Effects of metformin versus ethinyl-estradiol plus cyproterone acetate on ambulatory blood pressure monitoring and carotid intima media thickness in women with the polycystic ovary syndrome

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Objective: To compare the effects of metformin versus an antiandrogenic contraceptive pill on ambulatory blood pressure monitoring (ABPM) and carotid intima media thickness (CIMT) in women with polycystic ovary syndrome (PCOS).
Design: Clinical randomized trial.
Setting: Academic hospital.
Patient(s): Thirty-four consecutive PCOS patients.
Intervention(s): PCOS patients randomized to oral treatment with metformin (n = 19) or with Diane[35] Diario pill (n = 15) for 24 weeks.
Main Outcome Measure(s): ABPM recordings and ultrasound measurements of CIMT as marker of subclinical atherosclerosis obtained at baseline and after treatment.
Result(s): Metformin resulted in reductions in daytime and 24-hour average systolic and diastolic blood pressure whereas Diane[35] Diario induced a slight increase in these parameters. Compared with a nonhyperandrogenic control group, the increased CIMT values of PCOS patients decreased to the normal range after treatment with either metformin or Diane[35] Diario.
Conclusion(s): Metformin treatment decreased daytime ABPM recordings whereas Diane[35] Diario exerted the opposite effect. The safer blood pressure profile of metformin should be considered in PCOS patients who present with a history of hypertension or who are at risk for this disorder. Treatment with either Diane[35] Diario or metformin improved CIMT mean values. (Fertil Steril® 2009;91:2527–36. ©2009 by American Society for Reproductive Medicine.)

Key Words: Androgens, insulin resistance, hypertension, metformin, oral contraceptives, atherosclerosis

Polycystic ovary syndrome (PCOS) is associated with classic and nonclassic cardiovascular risk markers (1). Obesity and insulin resistance play a major role in the clustering of cardiovascular risk factors in the PCOS patient, including hypertension and abnormalities in the regulation of blood pressure (2); an increased prevalence of glucose intolerance (3), type 2 diabetes (3), and dyslipidemia (4); and an association with chronic low-grade inflammation (5). Androgen excess also contributes to the association of PCOS with cardiovascular risk factors, independent of insulin resistance and obesity. On the one hand, PCOS is characteristically associated with the loss of the physiologic nocturnal decrease in blood pressure in adults and adolescents (2, 6). On the other hand, hyperandrogenemia, not obesity or insulin resistance, is the major determinant of the increased carotid intima-media thickness (CIMT, an early marker of atherosclerosis) found in women with PCOS (7).

Lifestyle recommendations and diet in women with weight excess are essential for cardiovascular disease prevention in PCOS patients (8). But, aside from therapeutic strategies directed toward restoration of fertility, chronic pharmacologic treatment of PCOS is based on the use of two families of drugs: oral contraceptives containing a progestin of low androgenicity or even antiandrogenic properties, or insulin sensitizers. Because oral contraceptives might adversely influence insulin resistance and glucose tolerance in nonhyperandrogenic women, the possible worsening of the already unfavorable cardiovascular risk profile of PCOS patients by the administration of oral contraceptives has been raised by

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reputed investigators in the field (9), who advocate the use of the metabolically safer insulin-sensitizing drugs (10). However, this recommendation is not supported by scientifically convincing evidence (11).

We report the results of a randomized clinical trial on the effects on the ambulatory blood pressure monitoring (ABPM) recordings and CIMT of an antiandrogenic low-dose oral contraceptive pill compared with the insulin sensitizer metformin in PCOS patients.

MATERIALS AND METHODS

Our study was derived from a more ample randomized, controlled, open-label clinical trial that addressed the effects of treatment with an antiandrogenic oral contraceptive compared with the insulin sensitizer metformin on classic and nonclassic cardiovascular risk factors ClinicalTrials.gov Identifier NCT00428311). The precise description of the clinical trial as well as the results regarding the effects of these treatments on hyperandrogenism, insulin resistance, glucose tolerance, and the lipid profile were reported previously elsewhere (12).

In brief, 34 consecutive PCOS patients were recruited. The diagnosis of PCOS was based on the presence of clinical and/or biochemical hyperandrogenism, oligo-ovulation, and exclusion of secondary etiologies (13). The methods used to evaluate each particular criterion have been described in detail elsewhere (12).

None of the patients had a personal history of hypertension, diabetes mellitus, or cardiovascular events, or had received treatment with oral contraceptives, antiandrogens, insulin sensitizers, or drugs that might interfere with blood pressure regulation for the previous 6 months. Family history of hypertension or cardiovascular events in first-degree relatives was collected. Written informed consent was obtained from all the participants, and the study was approved by the local ethics committee and by the Spanish Agency of Medicines.

After giving informed consent, the 34 patients were randomized to receive an antiandrogenic oral contraceptive containing 35 μg of ethinyl-estradiol plus 2 mg of cyproterone acetate (Diane^{35} Diario; Schering España S.A., Madrid, Spain) or 850 mg of metformin (Dianben; Merck Farma y Química S.A., Mollet del Vallés, Spain) twice daily for 24 weeks. Simple randomization was conducted using blocks of 10 sealed opaque envelopes assigning five patients to receive Diane^{35} Diario and five patients to receive metformin. Randomization allocated 15 patients to Diane^{35} Diario and 19 patients to metformin.

Treatment was started the first day of a spontaneous menstrual cycle, or, in women with amenorrhea, after excluding pregnancy by proper testing. Patients were instructed to maintain a diet containing 25 to 30 kcal per kg of body weight per day and continue to perform moderate physical activity throughout the trial. Patients were given a complete evaluation at baseline and after 12 and 24 weeks of treatment that included anthropometric and laboratory measurements (12), and several tests of cardiovascular performance. The ABPM profiles and CIMT measurements were performed at baseline and at the end of the study.

Ambulatory Blood Pressure Monitoring

Twenty-four hour recordings were obtained in the 15 patients allocated to the Diane^{35} Diario group. Recordings were performed for 18 of the 19 patients allocated to the metformin group because the baseline recordings of one patient were erased by a malfunction of the A&D TM2430EX oscillometric device (A&D Company, Ltd., Tokyo, Japan). The cuff (12 $\times$ 22 cm for lean patients, and 14 $\times$ 30 cm for overweight or obese patients) was placed on the nondominant arm in every woman. The period from 07:00 to 23:00 was considered daytime, and from 23:00 until 07:00 the next day was considered nighttime, reflecting the usual sleeping habits of Spaniards. Systolic, diastolic, and mean blood pressure as well as heart rate were measured every 20 minutes during daytime and every 30 minutes during nighttime. The nocturnal decreases in systolic and diastolic blood pressure were calculated using this equation: [(Mean of diurnal blood pressure $-$ Mean of nocturnal blood pressure)/Mean of diurnal blood pressure] $\times$ 100. Nondippers were defined as those patients who did not show a reduction in mean systolic and diastolic blood pressures by $\geq$10% from day to night; the remaining patients were considered dippers. For ABPM, we used the normative data for women in the 25 to 44 years age range derived from the PAMELA study (14). Women presenting with average 24-hour systolic and/or diastolic blood pressure values at or above the 95th percentile of the reference population were considered hypertensive, but the presence of isolated daytime or nighttime hypertension (values at or above the 95th percentile only during these periods, but not during the 24-hour period) were also noted. Systolic and diastolic blood pressure loads were defined as the percentage of blood pressure determinations above or equal to a reference range of 140/90 mm Hg and 125/75 mm Hg for daytime and nighttime periods, respectively.

Carotid Intima-Media Thickness Measurements

In all the patients, carotid imaging was obtained as previously reported elsewhere (7) by the same trained operator (C.M-A.) using a high-resolution 7.5-MHz phased-array transducer (Imagepoint-Hx; Hewlett-Packard, Andover, MA), according to the method described by Pignoli et al. (15).

The intraobserver coefficient of variation was 10.8%. The normal CIMT range was defined by the mean $\pm$ 2 standard deviations (0.17–0.49) of the CIMT measurements of 20 nonhyperandrogenic women in the same age and body mass index range of the PCOS patients studied here (7).

Statistical Analysis

Data are shown as mean $\pm$ standard deviation and raw numbers (percentages) unless otherwise stated. The sample size analysis for the clinical trial indicated adequate 0.80 power to detect differences in surrogates indexes of insulin resistance (the primary outcome of the clinical trial) with a total

of 22 patients, as described in detail in the previous report of the study protocol (12).

The differences in frequencies among groups were analyzed by chi-square or Fisher's exact tests, as appropriate. The changes in the frequencies of abnormalities in blood pressure and CIMT values during the study were analyzed by McNemar's test, applying a Bonferroni correction to the level of significance to compensate for the multiple comparisons conducted.

For continuous variables, the Kolmogorov-Smirnov test was applied, and logarithmic or square root transformations were used as needed to ensure normality. Then, the baseline characteristics of the patients randomized to receive Diane[35] Diario or metformin were compared by unpaired *t*-test. Data were submitted to a repeated-measures general lineal model including the arm of treatment as the between-subjects effect, and the visit of evaluation (baseline or 24 weeks) as the within-subjects effect. To evaluate the differences in the response to each treatment, the interaction among the between-subjects and within-subjects effects was calculated. The comparisons of the CIMT values of the nonhyperandrogenic control women with the PCOS women treated with metformin or Diane[35] Diario were analyzed by one-way analysis of variance followed by the least significant difference post hoc test.

Seven patients discontinued metformin for different reasons: mild to moderate gastrointestinal side effects in two women, pregnancy in one woman, protocol violation in three women, and lost to follow-up in one women. Diane[35] Diario was well tolerated by all of the women (12). Because of the high dropout rate in the metformin arm, the results obtained when considering only the patients completing the study were also confirmed by intention-to-treat analysis assuming, for patients who did not complete the 24 weeks of the study, that the dependent variables had not changed at the missing visit with respect to baseline. $P<.05$ was considered statistically significant. Analyses were performed using SPSS 10.0 for Macintosh (SPSS Inc, Chicago, IL).

RESULTS

The complete baseline clinical, anthropometric, biochemical, and hormone profiles of the PCOS patients randomized to treatment with Diane[35] Diario or metformin showed no differences between the groups, as previously reported elsewhere (12). Table 1 shows a selection of these variables as well as the baseline 24-hour blood pressure profiles and CIMT values.

There were no statistically significant differences in any variable between the women allocated to metformin and those taking Diane[35] Diario (see Table 1). However, when comparing the women allocated to metformin who dropped out with those who completed the study, the former had higher androstenedione levels, systolic and mean blood pressure values, and diastolic blood pressure loads during the nighttime and higher CIMT values (see Table 1).

Ambulatory Blood Pressure Monitoring Parameters

At baseline, hypertension according to ABPM recordings was found in seven of the 33 patients (21%), and another five had abnormal recordings but only during nighttime (Table 2). Furthermore, 15 patients (45%) presented with a nondipper pattern of nocturnal decrease in blood pressure. Of note, these baseline abnormalities were equally distributed among both arms of treatment (see Table 2).

After 24 weeks of treatment, and compared with baseline measurements, Diane[35] Diario increased whereas metformin decreased the average systolic, diastolic, and mean blood pressure daytime values (Fig. 1). These opposite effects of the drugs on blood pressure were not observed during the nighttime, but the effects during daytime explained the increase in systolic and diastolic blood pressure and the decrease in these values with metformin observed in the 24-hour recordings (see Fig. 1). The beneficial effect of metformin on blood pressure parameters was especially evident in the two hypertensive patients who completed the study and had normal blood pressure values after 24 weeks of treatment with this drug. In conceptual agreement, there was a statistically significant interaction between the changes during treatment in average systolic, diastolic, and mean blood pressure during daytime and the 24 hours periods, the arm of treatment, and the presence or absence of hypertension at baseline (daytime systolic blood pressure: Wilks' $\lambda = 0.842$, $F = 4.135$, $P=.054$; daytime diastolic blood pressure: Wilks' $\lambda = 0.631$, $F = 12.581$, $P=.002$; daytime mean blood pressure: Wilks' $\lambda = 0.637$, $F = 12.540$, $P=.002$; 24-hour systolic blood pressure: Wilks' $\lambda = 0.866$, $F = 3.404$, $P=.079$; 24-hour diastolic blood pressure: Wilks' $\lambda = 0.595$, $F = 14.947$, $P=.001$). However, the detrimental effects of Diane[35] Diario on blood pressure values were similar in hypertensive and nonhypertensive patients at baseline. Finally, these changes did not result in statistically significant differences in the frequencies of hypertension compared with those observed at baseline with either drug (see Table 2).

In conceptual agreement with the changes observed in average blood pressure values, the daytime blood pressure loads—but not those during the nighttime—tended to be increased with Diane[35] Diario (daytime systolic blood pressure load: $11 \pm 9\%$ at baseline versus $16 \pm 16\%$ at 24 weeks; daytime diastolic blood pressure load: $9 \pm 6\%$ at baseline versus $13 \pm 13\%$ at 24 weeks; nighttime systolic blood pressure load: $14 \pm 16\%$ at baseline versus $8 \pm 10\%$ at 24 weeks; nighttime diastolic blood pressure load: $7 \pm 11\%$ at baseline versus $12 \pm 15\%$ at 24 weeks) and to be decreased by metformin (daytime systolic blood pressure load: $11 \pm 15\%$ at baseline vs. $4 \pm 5\%$ at 24 weeks; daytime diastolic blood pressure load: $10 \pm 14\%$ at baseline vs. $4 \pm 4\%$ at 24 weeks; nighttime systolic blood pressure load: $8 \pm 9\%$ at baseline vs. $5 \pm 5\%$ at 24weeks; nighttime diastolic blood pressure load: $7 \pm 10\%$ at baseline vs. $6 \pm 6\%$ at 24 weeks), reaching statistical significance in the case of diastolic blood pressure (Wilks' $\lambda = 0.833$, $F = 4.824$, $P=.038$; intention-to-treat analysis, Wilks' $\lambda = 0.868$, $F = 4.567$, $P=.041$) and attaining borderline

TABLE 1

Baseline characteristics of the patients randomized to receive Diane[35] Diario or metformin.

	Diane[35] Diario (n = 15)	Metformin (intention-to-treat analysis, n = 19)[a]	Metformin (patients completing the study, n = 12)[a]	Metformin (lost to follow-up, n = 7)
Age (years)	23.4 ± 5.6	25.1 ± 6.6	24.8 ± 7.4	24.9 ± 6.1
Smokers, n (%)	6 (40)	8 (42)	5 (46)	3 (43)
Family history of hypertension, n (%)	6 (43)	12 (63)	7 (58)	5 (70)
Family history of CV events, n (%)	2 (14)	4 (21)	1 (8)	3 (43)
Body mass index (kg/m^2)	29.2 ± 5.7	30.5 ± 6.9	28.9 ± 6.0	34.1 ± 7.3
Waist circumference (cm)	83 ± 12	89 ± 18	88 ± 16	94 ± 21
Waist to hip ratio	0.79 ± 0.06	0.82 ± 0.11	0.80 ± 0.09	0.87 ± 0.14
Hirsutism score	11 ± 5	10 ± 6	9 ± 5	12 ± 6
Free testosterone (ng/dL)	1.1 ± 0.4	1.3 ± 0.6	1.2 ± 0.6	1.5 ± 0.7
Androstendione (ng/dL)	3.4 ± 0.9	4.0 ± 1.2	3.4 ± 0.9	4.6 ± 1.2[b]
DHEAS (ng/mL)	2,741 ± 1,037	2,259 ± 926	2,185 ± 667	2,407 ± 1333
Insulin sensitivity index	4.4 ± 3.5	3.8 ± 2.4	3.6 ± 2.2	3.2 ± 1.8
Daytime period				
Systolic blood pressure (mm Hg)	117 ± 10	118 ± 11	116 ± 12	121 ± 11
Diastolic blood pressure (mm Hg)	70 ± 7	72 ± 8	72 ± 8	72 ± 7
Mean blood pressure (mm Hg)	85 ± 7	87 ± 9	86 ± 10	88 ± 8
Heart rate (beats per minute)	79 ± 10	81 ± 9	81 ± 10	82 ± 9
Systolic blood pressure load (%)	11 ± 9	13 ± 14	11 ± 15	16 ± 14
Diastolic blood pressure load (%)	9 ± 6	11 ± 12	10 ± 14	12 ± 10
Nighttime period				
Systolic blood pressure (mm Hg)	106 ± 12	109 ± 13	104 ± 8	117 ± 15[b]
Diastolic blood pressure (mm Hg)	60 ± 7	63 ± 7	61 ± 6	66 ± 8
Mean blood pressure (mm Hg)	75 ± 8	78 ± 8	75 ± 7	83 ± 8[b]
Heart rate (beats per minute)	67 ± 9	73 ± 11	70 ± 7	76 ± 16
Systolic blood pressure load (%)	14 ± 16	15 ± 23	8 ± 9	28 ± 33
Diastolic blood pressure load (%)	7 ± 11	11 ± 10	7 ± 8	17 ± 11[b]
24-hour period				
Systolic blood pressure (mm Hg)	113 ± 9	115 ± 11	112 ± 9	120 ± 12
Diastolic blood pressure (mm Hg)	66 ± 7	69 ± 7	69 ± 7	70 ± 7
Mean blood pressure (mm Hg)	82 ± 7	83 ± 11	81 ± 12	86 ± 9
Heart rate (beats per minute)	75 ± 9	77 ± 11	75 ± 10	80 ± 12
Systolic blood pressure load (%)	11 ± 8	12 ± 14	8 ± 10	19 ± 19
Diastolic blood pressure load (%)	8 ± 6	10 ± 10	7 ± 9	13 ± 10

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TABLE 1

Continued.

	Diane[35] Diario (n = 15)	Metformin (intention-to-treat analysis, n = 19)[a]	Metformin (patients completing the study, n = 12)[a]	Metformin (lost to follow-up, n = 7)
Nocturnal decrease in blood pressure				
Systolic blood pressure (%)	9.4 ± 8.4	7.3 ± 9.3	10 ± 10	3 ± 7
Diastolic blood pressure (%)	14.0 ± 7.6	12.6 ± 9.4	16 ± 10	8 ± 5
Mean blood pressure (%)	12.0 ± 7.4	10.0 ± 9.1	13 ± 10	5 ± 4
Carotid intima media thickness (mm)	0.41 ± 0.01	0.40 ± 0.01	0.37 ± 0.08	0.46 ± 0.01[b]

Note: Data are mean ± SD, or raw numbers (%). Data were submitted to unpaired *t* test or to chi-square test, as appropriate.

[a] Ambulatory blood pressure recordings obtained from only 18 of the 19 patients allocated to metformin.

[b] $P<.05$ compared with patients who completed the metformin arm.

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statistical significance in the case of systolic blood pressure (Wilks' $\lambda = 0.851$, $F = 4.199$, $P=.052$; intention-to-treat analysis, Wilks' $\lambda = 0.883$, $F = 3.969$, $P=.056$).

As opposed to average blood pressure measurements, the average heart rate (see Fig. 1) and the average physiologic nocturnal decrease in systolic, diastolic, and mean blood pressure were not significantly influenced either by Diane[35] Diario or by metformin (Fig. 2), yet there was a borderline ($P<.10$) tendency toward an improvement in the later measurements in the women treated with Diane[35] Diario as compared with no change or a worsening in those receiving metformin ($P=.091$ for the physiologic nocturnal decrease in systolic blood pressure, $P=.095$ for the physiologic nocturnal decrease in diastolic blood pressure, and $P=.083$ for the physiologic nocturnal decrease in mean blood pressure) (see Fig. 2).

Carotid Intima-Media Thickness Measurements

At baseline, four (27%) of the patients allocated to Diane[35] Diario and three (16%) of the women allocated to metformin had increased CIMT values (see Table 2). Considered as a whole, both the patients allocated to Diane[35] Diario (0.41 ± 0.01 mm) and to metformin (0.40 ± 0.01 mm) had increased CIMT values compared with the nonhyperandrogenic control women (0.33 ± 0.08 mm) ($F = 4.205$, $P=.020$).

The mean CIMT values showed no statistically significant change with either drug (Fig. 3); however, a nonsignificant (Wilks' $\lambda = 0.915$, $F = 2.144$, $P=.157$; intention-to-treat analysis, Wilks' $\lambda = 0.944$, $F = 1.768$, $P=.194$) tendency toward a decrease in CIMT values was observed in the analysis of the patients who completed the study.

This tendency explained why the mean CIMT values of the PCOS patients after treatment were no longer increased when compared with the nonhyperandrogenic controls (nonhyperandrogenic controls: 0.33 ± 0.08 mm; patients on Diane[35] Diario: 0.39 ± 0.01 mm; patients on metformin completing the study: 0.36 ± 0.01 mm; patients on metformin by intention-to-treat analysis: 0.39 ± 0.01 mm; $F = 1.690$, $P=.196$ for the analysis of patients completing the study; $F = 2.737$, $P=.074$ in the intention-to-treat analysis). Furthermore, normalization of the increased CIMT at baseline was observed only in one of the women treated with Diane[35] Diario, whereas two of the patients presenting with normal CIMT at baseline developed increased CIMT measurements after treatment with metformin. Unfortunately, the three patients presenting with increased CIMT who were allocated to metformin were lost to follow-up evaluation, so we have no data on the possible changes in their CIMT in response to this drug (see Table 2).

DISCUSSION

To our best knowledge, ours is the first study to compare the impact of the two main drug therapies available for PCOS, namely, oral contraceptives and insulin sensitizers, on the

TABLE 2

Frequencies of abnormalities in ambulatory blood pressure monitoring (ABPM) recordings and carotid intima media thickness (CIMT).

	Diane[35] Diario (n = 15)		Metformin (patients completing the study, n = 12)[a]		Metformin (intention-to-treat analysis, n = 19)[a]	
	Baseline	24 weeks	Baseline	24 weeks	Baseline	24 weeks
Hypertension[b]	2 (13)	5 (33)	2 (18)	0 (0)	5 (28)	3 (17)
Isolated daytime hypertension[c]	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Isolated nighttime hypertension[c]	2 (13)	2 (13)	1 (9)	1 (9)	3 (17)	3 (17)
Nondipper pattern	5 (33)	6 (40)	3 (27)	4 (36)	10 (56)	11 (61)
Increased CIMT values[d]	3 (20)	2 (13)	0 (0)	2 (17)	3 (16)	5 (26)

Note: Data are raw numbers (percentages). There were no statistically significant differences in the frequencies of blood pressure and CIMT abnormalities between the arms of treatment, nor were statistically significant changes found in these frequencies during treatment when considering all patients as a whole or when considering each arm of treatment separately.

[a] Baseline ambulatory blood pressure recordings were not obtained in one woman allocated to metformin, so only 11 of the 12 patients completing the study and 18 of the 19 patients included in the intention-to-treat analysis had blood pressure profiles available for analysis.

[b] Hypertension was defined by average 24-hour systolic and/or diastolic blood pressure values in ABPM at or above the 95th percentile of women in the 25 to 44 year age range derived from the PAMELA study (14).

[c] Isolated daytime or nighttime hypertension was defined by values at or above the corresponding 95th percentile of women from the PAMELA study only during daytime or only nighttime, but not during the 24-hour period.

[d] Increased CIMT was defined by values above the mean + 2 standard deviations of the CIMT mean of a control group of nonhyperandrogenic women in the same range of age and body mass index.

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24-hour ambulatory blood pressure profiles and CIMT of these patients.

Although the issue of hypertension in PCOS is still controversial (16), we have recently shown that abnormalities in the 24-hour ABPM recordings are present in as many as 30% of PCOS patients, and that abnormalities are mostly dependent on the concurrence of obesity in these women (2). Also, PCOS patients present with increased CIMT when compared with nonhyperandrogenic women, indicating the presence of subclinical atherosclerosis, but this particular finding is associated with androgen excess and not with obesity or insulin resistance (7). Therefore, oral contraceptives and insulin sensitizers might have completely different effects on these cardiovascular risk factors arising from the marked differences in their mechanisms of action (amelioration of androgen excess as opposed to improvement in insulin resistance) and from the possible cardiovascular adverse effects of oral contraceptives previously reported in nonhyperandrogenic women (17).

Our results clearly show that the antiandrogenic oral contraceptive Diane[35] Diario induces a mild increase in the average daytime systolic, diastolic, and mean blood pressure values of PCOS patients, whereas metformin decreases them. The increase in daytime blood pressure resulted also in an increase in the average 24-hour systolic and diastolic blood pressure; however, on the contrary, such differential effects of these drugs on blood pressure were not observed during the nighttime. In conceptual agreement, the systolic and diastolic blood pressure loads during the daytime were increased after treatment with Diane[35] Diario and were decreased after treatment with metformin, reaching statistical significance in the case of diastolic blood pressure. Therefore, our results suggest that oral contraceptives induce a mild yet significant worsening in the blood pressure profiles of PCOS patients whereas metformin administration results in an improvement in them, but only during the daytime.

The possible beneficial effects of metformin on blood pressure have been previously reported not only in PCOS women (18) but also in patients with type 2 diabetes (19), a finding that might be related, at least partly, to the decrease in vasoconstrictor molecules such as endothelin-1 observed in PCOS patients treated with metformin (20). However, it must be noted that a recent study in lean PCOS patients failed to demonstrate any beneficial effect of metformin on ABPM recordings (21). Nevertheless, the lower body mass indexes and the absence of baseline insulin resistance in that study compared with those of the PCOS patients we studied may account for the discrepant findings.

On the contrary, the worsening of the blood pressure profiles of our PCOS patients when on oral contraceptives, although in conceptual agreement with the findings observed in the general population (22), had not been reported previously in PCOS. Furthermore, a previous clinical trial

FIGURE 1

Changes in average daytime, nighttime, and 24-hour blood pressure and heart rate recordings of polycystic ovary syndrome patients treated with Diane[35] Diario or metformin for 24 weeks. Data are mean ± standard error of the mean. Eleven patients completed the metformin arm (this arm had a large dropout rate, and the baseline ambulatory blood pressure monitoring recording was missing for one patient); the intention-to-treat analysis included all of the patients treated with metformin (n = 18). *$P<.05$ for the interaction between the arms of treatment, in both analysis of patients who completed the study and in intention-to-treat analysis.

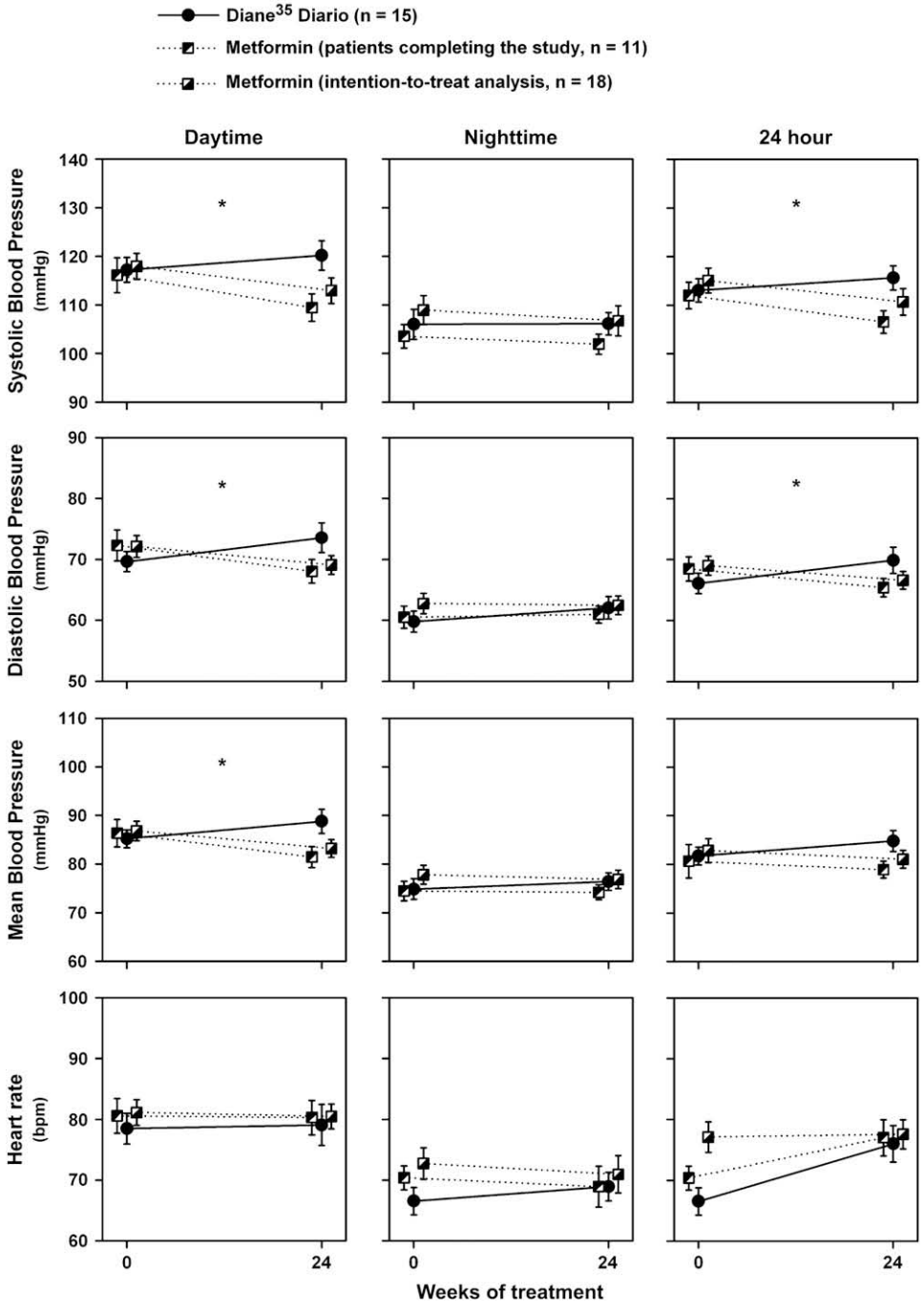

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FIGURE 2

Changes in the physiologic nocturnal decrease in systolic, diastolic, and mean blood pressure of polycystic ovary syndrome patients treated with Diane[35] Diario or metformin for 24 weeks. Data are mean ± SEM. Eleven patients completed the metformin arm (this arm had a large dropout rate, and the baseline ambulatory blood pressure monitoring recording was missing for one patient); the intention-to-treat analysis included all of the patients treated with metformin (n = 18).

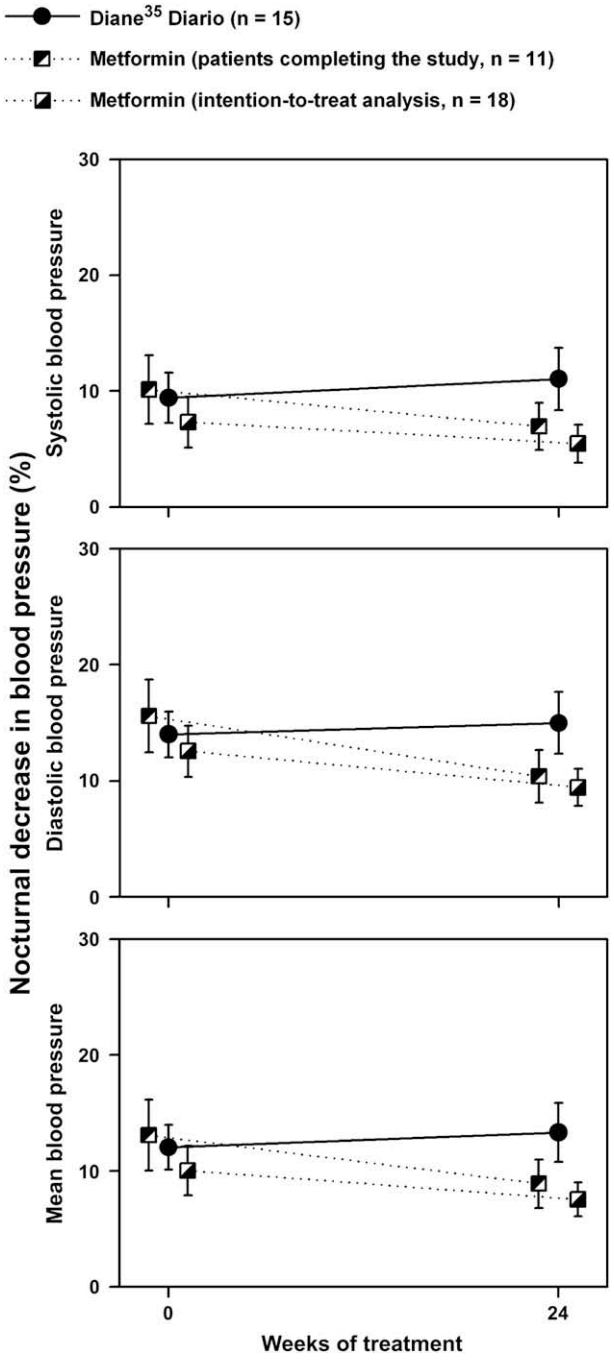

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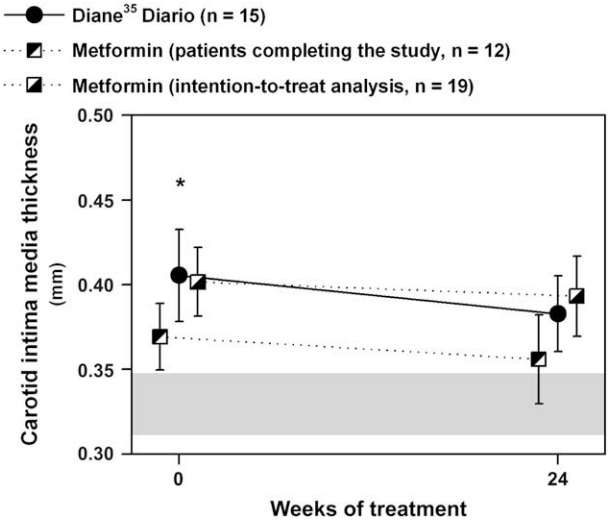
FIGURE 3

Changes in common carotid intima-media thickness of PCOS patients submitted to treatment with Diane[35] Diario (n = 15) or metformin (n = 19) for 24 weeks in the whole of patients. Symbols and error bars are mean ± SEM. The shaded area represents the mean ± SEM of the nonhyperandrogenic control group. Because of the large drop-out rate in the metformin arm, the Figures show the analysis of the patients completing the study (n = 12) and an intention-to-treat analysis including all the patients treated with metformin (n = 19). *$P<.05$ compared with the control group of healthy women irrespective of the arm of treatment.



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comparing Diane[35] Diario with metformin concluded that this particular oral contraceptive pill even resulted in a decrease of diastolic blood pressure office measurements (23). Considering that most of the abnormalities in the regulation of blood pressure of PCOS patients require 24-hour ABPM to be detected (2), the apparent discrepancies between our present results and those reported elsewhere (23) possibly result from the much more sensitive methods we used to detect the subtle derangements in the regulation of blood pressure characteristic of PCOS and the minor changes induced by drug treatment.

Because a recent study (24) found an independent direct relationship between serum androgen concentrations and office systolic and diastolic blood pressure in women with PCOS, the marked decrease in serum androgen levels in response to Diane[35] Diario would have been expected to result in a decrease in blood pressure parameters, yet the opposite effect was actually observed on the daytime blood pressure recordings of our patients. This apparent discrepancy might have been related to the estrogenic component of Diane[35] Diario because oral estrogens in supraphysiologic doses induce hepatic synthesis of vasoconstrictor molecules

(25) and oral contraceptives have been shown to increase blood pressure in the general female population (26). We might speculate that the undesirable effects on blood pressure of oral estrogens may have predominated in our PCOS patients over the putative beneficial effects of lowering serum androgen concentrations.

We have previously noted that the lack of the physiologic nocturnal decrease in blood pressure, the so-called "nondipper pattern," is the only blood pressure derangement that appears to be specific of PCOS patients (2). If the hyperandrogenemia of PCOS plays a putative role on this association, the antiandrogenic effect of Diane[35] Diario might account for the borderline downward trend in the nocturnal decrease in systolic, diastolic, and mean blood pressure seen in our study.

Although we have previously reported that the major determinant of the abnormalities in blood pressure in our PCOS population was the heart rate (2), neither Diane[35] Diario nor metformin induced statistically significant changes in this variable, suggesting that the effects of both drugs on blood pressure observed in our study were not mediated by a clinically relevant influence on sympathetic activity, even when both androgens (27) and insulin resistance (28) have been proposed to influence the cardiovascular autonomic system.

However, it must be highlighted that neither Diane[35] Diario nor metformin actually induced a change in the number of patients presenting with hypertension according to ABPM, or in the number of women presenting with a nondipper pattern in the nocturnal decrease of blood pressure, which casts a reasonable doubt upon the long-term clinical consequences of the mild changes in daytime blood pressure values observed after administration of these drugs. However, this lack of changes also could have been related to the relatively insensitivity of the chi-square and McNemar's tests used here and to the small sample size of our trial, which might have been underpowered to detect such small differences in frequencies.

Administration of either Diane[35] Diario or metformin for 24 weeks only induced a modest, not statistically significant decrease in the CIMT of our PCOS patients; however, after this small decrease, the mean CIMT values of the PCOS patients was no longer increased when compared with that of their nonhyperandrogenic counterparts. The antiandrogenic effect of Diane[35] Diario, and the amelioration of both hyperandrogenemia and insulin resistance with metformin, may explain the improvement of CIMT in our PCOS patients because androgen excess is the main determinant of the increased CIMT of PCOS patients, independent from obesity and insulin resistance (7). Also, treatment of PCOS with metformin results in a decrease in the serum levels of advanced glycated end products (AGEs) (29); because these molecules facilitate the proliferation and migration of smooth muscle cells toward the vascular intima (30), the decrease in the serum concentration of advanced glycated end products also might have been related to the decrease in CIMT observed in our study. Yet it must be noted that the decrease in CIMT in the women treated with metformin may be related to the dropping out of the PCOS patients who presented with higher CIMT values and not to the effects of the drug. Of note, two patients presented with normal CIMT at baseline, and this marker of atherosclerosis became abnormal during treatment with metformin, as has been also reported in metformin-treated type 2 diabetic patients (31). Nevertheless, previous studies have shown a decrease in CIMT during treatment of PCOS with metformin (32).

Our study was not free of limitations, of which the most important was probably the large dropout rate that occurred in the metformin treatment arm (12); however, that rate was in line with those observed in similar clinical trials conducted in the past (23, 33). Aside from side effects, the relatively poor response of hyperandrogenic symptoms and menstrual dysfunction to metformin might underlay the large dropout rate we observed (12) because the women who did not complete the study were more hyperandrogenic and insulin-resistant than those who completed the metformin arm, and metformin is less effective in the more obese PCOS patients (34). Our study was also limited by the unfortunate loss to follow-up of the three patients who presented with increased CIMT who had been allocated to treatment with metformin, preventing us from reaching any definite conclusion about the previously suggested possible beneficial effect of this drug on increased CIMT (32). Although we instructed all of the PCOS patients at baseline to maintain stable caloric intake and moderate physical activity throughout the trial, these recommendations were not stressed thereafter; therefore, we cannot rule out that dietary modifications may have contributed at least in part to the observed results. And, finally, the relatively short duration of the study possibly precluded the occurrence of larger changes in CIMT that might have become apparent had the trial been longer.

Metformin and Diane[35] Diario exerted opposite effects on the daytime blood pressure values of PCOS patients: decreasing with the former and increasing with the latter. The possibility of an increase in daytime blood pressure values should be considered when administering oral contraceptives to women with a personal or family history of hypertension, in whom metformin appears to be a safer therapeutic option. Finally, both drugs resulted in a normalization of CIMT values in PCOS women, suggesting that amelioration of androgen excess and of insulin resistance should be considered for cardiovascular risk prevention in these patients.

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REFERENCES

1. Legro RS. Polycystic ovary syndrome and cardiovascular disease: a premature association? Endocr Rev 2003;24:302–12.
2. Luque-Ramirez M, Alvarez-Blasco F, Mendieta-Azcona C, Botella-Carretero JI, Escobar-Morreale HF. Obesity is the major determinant of the abnormalities in blood pressure found in young women with the polycystic ovary syndrome. J Clin Endocrinol Metab 2007;92:2141–8.

3. Ehrmann D, Barnes R, Rosenfield R, Cavaghan M, Imperial J. Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. Diabetes Care 1999;22:141–6.
4. Legro RS, Kunselman AR, Dunaif A. Prevalence and predictors of dyslipidemia in women with polycystic ovary syndrome. Am J Med 2001;111:607–13.
5. Escobar-Morreale HF, Villuendas G, Botella-Carretero JI, Sancho J, San Millan JL. Obesity, and not insulin resistance, is the major determinant of serum inflammatory cardiovascular risk markers in pre-menopausal women. Diabetologia 2003;46:625–33.
6. Arslanian SA, Lewy VD, Danadian K. Glucose Intolerance in obese adolescents with polycystic ovary syndrome: roles of insulin resistance and β-cell dysfunction and risk of cardiovascular disease. J Clin Endocrinol Metab 2001;86:66–71.
7. Luque-Ramirez M, Mendieta-Azcona C, Alvarez-Blasco F, Escobar-Morreale HF. Androgen excess is associated with the increased carotid intima-media thickness observed in young women with polycystic ovary syndrome (PCOS). Hum Reprod 2007;22:3197–203.
8. Norman RJ, Davies MJ, Lord J, Moran LJ. The role of lifestyle modification in polycystic ovary syndrome. Trends Endocrinol Metab 2002;13:251–7.
9. Diamanti-Kandarakis E, Baillargeon J-P, Iuorno MJ, Jakubowicz DJ, Nestler JE. A modern medical quandary: polycystic ovary syndrome, insulin resistance, and oral contraceptive pills. J Clin Endocrinol Metab 2003;88:1927–32.
10. Cheang KI, Nestler JE. Should insulin-sensitizing drugs be used in the treatment of polycystic ovary syndrome? Reprod Biomed Online 2004;8:440–7.
11. Costello M, Shrestha B, Eden J, Sjoblom P, Johnson N. Insulin-sensitising drugs versus the combined oral contraceptive pill for hirsutism, acne and risk of diabetes, cardiovascular disease, and endometrial cancer in polycystic ovary syndrome. Cochrane Database Syst Rev 2007;1:CD005552.
12. Luque-Ramirez M, Alvarez-Blasco F, Botella-Carretero JI, Martinez-Bermejo E, Lasuncion MA, Escobar-Morreale HF. Comparison of ethinyl-estradiol plus cyproterone acetate versus metformin effects on classic metabolic cardiovascular risk factors in women with the polycystic ovary syndrome. J Clin Endocrinol Metab 2007;92:2453–61.
13. Zawadzki JK, Dunaif A. Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In: Dunaif A, Givens JR, Haseltine FP, Merriam GR, eds. Polycystic ovary syndrome. Vol. 4. Boston: Blackwell Scientific, 1992:377–84.
14. Mancia G, Sega R, Bravi C, De Vito G, Valagussa F, Cesana G, et al. Ambulatory blood pressure normality: results from the PAMELA study. J Hypertens 1995;13:1377–90.
15. Pignoli P, Tremoli E, Poli A, Oreste P, Paoletti R. Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. Circulation 1986;74:1399–406.
16. Wild RA. Polycystic ovary syndrome: a risk for coronary artery disease? Am J Obstet Gynecol 2002;186:35–43.
17. Baillargeon JP, McClish DK, Essah PA, Nestler JE. Association between the current use of low-dose oral contraceptives and cardiovascular arterial disease: a meta-analysis. J Clin Endocrinol Metab 2005;90:3863–70.
18. Lord JM, Flight IH, Norman RJ. Insulin-sensitising drugs (metformin, troglitazone, rosiglitazone, pioglitazone, D-chiro-inositol) for polycystic ovary syndrome. Cochrane Database Syst Rev 2003;3:CD003053.
19. Saenz A, Fernandez-Esteban I, Mataix A, Ausejo M, Roque M, Moher D. Metformin monotherapy for type 2 diabetes mellitus. Cochrane Database Syst Rev 2005;3:CD002966.
20. Diamanti-Kandarakis E, Spina G, Kouli C, Migdalis I. Increased endothelin-1 levels in women with polycystic ovary syndrome and the beneficial effect of metformin therapy. J Clin Endocrinol Metab 2001;86:4666–73.
21. Sahin Y, Unluhizarci K, Yilmazsoy A, Yikilmaz A, Aygen E, Kelestimur F. The effects of metformin on metabolic and cardiovascular risk factors in nonobese women with polycystic ovary syndrome. Clin Endocrinol (Oxf) 2007;67:904–8.
22. Dong W, Colhoun HM, Poulter NR. Blood pressure in women using oral contraceptives: results from the Health Survey for England 1994. J Hypertens 1997;15:1063–8.
23. Harborne L, Fleming R, Lyall H, Sattar N, Norman J. Metformin or antiandrogen in the treatment of hirsutism in polycystic ovary syndrome. J Clin Endocrinol Metab 2003;88:4116–23.
24. Chen MJ, Yang WS, Yang JH, Chen CL, Ho HN, Yang YS. Relationship between androgen levels and blood pressure in young women with polycystic ovary syndrome. Hypertension 2007;49:1442–7.
25. Ashraf MS, Vongpatanasin W. Estrogen and hypertension. Curr Hypertens Rep 2006;8:368–76.
26. Atthobari J, Gansevoort RT, Visser ST, de Jong PE. de Jong-van den Berg LT. The impact of hormonal contraceptives on blood pressure, urinary albumin excretion and glomerular filtration rate. Br J Clin Pharmacol 2007;63:224–31.
27. Yildirir A, Aybar F, Kabakci G, Yarali H, Oto A. Heart rate variability in young women with polycystic ovary syndrome. Ann Noninvasive Electrocardiol 2006;11:306–12.
28. Muniyappa R, Montagnani M, Koh KK, Quon MJ. Cardiovascular actions of insulin. Endocr Rev 2007;28:463–91.
29. Diamanti-Kandarakis E, Alexandraki K, Piperi C, Aessopos A, Paterakis T, Katsikis I, et al. Effect of metformin administration on plasma advanced glycation end product levels in women with polycystic ovary syndrome. Metabolism 2007;56:129–34.
30. Hattori Y, Suzuki M, Hattori S, Kasai K. Vascular smooth muscle cell activation by glycated albumin (Amadori adducts). Hypertension 2002;39:22–8.
31. Stocker DJ, Taylor AJ, Langley RW, Jezior MR, Vigersky RA. A randomized trial of the effects of rosiglitazone and metformin on inflammation and subclinical atherosclerosis in patients with type 2 diabetes. Am Heart J 2007;153:445.e1–6.
32. Orio F, Palomba S, Cascella T, De Simone B, Manguso F, Savastano S, et al. Improvement in endothelial structure and function after metformin treatment in young normal-weight women with polycystic ovary syndrome: results of a 6-month study. J Clin Endocrinol Metab 2005;90:6072–6.
33. Rautio K, Tapanainen JS, Ruokonen A, Morin-Papunen LC. Effects of metformin and ethinyl estradiol-cyproterone acetate on lipid levels in obese and non-obese women with polycystic ovary syndrome. Eur J Endocrinol 2005;152:269–75.
34. de Leo V, La Marca A, Petraglia F. Insulin-lowering agents in the management of polycystic ovary syndrome. Endocr Rev 2003;24:633–67.