Metabolic and ovarian effects of rosiglitazone treatment for 12 weeks in insulin-resistant women with polycystic ovary syndrome

Nicholas A. Cataldo¹,⁵, Fahim Abbasi², Tracey L. McLaughlin³, Marina Basina³, Patricia Y. Fechner⁴, Linda C. Giudice¹ and Gerald M. Reaven²

¹Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Divisions of ²Cardiovascular Medicine and ³Endocrinology, Gerontology and Metabolism, Department of Medicine and ⁴Division of Endocrinology, Department of Pediatrics, Stanford University School of Medicine, Stanford, CA 94305, USA
⁵To whom correspondence should be addressed at: Department of Obstetrics and Gynecology, Stanford University Medical Center, 300 Pasteur Drive, MC5317, Stanford, CA 94305-5317, USA. E-mail: nixie54@yahoo.com

BACKGROUND: Insulin sensitizers have favourable metabolic and ovarian effects in polycystic ovary syndrome (PCOS). This study examined rosiglitazone, a thiazolidinedione, in PCOS.

METHODS: In a prospective, open-label study, the effects of rosiglitazone on metabolism and ovarian function were examined in 42 non-diabetic women with PCOS classified according to the National Institute of Child Health and Human Development criteria and insulin resistance (IR) by steady-state plasma glucose (SSPG) ≥10 mmol/l on octreotide-modified insulin suppression testing.

Participants were randomized to rosiglitazone 2, 4 or 8 mg daily for 12 weeks. Endpoints included ovulation and menstrual pattern; serum testosterone, sex hormone-binding globulin (SHBG), and LH; and changes in IR and glucose–insulin responses on 8 h mixed-meal profile.

RESULTS: After rosiglitazone 8 mg daily for 12 weeks, SSPG declined and insulinaemia fell by 46%; lower doses gave lesser effects. Serum LH, total and free testosterone were unchanged; SHBG increased. With rosiglitazone, ovulation occurred in 23/42 women (55%), without significant dose dependence. Both before and during treatment, ovulators on rosiglitazone had lower circulating insulin and free testosterone and higher SHBG than non-ovulators. Testosterone declined only in a subgroup of ovulators with early vaginal bleeding after starting rosiglitazone.

CONCLUSIONS: Rosiglitazone in insulin-resistant PCOS promoted ovulation and dose-dependently decreased IR and insulinaemia; ovulators had lower circulating insulin and testosterone.

Key words: hyperandrogenism/insulin resistance/ovulation/polycystic ovary syndrome/rosiglitazone

Introduction

Polycystic ovary syndrome (PCOS), a heterogeneous disorder which according to the 1990 NIH consensus definition (Zawadzki and Dunai, 1992) includes both anovulation and ovarian hyperandrogenism in the absence of other, specific aetiology, is associated with resistance to the metabolic actions of insulin in an estimated 50–70% of cases (Dunai, 1997). The resulting compensatory hyperinsulinaemia, acting on a normally insulin-sensitive ovary (Franks et al., 1999), has been implicated in the ovarian pathophysiology of PCOS, given that both weight loss and medications which reduce circulating insulin can reduce hyperandrogenism and promote ovulation (Nestler et al., 1989; Fulghesu et al., 1995; Dunai et al., 1996; Ehrmann et al., 1997b; Pasquali et al., 1997, 2000; Azziz et al., 2001; Costello and Eden, 2003; Haas et al., 2003; Lord et al., 2003).

The insulin-sensitizing agents metformin and troglitazone have been found to improve both hyperandrogenism and ovulatory function in PCOS, while decreasing circulating insulin (Dunaif et al., 1996; Ehrmann et al., 1997b; Azziz et al., 2001; Lord et al., 2003; Haas et al., 2003; Costello and Eden, 2003).

Metformin, a biguanide, is associated with frequent gastrointestinal side-effects and has not shown effectiveness in all reported studies of PCOS (Açbay and Gündogdu, 1996; Ehrmann et al., 1997a), while troglitazone, the first approved thiazolidinedione (TZD), has been removed from the market because of sporadic severe hepatotoxicity.

Rosiglitazone, like troglitazone, is a peroxisome proliferator-activated receptor (PPAR-γ) agonist TZD with insulin-sensitizing and antidiabetic properties. Unlike troglitazone, it does not present an excess risk of liver toxicity (Wagstaff and Goa, 2002). Although all TZD are PPAR-γ ligands, distinct agonistic profiles of troglitazone and rosiglitazone have been reported (Camp et al., 2000), giving the possibility of distinct effects in PCOS.

The present study was designed to examine in parallel the metabolic and ovarian actions of rosiglitazone in non-diabetic...
women with PCOS prospectively identified as insulin resistant by preset criteria. Qualifying women were given one of three rosiglitazone doses for 12 weeks in a randomized, open-label protocol. Our study differs from those previously reported in that it compared the effects of three rosiglitazone doses, it assessed the effects of treatment on measures of both dynamic insulin action (insulin sensitivity) and day-long circulating insulin levels, and it specifically examined the insulin-resistant subgroup of women with PCOS.

Materials and methods

Human subjects approval
The study protocol was approved by the Stanford University Medical Human Subjects Committee and the Stanford General Clinical Research Center (GCRC) Committee. Each subject gave written, informed consent to participate in the study prior to screening.

Subjects
Subjects were recruited by advertisement beginning in June, 2001 in newspapers, on internet sites, and on bulletin boards at Stanford. Prospective subjects were women in general good health aged 18–45 years with PCOS, defined according to the 1990 National Institute of Child Health and Human Development (NICHD) consensus criteria (Zawadzki and Dunai, 1992) as having oligomenorrhea or amenorrhea (eight or fewer menses per year, or ≥45 mean days between bleeding episodes) and either clinical or biochemical evidence of hyperandrogenism, with normal serum thyroid-stimulating hormone and prolactin levels. No use of potentially confounding medications was permitted within 1 month of initial metabolic screening. These medications included estrogens and selective estrogen-receptor modulators (including clomiphenone), progestins, gonadotrophins or GnRH agonists, antiandrogens, bromocriptine, systemic corticosteroids, metformin and TZD. Depot medroxyprogesterone acetate was not permitted within 1 year of entry. Initial screening assured normal packed-cell volume (haematocrit), fasting plasma glucose, and serum creatinine; and levels of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) that were <150% of the upper limit of the reference range.

Octreotide-modified insulin suppression test (IST)
After initial screening, an IST was performed as described (Greenfield et al., 1981; Pei et al., 1994) to determine insulin sensitivity. After an overnight fast of 12–14 h, subjects were admitted to the Stanford GCRC. Vital signs, height, and weight were obtained. After fasting, venous blood was drawn through an antecubital line, a constant infusion of glucose (13.3 mmol/min·m²), insulin (172 pmol/min·m²) and octreotide (250 μg/h), calculated to achieve a steady-state plasma insulin level of 347–417 pmol/l, was continued for 180 min, while plasma glucose was monitored every 30 min on a Beckman autoanalyzer using blood drawn from a contralateral antecubital line. The mean plasma glucose at 150, 160, 170 and 180 min, termed the steady-state plasma glucose (SSPG), has been characterized as an index of insulin sensitivity (Greenfield et al., 1981; Pei et al., 1994; Yeni-Komshian et al., 2000). By design, only women with SSPG ≥10 mmol/l were eligible to receive rosiglitazone in this study; this SSPG range characterizes the most insulin-resistant tertile of a previously reported unselected non-diabetic adult volunteer population at Stanford (Yeni-Komshian et al., 2000). Six subjects underwent IST >6 months prior to beginning rosiglitazone; given the reported reproducibility of SSPG (Facchini et al., 1999), the IST was not repeated if the subject’s current weight was within 5% of her prior weight and her lifestyle was judged not to be significantly different. The remaining subjects completed all baseline metabolic tests in the 6 weeks prior to starting rosiglitazone.

Oral glucose tolerance test (OGTT) and meal profile test
Subjects also underwent OGTT in the Stanford GCRC after an overnight fast. After fasting plasma glucose <7.0 mmol/l was confirmed, 75 g glucose syrup was ingested and venous blood drawn from an indwelling catheter at 30, 60, 120 and 180 min. A 120 min plasma glucose >11.1 mmol/l prompted exclusion for possible diabetes. Qualifying subjects then underwent a mixed-meal profile study on a separate day. For this study, again in the GCRC after an overnight fast, blood was drawn from an indwelling catheter, then hourly for 8 h, with breakfast and lunch being served at time 0 and 4 h respectively. The meals were prepared in the GCRC metabolic kitchen and consisted of 20% of total recommended daily calories at breakfast and 40% at lunch, with a composition of 15% protein, 45% carbohydrate, and 40% fat. Plasma was separated from all samples obtained during OGTT and meal profile and stored at −70°C until later analysis of glucose and insulin as described below. Integrated glucose and insulin levels were calculated by the trapezoidal rule and reported as area under curve (AUC). Fasting lipoprotein profiles were obtained on two occasions during initial evaluation and results averaged; these consisted of total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, and total triglycerides.

Randomization
Subjects who completed the IST, OGTT and meal profile and who had a normal physical and gynaecological examination and a negative serum pregnancy test on the last day of these procedures were randomized to begin taking 2, 4 or 8 mg rosiglitazone once daily (Avandia®; Glaxo SmithKline). Randomization was accomplished by sealed envelopes and blocked for body mass index (BMI; <27, 27–32, >32 kg/m²), with an equal number of envelopes prepared for each rosiglitazone dose in each BMI category. Subjects were instructed to complete a diary by marking each rosiglitazone dose as taken, as well as the character of each day of vaginal bleeding they experienced (spotting, light flow, normal menstrual flow, heavy flow). They were instructed to avoid pregnancy, using non-hormonal contraception if needed, and to not purposely modify their diet or exercise habits or purposely attempt to lose weight.

Evaluation of ovulation
Subjects were instructed to report for weekly (every 7 ± 1 days) venipunctures beginning after 4 weeks of rosiglitazone, from which serum progesterone was determined concurrently by the Reproductive Endocrinology Laboratory (REL), Department of Ob/Gyn at Stanford. Recent ovulation was inferred from a progesterone level ≥12.7 nmol/l. Two such consecutive weekly levels prompted serum pregnancy testing, and a positive pregnancy test led to prompt discontinuation of rosiglitazone and removal from the study. Progesterone levels <6.36 nmol/l were taken as evidence of no recent ovulation, and values between 6.36 and 12.7 nmol/l were considered indeterminate. Serum obtained at baseline and at 4, 8, and 12 weeks was analysed for total testosterone, free testosterone, and sex hormone-binding globulin (SHBG), and for LH (omitted for the last seven subjects after analysis revealed no treatment effect). At 8 weeks of treatment, serum ALT was determined. Subjects were permitted to miss a single weekly draw but were advised that missing two consecutive weekly draws could result in their removal from the study.
**Rosiglitazone in PCOS**

After 78–90 days (12 weeks ± 6 days) of rosiglitazone, subjects were readmitted to the GCRC for repeat IST and meal profile testing, performed on separate days exactly as previously. Fasting lipoprotein profiles were repeated on each occasion and results averaged. At this time, a pill count was obtained, the menstrual and medication diary reviewed, and a physical and gynaecological examination performed. Rosiglitazone was continued until both metabolic studies and 12 weeks of treatment were completed.

**Assays**

Plasma glucose analyses for the IST and OGGT were performed concurrently in the GCRC with a Beckman autoanalyzer using the glucose oxidase technique. Plasma glucose was analysed on thawed samples from the meal profiles by a modification of the Trinder oxidase/peroxidase method. Plasma insulin was determined by enzyme immunoassay (EIA; Diagnostic Systems Laboratories, Inc., Webster, TX, USA). Lipoproteins were measured in fresh plasma by standard clinical assays in use at Stanford University Medical Center. Serum progesterone and LH were analysed in the Stanford REL by EIA (Inmulite; Diagnostic Products Corp., Los Angeles, CA, USA). The serum pregnancy test used was the Nimbus Plus hCG EIA (Biomerica, Newport Beach, CA, USA; sensitivity, 25 IU/l). Total testosterone and SHBG were determined by standard clinical EIA at ARUP Laboratories (Salt Lake City, UT, USA), and free testosterone was calculated by that laboratory using the Sodergard equation (Sodergard et al., 1982). The intra- and inter-assay coefficients of variation for total testosterone were 3% and 8%, for progesterone 10% and 11%, and for LH 4% and 7% respectively. Results were reported in conventional units and converted to SI units as follows: for progesterone, (ng/ml) × 3.18 = (nmol/l); for testosterone, (ng/dl) × 0.0347 = (nmol/l) and (pmol/l); for glucose, (mg/dl) × 0.0555 = (mmol/l); for insulin, (pmol/l) × 6.945 = (mmol/l); for cholesterol, (mg/dl) × 0.0259 = (mmol/l); for triglycerides, (mg/dl) × 0.0113 = (mmol/l).

**Power calculation and statistical analysis**

The primary endpoint of this study, for which a power calculation was used to determine the sample size, was an ovulatory response; it was determined that 15 subjects per dose group would be needed to give 80% power to detect significance at α = 0.05 of a 50% ovulation rate with treatment by comparison with a pre-treatment ovulation prevalence projected as 5% based on the inclusion criterion of oligomenorrhea. Comparisons of data from groups of subjects (defined, e.g., by dose assignment or ovulator status) were performed by ANOVA. Post-hoc testing was carried out using Fisher’s protected least significant difference (PLSD) if the overall ANOVA reached significance. Proportions were compared by χ² or Fisher’s exact test as appropriate, StatView (Abacus Concepts, Berkeley, CA, USA) or Primer of Biostatistics software (S.Glantz, University of California San Francisco; McGraw-Hill, 1992) and a Macintosh computer were used for these analyses. A level of P < 0.05 was taken as significant.

**Results**

**Subject screening**

A total of 95 subjects came to Stanford, consented to participate, and underwent initial screening. This screening resulted in disqualification of six women (6%) for transaminase (ALT) elevation and three (3%) for elevated fasting or 2 h glucose on OGGT. Eleven women did not participate further for other reasons, including personal preference. Including one with elevated ALT, 76 women underwent screening IST, of whom 58 (76%) were Caucasian, two (3%) African American, and 15 (20%) Asian; one (1%) was biracial. Seven (9%) identified themselves as Hispanic. A SSPG ≥10 mmol/l was found in 44 (58%) of the 76 women tested. Of these, 42 received rosiglitazone; their racial and ethnic distribution (33 Caucasian, six Asian, two African American, one biracial) was indistinguishable from that of the 76 women undergoing IST. Among the 42 women who received rosiglitazone, impaired glucose tolerance (IGT; 2 h glucose on OGGT of 7.78–11.1 mmol/l) was found in 15 (36%).

**Randomization**

Subjects were randomly assigned to daily rosiglitazone doses of 2 mg (n = 15), 4 mg (n = 11), or 8 mg (n = 16). As shown in Table I, the groups assigned to each of these doses did not differ at baseline in age or BMI; in metabolic assessments including SSPG, plasma glucose and insulin fasting and integrated on OGGT and meal profile, and prevalence of impaired glucose tolerance; or in reproductive hormone levels, including serum total testosterone, free testosterone, SHBG and LH.

**Compliance with protocol**

Of the 42 subjects who began rosiglitazone, three were removed from the study because of pregnancies detected after 9, 10 and 10 weeks of treatment. All these pregnancies were detected and rosiglitazone discontinued within a calculated maximum of 21 days after the time of ovulation. One of the pregnant subjects experienced a first-trimester spontaneous abortion and subsequently re-entered the study, completing the protocol; only data from her completed study are included in the analysis. The other two delivered healthy infants at or near term. One other subject failed to complete her 12 week metabolic and hormonal studies, and another was unable to have a repeat IST because of inadequate venous access. Compliance with blood draws for progesterone levels was 97%, and only one subject missed two consecutive weekly blood draws. Two other subjects failed to return their diaries. Rosiglitazone was well tolerated, and medication compliance exceeded 98% of prescribed doses; no subject discontinued rosiglitazone because of side-effects.

**Metabolic effects of rosiglitazone**

Subjects who completed the study did not differ among dose groups in baseline weight, serum ALT, blood pressure, or plasma lipoprotein levels (Table II). Treatment with each of the tested rosiglitazone doses for 12 weeks led to a significant decrease in SSPG, indicating decreased insulin resistance (Figure 1, top). The 8 mg dose produced a significantly greater decline in SSPG (by 4.17 mmol/l) than the lower doses (by 1.61 and 2.06 mmol/l). Fasting glucose and insulin were significantly lowered only by the 8 mg dose (Figure 1, middle row). Integrated day-long glycaemia on meal profile was reduced by 13% on the 8 mg dose (P < 0.0001; Figure 1, bottom left). Integrated day-long insulinemia was decreased by rosiglitazone in a dose-dependent fashion: the decrease with 8 mg daily (by 46%)
Baseline characteristics of study subjects assigned to each rosiglitazone dose

<table>
<thead>
<tr>
<th>Variable</th>
<th>2 mg</th>
<th>4 mg</th>
<th>8 mg</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>101.4 ± 3.7</td>
<td>100.8 ± 6.3</td>
<td>109.7 ± 7.2</td>
<td>96.2 ± 5.9</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>31.5 ± 1.7</td>
<td>31.5 ± 2.7</td>
<td>26.5 ± 1.8</td>
<td>32.4 ± 3.5</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>5.07 ± 0.14</td>
<td>5.14 ± 0.19</td>
<td>5.17 ± 0.28</td>
<td>4.91 ± 0.26</td>
</tr>
<tr>
<td>2 h glucose (OGTT) (mmol/l)</td>
<td>1.78 ± 0.20</td>
<td>2.16 ± 0.45</td>
<td>1.64 ± 0.28</td>
<td>1.47 ± 0.20</td>
</tr>
<tr>
<td>Total testosterone (nmol/l)</td>
<td>1.86 ± 0.14</td>
<td>1.90 ± 0.09</td>
<td>1.88 ± 0.09</td>
<td>1.90 ± 0.09</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>3.26 ± 0.11</td>
<td>3.26 ± 0.20</td>
<td>3.32 ± 0.18</td>
<td>3.23 ± 0.20</td>
</tr>
</tbody>
</table>

Values are mean ± SE.

SSPG = steady-state plasma glucose on insulin suppression test; OGTT = oral glucose tolerance test; MP = meal profile; AUC = area under curve.

Ovulation and vaginal bleeding during rosiglitazone treatment

Immediately before starting rosiglitazone, two of 42 subjects (5%) had post-ovulatory serum progesterone levels (≥2.7 mmol/l). In 16 subjects (termed ‘non-ovulators’), all progesterone levels from 4 to 12 weeks of rosiglitazone were <6.36 mmol/l. Three subjects’ ovulation status was indeterminate; two of these had a single progesterone elevation to between 6.36 and 12.7 mmol/l during treatment, and the third missed two consecutive weekly tests. In the remaining 23 subjects (55% of the total treated), at least one ovulation was detected during treatment; the prevalence of ovulators among subjects taking each rosiglitazone dose is shown in Table III.

The timing of all detected ovulations is shown in Figure 3 (top panel). First detected ovulation events occurred between weeks 4 and 11, with the timing not affected by rosiglitazone dose (not shown). A total of 33 ovulations were detected by progesterone assays, with second ovulations in nine subjects (47% of the 19 women with first ovulation at or before 8 weeks) and a third ovulation in one. Second ovulations were detected a median of 5 (range 4–7) weeks after the first. Two additional subjects had apparent impending second ovulations at 12 weeks, indicated by serum LH elevation to 30 and 33 IU/l, while another ovulator had a borderline progesterone level of 10.5 mmol/l at 12 weeks. The mean luteal progesterone level measured in ovulatory cycles was 28.0 mmol/l. All ovulations were greater than that with 2 mg daily (by 17%, P < 0.05; Figure 1, bottom right).
detected at or before 10 weeks were followed by either reported menses or pregnancy.

The timing of episodes of bleeding described as greater than spotting is shown in Figure 3 (bottom panel). Six subjects had bleeding episodes documented by progesterone levels as anovulatory, two of whom were ovulators. Of the 22 ovulators who returned their menstrual diary, 14 (64%) had a bleeding episode which began after 14–30 days of rosiglitazone (termed ‘early bleeding’). Early bleeding began after a median of 24 (range 16–28) days of treatment and lasted 2–6 days; the subsequent menses began 32 (median; range 16–50) days later, always after a detected ovulation. Of the 16 non-ovulators, only three (19%) had early bleeding ($P < 0.01$ for prevalence, compared to ovulators). Two of these three also had a subsequent bleed documented as anovulatory. The incidence of bleeding beginning after 14–30 days of rosiglitazone (17/42, 40%) exceeded that of bleeding in the first 14 days of treatment (4/42, 10%; $P < 0.01$).

**Effects of rosiglitazone on LH and testosterone**

Serum LH was unaltered by rosiglitazone for 4, 8 or 12 weeks either in the entire study group or in any dose group (data not shown). Neither total nor free serum testosterone was significantly changed by rosiglitazone in the entire study group (Figure 4, top row) or in any dose group (not shown). In the entire study group (Figure 4, top right) and in subjects taking 8 mg rosiglitazone (not shown), SHBG levels rose significantly at 4 weeks and remained higher than baseline at 8 and 12 weeks.

**Differences between ovulators and non-ovulators**

At baseline (Table IV), when compared with non-ovulators, ovulators had fasting plasma insulin 34% lower, 2 h and integrated insulin on OGTT both 47% lower, and day-long glucose and insulin on meal profile 11 and 39% lower, respectively. The ovulators also had lower free testosterone and higher SHBG than the non-ovulators. A trend to higher serum LH in ovulators did not reach significance ($P = 0.08$). At baseline, there was no significant difference between the two groups in age, BMI, SSPG, plasma glucose either fasting or integrated on OGTT, prevalence of IGT, or serum total testosterone.

After 12 weeks of rosiglitazone treatment (Table V), ovulators showed a trend ($P = 0.07$) toward lower SSPG than non-ovulators. While SSPG declined significantly with rosiglitazone in both groups, its decline was greater in ovulators ($P < 0.05$). After treatment, ovulators had lower day-long insulin (by 33%) than non-ovulators; the decline in insulinemia with treatment did not differ between the groups. As shown in Figure 4 (middle
row), in ovulators both total and free testosterone declined after 4 weeks of rosiglitazone and were lower than in non-ovulators, in whom no changes occurred. In ovulators, the decline in free but not total testosterone persisted at 8 weeks, but at 12 weeks both total and free testosterone were indistinguishable from baseline. In ovulators, SHBG during rosiglitazone treatment was increased over baseline at 4, 8 and 12 weeks and was higher than in non-ovulators at all three times; in non-ovulators, a significant increase in SHBG was found only at 4 and 8 weeks (Figure 4, middle right).

**Table III.** Prevalence of ovulatory response to rosiglitazone, by dose

<table>
<thead>
<tr>
<th>Dose group</th>
<th>n</th>
<th>Ovulators</th>
</tr>
</thead>
<tbody>
<tr>
<td>All doses</td>
<td>42</td>
<td>23 (55)</td>
</tr>
<tr>
<td>2 mg</td>
<td>15</td>
<td>6 (40)</td>
</tr>
<tr>
<td>4 mg</td>
<td>11</td>
<td>6 (55)</td>
</tr>
<tr>
<td>8 mg</td>
<td>16</td>
<td>11 (69)</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages. The prevalence of ovulators does not differ significantly between dose groups ($P = 0.11$, $\chi^2$ for trend).

**Differences among ovulators in response to rosiglitazone**

Although not specifically demonstrated, early bleeding (after 14–30 days of treatment) in women who subsequently had a detected ovulation while taking rosiglitazone suggests the occurrence of early, treatment-dependent ovulation prior to the first scheduled weekly progesterone assay. We compared the
Thiazolidinediones are insulin sensitizers which are ligands of PPAR-γ with mechanisms of action distinct from metformin (Fonseca et al., 2000). In women with PCOS, the first marketed TZD, troglitazone, showed efficacy in short-term studies similar to that of metformin (Dunaif et al., 1996; Ehrmann et al., 1997b). In the largest reported study (Azziz et al., 2001), troglitazone reduced circulating testosterone and insulin and promoted ovulation in dose-dependent fashion. Now that troglitazone is unavailable, the efficacy in PCOS of the newer TZD rosiglitazone is of great interest.

Reported here is a 12 week, open-label, dose-finding study of rosiglitazone in 42 insulin-resistant women with PCOS. Endpoints encompassed ovarian function, including testosterone levels, ovulation, and menstrual pattern; insulin resistance, insulinemia, and glycaemia; weight, serum ALT, blood pressure, and plasma lipoproteins. At randomization, the three dose groups were shown to be statistically indistinguishable on all metabolic and reproductive hormonal parameters assessed.

In this study, insulin resistance was determined by the octreotide-modified insulin suppression test (IST) (Pei et al., 1994), which measures insulin-mediated glucose disposal principally by skeletal muscle. The SSPG is an index of insulin resistance derived from the IST which is correlated in non-diabetics with measures derived from the euglycaemic clamp (Greenfield et al., 1981). We chose the IST because it is simple to perform and provides a measure of insulin resistance that is stable in individuals over time (Facchini et al., 1999). A previous study (Yeni-Komshian et al., 2000) had revealed the continuous distribution of SSPG in a non-diabetic adult volunteer population in our community, and for the present study an SSPG threshold (10 mmol/l) was chosen at the upper tertile of these volunteers. This study (Yeni-Komshian et al., 2000) also showed a correlation in non-diabetics between SSPG and day-long insulinemia, as determined by the mixed-meal profile (MP) test used in this study. We chose meal profile rather than oral glucose tolerance testing to assess changes in integrated insulinemia with rosiglitazone because MP is more physiological and reproducible.

Our recruited subjects were metabolically similar to those in survey studies of PCOS (Ehrmann et al., 1999; Legro et al., 1999), with 36% of those with SSPG >10 mmol/l having impaired glucose tolerance and 3% undiagnosed diabetes by glucose tolerance test criteria. Using our prospectively set SSPG threshold, we characterized 58% of our non-diabetic subjects as significantly insulin resistant, a proportion consistent with literature estimates (Dunaif, 1997).

In the present study, rosiglitazone decreased insulin resistance and day-long insulinemia and glycaemia most effectively at the 8 mg daily dose, with a significantly lesser metabolic effect of the lower doses. This dose-dependence parallels that reported for improvement of glycaemic control in diabetics (Nolan et al., 2000; Phillips et al., 2001). The reduction of day-long insulinemia on the 8 mg dose is of the same relevance to patients with PCOS that have insulin resistance and impaired glucose tolerance.

**Discussion**

Although the best definition of polycystic ovary syndrome continues to be debated (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004; Azziz, 2005), the present study recruited women fitting the NICHD ‘consensus’ definition of PCOS, which includes irregular menses, indicating an ovulation disorder, along with ovarian hyperandrogenism (Zawadzki and Dunai, 1992). Many, but not all, women with PCOS have a significant degree of insulin resistance and compensatory hyperinsulinemia (Dunaif, 1997). While the majority of previous studies of insulin sensitizers, chiefly metformin, in PCOS enrolled women regardless of metabolic subphenotype, studies have been reported (Nestler and Jakubowicz, 1997; Ibanez et al., 2001) of non-obese women, who are presumptively more insulin sensitive, and of insulin-sensitive women (Bailargeon et al., 2004). The present study prescreened subjects and treated only insulin-resistant women with rosiglitazone in order to maximize the potential for metabolic improvement.

Figure 3. Timing of detected ovulation and vaginal bleeding with rosiglitazone treatment. In the top panel, the week of treatment at the end of which a progesterone level >12.7 nmol/l was first measured is shown for each detected ovulatory event in study subjects. In the bottom panel, the week in which each reported episode of vaginal bleeding began is shown. In both panels, the open bars denote first ovulations or initiations of bleeding, the stippled bars second such events for each subject, and the solid bars third such events.
magnitude as the reduction of insulinaemia on OGTT reported in PCOS with troglitazone at 600 mg (Azziz et al., 2001). The small but significant weight gain with rosiglitazone also parallels reported effects in diabetics (Phillips et al., 2001). The small but significant reduction in ALT with rosiglitazone in the present study may reflect a reduction of subclinical steatohepatitis, as has been reported to occur with troglitazone and metformin in studies not limited to women with PCOS (Caldwell et al., 2001; Marchesini et al., 2001). The finding of baseline ALT elevation (>150% of the upper limit of normal) in 6% of screened women with PCOS parallels a recent report (Schwimmer et al., 2005) and points to the value of routine transaminase screening of women with PCOS.

We found modest but significant improvement with rosiglitazone in plasma HDL- and LDL-cholesterol, but not triglycerides, when the entire study group was considered. Previous studies of rosiglitazone have shown non-significant trends towards lipoprotein improvement in women with PCOS (Ghazeeri et al., 2003; Dereli et al., 2005), and significant improvement in HDL- and LDL-cholesterol, but a trend toward increased triglycerides, in type 2 diabetics (Goldberg et al., 2005). A decline in systolic but not diastolic pressure in PCOS.
Comparison of baseline metabolic and reproductive hormone status in ovulators and non-ovulators

### Table IV. Comparison of baseline metabolic and reproductive hormone status in ovulators and non-ovulators

<table>
<thead>
<tr>
<th></th>
<th>Ovulators</th>
<th>Non-ovulators</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>23</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>30.5 ± 1.0</td>
<td>27.9 ± 1.6</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>37.0 ± 1.6</td>
<td>38.8 ± 1.9</td>
<td>NS</td>
</tr>
<tr>
<td>SSPG (mmol/l)</td>
<td>14.27 ± 0.38</td>
<td>14.76 ± 0.56</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>5.20 ± 0.08</td>
<td>5.49 ± 0.15</td>
<td>0.07</td>
</tr>
<tr>
<td>2 h glucose (OGTT) (mmol/l)</td>
<td>7.20 ± 0.29</td>
<td>7.32 ± 0.33</td>
<td>NS</td>
</tr>
<tr>
<td>Impaired glucose tolerance (%)</td>
<td>35</td>
<td>37</td>
<td>NS</td>
</tr>
<tr>
<td>AUC glucose (OGTT) (h * mmol/l)</td>
<td>21.69 ± 0.67</td>
<td>22.66 ± 0.90</td>
<td>NS</td>
</tr>
<tr>
<td>AUC glucose (MP) (h * mmol/l)</td>
<td>44.69 ± 1.26</td>
<td>50.41 ± 1.76</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)</td>
<td>170 ± 22</td>
<td>257 ± 29</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td>2 h insulin (OGTT) (pmol/l)</td>
<td>1064 ± 180</td>
<td>1998 ± 298</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>AUC insulin (OGTT) (h * pmol/l)</td>
<td>2856 ± 427</td>
<td>5350 ± 786</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>AUC insulin (MP) (h * pmol/l)</td>
<td>3405 ± 339</td>
<td>5620 ± 736</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Total testosterone (nmol/l)</td>
<td>1.48 ± 0.13</td>
<td>1.74 ± 0.09</td>
<td>NS</td>
</tr>
<tr>
<td>Free testosterone (pmol/l)</td>
<td>30.5 ± 3.4</td>
<td>40.8 ± 3.0</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>22.1 ± 2.4</td>
<td>15.1 ± 2.1</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>7.2 ± 1.0</td>
<td>5.3 ± 0.4</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Values are mean ± SE.

SPPG = steady-state plasma glucose on insulin suppression test; OGTT = oral glucose tolerance test; MP = meal profile; AUC = area under curve.

NS: P > 0.10.

### Table V. Comparison of metabolic status in ovulators and non-ovulators after rosiglitazone treatment

<table>
<thead>
<tr>
<th></th>
<th>Ovulators</th>
<th>Non-ovulators</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>21</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>37.4 ± 1.8*</td>
<td>39.5 ± 2.0**</td>
<td>NS</td>
</tr>
<tr>
<td>SSPG (mmol/l)</td>
<td>11.04 ± 0.68****</td>
<td>13.11 ± 0.86*</td>
<td>0.07</td>
</tr>
<tr>
<td>Δ SSPG (mmol/l)</td>
<td>3.30 ± 0.53</td>
<td>1.60 ± 0.65</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>4.72 ± 0.14</td>
<td>5.41 ± 0.25</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td>AUC glucose (MP) (h * mmol/l)</td>
<td>41.71 ± 1.44*</td>
<td>46.60 ± 2.24</td>
<td>0.07</td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)</td>
<td>143 ± 26</td>
<td>197 ± 23</td>
<td>NS</td>
</tr>
<tr>
<td>AUC insulin (MP) (h * pmol/l)</td>
<td>2503 ± 328***</td>
<td>3752 ± 474***</td>
<td>&lt; 0.04</td>
</tr>
<tr>
<td>Δ AUC insulin (h * pmol/l)</td>
<td>1051 ± 225</td>
<td>1868 ± 445</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean ± SE.

SSPG = steady-state plasma glucose on insulin suppression test; MP = meal profile; AUC = area under curve.

Δ denotes change from baseline.

P-Values are shown for comparisons between the two groups at 12 weeks. NS: P > 0.10. Asterisks indicate significant differences from baseline within each group after rosiglitazone treatment (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001).

with rosiglitazone has previously been reported (Baillargeon et al., 2004).

By intention-to-treat analysis, the overall prevalence in our study of at least one detected ovulation during rosiglitazone treatment, 55%, is comparable to that reported for women with PCOS taking metformin (Costello and Eden, 2003; Lord et al., 2003) or troglitazone (Azziz et al., 2001) as monotherapy. Our findings are also consistent with other studies of rosiglitazone (Ghazeeri et al., 2003; Baillargeon et al., 2004; Belli et al., 2004; Sepilian and Nagamani, 2005; Dereli et al., 2005) and pioglitazone (Brettenthaler et al., 2004). While pre-treatment ovulatory frequency was not directly measured in the present study, selection requiring a history of oligomenorrhea or amenorrhea (NICHD criteria) yielded a group of subjects of whom only two (5%) had luteal-range progesterone levels at baseline; this suggests an untreated ovulation incidence per month of ~10%. A population-based estimate of the incidence of ovulation in women with PCOS defined by NIH criteria has not been reported (Legro, 2003); but because some women with androgen excess are regularly ovulatory (Azziz et al., 2004), it cannot be assumed that over 12 weeks the prevalence of ovulators in our population left untreated would approach 30%. A more reasonable estimate for this prevalence would be 15–20%. Employing the binomial distribution, the 95% confidence interval (39–71%) around the observed ovulation prevalence of 55% during rosiglitazone treatment clearly allows rejection of the null hypothesis of a 15–20% prevalence.

While there was a trend towards a higher prevalence of ovulation on the higher rosiglitazone doses, with the present sample size the ovulation rates observed on the three doses tested were statistically indistinguishable. Other studies have employed daily doses of 2 mg (Dereli et al., 2005), 4 mg (Belli et al., 2004; Sepilian and Nagamani, 2005; Dereli et al., 2005) or 8 mg (Ghazeeri et al., 2003; Baillargeon et al., 2004), with favourable effects of all three doses found on menstrual pattern or ovulation. The occurrence of ovulations on the 2 mg and 4 mg doses in the present study in association with only modest changes in insulin resistance and insulinaemia suggests either that a small metabolic improvement is sufficient to promote pre-ovulatory follicular maturation or that rosiglitazone exerts
its effect, at least in part, independently of insulin. In support of a direct action of rosiglitazone on the ovary, other PPAR-γ agonists have been reported to inhibit thecal androgen and granulosa–luteal progesterone production in vitro (Gasic et al., 1998; Mitwally et al., 2002; Veldhuis et al., 2002).

Ovulation, once initiated with rosiglitazone, continued in a monthly pattern in about half of the participants, without regard to rosiglitazone dose. This lack of universal restoration of consistent ovulation following insulin reduction has also been reported with metformin (Fleming et al., 2002; Baillargeon et al., 2004). Unlike with metformin (Ibanez et al., 2001; Fleming et al., 2002), however, with short-term rosiglitazone treatment almost all the evaluable vaginal bleeding episodes were preceded by ovulation.

In our entire study population, total and free testosterone levels were not significantly lowered by rosiglitazone. While some previous studies of metformin (Pasquali et al., 2000; Baillargeon et al., 2004), troglitazone (Azziz et al., 2001) and rosiglitazone (Baillargeon et al., 2004; Sepilian and Nagamani, 2005; Dereli et al., 2005) have found reductions of total or free testosterone or both, our findings are consistent with other reported studies of rosiglitazone (Ghazeeri et al., 2003; Belli et al., 2004) and pioglitazone (Romualdi et al., 2003; Brettenthaler et al., 2004). Taken together, these findings suggest that the newer TZD may act differently from the other two insulin sensitizers. A possible explanation for the discrepant reported effects of rosiglitazone on testosterone may lie in the duration of treatment (reduction was seen after 6–8 months, but not 3 months) or in the degree of insulin resistance in the women studied. The increase in SHBG levels on rosiglitazone is consistent with release of the inhibitory influence of insulin on hepatic SHBG production (Pugeat et al., 1991). Our finding of unchanged serum LH with rosiglitazone is in agreement with one report (Sepilian and Nagamani, 2005) but contrasts with the finding by others of a modest decline in LH (Ghazeeri et al., 2003; Belli et al., 2004; Dereli et al., 2005). In this regard, differing criteria for patient selection may play a role, given the reported negative association between insulin resistance and LH elevation in PCOS (Mor et al., 2004).

Analysis of those subjects who ovulated on rosiglitazone in this study reveals a decline with treatment in both total and free testosterone, observed at 4 and 8 weeks but not at 12 weeks. In view of the persistence of the SHBG increase through 12 weeks, this testosterone reduction appears to be of ovarian origin rather than merely a consequence of down-regulation of SHBG by insulin. An explanation for the waning reduction of testosterone after 12 weeks of rosiglitazone, despite the observed decline in insulin levels at this time, may lie in a possible loss of direct inhibition by rosiglitazone of thecal androgen production (Veldhuis et al., 2002) or in an increase in thecal stimulation by LH. Although not detected by the single serum LH assays performed every 4 weeks in this study, an increase in day-long LH levels cannot be excluded by our data.

Although not the outcome of a planned analysis, the effect of rosiglitazone to transiently depress serum testosterone was found to be restricted to a subgroup of ovulators who experienced early vaginal bleeding with a timing suggestive of ovulation during the first 2 weeks of treatment. While the ovulators who experienced this early bleeding appear to have responded robustly to rosiglitazone, achieving both ovulation and a decline in testosterone by 4 weeks of treatment, the latter might have occurred as the consequence of the former (Taylor et al., 1997). Against this possibility, however, is that no similar decline in testosterone was observed following the first detected ovulation in those ovulators without early bleeding.

The ovulators in this study had lower insulin levels than the non-ovulators, both before and after rosiglitazone treatment. In the non-ovulators, mean day-long insulinaemia declined after 12 weeks of treatment but failed to reach even the mean baseline level of the ovulator group. While the ovulatory response to rosiglitazone may depend on suppression of day-long insulinaemia below a critical level, it is also possible that sufficiently low insulin levels are merely permissive for the promotion by rosiglitazone of ovarian follicle maturation; lower intraovarian androgen levels or a direct action of rosiglitazone on the ovary may also play a role. The finding of lower insulin levels in ovulators parallels previous studies of troglitazone (Azziz et al., 2001) and metformin (Pirowsky et al., 1999), but contradicts another study of metformin (Moghetti et al., 2000).

At baseline, ovulators had lower free testosterone and higher SHBG than non-ovulators, with a trend to lower total testosterone as well. This finding parallels that of the large troglitazone study (Azziz et al., 2001). The greater efficacy of rosiglitazone in promoting ovulation in the presence of lower androgenemia, taken together with the demonstrated efficacy of rosiglitazone in reducing insulinaemia, suggests that insulin excess and androgen excess may be distinct functional blocks to ovulation in PCOS, such that for ovulation to occur, both insulin and androgen levels must be sufficiently low.

The mechanism whereby hyperinsulinaemia leads to ovulation failure in PCOS is not well understood, but may primarily involve promotion of androgen excess. Insulin is a well-established stimulus to androgen secretion by the ovarian theca, and in so doing may act both through its own receptor (Nestler et al., 1998) and through an increase in circulating unbound insulin-like growth factors (IGF) mediated by a reduction of circulating IGF-binding protein (IGFBP)-1 (van Dessel et al., 1999). Androgen excess may, in turn, act within the ovary to contribute to ovulatory failure, possibly by promoting the excessive recruitment of preantral follicles, resulting in an excess of small antral follicles (Hughesdon, 1982; Vendola et al., 1999; Webber et al., 2003; Maciel et al., 2004). While previous studies of metformin and troglitazone have found concomitant reductions of insulin and testosterone in their study populations, and so have failed to dissociate androgen reduction from restoration of ovulation, our finding with rosiglitazone suggests that insulin reduction without androgen reduction may promote ovulatory follicular maturation in some women with PCOS.

The fecundity of ovulatory cycles on rosiglitazone monotherapy is as yet unknown. Mean luteal serum progesterone in this study appears adequate, and the occurrence of three pregnancies among 23 ovulators (9% of ovulatory cycles), despite instructions to contracept, also suggests that luteal function is not significantly impaired by rosiglitazone. It is noteworthy that all three subjects who conceived experienced early bleeding
prior to their conceptional cycle. Given the lack of teratogenicity of rosiglitazone in laboratory species (Glaxo SmithKline, 2001), the use of rosiglitazone in women seeking pregnancy appears justified in controlled clinical settings in which patients are carefully monitoring their cycles and can discontinue the drug within a week of their missed menses.

There are some limitations to our study design. The study would have been more robust with inclusion of a placebo arm and dose blinding; the actual design was selected because the expense of overencapsulation of rosiglitazone tablets would have necessitated a severe reduction in sample size. An increase in the sample size could have improved power to detect differences in ovulation rate between dose groups; however, 46 women in each group would be needed to have 80% power to find a significant difference between the observed prevalences of ovulation in the 2 mg and 8 mg groups. An observational run-in period prior to treatment could have better assessed the incidence of spontaneous ovulation. Despite these limitations, the study found significant metabolic changes with rosiglitazone in all dose groups, significant androgen reduction limited to ovulators, and significant differences between ovulatory responders and non-responders to rosiglitazone in both metabolic and androgen assessments at baseline as well as after treatment.

In summary, rosiglitazone is a well-tolerated agent effective in improving insulin sensitivity, reducing insulinemia, and promoting ovulation in insulin-resistant women with PCOS. The full range of benefits is seen at a daily dose of 8 mg. The effects of rosiglitazone are similar but not identical to those of metformin; side-effects were not found to limit compliance. Given the apparent lesser effectiveness of rosiglitazone than metformin in reducing serum testosterone, rosiglitazone treatment appears well suited not to a woman primarily seeking reduction of hyperandrogenic symptoms, but rather to one who is seeking fertility and/or has insulin excess with only modest serum testosterone elevation. Restoration of ovulation in women with more severe insulin excess coupled with greater androgen excess may require longer treatment, combination sensitizer therapy, or combinations of sensitizers and conventional ovulation induction agents. The development of criteria for the individualization of such treatment, as well as the rational use of rosiglitazone in less-insulin-resistant women with PCOS, who were not the subject of this study, will be possible only after appropriately designed specific studies.

Acknowledgements

This work was supported by NICHD R03 HD39826 (to N.A.C.) and by the Stanford General Clinical Research Center (NIH 5M01 RR000070), whose entire staff is gratefully acknowledged for their extensive assistance. The assistance of Drs James Chu and Christian Tuan; Cynthia Lamendola, MSN, RNP; Gail Wu; Annie Halstead; and Kathy Turner is greatly appreciated. The support of the staff of the Reproductive Endocrinology Laboratory is also gratefully acknowledged.

References


Nestler JE, Jakubowicz DJ, de Vargas AF, Brik C, Quintero N and Medina F


Submitted on April 30, 2005; resubmitted on July 29, 2005; accepted on August 5, 2005.