

The effects of rosiglitazone and metformin on oxidative stress and homocysteine levels in lean patients with polycystic ovary syndrome

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BACKGROUND: Oxidative stress and hyperhomocysteinaemia are risk factors for cardiovascular diseases. The aim of this study was to assess the effects of rosiglitazone and metformin on cardiovascular disease risk factors such as insulin resistance, oxidative stress and homocysteine levels in lean patients with polycystic ovary syndrome (PCOS). **MEHODS:** Fifty lean patients (BMI <25 kg/m²) with PCOS and 35 healthy subjects were included this study. Serum homocysteine, sex steroids, fasting insulin, fasting glucose and lipid levels were measured. Total antioxidant status (TAS; combines concentrations of individual antioxidants) and malonyldialdehyde concentration (MDA) were determined. Insulin resistance was evaluated by using the homeostasis model insulin resistance index (HOMA-IR), quantitative insulin sensitivity check index (QUICKI), Area under the curve insulin (AUCI) and the insulin sensitivity index (ISI). Patients were divided into two groups. One group was treated with metformin ($n = 25$) and the other received rosiglitazone ($n = 25$) for 12 weeks. All measurements were repeated at the end of 12 weeks. **RESULTS:** Compared with healthy women, those with PCOS had significantly elevated serum MDA, homocysteine, HOMA-IR, AUCI and lipoprotein a levels, and significantly decreased serum TAS, QUICKI and ISI. Serum free testosterone levels showed a significant positive correlation with MDA, AUCI and HOMA-IR, and a negative correlation with TAS, ISI and QUICKI in PCOS patients. HOMA-IR and AUCI significantly decreased, while QUICKI and ISI significantly increased after treatment in both groups. Serum TAS level increased and serum MDA level decreased after the rosiglitazone treatment, but these parameters did not change after the metformin treatment. Serum homocysteine and lipid levels did not change in either group, while serum androgen levels and LH/FSH ratio significantly decreased after the treatment period in only the rosiglitazone-treated group. **CONCLUSION:** Elevated insulin resistance, oxidative stress and plasma homocysteine levels and changes in serum lipid profile (risk factors for cardiovascular disease) were observed in lean PCOS patients. Rosiglitazone seemed to decrease elevated oxidative stress when compared with metformin treatment in lean PCOS patients.

Key words: insulin resistance/metformin/oxidative stress/PCOS/rosiglitazone

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathies in women, affecting 5–10% of the population (Frank, 1995). PCOS is a heterogeneous disorder characterized by menstrual irregularities, clinical and/or biochemical hyperandrogenism, and hyperinsulinaemia secondary to reduced insulin sensitivity (Homburg, 2003). Approximately half of all women with PCOS are overweight or obese. Independently of the presence of obesity, these women are frequently insulin-resistant and therefore they have hyperinsulinaemia, which may play a pathogenic role in the disease (Dunaif *et al.*, 1989; Dunaif, 1997). Insulin resistance and the resulting hyperinsulinaemia lead patients to a cluster of long-term metabolic disorders, including impaired glucose tolerance, type 2 diabetes

mellitus (DM) (Ehrmann *et al.*, 1999) and cardiovascular disease (CVD) (Guzick, 1996; Legro, 2003).

Oxidative stress means an imbalance between the production of reactive oxygen species and the antioxidant defence system, which buffers the oxidative damage. Oxidative stress is implicated in the pathogenesis of several diseases, such as atherosclerosis, DM and carcinogenesis (Betteridge *et al.*, 2000). Oxidative stress also impairs insulin action, as has been demonstrated in type 2 diabetics, and this impairment might be due to several factors, such as membrane fluidity alterations, decreased availability of nitric oxide and increased intracellular calcium content (Caimi *et al.*, 2003). Serum total antioxidant status (TAS) combines the concentrations of individual antioxidants, such as vitamin C and E, β -carotene and thiol groups, and also their synergists. TAS is

sensitive to the changes in plasma antioxidant levels and degrees of oxidative stress (Garibaldi *et al.*, 2001). Malonyldialdehyde (MDA) is a marker of lipid peroxidation and increases in oxidative stress states (Knight *et al.*, 1987; Betteridge, 2000). Recent studies have documented increased oxidative stress in patients with PCOS (Sabuncu *et al.*, 2001; Fenkçi *et al.*, 2003), which may increase the risk of CVD in such patients.

Homocysteine (Hcy) is an intermediate product formed during the breakdown of the amino acid methionine, and may undergo remethylation to methionine or trans-sulphuration to cystathione and cysteine. Elevated serum levels of Hcy, called hyperhomocysteinaemia, have adverse effects on the cardiovascular system (Fonseca *et al.*, 1999). Elevated plasma Hcy levels are considered to be an independent risk factor for CVD (Clarke *et al.*, 1991). In recent studies, serum Hcy concentrations were found to be elevated in PCOS women, suggesting that an alteration in Hcy metabolism may play a role in the increased cardiovascular risk associated with PCOS (Loverro *et al.*, 2002; Randeva *et al.*, 2002; Vrbikova *et al.*, 2002; Schachter *et al.*, 2003; Wijeyaratne *et al.*, 2004).

Experimental and clinical evidence suggest indirectly that moderate hyperhomocysteinaemia may predispose affected individuals to endothelial dysfunction through a mechanism that involves the generation of reactive oxygen species (Kanani *et al.*, 1999). Hyperhomocysteinaemia-induced oxidative stress may occur as a consequence of either decreased expression and activity of key antioxidant enzymes or increased enzymatic generation of superoxide anions (Eberhardt *et al.*, 2000; Lentz *et al.*, 2000; Faraci, 2003; Loscalzo, 2003; Ungvari *et al.*, 2003).

Use of oral anti-hyperglycaemic drugs, predominantly metformin and thiazolidinediones, have been shown to improve insulin sensitivity and ovarian function of women with PCOS. Metformin inhibits hepatic glucose production and enhances peripheral tissue sensitivity to insulin, resulting in a decrease in insulin secretion (Bailey and Turner, 1996). In women with PCOS, many studies have demonstrated the efficacy of metformin in improving menstrual cycle pattern, ovulation and pregnancy outcomes (Morin-Papunen *et al.*, 1998; Unluhizarci *et al.*, 1999; La Marca *et al.*, 2000; Moghetti *et al.*, 2000; Kocak *et al.*, 2002; Baillargeon *et al.*, 2004; Hoeger, *et al.*, 2004). Rosiglitazone, an agonist of peroxisome proliferator-activating receptor- γ (PPAR- γ), increases the uptake and utility of glucose in the periphery and decreases hepatic gluconeogenesis (Goldstein, 1999). Most of the studies have shown that rosiglitazone reduces plasma androgen levels, with consequent improvement in ovulatory reproductive function in PCOS patients (Cataldo *et al.*, 2001; Ghazeeri *et al.*, 2003; Shobokshi and Shaarawy, 2003; Baillargeon *et al.*, 2004; Belli *et al.*, 2004; Kilicdag *et al.*, 2005a; Sepilian and Nagamani, 2005).

The aim of this study was to evaluate the effects of rosiglitazone and metformin on cardiovascular risk factors, such as insulin resistance, homocysteine levels and oxidative stress in lean PCOS patients.

Materials and methods

Fifty lean patients with PCOS (BMI <25 kg/m²) and 35 age- and weight-matched healthy subjects were included in the study. PCOS

was defined according to the 2003 Rotterdam consensus criterion (Rotterdam ESHRE/ASRM, 2004). The ethics committee of Gazi University, Faculty of Medicine, Ankara, approved the study protocol. All patients gave their signed informed consent.

Patients who had DM, hyperprolactinaemia, congenital adrenal hyperplasia, thyroid disorders, Cushing's syndrome (1 mg dexamethasone suppression test), hypertension, vitamin B12 and folate deficiency, hepatic or renal dysfunction were excluded from the study. Confounding medications, including oral contraceptive agents, anti-lipidaemic drugs, hypertensive medications, and insulin-sensitizing drugs which may affect the metabolic criteria, were questioned. Patients were also excluded if they had used any anti-androgen agent, combined estrogen-progestin or an oral hypoglycaemic agent within 90 days prior to enrolment.

BMI (kg/m²) and waist to hip (W/H) ratio were calculated. Weight and height were measured in light clothing without shoes. Waist circumference was measured at the narrowest level between the costal margin and the iliac crest, and the hip circumference was measured at the widest level over the buttocks while the subject was standing and breathing normally. Degree of hirsutism was determined by Ferriman-Gallwey scoring (Ferriman and Gallwey, 1961).

Menstrual cyclicity was assessed by recording the menses in the last 6 months prior to the study. The menstrual patterns were defined as follows. Cycles between 22 and 45 days were noted as regular, those between 46 and 180 days as irregular, those lasting 21 days or less as polymenorrhoea, those between 46 and 180 days as oligomenorrhoea, and those lasting 180 days or more as amenorrhoea.

Serum levels of FSH, LH, prolactin, dehydroepiandrosterone sulphate (DHEA-S), insulin, cortisol and thyroid-stimulating hormone (TSH) were measured with specific chemiluminescence assays from the Abbott Architect system (Chicago, USA). Serum levels of 17 OH-progesterone, free testosterone, androstenedione were measured by radioimmunoassay (RIA). Serum levels of total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol and triglyceride were measured using an Abbott-Aeroset (Chicago, IL, USA) autoanalyser with original kits. Lipoprotein a [Lp (a)], apoprotein A (Apo A) and apoprotein B (Apo B) levels were determined by nephelometric assay using a Beckman 360 protein array system. Serum vitamin B12 level was measured using an Immulyte 2000 (Los Angeles, CA, USA) analyser with the chemiluminescence method. Folate level was determined with a Tosoh (Tokyo, Japan) analyser. Plasma Hcy levels were measured by HPLC using Chromsystems kits with fluorescence detector (München, Germany).

A standard 75 g oral glucose tolerance test (OGTT) and a test of the insulin response to oral glucose loading were performed after 10–12 h of fasting between 8.30 and 10.30 a.m. Glucose tolerance state was evaluated using the criteria of American Diabetes Association (Expert Committee on the Diagnosis and Classification Follow-up Report on the Diagnosis of Diabetes Mellitus, 2003). The responses of glucose and insulin to the OGTT were analysed by calculating the area under the curve insulin (AUCI) and area under the curve glucose (AUCG) using the trapezoidal method. The insulin sensitivity index (ISI) was calculated by the Matsuda method (Matsuda and DeFronzo, 1999). The homeostasis model (HOMA) insulin resistance (HOMA-IR) index was calculated according to the following formula: fasting glucose (mmol/l) $\times$ fasting insulin (μ U/ml)/22.5 (Matthews *et al.*, 1985). The quantitative insulin sensitivity check index (QUICKI) was calculated according to the following formula: $1/[\log \text{fasting serum insulin } (\mu\text{U/ml}) + \log \text{fasting plasma glucose (mg/dl)}]$ (Katz *et al.*, 2000).

Serum TAS was measured spectrophotometrically using ABTS [2,2'-azino-di-(3-ethyl benzthiazoline sulphate)] method (Total Anti-oxidant Status kit; Randox, Antrim, UK). Serum MDA levels were determined by spectrophotometry at 532 nm after boiling the sample

and condensing it with thiobarbituric acid. The results were expressed as nmol/ml (Yoshioka *et al.*, 1979).

After the screening period, patients were assigned to metformin and rosiglitazone treatment groups consecutively. The study was designed as open-labelled. The first patient recruited into the study was treated with rosiglitazone, then metformin was given to the second patient. Then, the patients were quasi-randomized, i.e. subjects with odd numbers received rosiglitazone ($n = 25$) 4 mg/day for 12 weeks, whilst those with even numbers received metformin ($n = 25$) 850 mg twice daily.

Effects of metformin and rosiglitazone on menstrual cyclicity were evaluated by accessing the changes in frequency of cycles after treatment. These assessments were performed by two independent obstetricians. Liver enzymes were measured monthly during the study. At the end of 12 weeks all laboratory and anthropometric measurements were repeated.

Statistical analysis

For comparison of all the variables between the PCOS and control groups, the unpaired *t*-test was used. For comparison of all quantitative variables, clinical, biochemical and hormonal parameters between rosiglitazone and metformin groups the unpaired *t*-test was used. Menstrual cycle rates were compared by using the χ^2 test. The paired *t*-test was performed to compare the basal and end values within the same group. Multiple regression analysis was conducted with HOMA, AUCI, ISI, QUICKI, BMI, TAS, MDA, lipids, Hcy and androgens. Pearson correlation analysis was used to explore correlations between the variables. Results are shown as mean $\pm$ SD. Statistical significance was accepted at $P < 0.05$. Data analysis was performed using the statistical software package SPSS for Windows (Version 10.0 for Windows; SPSS, Inc., Chicago, IL).

Results

Mean age, BMI and smoking were similar between PCOS and control groups. The clinical and endocrinological parameters

of PCOS patients and control subjects are shown in Table I. The clinical and endocrinological parameters of the metformin and rosiglitazone groups are shown in Table II. The clinical and endocrinological parameters after the rosiglitazone and metformin treatments are shown in Table III.

Insulin resistance, BMI and W/H ratio

Compared with healthy women, those with PCOS had significantly elevated HOMA-IR ($P < 0.001$) and AUCI ($P < 0.001$) levels, and significantly decreased QUICKI ($P < 0.001$) and ISI ($P < 0.001$) levels. After treatment, the HOMA-IR and AUCI levels declined significantly ($P < 0.001$), while QUICKI and ISI levels significantly increased ($P < 0.001$). BMI increased in the rosiglitazone group ($P < 0.05$) but decreased in the metformin group ($P < 0.05$). W/H ratio decreased in the metformin group ($P < 0.05$) but did not change in the rosiglitazone group ($P > 0.05$). With multiple regression analysis, the reduction in insulin resistance was calculated to be independent from the decrease in BMI in the metformin groups (HOMA-IR, $\beta = 0.123$; AUCI, $\beta = 0.136$; QUICKI, $\beta = 0.119$; ISI, $\beta = 0.127$; $P > 0.05$).

Oxidative stress

Women with PCOS had significantly higher serum MDA levels than healthy women ($P < 0.001$). However, TAS levels were significantly lower in women with PCOS compared with healthy women ($P < 0.005$). TAS levels ($P < 0.005$) increased and MDA levels ($P < 0.001$) declined after the rosiglitazone treatment, but these parameters did not change after the metformin treatment ($P > 0.05$).

Table I. Basal clinical and endocrinological parameters in patients with PCOS and control subjects

	PCOS (mean $\pm$ SD) ($n = 50$)	Controls (mean $\pm$ SD) ($n = 35$)	<i>P</i>
Age (years)	24.12 $\pm$ 4.89	24.35 $\pm$ 5.02	NS
BMI (kg/m ²)	21.92 $\pm$ 3.21	22.45 $\pm$ 4.89	NS
W/H ratio	0.83 $\pm$ 0.08	0.83 $\pm$ 0.07	NS
Smoking	10/40	6/29	NS
LH (mIU/ml)	9.96 $\pm$ 4.76	5.89 $\pm$ 1.99	<0.001
FSH (mIU/ml)	6.04 $\pm$ 2.18	5.82 $\pm$ 1.88	NS
LH/FSH ratio	1.65 $\pm$ 0.18	1.01 $\pm$ 0.14	<0.001
Free testosterone (pg/ml)	2.99 $\pm$ 1.06	2.05 $\pm$ 0.55	<0.001
DHEA-S (μ g/dl)	312.63 $\pm$ 108.56	182.73 $\pm$ 63	<0.001
Androstenedione (ng/ml)	2.96 $\pm$ 1.62	1.58 $\pm$ 0.51	<0.001
HOMA-IR	3.20 $\pm$ 1.52	2.19 $\pm$ 0.98	<0.001
QUICKI	0.315 $\pm$ 0.02	0.378 $\pm$ 0.03	<0.001
ISI _{OGTT}	4.25 $\pm$ 2.64	6.39 $\pm$ 3.76	<0.001
TAS (mmol/l)	0.98 $\pm$ 0.08	1.25 $\pm$ 0.12	<0.005
MDA (nmol/ml)	7.31 $\pm$ 3.07	3.44 $\pm$ 1.43	<0.001
Hcy (μ mol/l)	13.76 $\pm$ 6.28	9.32 $\pm$ 4.52	<0.005
Vitamin B12 (pg/ml)	312.87 $\pm$ 72.61	306.12 $\pm$ 68.35	NS
Folic acid (ng/ml)	11.52 $\pm$ 3.14	11.83 $\pm$ 3.39	NS
AUCI (μ IU/ml/min)	7012.35 $\pm$ 812.98	4487.12 $\pm$ 619.4	<0.001
AUCG (mg/dl/min)	13 984.63 $\pm$ 1718.6	13 523.98 $\pm$ 1611.12	NS
Total cholesterol (mg/dl)	163.62 $\pm$ 59.35	166.71 $\pm$ 63.23	NS
LDL cholesterol (mg/dl)	100.63 $\pm$ 26.35	93.68 $\pm$ 23.11	NS
HDL cholesterol (mg/dl)	48.36 $\pm$ 11.56	59.87 $\pm$ 9.48	<0.05
Triglyceride (mg/dl)	78.01 $\pm$ 32.67	72.87 $\pm$ 45.59	NS
Apo A (mg/dl)	110.21 $\pm$ 27.81	136.15 $\pm$ 23.85	<0.05
Apo B (mg/dl)	85.19 $\pm$ 21.87	84.92 $\pm$ 21.66	NS
Lp(a) (mg/dl)	21.98 $\pm$ 12.76	17.38 $\pm$ 10.3	<0.05

Table II. Basal clinical and endocrinological parameters of PCOS patients in rosiglitazone and metformin groups

	Rosiglitazone group (mean ± SD) (n = 25)	Metformin group (mean ± SD) (n = 25)	P
Age (years)	23.92 ± 4.64	24.31 ± 4.75	NS
BMI (kg/m ²)	21.82 ± 3.44	22.16 ± 3.57	NS
W/H ratio	0.83 ± 0.08	0.83 ± 0.09	NS
LH (mIU/ml)	10.08 ± 7.81	9.89 ± 5.62	NS
FSH (mIU/ml)	6.11 ± 2.27	5.96 ± 2.47	NS
LH/FSH ratio	1.65 ± 0.18	1.65 ± 0.12	NS
Free Testosterone (pg/ml)	2.95 ± 1.06	3.01 ± 1.34	NS
DHEA-S (µg/dl)	308.11 ± 112.56	317.25 ± 131.38	NS
Androstenedione (ng/ml)	2.95 ± 1.58	2.96 ± 1.46	NS
Hcy (µmol/l)	13.94 ± 6.72	13.52 ± 5.98	NS
QUICKI	0.321 ± 0.023	0.313 ± 0.021	NS
ISI _{OGTT}	4.26 ± 2.61	4.23 ± 2.58	NS
HOMA-IR	3.21 ± 1.45	3.18 ± 1.19	NS
TAS (mmol/l)	0.95 ± 0.07	1.00 ± 0.12	NS
MDA (nmol/ml)	7.46 ± 3.07	7.10 ± 3.72	NS
AUCI (µIU/ml/min)	6973.81 ± 856.12	7061.11 ± 912.87	NS
AUCG (mg/dl/min)	14 312.82 ± 1718.61	13 767.32 ± 1542.17	NS
Total C (mg/dl)	161.16 ± 31.85	166.68 ± 36.43	NS
LDL cholesterol (mg/dl)	101.83 ± 25.55	99.72 ± 26.34	NS
HDL cholesterol (mg/dl)	48.22 ± 12.47	49.31 ± 16.01	NS
Triglyceride (mg/dl)	77.48 ± 34.43	78.47 ± 29.87	NS
Apo A (mg/dl)	110.27 ± 28.84	108.73 ± 25.67	NS
Apo B (mg/dl)	84.68 ± 21.73	86.18 ± 20.60	NS
Lp(a) (mg/dl)	21.54 ± 13.61	22.06 ± 14.87	NS

There was no correlation between oxidative stress and Hcy levels, lipid levels and insulin resistance parameters ($P > 0.05$). Serum free testosterone showed a significant positive correlation with MDA ($P < 0.05$, $r = 0.41$) and a negative correlation with TAS ($P < 0.05$, $r = -0.33$) in PCOS patients.

Homocysteine and lipid levels

Serum Hcy levels were significantly elevated in women with PCOS compared with healthy women ($P < 0.005$). No significant difference in serum fasting total cholesterol, LDL cholesterol, triglyceride and Apo B levels were observed between PCOS

Table III. Clinical and endocrinological parameters after the rosiglitazone and metformin treatments

	Metformin			Rosiglitazone		
	Before treatment (n = 25)	After treatment (n = 25)	P	Before treatment (n = 25)	After treatment (n = 25)	P
Age (years)	24.31 ± 4.75			23.92 ± 4.64		
BMI (kg/m ²)	22.16 ± 3.57	20.08 ± 3.14	<0.05	21.82 ± 3.44	23.13 ± 3.70	<0.05
W/H ratio	0.83 ± 0.09	0.82 ± 0.08	<0.05	0.83 ± 0.08	0.83 ± 0.08	NS
LH (mIU/ml)	9.89 ± 5.62	8.12 ± 4.62	NS	10.08 ± 7.81	7.47 ± 6.06	<0.005
FSH (mIU/ml)	5.96 ± 2.47	5.12 ± 2.38	NS	6.11 ± 2.27	5.75 ± 2.18	NS
LH/FSH ratio	1.65 ± 0.12	1.58 ± 0.11	NS	1.65 ± 0.18	1.28 ± 0.12	<0.005
Free testosterone (pg/ml)	3.01 ± 1.34	2.76 ± 1.27	NS	2.95 ± 1.06	2.04 ± 0.92	<0.005
DHEA-S (µg/dl)	317.25 ± 131.38	282.61 ± 121.56	NS	308.11 ± 112.56	218.73 ± 78.05	<0.005
Androstenedione (ng/ml)	2.96 ± 1.46	2.65 ± 1.41	NS	2.95 ± 1.58	2.17 ± 0.51	<0.005
Hcy (µmol/l)	13.52 ± 5.98	13.46 ± 6.01	NS	13.94 ± 6.72	13.02 ± 5.88	NS
HOMA-IR	3.18 ± 1.19	2.32 ± 0.87	<0.001	3.21 ± 1.45	2.02 ± 0.91	<0.001
AUCI (µIU/ml/min)	7061.1 ± 912.8	4412.6 ± 764.9	<0.001	6973.81 ± 856.12	4118.3 ± 652.95	<0.001
QUICKI	0.313 ± 0.021	0.382 ± 0.031	<0.001	0.321 ± 0.023	0.392 ± 0.031	<0.001
ISI _{OGTT}	4.23 ± 2.58	6.19 ± 3.36	<0.001	4.26 ± 2.61	6.78 ± 3.75	<0.001
AUCG (mg/dl/min)	13767.3 ± 1542.1	13654.1 ± 1586.2	NS	14312.8 ± 1718.6	13832.1 ± 1705.4	NS
MDA (nmol/ml)	7.10 ± 3.72	6.35 ± 2.98	NS	7.46 ± 3.07	4.02 ± 2.34	<0.001
TAS (mmol/l)	1.00 ± 0.12	1.11 ± 0.13	NS	0.95 ± 0.07	1.21 ± 0.22	<0.005
Total cholesterol (mg/dl)	166.68 ± 36.43	156.72 ± 32.98	NS	161.16 ± 31.85	164.35 ± 32.09	NS
LDL cholesterol (mg/dl)	99.72 ± 26.34	95.36 ± 25.12	NS	101.83 ± 25.55	97.13 ± 27.42	NS
HDL cholesterol (mg/dl)	49.31 ± 16.01	51.54 ± 15.87	NS	48.22 ± 12.47	54.56 ± 16.01	NS
Triglyceride (mg/dl)	78.47 ± 29.87	73.71 ± 27.98	NS	77.48 ± 34.43	78.47 ± 29.87	NS
Apo A (mg/dl)	108.73 ± 25.67	110.86 ± 26.52	NS	110.27 ± 28.84	116.73 ± 22.85	NS
Apo B (mg/dl)	86.18 ± 20.60	78.94 ± 19.43	NS	84.68 ± 21.73	80.38 ± 20.60	NS
Lp(a) (mg/dl)	22.06 ± 14.87	21.19 ± 13.85	NS	21.54 ± 13.61	21.60 ± 19.44	NS
Vitamin B12 (pg/ml)	311.19 ± 71.74	309.23 ± 71.12	NS	313.62 ± 72.38	314.48 ± 73.92	NS
Folic acid (ng/ml)	11.19 ± 3.09	11.58 ± 3.17	NS	11.57 ± 3.26	11.62 ± 3.29	NS

and control subjects ($P > 0.05$). In PCOS patients, Lp(a) levels were significantly elevated ($P < 0.05$) while HDL cholesterol and Apo A levels were lower when compared with the control group ($P < 0.05$). Serum Hcy, vitamin B12, folic acid and lipid levels did not change after treatment in either group ($P > 0.05$). There was no correlation between Hcy and lipids, TAS, MDA, serum androgens and insulin resistance ($P > 0.05$).

Menstrual disturbance and androgen levels

Compared with controls, women with PCOS had significantly elevated free testosterone, androstenedione, DHEA-S and LH levels ($P < 0.001$). Serum androgen levels and LH/FSH ratio decreased significantly in the rosiglitazone group ($P < 0.005$) but did not change in the metformin group after treatment ($P > 0.05$). The decrease in serum androgen levels in rosiglitazone-treated patients was found to be independent of the decrease in insulin resistance ($\beta = 0.134$, $P > 0.05$).

Nineteen women had menstrual disturbance (13 oligomenorrhoea, three secondary amenorrhoea, three polymenorrhoea) in the metformin group before treatment. Nine (47.3%) of the 19 women with menstrual disturbance achieved regular cycles after the therapy. Twenty women had menstrual disturbance (13 oligomenorrhoea, four secondary amenorrhoea, three polymenorrhoea) in the rosiglitazone group before treatment. Eleven (55%) of the 20 women with menstrual disturbance achieved regular cycles after rosiglitazone therapy. This difference did not reach significance ($P > 0.05$).

Comparison between post-treatment values of metformin and rosiglitazone groups

After treatment, serum LH, free testosterone, DHEA-S, androstenedione and MDA levels were significantly higher in the metformin group compared with the rosiglitazone group ($P < 0.05$). HOMA-IR, AUCI, ISI, QUICKI, TAS, Hcy and lipoprotein levels were similar in the two groups after treatment ($P > 0.05$). Although pre- and post-treatment TAS values in each group were statistically similar ($P > 0.05$), in the rosiglitazone group TAS was found to have increased following treatment compared with the pretreatment levels ($P < 0.005$). BMI was lower in the metformin group compared with the rosiglitazone group after treatment ($P < 0.05$), although W/H ratio was similar ($P > 0.05$).

Side-effects

All patients completed the study and were analysed for the primary outcome after 12 weeks of follow-up. Ten patients (40%) in metformin group had gastrointestinal side-effects, such as nausea, vomiting, abdominal pain and diarrhoea. None of the subjects had elevated liver enzymes.

Discussion

Recent studies have shown that PCOS is not only a gynaecological condition affecting women of reproductive age but also a comprehensive syndrome with a variety of associated metabolic disorders, such as insulin resistance and dyslipidaemia (Wild *et al.*, 2000; Dunaif *et al.*, 1989; Dunaif, 1997;

Talbott *et al.*, 1998). The present study investigated both classical CVD risk factors, such as insulin resistance and dyslipidaemia, and more recently emerging risk factors, such as serum Hcy and oxidative stress.

Although the mechanisms leading to the development of PCOS are still not completely understood, it has become apparent that insulin resistance and hyperinsulinaemia may play pivotal roles in the pathophysiology of PCOS (Dunaif *et al.*, 1989; Dunaif, 1997). Insulin resistance is associated with obesity. However, hyperinsulinaemia and insulin resistance are not considered to be related to obesity in patients with PCOS (Dunaif *et al.*, 1989) and insulin secretion is defective also in lean patients with PCOS (Holte *et al.*, 1994; Ehrmann *et al.*, 1995; Holte *et al.*, 1995; Cibula, 2004). In the present study, insulin resistance, as detected by the markers HOMA-IR, AUCI, QUICKI and ISI, was significantly higher in lean patients with PCOS than in healthy controls.

A few previous studies have assessed the effects of metformin and rosiglitazone in lean women with PCOS. In a recent study, lean PCOS patients without insulin resistance were given separate and combined treatments of metformin and rosiglitazone. After the treatment, insulin resistance improved only in the metformin group. Compared with the placebo group, those patients who received metformin and rosiglitazone had a significantly higher rate of menstrual regularization, increased ovulation and decreased androgen levels (Baillargeon *et al.*, 2004). Maciel *et al.* demonstrated that lean women with PCOS respond better to treatment with metformin, compared with obese women (Maciel *et al.*, 2004). In the present study, insulin resistance improved, although serum androgen levels did not significantly change in the metformin-treated subjects. However, both insulin resistance and hyperandrogenism improved in the rosiglitazone-treated subjects. Both groups displayed a similar increase in menstrual cycles.

Oxidative stress is an accepted risk factor for the development of CVD (Betteridge, 2000). PCOS is also associated with an increased risk of CVD. However, the oxidative stress state and the effects of current therapies on this state are not well established in PCOS patients. There are only two studies showing that oxidative stress is increased, and these studies concerned obese PCOS patients (Sabuncu *et al.*, 2001; Fenkçi *et al.*, 2003). In our study, serum TAS levels were significantly lower in lean women with PCOS compared with healthy controls. Higher serum MDA levels were found in patients than in controls. These findings implicate the presence of increased oxidative stress in lean patients with PCOS. As the first derivative of thiazolidinedione used to treat DM, troglitazone is chemically related to α -tocopherol, which is known as an antioxidant (Garg *et al.*, 2000). Hydroxylation of the phenyl and pyridine rings in the chemical structure of rosiglitazone may facilitate the scavenging of hydroxyl radicals. In animal models, rosiglitazone has decreased oxidative stress, but this issue has not been investigated in human studies (Sivarajah *et al.*, 2003; Bagi *et al.*, 2004). In some studies, the effects of metformin on oxidative stress have been evaluated in diabetic patients, and conflicting data were obtained. Bonnefont-Rousselot *et al.* demonstrated that metformin reduced oxidative stress (Bonnefont-Rousselot *et al.*, 2003), while

Pavlović *et al.* showed that it increases oxidative stress (Pavlović *et al.*, 2000). We observed a significant increase in the serum TAS level and a decrease in serum MDA level with rosiglitazone but not with metformin treatment. These results suggest that rosiglitazone is capable of reducing oxidative stress in patients with PCOS.

Oxidative stress is known to increase in parallel with factors such as age, smoking, hyperhomocysteinaemia, hyperandrogenaemia and insulin resistance. In one study, slightly higher plasma MDA concentrations were reported in males than in females in a healthy population (Bolzan *et al.*, 1997). Another study, also on antioxidant enzymes in healthy individuals, showed higher superoxide dismutase and lower glutathione peroxidase activities in women than in men (Knight *et al.*, 1987). It is not known whether hyperandrogenaemia has any effect on oxidant and antioxidant status in women with PCOS. As for studies regarding PCOS, no correlation was found between MDA, antioxidants and serum androgens (Sabuncu *et al.*, 2001; Fenkçi *et al.*, 2003). In contrast with previous studies, we found a significant positive correlation between MDA and serum free testosterone and a significant negative correlation between TAS and serum free testosterone in lean patients with PCOS. The present study has not shown a significant relationship between oxidative stress and age, insulin resistance or Hcy levels. The proportion of smokers was similar between PCOS and control subjects (20 and 17% respectively).

Metformin and glitazones may reduce androgen levels by reducing pituitary gonadotrophin secretion, ovarian androgen secretion and adrenal androgen secretion, and by increasing the plasma levels of sex hormone binding globulin (SHBG) (De Leo *et al.*, 2003). The decrease in the circulating adrenal androgen levels might be secondary to the direct inhibitory effect of glitazones on the adrenal cortex (Azziz *et al.*, 2003). Rosiglitazone has also a direct but weaker inhibitory effect on both P450c17 and 3 β -Hydroxy steroid dehydrogenase II (3 β -OH steroid DH II) than troglitazone (Arlt *et al.*, 2001). Rosiglitazone inhibits two key enzymes involved in androgen biosynthesis; this effect is independent of its insulin-lowering effect. Metformin has no direct effect on these enzymes, although it may decrease androgen levels by decreasing insulin levels (La Marca *et al.*, 2000). The present study concluded that a decrease in serum androgen levels in patients treated with rosiglitazone is independent of the decrease in insulin resistance. As already described, men appear to have higher oxidative stress than women in healthy populations, and we found a positive correlation between MDA levels and serum free testosterone levels and a significant negative correlation between TAS levels and serum free testosterone levels. The reduction in oxidative stress in the rosiglitazone group may be explained by the decrease in serum testosterone levels as a result of rosiglitazone therapy.

Recently, early atherosclerosis and increased risk of CVD have been reported in patients with PCOS compared with healthy controls (Loverro *et al.*, 2002; Randeva *et al.*, 2002; Vrbikova *et al.*, 2002; Schachter *et al.*, 2003; Wijeyaratne *et al.*, 2004). Increased Hcy levels in patients with PCOS may partly explain these findings. In the present study we found higher Hcy levels in lean PCOS patients than control subjects. However, Orio *et al.* (Orio *et al.*, 2003) and Boulman *et al.*

(Boulman *et al.*, 2004) did not detect a significant difference between patients with PCOS and healthy controls in terms of Hcy levels. Increased serum Hcy level is an independent risk factor for CVD, which may put these subjects at a higher risk of developing CVD. On the other hand, lowering plasma Hcy improves endothelial function in individuals with coronary artery disease and decreases the incidence of major cardiac events (Schnyder *et al.*, 2001). Four studies have elucidated the impact of PCOS treatment on Hcy. Randeva *et al.* showed that regular exercise significantly lowers plasma Hcy in young, overweight or obese women with PCOS (Randeva *et al.*, 2002). Vrbikova *et al.* reported that metformin treatment in women with PCOS may increase Hcy levels (Vrbikova *et al.*, 2002). Kilicdag *et al.*, 2005a found the same results with metformin and rosiglitazone treatment in a similar study population. They also showed that neither drug improved insulin resistance and serum androgen levels (Kilicdag *et al.*, 2005a). The same authors also reported that metformin treatment in PCOS patients can lead to increase in Hcy levels and B-group vitamins and folic acid administration decreased Hcy levels in these patients (Kilicdag 2005b). In our study, there was no change in Hcy levels in either treatment group.

Dyslipidaemia has been reported in women with PCOS. In accordance with previous studies, we noted an unfavourable lipid profile, including increased Lp(a) levels and decreased HDL cholesterol and Apo A levels. These are considered to be risk factors for CVD (Wild *et al.*, 2000; De Leo *et al.*, 2003; Legro, 2003). No significant change in total cholesterol, LDL cholesterol, HDL cholesterol, triglyceride, Apo A, Apo B or Lp(a) was found after rosiglitazone and metformin therapies.

Oxidative stress, hyperhomocysteinaemia, insulin resistance and hyperlipidaemia are accepted risk factors for CVD. The present study confirmed that such risk factors are present in patients with PCOS. These findings show that the risk of development of CVD is increased in PCOS. A decrease in insulin resistance could be reduced by both metformin and rosiglitazone. However, only rosiglitazone was able to lower oxidative stress in patients with PCOS. Studies performed on PCOS have indicated that metformin could increase Hcy levels, although our study did not support this. These findings suggest that metformin may not facilitate the development of CVD by increasing Hcy levels and rosiglitazone may protect against CVD by lowering oxidative stress.

In the present study, we have evaluated the effects of these drugs on metabolic and hormonal parameters for 12 weeks, although this was a short follow-up period for such a long-lasting illness. Treatment for PCOS may continue for years because it is a chronic disorder, and these drugs are efficient as long as they are administered. After re-evaluation of the patients at the end of 12 weeks, they were advised to continue their treatments in the same manner.

In conclusion, in the present study elevated insulin resistance, oxidative stress and plasma Hcy levels and changes in serum lipid profile were found in our lean PCOS patients. These findings show that the risk of development of CVD is increased in lean PCOS patients. Furthermore, we found that rosiglitazone seemed to decrease elevated oxidative stress when compared with metformin treatment. However, neither

rosiglitazone nor metformin therapy for 12 weeks had any effect on serum lipid and plasma Hcy levels in lean PCOS patients. Our results suggest that metformin may not facilitate the development of CVD by increasing Hcy levels and that rosiglitazone may protect against CVD by lowering oxidative stress.

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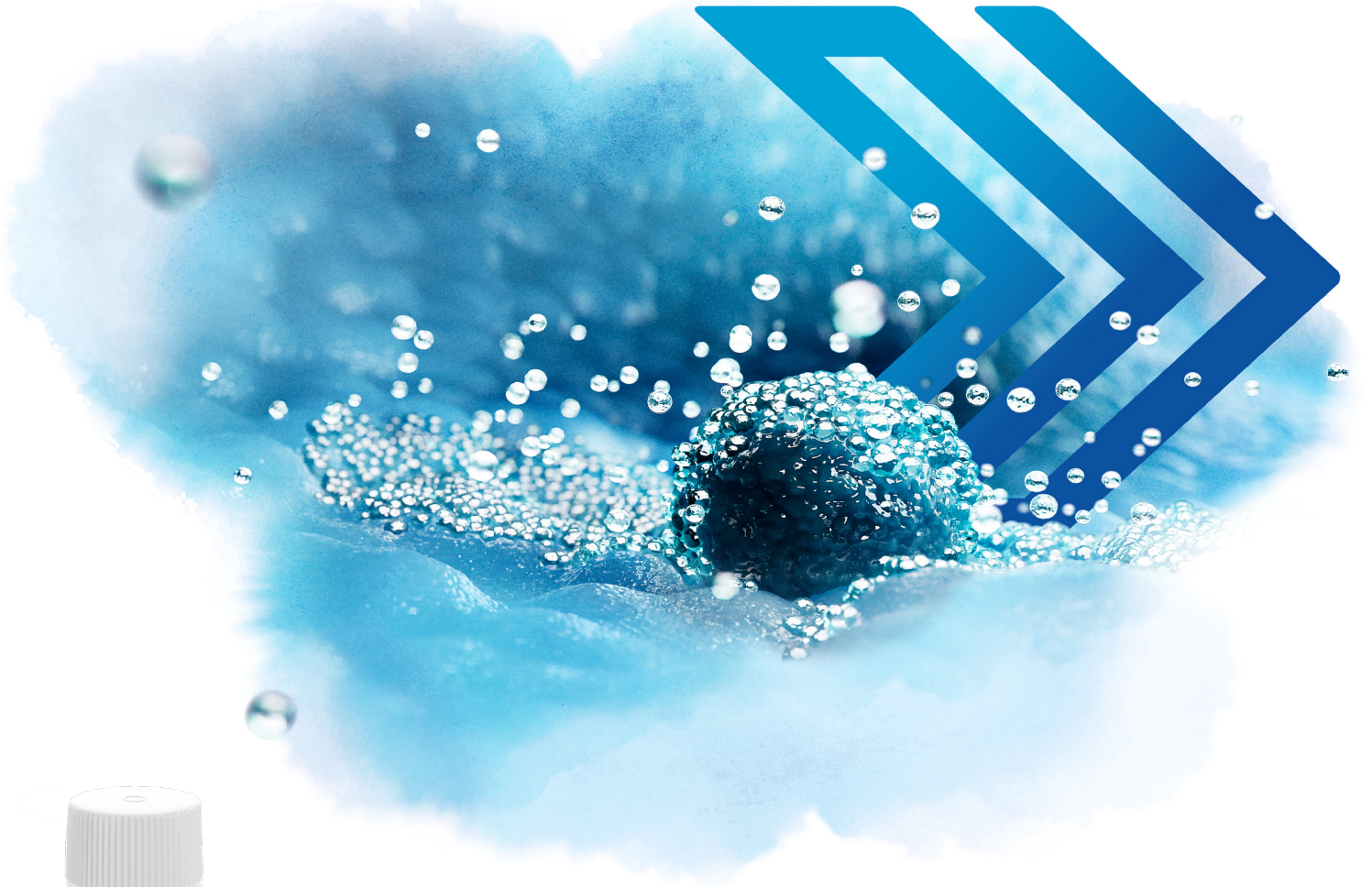
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