

Co-administration of metformin during rFSH treatment in patients with clomiphene citrate-resistant polycystic ovarian syndrome: a prospective randomized trial

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BACKGROUND: This study aims to evaluate the impact of metformin on ovarian response when co-administered during recombinant (r)FSH using the low-dose step-up protocol in clomiphene citrate-resistant polycystic ovarian syndrome (PCOS) patients with normal glucose tolerance. **METHODS AND RESULTS:** Thirty-two patients were randomized to metformin ($n = 16$) and placebo ($n = 16$) groups. Hormonal assessment, a 75 g oral glucose tolerance test (OGTT) and a frequently sampled i.v. glucose tolerance test (FSIGTT) were performed before and after oral administration of metformin (850 mg twice daily) or placebo for 6 weeks. Recombinant FSH treatment was undertaken, thereafter, in women who did not ovulate on metformin ($n = 10$) or placebo ($n = 15$). There was no significant change in all insulin sensitivity indices in both groups. The only change noted was a decline in mean serum free testosterone concentration in the metformin group ($P = 0.049$). One patient on placebo and six patients on metformin ovulated spontaneously ($P < 0.05$). All parameters of ovarian response were comparable between the two groups during rFSH treatment. Combining the 6 week placebo or metformin-only period with a single rFSH treatment cycle, the overall ovulation rates were 75 and 94% in the placebo and metformin groups respectively ($P > 0.05$). The respective figures for pregnancy were 6.3 and 31.3% ($P > 0.05$). **CONCLUSIONS:** Metformin may restore ovulation with no improvement on insulin resistance in clomiphene citrate-resistant PCOS patients with normal glucose tolerance, but has no significant effect on ovarian response during rFSH treatment.

Key words: insulin resistance/low-dose step-up protocol/metformin/polycystic ovary syndrome/rFSH

Introduction

Polycystic ovarian syndrome (PCOS) is the most common reproductive endocrinopathy, affecting ~5% of the population of reproductive-aged women and is a major cause of infertility (Knochenhauer *et al.*, 1998). Insulin resistance and consequent hyperinsulinaemia, independent of obesity, are considered to be among the most prominent features of PCOS (Dunaif, 1987, 1997; Franks *et al.*, 1997). Insulin resistance is a primary aetiological factor precipitating a broad spectrum of endocrine abnormalities characteristic of PCOS. There is ample evidence that hyperinsulinaemia results in increased ovarian androgen biosynthesis *in vivo* and *in vitro* (Adashi *et al.*, 1985; Barbieri *et al.*, 1986) and decreased sex hormone-binding globulin (SHBG) synthesis from the liver (Nestler *et al.*, 1991), leading to increased bioavailability of free androgens. Although controversial, hyperinsulinaemia may have a direct effect on hypothalamus and/or pituitary to increase serum LH concentra-

tions and therefore indirectly increase LH-dependent ovarian androgen biosynthesis (Dunaif, 1997). Hyperinsulinaemia may also directly affect folliculogenesis and may arrest growth of antral follicles after reaching a diameter between 5 and 8 mm (Franks *et al.*, 1999); however, this effect has not yet been proven.

Clomiphene citrate is the primary agent used for ovulation induction in infertile patients with PCOS. Clomiphene citrate will induce ovulation in ~80% but will achieve pregnancy in only 35% of these patients (Macgregor *et al.*, 1968). Exogenous gonadotrophins are traditionally employed to restore ovulation in clomiphene citrate-resistant cases. Low-dose step-up protocol is the treatment of choice and yields a high rate of monofollicular growth and ovulation (White *et al.*, 1996; Yaralı *et al.*, 1999).

On the basis of the theory that insulin resistance and resultant hyperinsulinaemia impede ovulation and may be important

contributors to the pathophysiology of PCOS, it may be postulated that insulin sensitizers might improve the abnormal endocrine milieu associated with PCOS, resulting in an increase in ovulatory cycles and pregnancy. Metformin is a biguanide that has been used for many years in Europe for the treatment of diabetes mellitus. Metformin has been reported to improve insulin resistance (Velazquez *et al.*, 1994; Diamanti-Kandarakis *et al.*, 1998; Nestler *et al.*, 1998; Glueck *et al.*, 1999; Kolodziejczyk *et al.*, 2000; Moghetti *et al.*, 2000), decrease serum total testosterone (Pirwany *et al.*, 1999; Kolodziejczyk *et al.*, 2000) and free testosterone concentrations (Nestler *et al.*, 1998; Moghetti *et al.*, 2000), increase SHBG levels (Nestler and Jakubowicz, 1996), improve menstrual cyclicality (Velazquez *et al.*, 1997; Diamanti-Kandarakis *et al.*, 1998; Morin-Papunen *et al.*, 1998) and documented ovulation (Nestler *et al.*, 1998; Glueck *et al.*, 1999) in patients with PCOS. However, in other studies, metformin had no beneficial effect on insulin resistance (Crave *et al.*, 1995; Acbay and Gundogdu, 1996; Ehrmann *et al.*, 1997; Pirwany *et al.*, 1999) and various endocrine parameters (Crave *et al.*, 1995; Acbay and Gundogdu, 1996; Ehrmann *et al.*, 1997).

There is a paucity of data on the use of metformin as an adjunct to ovulation induction in infertile patients with PCOS. Nestler *et al.* reported a significant increase in the number of ovulatory cycles in conjunction with clomiphene citrate compared with placebo (Nestler *et al.*, 1998). De Leo *et al.*, using the conventional protocol for exogenous gonadotrophin treatment in clomiphene citrate-resistant PCOS patients, reported an orderly follicular growth and a reduction in multifollicular development with co-administration of metformin (De Leo *et al.*, 1999). To our knowledge there are no data evaluating the effect of metformin on ovarian response when co-administered during exogenous gonadotrophin treatment using the low-dose step-up protocol.

The aim of this prospective randomized, placebo-controlled study is to analyse the impact of metformin on ovarian response when co-administered during a low-dose step-up protocol using recombinant (r)FSH.

Materials and methods

Subjects and study protocol

Thirty-two consecutive infertile women with PCOS and normal glucose tolerance were recruited. The diagnosis of PCOS was based on peripubertal onset of oligo-amenorrhoea, elevated serum testosterone levels (>80 ng/dl; conversion factor = 0.03467; >2.4 nmol/l) and ultrasonographic evidence of polycystic ovaries (PCO). Oligomenorrhoea was defined as bleeding episode occurring fewer than six times per year. Anovulation was confirmed in all patients with serial serum progesterone levels <5 ng/ml (conversion factor = 3.18; <15.9 nmol/l). All 32 patients were clomiphene citrate-resistant, defined as failure to ovulate with incremental doses of clomiphene citrate up to 250 mg/day for 5 days, if necessary, for 6 months. None of the patients had undergone any previous exogenous gonadotrophin treatment cycle. Inclusion criteria included normal semen analysis according to World Health Organization criteria (World Health Organization, 1999), normal hysterosalpingography and/or laparoscopy within the preceding 6 months and no history of previous genital surgery. Before entry into the study, all patients

underwent a 75 g oral glucose tolerance test (OGTT) and were shown to have normal glucose tolerance.

Exclusion criteria included the presence of any infertility factor other than PCOS, the use of medications known to alter insulin secretion or action, endocrinopathies, including non-classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency, Cushing's syndrome, hyperprolactinaemia or thyroid dysfunction. Patients with impaired glucose tolerance or type 2 diabetes were excluded. No patient was excluded on the basis of body mass index (BMI).

Informed consent was obtained from all patients and the Institutional Review Board approved the study.

Experimental protocol

At the time of entry into the study, all the women were in the equivalent of the follicular phase of the menstrual cycle and they had either spontaneous or progesterone-induced menses during the preceding week. At first visit, patients' weight, height, waist and hip circumferences were measured. The BMI [weight (kg)/height² (m²)] and the waist:hip ratio (WHR) were calculated.

An i.v. cannula was inserted into a forearm or antecubital fossa vein between 0800–0900 h and blood samples were obtained for baseline serum concentrations of LH, FSH, testosterone, free testosterone, androstenedione, estradiol (E₂), 17-OH progesterone, dehydroepiandrosterone sulphate (DHEAS) and leptin.

Oral glucose tolerance test

OGTT was performed after a 3 day, 300 g carbohydrate diet and 12 h overnight fasting. A 75 g oral glucose load was administered and blood samples were collected through an intravenous cannula for plasma glucose and insulin concentrations at 0, 30, 60, 90 and 120 min.

Frequently sampled i.v. glucose tolerance test (FSIGTT)

Intravenous cannulas were placed in both antecubital veins. A bolus of 50% glucose solution (0.3 gr/kg) was injected over 1 min at time zero. Regular human insulin (0.03 IU/kg Humulin®; Eli Lilly and Corp., IN, USA) was administered as an i.v. bolus at 20 min. Blood samples were collected at -15, -10, -5, -1, 2, 3, 4, 5, 6, 8, 10, 14, 19, 22, 25, 30, 50, 70, 100, 140 and 180 min for determination of plasma glucose and insulin concentrations.

Following the completion of baseline studies, 32 women were randomized to oral placebo ($n = 16$) or metformin (Glucophage Retard; Ilsan-Iltas Pharmaceuticals, Istanbul, Turkey) ($n = 16$) groups using computer-generated numbers. The study was double-blinded. The women took metformin or placebo alone for 6 weeks to allow metformin to exert its putative insulin-sensitizing effect before exogenous gonadotrophin treatment. The dose of metformin was 850 mg (two tablets daily). Patients were instructed not to modify their usual eating habits throughout the study.

Restoration of spontaneous ovulation was monitored by weekly serum progesterone levels and ovulation was assumed to have occurred when this was >5 ng/ml. Those who spontaneously ovulated on placebo or metformin did not undergo subsequent ovulation induction with rFSH.

The pretreatment studies were repeated after 6 weeks in all subjects excluding the two patients who spontaneously conceived. Metformin or placebo was continued during ovulation induction with rFSH until the day of HCG administration.

Ovulation induction with rFSH (Follitropin alpha, Gonal-F; Ares-Serono, Geneva, Switzerland) using the low-dose step-up protocol was performed as previously described (White *et al.*, 1996; Yarali *et al.*, 1999). Treatment was commenced on day 3–5 of a spontaneous or progesterone-induced menstrual bleeding. The starting dose was 75 IU of FSH s.c. daily. The initial dose of 75 IU/day was

maintained for up to 14 days unless follicle maturity was reached so that HCG would be administered. Ovarian response was initially monitored by serum E_2 levels every 2–3 days. When serum E_2 level was >100 pg/ml (conversion factor = 3.671; 367 pmol/l), monitoring was continued by daily transvaginal ultrasonography and serum E_2 levels. If no ovarian response was noted after 14 days of 75 IU/day therapy, the daily dose was increased by half a vial (37.5 IU). Any further increment, if necessary, was made by half a vial (37.5 IU) at weekly intervals to a maximum of 187.5 IU/day. If a dominant follicle emerged, the dose of FSH (threshold dose) was maintained until the follicle reached a mean diameter of 17 mm. At that point, HCG (Profasi; Serono, Aubonne, Switzerland) at a dose of 10 000 IU was administered by i.m. injection. If there were more than three follicles of ≥ 15 mm in diameter, the cycle was cancelled due to the risk of multiple pregnancy and/or ovarian hyperstimulation syndrome (OHSS). If there was no ovarian response after 35 days of treatment, the cycle was cancelled. Blood was taken for measurement of progesterone 6–8 days after HCG administration and ovulation was assumed to have occurred when progesterone level was >5 ng/ml (conversion factor = 3.18; 15.9 nmol/l). A serum pregnancy test was performed 13–15 days after administration of HCG.

Assay methods

Blood samples were centrifuged immediately, and serum was stored at -20°C until assayed. Plasma glucose was measured by the glucose oxidase technique (Boehringer, Mannheim, Germany). LH, FSH, E_2 , total testosterone and DHEAS were measured by chemiluminescent enzyme immunoassay (Immulate 2000; Diagnostic Products Corp., Los Angeles, CA, USA) with an average inter-assay coefficient of variation (CV) of 8% and intra-assay CV of 7%. Leptin was measured by using an immunoradiometric assay (Active Human Leptin IRMA, DSL-23100; Diagnostic Systems Laboratories Inc., Webster, TX, USA) with intra-assay and inter-assay CV of 4.9 and 6.6% respectively. Free testosterone, androstenedione and insulin were measured by radioimmunoassay (Diagnostic Systems Laboratories). The average inter-assay and intra-assay CV were 6.7 and 6.4% respectively. Plasma 17-OH progesterone was measured by radioimmunoassay (Immunotech, Marseille, France) with an intra-assay CV of 5.4% and inter-assay CV of 4.5%. To avoid inter-assay variation, all samples were analysed in duplicate in a single assay.

Data analysis

Using glucose and insulin data from the OGTT and FSIGTT, the following parameters were calculated: fasting glucose to insulin ratio [FG:I ratio ($\text{mg}/10^{-4}$ IU)], glucose and insulin area under curves during OGTT ($\text{AUC}_{\text{glucose}}$ and $\text{AUC}_{\text{insulin}}$), the first phase insulin secretion [area in response to glucose: AIRg ($\mu\text{IU}/\text{ml min}$)] in response to glucose, calculated as the mean increment above basal of insulin values measured at 2, 3, 4, 5, 6, 8 and 10 min during FSIGTT, the insulin sensitivity index [$\text{SI} \times 10^{-4}$ ($\text{min}^{-1} \cdot \text{pmol}^{-1} \cdot \text{l}^{-1}$)] calculated using the minimal model analysis (MINMOD) program (Bergman *et al.*, 1981). The SI represents the increase in net fractional glucose clearance rate per unit change in plasma insulin concentration after the i.v. glucose load.

Statistical analysis

SPSS for Windows for 9.0 (SPSS, Chicago, IL, USA) was used for statistical analysis. Student's *t*-test, Mann–Whitney *U*-test, χ^2 -test, Fisher's exact test were used where appropriate. Areas under curves for glucose and insulin were calculated using the trapezoidal formula. Data are given as mean \pm SD. $P \leq 0.05$ was considered statistically significant.

Results

The mean ages of the metformin and placebo groups were 29.7 ± 5.6 and 28.4 ± 5.1 years respectively ($P > 0.05$). The mean duration of infertility was 57.8 ± 37.9 months in the former and 62.3 ± 41.9 months in the latter group respectively ($P > 0.05$).

One patient on placebo and six patients on metformin ovulated spontaneously ($P < 0.05$). In the metformin group, ovulation was noted at week 3 in five patients and at week 6 in one patient. The only ovulation with placebo was noted at week 6. All these seven patients did not undergo subsequent ovulation induction with rFSH. Two patients on metformin conceived spontaneously. Of these two patients, one has an ongoing pregnancy and the other had a miscarriage.

On entry into the study, the metformin and placebo groups did not differ with respect to the anthropometric variables (Table I). BMI and WHR remained stable over the course of the study in both groups.

There was no drug discontinuation due to side-effects. Only one patient in the metformin group reported nausea; however, this complaint resolved following intake of metformin with meals.

Hormonal and metabolic characteristics of study subjects

Responses during OGTT and IVGTT

By design, no subject had impaired glucose tolerance or type 2 diabetes. Before treatment with placebo or metformin for 6 weeks, all parameters of insulin resistance, including the baseline mean fasting insulin level, FG:I ratio, $\text{AUC}_{\text{insulin}}$, SI and AIRg values were comparable between the two groups (Table II). In neither group was there any change in mean fasting insulin, fasting glucose/insulin ratio, $\text{AUC}_{\text{glucose}}$, $\text{AUC}_{\text{insulin}}$, SI and AIRg values after 6 weeks of treatment with placebo or metformin (Table II). In the metformin group, $\text{AUC}_{\text{insulin}}$ decreased from $50\,040 \pm 21\,918$ to $43\,806 \pm 17\,064$ pmol/l min, and AIRg decreased from 4016 ± 2484 to 3065 ± 1690 pmol/l min; however, these differences did not reach statistical significance.

Endocrine changes

On entry into the study, the metformin and placebo groups did not differ with respect to the baseline endocrine parameters (Table I). The only significant change after treatment was a decline in mean serum free testosterone concentration in the metformin group ($P = 0.049$) (Table I). There was no significant change in all other hormones studied in both metformin and placebo groups.

Data on ovulation induction with rFSH

The baseline characteristics of the placebo ($n = 15$) and metformin ($n = 10$) groups undergoing ovulation induction with rFSH, including the mean age, duration of infertility, all insulin resistance indices and endocrine parameters, were comparable (data not shown).

All parameters of ovarian response, including the mean duration of stimulation, total dose of rFSH used, E_2 on the day of HCG and endometrial thickness on the day of HCG were comparable between the two groups (not significant) (Table III). Monofollicular development and ovulation rates

Table I. The anthropometric and endocrine parameters before and after 6 week treatment with placebo or metformin

	Placebo		Metformin	
	Before (n = 16)	After (n = 16)	Before (n = 16)	After (n = 16)
Waist:hip ratio	0.8 ± 0.1	0.8 ± 0.2	0.8 ± 0.1	0.8 ± 0.1
Body mass index (kg/m ²)	29.6 ± 4.8	29.8 ± 4.9	28.6 ± 4.0	28.0 ± 3.4
FSH (mIU/ml)	7.3 ± 1.9	7.3 ± 2.4	6.3 ± 2.0	6.7 ± 2.5
LH (mIU/ml)	10.9 ± 5.0	11.7 ± 7.1	14.2 ± 6.5	13.3 ± 5.3
LH/FSH	1.6 ± 0.7	1.5 ± 0.7	2.4 ± 1.0	2.1 ± 0.8
Estradiol (pg/ml)	47.2 ± 13.7	47.4 ± 19.4	75.7 ± 53.6	53.3 ± 43.5
Testosterone (ng/dl)	173.3 ± 84.5	148.8 ± 78.9	178.4 ± 103.0	168.2 ± 45.0
Free testosterone (pg/ml)	2.9 ± 1.1	3.6 ± 2.3	4.3 ± 1.9*	1.2 ± 0.4*
Androstenedione (ng/ml)	2.3 ± 0.9	2.4 ± 1.4	3.1 ± 1.4	2.2 ± 0.6
DHEAS (µg/dl)	282.0 ± 90.1	250.2 ± 109.8	233.9 ± 123.5	259.2 ± 131.9
Leptin (ng/ml)	31.3 ± 18.8	34.5 ± 17.7	31.9 ± 15.3	30.6 ± 15.0
17-OH progesterone (ng/ml)	1.7 ± 0.7	1.6 ± 0.9	2.3 ± 1.9	1.2 ± 0.4

*P = 0.049; all other comparisons between and within the placebo and metformin groups are not significant.
DHEAS = dehydroepiandrosterone sulphate.

Table II. The indices of insulin resistance before and after 6 week treatment of metformin or placebo

	Placebo		Metformin	
	Before (n = 16)	After (n = 16)	Before (n = 16)	After (n = 14)
Fasting insulin (pmol/l)	66.0 ± 33.0	73.2 ± 42.0	93.0 ± 128.4	98.4 ± 196.2
Fasting glucose/insulin (mmol/pmol)	0.09 ± 0.04	0.10 ± 0.06	0.10 ± 0.03	0.120 ± 0.07
Glucose AUC (mmol/l min)	832.8 ± 137.1	890.9 ± 156.8	858.1 ± 152.5	912.6 ± 143.0
Insulin AUC (pmol/l min)	48 414 ± 41 400	52 788 ± 47 718	50 040 ± 21 918	43 806 ± 17 064
AIRg (pmol/l min)	4058 ± 3313	4019 ± 2466	4016 ± 2484	3065 ± 1690
SI × 10 ⁻⁴ (min ⁻¹ .pmol ⁻¹ .l ⁻¹)	21.0 ± 21.6	18.6 ± 9.0	15.6 ± 15.6	16.2 ± 8.4

All comparisons between and within the placebo and metformin groups are not significant.
AUC = area under the curve; AIRg = area in response to glucose; SI = insulin sensitivity index.

Table III. The ovarian response and pregnancy rates during rFSH treatment using low dose the step-up protocol

	Placebo (n = 15)	Metformin (n = 10)
Duration of stimulation (day)	19.7 ± 10.8	17.1 ± 7.8
Total dose (IU)	2062.5 ± 1397.1	1607.8 ± 887.1
No. of vials	27.5 ± 18.6	21.4 ± 11.8
Estradiol on the day of HCG (pg/ml)	543.5 ± 619.9	509.1 ± 246.0
Endometrial thickness on the day of HCG (mm)	9.7 ± 1.7	10.0 ± 1.3
Monofollicular development (%)	8 (53.3)	5 (50.0)
Multifollicular development (%)	7 (46.7)	5 (50.0)
Ovulation (%)	11 (73.3)	9 (90.0)
Pregnancy per cycle (%)	1 (6.7)	3 (30.0)

All comparisons between the placebo and metformin groups are not significant.

were also comparable between the two groups (not significant). Two cycles were cancelled in the placebo group; one due to no ovarian response and the other due to development of more than three follicles of ≥15 mm diameter. One patient in the placebo group and three patients in the metformin group conceived. Of the three patients who conceived in the metformin group, two have an ongoing pregnancy and the remaining had a miscarriage. The only patient who conceived in the placebo group has an ongoing pregnancy.

The numbers of women who ovulated and/or conceived combining the 6 week placebo or metformin-only period with

a single rFSH treatment cycle are shown in Figure 1. The overall ovulation rates were 75 and 94% in the placebo and metformin groups respectively (P > 0.05). The respective figures for pregnancy were 6.3 and 31.3% (P > 0.05).

Discussion

In this double-blind, placebo-controlled, prospective randomized trial, we did not note a significant improvement in peripheral insulin resistance with metformin at a dose of 850 mg twice daily for 6 weeks in clomiphene citrate-

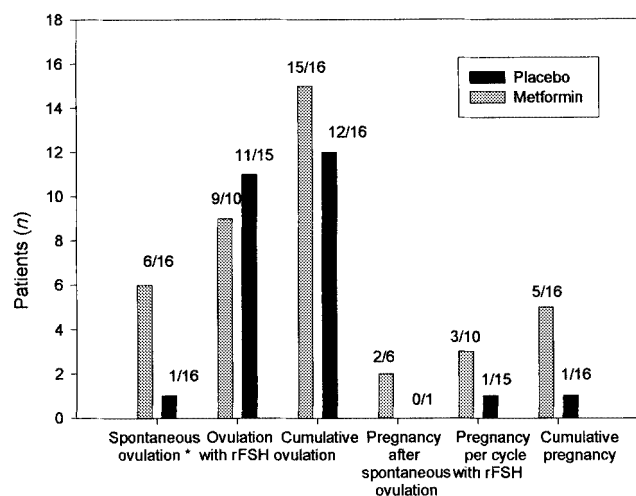


Figure 1. Numbers of women who ovulated and/or conceived in response to metformin/placebo only or combined with rFSH treatment. * $P < 0.05$.

resistant PCOS women with normal glucose tolerance. In the metformin group, there was no significant improvement in all insulin SI, including fasting insulin concentration, FG:I ratio, AUC_{insulin} , Si and AIRg. After the 6 week priming period, the only significant change noted was a decrease in free testosterone concentrations in the metformin group. Despite a lack of improvement in insulin resistance with metformin, there was a marked and significant increase in spontaneous ovulation rate to 38%. These findings suggest that the mode of action of metformin to restore spontaneous ovulation in our patient population may not be mediated directly through an improvement in insulin resistance. Rather, metformin may act directly within the ovary by modulating the sensitivity of ovarian follicles to circulating insulin, perhaps by augmenting the post-receptor mechanism of action of insulin within the follicular apparatus (Pirwany *et al.*, 1999). An indirect effect of metformin through alteration of gonadotrophins is unlikely, since there was no change in both FSH and LH concentrations. However, the effect of an improvement in androgenic microenvironment within the ovary due to decreased free testosterone concentrations could not be ruled out. The lack of data on SHBG is a limitation of our study. We may postulate that the decrease in free testosterone levels in the metformin group is possibly due to increased SHBG concentrations.

Previous studies have reported conflicting data on the effect of metformin on insulin resistance and endocrine parameters in patients with PCOS. Although some studies reported an improvement in insulin resistance (Velazquez *et al.*, 1994; Diamanti-Kandarakis *et al.*, 1998; Nestler *et al.*, 1998; Glueck *et al.*, 1999; Kolodziejczyk *et al.*, 2000; Moghetti *et al.*, 2000), others failed to observe any statistically significant effect (Crave *et al.*, 1995; Acbay and Gundogdu, 1996; Ehrmann *et al.*, 1997; Pirwany *et al.*, 1999), in agreement with the present study. The effect of metformin on endocrine parameters has also been variable (Taylor, 2000). The three studies reporting no effect of metformin on insulin resistance have not provided data on restoration of ovulation (Crave *et al.*, 1995; Acbay and Gundogdu, 1996; Ehrmann *et al.*, 1997). However, in 15 obese patients with PCOS,

despite no change in fasting insulin concentration, a significant improvement in spontaneous ovulation was reported with metformin (Pirwany *et al.*, 1999).

Several hypotheses have been proposed to explain why metformin successfully lowers insulin and androgen levels in some studies but not in others. These include variations in patient inclusion criteria, BMI, dosing and duration of metformin therapy, and genetic background. In the study in which metformin had no effect on hyperinsulinaemia and androgen excess (Ehrmann *et al.*, 1997), the mean BMI was 39 kg/m². However, BMI is not the sole predictor of success of metformin therapy, since the mean BMI of the current study and another negative study (Acbay and Gundogdu, 1996) are ~27 and ~30 kg/m² respectively. The variability of responses to comparable regimens of metformin in individual studies underscores the role of ethnic background and other factors contributing to a great diversity of clinical and hormonal profiles found among women with PCOS.

There is a lack of data on the effect of metformin therapy when used as an adjunct to ovulation induction in infertile patients with PCOS. To our knowledge, there is only one study evaluating the effect of pretreatment with metformin on exogenous gonadotrophin treatment in infertile women with clomiphene citrate-resistant PCOS (De Leo *et al.*, 1999). Twenty women were randomized to two groups. The first group consisted of 10 patients treated with urinary (u)FSH alone for two cycles and then for 1 month with metformin, after which they underwent a third cycle of combined metformin and uFSH stimulation. The second group ($n = 10$) was treated with metformin for 1 month before undergoing ovulation induction with combined metformin and uFSH for one cycle. Metformin was given at a dose of 500 mg three times daily. Conventional protocol was employed for uFSH treatment. In the first group, two women conceived; hence eight women in the first group were treated with metformin before the final stimulation cycle with uFSH. Therefore, a total of 19 cycles with uFSH alone and 18 cycles with uFSH and metformin were performed and compared. The number of follicles >15 mm in diameter on the day of HCG administration, the percentage of cycles with HCG withheld because of excessive follicular development and plasma levels of E₂ on the day of HCG administration were significantly lower in cycles performed after metformin treatment. Two pregnancies occurred in women treated with uFSH alone and three pregnancies occurred in those treated also with metformin. The authors concluded that co-administration of metformin led to an orderly induced ovulation with uFSH and lowered the risk of multifollicular development and hence OHSS. There are two drawbacks of this study. First, by design, crossing-over of the first group to the second group may create selection bias and should be avoided in studies with terminal events such as pregnancy (Daya, 1993). Second, there is no data on the effect of metformin on insulin resistance and endocrine parameters. Of interest, no patient ovulated in response to metformin only.

Multiple follicular development resulting in high rates of multiple pregnancy and OHSS is the major drawback of conventional protocols. Low-dose step-up protocol is the treatment of choice in clomiphene citrate-resistant cases and yields a high rate of monofollicular growth and ovulation (White *et al.*, 1996; Yarali *et al.*, 1999). The principle behind the low-dose

step-up protocol is to find the threshold level of FSH that will lead to the development of a single pre-ovulatory follicle, thereby avoiding multiple pregnancy and OHSS. Dale *et al.* examined the impact of insulin resistance on the outcome of ovulation induction with the low-dose step-up protocol in 42 clomiphene citrate-resistant women with PCOS (Dale *et al.*, 1998). The insulin-resistant women required a higher gonadotrophin dose and a longer time to achieve follicular maturation. Cycle cancellation due to multifollicular development was significantly greater in the insulin-resistant cases. Although ovulation rates were similar between the insulin-resistant and non-insulin-resistant groups, the conception rate was significantly better in the non-insulin-resistant PCOS women.

To our knowledge, there are no data on the effect of co-administration of metformin during a low-dose step-up protocol using rFSH in clomiphene citrate-resistant PCOS patients. In contrast to the study by De Leo (De Leo *et al.*, 1999), all parameters of ovarian response were comparable between the placebo and metformin groups in our study.

We should stress that our study is a pilot study, and a lack of difference in insulin sensitivity and ovulation induction parameters may result from Type 2 statistical error due to limited sample size and high variation. Further well-designed, powerful, prospective randomized studies are warranted to delineate the role of metformin for co-administration during low-dose step-up protocol using exogenous gonadotrophins.

We conclude that in clomiphene citrate-resistant PCOS women with normal glucose tolerance: (i) metformin may markedly restore spontaneous ovulation with no improvement in insulin resistance; and (ii) metformin has no significant effect on ovarian response during low-dose step-up protocol using rFSH.

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References

Acbay, O. and Gundogdu, S. (1996) Can metformin reduce insulin resistance in polycystic ovary syndrome? *Fertil. Steril.*, **65**, 946–949.

Adashi, E.Y., Resnick, C.E., D'Ercole, A.J. *et al.* (1985) Insulin-like growth factors as intraovarian regulators of granulosa cell growth and function. *Endocr. Rev.*, **6**, 400–420.

Barbieri, R.L., Makris, A., Randall, R.W. *et al.* (1986) Insulin stimulates androgen accumulation in incubations of ovarian stroma obtained from women with hyperandrogenism. *J. Clin. Endocrinol. Metab.*, **62**, 904–910.

Bergman, R.N., Phillips, L.S. and Cobelli, C. (1981) Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and beta-cell glucose sensitivity from the response to intravenous glucose. *J. Clin. Invest.*, **68**, 1456–1467.

Crave, J.C., Fimbel, S., Lejeune, H. *et al.* (1995) Effects of diet and metformin administration on sex hormone-binding globulin, androgens, and insulin in hirsute and obese women. *J. Clin. Endocrinol. Metab.*, **80**, 2057–2062.

Dale, P.O., Tanbo, T., Haug, E. *et al.* (1998) The impact of insulin resistance on the outcome of ovulation induction with low-dose follicle stimulating hormone in women with polycystic ovary syndrome. *Hum. Reprod.*, **13**, 567–570.

Daya, S. (1993) Is there a place for the crossover design in infertility trials? [editorial] [see comments]. *Fertil. Steril.*, **59**, 6–7.

De Leo, V., la Marca, A., Ditto, A. *et al.* (1999) Effects of metformin on gonadotropin-induced ovulation in women with polycystic ovary syndrome. *Fertil. Steril.*, **72**, 282–285.

Diamanti-Kandarakis, E., Kouli, C., Tsianeteli, T. *et al.* (1998) Therapeutic effects of metformin on insulin resistance and hyperandrogenism in polycystic ovary syndrome [see comments]. *Eur. J. Endocrinol.*, **138**, 269–274.

Dunaif, A. (1997) Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. *Endocr. Rev.*, **18**, 774–800.

Dunaif, A., Graf, M., Mandeli, J. *et al.* (1987) Characterization of groups of hyperandrogenic women with acanthosis nigricans, impaired glucose tolerance, and/or hyperinsulinemia. *J. Clin. Endocrinol. Metab.*, **65**, 499–507.

Ehrmann, D.A., Cavaghan, M.K., Imperial, J. *et al.* (1997) Effects of metformin on insulin secretion, insulin action, and ovarian steroidogenesis in women with polycystic ovary syndrome. *J. Clin. Endocrinol. Metab.*, **82**, 524–530.

Franks, S., Gilling-Smith, C., Watson, H. *et al.* (1999) Insulin action in the normal and polycystic ovary. *Endocrinol. Metab. Clin. North Am.*, **28**, 361–378.

Glueck, C.J., Wang, P., Fontaine, R. *et al.* (1999) Metformin-induced resumption of normal menses in 39 of 43 (91%) previously amenorrhic women with the polycystic ovary syndrome. *Metabolism*, **48**, 511–519.

Knochenhauer, E.S., Key, T.J., Kahsar-Miller, M. *et al.* (1998) Prevalence of the polycystic ovary syndrome in unselected black and white women of the southeastern United States: a prospective study. *J. Clin. Endocrinol. Metab.*, **83**, 3078–3082.

Kolodziejczyk, B., Duleba, A.J., Spaczynski, R.Z. *et al.* (2000) Metformin therapy decreases hyperandrogenism and hyperinsulinemia in women with polycystic ovary syndrome. *Fertil. Steril.*, **73**, 1149–1154.

Macgregor, A.H., Johnson, J.E. and Bunde, C.A. (1968) Further clinical experience with clomiphene citrate. *Fertil. Steril.*, **19**, 616–622.

Moggetti, P., Castello, R., Negri, C. *et al.* (2000) Metformin effects on clinical features, endocrine and metabolic profiles, and insulin sensitivity in polycystic ovary syndrome: a randomized, double-blind, placebo-controlled 6-month trial, followed by open, long-term clinical evaluation. *J. Clin. Endocrinol. Metab.*, **85**, 139–146.

Morin-Papunen, L.C., Koivunen, R.M., Tomas, C. *et al.* (1998) Decreased serum leptin concentrations during metformin therapy in obese women with polycystic ovary syndrome. *J. Clin. Endocrinol. Metab.*, **83**, 2566–2568.

Nestler, J.E. and Jakubowicz, D.J. (1996) Decreases in ovarian cytochrome P450c17 alpha activity and serum free testosterone after reduction of insulin secretion in polycystic ovary syndrome [see comments]. *N. Engl. J. Med.*, **335**, 617–623.

Nestler, J.E., Powers, L.P., Matt, D.W. *et al.* (1991) A direct effect of hyperinsulinemia on serum sex hormone-binding globulin levels in obese women with the polycystic ovary syndrome. *J. Clin. Endocrinol. Metab.*, **72**, 83–89.

Nestler, J.E., Jakubowicz, D.J., Evans, W.S. *et al.* (1998) Effects of metformin on spontaneous and clomiphene-induced ovulation in the polycystic ovary syndrome. *N. Engl. J. Med.*, **338**, 1876–1880.

Pirwany, I.R., Yates, R.W., Cameron, I.T. *et al.* (1999) Effects of the insulin sensitizing drug metformin on ovarian function, follicular growth and ovulation rate in obese women with oligomenorrhoea. *Hum. Reprod.*, **14**, 2963–2968.

Taylor, A.E. (2000) Insulin-lowering medications in polycystic ovary syndrome [In Process Citation]. *Obstet. Gynecol. Clin. North Am.*, **27**, 583–595.

Velazquez, E.M., Mendoza, S., Hamer, T. *et al.* (1994) Metformin therapy in polycystic ovary syndrome reduces hyperinsulinemia, insulin resistance, hyperandrogenemia, and systolic blood pressure, while facilitating normal menses and pregnancy. *Metabolism*, **43**, 647–654.

Velazquez, E., Acosta, A. and Mendoza, S.G. (1997) Menstrual cyclicality after metformin therapy in polycystic ovary syndrome. *Obstet. Gynecol.*, **90**, 392–395.

White, D.M., Polson, D.W., Kiddy, D. *et al.* (1996) Induction of ovulation with low-dose gonadotropins in polycystic ovary syndrome: an analysis of 109 pregnancies in 225 women. *J. Clin. Endocrinol. Metab.*, **81**, 3821–3824.

World Health Organization (1999) *WHO Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction*. Cambridge University Press, Cambridge.

Yarali, H., Bukulmez, O. and Grgan, T. (1999) Urinary follicle-stimulating hormone (FSH) versus recombinant FSH in clomiphene citrate-resistant, normogonadotropic, chronic anovulation: a prospective randomized study. *Fertil. Steril.*, **72**, 276–281.

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