Efficacy of metformin in obese and non-obese women with polycystic ovary syndrome: a randomized, double-blinded, placebo-controlled cross-over trial

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BACKGROUND: Our aim was to assess the effects of metformin on menstrual frequency, fasting plasma glucose (FPG), insulin resistance assessed as HOMA-index, weight, waist/hip ratio, blood pressure (BP), serum lipids, and testosterone levels in women with polycystic ovary syndrome (PCOS) METHODS: In a randomized, controlled, double-blinded setup, 56 women aged 18–45 with PCOS were treated with either metformin 850 mg or placebo twice daily for 6 months. After a wash-out period of 3 months participants received the alternate treatment for 6 months. The changes in the measured parameters were analysed by intention-to-treat and per protocol RESULTS: There were no changes in menstrual frequency. In the intention-to-treat analysis, weight and systolic BP were reduced on metformin treatment (p < 0.009 and 0.047, respectively), while high-density lipoprotein (HDL) increased (p = 0.001). On placebo, weight and FPG increased (p<0.05). Post-hoc subgrouping according to BMI revealed reductions in testosterone (p = 0.013), FPG (p = 0.018), insulin (p = 0.045) and HOMA-index (p = 0.022) in obese women. Per protocol analysis showed the following differences between the changes on placebo and metformin (mean (5 - 95 % percentiles): weight (-4.2 (-7.0, -1.9) kg, p<0.001), FPG (-0.23 (-0.44, -0.01) mmol/l, p = 0.041), insulin (-4.17 (-8.10, -0.23) mIU/l, p = 0.039) and HOMA index (-1.50 (-2.53, -0.47) mIU/l*mmol/l, p = 0.006). Weight, FPG and HOMA index were lower after metformin than after placebo. CONCLUSIONS: Metformin treatment lowered weight and systolic blood pressure and increased HDL in women with PCOS. In post-hoc analysis it increased insulin sensitivity and lowered testosterone in obese women. Non-obese women did not benefit from metformin.

Keywords: polycystic ovary syndrome; metformin; obesity; randomized controlled trial

Introduction

Polycystic ovary syndrome (PCOS) is characterized by oligo- or anovulation, clinical and/or biochemical signs of hyperandrogenism and polycystic ovaries. The severity of the symptoms is weight dependent (Vanky et al., 2004). Women with PCOS show features of the metabolic syndrome (Holte et al., 1998; Legro et al., 1999; Glueck et al., 2003), a constellation of metabolic abnormalities associated with an increased risk of type 2 diabetes mellitus and cardiovascular disease. A disturbed balance between insulin sensitivity and β-cell activity has been described in both obese and non-obese women with PCOS (Dunaif et al., 1989) and >50% have hyperinsulinemia and are at increased risk of developing type 2 diabetes.

Metformin reduces the incidence of diabetes in pre-diabetic subjects (Diabetes Prevention Program Research Group, 2002) and lowers body weight in patients with and without type 2 diabetes. When metformin was introduced as treatment for PCOS, observational studies showed encouraging results on menstrual pattern and infertility (Nestler et al., 1998; Moghetti et al., 2000), body weight (Fleming et al., 2002; Gambineri et al., 2004), insulin sensitivity, androgen levels and hirsutism (Kelly and Gordon, 2002; Harborne et al., 2003b; Gambineri et al., 2004; Ganie et al., 2004). A recent meta-analysis concluded that metformin is an effective treatment for anovulation...
Materials and Methods

Study population
The study was performed at the Department of Gynaecology and Obstetrics, Holstebro Hospital, Holstebro, Denmark. Women 18–45 years of age, referred to the outpatient clinic in Holstebro from September 2001 to December 2002 with symptoms indicating PCOS were invited to participate in the study. Blood tests were performed for fasting venous plasma glucose (FPG), gonadotrophins, total testosterone, dehydroepiandrosterone sulphate, androstenedione, prolactin, and for thyroid, renal and hepatic function. Women were considered eligible for the trial if they had a testosterone value above the upper normal limit and oligo- or amenorrhoea, and did not meet any of the exclusion criteria. Oligomenorrhoea was defined as irregular bleeding periods with an interval varying between 5 weeks and 6 months and amenorrhoea as absent bleedings for at least 6 months. Ovarian morphology or clinical hyperandrogenism were not diagnostic criteria and were not registered in all cases. Exclusion criteria were periclinal sterility, gonadotrophin values, hyperprolactinaemia, diabetes mellitus, impaired thyroid, renal or hepatic function, hormonal treatment, pregnancy, lactation or a wish for fertility treatment. Women taking antihypertensive agents were included and their medication was unchanged throughout the study.

Protocol
Eligible women who gave written informed consent were assigned to 6 months of treatment with either 850 mg of metformin or placebo twice daily, followed by a wash-out period of 3 months before cross-over to the alternate treatment for another 6 months. Randomization defining treatment sequence was done at inclusion by random number tables (Dien, 1963). The appearance of the tablets was identical, and patients and investigators were blinded to treatment allocation.

The randomization code was stored in a closed envelope until all participants had finished the treatment.

Participants were seen by one of the investigators before inclusion and every second month during treatment periods, always in the morning after an overnight fast of at least 8 h. They were weighed wearing light clothing. Waist circumference was measured at the umbilical level and hip circumference at the trochanter region. Systolic (SBP) and diastolic blood pressure (DBP) was measured with a semiautomatic blood pressure monitor (NA-777, A&D Instruments Ltd., Abingdon, Oxford, UK), and a blood sample was drawn for immediate analysis without respect to bleeding periods. All participants registered their bleeding periods in a calendar during both study periods and the 3 months wash-out period.

The participants were informed to contact the investigators in case of adverse events or pregnancy. Sexually active women were asked to use barrier contraception during the study. They were asked to do a home ovulation test in case of abdominal discomfort and a pregnancy test if they suspected pregnancy.

The efficiency of blinding was evaluated by telephone interview of a group of study participants after the study, asking them during which treatment period they assumed that they had received metformin.

Statistics
Calculation of sample size was based on the assumption that at least 50% of the women would experience at least 30% more menstrual periods on metformin than on placebo. Based on a power of 90 (% = 0.10) to detect a significant difference (two-sided $P$-value ($\alpha$) of 0.05), the minimum sampling size was calculated to 44 subjects. We also assumed a 10% drop-out rate, and thus aimed at including 50 women. As drop-out rate quickly rose higher than expected, we decided to include 60 women.

In the intention-to-treat analysis, the values of each participant after 6 months of metformin or placebo were compared with the baseline values. Linear regression analysis with the changes in testosterone and homeostasis model assessment (HOMA) index as dependent variables was performed to examine potential relations between the changes.

The per protocol analysis included data from participants completing both study periods, i.e. the difference between the values of each participant after placebo and metformin, respectively, was calculated, and a significance test performed on the differences.

A paired Student’s $t$-test was performed if data followed Gaussian distribution evaluated by the Kolmogorov–Smirnov test; otherwise a Wilcoxon paired sample test was applied. An unpaired $t$-test was used for testing differences between means.

A post-hoc analysis was performed by subgrouping the data according to body mass index (BMI), as BMI was considered the most important potential confounder.

As the data are not complete for all women at all times, the number of analysed pairs varies. Some controls were cancelled because the women failed to turn up or wanted to skip a control for personal reasons. At some occasions, blood tests were not done because the woman had not been fasting for at least 8 h.

The statistical software program Stata, version 9.2 (StataCorp 2005) was used for the statistical evaluation.

The tests used in this study have the following normal values: FPG < 6.1 mmol/l, fasting insulin <40 mIU/l, total cholesterol 3.4–7.0 mmol/l, high-density lipoprotein (HDL)-cholesterol 0.8–1.7 mmol/l, low-density lipoprotein (LDL)-cholesterol 1.5–4.9 mmol/l, triglycerides 0.5–2.3 mmol/l, testosterone 0.6–1.8 mmol/l and sex hormone-binding globulin (SHBG) 41–169 nmol/l.

Insulin sensitivity was evaluated by HOMA, calculated as fasting serum insulin (mIU/l) × FPG (mmol/l) / 22.5. Obesity was defined as BMI ≥ 30 kg/m². Adherence to therapy was assessed by counting the tablets returned by the participants after each treatment period.

The study was approved by the regional Ethics Committee, the Danish Medicines Agency, the Danish Data Protection Agency, and conducted in accordance with the Helsinki Declaration and the guidelines for Good Clinical Practice.

Role of the pharmaceutical companies providing the study medication
Metformin and placebo was donated by GEA A/S, Hvidovre, Denmark. The pregnancy and ovulation tests were donated by
Unipath Limited, UK. The companies did not participate in or influence study design, collection, analysis or interpretation of data; in the writing of the report or the decision to submit the paper for publication. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

Results
Sixty women were included. All had both elevated testosterone levels and oligo- or amenorrhoea and thus had PCOS according to the Rotterdam definition. Fifty-six remained for the intention-to-treat analysis and 38 for the per protocol analysis (Fig. 1). There was no difference in drop-out rate between obese and non-obese women.

Subjects included in the per protocol analysis did not differ from the intention-to-treat group in any of the studied parameters (Table 1).

There was no difference in compliance between the two periods (data not shown). Of 50 women taking metformin, 29 (58%) reported side effects, mostly gastrointestinal. Two women reported side effects on placebo. There were no serious adverse events. Thirty-one women were interviewed after the study and 24 (77%) guessed correctly the sequence of metformin and placebo. Their guesses were made mainly on the basis of side effects.

Effect of treatment
Bleeding calendars were available for 36 women who completed both study periods. There were more bleeding periods on metformin than on placebo, but the difference did not reach statistical significance ($P = 0.063$, Wilcoxon signed-rank test). Nineteen had more frequent periods on metformin than on placebo (4.8 against 3.2), while 11 had more frequent periods on placebo (5.6 against 4.5) and 7 did not register any change. There was no difference between obese and non-obese women.

The intention-to-treat analysis showed significant results for both metformin and placebo (Table 2). All changes occurred in the total group of participants or the obese group, while there were no significant changes in non-obese women. The decrease in testosterone levels was close to significance during the placebo as well as the metformin periods. DBP, waist-hip ratio, triglyceride, LDL, cholesterol and SHBG did not change.

The changes during metformin and placebo periods, respectively, were compared and significant differences were found in the total group of participants and the obese group. The results are shown in Table 3. Only the differences in change in HOMA index reached significance, when obese and non-obese women were compared.

The per protocol analysis showed that after 6 months, body weight, FPG and HOMA index were significantly lower on metformin than on placebo ($P < 0.001$, $P = 0.022$ and $P = 0.032$, respectively). Obese women also had lower

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**Figure 1:** Flow chart of participants during the study
values of insulin \((P = 0.012)\) and testosterone \((P = 0.039)\). No difference between metformin and placebo was seen in non-obese women.

When regression analysis was performed with baseline values of testosterone as the dependent variable and insulin, glucose and weight as independent variables, insulin was the only significant predictor \([\beta = 0.04, \ P < 0.001, \ 95\% \ CI \ 0.019–0.060]\). The change in insulin was also the only significant predictor of the change in testosterone after the metformin period \((\beta = 0.08, \ P < 0.001, \ 95\% \ CI \ 0.048–0.118)\), while the change during the placebo period was not associated with any of the other parameters. At baseline, the HOMA-index was significantly associated with weight \((\beta = 0.045, \ P = 0.035, \ 95\% \ CI \ 0.003–0.087)\) and with testosterone \((\beta = 2.21, \ P < 0.001, \ 95\% \ CI \ 1.21–3.21)\). The change on metformin was associated with the change in testosterone \((\beta = 1.22, \ P = 0.001, \ 95\% \ CI \ 0.58–1.87)\), but not significantly with the change in weight.

### Discussion

The present study shows a clear effect of metformin on weight, testosterone and several metabolic factors in obese women with PCOS. Further, the data indicate that there may not be an effect

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**Table 2:** Changes from baseline to 6 months (intention-to-treat analysis)

<table>
<thead>
<tr>
<th>All participants</th>
<th>Per protocol</th>
<th>Non-obese</th>
<th>Obese (n = 40)</th>
<th>(P)-value for obese versus non-obese</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Change</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metformin</td>
<td>-2.3 (3.9, -0.6)</td>
<td>0.009 (42)</td>
<td>-2.7 (5.0, -0.4)</td>
<td>0.02 (30)</td>
</tr>
<tr>
<td>Placebo</td>
<td>1.5 (2.7, 0.2)</td>
<td>0.019 (45)</td>
<td>2.1 (6.3, 3.7)</td>
<td>0.008 (32)</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>-5.4 (-10.8, -0.1)</td>
<td>0.047 (40)</td>
<td>-5 (-12, 1)</td>
<td>0.126 (29)</td>
</tr>
<tr>
<td>Metformin</td>
<td>1 (-3.5)</td>
<td>0.529 (43)</td>
<td>0 (-6.5)</td>
<td>0.921 (31)</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>0.11 (0.05, 0.18)</td>
<td>0.001 (42)</td>
<td>0.14 (0.06, 0.22)</td>
<td>0.002 (30)</td>
</tr>
<tr>
<td>Metformin</td>
<td>0.04 (0.01, 0.08)</td>
<td>0.116 (43)</td>
<td>0.02 (-0.03, 0.08)</td>
<td>0.434 (31)</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td>-0.30 (-0.61, 0.01)</td>
<td>0.055 (42)</td>
<td>-0.46 (-0.82, -0.1)</td>
<td>0.013 (30)</td>
</tr>
<tr>
<td>Metformin</td>
<td>-0.24 (-0.46, 0.00)</td>
<td>0.054 (45)</td>
<td>-0.2 (-0.5, 0.1)</td>
<td>0.178 (32)</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPG (mmol/l)</td>
<td>-0.13 (-0.26, 0.00)</td>
<td>0.058 (38)</td>
<td>-0.18 (-0.33, -0.03)</td>
<td>0.018 (27)</td>
</tr>
<tr>
<td>Metformin</td>
<td>0.16 (0.01, 0.30)</td>
<td>0.031 (41)</td>
<td>0.22 (0.03, 0.40)</td>
<td>0.024 (29)</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin (mIU/l)</td>
<td>-1.48 (-18.38, 9.52)</td>
<td>0.101 (35)</td>
<td>-2.09 (-18.38, 8.45)</td>
<td>0.045 (25)</td>
</tr>
<tr>
<td>Metformin</td>
<td>0.45 (-7.77, 12.96)</td>
<td>0.379 (34)</td>
<td>0.77 (-7.77, 12.96)</td>
<td>0.412 (23)</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA index</td>
<td>-0.48 (-5.96, 1.89)</td>
<td>0.070 (33)</td>
<td>-0.66 (-5.96, -1.54)</td>
<td>0.022 (23)</td>
</tr>
<tr>
<td>Metformin</td>
<td>0.19 (-2.10, 3.62)</td>
<td>0.092 (32)</td>
<td>0.38 (-2.10, 3.62)</td>
<td>0.068 (21)</td>
</tr>
</tbody>
</table>

\(\text{Gaussian distributed parameters (*) are given as mean (5–95\% \text{ percentiles}) and tested by paired } t\text{-test, otherwise values are given as median (5–95\% \text{ percentiles}) and tested by Wilcoxon signed rank-sum test. } \text{n: number analysed.}\)
in non-obese subjects. This finding must be interpreted with caution because of the small number of non-obese patients completing both study periods. It is still striking, however, that metformin had no effect on any of the studied parameters in this group, and it emphasizes the heterogeneity of PCOS and the importance of testing treatments on clearly defined subgroups of patients. It must also be taken into account that obese and non-obese women differ at baseline. The obese group generally have values more distant from the median of the normal area, which makes the room for improvement and group generally have values more distant from the median of

## Table 3: Mean differences between changes during metformin and placebo periods (intention-to-treat analysis), paired t-test (5–95% percentiles)

<table>
<thead>
<tr>
<th>All participants</th>
<th>Obese</th>
<th>Non-obese</th>
<th>Obese versus non-obese</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>-4.2</td>
<td>-5.2</td>
<td>-1.6</td>
</tr>
<tr>
<td><strong>SBP (mmHg)</strong></td>
<td>-5.0</td>
<td>-4.4</td>
<td>-6.6</td>
</tr>
<tr>
<td><strong>HDL (mmol/l)</strong></td>
<td>0.07</td>
<td>0.11</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Testosterone (nmol/l)</strong></td>
<td>-0.09</td>
<td>-0.24</td>
<td>0.34</td>
</tr>
<tr>
<td><strong>FPG (mmol/l)</strong></td>
<td>-0.23</td>
<td>-0.34</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>Insulin (mIU/l)</strong></td>
<td>-4.17</td>
<td>-6.16</td>
<td>0.32</td>
</tr>
<tr>
<td><strong>HOMA index (mIU/l mmol/l)</strong></td>
<td>-1.50</td>
<td>-2.28</td>
<td>-0.04</td>
</tr>
</tbody>
</table>

*n* value number analysed. An unpaired *t*-test was used for obese vs. non-obese.

in the intention-to-treat analysis of our results, we could not demonstrate any significant difference between the changes in testosterone levels during metformin and placebo periods. In fact, the trend towards a significant reduction in testosterone was surprisingly seen both in metformin and placebo treated subjects. We speculate that the decline in testosterone levels may be a result of care itself. Most participants had never received any information or treatment of their condition before entering the study, and they were generally pleased to be subject to care. There are reports of a decreased quality of life in PCOS (Elsenbruch et al., 2003) and a higher frequency than expected of metabolic and hormonal changes in women suffering from fear and anxiety (Rosmond et al., 2001). The metabolic syndrome shows an association with chronic stress and dysregulation of the hypothalamic–pituitary–adrenal axis (Bjorntorp and Rosmond, 2000; Tsigos and Chrousos, 2002). Thus, psychosocial stress may influence the manifestations of PCOS and render the condition susceptible to the effect of care. The participants may also have changed their life style beyond our knowledge as a result of the information they received, but the fact that the testosterone changes were not associated with changes in BMI, insulin or HOMA-index makes this explanation less plausible.
The blood tests were done without respect to bleeding pattern. We aimed to include only anovulatory women with a steady-state hormonal pattern in our study, but there may have been temporary hormonal fluctuations interfering with the results.

In obese participants, HOMA-index and FPG levels were significantly reduced on metformin treatment, whereas in the placebo FPG increased significantly. FPG levels tend to increase over time in PCOS women, and in our Danish population 38% of the PCOS patients 35 years or older have impaired FPG values (Trolle and Lauszus, 2005). The effect of metformin on FPG is well known and consistent with the observation that metformin suppresses endogenous glucose production (DeFronzo, 1999). We speculate that the lowering of FPG may postpone or abolish deterioration of glucose metabolism in obese PCOS subjects, but long-term studies are necessary to prove this. The same holds true for the development of cardiovascular disease. So far, there is no clinical evidence that metformin treatment reduces the development of cardiovascular disease in PCOS patients.

The number of patients included in this study and the cross-over design gives this study a considerable strength in spite of the high percentage of drop-outs (32%). Thirty-eight subjects were still available for per protocol analysis and acted as their own controls, thus minimizing influence of possible confounders such as age, exercise and smoking habits. We did not demonstrate a significant carry-over effect. An order effect cannot be ruled out considering the frequent side effects of metformin, which hampered the binding to the patients. The supposition of receiving active or inactive treatment may have influenced eating pattern, interfered with adherence or otherwise affected test results. However, adherence to treatment was high despite the re-called insufficient binding.

Very few studies on metformin have been paired studies. The unpaired study design has a larger risk of potential bias in the comparison of efficacy of spironolactone with metformin in the management of polycystic ovary syndrome: an open-labeled study. J Clin Endocrinol Metab 2003;88:5801–5807.

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Submitted on January 24, 2007; resubmitted on July 4, 2007; accepted on July 19, 2007