Acute effects of metformin therapy include improvement of insulin resistance and ovarian morphology

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Objective: To evaluate the acute effects of metformin therapy on biochemical markers and polycystic ovarian morphology among insulin-resistant (IR) and noninsulin-resistant (NIR) patients with polycystic ovary syndrome (PCOS).

Design: Prospective interventional study.

Setting: Reproductive endocrinology clinic in a university hospital.

Patient(s): Five IR and five NIR patients with PCOS. The mean age of patients was 29.5 ± 4.8 years (range, 23–36 years).

Intervention: Metformin therapy, using 850 mg orally per day for 1 week.

Main Outcome Measure(s): Serum levels of T, FSH, and LH, fasting glucose/insulin ratio, levels of anti-Müllerian hormone (AMH) and inhibin B, and antral follicle count.

Result(s): Levels of AMH and inhibin B were statistically significantly higher in patients with PCOS compared to controls (2.81 ± 1.79 ng/mL versus 0.95 ± 1.17 ng/mL, and 56.24 ± 29.39 pg/mL versus 17.89 ± 12.87 pg/mL, respectively). Levels of AMH and inhibin B were similar among IR and NIR patients with PCOS (2.77 ± 1.92 ng/mL versus 2.85 ± 1.89 ng/mL, and 53.96 ± 28.58 pg/mL versus 58.51 ± 33.28 pg/mL, respectively). One week of metformin therapy did not alter either AMH or inhibin B levels. However, there was a statistically significant increase in glucose/insulin ratios (4.59 ± 1.57 versus 6.35 ± 3.6), and a significant decrease in the number of antral follicles after 1 week of metformin therapy (38.8 ± 19.3 versus 23.1 ± 7.4).

Conclusion(s): Levels of AMH and inhibin B are significantly increased in patients with PCOS compared to controls, but are not associated with insulin resistance. Low-dose metformin therapy improves IR and polycystic ovary morphology, even though levels of T, AMH, and inhibin B remain unchanged. (Fertil Steril 2007;87: 870–5. ©2007 by American Society for Reproductive Medicine.)

Key Words: Polycystic ovary syndrome (PCOS), anti-Müllerian hormone, inhibin B, antral follicle count, ovarian morphology

Polycystic ovary syndrome (PCOS) is the most common cause of anovulatory infertility, and affects 4%–6% of reproductive-age women (1). Polycystic ovary syndrome is a heterogeneous syndrome characterized by ovulatory dysfunction along with features of hyperandrogenism and polycystic ovarian morphology (2). Although the exact etiology of PCOS is unknown, it is widely accepted that insulin resistance (IR) and compensatory hyperinsulinemia play a major role in the pathophysiology of PCOS (3). It was reported that about 50%–70% of patients with PCOS have variable degrees of IR, which may explain the abnormal clinical and biochemical findings associated with this syndrome (4). Patients with IR represent a unique subgroup of PCOS that demonstrates clinical and biochemical characteristics that are different from noninsulin-resistant (NIR) patients with PCOS (3, 5, 6). In patients with PCOS, whether IR or hyperinsulinemia affects polycystic ovarian morphology is not well-documented.

Metformin, a biguanide drug, is frequently used in patients with noninsulin-dependent diabetes mellitus, and was recently utilized extensively in the treatment of patients with PCOS. A majority of studies demonstrated that metformin therapy reduced serum levels of insulin, T, and LH, improved the parameters of IR, and achieved ovulatory cycles in patients with PCOS (7–9). Although it could be assumed that these effects would be exclusive to those patients with PCOS who are obese and have IR, a recent study by Bailargeon et al. suggested that metformin therapy is also effective in reducing androgen levels and restoring ovulatory function in nonobese patients with PCOS with normal indices of insulin sensitivity (10).

In the treatment of patients with PCOS, the effects of metformin on clinical and biochemical parameters were reported based on administration of the drug at a dose of...
1,500–2,000 mg per day for a minimum duration of 4–6 weeks. This dosage was based on dose-response studies of type II diabetic patients (11, 12). Whereas these doses and durations have proven effective in inducing ovulatory cycles and improvements in biochemical markers of PCOS, the immediate response to short-course and low-dose metformin therapy has not been investigated. Furthermore, the acute effects of metformin on polycystic ovarian morphology have not been reported.

The objective in this study was to evaluate the acute effects of metformin therapy on biochemical markers of PCOS and polycystic ovarian morphology among IR and NIR patients with PCOS.

**MATERIALS AND METHODS**

In this prospective trial, we studied 5 insulin-resistant (IR) and 5 NIR patients with PCOS who were being evaluated for infertility at the Reproductive Endocrinology and Infertility Clinic at the Los Angeles County-University of Southern California Medical Center, Los Angeles, California. The mean (range) age in the population with PCOS was 29.5 ± 4.8 years (range, 23–36 years). This study was approved by the University of Southern California Health Sciences Institutional Review Board.

The diagnosis of PCOS was made on the basis of the following criteria: chronic oligoovulation (≤6 menstrual cycles per year), hyperandrogenemia (total T, ≥60 ng/dL), or clinical hyperandrogenism, as evidenced by hirsutism (Ferriman-Gallwey score, ≥8) and/or persistent acne, and exclusion of adrenal hyperplasia (17-OH P, <200 ng/dL), hyperprolactinemia (PRL, <25 ng/mL), and hypothyroidism (TSH, <3.5 µIU/mL). Hyperprolactinemia was ruled out by a PRL level of <25 ng/mL, and hypothyroidism by a TSH level of <3.5 µIU/mL. Patients receiving metformin or any ovulation-induction agents within the past 3 months were excluded from the study. As a control group, body mass index (BMI)-matched and age-matched patients who had regular menstrual cycles and no evidence of hyperandrogenism were included (n = 4).

Blood samples were obtained after an overnight fast for the measurement of levels of serum glucose (G), insulin (I), FSH, LH, anti-Müllerian hormone (AMH), inhibit B, and T. Patients were categorized into two subgroups, based on IR (fasting G/I, <4.5, and/or fasting I level, >20 µIU/mL). All patients who were found to be IR on initial evaluation were further evaluated with a glucose tolerance test; none of these patients had diabetes mellitus.

On the day of initial blood draw, the number of antral follicles in all patients was counted and recorded by a single ultrasonographer (A.B.) to limit interobserver variability; A.B. was blinded to the status of the patients’ IR. After this initial evaluation, all patients were administered 850 mg of metformin orally once a day for 1 week. All patients were followed by telephone calls by one of the investigators to ensure compliance with the medication. After 1 week of treatment, patients returned to the reproductive endocrinology clinic after an overnight fast for a repeat blood draw for hormone analysis and ultrasound examination. At time of follow-up evaluation, the ultrasonographer did not have access to the patients’ status of IR and the pretreatment antral follicle counts (AFCs).

Serum and plasma samples were analyzed for G and I immediately following separation from blood, and the remainder of the serum was stored at –20°C for later analysis. Levels of inhibit B and AMH were measured using ELISA kits (DSL, Webster, TX). Commercial immunoassay kits were used for the analysis of FSH, LH, and T (ACS-180; Bayer, Norwood, MA). All hormone analyses were batchmatched to minimize interassay variability.

There were three parts in the study, in which comparisons were made between patients with PCOS and controls, and between IR and NIR patients with PCOS. In the first part of the study, AMH and inhibit B levels were compared between patients with PCOS and controls at baseline. Parameters of IR, gonadotropins, and androgens were not measured in controls and not compared to patients with PCOS because these changes are already extensively established in the medical literature. In the second part of the study, levels of fasting G, fasting I, G/I ratio, FSH, LH, LH/FSH ratio, T, endometrial thickness, AFC, AMH, and inhibit B were compared between IR and NIR patients with PCOS at baseline. In the third part of the study, the acute effects of metformin were assessed after 1 week of treatment in patients with PCOS.

Continuous data were analyzed using the paired *t*-test. Pearson’s correlation analysis was used when applicable. All statistical calculations were performed with SPSS software (SPSS, Chicago, IL).

**RESULTS**

Patient characteristics at baseline are summarized in Table 1. Patients were, on average, obese, with a mean BMI of 30.2 ± 4.5 kg/m², and they had high LH and T levels at baseline. Mean fasting I level was elevated, with a normal endometrial thickness at baseline. Anti-Müllerian hormone and inhibit B were detectable in the serum of all patients with PCOS, as well as controls. Most patients had polycystic-appearing ovaries, with a mean AFC of 38.8 ± 19.3 (range, 14–71).

In the first part of the study, when AMH and inhibit B levels were compared between patients with PCOS and controls at baseline, we found that both hormone levels were statistically significantly elevated in patients with PCOS (2.81 ± 1.79 ng/mL versus 0.95 ± 1.17 ng/mL, *P* < .04, and 56.24 ± 29.39 pg/mL versus 17.89 ± 12.87 pg/mL, *P* < .01, respectively).

In the second part of the study, when the biochemical markers of PCOS were compared between IR and NIR
patients with PCOS at baseline, we found that IR patients were significantly more obese than NIR patients with PCOS, as shown in Table 2. As expected, IR patients with PCOS had higher mean fasting I and lower G/I ratios by definition. Insulin-resistant patients with PCOS, compared to NIR patients with PCOS, had significantly higher mean T levels (76 ± 17.2 ng/dL versus 53.2 ± 14.06 ng/dL, P < .02) and AFCs (50.75 ± 19.26 versus 29.4 ± 14.7, P < .04). Levels of AMH and inhibin B were similar between IR and NIR patients with PCOS. Furthermore, AMH and inhibin B did not correlate with fasting G, fasting I, G/I ratio, FSH, LH, T, endometrial thickness, or AFC (data not shown). However, a significant positive correlation was found between BMI and T levels (r = 0.702, P < .02, Fig. 1). Additionally, the number of antral follicles positively correlated with fasting G levels (r = 0.859, P < .003, data not shown), and negatively correlated with fasting G/I ratios (r = −0.770, P < .01, Fig. 2).

In the third part of the study, when acute effects of metformin therapy were assessed in patients with PCOS, there were no statistically significant differences in mean levels of FSH (5.56 ± 2.06 IU/L versus 5.53 ± 1.06 IU/L, P = .9), LH (9.42 ± 4.61 IU/L versus 9.11 ± 3.9 IU/L, P = .7), LH/FSH ratio (1.73 ± 0.73 versus 1.62 ± 0.69, P = .5), T (64.6 ± 19.1 ng/dL versus 58.1 ± 14.2 ng/dL, P = .1), or endometrial thickness (6.9 ± 2.2 mm versus 6.7 ± 1.4 mm,

### Table 1

**Clinical and endocrine characteristics of women with PCOS at baseline.**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean ± SD (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>29.5 ± 4.8 (23–36)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.2 ± 4.5 (23.4–36)</td>
</tr>
<tr>
<td>FSH (IU/L)</td>
<td>5.56 ± 2.06 (1.6–8.1)</td>
</tr>
<tr>
<td>LH (IU/L)</td>
<td>9.42 ± 4.61 (2.5–15.7)</td>
</tr>
<tr>
<td>LH/FSH ratio</td>
<td>1.73 ± 0.73 (0.93–3.34)</td>
</tr>
<tr>
<td>T (ng/dL)</td>
<td>64.6 ± 19.1 (32–95)</td>
</tr>
<tr>
<td>AMH (ng/mL)</td>
<td>2.81 ± 1.79 (0.58–6.05)</td>
</tr>
<tr>
<td>Inhibin B (pg/mL)</td>
<td>56.24 ± 29.39 (20.48–105.67)</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>84.7 ± 16.8 (58–113)</td>
</tr>
<tr>
<td>Insulin (µIU/mL)</td>
<td>22.1 ± 14.3 (8–57)</td>
</tr>
<tr>
<td>Endometrial thickness (mm)</td>
<td>6.9 ± 2.2 (4.3–11.6)</td>
</tr>
<tr>
<td>AFC</td>
<td>38.8 ± 19.3 (14–71)</td>
</tr>
</tbody>
</table>

### Table 2

**Comparison of patient characteristics and hormone analysis at baseline in IR and NIR patients with PCOS.**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>IR patients with PCOS</th>
<th>NIR patients with PCOS</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>33.52 ± 1.78</td>
<td>27.04 ± 4.12</td>
<td>.006</td>
</tr>
<tr>
<td>Fasting G (mg/dL)</td>
<td>92 ± 16.4</td>
<td>77.4 ± 14.69</td>
<td>.08</td>
</tr>
<tr>
<td>Fasting I (µIU/mL)</td>
<td>30.2 ± 16.46</td>
<td>14 ± 4.74</td>
<td>.03</td>
</tr>
<tr>
<td>G/I ratio</td>
<td>3.42 ± 1.1</td>
<td>5.75 ± 0.98</td>
<td>.003</td>
</tr>
<tr>
<td>FSH (IU/L)</td>
<td>5.96 ± 1.59</td>
<td>5.16 ± 2.56</td>
<td>.28</td>
</tr>
<tr>
<td>LH (IU/L)</td>
<td>9.1 ± 4.7</td>
<td>9.7 ± 5.06</td>
<td>.42</td>
</tr>
<tr>
<td>LH/FSH ratio</td>
<td>1.46 ± 0.46</td>
<td>1.47 ± 0.57</td>
<td>.26</td>
</tr>
<tr>
<td>T (ng/dL)</td>
<td>76 ± 17.2</td>
<td>53.2 ± 14.06</td>
<td>.02</td>
</tr>
<tr>
<td>AMH (ng/mL)</td>
<td>2.77 ± 1.92</td>
<td>2.85 ± 1.89</td>
<td>.47</td>
</tr>
<tr>
<td>Inhibin B (pg/mL)</td>
<td>53.96 ± 28.58</td>
<td>58.51 ± 33.28</td>
<td>.41</td>
</tr>
<tr>
<td>Endometrial thickness (mm)</td>
<td>5.96 ± 0.82</td>
<td>7.87 ± 2.92</td>
<td>.10</td>
</tr>
<tr>
<td>AFC</td>
<td>50.75 ± 19.26</td>
<td>29.4 ± 14.7</td>
<td>.04</td>
</tr>
</tbody>
</table>
Levels of AMH and inhibin B were also not altered by 1 week of metformin therapy (2.82 ± 1.8 ng/mL versus 2.92 ± 1.6 ng/mL, P = .7, and 56.24 ± 29.34 pg/mL versus 52.41 ± 23.85 pg/mL, P = .2, respectively). However, a significant increase in G/I ratios after 1 week of metformin therapy was observed, as shown in Figure 3A (4.59 ± 1.57 versus 6.35 ± 3.6, P < .05). Furthermore, there was a statistically significant decrease in mean AFC after metformin therapy (38.8 ± 19.3 versus 23.1 ± 7.4, P < .005), as shown in Figure 3B.

DISCUSSION

In the current study, we assessed specific clinical and biochemical parameters associated with PCOS, including AMH and inhibin B levels and polycystic ovarian morphology, as determined by AFC. We compared these parameters among IR and NIR patients with PCOS and controls at baseline, and after a short course (1 week) of a relatively low dose (850 mg) of metformin therapy.

Patients in our study constituted an obese PCOS population, with most patients exhibiting polycystic-appearing ovaries characterized by an increased AFC at baseline ultrasonography. Levels of LH and T were elevated in most patients with PCOS. Levels of AMH and inhibin B were detectable in all patients with PCOS and controls.

Anti-Müllerian hormone plays a regulatory role in folliculogenesis (13–15), and recent observations indicate that it may be considered a novel marker for ovarian aging and reserves (16, 17). It has been suggested that AMH is a strong predictor of ovarian follicular status and response to controlled ovarian hyperstimulation (18, 19). Whether there is an association between AMH and IR in patients with PCOS is controversial (20, 21).

Our findings indicate that patients with PCOS have significantly higher levels of AMH compared to controls, in agreement with previous reports (22–24). However, when we compared AMH levels among IR patients with PCOS and NIR patients with PCOS, we did not find a significant difference between the two subgroups. We also did not observe a correlation between AMH levels and fasting I levels or G/I ratios. Anti-Müllerian hormone is thought to be primarily secreted by the preantral and early antral follicles (25, 26), and elevated levels of AMH in patients with PCOS are attributed to an increased number of antral follicles (polycystic ovaries).

In our study, IR patients with PCOS, who had a significantly greater number of antral follicles compared to NIR patients with PCOS, actually had similar AMH levels. Furthermore, we did not find any association between AMH levels and AFC. This is further substantiated by the fact that, even though the mean AFC decreased following a short course of metformin treatment, there was no significant decrease in AMH levels. Therefore, there may be other factors that play a role in the increased levels of AMH in patients with PCOS, such as the pool of preantral follicles.

Inhibin B selectively suppresses FSH in the pituitary gland, and its levels are maximal in the early to midfollicular
phase (27). Controversy exists regarding inhibin B levels in patients with PCOS compared to controls. Furthermore, there are limited data on whether inhibin B levels are influenced by IR or hyperinsulinemia (28).

Based on our data, levels of serum inhibin B in patients with PCOS are significantly higher than in controls, in agreement with previous reports (29, 30), and in contrast to others (31–35). Inhibin B levels were similar among IR and NIR patients with PCOS, and we also did not find an association with IR. In addition, inhibin B levels did not correlate with AFC, and were similar in patients with significantly higher AFCs, such as IR patients with PCOS. It was previously reported that inhibin B levels are similar in size-matched antral follicles from women with PCOS and controls (36). Whether an increased number of antral follicles influences inhibin B levels remains a subject of debate.

In the second part of the study, when IR and NIR patients with PCOS were compared by clinical and biochemical parameters at baseline, IR patients were found to be more obese, with higher T levels and a significantly higher mean AFC. Additionally, there was a positive correlation between BMI and T levels, which may explain the elevated levels of T in patients with IR who were significantly more obese than NIR patients.

Interestingly, IR patients with PCOS had significantly higher AFCs compared to NIR patients with PCOS. This may be secondary to the degree of the IR, which may influence polycystic ovarian morphology. This statement is supported by our data in a significant positive correlation between G levels and AFC. Additionally, a significant negative correlation was found between G/I ratios and AFC. Therefore, based on our data, IR has a significant impact on polycystic ovarian morphology.

Our data indicate that the immediate effects of metformin can be observed by a low dose and short course of treatment. These effects include an improvement in IR, demonstrated by an increase in G/I ratios and a decrease in T levels. Whereas levels of AMH and inhibin B were elevated in patients with PCOS compared to controls, these levels were unchanged following a short course of metformin therapy.

An intriguing finding in our study was a significant decrease in AFC following a short course of metformin treatment. Whether this effect was due to an improvement in IR is unclear, but plausible. Therefore, a lower dose of metformin may be sufficient to achieve improvements in biochemical markers and polycystic ovarian morphology. The effects of this dose in achieving ovulatory cycles and pregnancy have not yet been studied. We speculate that these observed changes may provide a mechanism for the previously observed improvement in response to ovulation-inducing agents.

The number of subjects in this study was small because this was a preliminary study to determine the effects of metformin therapy used for a short period at a lower dose. Larger-scale studies are needed not only to confirm our findings, but also to define the lowest dose of metformin that could achieve biochemical and clinically significant outcomes.

In conclusion, levels of AMH and inhibin B are increased in patients with PCOS compared to controls, but are not associated with IR. Insulin resistance positively correlates with severity of polycystic ovarian morphology in patients with PCOS. Although a short course and a low dose of metformin therapy do not alter levels of AMH or inhibit B, they do result in significant improvement in IR and polycystic ovarian morphology.

REFERENCES


