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Homocysteine levels in women with polycystic ovary syndrome treated with metformin versus rosiglitazone: a randomized study

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BACKGROUND: Elevated levels of plasma homocysteine (Hcy) have been implicated as a significant risk factor for cardiovascular disease. Although long-term treatment with metformin can increase Hcy levels in patients with type II diabetes mellitus or coronary heart disease, it is becoming an increasingly accepted and widespread medication in polycystic ovary syndrome (PCOS). In the literature, only one study has demonstrated that metformin increases Hcy levels in PCOS patients, but the effect of other insulin sensitizers on Hcy levels have not been reported previously in women with PCOS. We aimed to assess the effects of metformin and rosiglitazone on plasma Hcy levels in patients with PCOS. METHODS: Thirty women were randomized to two groups: 15 women in group 1 received 850 mg of metformin twice daily for 3 months. In group 2, 15 women received 4 mg of rosiglitazone for 3 months. In both groups, body mass index, menstrual pattern, and plasma total Hcy, insulin, glucose and lipid metabolism parameters were recorded at baseline and at 3 months. RESULTS: Hcy levels increased from 8.93 ± 0.49 to 11.26 ± 0.86 μmol/l ($P = 0.002$) and from 10.70 ± 0.86 to 12.36 ± 0.81 μmol/l ($P = 0.01$) in the metformin and rosiglitazone groups, respectively. Apolipoprotein (Apo) A1 levels increased from 127.10 ± 6.85 to 145.7 ± 7.18 mg/dl ($P = 0.018$) in the metformin group. Total cholesterol (total-C), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), lipoprotein (a) and Apo B levels decreased in the metformin group, but the change was not significant. Total-C levels decreased from 161.15 ± 8.94 to 150.23 ± 8.73 mg/dl ($P = 0.026$), HDL-C decreased from 43.13 ± 2.65 to 39.15 ± 2.52 mg/dl ($P = 0.005$) and LDL-C levels decreased from 93.83 ± 6.06 to 80.7 ± 2.30 mg/dl ($P = 0.021$) in the rosiglitazone group. CONCLUSION: Treatment with insulin sensitizers in women with PCOS may lead to increases in Hcy levels.

Key words: hyperhomocysteinaemia/metformin/PCOS/rosiglitazone

Introduction

Polycystic ovary syndrome (PCOS), defined as ovarian hyperandrogenism and oligo-anovulation in the absence of another specific disorder, is frequently associated with peripheral insulin resistance and compensatory hyperinsulinaemia. Hyperinsulinamia in PCOS has been implicated in promoting ovarian androgen hypersecretion (Nestler, 1997). Evidence for a causal role for insulin in the ovarian abnormalities of PCOS is derived from the clinical use of agents that reduce circulating insulin, either by directly diminishing its secretion or by improving peripheral insulin sensitivity (Ehrmann, 1999; Dahlgren and Janson, 2000; Seli and Duleba, 2002, 2004).

Late complications of PCOS commonly include non-insulin-dependent diabetes mellitus, hypertension, dyslipidaemia, atherosclerosis and vascular disease (Wild *et al.*, 1985; Talbot *et al.*, 1995; Legro *et al.*, 2001). PCOS is also associated with an increased cardiovascular disease (CVD) risk. Because elevated levels of homocysteine (Hcy) have been considered a risk factor for CVD, these elevated levels may provide an explanation for the increased CVD risk found in insulin-resistant patients with PCOS (Wild *et al.*, 1985; Fonseca *et al.*, 1999; Legro *et al.*, 2001; Yarali *et al.*, 2001). Use of insulin sensitizers for long-term medical benefits requires further prospective studies to confirm their clinical significance; however, there is strong evidence for the use of metformin and rosiglitazone for short-term therapy to regulate cycle and ovulation induction among patients with PCOS (Stadtmauer *et al.*, 2002; Belli *et al.*, 2004). However, there is evidence that metformin may increase Hcy levels in patients with type II diabetes mellitus and coronary heart disease on long-term treatment (Carlsen *et al.*, 1997; Wulffele

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et al., 2003). Therefore, it is important to assess the effect of short-term insulin sensitizer administration on Hcy levels in patients with PCOS and insulin resistance.

This study was designed to examine the effects of short-term metformin and rosiglitazone therapy, especially on serum levels of Hcy and other cardiovascular factors such as lipid profile and insulin resistance, in patients with PCOS.

Materials and methods

This study was conducted in the Department of Obstetrics and Gynecology of Baskent University School of Medicine, Adana, Turkey. Between April 2002 and June 2003, 30 women with PCOS participated in this prospective randomized study. The study was approved by the Ethical Committee of Baskent University. Informed consent was obtained from each patient before entering the study.

All patients with oligomenorrhoea (a cycle length of 45 days or six periods per year) or amenorrhoea, who also had evidence of hyperandrogenism [a hirsutism score >7, according to Ferriman and Gallway (1961)] and/or an elevated serum testosterone level, were diagnosed as having PCOS, after all the other causes of hyperandrogenism had been excluded. Subjects treated with hormonal medications within 3 months were also excluded from the study. Sonographic diagnosis of PCOS was confirmed if there were 10 or more subcapsular follicular cysts, 2–8 mm in diameter, arranged around a thickened ovarian stroma (Adams *et al.*, 1986). Patients were randomized to two groups by an allocation sequence generated from a random number table and assigned through consecutively numbered opaque, sealed envelopes. The first group ($n = 15$) received metformin (850 mg twice daily; Glucophage®, 850 mg, Eczacibasi, Kücükkaristiran, Lüleburgaz); the second group ($n = 15$) received rosiglitazone maleate (4 mg; Avandia®, 4 mg, GlaxoSmithKline, SB Pharmaco PR, Inc., Puerto Rico) for 3 months. Husbands of patients were advised to use barrier methods of contraception because the safety of rosiglitazone administration during pregnancy has yet to be confirmed. Hormonal parameters [FSH, LH, estradiol (E_2), total testosterone, free testosterone, dehydroepiandrosterone sulphate (DHEAS), and prolactin], lipid profile [total cholesterol (total-C), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), triglyceride (TG), lipoprotein (Lp) (a), apolipoprotein (Apo) A1 and Apo B], Hcy concentrations, vitamin B_{12} and folic acid levels, and basal insulin levels were assessed. A 75 g oral glucose tolerance test was performed, before and after treatment, in all patients. Blood samples were obtained on the third day of menstruation or at any time after a spontaneous luteal phase was excluded by serum progesterone measurements (serum progesterone measurements <3 ng/ml) in patients with delayed menstruation.

Blood samples were collected at 8 a.m. (12 h fasting state) and at 120 min after 75 g glucose ingestion. Plasma glucose levels were measured using the glucose oxidase method; plasma insulin concentrations were measured by the microparticle enzyme immunoassay method (Ax SYM insulin assay; Abbott, Tokyo, Japan).

Hcy levels are influenced by several variables, including smoking, renal function, vitamin B status and enzyme dysfunction states. Renal status was examined before the women entered the study. Vitamin B_{12} and folate levels were examined before and after treatment. All women were non-smokers. Patients with folic acid and vitamin B_{12} deficiencies were excluded from the study. None of the patients had used metformin or rosiglitazone before this trial.

Levels of plasma fasting glucose, total-C, HDL-C and TG were determined by the calorimetric method using a Cobas Mira Plus autoanalyser (Roche Diagnostics, Mannheim, Germany). LDL-C and VLDL-C levels were calculated by the Friedwald formula (Friedwald *et al.*, 1972). Apo A_1, Apo B and Lp (a) were quantitated by the immunoturbidimetric method in a Roche/Hitachi 912 autoanalyser. Insulin, LH, FSH, E_2, prolactin and Hcy concentrations were measured using an Ax SYM hormone autoanalyser (Abbott Laboratories, Abbott Park, IL) using the microparticle enzyme immunoassay method. Total testosterone and DHEAS were measured in an Immulite One auto analyser (Bio Diagnostic Products Corp., Los Angeles, CA) using the chemiluminescent method. Insulin sensitivity was calculated using the HOMA (homeostasis model assessment) [(formula: fasting glucose (mmol/l) × fasting insulin (μU/ml)/22.5)] and QUICKI (quantitative insulin sensitivity check index) [(formula: 1/(log fasting insulin (μU/ml) + log fasting glucose (mg/dl)] indexes (Matthews *et al.*, 1985; Belli *et al.*, 2004). The intra- and inter-assay coefficients of variation of glucose were 0.4 and 1.2% at 82.2 mg/dl, 0.6 and 1.3% at 81.4 mg/dl, and 0.7 and 1.1% at 80.5 mg/dl, respectively. The intra- and inter-assay coefficients of variation of insulin were 2.6 and 1.8% at 8.7 μU/ml, 4.1 and 2.5% at 42.2 μU/ml, and 2.9 and 2% at 126.2 μU/ml, respectively.

Hcy, mixed disulphide, and protein-bound forms of Hcy in the sample were reduced to form free Hcy using dithiothreitol (DTT). Free Hcy was converted to *S*-adenosyl-L-homocysteine (SAH) using SAH hydrolase and excess adenosine. SAH and labelled fluorescein tracer compete for sites on the monoclonal antibody molecule. The intensity of polarized florescent light was measured using a fluorescence polarization immunoassay (FPIA) optical unit. The coefficient of variation of FPIA was 4.6% at 7.99 μmol/l, 3.1% at 13.71 μmol/l and 2.8% at 26.67 μmol/l.

Plasma folic acid and vitamin B_{12} concentrations were measured with the chemiluminescent method using an E170 immunoassay analyser (Roche Diagnostics Corp., Indianapolis, IN). The electrochemiluminescence immunoassay was used on a Roche Modular Analytics E170 immunoassay analyser (Roche Diagnostics).

The primary outcome was elevation in Hcy levels. Secondary outcomes included changes in body mass index (BMI), folic acid, vitamin B_{12}, lipid profile, and QUICKI and HOMA levels with metformin and rosiglitazone therapy.

Statistical analysis

Sample size calculations, assuming 80% power to detect 2 μmol/l changes in Hcy levels between groups, indicated the need for 15 patients in each group.

Data are expressed as mean ± SEM. An analysis of variance test was used to analyse differences between groups. Differences between the two groups were analysed using the independent Student *t*-test and the Mann–Whitney U-test. The paired-samples *t*-test was used to analyse changes in variables before and after treatment in groups 1 and 2 overall. Data were analysed using SPSS software for Windows® (Statistical Package for the Social Sciences, version 9.05, SSPS Inc, Chicago, IL). Correlations between parametric variables and nominal parametric data sets at baseline were assessed by Pearson and Spearman correlation coefficients, respectively. Differences were considered statistically significant at a level of $P < 0.05$.

Results

All patients completed the study and were analysed for the primary outcome after 3 months follow-up. Baseline characteristics of the patients are given in Table I. HDL-C levels

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Table I. Baseline characteristics of patients with polycystic ovary syndrome before treatment with metformin or rosiglitazone

	Group 1 ($n = 15$)	Group 2 ($n = 15$)	*P*
Age (years)	24.13 ± 1.42	25.53 ± 1.68	NS
Parity	0.53 ± 0.19	0.40 ± 0.24	NS
BMI (kg/m^2)	26.17 ± 1.44	29.32 ± 1.58	NS
Weight (kg)	67.77 ± 3.38	77.80 ± 4.65	NS
Hcy (μmol/l)	8.93 ± 0.49	10.70 ± 0.86	NS
Folic acid (ng/ml)	7.72 ± 0.52	8.86 ± 1.10	NS
Vitamin B_{12} (pg/ml)	288.68 ± 24.10	309.38 ± 31.95	NS
FSH (mIU/l)	6.42 ± 0.40	5.48 ± 0.35	NS
LH (mIU/l)	13.65 ± 3.31	8.27 ± 2.02	NS
DHEAS (ng/ml)	2851 ± 389.48	2624.93 ± 336.91	NS
17-OH-progesterone (ng/ml)	1.10 ± 0.17	1.11 ± 0.15	NS
Total testosterone (ng/ml)	0.85 ± 0.11	0.71 ± 0.12	NS
Free testosterone (pg/ml)	2.27 ± 0.15	2.36 ± 0.14	NS
HOMA >2.16 (%)	60	66.7	NS
QUICKI <0.34 (%)	53.3	60	NS
Total-C (mg/dl)	170.87 ± 7.62	160.7 ± 8.28	NS
HDL-C (mg/dl)	57.87 ± 3.69	43.13 ± 2.65	0.003
LDL-C (mg/dl)	101.33 ± 7.7	94.57 ± 5.48	NS
VLDL-C (mg/dl)	15.33 ± 1.92	23.57 ± 4.74	NS
TG (mg/dl)	76.8 ± 9.66	144.92 ± 45.92	NS
Apo A1 (mg/dl)	137.46 ± 7.73	132.47 ± 5.07	NS
Apo B (mg/dl)	72.8 ± 7.51	76.78 ± 7.04	NS
Lip (a) (mg/dl)	54.63 ± 19.61	42.70 ± 15.06	NS

Group 1 = metformin therapy; group 2 = rosiglitazone therapy.
BMI = body mass index; Hcy = homocysteine;
DHEAS = dehydroepiandrosterone sulphate; HOMA = homeostasis model assessment; QUICKI = quantitative insulin sensitivity check index;
total-C = total cholesterol; HDL-C = high-density lipoprotein cholesterol;
LDL-C = low-density lipoprotein cholesterol; VLDL-C = very low-density lipoprotein cholesterol; TG = triglyceride; Apo A1 = apolipoprotein;
Apo B = apolipoprotein B; Lip (a) = lipoprotein (a).

were 57.87 ± 3.69 and 43.13 ± 2.65 mg/dl in the metformin and rosiglitazone groups, respectively ($P = 0.003$). At baseline, patients in the rosiglitazone group had higher weights and BMIs, but these differences did not reach statistical significance. There were no statistically significant differences in the other parameters.

All of the women in the study had oligo-amenorrhoea and PCOS appearance on sonography. Ten patients in the metformin group and 11 patients in the rosiglitazone group had clinical and/or biochemical signs of hyperandrogenism. Seven patients in the metformin group and three patients in the rosiglitazone group had clinical hirsutism. Elevated total testosterone levels were detected in six patients and in four patients, and elevated DHEAS levels were detected in five patients and in four patients in the metformin and rosiglitazone groups, respectively. An elevated free testosterone level was detected only in one patient out of the 15 in the rosiglitazone group.

Hcy and vitamin B assessments

Hcy levels were increased from 8.93 ± 0.49 to 11.26 ± 0.86 μmol/l ($P = 0.002$) and from 10.70 ± 0.86 to 12.36 ± 0.81 μmol/l ($P = 0.01$) in the metformin and rosiglitazone groups, respectively (Table II). There were no statistically significant changes recorded for folic acid and vitamin B_{12} in the two groups.

In the Pearson correlation test, no correlation was found between Hcy levels and age, BMI, insulin, HOMA, QUICKI, blood glucose level after 50 g glucose screening, total testosterone, DHEAS, vitamin B_{12} and folate levels. There was a correlation between free testosterone and Hcy levels ($r = -0.541$, $P = 0.037$) at baseline in the metformin group. In the Spearman correlation test, there was a negative correlation between the presence of insulin resistance and Hcy levels (according to QUICKI and HOMA: $r = -0.525$, $P = 0.044$ and $r = -0.541$, $P = 0.037$, respectively) at baseline in the rosiglitazone group.

Body weight, glucose and lipid metabolism assessments

Insulin resistance (IR) was defined as an abnormal result in HOMA and QUICKI values (HOMA >2.16, QUICKI <0.34) (Belli *et al.*, 2004). The IR ratio was similar in both groups. There were no statistically significant differences recorded in the HOMA and QUICKI levels in either group (Table II). BMI and weight were not altered with treatment (Table III).

Apo A1 levels increased from 127.10 ± 6.85 to 145.7 ± 7.18 mg/dl ($P = 0.018$) in the metformin group. However, Apo A1 levels in the rosiglitazone group showed no significant change.

Total-C levels decreased from 161.15 ± 8.94 to 150.23 ± 8.73 mg/dl ($P = 0.026$), HDL-C decreased from 43.13 ± 2.65 to 39.15 ± 2.52 mg/dl ($P = 0.005$) and LDL-C

Table II. Homocysteine levels, vitamin B status and insulin sensitivity before and after treatment in both the metformin and rosiglitazone groups

Variable	Group	Before treatment	After treatment	3 Months (95% CI)	*P*
Hcy (μmol/l)	1	8.93 ± 0.49	11.26 ± 0.86	2.33 (1.04 to 3.61)	0.002
	2	10.70 ± 0.86	12.36 ± 0.81	1.66 (+0.46 to 2.85)	0.01
Folic acid (ng/ml)	1	7.72 ± 0.52	6.65 ± 0.78	−1.08 (−2.32 to 0.17)	NS
	2	8.86 ± 1.10	7.76 ± 0.52	−0.31 (−1.88 to 1.26)	NS
Vitamin B_{12} (pg/ml)	1	288.68 ± 24.10	328.56 ± 136.81	39.88 (−06.86 to 386.62)	NS
	2	309.38 ± 31.95	321.76 ± 76.10	12.38 (−46.02 to 170.78)	NS
HOMA	1	2.6637 ± 0.48613	2.564 ± 0.3059	−0.10 (−1.14 to 0.94)	NS
	2	3.9567 ± 1.14612	3.230 ± 0.6148	−0.73 (−3.21 to 1.76)	NS
QUICKI	1	0.3396 ± 0.00987	0.3397 ± 0.0066	0.00 (−0.01 to 0.02)	NS
	2	0.3360 ± 0.0147	0.3340 ± 0.0019	−0.01 (−0.03 to 0.02)	NS

Group 1 = etformin therapy ($n = 15$); group 2 = rosiglitazone therapy ($n = 15$).
CI = confidence interval.

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Table III. Parameters related to lipid metabolism and body weight before and after treatment with metformin or rosiglitazone

Variable	Group	Before treatment	After treatment	3 Months (95% CI)	*P*
Weight (kg)	1	67.77 ± 3.38	66.88 ± 3.62	−0.35 (−0.98 to 1.67)	NS
	2	77.80 ± 4.65	76.73 ± 4.52	−1.07 (−2.16 to 0.03)	NS
BMI (kg/m^2)	1	26.17 ± 1.44	25.82 ± 1.49	−0.34 (−1.68 to 0.98)	NS
	2	29.32 ± 1.58	28.43 ± 1.46	−0.59 (−1.27 to 0.08)	NS
Total-C (mg/dl)	1	170.87 ± 7.62	163.67 ± 6.46	−7.2 (−17.59 to 3.19)	NS
	2	161.15 ± 8.94	150.23 ± 8.73	−10.92 (−20.32 to 1.52)	0.026
HDL-C (mg/dl)	1	57.87 ± 3.69	56.8 ± 4.09	−1.07 (−3.95 to 1.81)	NS
	2	43.13 ± 2.65	39.15 ± 2.52	−4.62 (−1.64 to −7.59)	0.005
LDL-C (mg/dl)	1	101.33 ± 7.77	92.20 ± 6.55	−9.13 (−19.16 to 0.89)	NS
	2	93.83 ± 6.06	80.7 ± 2.30	−13.13 (−2.42 to −23.85)	0.021
VLDL- C (mg/dl)	1	15.57 ± 2.05	15.59 ± 1.67	0.01 (−0.25 to 2.57)	NS
	2	20.00 ± 2.09	21.22 ± 3.08	1.22 (−5.51 to 7.94)	NS
TG (mg/dl)	1	76.8 ± 9.66	78.87 ± 7.71	0.06 (−11.58 to 11.72)	NS
	2	144.92 ± 45.92	152.46 ± 48.42	7.54 (−22.62 to 37.70)	NS
APO 1(mg/dl)	1	127.10 ± 6.85	145.7 ± 7.18	18.60 (4.01 to 33.20)	0.018
	2	130.80 ± 6.86	115.20 ± 15.99	−15.60 (−46.36 to 5.16)	NS
APO B (mg/dl)	1	72.8 ± 7.51	69.2 ± 6.83	−3.60 (−12.47 to 5.27)	NS
	2	76.78 ± 7.04	69.67 ± 7.01	−7.11 (−14.72 to 0.50)	NS
Lp (a) (mg/dl)	1	54.63 ± 19.61	40.00 ± 19.47	−14.63 (−50.81 to 21.56)	NS
	2	42.70 ± 15.06	51.3 ± 16.26	8.60 (15.18 to 32.38)	NS

Group 1 = metformin therapy ($n = 15$); group 2 = rosiglitazone therapy ($n = 15$).

levels decreased from 93.83 ± 6.06 to 80.7 ± 2.30 mg/dl ($P = 0.021$) in the rosiglitazone group. However, the decreases in total-C, HDL-C and LDL-C levels in the metformin group were not significant.

Hormonal parameters

Total testosterone, free testosterone and LH levels were not altered with treatment (data not shown).

Ovulation and menstrual pattern

Before treatment, all patients had anovulation and oligoamenorrhoea. Two patients of the 15 patients given metformin and three of the 15 patients given rosiglitazone ovulated following treatment. Seven patients in the metformin group and five patients in the rosiglitazone group had regular menses after treatment.

Adverse effects

None of the patients reported any adverse effects with rosiglitazone throughout the treatment period. Three of the 15 patients given metformin had problems with nausea and vomiting. None of the 30 patients had to stop therapy early.

Discussion

The most important finding of our study is that 3 months of metformin or rosiglitazone therapy result in a significant increase in plasma Hcy concentrations, without significant changes in BMI and IR parameters. Another interesting finding of this study was the negative correlation between the presence of IR and Hcy levels in some patients.

In our study, neither metformin therapy nor rosiglitazone therapy changed the BMI significantly. Although a small but significant decrease in weight during metformin treatment has been described previously (Harborne *et al.*, 2003), at least five studies failed to demonstrate any significant weight or waist–hip ratio reduction during the treatment period (Crave *et al.*, 1995; Acbay and Gundogdu, 1996; Moghetti *et al.*, 2000; Vandermolen *et al.*, 2001; Chou *et al.*, 2003). A small number of patients in the studies and differences in the treatment periods may explain these discrepancies.

Some studies have suggested a possible relationship between Hcy and IR (Schachter *et al.*, 2003); however, others have not (Randeva *et al.*, 2002). One author has reported a negative correlation between Hcy and insulin levels (Bar-On *et al.*, 2000). We found a significant negative correlation between Hcy levels and the presence of IR according to QUICKI only in the rosiglitazone group. There is no immediate explanation for this finding. First, IR and elevated Hcy are two factors that may act separately and probably do not potentiate each other; secondly, the relatively limited number of patients in each group might explain this discrepancy.

Wulffele and colleagues have demonstrated that 16 weeks of treatment with metformin in patients with type 2 diabetes mellitus reduces folic acid and vitamin B_{12} and increases Hcy levels (Wulffele *et al.*, 2004). Another study has shown that metformin increases total serum Hcy levels in non-diabetic male patients with coronary heart disease (Carlsen *et al.*, 1997). Recently, Vrbikova *et al.* (2002) showed that metformin (1000 mg/day) increases serum Hcy levels from 10.1 ± 2.6 to 13.4 ± 5.1 μmol/l in patients with PCOS after 4 weeks of treatment. In our study, mean increases in Hcy concentrations were 2.33 ± 0.60 and 1.66 ± 0.56 μmol/l in the metformin and rosiglitazone groups, respectively. Because of chronic use of this medication from as early as the teenage years in patients with PCOS, the safety of long-term metformin therapy must be studied. Future studies should investigate the duration of therapy and the benefit or detrimental effects of the medication on cardiovascular risk factors.

Studies on the effects of metformin on the lipid profile offer conflicting results. Crave *et al.* (1995) have demonstrated that metformin administration has no additional

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benefit over the effect of diet on the lipid profile. Fleming *et al.* (2002) have demonstrated an increase in HDL-C and a decrease in LDL-C within 14 weeks of metformin treatment. In our study, Apo A1 levels increased with metformin therapy, while no changes were observed in any other lipid profile parameters.

Other insulin-sensitizing agents that have been studied in women with PCOS include troglitazone, rosiglitazone, pioglitazone and D-chiro-inositol. There are several studies examining the effects of rosiglitazone in patients with PCOS. It has been suggested that rosiglitazone improves IR parameters, normalizes the menstrual cycle and enhances both spontaneous and clomiphene-induced ovulation in overweight and obese women with PCOS (Ghazeeri *et al.*, 2003; Belli *et al.*, 2004). To our knowledge, there are no data regarding the effects of rosiglitazone on Hcy levels and lipid profiles in patients with PCOS. In our study, the mean Hcy levels increased, and total-C, HDL-C and LDL-C levels decreased significantly with rosiglitazone treatment.

The mechanisms by which drugs alter the plasma Hcy level vary. Any drug, such as methotrexate, nitrous oxide or azaribine, that reacts with folic acid, vitamin B_{12} or B_6 can cause hyperhomocysteinaemia (de la Calle *et al.*, 2003). Interference with vitamin absorption from the gut may lead to increased plasma Hcy levels (Desouza *et al.*, 2002). In patients treated with metformin, a decrease in vitamin B_{12} and folate levels has also been found; however, serum methylmalonic acid, usually a more sensitive marker of functional vitamin B_{12} deficiency than elevated Hcy levels, remained unchanged (Savage *et al.*, 1994; Carlsen *et al.*, 1997). In our study, although the folic acid levels tended to decrease, vitamin B_{12} and folic acid levels did not change significantly from baseline values after treatment in both groups. Therefore, it remains an open question whether the metformin-induced increase in Hcy is secondary to reduced vitamin B_{12} or folate levels, a combination of both, or if metformin increases Hcy by an as yet unknown mechanism.

In conclusion, our results suggest that 3 months of metformin and rosiglitazone therapy result in a significant increase in plasma Hcy concentrations, without significant changes in BMI and IR parameters that could result in increased cardiovascular risk. We believe that in patients with PCOS, additional treatment modalities that could decrease Hcy levels (which are increased during metformin or rosiglitazone therapy) might be an appropriate management option. Studies with control groups and long-term follow-up are necessary to determine whether PCOS is associated with an increase in cardiovascular events and whether insulin sensitizer therapy can be useful in modifying cardiovascular risk factors.

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