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Endocrine and metabolic effects of rosiglitazone in overweight women with PCOS: a randomized placebo-controlled study

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BACKGROUND: The objective of the study was to assess the therapeutic effects of rosiglitazone in overweight women with polycystic ovary syndrome (PCOS). METHODS: A double-blind, placebo-controlled study was conducted on 30 (BMI > 25 kg/m^2, mean age 29.1 ± 1.2 years) overweight women with PCOS treated with rosiglitazone or placebo for 4 months. Waist-to-hip ratios (WHRs), serum concentrations of sex hormones and binding proteins, blood glucose, serum insulin and serum C-peptide during a 75-g oral glucose tolerance test (OGTT), first-phase insulin secretion as determined by an intravenous glucose tolerance test (IVGTT), *M* values (expressing insulin sensitivity using a euglycaemic clamp) and calorimetric data were assessed at 0 and 4 months of treatment. RESULTS: Rosiglitazone improved menstrual cyclicity, increased serum sex hormone-binding globulin (SHBG) levels and decreased serum levels of androstenedione, 17-hydroxyprogesterone (17-OHP), dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulphate (DHEA-S). Glucose tolerance [expressed as $AUC_{glucose}$ during the OGTT] improved (*P* = 0.002) and peripheral insulin response (expressed as $AUC_{insulin}$) decreased (*P* = 0.004) in the rosiglitazone group (ROSI group). *M* value improved in the ROSI group from 33.4 ± 3.27 to 40.0 ± 5.51 μmol/kg min (*P* = 0.04). CONCLUSION: Rosiglitazone, by improving menstrual cyclicity, hyperandrogenism, insulin resistance and hyperinsulinaemia, represents an alternative treatment for overweight anovulatory women with PCOS and no pregnancy desire.

Key words: insulin resistance/PCOS/rosiglitazone

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrinopathy among women of reproductive age (Azziz *et al*., 2004). It is characterized by menstrual abnormalities, symptoms of hyperandrogenism such as hirsutism and acne, enlarged, rounded ovaries with numerous subcapsular follicles, and infertility (Franks, 1995). Abdominal obesity, hyperinsulinaemia and insulin resistance have been shown to play a central role in the pathogenesis of PCOS (Burghen *et al*., 1980; Dunaif *et al*., 1989), which predisposes these women to risks of type 2 diabetes mellitus (DM) and cardiovascular disease (Dunaif *et al*., 1987; Guzick, 1996).

Two classes of insulin-lowering drugs are currently available for the treatment of insulin resistance in PCOS: the biguanide metformin and the thiazolidinediones. Metformin has been widely used in the treatment of PCOS and has been shown to improve the metabolic and hormonal disturbances of PCOS (Velazquez *et al*., 1994; Diamanti-Kandarakis *et al*., 1998; Moghetti *et al*., 2000), but its exact mechanism of action is still under debate. It lowers blood glucose mainly by increasing the intestinal use of glucose, enhancing insulin sensitivity and peripheral glucose uptake and inhibiting hepatic glucose production (Kirpichnikov *et al*., 2002). Moreover, results of some studies on subjects with type 2 DM and PCOS have suggested that the antihyperglycaemic effect of metformin might primarily be the result of decreased release of free fatty acids (FFAs) from adipose tissue, i.e. decreased lipolysis (Abbasi *et al*., 1997; Morin-Papunen *et al*., 2000; Pasquali *et al*., 2000).

Recently, rosiglitazone, a peroxisome-proliferator-activated receptor-γ (PPRAγ) agonist belonging to the thiazolidinedione group, has attracted increasing interest in the treatment of type 2 DM. Unlike metformin, rosiglitazone is a 'true' insulin sensitizer, which binds to and activates PPRAγ, a hormone receptor located in the cell nucleus (Lehmann *et al*., 1995). Rosiglitazone acts by increasing insulin sensitivity in a novel manner through its effects on PPRAγ. By this mechanism, it influences the transcription of a variety of genes implicated in carbohydrate and lipid homeostasis, thereby increasing peripheral glucose uptake and improving pancreatic β-cell function (either directly or through reduction of insulin resistance). In diabetic subjects, rosiglitazone significantly reduces plasma levels of glucose by improving insulin sensitivity and enhances insulin

effects at peripheral target sites such as skeletal muscle and adipose tissue, without any direct effect on pancreatic insulin secretion (Saltiel and Olefsky, 1996). In women with PCOS, rosiglitazone has also been shown to improve insulin sensitivity and ameliorate hyperinsulinaemia (Cataldo *et al*., 2001; Ghazeeri *et al*., 2003; Belli *et al*., 2004; Sepilian and Nagamani, 2005). However, only one of these studies has been randomized and placebo controlled (Baillargeon *et al*., 2004), and none has involved examination of insulin and glucose metabolism in detail, with calorimetry, the euglycaemic hyperinsulinaemic clamp technique and intravenous glucose tolerance tests (IVGTTs).

The aim of our study was to assess the effects of rosiglitazone therapy on clinical symptoms, insulin and glucose metabolism and hormonal parameters in overweight women with PCOS.

Subjects and methods

Subjects

Thirty women with PCOS (BMI > 25 kg/m^2, mean age 29.1 ± 1.2 years, range 18–41) were recruited from the Reproductive Endocrine Unit at Oulu University Hospital between February 2002 and April 2004. Criteria for PCOS were defined according to the new consensus criteria (Anonymous, 2004). All subjects had polycystic ovaries (in vaginal ultrasonography) and at least one of the following symptoms: oligomenorrhoea or amenorrhoea, clinical manifestations of hyperandrogenism, such as a hirsutism score of more than 7, according to Ferriman and Gallwey (1961), and/or an elevated serum testosterone level (>2.7 nmol/l). Diabetic subjects, subjects who had signs of liver or renal failure or active liver disease [alanine aminotransferase (ALT) > 2.5× the upper limit of normal values], smokers, alcohol users and those taking sex hormones or drugs known to affect lipid metabolism during the period of 2 months preceding the study were excluded.

The study was approved by the Ethics Committee of Oulu University Hospital, and informed written consent was obtained from each subject.

Protocol

Using computer-generated assignment, the subjects were randomly and blindly allocated to either a placebo group (PLA group) or a rosiglitazone group (ROSI group) (Avandia®, GlaxoSmithKline, Philadelphia, PA, USA: 4 mg once daily for 2 weeks, then 4 mg twice daily for 4 months) (Figure 1). This dosage is the most commonly used dosage in diabetic patients (Raskin *et al*., 2000; Lebovitz *et al*., 2001; Phillips *et al*., 2001). All subjects were examined 1–7 days after spontaneous or progestin-induced (oligomenorrhoeic and amenorrhoeic subjects) menstruation before the treatment and after 4 months of the treatment. The aim of using progestin in these subjects was to avoid examinations (ultrasonography and hormone assays) during a spontaneous luteal phase. We used dydrogesterone (10 mg/day for 10 days), which has only a negligible effect on insulin sensitivity (Cucinelli *et al*., 1999). Furthermore, to assure a minimal progestin effect, the examinations were performed at least 7 days after the last progestin pill intake.

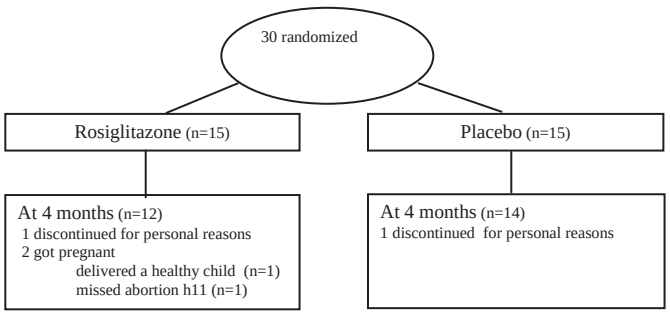

Figure 1. Flow chart of the study.

Because rosiglitazone is a category C drug that has been shown to retard fetal development in animal studies, pregnancy was contraindicated during the treatment. All the subjects were advised to use some form of non-hormonal contraception during the study. They were also advised not to modify their usual eating or exercise habits throughout the study. The weight of the subjects, fasting glucose, haemoglobin, hematocrit, liver enzymes [ALT and alkaline phosphatase (ALP)] and urinary pregnancy test results were assessed monthly during the study.

Clinical parameters and ultrasonography

Waist and hip circumferences were measured to the nearest centimetre with a soft tape at the narrowest part of the torso and the widest part of the gluteal region. Transvaginal ultrasonography (General Electric RT-X200, Milwaukee, WI, USA, with a 6.5-MHz probe) was carried out to measure ovarian volumes and the number of follicles. Volume determinations were carried out using the formula for the volume of an ellipsoid: 0.523 × length × width × thickness (Robert *et al*., 1995).

Oral glucose tolerance test

After an overnight fast of 10–12 h, all subjects underwent an oral glucose tolerance test (OGTT) (a load of 75 g of glucose in 300 ml of water). Venous blood samples for blood glucose, serum insulin and serum C-peptide assays were drawn at 0, 15, 30, 60 and 120 min. A glycaemic response to the OGTT was defined according to World Health Organization (WHO) criteria of 1999 (WHO, 1999). A diagnosis of impaired fasting glycaemia (IFG) or impaired glucose tolerance (IGT) was assigned if the fasting glucose level was between 6.1 and 6.9 mmol/l or if the glucose level at 2 h was between 7.8 and 11.1 mmol/l, respectively (WHO, 1999).

The incremental insulin ($AUC_{insulin}$) and glucose ($AUC_{glucose}$) areas under the curve (AUCs) were calculated by the trapezoidal method.

IVGTT

First-phase insulin secretion capacity was determined by an IVGTT. At 8 a.m. after a 12-h overnight fast, an intravenous catheter was placed in an antecubital vein for infusion of glucose. Another cannula for blood sampling was inserted into a wrist vein surrounded by a heated box (40°C). After baseline blood collection and measurement of gas exchange (see *Calorimetry*), a bolus of glucose (300 mg/kg in a 50% solution) was administered (within 60 s) into the antecubital vein to increase the blood glucose level acutely. Samples for the measurement of blood glucose and serum insulin were drawn at –5, 0, 2, 4, 6, 8 and 10 min. The acute insulin response was calculated as the ratio of the increment of serum insulin (pmol/l) to that of blood glucose (mmol/l) during the 10 min of IVGTT.

Euglycaemic hyperinsulinaemic clamp

The euglycaemic hyperinsulinaemic clamp technique was used for the assessment of insulin peripheral sensitivity (DeFronzo *et al*., 1979). After the IVGTT, a priming dose of insulin infusion (Actrapid, 100 IU/ml; Novo Nordisk, Gentofte, Denmark) was administered during the initial 10 min to raise the serum insulin concentration acutely to the desired level, where it was maintained by continuous insulin infusion (80 mU/m^2 body surface area per minute). The blood glucose level was clamped at 5 mmol/l for the next 180 min by adjusting the

rate of 20% glucose infusion according to blood glucose measurements performed every 5 min using a photometric assay (HemoCue AB, Ängelholm, Sweden). The *M* value (expressed as μmol/kg min) was calculated as the mean value for each 20-min interval during the last 60 min of the clamping. The higher the *M* value, the better the peripheral insulin sensitivity. As it has been previously shown that in nondiabetic hyperandrogenic subjects endogenous glucose production is negligible at this insulin infusion rate, the amount of glucose infused may be considered to be equivalent to whole-body glucose uptake, i.e. whole-body glucose disposal (Moghetti *et al*., 1996). Blood samples for the assay of serum lactate, insulin and FFAs were drawn at 0, 120, 140, 160 and 180 min.

Calorimetry

Indirect calorimetry was performed with a computerized flow-through canopy gas analyser system (Deltatrac®, TM Datex, Helsinki, Finland) in connection with the euglycaemic clamp, as previously described (Laakso *et al*., 1988). This device has a precision of 2.5% for O_2 consumption and 1.0% for CO_2 production. On the day of the experiment, gas exchange (O_2 consumption and CO_2 production) was measured for 30 min after a 12-h fast before and during the last 30 min of the clamping. The values obtained during the first 10 min of both time periods were discarded, and the mean value for the remaining 20 min of data was used for calculation. Protein, glucose and lipid oxidations were calculated according to Ferrannini (1988). Protein oxidation was calculated on the basis of the urinary nonprotein nitrogen excretion rate (Ferrannini, 1988). The fraction of carbohydrate nonoxidation during the euglycaemic clamp was estimated by subtracting the carbohydrate oxidation rate (determined by indirect calorimetry) from the glucose infusion rate (determined by the euglycaemic clamp).

Assays and calculations

Blood samples were processed by centrifugation, and serum was stored at –20°C until assayed.

The concentrations of sex hormone-binding globulin (SHBG), LH and FSH were analysed by fluoroimmunoassays (Wallac, Turku, Finland), and radioimmunoassays were used for dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulphate (DHEA-S), androstenedione and 17-hydroxyprogesterone (17-OHP) (Diagnostic Products Corporation, Los Angeles, CA, USA), following the instructions of the manufacturers. Concentrations of human serum insulin-like growth factor-binding protein-1 (IGFBP-1) were determined by immunoenzymometric assay using commercial reagents (Medix Biochemica, Kauniainen, Finland) and concentrations of testosterone, insulin and C-peptide by using an automated chemiluminescence system (Advia Centaur, Bayer Corporation, New York, NY, USA). The free androgen index (FAI) was calculated according to the following equation: (testosterone × 100)/SHBG, with testosterone and SHBG expressed as nmol/l. Levels of blood glucose and serum lactate, ALT and FFAs were determined by standard methods.

The intra- and inter-assay coefficients of variation were, respectively, 1.3% and 5.1% for SHBG, 4.9% and 6.5% for LH, 3.8% and 4.3% for FSH, 6.5% and 7.9% for DHEA, 5.3% and 7.0% for DHEA-S, 5.0% and 8.6% for androstenedione, 5.0% and 5.4% for 17-OHP, 5.3% and 7.2% for C-peptide, 4.0% and 5.1% for insulin, 4.3% and 7.5% for IGFBP-1, 4.7% and 6.6% for testosterone, 1.5% and 2.3% for blood glucose, 2.2% and 3.8% for lactate, 2.8% and 4.3% for ALT and 3.8% and 5.5% for FFAs.

Statistical methods

Where there were normally distributed variables (without or with logarithmic transformation), the paired *t*-test was used to compare the clinical, metabolic and hormonal parameter changes within the rosiglitazone and PLA groups during the treatment. Wilcoxon's unpaired test was used for variables with persisting skewed distribution after log transformation.

For comparison between the rosiglitazone and PLA groups before and at 4 months of treatment, two-tailed Student's *t*-test was used for normally distributed variables, either without or with log transformation. The Mann–Whitney *U*-test was used for variables with persisting skewed distribution after log transformation.

It was clear already before the study that it is difficult to obtain a sufficient sample size to reach enough power. In addition, there was only one case report on the effects of rosiglitazone in PCOS (Cataldo *et al*., 2001), but there were no studies using calorimetry and euglycaemic hyperinsulinaemic clamp. Thus, the sample size was based on our previous studies on metformin using the same investigation methods (Morin-Papunen *et al*., 1998, 2000).

Results

Baseline characteristics

At baseline, the ROSI and PLA groups did not differ with respect to age, BMI, waist-to-hip ratio (WHR), hirsutism score, menstrual cycle (Table I), fasting glucose and insulin (Table II), serum levels of SHBG, IGFBP-1, androstenedione, DHEA, 17-OHP, LH or FSH (Table III). Serum fasting C-peptide levels ($P = 0.04$) and serum testosterone levels ($P = 0.04$) were significantly higher, and serum DHEA-S concentrations ($P = 0.01$) were significantly lower in the PLA group. In the ROSI group, one subject had IFG, which normalized at 4 months of treatment. One of the subjects in the PLA group had IGT before the treatment, which had worsened at 4 months. The two groups were comparable with respect to *M* values, lipid and glucose oxidation and serum FFA levels.

Oligo/amenorrhoea and hyperandrogenism were diagnosed at baseline in 9 and 10 subjects in ROSI group and 10 and 13 subjects in PLA group, respectively.

Changes during the treatment

Clinical and hormonal parameters

The BMI had increased significantly ($P = 0.006$), and menstrual cycles had become more regular ($P = 0.005$) in the ROSI group at 4 months of treatment. The WHR and the hirsutism score did not change during either treatment (Table I).

Table I. Clinical parameters of the subjects before and during treatment

	Rosiglitazone		Placebo	
	Before ($n = 12$)	4 months ($n = 12$)	Before ($n = 14$)	4 months ($n = 14$)
Age (years)	26.7 ± 1.1		30.1 ± 2.1	
BMI (kg/m^2)	33.1 ± 1.7	34.1 ± 1.8*	33.6 ± 1.0	34.1 ± 1.2
Waist-to-hip ratio	0.87 ± 0.12	0.80 ± 0.06	0.88 ± 0.01	0.88 ± 0.02
Hirsutism	8.92 ± 0.9	8.45 ± 0.9	9.86 ± 1.5	10.29 ± 1.6
Menstrual cycle length (days)	125 ± 35.9	39 ± 7.5**,***	93 ± 24.4	65 ± 10.2**

Data are shown as mean ± SE.
*$P < 0.05$ compared with the level before treatment.
**$P < 0.05$ between rosiglitazone and placebo groups.
***$P < 0.01$ compared with the level before treatment.

Table II. Metabolic parameters of the subjects before and during treatment

	Rosiglitazone		Placebo	
	0 month (n = 12)	4 months (n = 12)	0 month (n = 14)	4 months (n = 14)
Fasting blood glucose (mmol/l)	5.4 ± 0.2	5.2 ± 0.1*	5.4 ± 0.1	5.5 ± 0.1*
Fasting insulin (pmol/l)	12.4 ± 1.9	12.9 ± 3.0	15.0 ± 2.6	17.2 ± 2.7
Fasting C-peptide (nmol/l)	0.4 ± 0.06*	0.3 ± 0.08**	0.2 ± 0.04*	0.3 ± 0.05**
First-phase insulin secretion (pmol/mmol)	8.6 ± 1.16	10.3 ± 1.65	7.6 ± 1.85	8.1 ± 2.30
Fasting glucose oxidation (μmol/kg/min)	10.5 ± 1.06	9.33 ± 0.88	9.85 ± 1.12	9.5 ± 1.02
Fasting				
FFAs (mmol/l)	0.53 ± 0.06	0.45 ± 0.04**	0.51 ± 0.45	0.53 ± 0.06
Lipid oxidation (mg/kg/min)	2.7 ± 0.45	2.9 ± 0.41	3.2 ± 0.41	3.4 ± 0.39
Clamp				
FFAs (mmol/l)	0.06 ± 0.01	0.06 ± 0.02	0.07 ± 0.02	0.15 ± 0.07
Lipid oxidation (mg/kg/min)	2.0 ± 0.41	0.7 ± 0.28*	2.6 ± 0.31	2.4 ± 0.35*

Data are shown as mean ± SE. Results concerning clamp and calorimetry are given for n = 10 in the rosiglitazone group and n = 14 in the placebo group. FFA, free fatty acid.
*P < 0.05 between rosiglitazone and placebo groups.
**P < 0.05 compared with the level before treatment.

Table III. Hormonal parameters of the subjects before and during treatment

	Rosiglitazone		Placebo	
	0 month (n = 12)	4 months (n = 12)	0 month (n = 14)	4 months (n = 14)
Testosterone (nmol/l)	2.7 ± 0.1*	2.7 ± 0.2	3.5 ± 0.3*	3.2 ± 0.3
Sex hormone-binding globulin (nmol/l)	30.3 ± 3.4	36.9 ± 5.2**	38.6 ± 5.3	36.2 ± 4.6
Free androgen index	10.3 ± 1.4	9.2 ± 1.6	11.4 ± 1.6	12.2 ± 2.5
Insulin-like growth factor-binding protein-1 (μg/l)	2.5 ± 0.5	3.3 ± 0.6	2.1 ± 0.3	2.2 ± 0.3
Androstenedione (nmol/l)	16.6 ± 1.8	13.9 ± 1.8**	16.3 ± 1.7	16.8 ± 1.5
Dehydroepiandrosterone (nmol/l)	58.2 ± 10.7	46.5 ± 9.0**	42.3 ± 5.7	46.2 ± 7.4
Dehydroepiandrosterone sulphate (μmol/l)	8.18 ± 0.9*	7.4 ± 1.3**	5.2 ± 0.7**	5.4 ± 0.8
17-Hydroxyprogesterone (nmol/l)	5.9 ± 1.1	5.06 ± 1.0**	4.7 ± 0.6	4.8 ± 0.6
LH (IU/l)	6.4 ± 0.8	6.2 ± 1.2	6.9 ± 0.9	7.3 ± 0.9
FSH (IU/l)	4.8 ± 0.4	4.9 ± 0.4	4.9 ± 0.4	5.2 ± 0.4

Data are shown as mean ± SE.
*P < 0.05 between rosiglitazone and placebo groups.
**P < 0.05 compared with the level before treatment.

Despite the requirement of use of contraception, two women became pregnant during rosiglitazone treatment. Both subjects stopped the medication when the pregnancy test result was positive. One of them delivered a healthy child, and the other woman had missed abortion at 10 gestational weeks, and pregnancy was terminated by evacuation and abrasion. Two women (one in each group) discontinued the study for personal reasons, and two subjects (both in the ROSI group) did not wish to undergo the clamp test at 4 months of treatment. None of the subjects reported drug-related adverse events (Figure 1).

Serum concentrations of testosterone, LH, FSH and IGFBP-1 did not change during either treatment. Serum SHBG levels increased significantly in the ROSI group (P = 0.04), and serum levels of androstenedione (P = 0.04), 17-OHP (P = 0.04), DHEA (P = 0.01) and DHEA-S (P = 0.05) decreased significantly during rosiglitazone treatment, but the FAI did not change in either group (Table III).

Glucose metabolism

Despite no significant changes during either treatment, fasting plasma glucose concentrations were significantly lower in the ROSI group than in the PLA group at 4 months of treatment (P = 0.03). Glucose tolerance, expressed as $AUC_{glucose}$ during the OGTT, improved significantly during rosiglitazone treatment (P = 0.002) and worsened significantly in the PLA group (P = 0.05) (Figure 2).

Insulin metabolism and peripheral insulin response

OGTT. Serum fasting insulin concentrations did not change during either treatment, but fasting C-peptide concentrations had decreased significantly in the ROSI group (P = 0.01) (Table II). However, the peripheral insulin response, expressed as $AUC_{insulin}$, had decreased significantly at 4 months of treatment in the ROSI group (P = 0.004) (Figure 2).

IVGTT. Values of $AUC_{glucose}$ (P = 0.02) and $AUC_{insulin}$ (P = 0.04) decreased significantly during the IVGTT in the ROSI group, but first-phase insulin secretion did not change in either group during treatment (Table II).

Insulin sensitivity, lipid and glucose oxidation and serum FFA levels. Treatment with rosiglitazone increased the M value (from 33.4 ± 3.27 to 40.0 ± 5.51 μmol/kg min, P = 0.04), although rates of glucose oxidation (from 13.42 ± 1.01 to 16.48 ± 1.23 μmol/kg min, P = 0.052) and rates of glucose nonoxidation

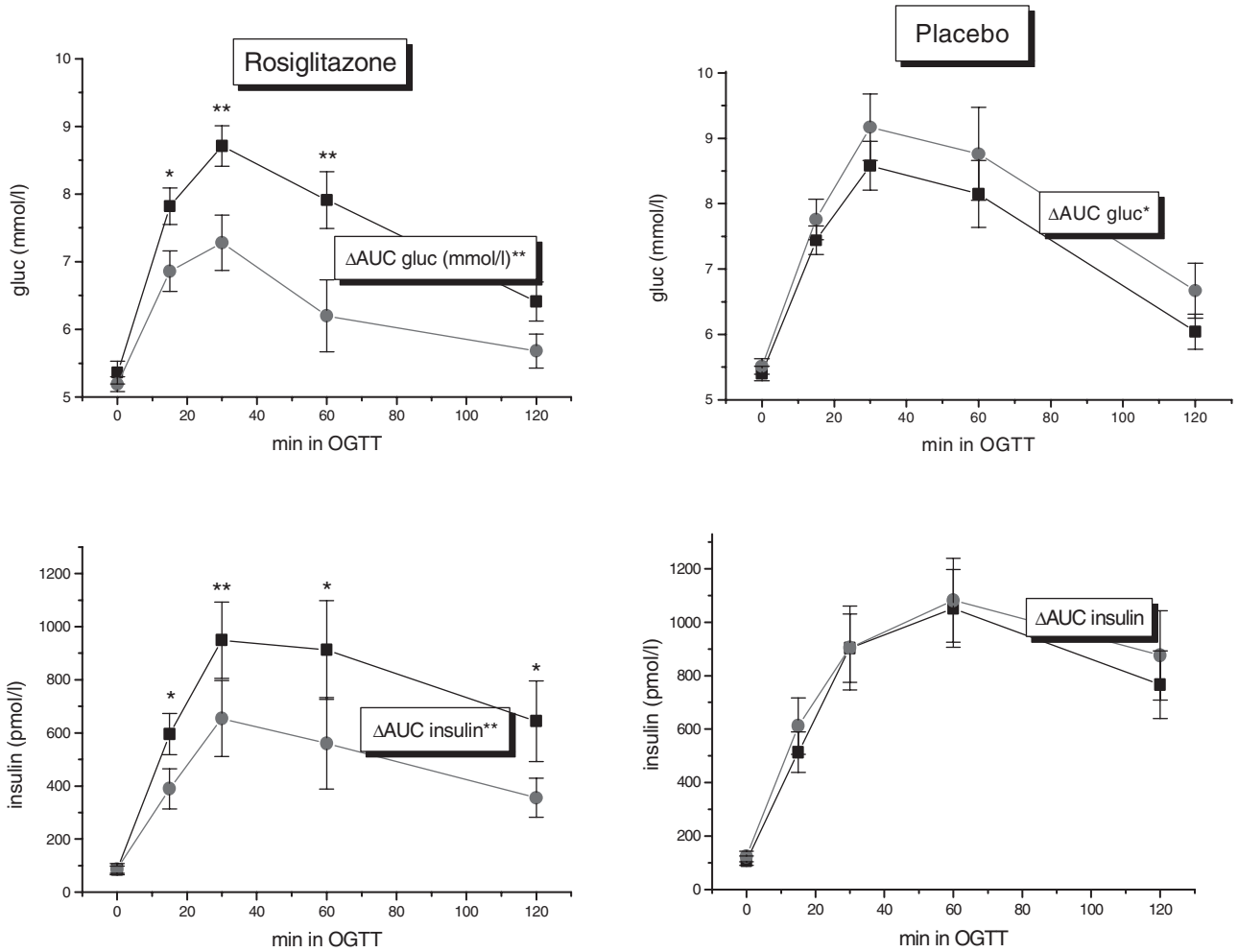

Figure 2. Serum glucose and insulin concentrations during oral glucose tolerance test (OGTT), before (■) and at four months (●) of the treatment. *$P < 0.05$, **$P < 0.01$ compared with level before the treatment.

(from 19.98 ± 2.74 to 26.38 ± 4.44 μmol/kg min, $P = 0.07$) did not change statistically significantly (Figure 3).

Glucose oxidation rates during the hyperinsulinaemic euglycaemic clamp increased during rosiglitazone treatment when compared with those in the PLA group ($P = 0.05$). Serum fasting FFA levels had decreased significantly in the ROSI group at 4 months of treatment ($P = 0.05$), and the rates of lipid oxidation during the hyperinsulinaemic euglycaemic clamp tended to be decreased at 4 months in the ROSI group ($P = 0.09$) (Table II).

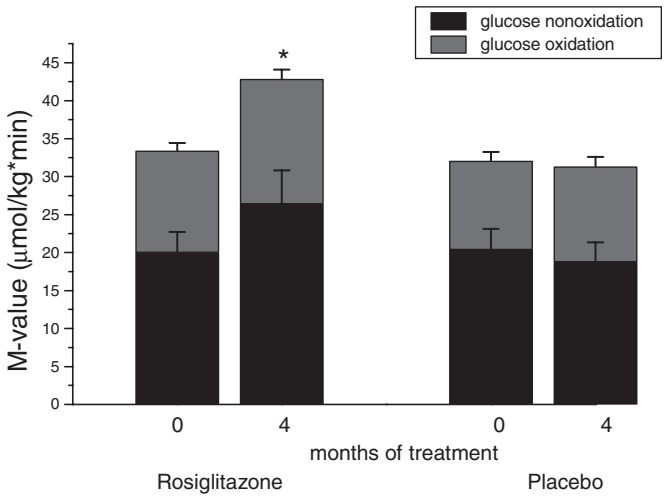

Figure 3. Changes in *M* value (including glucose oxidation and nonoxidation) during the clamp in rosiglitazone and placebo groups. *$P = 0.05$ compared with the level before the treatment.

Discussion

Rosiglitazone treatment resulted in a significant improvement in insulin resistance, hyperinsulinaemia and menstrual cyclicity and, to a lesser extent, hyperandrogenism in overweight women with PCOS.

Despite no decrease in fasting insulin serum levels during the treatment, the improvement in insulin resistance (shown by the significant increase in *M* values during the euglycaemic hyperinsulinaemic clamp) was followed by a decrease in compensatory hyperinsulinaemia, reflected by an improvement in $AUC_{insulin}$ during the OGTT. These findings are in line with those in studies on diabetic patients, in which rosiglitazone has been shown to improve glycaemic control by increasing insulin

sensitivity and enhancing insulin effects at peripheral target sites such as skeletal muscle and adipose tissue, without any direct effect on pancreatic insulin secretion (Saltiel and Olefsky, 1996; Zinman, 2001). Likewise, in diabetic subjects, rosiglitazone has been shown to decrease the fasting (Raskin *et al*., 2000; Lebovitz *et al*., 2001) and postprandial levels of insulin (Raskin *et al*., 2000) and to improve insulin sensitivity, as measured by the homeostasis model assessment (HOMA) (Raskin *et al*., 2000; Lebovitz *et al*., 2001; Phillips *et al*., 2001). In the few previous studies on subjects with PCOS, rosiglitazone and pioglitazone have induced similar improvements in insulin sensitivity, as calculated by HOMA and the quantitative insulin sensitivity check index (QUICKI) methods (Cataldo *et al*., 2001; Ghazeeri *et al*., 2003; Belli *et al*., 2004; Ortega-Gonzalez *et al*., 2005; Sepilian and Nagamani, 2005). To our knowledge, this is the first study involving detailed investigation of the mechanisms of action of rosiglitazone in PCOS using calorimetry and the clamp technique, the gold standard for the measurement of insulin sensitivity (DeFronzo *et al*., 1979). Both rates of glucose oxidation and glucose non-oxidation improved slightly, resulting in a significant increase in *M* value, i.e. peripheral insulin sensitivity. Moreover, serum fasting FFA concentrations and the rates of lipid oxidation during the clamp decreased, a finding in line with that from previous results in diabetic patients, in which rosiglitazone decreased serum FFA levels by increasing FFA uptake and oxidation in skeletal muscle (Raskin *et al*., 2000; Lebovitz *et al*., 2001; Phillips *et al*., 2001; De Leo *et al*., 2003). This effect contributes to lower circulating FFA levels and to decreased competition between glucose and FFAs, thus improving insulin sensitivity (Randle *et al*., 1963; Bevilacqua *et al*., 1987; Spiegelman and Flier, 1996; Wilmsen *et al*., 2003).

The positive effect of rosiglitazone on insulin sensitivity was not related to weight reduction, as BMI increased during the treatment, a finding which is in line with the results of studies on diabetic subjects (Spiegelman, 1998; Phillips *et al*., 2001). Weight gain during rosiglitazone treatment is likely to be multifactorial, and it has been associated with fluid retention (Song *et al*., 2004) and increased appetite in diabetic subjects (Shimizu *et al*., 1998). In previous studies on women with PCOS, thiazolidinediones (rosiglitazone, troglitazone and pioglitazone) have been found to have no effect on BMI (Ehrmann *et al*., 1997; Ghazeeri *et al*., 2003; Belli *et al*., 2004; Ortega-Gonzalez *et al*., 2005; Sepilian and Nagamani, 2005) or to increase BMI (Baillargeon *et al*., 2004). In some diabetic subjects, rosiglitazone seems to induce a reduction in WHR, suggesting a shift of fat distribution from visceral to subcutaneous adipose depots (Kelly *et al*., 1999; Akazawa *et al*., 2000; Lebovitz *et al*., 2001). However, in our study, WHR did not change, a finding which is in line with the results of some (Ehrmann *et al*., 1997; Sepilian and Nagamani, 2005) but not all (Belli *et al*., 2004; Ortega-Gonzalez *et al*., 2005) previous studies on women with PCOS treated with thiazolidinediones.

First-phase insulin secretion during the IVGTT, reflecting the β-cell secretory capacity of the pancreas, did not change during rosiglitazone treatment. Rosiglitazone is an insulin sensitizer with no stimulatory effect on insulin pancreatic secretion (Saltiel and Olefsky, 1996), but it has been shown to have a β-cell-sparing effect in diabetic patients with impaired β-cell function (Phillips *et al*., 2001). Moreover, in women with PCOS, abnormalities in first-phase insulin secretion have been shown to be reversed with the use of troglitazone, but the women concerned were significantly more obese than those in our study (Ehrmann *et al*., 1997). The lack of effect of rosiglitazone on first-phase insulin secretion in this study could be the result of differences in study populations. Our subjects were only moderately obese, and all except one had normal glucose tolerance at baseline, suggesting less disturbed β-cell function compared with diabetic subjects or extremely obese women with PCOS.

The decrease in testosterone during treatment with thiazolidinediones in some studies has been associated with improvement in hyperinsulinaemia (Ehrmann *et al*., 1997; Azziz *et al*., 2001; Cataldo *et al*., 2001; Shobokshi and Shaarawy, 2003; Ortega-Gonzalez *et al*., 2005; Sepilian and Nagamani, 2005) and/or with the direct effect of these agents on ovarian/adrenal steroidogenesis (Arlt *et al*., 2001; Guido *et al*., 2004; Seto-Young *et al*., 2005). Despite a significant improvement in hyperinsulinaemia in this study, rosiglitazone did not affect serum testosterone levels. This is difficult to explain, especially while other androgens (androstenedione, 17-OHP, DHEA and DHEA-S) decreased significantly. Both unchanged (Ghazeeri *et al*., 2003; Belli *et al*., 2004; Cataldo *et al*., 2006) and decreased (Cataldo *et al*., 2001; Shobokshi and Shaarawy, 2003; Sepilian and Nagamani, 2005) serum androgen levels have been observed in earlier studies with rosiglitazone. Despite increased serum SHBG concentrations in this study, the FAI did not change; neither did hirsutism scores improve.

Treatment with rosiglitazone resulted in more regular menstrual cycles in most of the women (88%) with menstrual disturbances, a finding which is in accordance with the results of previous studies on rosiglitazone (Cataldo *et al*., 2001; Baillargeon *et al*., 2004; Belli *et al*., 2004; Sepilian and Nagamani, 2005), troglitazone (Azziz *et al*., 2001) and metformin (Velazquez *et al*., 1994; Morin-Papunen *et al*., 1998). The occurrence of ovulation was not assessed in this study, but two of the subjects in the ROSI group became pregnant. However, as rosiglitazone has been suspected of retarding fetal development in animal studies, its use in restoring fertility in anovulatory infertile women cannot be recommended.

In conclusion, by improving menstrual cyclicity, hyperandrogenism, insulin resistance and hyperinsulinaemia, rosiglitazone offers a well-tolerated and useful alternative treatment for overweight anovulatory women with PCOS with no pregnancy desire.

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