Effects of metformin and ethinyl estradiol–cyproterone acetate on lipid levels in obese and non-obese women with polycystic ovary syndrome

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Abstract
Objective: Women with polycystic ovary syndrome (PCOS) exhibit risk factors for cardiovascular diseases such as abdominal obesity, insulin resistance and dyslipidemia. Insulin sensitizers, especially metformin, have been shown to improve these metabolic disturbances, but there are only a few studies on their effects on serum lipids in polycystic ovary syndrome.

Methods: Thirty-five women with PCOS (18 obese and 17 non-obese) were randomized to 6-month treatments with metformin or ethinyl estradiol–cyproterone acetate oral contraceptive pills.

Results: In the whole-study population (non-obese and obese women) serum levels of high-density lipoprotein cholesterol increased from 1.4±0.2 to 1.6±0.1 mmol/l (means±S.E. throughout) at 3 and 6 months (P < 0.001), the total cholesterol/high-density lipoprotein cholesterol ratio decreased significantly from 3.8±0.3 to 3.3±0.2 at 6 months (P < 0.001) and a similar trend was observed in serum triglyceride levels during metformin treatment. In the oral contraceptive group, serum levels of total cholesterol increased from 4.9±0.3 to 5.4±0.3 mmol/l (P < 0.05), high-density lipoprotein cholesterol increased from 1.2±0.1 to 1.5±0.1 mmol/l (P < 0.001), the total cholesterol/high-density lipoprotein cholesterol ratio decreased from 4.6±0.4 to 3.7±0.2 (P < 0.001) and triglycerides increased from 1.3±0.1 to 1.9±0.2 mmol/l at 6 months of treatment (P < 0.001). Serum low-density lipoprotein cholesterol levels remained unchanged during both treatments. Milder but similar changes in the subgroups of obese and non-obese women were observed during both treatments. Moreover, in the whole-study population both systolic (P = 0.02) and diastolic (P = 0.05) blood pressures decreased over the 6 months of metformin treatment.

Conclusion: In women with PCOS, metformin treatment had beneficial effects on lipid profile and blood pressure, and therefore it could be useful in the prevention of cardiovascular complications in these women.

European Journal of Endocrinology 152 269–275

Introduction
Polycystic ovary syndrome (PCOS) is a heterogeneous condition characterized by chronic anovulation and hyperandrogenism, and it is typically associated with insulin resistance and hyperinsulinemia – especially in obese women (1, 2). The impaired insulin sensitivity in PCOS has been shown to be closely associated with an increased amount of abdominal fat, independently of body mass index (BMI) (3, 4). Furthermore, women with PCOS have an atherogenic lipid profile characterized by lower high-density lipoprotein (HDL) cholesterol and/or HDL2 cholesterol levels, and higher triglyceride and low-density lipoprotein (LDL) cholesterol levels than the age- and weight-matched control women (5–7). Thus, the presence of abdominal obesity, insulin resistance and dyslipidemia predisposes women with PCOS to cardiovascular diseases (CVDs) (8). Metformin, a biguanide antihyperglycemic drug, has been used for decades for the treatment of type 2 diabetes mellitus. In diabetic patients, metformin treatment has been shown to have a beneficial effect on circulating lipid levels by decreasing the concentrations of plasma triglycerides and total and LDL cholesterol, and by increasing the levels of HDL cholesterol and the HDL:LDL cholesterol ratio, independently of the improvement of glycemic control (9). However, there are only a few studies specifically concerning the effects of metformin therapy on the lipid profile in women with PCOS.

Oral contraceptive (OC) pills are commonly used in the treatment of menstrual disturbances and hyperandrogenism in women with PCOS. This treatment may have negative effects on glucose tolerance and the lipid profile (10, 11); however, these effects depend on the dose of estrogen, and the dose and type of progestin (12, 13).
As both metformin and OC pills containing ethinyl estradiol–cyproterone acetate (EE–CA) are now commonly used in the treatment of PCOS, it was of particular interest to examine the effects of these two types of medication on the lipid profile in obese and non-obese women with PCOS.

Subjects and methods

Subjects

The subjects included in this study had participated in two previous studies on the effects of metformin and the EE–CA pill on insulin sensitivity, glucose tolerance and hormonal parameters in PCOS (3, 11). Twenty non-obese (BMI < 27 kg/m²) and 32 obese (BMI ≥ 27 kg/m²) women with PCOS were investigated. Seventeen non-obese (mean age, 28.2 ± 1.2 years (means ± S.E. throughout); mean BMI, 22.2 ± 0.5 kg/m²) and 18 obese (mean age, 29.6 ± 1.1 years; mean BMI, 35.1 ± 1.2 kg/m²) women completed the 6-month study (Fig. 1).

Criteria for PCOS were as defined by Homburg (14). All subjects had polycystic ovaries as shown by vaginal ultrasonography (eight or more subcapsular follicles of 3–8 mm diameter in one plane in one ovary and increased stroma) and at least one of the following symptoms: oligomenorrhea or amenorrhea, clinical manifestations of hyperandrogenism such as hirsutism scored (> 7) according to Ferriman and Gallwey, acne and/or an elevated serum testosterone level (> 2.7 nmol/l). Diabetic subjects, smokers, alcohol users and those taking sex hormones or drugs known to affect lipid metabolism during the 2 months preceding the study were excluded.

The study was approved by the Ethics Committee of the University of Oulu, Finland, and informed written consent was obtained from each subject.

Protocol of the study

The subjects were randomized to either the metformin or the oral contraceptive (OC) pill group (OC: EE 35 μg, CA 2 mg; Diane Nova, (Shering, Germany); 21 days per

Figure 1 Flow chart of the study.

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month followed by a 7-day pill-free period). The metformin dose was doubled after 3 months of treatment (metformin hydrochloride; Diformin, Leiras, Finland: 500 mg × 2 for 3 months, then 1000 mg × 2 for 3 months) in both non-obese and obese groups to study the effects of different doses.

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. Blood pressure was measured after a 20-min rest in a sitting position. Systolic and diastolic blood pressure were measured with a calibrated manometer from the right arm and recorded to the nearest 2 mmHg. Blood pressures were defined as the points of appearance and disappearance of Korotkoff sounds respectively (level V). Transvagal ultrasonography, the oral glucose tolerance test (OGTT) and the euglycemic hyperinsulinemic clamp were performed as described previously (3, 11). These examinations were performed and venous blood samples were drawn at the following times: 1–7 days after spontaneous, or progestin-induced (hydrogesterone, 10 mg/day for 10 days, four amenorrheic subjects in the obese group and three in the non-obese group) or EE–CA pill-induced menstruation; before the treatment; and at 3 and 6 months of treatment.

All samples were handled similarly and frozen at −20 °C for 1–3 years.

Assays
Total serum cholesterol (15), triglycerides (16), HDL cholesterol (17) and LDL cholesterol (18) were determined using Cobas Integra 700 automatic analyzer (Hoffman-La Roche, Basel, Switzerland). Serum LDL levels were determined as published previously (19) using a Hitachi 911 automatic analyser (Boehringer Mannheim, Germany).

The intra- and interassay coefficients of variation were respectively: 0.7 and 2.3% for cholesterol, 0.9 and 2.1% for triglycerides, 0.5 and 3.6% for HDL cholesterol, and 2.0 and 2.9% for LDL cholesterol.

Statistical analysis
Student’s two-tailed t-test was used for comparison of normally distributed variables, with or without log transformation. The Mann–Whitney U-test was used for variables with a persisting skewed distribution after log transformation.

Where there were normally distributed variables, ANOVA for repeated measures was used to study the clinical, metabolic and hormonal changes within the metformin and EE–CA groups during the treatment, either without or with logarithmic transformation. The Wilcoxon unpaired test was used for variables with persisting skewed distribution after log transformation.

Analysis of correlation between parameters was performed by calculating Pearson’s bivariate correlation coefficient.

The stepwise method was used to identify the significant predictors (independent variables) of serum lipid levels at baseline. Serum lipid level at baseline was entered as a dependent variable, and BMI, waist:hip ratio (WHR), M-value (expressing the level of insulin sensitivity as assessed during the euglycaemic clamp), serum fasting insulin, sex hormone binding globulin (SHBG) and androgen levels, and free androgen index (FAI) were used as independent variables in the stepwise regression analysis.

Previous studies have indicated that a 30% increase in serum triglyceride levels could be expected during OC treatment (20). No consistent data on lipid values in PCOS women during metformin treatment were available for power analysis. The power analysis revealed that an estimated group size of 16 subjects was required to obtain a significant difference with a power of 0.8 (α = 0.05).

Results
At baseline, serum HDL cholesterol levels were significantly lower (1.0 ±0.1 vs 1.5 ±0.1, P = 0.001) and serum triglyceride concentrations and the total cholesterol:HDL cholesterol ratio were significantly higher in the obese than in the non-obese subjects (Table 1). In the combined group of non-obese and obese women the levels of HDL cholesterol (r = −0.69, P < 0.0001), LDL cholesterol (r = 0.41, P = 0.015) and triglycerides (r = 0.64, P < 0.0001), and the total cholesterol:HDL cholesterol ratio (r = 0.68, P < 0.0001) correlated significantly with the WHR.

In a stepwise regression analysis, WHR (P = 0.001) and SHBG (P = 0.001) were significant determinants of serum HDL cholesterol levels at baseline. The only significant determinant of serum LDL cholesterol (P = 0.02) and triglyceride levels (P = 0.001) was WHR, and BMI (P < 0.001) was the only significant predictor of serum triglycerides.

Table 1 Lipid profile of the obese and non-obese women at baseline.

<table>
<thead>
<tr>
<th></th>
<th>Obese (BMI ≥27)</th>
<th>Non-obese (BMI &lt;27)</th>
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<tbody>
<tr>
<td></td>
<td>(n = 18)</td>
<td>(n = 17)</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.0–5.0 mmol/l</td>
<td>4.9 ±0.2</td>
<td>5.0 ±0.2</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.0 ±0.1</td>
<td>1.5 ±0.1</td>
</tr>
<tr>
<td>1.0–2.5 mmol/l</td>
<td>3.1 ±0.2</td>
<td>2.7 ±0.2</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>1.6 ±0.2</td>
<td>1.1 ±0.1†</td>
</tr>
<tr>
<td>&lt; 3.5 mmol/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>4.9 ±0.4</td>
<td>3.5 ±0.3‡</td>
</tr>
<tr>
<td>0.4–1.7 mmol/l</td>
<td></td>
<td></td>
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</tbody>
</table>

Data are shown as means ± S.E. and the reference ranges are shown in brackets.

* P < 0.001, †P < 0.05 and ‡P < 0.005 between groups at baseline.
determinant for cholesterol:HDL ratio, whereas the effect of other parameters (M-value, serum fasting insulin, SHBG and androgen levels, and FAI) on lipid levels remained non-significant.

At baseline the two treatment groups did not differ as regards BMI, waist, WHR, M-value, and fasting serum glucose, insulin (21) and lipid levels (Table 2).

In the combined group of non-obese and obese women the mean BMI decreased slightly at 3 months and significantly at 6 months, and WHR decreased significantly during metformin treatment, as published previously (21). Similarly, serum levels of HDL cholesterol increased and the cholesterol:HDL ratio decreased significantly at 3 and 6 months of metformin treatment, and serum triglyceride levels tended to decrease ($P = 0.09$, Table 2).

In the EE–CA group, the cholesterol:HDL ratio decreased and serum levels of total cholesterol, HDL and triglycerides increased significantly at 3 and 6 months of treatment (Table 2). Serum LDL cholesterol levels remained unchanged during both treatments.

In the obese women, serum total cholesterol and LDL cholesterol levels remained unchanged, and the total cholesterol:HDL cholesterol ratio decreased significantly in both treatment groups (Fig. 2). Serum HDL cholesterol concentrations increased significantly in both the metformin (from $1.1 \pm 0.1$ to $1.4 \pm 0.1$ mmol/l at 3 and 6 months, $P < 0.005$) and the EE–CA group (from $1.0 \pm 0.1$ to $1.3 \pm 0.1$ mmol/l at 3 and 6 months, $P < 0.001$). Serum triglyceride concentrations did not change significantly in the metformin group, but increased during EE–CA treatment (from $1.5 \pm 0.1$ to $2.0 \pm 0.2$ mmol/l at 3 months ($P < 0.05$) and to $1.9 \pm 0.1$ mmol/l at 6 months ($P < 0.01$)).

In the non-obese women, serum total cholesterol levels increased significantly in the EE–CA group (Fig. 2). Serum LDL cholesterol levels remained unchanged in both groups. Serum HDL cholesterol concentrations did not change significantly in the metformin group, but increased from $1.4 \pm 0.1$ to $1.7 \pm 0.1$ mmol/l at 3 months ($P < 0.01$) and to $1.8 \pm 0.1$ mmol/l at 6 months ($P < 0.001$) in the EE–CA group. The total cholesterol:HDL cholesterol ratio decreased significantly during both treatments (Fig. 2). Serum triglyceride concentrations increased significantly in the EE–CA group, as previously reported (3).

In the whole-study population, both systolic (from $126 \pm 3.6$ to $117 \pm 2.8$ mmHg, $P = 0.02$) and diastolic (from $81 \pm 2.0$ to $78 \pm 2.0$ mmHg, $P = 0.05$) blood pressures were decreased at 6 months of metformin treatment, but did not change during EE–CA treatment. In the subgroup of obese subjects, systolic blood pressure was decreased slightly (from $133 \pm 5.1$ to $121 \pm 4.0$ mmHg, $P = 0.09$) after 6 months of metformin treatment.

### Discussion

The present results demonstrate that metformin treatment improves the lipid profile and decreases blood pressure in women with PCOS, whereas the EE–CA OC pill has both beneficial and negative effects on serum lipids over 6 months of treatment.

At baseline, the obese women had a more atherogenic lipid profile than the non-obese subjects. Previous studies have shown that both non-obese and obese subjects with PCOS have higher WHR values (i.e. greater abdominal obesity) and greater insulin resistance than their healthy controls (1, 22, 23). Thus, the present observations that the serum levels of HDL cholesterol, LDL cholesterol, triglycerides and the total cholesterol:HDL cholesterol ratio correlated significantly to well-known indicators of insulin resistance, i.e. BMI and WHR, strengthen the concept that obesity (mostly abdominal obesity) and insulin resistance are the main contributors to the development of lipid and metabolic disturbances in PCOS (4, 23, 24). Accordingly, women with PCOS have been shown to be at greater risk of CVD compared with age-matched controls (25, 26).

Metformin treatment significantly increased serum HDL cholesterol concentrations and decreased the cholesterol:HDL cholesterol ratio in the whole-study population.

### Table 2 Lipid profile of the whole group of women before and during the treatment.

<table>
<thead>
<tr>
<th></th>
<th>Metformin</th>
<th>EE–CA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 month ($n = 16$)</td>
<td>3 months ($n = 16$)</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(3.0–5.0 mmol/l)</td>
<td>$4.9 \pm 0.2$</td>
<td>$5.1 \pm 0.2$</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>$1.4 \pm 0.1$</td>
<td>$1.6 \pm 0.1^\dagger$</td>
</tr>
<tr>
<td>(1.0–2.5 mmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>$2.7 \pm 0.2$</td>
<td>$2.9 \pm 0.2$</td>
</tr>
<tr>
<td>(&lt;3.5 mmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>$1.4 \pm 0.3$</td>
<td>$1.2 \pm 0.2$</td>
</tr>
<tr>
<td>(0.4–1.7 mmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol:HDL ratio</td>
<td>$3.8 \pm 0.3$</td>
<td>$3.4 \pm 0.3^\dagger$</td>
</tr>
</tbody>
</table>

Data are shown as means±S.E. and the reference ranges are shown in brackets.

$^*P < 0.05$, $^1P \leq 0.001$ and $^1P < 0.01$ compared with the level before treatment.

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population and in the obese subjects. The milder metabolic abnormalities in non-obese PCOS women and the small number of subjects continuing the study up to 6 months may explain the lack of a significant effect of metformin on lipid profile in these subjects. Our results are in line with previous studies on PCOS, where metformin has been shown to improve the lipid profile, mainly by increasing serum HDL cholesterol concentrations (27, 28). On the other hand, some other studies have shown only a negligible or no effect on lipids in women with PCOS (29, 30). These discrepancies could be explained by differences in study populations or by a shorter duration of treatment compared with the present study.

We have shown previously that beneficial effects of metformin were observed already at 3 months of treatment and at a dosage of 1 g/day, but most of the hormonal and metabolic parameters improved further after 3 months of treatment when the dose was increased (3, 11). Whether this improvement is due to a longer treatment or to an increase of the metformin dose remains uncertain. In both non-obese and obese groups, one subject stopped the treatment because of side-effects after increasing the metformin dose. Because of the small number of subjects continuing the treatment up to 6 months, it is difficult to draw reliable conclusions as regards a difference of compliance between the two doses. It has to be noted, however, that most of the significant changes in serum lipids were already obtained at 3 months of treatment with a lower dose (1000 mg/day) than routinely used in obese women (30–33). Thus, this dose could be recommended for subjects suffering from side-effects with higher doses of metformin.

The mechanisms by which metformin improves the lipid profile are not clear. Metformin has been suggested to reduce lipid uptake or synthesis in the intestine and in the hepatocytes (34, 35). The improvement of obesity and especially abdominal obesity with a subsequent decreased release of free fatty acids (FFAs) from adipose tissue observed during metformin therapy (3, 11, 36) could also partly explain the improvement of lipid profile during metformin treatment, at least in obese women.

Blood pressure decreased in the whole-study population during metformin treatment. Although the change was modest it may have clinical significance, since reduction of mild-to-moderate raised blood pressure has been associated with a reduced risk of CVD in large placebo-controlled studies (37, 38). Moreover, a decrease in blood pressure, and decreases in plasma levels of triglycerides, and total and LDL cholesterol

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**Figure 2** Serum total cholesterol levels and cholesterol:HDL-cholesterol ratios of the subgroups of obese and non-obese PCOS women before and during the treatments. *P < 0.05 and †P < 0.01 compared with the level before treatment.
concentrations, and an increase in HDL cholesterol levels have been observed in diabetic patients on metformin independently of improved glycemic control (9). Thus, metformin, by way of its beneficial effects on lipids and blood pressure, could be useful in the prevention of cardiovascular disease, especially in obese women with PCOS.

The EE–CA pill was associated with increased serum HDL cholesterol and triglyceride concentrations in both non-obese and obese subjects, as shown previously (20, 38–41), and the total cholesterol:HDL cholesterol ratio decreased. OCs have been shown to induce adverse effects on carbohydrate and lipid metabolism in healthy women and those with PCOS, but these effects depend on the dose of estrogen and the type and dose of progestin used (12). Although the progestin used in the present study, cyproterone acetate, is a pregnane-derived progestogen without androgenic activity, it has been shown to have both HDL2 cholesterol- and LDL cholesterol-lowering properties (42). The results of an earlier study suggest that the slight estrogen dominance of EE–CA treatment could account for some of the beneficial effects of EE–CA treatment on the lipid profile in women with PCOS (39). On the other hand, the slight worsening of glucose tolerance with compensatory hyperinsulinemia and the increase in serum FFA levels induced by the EE–CA pill (3, 10, 11) could explain some of the negative effects of this treatment on the lipid profile, such as the increase in serum triglyceride levels. Recently, the addition of metformin to EE–CA OC pill treatment has been shown to improve the lipid profile and insulin sensitivity in non-obese women with PCOS, compared with EE–CA treatment alone, suggesting that at least some women with PCOS could benefit from the combination of OC pill and metformin (43).

In conclusion, the beneficial effects of metformin on the lipid profile and blood pressure strength earlier data suggesting that metformin could be an effective drug in the prevention of CVD in insulin-resistant women with PCOS. The EE–CA OC pill is a good alternative in the treatment of hirsutism and irregular menstruation problems in women with PCOS, but, especially in obese women, its overall effect on the lipid profile and its impact on CVD risks need to be clarified in larger follow-up studies.

Acknowledgements

We thank nurses Mirja Ahvensalmi (Research Unit of the Department of Obstetrics and Gynecology) and Anja Heikkinen (Clinical Chemistry Laboratory, Oulu University Hospital) for expertise in the technical work and the care of the patients. This research has been supported by grants provided by University of Oulu, the Finnish Gynecologic Association, the Sigrid Juselius Foundation and the Academy of Finland.

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Received 27 April 2004
Accepted 27 October 2004