**Metformin administration improves leukocyte count in women with polycystic ovary syndrome: a 6-month prospective study**

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**Abstract**

**Introduction:** Polycystic ovary syndrome (PCOS) is a common disorder associated with a wide range of endocrine and metabolic abnormalities. Low-grade chronic inflammation is a related complication recently observed in PCOS. Increased white blood cell (WBC) count was previously reported in PCOS women.

**Objective:** To evaluate the effects of six months metformin administration on WBC count in PCOS women.

**Patients and methods:** Fifty normal-weight PCOS women without additional metabolic or cardiovascular diseases were enrolled and treated with metformin (850 mg twice daily) for 6 months in a prospective baseline-controlled clinical study. At baseline and after treatment, WBC count and C-reactive protein (CRP) were evaluated in each patient. The whole hormonal profile, serum insulin and glucose levels (at fasting and during a 75 g 2-h oral glucose tolerance test), serum lipid profile were also assessed.

**Results:** A significant difference was observed in WBC count (7050 ± 552 vs 6080 ± 577 cell/mm\(^3\)) and CRP levels (1.8 ± 0.9 vs 1.1 ± 0.6 mg/l) after metformin treatment in comparison with baseline values. SHBG levels and the free androgen index also changed significantly. Finally, high-density lipoproteins and the area under curve for glucose/area under curve for insulin ratio also significantly increased, whereas low-density lipoproteins and area under curve for insulin were significantly reduced. No other change was found in any of the biochemical parameters evaluated.

**Conclusion:** A six-month course of metformin reduces WBC count in PCOS women.
Based on these considerations, the present study was carried out to evaluate the effects of metformin administration on WBC count in PCOS patients.

Patients and methods

The institutional review board of University ‘Federico II’ of Naples approved the study. The purpose of the protocol was explained to each subject and written consent was obtained from each before beginning the study.

Patients

Fifty normal-weight women with PCOS were screened from the patient population of the Department of Molecular and Clinical Endocrinology and Oncology in Naples. The diagnosis of PCOS was made according to the Rotterdam criteria (26). Specifically, patients with anovulation and clinical and/or biochemical hyperandrogenism were enrolled.

Exclusion criteria included: age < 18 or > 35 years, pregnancy, hypothyroidism, hyperprolactinemia, Cushing’s syndrome, nonclassical congenital adrenal hyperplasia, and use of oral contraceptives, glucocorticoids, anti-androgens, ovulation induction agents, anti-diabetic or anti-obesity drugs, or any other hormonal drugs within the previous 6 months. Subjects with neoplastic, metabolic (including glucose intolerance), hepatic, inflammatory, cardiovascular, and hematological disorder or other concurrent medical illness (i.e. diabetes, renal disease, and malabsorptive disorders) were also excluded from the study. All subjects were nonsmokers and had normal physical activity, and none drank alcoholic beverages.

Treatment

All subjects received metformin (Glucophage, Merck) at a dosage of 850 mg twice daily for 6 months, as previously described (27). In addition, standard clinical evaluations and laboratory analyses, including hematological, renal function, and liver function tests, were performed at baseline and after 3 and 6 months of treatment as safety measures.

Throughout the study, no changes in lifestyle were implemented, and subjects were instructed to follow their usual diet and physical activity and to use barrier contraceptives.

After the treatment period, in each patient all of the above parameters were reevaluated as at baseline.

Methods

At study entry, all subjects underwent blood sampling for WBC count, hormonal assessment, lipid profile, and fasting glucose and insulin levels. All blood samples were obtained in the morning between 0800 and 0900 h after an overnight fast during the early follicular phase (second to fourth day) of a spontaneous or progesterone-induced menstrual cycle. Blood samples were collected into tubes containing EDTA after a 30-min resting period in the supine position. Each subject underwent an oral glucose tolerance test for which they received 75 g glucose orally, and blood samples were obtained before and at 30-min intervals for 2 h (at 0, 30, 60, 90, and 120 min). All blood samples were immediately centrifuged at 4 °C for 20 min at 1600 g and stored at −20 °C until assayed.

During the same visit, all subjects underwent transvaginal ultrasonography (TV-USG); anthropometric measurements (including height, weight, body mass index (BMI, ratio between the weight and the square of the height), and waist-to-hip ratio (WHR, ratio between the smallest circumference at the torso and the widest circumference at the hip)); evaluation of heart rate, and diastolic and systolic blood pressures; and assessments of daily physical activity at their job and at home using a well-validated semi-quantitative questionnaire (15, 16).

The estimate of IR by the homeostasis model assessment (HOMA) score [fasting serum insulin (µU/ml) × fasting plasma glucose (mmol/l)/22.5] was calculated in all subjects. Plasma luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin (PRL), E2, P, 17-OHP, T, DHEA-S, and androstenedione levels were measured by specific radioimmunoassays, as previously described (15, 16). SHBG levels were measured using an IRMA (15, 16), and the free androgen index (FAI) was calculated (T (nmol/l)/SHBG (nmol/l)×100). Blood insulin and glucose levels were measured by a solid-phase chemiluminescent enzyme immunoassay and the glucose oxidase method respectively (15, 16). The glucose and insulin areas under curve (AUCs) and the AUCglucose/AUCinsulin ratio (28) in response to the oral glucose tolerance test were calculated. The lipid profile consisted of serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) levels (9, 10).

As previously detailed (5), leukocyte count was determined within 2 h after venepuncture with an automatic workstation cell-counter (Technicon H3; Bayer Diagnostics).

Statistical analysis

Continuous data are expressed as mean ± S.D. Clinical and biochemical data were compared before and after treatment using the general linear model repeated measures procedure. A value of \( P < 0.05 \) was considered statistically significant. All analyses were run using SPSS 15.0.0 (SPSS Inc., Chicago, IL, USA).
Table 1 Clinical and hormonal profile in polycystic ovary syndrome before and after metformin administration.

<table>
<thead>
<tr>
<th></th>
<th>Baseline (n = 50)</th>
<th>After treatment (n = 50)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28.5 ± 3.1</td>
<td>29.1 ± 3.2</td>
<td>0.34</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.6 ± 4.2</td>
<td>24.5 ± 4.5</td>
<td>0.82</td>
</tr>
<tr>
<td>WHR</td>
<td>0.85 ± 0.05</td>
<td>0.84 ± 0.3</td>
<td>0.90</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>85.2 ± 2.5</td>
<td>84.8 ± 2.3</td>
<td>0.40</td>
</tr>
<tr>
<td>Ferriman–Gallwey score</td>
<td>12 ± 3.2</td>
<td>11 ± 3.8</td>
<td>0.16</td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>10.3 ± 1.6</td>
<td>10.1 ± 1.4</td>
<td>0.50</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>25.2 ± 3.9</td>
<td>24.6 ± 3.5</td>
<td>0.41</td>
</tr>
<tr>
<td>PRL (ng/ml)</td>
<td>10.1 ± 1.1</td>
<td>9.8 ± 1.2</td>
<td>0.19</td>
</tr>
<tr>
<td>E₂ (pmol/l)</td>
<td>119 ± 31</td>
<td>115 ± 29</td>
<td>0.50</td>
</tr>
<tr>
<td>P (nmol/l)</td>
<td>1.3 ± 0.6</td>
<td>1.5 ± 0.8</td>
<td>0.16</td>
</tr>
<tr>
<td>17-OHP (nmol/l)</td>
<td>1.8 ± 0.5</td>
<td>1.7 ± 0.6</td>
<td>0.36</td>
</tr>
<tr>
<td>T (nmol/l)</td>
<td>2.8 ± 0.5</td>
<td>2.6 ± 0.8</td>
<td>0.13</td>
</tr>
<tr>
<td>A (nmol/l)</td>
<td>5.1 ± 0.8</td>
<td>4.8 ± 0.9</td>
<td>0.08</td>
</tr>
<tr>
<td>DHEAS (μmol/l)</td>
<td>4310 ± 545</td>
<td>4290 ± 510</td>
<td>0.85</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>26.1 ± 5.4</td>
<td>37.4 ± 5.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FAI</td>
<td>9.8 ± 2.3</td>
<td>6.7 ± 1.6</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

See text for key to abbreviations. Data expressed as mean ± S.D.

Results

The patients’ characteristics and hormonal profiles at baseline and after treatment are presented in Table 1. At study entry, the mean age of the PCOS patients was 22.8 ± 3.1 years. Forty (80%) PCOS patients showed polycystic ovaries at TV-USG. 45 (90%) had chronic anovulation, 47 (94%) and 42 (84%) showed clinical and biochemical hyperandrogenism respectively.

No patient dropped out of the study. The treatment was well tolerated and only five patients reported adverse experiences, primarily gastrointestinal discomfort, which spontaneously disappeared after the first 3 weeks of treatment.

After 6 months of metformin administration, 41 out 45 patients (91.1%) demonstrated normal ovulatory menstrual cycles. SHBG and FAI significantly increased and decreased respectively (P < 0.001). No other significant change in hormonal levels was observed when compared with baseline values (Table 1).

Table 2 shows the metabolic and inflammatory profile of subjects before and after treatment.

WBC count was significantly reduced (P < 0.001) after metformin treatment with a net change of −9.70 ± 2.97. In particular lymphocytes (change of −0.27 ± 0.11) and monocytes (change of −0.1 ± 0.06) were significantly reduced (P < 0.05); while no difference before and after therapy was observed in neutrophiles and basophiles and in neutrophiles to lymphocytes (N:L) ratio. The percentage variation of WBC count, leukocytes, and monocytes after six months of metformin treatment were: −13.78 ± 4.710, −12.68 ± 5.486, and −25.0 ± 19.5 respectively. In addition, six months of metformin therapy significantly reduced C-reactive protein (CRP) levels (P < 0.001) (Table 2).

Table 2 Metabolic and inflammatory profile in polycystic ovary syndrome before and after metformin administration.

<table>
<thead>
<tr>
<th></th>
<th>Baseline (n = 50)</th>
<th>After treatment (n = 50)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>93 ± 8.2</td>
<td>92 ± 6.8</td>
<td>0.99</td>
</tr>
<tr>
<td>Fasting insulin (μU/ml)</td>
<td>21.5 ± 4.2</td>
<td>12.2 ± 2.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA (μU/ml)</td>
<td>4.8 ± 1.3</td>
<td>2.6 ± 0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AUCGLU</td>
<td>12123 ± 2360</td>
<td>11 760 ± 1852</td>
<td>0.39</td>
</tr>
<tr>
<td>AUCINS</td>
<td>15 956 ± 850</td>
<td>4620 ± 1132</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AUCGLU/AUCINS ratio</td>
<td>0.76 ± 0.3</td>
<td>2.5 ± 0.45</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>155 ± 8.2</td>
<td>152 ± 7.5</td>
<td>0.06</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>87.2 ± 7.6</td>
<td>81.6 ± 5.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>45.4 ± 4.2</td>
<td>48.4 ± 4.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>114 ± 21</td>
<td>112 ± 26</td>
<td>0.67</td>
</tr>
<tr>
<td>WBC count (cell/mm³)</td>
<td>7050 ± 562</td>
<td>6080 ± 577</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Neutrophiles (×1000 μl)</td>
<td>4.4 ± 1.6</td>
<td>4.2 ± 1.2</td>
<td>0.48</td>
</tr>
<tr>
<td>Lymphocytes (×1000 μl)</td>
<td>2.2 ± 0.3</td>
<td>1.9 ± 0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Monocytes (×1000 μl)</td>
<td>0.5 ± 0.2</td>
<td>0.4 ± 0.2</td>
<td>0.001</td>
</tr>
<tr>
<td>Basophiles (×1000 μl)</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>1.00</td>
</tr>
<tr>
<td>N:L ratio</td>
<td>2.0 ± 1.3</td>
<td>2.2 ± 1.2</td>
<td>0.42</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>1.8 ± 0.9</td>
<td>1.1 ± 0.6</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± S.D.

Discussion

Low-grade chronic inflammation is a recently discovered feature related to PCOS (5, 24, 29). This appears as a further health consequence of PCOS, which is now recognized a multi-faceted disease (4).

This is the first study aimed at demonstrating a reduction of WBC count in PCOS women after six months of metformin treatment and that suggests an important role for metformin as a first-line therapeutic strategy for improving the chronic inflammatory state in PCOS. The PCOS-related low-grade chronic inflammation has been linked to IR and early development of atherosclerosis (5, 16, 30). As previously reported (24, 28), metformin therapy is able to reduce not only endothelial dysfunction and the intima-media thickness (27), but also CRP (31) and the levels of plasma inflammatory indices (32) in PCOS women.
The positive consequences on the CVR (33) and the anti-inflammatory property (34) of an insulin sensitizing drug, such as metformin, are extensively well known (33, 34). In fact, metformin suppresses plasma migration inhibitor factor, suggesting an anti-inflammatory effect of this drug (35). Furthermore, this effect of metformin may contribute to a potential antiatherogenic action, which may have implications for the reduced cardiovascular mortality observed with metformin therapy in type-2 diabetes mellitus (35).

Previously, Ibanez et al. (24) clearly demonstrated that a high leukocyte count was already present in girls with hyperinsulinemic hyperandrogenism, and metformin therapy decreased leukocyte count and N:L ratio (24). In addition, an increased leukocyte count was also demonstrated in a small-for-gestational age children (25) and this was due to a raised neutrophil count. Therefore, this hyperneutrophilia was attenuated by metformin therapy (24, 25). These two studies were performed in two different populations. In the first, children with a mean age of 12.5 years were evaluated (24), while the latter included prepubertal girls with a mean age of 8.0 years who had a low weight at term birth (25).

Lymphocytes and monocytes can be considered the most typical cells involved in the chronic low-grade inflammation (36) directly associated with increased incidence of coronary heart disease, ischemic stroke, and mortality from CVD (8). Thus, here we demonstrate for the first time a positive effect of metformin on the low-grade chronic inflammation, both leukocyte counts and CRP in PCOS women. The decrease in serum CRP levels during metformin therapy is in accordance with the known beneficial metabolic effects of this drug and suggests that CRP or other inflammatory parameters could be used as markers for the efficiency of therapy in PCOS (37). However, our group recently demonstrated that CRP levels also improved after a 3-month well-structured exercise training program in PCOS patients, showing the beneficial effects of a nonpharmacological therapeutic strategy (18). Further studies have been scheduled in order to evaluate the effects of a regular exercise training program on the leukocytes and other inflammatory markers.

As already shown (5), the WBC increase was not related to BMI and, therefore, we can speculate that metformin therapy can also be useful in nonobese PCOS women, clearly improving cardiovascular and inflammatory pattern.

Also in the present study lipid profile improved after metformin therapy and these data partially agree with Rautio et al. (37) who demonstrated beneficial effects not only for HDL levels but also for TC and TG; however, other contrasting evidences were reported on the same issue, showing some improvement (38) or no difference (39) in lipid profile after metformin therapy in PCOS women.

Conclusions

The results of the present work demonstrate that a six-month course of metformin improves WBC count in PCOS women. Further studies evaluating the other insulin-sensitizing drugs on low-grade chronic inflammation should be advised for choosing the most efficacious and safe treatment in PCOS women and for preventing any event or sign of early CVR.

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Metformin and leukocyte count in polycystic ovary syndrome


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