Metformin effects on ovarian ultrasound appearance and steroidogenic function in normal-weight normoinsulinemic women with polycystic ovary syndrome: a randomized double-blind placebo-controlled clinical trial

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Objective: To investigate metformin effects on the endocrine-metabolic parameters and ovarian morphology in normoinsulinemic women with polycystic ovary syndrome (PCOS).
Design: Randomized double-blind study.
Setting: Operative Division of Endocrinological Gynecology, Università Cattolica del Sacro Cuore.
Patient(s): Twenty-eight normal-weight normoinsulinemic PCOS women.
Intervention(s): Patients were randomized to receive metformin 500 mg twice a day (group A, 15 subjects) or placebo (group B, 13 subjects) for 6 months. Ultrasonographic pelvic exams, hormonal and lipid features, and oral glucose tolerance test were performed at baseline and after 3 and 6 months of treatment.
Main Outcome Measure(s): Hormonal and glycoinsulinemic assessment, ovarian ultrasound appearance.
Result(s): Glycoinsulinemic assessment remained unvaried in both groups. About 70% of patients in group A experienced a restoration of menstrual cyclicity. Metformin significantly decreased testosterone levels at 3 and 6 months) and 17-hydroxyprogesterone levels at 6 months, and improved hirsutism score at 6 months. No clinical or hormonal modifications occurred in group B. Metformin, but not placebo, reduced ovarian volume and stromal/total area ratio at 3 and 6 months.
Conclusion(s): Metformin seems to improve the menstrual pattern and ultrasonographic ovarian features in normoinsulinemic PCOS women. These effects seem to be, at least in part, independent of the insulin-lowering properties of the drug. (Fertil Steril® 2010;93:2303–10. ©2010 by American Society for Reproductive Medicine.)

Key Words: Metformin, PCOS, ovaries, normoinsulinemic, normal-weight

Polycystic ovary syndrome (PCOS) is a common endocrine-metabolic disorder occurring in ~5%–10% of women in reproductive age. Hyperandrogenism, chronic anovulation, and infertility are the main features of this heterogeneous condition (1). Although the pathogenesis of the syndrome is still unclear, several authors have suggested that insulin resistance, hyperinsulinemia, and obesity, which affect most PCOS patients, may play a pivotal role (2). The increased insulin circulating concentrations, indeed, seem to contribute to the etiology of hyperandrogenism by acting at several levels of the hypothalamic-pituitary-ovarian axis as well as on the hepatic production of sex hormone–binding globulin (SHBG). Actually, at ovarian level, insulin promotes androgen secretion by playing a synergistic role with gonadotropins both directly and by stimulating insulin-like growth factor I (IGF-I) secretion (3); in the liver, it decreases serum SHBG synthesis (4).

It is well known that there is a close relationship between elevated androgen plasma levels and the ultrasound finding of stromal hypertrophy (5). In particular, in previous studies, we demonstrated that the polycystic ovary is characterized by a higher stromal area/total area (S/A) ratio compared with multifollicular and normal ovaries, thus suggesting that an augmented ovarian stroma could represent the morphologic expression of increased androgen synthesis (6–7).

The evidence that the main metabolic imbalance of PCOS is represented by insulin resistance has led to a growing interest in insulin-sensitizing drugs as the preferable therapeutic approach to the syndrome. Metformin is a drug belonging

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to the biguanide class and, previously used in obese patients with type 2 diabetes mellitus, has been recently proposed as a first-line treatment in obese or overweight PCOS women with hyperinsulinemia. In randomized trials, the administration of metformin was followed by an improvement in insulin sensitivity, body mass index (BMI), and menstrual pattern and a decrease of androgen levels in most women under treatment (8).

Based on the favorable profile of action in obese PCOS patients, the effects of metformin were also investigated in normal-weight patients affected by the syndrome: most studies documented a significant decrease in fasting insulin and androgen levels, as well as a restoration of menstrual cyclicity (9–10).

Because in normoinsulinemic women the beneficial effect on the hormonal pattern was observed even after minimal metabolic changes (11), it could be hypothesized that metformin may also modulate the ovarian androgen discharge through an insulin-independent route of action on the gonad.

The possible clinical impact of such a mechanism on the reproductive function and, especially, the consequences on the therapeutic strategy were never previously evaluated in literature.

In the present study, we sought to investigate the effect, if any, of metformin administration in normal-weight PCOS women on the clinical-endocrine parameters and on the ultrasound appearance of the ovary, and the possible correlation between these two aspects.

MATERIALS AND METHODS

Patients

We enrolled 28 normal-weight women with PCOS (BMI 22.4 $\pm$ 3.9 kg/m^2, age range 19–32 yrs) attending our divisional outpatient services. All women had spontaneous onset of puberty and normal sexual development, and all had oligomenorrhea with chronic anovulation variably associated with mild to moderate hirsutism. All of the women were euthyroid, and none had taken medications known to affect plasma sex steroids for at least 3 months before the study. In accordance with the Rotterdam Consensus criteria (12), PCOS was diagnosed in the presence of at least two of the three following clinical findings: chronic anovulation, clinical and/or biochemical evidence of hyperandrogenism, and ultrasonographic appearance of polycystic ovaries. The menstrual patterns were defined according to van Hooff et al. (13). A normal LH/FSH ratio was not considered to be an exclusion criterion (7). The presence of a late-onset adrenal enzyme defect was excluded by an ACTH test (250 μg IV Synachten; Ciba-Geigy, Basel, Switzerland) according to published criteria of New et al. (14). Significant liver (aspartate aminotransferase, alanine aminotransferase, total bilirubin, or alkaline phosphatase >2 times the upper limit of normal) or renal (serum creatinine >1.8 ng/dL) impairment, neoplasm, cardiovascular disease, diabetes mellitus, and unstable mental illness were considered to be exclusion criteria.

Informed consent was obtained from each patient, and the study protocol was approved by our Institutional Review Board.

Because of the impact of body fat distribution on androgen levels and glucose metabolism (15–16), waist-to-hip ratios (WHRs) were measured. Waist circumference was determined as the minimum value between the iliac crest and the lateral costal margin, whereas hip circumference was determined as the maximum value over the buttocks. Cut-off point for high WHR for women was set at 0.80 (17).

The grade of hirsutism was established using the Ferriman-Gallwey (FG) score (18), in which hair growth in each of 11 androgen-sensitive zones is graded from 0 (none) to 4 (frankly virile). On the basis of this method, four hirsutism levels were identified: score <8, no hirsutism; score of 8–16, low hirsutism; score of 17–25, moderate hirsutism; and score >25, severe hirsutism. We considered the change from one level to a lower one to be clinically meaningful.

The ratio of testosterone (T)/SHBG $\times$ 100 was used to calculate the free androgen index (FAI).

At baseline, during the early follicular phase of a spontaneous or induced (medroxyprogesterone acetate, 10 mg/d for 7 days) menstrual cycle (day 3–7), the patients were hospitalized and underwent gynecologic and medical examinations. The same day, a transvaginal pelvic ultrasoundwas performed on each patient using a 6.5 MHz endovaginal probe (AUC5; Esaote, Genova, Italy). The ultrasound examinations were performed by one of three well trained observers who were not aware of the patient's endocrine profiles.

Ovarian volume was calculated for each ovary using the formula for a prolate ellipsoid: (π/6 $\times$ (D1 $\times$ D2 $\times$ D3), where D1–D3 represent the maximum diameter in the transverse, anteroposterior, and longitudinal axes) (19).

The S/A ratio was calculated as ovarian stromal area, evaluated by outlining with the caliper the peripheral profile of the stroma identified by a central area slightly hyperechoic, with respect to the total area of the ovary evaluated by outlining with the caliper the external limits of the ovary in the maximum plane section.

The mean ovarian volume, area, stroma, and S/A ratio for each individual patient were calculated by adding the values for each ovary and dividing by 2. For the echographic diagnosis of PCOS, we adapted the criteria of Adams to the quantitative evaluation of stroma: a threshold value for S/A ratio of 0.34 (7) associated with $\geq$10 follicles with a diameter of 2–8 mm, arranged around an echodense central stroma (20), has been considered to be positive for PCO.

After following a standard carbohydrate diet (300 g/d) for 3 days and fasting overnight for 10–12 hours, blood samples were collected to perform the basal hormone assessment (T, DHEAS, A, 17OH-P, P, FSH, LH, SHBG, PRL, cortisol,