

# Comparative effects of atorvastatin and simvastatin on the plasma total homocysteine levels in women with polycystic ovary syndrome: a prospective randomized study

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**Objective:** To test the hypothesis that statins improve hyperhomocysteinemia in women with polycystic ovary syndrome (PCOS).

**Design:** A prospective randomized study.

**Setting:** University Hospital.

**Patient(s):** Fifty-two women with PCOS and 52 women matched for age and body mass index as controls.

**Intervention(s):** Patients were randomly divided into two groups for treatment: group 1, atorvastatin, 20 mg daily (n = 26), and group 2, simvastatin, 20 mg daily (n = 26). Blood samples were obtained before and after treatment.

**Main Outcome Measure(s):** Serum homocysteine levels.

**Result(s):** After 12 weeks of treatment, serum homocysteine levels in group 1 had decreased from  $14.3 \pm 2.9$  to  $10.6 \pm 1.7$   $\mu\text{mol/L}$ ; in group 2, the levels decreased from  $13.6 \pm 2.1$  to  $11.1 \pm 1.9$   $\mu\text{mol/L}$ . Both two groups, free testosterone and total testosterone declined statistically significantly (38.3% and 36.5%; and 40.6% and 46.0%, respectively). In group 1, vitamin B<sub>12</sub> increased from  $362.1 \pm 107$  to  $478.7 \pm 267$  pg/mL; in group 2, it increased from  $391.3 \pm 107$  to  $466 \pm 211$  pg/mL, but the change did not reach statistical significance. There was a considerable decline in the homeostatic model assessment index in group 1 (40.0% to 32.1%).

**Conclusion(s):** Treatment with statins in women with PCOS leads to decreases in serum homocysteine levels. (Fertil Steril® 2008; ■: ■–■. ©2008 by American Society for Reproductive Medicine.)

**Key Words:** Polycystic ovary syndrome, statin therapy, homocysteine, insulin resistance, testosterone

Polycystic ovary syndrome (PCOS), one of the most common endocrinopathies, is seen in approximately 5% to 7% of women in reproductive age (1). A significant majority of women have multiple cardiovascular risk factors associated with insulin resistance, including hypertension and dyslipidemia (2, 3). Obesity and hyperinsulinemia are also frequently encountered in PCOS (3–5). Other markers of cardiovascular disease, such as C-reactive protein and homocysteine (Hcy), have been found to be elevated in women with PCOS (6, 7). Insulin resistance in patients with PCOS is associated with raised plasma Hcy (7–9).

Statins have multiple actions, independent of their classic effects on lipoproteins (10). The use of statins has resulted in significant improvement in insulin sensitivity in patients with the metabolic syndrome (11–14). Recently, several studies have reported that simvastatin decreases in serum androgen levels in women with PCOS (15–18). Gül et al. (19) also

showed a positive correlation between serum androgen levels and in Hcy levels in PCOS patients. However, less is known about the effect of statin therapy on Hcy levels. Luftjohann et al. (20) demonstrated that higher doses of simvastatin (i.e., 80 mg daily) produces a significant reduction in serum Hcy levels in patients with hypercholesterolemia.

At present, no data are available on the influence of statins on serum Hcy levels in women with PCOS. We proposed that the use of statins might be beneficial in women with PCOS by improving hyperinsulinemia, hyperandrogenemia, and hyperhomocysteinemia, and we investigated the effects of treatment with a synthetic and a natural statin (atorvastatin, simvastatin) on serum Hcy levels in women with PCOS. Specifically, we tested whether the serum Hcy level was higher in the PCOS group than the control group, and investigated which factors were related to any level of difference. When hyperhomocysteinemia was found, we clarified on what terms and how statins affected the serum Hcy levels.

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## MATERIALS AND METHODS

### Patients

The study took place at the University of Ufuk and University of Ankara, and all participants were asked to give written

consent. The institutional review board of Ufuk University approved the study protocol.

The study group consisted of 52 PCOS patients (body mass index [BMI]  $24.7 \pm 6.2$ , range: 20.9 to 33.4 kg/m<sup>2</sup>). The diagnosis of PCOS was made according to the Rotterdam European Society of Human Reproduction and Embryology (ESHRE)/American Society for Reproductive Medicine (ASRM)-sponsored PCOS Consensus Workshop Group guidelines (21). Specifically, all eligible patients presented at least two of the three following criteria: [1] chronic anovulation, [2] hyperandrogenism (hirsutism, acne) and/or hyperandrogenemia, and [3] polycystic ovaries. The presence of polycystic ovarian appearance was determined by ultrasonography (22).

We recruited 52 healthy women matched for age and BMI as controls. The control group women were healthy volunteers, students, and hospital staff with normal menstrual cycles and without clinical or biochemical symptoms of hyperandrogenism. None of controls smoked, had any systemic diseases such as hypertension, had endocrinopathies comprising diabetes mellitus, or had a family history of cardiovascular disease.

Any patients or controls who had diabetes mellitus, hyperprolactinemia, congenital adrenal hyperplasia, thyroid disorders, Cushing syndrome (determined by 1-mg dexamethasone suppression test), hypertension, smoking, vitamin B<sub>12</sub> and folate deficiency, or hepatic or renal dysfunction were excluded from the study. Patients who had been treated with any hormone medications, vitamins, or drugs that increase Hcy levels within the previous 3 months and those with folic acid, vitamin B<sub>12</sub>, or vitamin B<sub>6</sub> deficiencies were excluded from the study. Patients were also excluded if they had used any confounding medications, including oral contraceptive agents, antilipidemic drugs, and insulin-sensitizing drugs that might affect the metabolic criteria, within 3 months before enrollment.

Eligible PCOS patients were divided into two groups by an allocation sequence generated from a random-number table, assigned through consecutively numbered opaque, sealed envelopes. The first group (n = 26) received atorvastatin (Tarden, 20 mg daily; Abdi İbrahim, Zincirlikuyu, Istanbul); and the second group (n = 26) received simvastatin (Zocor, 20 mg daily; Merck, İstinye, Istanbul) for 12 weeks. The daily dose of 20 mg for atorvastatin and simvastatin was determined on the basis of previous studies. Duleba et al. (12) had demonstrated that simvastatin at 20 mg/day decreases testosterone levels and normalizes gonadotropin levels in women with PCOS. In addition to this, administration of low-dose atorvastatin at 10 mg/day also had been found to have a positive effect on glucose metabolism patients with metabolic syndrome (15). Taking the previous studies into account, we decided to use the dose of 20 mg per day in our study.

None of the women contemplated pregnancy during the study period. Husbands of patients were advised to use barrier methods of contraception because of concerns regarding

the potential teratogenic actions of statins. None of the women dropped out during the study. The primary end point was a change in total plasma Hcy levels, and the secondary end points are listed in Table 1. Weight and height were measured in light clothing without shoes, and the BMI was calculated as follows: BMI = weight (kg)/height (m<sup>2</sup>). Waist circumference was measured at the narrowest level between the costal margin and iliac crest, and the hip circumference was measured at the widest level over the buttocks while the women stood and breathed normally; the waist-to-hip ratio (WHR) was calculated (23), and WHR >0.72 was considered abnormal. The degree of hirsutism was assessed through the Ferriman and Gallwey method (24). The BMI, WHR, and hirsutism scores were assessed by a single investigator (C.K.) in all the participants.

The following hormone parameters were assessed: follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), total testosterone (T), dehydroepiandrosterone sulphate (DHEA-S), and thyroid-stimulating hormone (TSH). We also determined the lipid profile, total cholesterol (total-C), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), Hcy concentrations, vitamin B<sub>12</sub> and folate levels, and basal insulin levels. A standard 75 g oral glucose tolerance test (OGTT) was performed before treatment in all patients. Venous blood samples were obtained in the follicular phase of a spontaneous or progesterone-induced menstrual cycle.

Blood samples were collected after 10 to 12 hours of fasting between 8:30 and 10:30 AM; then, a standard 75 g OGTT was performed. Impaired fasting glucose, impaired glucose tolerance, and diabetes mellitus were evaluated by using the criteria set by the American Diabetes Association (25). Plasma glucose was determined with the glucose hexokinase (Cobas Integra 400 Plus; Roche Diagnostics, Mannheim, Germany). The presence of insulin sensitivity index (ISI) was investigated by using basal insulin levels, fasting glucose, and the homeostasis model assessment (HOMA index >2.1). The HOMA index was calculated fasting glucose (mg/dL) × fasting insulin (μIU/mL) × 0.055/22.5 (26, 27).

Serum levels of FSH, LH, PRL, DHEAS-S, insulin, and TSH were measured with specific chemiluminescence assays from Roche Diagnostic (ELECYS 2010 Hitachi, Roche Diagnostics). Serum levels of 17OH-progesterone and free testosterone were measured by radioimmunoassay (RIA). Levels of total cholesterol (total-C), high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C), and triglycerides (TG) were determined with enzymatic colorimetric assays (Roche Diagnostics). Samples were immediately centrifuged, and serum was separated and frozen at -20°C until assayed. The intra-assay and interassay coefficients of variation were <5% for all assays performed.

Plasma Hcy levels were measured as total Hcy by high-performance liquid chromatography using a Chromsystems kit with fluorescence detector (Mannheim, Germany). The intra-assay and interassay coefficients of variation were <2%.

Serum vitamin B<sub>12</sub> and folate levels were measured with specific electrochemiluminescence immunoassay (ELECYS 2010 Hitachi; Roche Diagnostics). Mean intra-assay and interassay coefficients of variance for vitamin B<sub>12</sub> and folate were 5.2% to 3.4%, and 6.8% to 7.9% respectively.

### Statistical Analysis

Data are shown as mean  $\pm$  standard deviation, or median. Data analysis was performed using SPSS for Windows, version 11.5 (SPSS Inc., Chicago, IL). Groups were compared using Student's *t*-test or Mann-Whitney *U* test, where appropriate. Shapiro-Wilk test was used to detect whether the continuous variables were normally distributed. Descriptive statistics were shown as mean  $\pm$  standard deviation for continuous data and percentages for categorical ones. Paired *t*-test was performed to compare the basal and end values within the same group. Degree of association between continuous and categorical variables were calculated through Pearson's correlation coefficient. *P* < .05 was considered statistically significant.

### RESULTS

The clinical and endocrinologic parameters of PCOS patients and controls are shown in Table 1. Tables 2 and 3

show the clinical and endocrinologic parameters before and after atorvastatin and simvastatin treatments. None of the patients had impaired glucose tolerance or diabetes mellitus.

Compared with healthy women, patients with PCOS had statistically significantly elevated levels of Hcy, total T, free T, fasting insulin, and HOMA index (*P* < .01). The levels of Hcy, total T, and free T statistically significantly declined (*P* < .01) in the atorvastatin and simvastatin groups. In the atorvastatin group, serum Hcy levels showed a greater reduction than in the simvastatin group (−3.7 vs. −2.5; see Table 2). In the atorvastatin group, the fasting insulin and HOMA index statistically significantly decreased; whereas in the simvastatin group the decrease in the fasting insulin and HOMA index did not reach statistical significance (see Table 2). In the atorvastatin group, serum vitamin B<sub>12</sub> levels statistically significantly increased (*P* < .01); in the simvastatin group, the increase did not reach statistical significance. There were no statistically significant changes recorded for the serum folate levels of either group (see Table 2).

Women with PCOS had statistically significantly higher serum total-C and LDL-C levels than healthy women (*P* < .05). Total-C and LDL levels decreased after both atorvastatin and simvastatin therapies (*P* < .05). Triglyceride

**TABLE 1**

**Hormone, metabolic, and lipid profiles in women with polycystic ovary syndrome and controls.**

	PCOS (n = 52)	Control (n = 52)	P value
Age (years)	23.4 $\pm$ 6.2	24.8 $\pm$ 7.1	NS
FSH (IU/L)	4.7 $\pm$ 3.4	5.9 $\pm$ 6.7	NS
LH (IU/L)	9.4 $\pm$ 2.2	6.9 $\pm$ 3.7	< .05
BMI (kg/m <sup>2</sup> )	24.7 $\pm$ 6.2	24.0 $\pm$ 6.8	NS
WHR	0.83 $\pm$ 0.09	0.77 $\pm$ 0.07	< .05
Homocysteine ( $\mu$ mol/L)	14.1 $\pm$ 3.1	9.4 $\pm$ 1.8	< .05
Fasting insulin ( $\mu$ IU. min/mL)	14.6 $\pm$ 7.2	8.2 $\pm$ 6.6	< .01
HOMA >2.1	2.9 $\pm$ 1.3	1.9 $\pm$ 1.2	< .01
Total-C (mg/dL)	196 $\pm$ 53.2	182 $\pm$ 32	< .05
LDL-C (mg/dL)	114 $\pm$ 30	93 $\pm$ 19	< .01
HDL-C (mg/dL)	44 $\pm$ 12	47 $\pm$ 16	NS
TG (mg/dL)	91 $\pm$ 74	87 $\pm$ 36	NS
DHEAS ( $\mu$ g/dL)	644.2 $\pm$ 618	523.2 $\pm$ 414	NS
17-OH-progesterone (ng/mL)	1.4 $\pm$ 1.7	1.2 $\pm$ 0.89	NS
Total T (ng/mL)	0.60 $\pm$ 0.56	0.36 $\pm$ 0.12	< .05
Free T (pg/mL)	3.2 $\pm$ 1.7	1.6 $\pm$ 0.51	< .05
Vitamin B <sub>12</sub> (pg/mL)	369.3.1 $\pm$ 117	371.4 $\pm$ 116	NS
Folic acid (ng/mL)	8.2 $\pm$ 1.3	8.4 $\pm$ 1.4	NS

Notes: *P* < .05 was considered statistically significant. BMI, body mass index; DHEAS, dehydroepiandrosterone sulfate; Free T, free testosterone; FSH, follicle-stimulating hormone; HDL-C, high-density lipoprotein cholesterol; HOMA, homeostatic model assessment; LDL-C, low-density lipoprotein cholesterol; LH, luteinizing hormone; NS, not statistically significant; PCOS, polycystic ovary syndrome; TG, triglycerides; Total C, total cholesterol; Total T, total testosterone; WHR, waist-to-hip ratio.

Kaya. Statins, serum homocysteine in PCOS. Fertil Steril 2008.

TABLE 2

Baseline and posttreatment homocysteine levels, insulin sensitivity, androgens levels, vitamin B<sub>12</sub> status in both atorvastatin and simvastatin groups.

	Baseline	3rd month	P value <sup>a</sup>	Actual difference	
				Mean	95% CI
Homocysteine ( $\mu\text{mol/L}$ )					
1	14.3 $\pm$ 2.9	10.6 $\pm$ 1.7	<.01	-3.7	-3.1 to 4.2
2	13.6 $\pm$ 2.1	11.1 $\pm$ 1.9	<.05	-2.5	-1.9 to 2.8
P value <sup>b</sup>	NS				
Fasting insulin ( $\mu\text{IU min/mL}$ )					
1	14.5 $\pm$ 7.1	8.7 $\pm$ 4.3	<.01	-5.8	-2.7 to 6.9
2	14.2 $\pm$ 6.4	12.9 $\pm$ 5.1	NS	-1.3	-1.1 to 2.6
P value <sup>b</sup>	NS				
HOMA index					
1	2.8 $\pm$ 0.9	1.9 $\pm$ 0.56	.01	0.9	+0.1 to 2.6
2	2.9 $\pm$ 0.9	2.8 $\pm$ 0.91	NS	0.1	+0.04 to 1.7
P value <sup>b</sup>	NS				
Total testosterone (ng/mL)					
1	0.60 $\pm$ 0.56	0.37 $\pm$ 0.26	<.01	-0.23	-0.13 to 1.2
2	0.52 $\pm$ 0.22	0.33 $\pm$ 0.17	<.01	-0.19	-0.11 to 1.4
P value <sup>b</sup>	NS				
Free testosterone (pg/mL)					
1	3.2 $\pm$ 1.7	1.9 $\pm$ 0.5	.05	-1.3	-0.11 to 1.2
2	3.0 $\pm$ 0.6	1.6 $\pm$ 0.4	<.01	-1.4	0.86 to 1.4
P value <sup>b</sup>	NS				
Vitamin B <sub>12</sub> (pg/mL)					
1	362.1 $\pm$ 107	478.7 $\pm$ 267	.01	116.6	79 to 157
2	391.3 $\pm$ 107	466 $\pm$ 211	NS	74.7	11 to 98
P value <sup>b</sup>	NS				
Folic acid (ng/mL)					
1	8.2 $\pm$ 1.3	8.5 $\pm$ 1.4	NS	0.3	+0.2 to 1.2
2	8.5 $\pm$ 1.5	8.9 $\pm$ 1.8	NS	0.4	+0.3 to 1.8
P value <sup>b</sup>	NS				

Notes: Group 1: atorvastatin therapy (n = 26); group 2: simvastatin therapy (n = 26).  $P < .05$  was considered statistically significant. HOMA, homeostatic model assessment; NS, not statistically significant.

<sup>a</sup> Comparisons for repeated measurements within groups.

<sup>b</sup> The difference between group baselines.

Kaya. Statins, serum homocysteine in PCOS. Fertil Steril 2008.

and HDL levels did not change after treatment in either group ( $P > .05$ ) (see Table 3).

In the Pearson correlation test, the increase in Hcy levels was positive and statistically significantly correlated with the HOMA index, total T, and free T ( $r = 0.37$ ,  $P < .05$ ;  $r = 0.51$ ,  $P < .01$ ;  $r = 0.27$ ,  $P < .05$ ; respectively) (Table 4). There was a weak negative correlation between plasma Hcy levels and vitamin B<sub>12</sub> ( $r = -24.1$ ,  $P < .05$ ). There was a negative correlation between plasma Hcy levels and folate, but it did not reach statistical significance.

Uric acid and creatinine levels were not altered with treatment (data not shown). None of the patients in two study groups reported any adverse effects throughout the treatment period.

## DISCUSSION

This is the first study to demonstrate that treatment with atorvastatin or simvastatin produces significant changes in Hcy levels in PCOS patients. In this study, we confirmed that plasma concentrations of Hcy were elevated in PCOS patients compared with healthy controls (8–12). Orio et al. (28) and Boulman et al. (29), however, did not detect a significant difference between patients with PCOS and healthy women in terms of Hcy levels. Orio et al. (28) reported that their study population was based on a sample of women in Hardy-Weinberg equilibrium for allelic distribution of MTHFR genotypes, whereas no genetic evaluation was performed in our study. The study by Boulman et al. (29) was a retrospective one, and it differed from ours in the sense that it followed a different methodology and did not measure

TABLE 3

Baseline and posttreatment clinical and lipid parameters in both atorvastatin and simvastatin groups.

	Baseline	3rd month	P value <sup>a</sup>	Actual difference	
				Mean	95% CI
BMI (kg/m <sup>2</sup> )					
1	24.7 ± 6.2	24.2 ± 6.6	NS	-0.5	-1.2 to 0.8
2	25.2 ± 6.8	24.2 ± 4.8	NS	-1.0	-2.2 to 1.7
P value <sup>b</sup>	NS				
Total-C (mg/dL)					
1	198 ± 32	156 ± 31.4	< .01	-22	-5.1 to 31.4
2	191 ± 26	143 ± 27.6	< .01	-24	-6.7 to 32.6
P value <sup>b</sup>	NS				
LDL-C (mg/dL)					
1	112 ± 31	83 ± 73	< .05	-19	-3.4 to 27.6
2	114 ± 41	87 ± 21	< .05	-14	-4.6 to 18.2
P value <sup>b</sup>	NS				
HDL-C (mg/dL)					
1	44 ± 12	56 ± 11	< .05	12	2.6 to 18.6
2	47 ± 16	53 ± 16	.11	6	1.9 to 9.9
P value <sup>b</sup>	NS				
TG (mg/dL)					
1	91 ± 82	101 ± 82	.42	4	0.4 to 6.4
2	92 ± 34	99 ± 74	.11	7	2.8 to 11.4
P value <sup>b</sup>	NS				

Notes: Group 1: atorvastatin therapy (n = 26); Group 2: simvastatin therapy (n = 26).  $P < .05$  was considered statistically significant. BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NS, not statistically significant; TG, triglycerides; Total-C, total cholesterol.

<sup>a</sup> Comparisons for repeated measurements within groups.

<sup>b</sup> The difference between group baselines.

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Hcy in all participants, including the control group. In our study, the assessment of Hcy concentrations was performed using high-performance liquid chromatography, a more sensitive and specific assay.

Compared with healthy women, women with PCOS had statistically significantly elevated fasting insulin and HOMA index ( $14.6 \pm 7.2$  vs  $8.2 \pm 6.6$ ;  $2.9 \pm 1.3$  vs  $1.9 \pm 1.2$ ;  $P < .01$ , respectively). Although BMI was in the normal range in each group, the WHR showed a substantial increase in PCOS patients ( $0.83 \pm 0.09$  vs  $0.77 \pm 0.07$ ,  $P < .05$ ); this increase in WHR may be related to the insulin resistance of these women. Insulin resistance in patients with PCOS is associated with elevated plasma Hcy levels (8–10). We also identified positive correlations of plasma Hcy with HOMA index in the patients with PCOS. This finding confirms that the increase in plasma Hcy is associated with insulin resistance in PCOS patients.

Pharmacologic agents are known to modify the concentration of plasma Hcy. There are conflicting data regarding the effects of metformin and rosiglitazone on Hcy levels in women with PCOS (30–33). The small number of patients in the studies as well as differences in the treatment periods

and drug doses may explain these discrepancies. Several different classes of lipid lowering drugs (i.e., bile acid resins, niacin, fish oils, and fenofibrate) have been shown to modify Hcy levels (34). Fenofibrate treatment resulted in a significant increase of serum Hcy levels in primary hyperlipidemic and dyslipidemic patients (34, 35). In contrast to the fenofibrate studies, Millionis et al. (36) proposed the neutral effect of therapeutic dosages of either atorvastatin or simvastatin on Hcy levels in patients with primary hyperlipidemia.

However, Luftjohann et al. (20) reported that higher doses of simvastatin (i.e., 80 mg daily) produced a significant reduction in serum Hcy levels in patients with primary hyperlipidemia. They found that plasma concentrations of Hcy statistically significantly decreased from  $13.0$  to  $11.7 \mu\text{mol/L}$  ( $P = .002$ ) after 24 weeks. Vitamin B<sub>12</sub> showed a small rise after 6 weeks, but the level decreased thereafter. The change in Hcy was related to the baseline Hcy but not to a reduction in cholesterol.

Our study did not use the same high dose as Luftjohann et al. (20). Serum levels of Hcy showed a decrease in the atorvastatin and simvastatin groups (25.8% and 19.8%, respectively). Patients treated with atorvastatin showed a greater

**TABLE 4**

**Correlation coefficient of plasma homocysteine with continuous and categorical variables.**

Variable	r	P value
BMI (kg/m <sup>2</sup> )	0.09	NS
HOMA index	0.37	<.05
Total-C (mg/dL)	0.19	NS
LDL-C (mg/dL)	0.12	NS
HDL-C (mg/dL)	0.19	NS
TG (mg/dL)	0.11	NS
Total T (ng/mL)	0.51	<.01
Free T (pg/mL)	0.27	<.05
Vitamin B <sub>12</sub> (pg/mL)	-0.24	<.05
Folic acid (ng/mL)	-0.08	NS

Notes:  $P < .05$  was considered statistically significant.

BMI, body mass index; Free T, free testosterone; HDL-C, high-density lipoprotein cholesterol; HOMA, homeostatic model assessment; LDL-C, low-density lipoprotein cholesterol; NS, not statistically significant; TG, triglycerides; Total-C, total cholesterol; Total T, total testosterone.

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reduction in Hcy levels than the simvastatin group (-3.7 vs -2.5,  $P < .05$ ).

Plasma levels of insulin seem to influence Hcy metabolism, possibly through effects on glomerular filtration, or by influencing activity of key enzymes in Hcy metabolism or cystathionine  $\beta$ -synthase (CBS) (11, 12, 14, 37). Insulin inhibits hepatic CBS activity (13, 37, 39). Insulin resistance in women with PCOS is associated with high plasma Hcy (7, 8). There is some evidence that the use of atorvastatin results in significant improvement in insulin sensitivity in patients with the metabolic syndrome (11–12). In our study, at the third month of atorvastatin treatment, the fasting insulin and HOMA index declined, but the uric acid and creatinine levels did not change. In the Pearson correlation test, there was a positive correlation between the HOMA index and Hcy levels in the atorvastatin group ( $r = 0.37$ ,  $P < .05$ ). Therefore, the reduction of Hcy levels may be related to the decrease in the HOMA index in the atorvastatin group.

In our study, compared with healthy controls, women with PCOS had a statistically significantly higher levels of free T ( $3.2 \pm 1.7$  vs.  $1.6 \pm 0.51$ , respectively,  $P < .05$ ) and total T ( $0.60 \pm 0.56$  vs.  $0.36 \pm 0.12$ , respectively,  $P < .05$ ) in either treatment group. We found a positive and statistically significant correlation between increased plasma total Hcy and free/total T levels in both groups ( $r = 0.27$ ,  $P < .05$ ;  $r = 0.51$ ,  $P < .001$ , respectively). This finding suggests that an increase of plasma Hcy is associated with elevated androgens in PCOS patients. Men have higher plasma levels of Hcy than women, but the mechanism of this sex-dependent difference is not known (9, 14, 38, 39).

More recently, Vitvitsky et al. (40) demonstrated that testosterone down-regulates CBS activity expression via a posttranscriptional mechanism in the androgen-responsive prostate cancer cell line. This diminution in CBS levels is accompanied by a decrease in flux through the transsulfuration pathway and by a lower intracellular glutathione concentration. Therefore, the increase in androgen levels in women with PCOS might cause the decrease in CBS activity and finally an increase in Hcy levels. Recent studies have likewise shown the androgen decreasing effects of statins in PCOS patients (15–18). In our study, we also achieved a statistically significant decrease in both free T and total T levels in the third month of atorvastatin and simvastatin treatment (38.3% and 36.5%; 40.6% and 46.0%, respectively). Thus, the decline in androgen levels might cause an increase in CBS activity. The shift to transsulfuration pathway of the Hcy metabolism might cause a decrease in serum Hcy levels. In another study that supports this hypothesis, Gül et al. (19) observed a significant decrease in Hcy levels at the third months of ethinyl estradiol–cyproterone acetate treatment in a similar study population. Gül et al. (19) also showed a positive correlation between androgens and in Hcy levels and a significant decrease in androgens levels at the third month of the treatment, which reinforces the relation between androgens and Hcy metabolism in PCOS patients.

The reason for the lower decrease in the levels of Hcy in the simvastatin group as compared with the atorvastatin group may be due to the fact that atorvastatin has a more dominant effect on the HOMA index. Atorvastatin and simvastatin on the other hand, the effect of statin on Hcy may also be dose related. In our study, Pearson correlation analysis revealed that increased Hcy was correlated to both insulin resistance (based on the HOMA index) and serum androgens. In the 3rd month of the treatment, this caused statistically significant reductions in serum Hcy levels in both the atorvastatin and simvastatin groups. Patients treated with atorvastatin showed a greater reduction in Hcy levels than the patients treated with simvastatin (-3.7 vs. -2.5,  $P < .05$ ). The total T and free T levels were found to be the same in both atorvastatin and simvastatin groups in the 3rd month of the treatment. If the decrease in serum Hcy levels were linked to androgens only, we would expect a somewhat similar amount of decrease in serum Hcy levels in both groups; however, the decrease in serum Hcy levels in the atorvastatin group was much higher although there had been no difference between the two groups in terms of baseline parameters. Although atorvastatin caused a statistically significant decrease in insulin resistance (based on the HOMA index), there was no statistically significant change observed in the HOMA index in the simvastatin group. When we take into consideration that the increased Hcy levels had a positive correlation with HOMA index, we can explain the greater decrease observed in Hcy levels in atorvastatin group only by means of relating it to the difference that atorvastatin created in insulin resistance. We do believe that the positive effect that atorvastatin had on insulin resistance played an important role in the difference in serum Hcy levels in the 3rd month of the treatment.

One of the interesting results of this study was that both atorvastatin and simvastatin caused the vitamin B<sub>12</sub> concentration to increase. In the atorvastatin group, vitamin B<sub>12</sub> increased by 32.2%; in the simvastatin group, it increased by 19%. The importance of vitamin B<sub>12</sub> in the remethylation of Hcy to methionine is well recognized, and hyperhomocysteinemia is a feature of vitamin B<sub>12</sub> deficiency (41). In our study, there was a weak negative correlation between plasma Hcy levels and vitamin B<sub>12</sub> ( $r = -24.1$ ,  $P < .05$ , respectively). This might confirm that the decrease of serum Hcy levels has no correlation with the increase of serum vitamin B<sub>12</sub> levels. The vitamin B<sub>12</sub> increase may be due to less usage of the demethylation pathway in Hcy metabolism secondary to the interactions of insulin and testosterone on this metabolism by the effect of statins. To explain these findings, we can hypothesize two different mechanisms. First, the fact that statins, by way of reducing hyperinsulinemia, turn Hcy metabolism in the direction of cystine amino acid, which may be causing the routes of vitamin B<sub>12</sub> and folate to be less used and may increase the levels of serum vitamin B<sub>12</sub>. In that case, an increase is expected in folate levels as well because folate employs the same routes as vitamin B<sub>12</sub>. However, our study detected no increase in the levels of serum folate, which may be related to the biological half-life of folate. The biological half-life of vitamin B<sub>12</sub> in plasma has been calculated to be around 12 months, but the biological half-life of folate is only 30 to 60 minutes (42). Hence, we may not have detected increases in the levels of folate because of its shorter biological half-life. Second, both the decrease in androgens and the shift to transsulfuration pathway of the Hcy metabolism might cause the lessened usage of demethylation pathway and finally an increase in vitamin B<sub>12</sub> levels.

Endothelial dysfunction in PCOS was documented by the decreased response to vasodilation, the finding of increased levels of endothelin-1 in insulin-resistant PCOS patients, and the increased oxidative stress markers (43). It is possible that these findings are due, in part, to increased Hcy levels. These findings may have important implications in the long term for cardiovascular complications associated with hyperhomocysteinemia PCOS (44, 45). Lowering plasma Hcy improves endothelial function in individuals with coronary artery disease and decreases the incidence of major cardiac events (46). Statin therapy could decrease Hcy levels and contribute to the reduction of cardiovascular events in PCOS patients.

Our study has clearly demonstrated that atorvastatin or simvastatin treatment decreases Hcy levels and increases vitamin B<sub>12</sub> in women with PCOS. Statins may be an appropriate management option for PCOS patients with hyperhomocysteinemia.

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