higher weight loss' patients had more improvement in insulin sensitivity than 'lesser weight loss' patients. However, the difference was not significant, possibly due to a type II error. Another way to address the question of the role of weight loss is to analyse the correlations between changes in BMI and insulin sensitivity. In the present study, we could not observe any correlation between percentage changes in BMI, WHR and insulin sensitivity.

In the present study, adding metformin to the OC treatment resulted in a greater reduction in androstenedione and a higher increase in SHBG. Similar changes in serum androgens and SHBG levels have been reported previously with metformin treatment in women with PCOS (Velazquez *et al.*, 1994;

Diamanti-Kandarakis *et al.*, 1998). The increase in SHBG in the present study may be partly explained by the weight loss observed in the OC + metformin group (Guzick *et al.*, 1994; Jakubowicz and Nestler, 1997). This further improvement of hyperandrogenaemia after adding an insulin-lowering drug to the OC supports the idea that insulin, in synergy with LH, has a direct effect to modulate serum androgen levels (Taylor, 2000; Attia *et al.*, 2001).

PCOS is commonly associated with low HDL and high triglyceride levels (Wild *et al.*, 1985; Talbott *et al.*, 1995). It has been previously reported that the OC containing EE and CA induced additional adverse effects on lipids in women with PCOS (Dahlgren *et al.*, 1998). In the present study, we observed a significant increase in total cholesterol levels in the OC group, but not in the OC + metformin group. Data on the effects of metformin on lipids in women with PCOS suggest either no effect or an improvement of the lipid profile (Velazquez *et al.*, 1994; Crave *et al.*, 1995; Morin-Papunen *et al.*, 1998; Ibanez *et al.*, 2000; Moghetti *et al.*, 2000). A well-controlled trial (Moghetti *et al.*, 2000) reported a 10% increase in serum HDL levels after 12 months of metformin treatment with only a minor change in BMI. According to one group (Crave *et al.*, 1995), diet, neither alone nor combined with metformin, caused any significant change in serum HDL, triglyceride and total cholesterol levels, despite a favourable change in BMI. Surprisingly, the impact of weight loss on lipids has not been well studied in subjects with PCOS (Hoeger, 2001). One study reported a 15% decrease in total cholesterol and a 10% increase in HDL-cholesterol levels after 6 months of very-low-calorie diet in obese PCOS women (Andersen *et al.*, 1995).

Recent findings regarding the long-term metabolic consequences of PCOS show that the optimal treatment should correct the hyperandrogenaemia, yet at the same time improve insulin sensitivity and dyslipidaemia. Given that there is not enough evidence to suggest metformin as first-line therapy for women with PCOS, especially for those whose chief complaints are cosmetic problems, e.g. hirsutism and acne, adding metformin to the OC treatment may result in additional benefits. This combination may be suggested for the treatment of women with PCOS, especially those who are at high risk for diabetes. In addition, the present study supports the suggestion that metformin has some benefits in non-obese women with PCOS. However, long-term clinical consequences of these mostly hormonal benefits need to be investigated.

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