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ORIGINAL ARTICLE

The effect of ethinyl estradiol–cyproterone acetate treatment on homocysteine levels in women with polycystic ovary syndrome

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Abstract
Objective Women with polycystic ovary syndrome (PCOS) have multiple risk factors for cardiovascular disease. The cardiovascular risk marker homocysteine (Hcy) is elevated in women with PCOS. This prospective study investigated the effect of oral contraceptives containing ethinyl estradiol–cyproterone acetate (EE–CA) on serum Hcy levels in women with PCOS.
Study design A total of 30 women with PCOS were enrolled in this prospective study. The diagnosis of PCOS was made according to the criteria of the Rotterdam PCOS consensus workshop group. All women took oral contraceptives containing EE/CA (35 μg/2 mg) for 3 months. Serum samples for Hcy, lipid profile and hormones were obtained during the early follicular phase (days 3–5) of the spontaneous or progestin-induced bleeding at baseline, and after the third treatment cycle.
Results Three months of EE–CA therapy significantly decreased the Hcy levels from 55.97 ± 16.04 to 54.03 ± 16.15 ($P = 0.01$). A significant correlation was observed between the Hcy and total and free testosterone levels ($r = 0.44$, $P = 0.015$ and $r = 0.46$, $P = 0.001$ respectively).
Conclusions Although the decrease in Hcy levels with EE–CA therapy was statistically significant, further studies are necessary to determine the clinical benefit of this treatment.

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Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine diseases affecting 5–10% of women during their reproductive years [1, 2]. PCOS is characterized by hyperandrogenism, chronic anovulation, hyperinsulinemia, and obesity [1]. The consequences of PCOS extend beyond the reproductive axis; women with this disorder are at substantial risk for developing metabolic and cardiovascular abnormalities similar to those that constitute the metabolic syndrome. [3] Women with PCOS have several well-recognized risk factors for cardiovascular disease, such as obesity, impaired glucose tolerance and dyslipidemia [4]. Several studies using surrogate endpoints for cardiovascular disease risk have suggested that women with PCOS are at increased risk [5–10, 11]. As a result, PCOS is one of the major subjects for preventive medicine, and the new trend in primary treatment focuses on the metabolic sequelae.

Homocysteine (Hcy) is a sulfhydryl-containing amino acid derived from the metabolic demethylation of methionine. Multiple prospective and case-control studies have suggested that moderately elevated plasma Hcy concentration is an independent risk factor for atherothrombotic vascular disease. Hcy concentrations are consistently higher in patients with peripheral, cerebrovascular and coronary artery disease than in those without such diseases [12]. A graded, linear relationship between homocysteine levels and vascular risk has been revealed, and a 5 μmol/l increase in total Hcy was associated with an increased vascular risk of about one-third in a metanalysis of 27 observational studies [13]. The serum Hcy levels were significantly

higher in PCOS patients than in normally cycling women in several studies [9, 14–16].

Ovarian suppression with oral contraceptives (OC) is frequently used to treat menstrual disturbances and hyperandrogenism in women with PCOS. This treatment confers benefit through a variety of mechanisms, including the suppression of pituitary luteinizing hormone secretion, suppression of ovarian androgen secretion and increased circulating sex hormone-binding globulin (SHBG). However, because OC use is associated with adverse effects on carbohydrate and lipid metabolism in normal women [17–19] concern exists that this form of therapy could aggravate hyperinsulinemia and dyslipidemia and further increase the risk of cardiovascular disease in women with PCOS.

Conflicting data have been reported regarding the effects of different types of combined OCs on Hcy levels in normally cycling women [20]. At present, no data are available on the influence of combined OCs on serum Hcy levels in women with PCOS. Therefore, we investigated the effect of ethinyl estradiol–cyproterone acetate (EE–CA) treatment on homocysteine levels in women with PCOS.

Materials and methods

The study group consisted of 30 consecutive women with PCOS who presented to our clinic with oligo/amenorrhea, dysfunctional uterine bleeding or hyperandrogenism between October 2004 and May 2005. The study protocol was approved by the medical ethics committee of Duzce Medical School, Duzce, Turkey, and conformed to ethical guidelines of the 1975 Helsinki Declaration. Informed written consent was obtained from each patient before enrollment in the study. In this prospective longitudinal study, the subjects served as their own controls.

The diagnosis of PCOS was made according to the tenets of the Rotterdam PCOS consensus workshop group, when two of the three criteria were present: oligomenorrhea (fewer than six menstrual periods in the preceding year) or amenorrhea ($\leq$2 uterine bleedings/year), clinical or biochemical signs of hyperandrogenism and the presence of $\geq$12 follicles in each ovary measuring 2–9 mm in diameter or increased ovarian volume (>10 ml) [21]. Related disorders were ruled out by measuring the basal serum 17OH–progesterone, prolactin (PRL), and dehydroepiandrosterone sulfate (DHEAS) levels. Subjects treated with any hormonal medications, vitamins or drugs that increase Hcy levels within the previous 6 months and those with folic acid, vitamin B_{12} or vitamin B_6 deficiencies were excluded from the study.

Weight and height were measured and the body mass index (BMI = weight (kg)/height $(m)^2$) was calculated. BMI values of 25.1–30 kg/m^2 were considered overweight, and >30 kg/m^2 as obese. Waist and hip circumferences were measured to the nearest centimeter with a soft tape at the narrowest part of the torso and at the widest part of the gluteal region, and the waist-to-hip ratio (WHR) was calculated [22]. A WHR >0.72 was considered abnormal. The degree of hirsutism was assessed using the method of Ferriman and Gallwey [23]. Normal glucose tolerance was diagnosed according to criteria of the American Diabetes Association [24] with a 2 h 75 g oral glucose tolerance test (OGTT).

All the patients received combined OC pills containing EE 35 μg + CA 2 mg (Diane-35 tablet; Schering, Istanbul, Turkey) for 21 days per month followed by a 7-day pill-free period, for three cycles. All laboratory tests including hormonal parameters [follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E_2), total testosterone, free testosterone, DHEAS, prolactin], lipid profile [total cholesterol (total-C), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), very-low-density lipoprotein cholesterol (VLDL-C), triglyceride (TG)], Hcy, folic acid, vitamin B_{12} levels and a 75 g oral glucose tolerance test were performed before treatment and after the third cycle of treatment. Venous blood samples were obtained in the morning (08:00–09:00 after an overnight fast) during the early follicular phase (days 3–5) of the spontaneous or progestin-induced bleeding at baseline, and after the third treatment cycle. Blood samples were collected in tubes and placed on ice. The blood was centrifuged immediately and the serum was stored directly at −80°C until analysis.

The serum glucose, total-C, LDL-C, HDL-C, VLDL-C, and triglyceride concentrations were measured using commercial kits (Olympus Diagnostica, Hamburg, Germany) on an automatic analyzer (Olympus AU 640). The serum FSH, LH, E_2, total testosterone, DHEAS, prolactin, folic acid and vitamin B_{12} levels were measured using an electrochemiluminescence immunoassay (ECLIA) on an Elecsys E-170 analyzer (Roche Diagnostics Systems, Basel, Switzerland). The free testosterone concentration in serum was assessed using a radioimmunoassay (RIA) method with a DPC set (Diagnostic Products Corporation, Los Angeles, CA, USA). The sensitivity of the method defined as the detectable concentration was 0.15 pg/ml.

The fasting serum total Hcy level concentrations were measured using an ELISA method (Axis Homocysteine EIA; Axis-Shield AS, Heidelberg, Germany).

A sample size of 22 participants was estimated as necessary to detect a 2 μmol/l change in Hcy levels with ($\alpha = 0.05$ and a power of 0.80. The power analysis was performed using MINITAB Statistical Software, version 14.20. First, the distribution of the variables was analyzed. Because the values were not normally distributed, non-parametric tests were used for statistical analysis. Wilcoxon's signed-ranks

test was used to compare baseline and post-treatment values. Correlations between Hcy levels and the lipid profile and androgen levels of the subjects were determined by using bivariate correlation statistics and are expressed as Spearman's correlation coefficients. The data are expressed as means ± SD. In all analyses, $P < 0.05$ was considered to indicate statistical significance. All the statistical evaluations were performed using SPSS software (version 11.0; SPSS, Chicago, IL, USA).

Results

All the enrolled women completed the study. All of the patients had apparent PCO on ultrasonography. Of all the patients, 24 (80%) patients had oligomenorrhea or amenorrhea, 17 (56.6%) had clinical or biochemical signs of hyperandrogenism and 12 (40%) patients had hirsutism. Although only six (20%) patients had a waist circumference >88 cm, central obesity as estimated by the WHR was present in 23 patients (76.6%). Seventeen patients were lean (57%) and 13 patients were obese (43%). The blood pressure did not change during the study period (diastolic BP = 73.68 ± 7.61 vs. 72.23 ± 6.56 mmHg; systolic BP = 117.90 ± 10.32 vs. 115.30 ± 9.18 mmHg).

None of the patients had diabetes or impaired glucose tolerance; the fasting and 2 h glucose levels on the OGTT were normal in all patients at baseline (91.17 ± 10.04 and 99.60 ± 20.91, respectively). The baseline demographic, endocrinologic and metabolic features of the patients and the post-treatment values are summarized in Table 1. No significant changes were observed in BMI and WHR during the treatment period. The vitamin B_{12} and folic acid levels were within the normal ranges for all patients, both at the beginning and end of the study.

The serum levels of total-C, HDL-C, LDL-C, VLDL-C and TG were normal at baseline. After 3 months of EE–CA therapy, the serum levels of total-C, HDL-C, LDL-C, VLDL-C and TG remained unchanged. During treatment, significant decreases occurred in the total testosterone (from 65 ± 45 to 49 ± 36, $P = 0.018$), DHEAS (from 222 ± 95 to 187 ± 64, $P = 0.013$), and free testosterone (from 13.0 ± 4.0 to 8.7 ± 3.7, $P = 0.01$) levels.

At baseline, the Hcy levels of the obese patients were significantly higher than those of the lean PCOS patients (66.44 ± 13.32 vs. 51.48 ± 15.21 µmol/l, respectively, $P = 0.001$). Three months of contraceptive therapy resulted in a statistically significant decrease in Hcy levels (from 55.97 ± 16.04 to 54.03 ± 16.15, $P = 0.01$). The data indicated that EE-CA treatment significantly improved the Hcy levels with respect to baseline in both lean and obese PCOS patients (from 51.48 ± 15.21 to 49.67 ± 15.10, $P = 0.01$ and from 66.44 ± 13.32 to 64.22 ± 14.45, $P = 0.03$, respectively; Fig. 1). Spearman's coefficient of correlation showed a statistically significant positive relationship between Hcy levels and total and free testosterone levels at baseline ($r = 0.41$, $P = 0.027$ and $r = 0.45$, $P = 0.001$, respectively). This positive correlation persisted at the third month ($r = 0.44$, $P = 0.015$ and $r = 0.46$, $P = 0.001$, respectively). No relationship between Hcy levels and any other parameter was seen.

Table 1 Demographic, endocrinological and metabolic characteristics of PCOS subjects at baseline and post-treatment ($n = 30$)

	Baseline	Post-treatment	*P*
Age (year)	25.10 ± 6.94		
BMI (kg/m^2)	23.85 ± 4.17	23.20 ± 4.01	NS
WHR	0.76 ± 0.05	0.75 ± 0.04	NS
FSH (mIU/ml)	6.7 ± 1.6	6.2 ± 2.1	NS
LH (mIU/ml)	8.0 ± 4.7	6.1 ± 3.1	0.05
E2 (pg/ml)	37.3 ± 14.5	35.6 ± 22.5	NS
PRL (ng/ml)	11.4 ± 5.5	10.6 ± 5.5	NS
DHEAS (µg/ml)	222 ± 95	187 ± 64	0.002
Total testosterone (ng/ml)	65 ± 45	49 ± 36	0.001
Free testosterone (pg/ml)	13.0 ± 4.0	8.7 ± 3.7	0.01
Total cholesterol (mg/dl)	189 ± 35	180 ± 30	NS
LDL (mg/dl)	105 ± 28	109 ± 20	NS
HDL (mg/dl)	62 ± 12	52 ± 15	NS
Triglyceride (mg/dL)	103 ± 79	123 ± 53	NS
Homocysteine (µmol/l)	55.97 ± 16.04	54.03 ± 16.15	0.01
Vitamin B_{12} (pg/ml)	270.6 ± 120.1	261.7 ± 112.5	NS
Folic acid (ng/ml)	9.5 ± 3.8	9.1 ± 3.0	NS

Results are expressed as mean ± SD

NS non-significant

Discussion

Prospective and retrospective studies have consistently shown that women with PCOS are at increased risk for metabolic syndrome [3, 25–27] and consequently for type 2 diabetes mellitus and cardiovascular disease. Women with PCOS are more likely than normally cycling women to have insulin resistance, [28] central adiposity, dyslipidemia and hypertension [29]. Other markers of cardiovascular disease, such as C-reactive protein [30] and Hcy, [9, 14–16] have also been found to be elevated in women with PCOS. Therefore, in addition to being one of the most common endocrine diseases, PCOS is also one of the major subjects for preventive medicine.

Several studies have examined the relationship between PCOS and serum Hcy levels, with most finding that the serum Hcy levels were significantly higher in PCOS subjects compared to age-, weight-, and ethnicity-matched

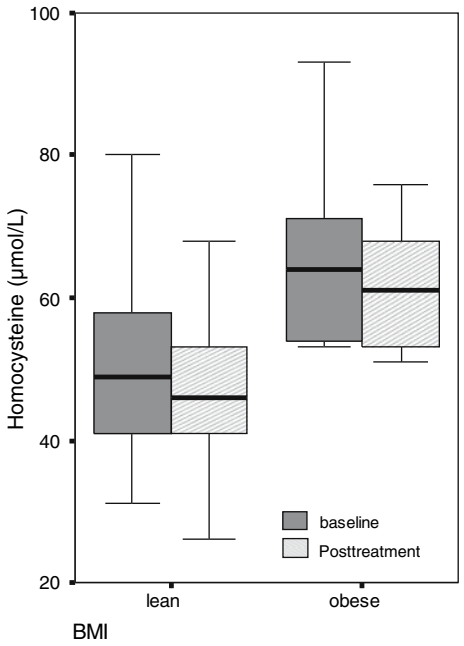

Fig. 1 Box and whisker plots depicting the baseline and post-treatment Hcy levels in lean and obese women with PCOS. *Solid lines* inside the boxes indicate the mean Hcy level, whereas the *upper* and *lower limits* of the boxes and whiskers indicate the 75th and 25th, and 95th and 5th percentiles, respectively

controls [4, 14–16, 31]. However, Orio et al. [32] failed to find any difference in the mean Hcy levels between PCOS and healthy women. In our study, the mean serum Hcy levels were elevated, which is in agreement with the other aforementioned studies. In addition, our findings indicated that EE–CA therapy decreases Hcy levels in patients with PCOS. Randeva et al. [31] achieved similar reductions in the serum Hcy concentrations of PCOS subjects with exercise. Although the decrease achieved with OCs was statistically significant, the serum Hcy levels were still elevated. Further studies are needed to determine whether this decrease has a clinical benefit.

In this study, the baseline Hcy levels were higher in obese PCOS patients than in the lean subjects. Our results did not support those of Loverro et al. [16] and Yilmaz et al. [33], who found no statistically significant difference in Hcy levels between obese and lean PCOS women, and who further stated that the elevated plasma Hcy levels were independent of the BMI. We observed a positive association between the serum total and free testosterone and Hcy levels, both before and after EE–CA treatment. The mechanisms by which hormonal contraception reduces hyperhomocysteinemia in PCOS are unclear, but may be a result of a decrease in hyperandrogenemia. However, this hypothesis needs further investigation.

Although several studies have suggested that women with PCOS have an atherogenic lipid profile characterized by lower HDL-C levels, and higher TG and LDL-C levels than age- and weight-matched control women, [8, 11, 29, 34, 35], the mean serum lipid levels were within the normal ranges in our study. Korytkowski et al. [36] found no differences in the baseline total-C, HDL-C and LDL-C levels between PCOS and control subjects, while they observed higher TG concentrations in women with PCOS. Some studies have found that obese women with PCOS had a more atherogenic lipid profile than lean subjects [37].

EE–CA therapy for 3 months neither increased nor decreased the lipid levels in our study. Conflicting data exist regarding the effect of OCs on the lipid profiles of both PCOS and normal cycling women. Various results have been obtained for the lipid profile following the use of OCs containing different types and doses of progestin. Rautio et al. [37] observed an increase in TG, total-C, and HDL-C, and no change in LDL-C levels after 3 months of EE-CA treatment; these changes were also observed at the sixth month in their study. No change in the lipid profile of PCOS subjects was observed with 3 months of triphasic OCs containing EE and norethindrone [36]. Cibula et al. [38] found an increase in all lipid parameters using pills containing EE and NGM. Although they observed no alteration in the HDL-C, LDL-C and TG levels, Elter et al. [39] reported a significant increase in the total-C levels of non-obese PCOS subjects, using the same drug as we used. The inconsistent data on the effects of OCs on the lipid profiles of women with PCOS may be attributable to the differences in the dose of estrogen, and the dose and type of progestin used in each study.

Many studies have shown that the plasma Hcy concentration is inversely related to the blood vitamin B_{12} and folic acid levels [40]. In our study, the serum vitamin B_{12} and folic acid levels were within the normal ranges at baseline and no significant changes from the baseline values were observed after treatment. Therefore, the initial higher Hcy concentrations in obese subjects and the final decrease in both obese and lean subjects were not related to the vitamin B_{12} and folic acid levels.

One limitation of this study was the absence of usage of different preparations. However, 60% of our patients had clinical or biochemical signs of hyperandrogenism, and OC was used to treat hyperandrogenism as well as menstrual disturbances in the current study. OCs are used to treat androgenic symptoms because of their ability to suppress the secretion of gonadotrophins, ovarian or adrenal androgens and to stimulate the hepatic synthesis of SHBG resulting in decreased free testosterone fraction. According to the relevant literature, a daily dose of 30–35 mg of EE guarantees sufficient suppression of ovarian follicular activity as well as effective stimulation of SHBG production [41]. Also, gestagens with low antiestrogenic (androgenic) activity, which do not decrease estrogen-stimulated overproduction of binding proteins, especially SHBG, are of favored

use [42]. Thus we used an EE–CPA combination. However, data from studies recruiting normally cycling women may indicate what could be expected in Hcy levels, if different gestagens, i.e., levonorgestrel containing drugs were used. Merki-Feld et al. [43] investigated the effect of OCs on Hcy level in a prospective cross-over study. Neither 30 μg EE/150 μg levonorgestrel nor 30 μg EE/75 μg gestodene affected Hcy level in their study. Another study by Seeger et al. [44] also confirmed that 20 μg EE/100 μg levonorgestrel and 30 μg EE/150 μg levonorgestrel did not change serum Hcy levels after 12 cycles.

Although they improve hirsutism and acne and protect against unopposed estrogenic stimulation of the endometrium, controversy persists regarding the use of OCs as first-line therapy in women with PCOS, particularly because of their potential adverse effects on insulin resistance, glucose tolerance, vascular reactivity and coagulability [45].

In conclusion, our data suggest that a 3-month EE–CA therapy is associated with a tendency to decrease serum Hcy levels. However, this observation merits further investigation. Moreover, large, prospective, randomized controlled studies, with longer treatment periods are necessary to determine whether Hcy levels can be lowered to within the normal range using OC treatment in women with PCOS.

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