Improved Efficacy of Low-Dose Spironolactone and Metformin Combination Than Either Drug Alone in the Management of Women With Polycystic Ovary Syndrome (PCOS): A Six-Month, Open-Label Randomized Study

Mohd Ashraf Ganie, Madan Lal Khurana, Sobia Nisar, Parvaiz Ahmed Shah, Zaffar Amin Shah, Bindu Kulshrestha, Nandita Gupta, Mohd Afzal Zargar, Tariq Ahmed Wani, Syed Mudasir, Farooq Ahmed Mir, and Shahnaz Taing*

Context: To improve the treatment outcomes in women with polycystic ovary syndrome (PCOS), various drugs like glitazones, oral contraceptive pills, or antiandrogens have been combined with metformin.

Objective: The aim of the study was to compare the efficacy of the combination of low-dose spironolactone and metformin with either drug alone in the management of women with PCOS.

Design and Setting: The present study was an open-label, randomized study conducted at a tertiary care referral center.

Patients and Intervention: Of 204 women who met the 2006 Androgen Excess-PCOS criteria for PCOS, 198 were randomized into 3 equal groups to receive metformin (1000 mg/d), low-dose spironolactone (50 mg/d), or a combination of both drugs for a period of 6 months. A total of 169 subjects (n = 56 metformin, 51 spironolactone, 62 combination) completed the study.

Main Outcome Measures: Menstrual cycle pattern, Ferriman-Gallwey score, body mass index (BMI), waist-hip ratio, blood pressure, LH, FSH, total T, glucose and insulin sensitivity indices were measured at baseline (0 mo) and 3 and 6 months after the intervention. Recording of adverse events and drug compliance was assessed at each of the visits.

Results: The 3 groups had comparable mean age and BMI at baseline. By 6 months, menstrual cycles/y increased, whereas Ferriman-Gallwey score, serum total T, and area under the curve-glucose and -insulin decreased significantly ($P < .05$) in the combination group as compared to either drug alone. There was no significant change in body weight, BMI, waist-hip ratio, and blood pressure in any of the 3 groups. The combination group had better compliance than either drug alone, and the adverse event rate was not higher.

Conclusion: The combination of low-dose spironolactone with metformin seems superior to either drug alone in terms of clinical benefits and compliance in women with PCOS. **(*J Clin Endocrinol Metab* 98: 3599–3607, 2013)**

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

Received January 3, 2013. Accepted July 5, 2013.
First Published Online July 11, 2013

* Author affiliations are shown at the bottom of the next page.

Abbreviations: AUC, area under the curve; AUC-G, AUC-glucose; AUC-I, AUC-insulin; BMI, body mass index; BP, blood pressure; DM, diabetes mellitus; EE, ethinyl estradiol; FG, Ferriman-Gallwey; FIGR, fasting glucose insulin ratio; HOMA-IR, homeostasis model of assessment for insulin resistance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; OCP, oral contraceptive pills; OGTT, oral glucose tolerance test; 17-OHP, 17-hydroxyprogesterone; PCOS, polycystic ovary syndrome; PRL, prolactin; QUICKI, quantitative insulin sensitivity check index; WHR, waist-hip ratio.

Polycystic ovary syndrome (PCOS), a common endocrine disorder associated with chronic anovulatory infertility and hyperandrogenism, is currently the focus of substantial research effort (1). Described by Stein and Leventhal (2) in 1935 as a gynecological disorder, it is now believed to be associated with a constellation of metabolic derangements such as obesity, type 2 diabetes mellitus (DM), dyslipidemia, hypertension, and cardiovascular disease (3). The underlying etiology of PCOS remaining elusive, most of the approaches to treat these cases in the past included inhibiting or decreasing androgen production (ovarian wedge resection, laparoscopic ovarian drilling, LHRH analogs, aromatase inhibitors, antiandrogens, oral contraceptive pills [OCPs]) (4). Spironolactone, chemically related to mineralocorticoid aldosterone, has been used as an antiandrogen. In addition, it acts as a steroid synthesis inhibitor (5). It has been used mostly in the treatment of hyperandrogenism (primarily hirsutism) in anovulatory women, besides being a diuretic (6–8). Although the experience of spironolactone in PCOS is limited (9–11), it is relatively safe in smaller doses (12). The drug has been used as a sole agent, in combination or in head-to-head comparison with other agents in hirsutism (13, 14).

Management of PCOS with insulin sensitizers like thiazolidinediones (15, 16) and metformin (17, 18) that target insulin resistance, the dominant pathogenic factor in DM, has generated significant interest in recent years. Metformin has also been shown to directly inhibit human theca cell androgen synthesis, suggesting an insulin-independent mechanism (19). A plethora of publications has demonstrated the efficacy of metformin in improving menstrual cyclicity, anovulation, cervical scores, spontaneous and assisted pregnancy outcomes, inflammatory markers, endothelial dysfunction, and various metabolic parameters (20–24).

To increase the treatment outcomes in women with PCOS, many investigators have attempted the addition of glitazones, OCPs, clomiphene citrate, or antiandrogens with metformin, alone or in combination (25–29). We previously reported the comparable efficacy of low-dose spironolactone to that of metformin in the management of women with PCOS (10).

In this open-label, randomized study, the efficacy of low-dose spironolactone and metformin combination was compared with either agent alone in the management of women with PCOS. To the best of our knowledge, after performing a systematic literature survey, this is the first study combining low-dose spironolactone with an insulin sensitizer (metformin) in the management of PCOS.

Subjects and Methods

Subjects

A total of 240 consecutive women presenting with menstrual disturbances such as oligo-/amenorrhea (menstrual interval ≥35 d or ≤8 cycles/y), amenorrhea (no menses in last ≥6 mo), hyperandrogenism (androgenic alopecia, male pattern hair growth, or moderate to severe acne vulgaris), infertility, or ultrasonographic evidence of polycystic ovaries to the Endocrine Clinic of our Institute between January 2009 and January 2011 and meeting the 2006 Androgen Excess Society criteria (30) for diagnosis of PCOS were enrolled for the study. Women having thyroid dysfunction, hyperprolactinemia, Cushing's syndrome, nonclassical congenital adrenal hyperplasia, and androgen-secreting tumors were excluded from the study. Women consuming any hormonal preparations or drug(s) known or suspected to affect reproductive or metabolic functions within 6 months of the study entry, or those having known DM, renal, hepatic, or cardiac dysfunction were also excluded. Of 204 women who consented to participate in the study, 198 with a suitable clinical profile were randomized equally (n = 66) into 3 groups by computer-generated random number allocation in an open-label manner. However, the allocation concealment was maintained until an oral glucose tolerance test (OGTT) was done. The study was approved by Institute's Ethics committee.

Study protocol

Anthropometric evaluation included measurement of body weight (kilograms), height (centimeters), body mass index (BMI; kilograms/meter^2), and waist-hip ratio (WHR). modified Ferriman-Gallwey score done by a single observer (M.A.G.) was used to assess the degree of hirsutism. A score of ≥ 7 out of 36 was taken as significant. A single observer (F.A.) performed transabdominal ultrasonography to demonstrate features of polycystic ovaries, ie, the presence of 10 or more peripheral follicles each measuring 2–8 mm in size with echogenic ovarian stroma and/or increased ovarian volume (31).

OGTT was performed from 8 to 9 AM after an overnight (10–12 h) fast. Blood samples were collected at 0, 60, and 120 min after an oral load of 75 g anhydrous glucose dissolved in 200–300 mL of water. Blood samples were separated in cold centrifuge at 4°C and aliqouted. The samples for glucose, electrolytes, lipids, liver and kidney function tests were analyzed on the same day. The samples for T_3, T_4, TSH, LH, FSH, prolactin (PRL), total T, insulin, and cortisol (morning) were stored at −70°C until analysis. Overnight dexamethasone-suppressed cortisol or ACTH-stimulated 17-hydroxyprogesterone (17-OHP) tests were done to rule out Cushing's syndrome or nonclassical congenital adrenal hyperplasia, after the baseline, if needed. The sampling was arranged in such a way that these were collected from days 3 to 7 (early follicular phase) of spontaneous or medroxyprogesterone-induced menstrual cycles.

Departments of Obstetrics and Gynecology (S.T.), Internal Medicine (S.N., P.A.S.), Government Medical College; Clinical Biochemistry, University of Kashmir (M.A.Z.); Clinical Biochemistry (S.M.), Biostatistics (T.A.W.), Radio Diagnosis (F.A.M.), Immunology and Molecular Medicine (Z.A.S.), Sheri-Kashmir Institute of Medical Sciences, Srinagar, Jammu & Kashmir; Departments of Endocrinology RML Hospital (B.K.); and, Endocrinology, Metabolism and Diabetes (M.A.G., M.L.K.), All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India

Intervention

Women were given standard diet counseling (30–35 kcal/kg, comprising 50–55% carbohydrate, 20–25% protein, and 15–20% fat with high fiber content) and lifestyle advice (25- to 35-min brisk walk per day) before randomization of the subjects and at each scheduled visit. The randomized groups were administered metformin 500 mg twice a day (Glycomet USV; India Ltd), spironolactone 50 mg/d (Aldactone; RPG Life Sciences Ltd), and the combination in the same doses. The 3-month supply of medication was provided at each visit in blister packing provided by the manufacturers. All women were subjected to clinical assessment, laboratory evaluation, and safety audit 3 and 6 months (±1 wk) after starting treatment. At each visit, diet and lifestyle advice was reinforced, and compliance of medication was checked by empty packs. All married/sexually active women were advised to use barrier contraception throughout the study. The CONSORT chart describes the flow of subjects in the study (Figure 1).

Laboratory analysis

Plasma insulin was measured by electrochemiluminescence (Cobas e411; Roche Diagnostics Limited). Estimations of serum T_3, T_4, cortisol, 17-OHP, and T were done by RIA using commercial kits in duplicate and according to supplier protocol. Measurement of TSH, PRL, LH, and FSH were done by immunoradiometric assay using commercial kits. The kits were supplied by: Diasorin, T_3 and T_4; Shinjin Medics Inc, TSH; Immunotech SA, T, 17OHP, and cortisol; and Diagnostic Products Corporation, PRL, LH, and FSH. Plasma glucose was measured by glucose oxidase peroxidase method (Roche Hitachi 912). Sensitivity, specificity, and interassay and intraassay coefficients of variation were within the prescribed limits prescribed in the manufacturer's protocol. Glucose tolerance was categorized according to 1999 World Health Organization criteria. Insulin resistance/insulin sensitivity indices (32) such as homeostasis model of assessment for insulin resistance (HOMA-IR), quantitative insulin sensitivity check index (QUICKI), area under the curve-insulin (AUC-I), area under curve-glucose (AUC-G), AUC-G/AUC-I, fasting glucose insulin ratio (FIGR), Reynaud's index, Drivsholm index, and Matsuda index were calculated. HOMA-IR was computed using the following formula: (fasting insulin in μIU/mL × fasting glucose in mmol/L)/22.5, whereas QUICKI was computed as 1/(log fasting insulin in μIU/mL + log glucose in mg/dL).

Statistical analysis

SPSS 16.0 version (SPSS Inc) was used for statistical analysis. The results are expressed as mean ± SD. Comparison of all clinical, biochemical, and hormonal quantitative variables within the groups and between the 3 groups at baseline and months 3 and 6 was done using ANOVA. ANOVA for repeated measures was used to compare the clinical, biochemical, and hormonal parameters within each group, and the Bonferroni test was used for multiple comparisons. Post hoc analysis was done, wherever necessary, to identify pairs of observations having significantly different levels of observations. Furthermore, to compare the distribution of qualitative variables between groups, χ^2 test was used. A P value of $<.05$ was considered statistically significant.

Results

Baseline parameters

Of the 204 subjects who gave consent, 6 women were excluded on the basis of abnormal biochemical profile. In

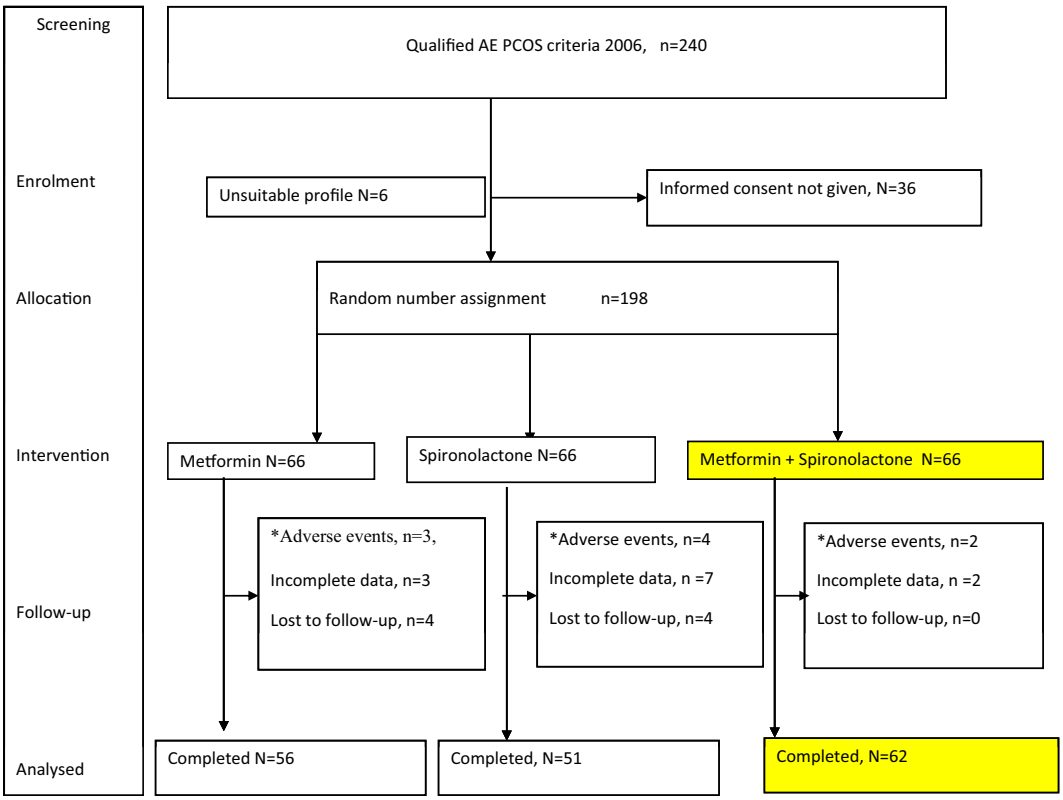

Figure 1. CONSORT chart showing progress of subjects in all 3 arms of the trial.

Table 1. Clinical, Anthropometric, and Hormonal Parameters of the Subjects Before and During Treatment

	Metformin (n = 56)			Spironolactone (n = 51)			Metformin + Spironolactone (n = 62)		
	0 mo	3 mo	6 mo	0 mo	3 mo	6 mo	0 mo	3 mo	6 mo
Age, y	22.42 ± 5.27 (range, 14–37)			23.57 ± 5.23 (range, 15–39)			23.56 ± 4.67 (range, 14–37)		
Age of menarche, y	12.96 ± 1.27			13.49 ± 4.73			12.80 ± 1.26		
Height, cm	157.64 ± 6.04			158.08 ± 5.73			156.69 ± 5.45		
Weight, kg	60.85 ± 11.21	60.03 ± 9.82	60.15 ± 8.95	60.91 ± 11.43	60.53 ± 9.79	61.14 ± 8.89	60.88 ± 9.10	60.90 ± 9.14	60.03 ± 7.53
BMI, kg/m^2	25.96 ± 4.09	25.41 ± 4.04	24.74 ± 3.11	24.26 ± 3.73	24.22 ± 3.32	24.46 ± 3.01	24.91 ± 4.91	24.69 ± 9.14	24.07 ± 3.36
WHR	0.90 ± 0.7	0.87 ± 0.06	0.87 ± 0.07	0.90 ± 0.07	0.90 ± 0.06	0.89 ± 0.06	0.90 ± 0.08	0.87 ± 0.07	0.84 ± 0.07[b]
No. of cycles/y	5.95 ± 1.96	8.80 ± 2.35[a]	10.02 ± 3.16[b,c]	6.45 ± 2.63	9.40 ± 2.45[a]	10.35 ± 2.8[b,c]	6.13 ± 2.54	9.30 ± 3.08[a]	10.86 ± 3.20[b,d]
FG score	13.27 ± 2.74	10.84 ± 2.42[a]	9.67 ± 2.19[b,c]	13.65 ± 2.72	10.27 ± 2.50[a]	9.56 ± 2.29[b,c]	13.11 ± 3.05	10.05 ± 2.9[a]	9.09 ± 2.29[b,c,d,e]
SBP, mm Hg	119.96 ± 7.9	121.50 ± 5.7	121.37 ± 6.5	123.13 ± 10.7	120.94 ± 8.2	119.16 ± 7.0[b]	123.92 ± 10.9	121.48 ± 7.4	120.13 ± 9.0
DBP, mm Hg	82.36 ± 5.50	81.90 ± 6.08	81.00 ± 5.1	82.02 ± 7.5	80.55 ± 5.1	79.19 ± 6.6	81.58 ± 5.5	80.05 ± 5.0	77.72 ± 6.0
LH, IU/L	6.13 ± 5.17	7.03 ± 4.15	5.69 ± 4.10	5.98 ± 4.11	7.43 ± 4.22	5.10 ± 2.84	6.01 ± 4.78	7.38 ± 4.07	4.95 ± 2.99
FSH, IU/L	5.39 ± 1.63	5.61 ± 1.82	5.83 ± 1.58	5.37 ± 1.44	5.87 ± 1.51	5.45 ± 1.91	5.99 ± 1.65	5.97 ± 1.47	6.02 ± 2.12
T, nmol/L	3.03 ± 1.58	2.40 ± 0.93[a]	1.89 ± 0.69[b,c]	2.98 ± 1.68	2.28 ± 1.27[a]	1.80 ± 1.11[b,c]	3.10 ± 1.55	2.19 ± 1.17[a]	1.58 ± 0.74[b,c,d,e]

Abbreviations: SBP, systolic BP; DBP, diastolic BP. Results are given as mean ± SD; $P < 0.05$. Conversion factors: LH and FSH, IU/L = mIU/mL × 1; T, nmol/L = ng/dL × 0.03467.

Comparison within the group: [a] month 0 vs month 3; [b] month 0 vs month 6; [c] month 3 vs month 6.

[d] Comparison of metformin with combination (metformin and spironolactone).

[e] Comparison of spironolactone with combination (metformin and spironolactone).

view of the incomplete data, dropouts, or adverse events, 29 (10 in the metformin group, 15 in the spironolactone group, and 4 in the combination group) subjects were not included in the final analysis. This left a complete data set of 169 women (n = 56 metformin, 51 spironolactone, and 62 combination) for statistical comparisons (Figure 1). Age, BMI, Ferriman-Gallwey (FG) score, anthropometric parameters and hormonal profiles were comparable in the groups (Table 1). The biochemical parameters and various insulin sensitivity indices were also comparable (Table 2).

Follow-up

Metformin group

Menstrual cycle frequency improved significantly (P = .01) with metformin, from 5.95 ± 1.96 to 8.8 ± 2.35 cycles/y at 3 months and to 10.02 ± 3.16 cycles/y at 6 months (Table 1). The cycles regularized in a majority of subjects, although 9 of 56 cases persisted with oligo-/amenorrhea. The hirsutism score decreased (P = .001) gradually from 13.27 ± 2.74 at baseline to 10.84 ± 2.42 and 9.67 ± 2.19 at 3 and 6 months of therapy, respectively (Table 1). Serum total T levels showed a significant de-

Table 2. Glycemic and Insulin Sensitivity Parameters of the Subjects Before and During Treatment

	Metformin (n = 56)		
	0 mo	3 mo	6 mo
Blood glucose-fasting (mg/dL)	90.79 ± 11.28	90.25 ± 9.84	88.44 ± 11.83
Blood glucose-1 h (mg/dL)	148.30 ± 19.02	147.62 ± 23.10	140.17 ± 24.69
Blood glucose-2 h (mg/dL)	135.08 ± 23.37	130.89 ± 15.87[a]	125.50 ± 20.06[b]
AUC-G (0–120)	15 671.6 ± 1991.9	15 451.3 ± 1575.0	14 841.0 ± 1904.8[c]
Serum insulin-fasting (μIU/mL)	15.71 ± 12.66	14.54 ± 10.98	10.51 ± 7.22[b]
Serum insulin-1 h (μIU/mL)	74.92 ± 59.07	56.96 ± 27.92[a]	55.38 ± 29.96[c]
Serum insulin-2 h (μIU/mL)	48.31 ± 31.50	38.69 ± 20.26[a]	35.86 ± 22.85[b]
AUC-I (0–120)	6466.81 ± 3790.29	5127.97 ± 2098.30[a]	5034.91 ± 2590.0[b]
AUC-G (0–120)/AUC-I (0–120)	3.30 ± 2.02	3.45 ± 1.37	4.00 ± 197[b]
HOMA-IR (mIU · mmol/L^2)	3.52 ± 2.73	3.15 ± 2.38	2.31 ± 1.5[c]
QUICKI	0.329 ± 0.030	0.335 ± 0.033	0.348 ± 0.034[b]
Matsuda index	4.76 ± 3.08	5.34 ± 2.52	7.05 ± 4.27[b]
Reynaud's index	3.78 ± 2.32	4.24 ± 3.08[a]	5.54 ± 3.88[b]
FGIR	8.55 ± 5.14	10.26 ± 6.07	11.85 ± 8.61[b]
Drivsholm index	3.30 ± 2.02	3.45 ± 1.37	4.00 ± 1.97[b]

Results are given as mean ± SD; $P < .05$. Conversion factors: insulin, pmol/L = μIU/mL × 7.175; glucose, mmol/L = mg/dL × 0.0555.

Comparison within the group: [a] month 0 vs month 3; [b] month 0 vs month 6; [c] month 3 vs month 6.

[d] Comparison of metformin with combination (metformin and spironolactone).

[e] Comparison of spironolactone with combination (metformin and spironolactone).

crease ($P = .01$) from baseline 3.03 ± 1.58 nmol/L to 2.40 ± 0.93 and 1.89 ± 0.69 at 3 and 6 months of therapy, respectively (Table 1). There was no significant effect on BMI, WHR, blood pressure (BP), LH, and FSH levels with 6 months of metformin therapy (Table 1). OGTT results revealed a nonsignificant decreasing trend in fasting and 1-hour plasma glucose values but a significant fall in 2-hour plasma glucose and AUC-G (Table 2). Plasma insulin levels during OGTT and the various insulin sensitivity parameters (AUC-I, AUC-G/AUC-I, HOMA-IR, QUICKI, Matsuda index, and FIGR) showed a significant improvement at 3 and 6 months of therapy.

Low-dose spironolactone group

Menstrual cyclicity increased from a baseline of 6.45 ± 2.63 cycles/y to 9.4 ± 2.45 at 3 months and to 10.35 ± 2.8 at 6 months ($P = .001$) of treatment (Table 1). The menstrual irregularity persisted or reappeared in 11 of 51 subjects. The irregularity, however, was significant enough to cause drug withdrawal in 2 patients only. The FG score decreased from 13.65 ± 2.72 at baseline to 10.27 ± 2.50 at 3 months and to 9.56 ± 2.29 at 6 months of therapy ($P = .02$) (Table 1). Serum total T levels showed a significant ($P = .001$) decrease from a baseline of 2.98 ± 1.86 nmol/L to 2.28 ± 1.27 at 3 months and to 1.80 ± 1.11 at 6 months (Table 1). Like metformin, fasting plasma glucose did not change significantly; however, post-OGTT glucose values and AUC-G showed a significant decrease at 6 months. Plasma insulin levels and various OGTT-derived insulin sensitivity indices (AUC-I, HOMA-IR, QUICKI, Matsuda index, and FIGR) showed a significant change (Table 2). There was no significant effect of spironolactone on BMI, WHR, LH, FSH, and diastolic BP (Table 1).

Combination group (low-dose spironolactone and metformin)

The combination of low-dose spironolactone and metformin increased the frequency of menstrual cycles from a baseline value of 6.13 ± 2.54 cycles/y to 9.30 ± 3.08 ($P = .004$) at 3 months and to 11.86 ± 3.20 ($P = .01$) at 6 months of therapy. The FG score showed a significant decrease at 3 and 6 months of treatment. The magnitude of the fall was significantly higher than in the metformin and spironolactone groups at 6 months of follow-up ($P < .05$). There was a fall in serum total T levels with the combination of both drugs, from a baseline value of 3.10 ± 1.55 nmol/L to 2.19 ± 1.17 at 3 months of treatment and 1.58 ± 0.74 at 6 months, which was significantly higher than the metformin group as well as the spironolactone group at 6 months of treatment (Table 1). There was no significant effect on BMI, BP, LH, and FSH levels, but there was a significant decrease in WHR by the end of 6 months (Table 1). The proportion of women with normal BMI (<25 kg/m^2) and obese women (≥25 kg/m^2) was similar in all 3 groups at baseline ($P = .61$). OGTT results revealed a significant decrease in all 3 glucose values and AUC-G by 6 months (Table 2). Plasma insulin levels during OGTT showed a significant fall at 3 and 6 months of treatment. Various insulin sensitivity parameters (AUC-I, AUC-G/AUC-I, HOMA-IR, QUICKI, Matsuda index, Reynaud's index, Drivsholm index, and FIGR) showed significant improvement at months 3 and 6. The combination was significantly superior to either drug alone in

Table 2. (Continued)

Spironolactone (n = 51)			Metformin + Spironolactone (n = 62)		
0 mo	3 mo	6 mo	0 mo	3 mo	6 mo
90.72 ± 11.73	87.97 ± 6.74	88.78 ± 8.84	92.30 ± 12.18	89.88 ± 11.97	86.14 ± 11.79[b]
152.61 ± 21.84	147.12 ± 19.39	139.71 ± 21.41[b]	145.37 ± 20.05	142.31 ± 31.39	135.49 ± 29.29[b]
133.55 ± 29.63	128.94 ± 17.73[a]	121.05 ± 16.75[b]	137.68 ± 20.86	128.39 ± 25.62[a]	120.23 ± 26.63[b]
15 960.0 ± 1796	15 377.5 ± 1356.0	14 720.0 ± 1571.6[b]	15 665.8 ± 1865.4	15 146.3 ± 1386.0[a]	14 400.5 ± 1413.6[b,c,e]
14.85 ± 10.15	12.12 ± 7.83	9.18 ± 5.56[b]	16.50 ± 9.32	12.07 ± 8.19[a]	8.9 ± 6.35[b,c]
80.23 ± 54.56	61.20 ± 24.01[a]	60.05 ± 25.77[b]	70.78 ± 45.07	60.44 ± 35.52[a]	46.80 ± 28.24[b,c,d,e]
45.94 ± 32.50	46.26 ± 34.46	38.59 ± 34.71[b,c]	47.26 ± 33.02	40.54 ± 28.02[b]	32.23 ± 20.82[b,c,d,e]
6568.14 ± 4002.5	5473.20 ± 2337.35[a]	5396.60 ± 2582.5[b]	6497.81 ± 3806.49	5413.80 ± 3052.2[a]	4051.46 ± 2191.5[b,d,e]
3.37 ± 1.88	3.42 ± 1.66	3.66 ± 2.48[b]	3.18 ± 1.77	3.38 ± 1.68	4.95 ± 2.73[b,c]
3.39 ± 2.08	2.61 ± 1.70[a]	2.56 ± 1.90[b]	3.67 ± 2.19	2.16 ± 1.80[a]	1.96 ± 1.47[b,d,e]
0.331 ± 0.028	0.341 ± 0.026	0.351 ± 0.034[b]	0.330 ± 0.022	0.349 ± 0.027[b]	0.358 ± 0.034[b,c]
4.83 ± 2.67	5.61 ± 2.73	6.46 ± 3.13[b]	4.70 ± 2.72	5.84 ± 2.98	8.04 ± 4.03[b,c,d,e]
3.82 ± 4.59	4.59 ± 2.85[b]	5.68 ± 3.01[c]	3.08 ± 1.51	4.42 ± 2.32[b]	6.27 ± 3.73[b,c]
8.68 ± 4.95	9.39 ± 6.86	11.85 ± 8.61[b]	8.32 ± 4.80	10.92 ± 6.07[b]	13.59 ± 9.04[b,c,d,e]
3.37 ± 1.88	3.42 ± 1.66	3.66 ± 2.48[b]	3.29 ± 1.77	3.83 ± 1.98	4.95 ± 2.73[b,c,e]

terms of some glycemic (AUC-G) or insulin sensitivity (AUC-I, HOMA-IR, Matsuda index, Drivsholm index) parameters at 6 months (Table 2).

Adverse events

Adverse events such as vomiting (1 of 66), nausea (3 of 66), diarrhea (9 of 66), and hyperadrenergic symptoms (2 of 66) causing drug withdrawal in 3 subjects were recorded in the metformin group. Polyuria (4 of 66), abdominal pain (1 of 66), menstrual irregularity (11 of 66), and dryness of the mouth (2 of 66) were noted in the spironolactone group, leading to drug withdrawal in 4 subjects. The increase in frequency of menses, although with persisting/new onset irregularity (n = 11) with 50 mg spironolactone used in the present study, was perceived positively by these oligo-amenorrheic women and therefore affected drug compliance in 2 cases only. None of the subjects had hyperkalemia. The adverse events of the combination were not significantly high (2 nausea, 3 metrorrhagia, 7 diarrhea, 1 abdominal pain), leading to drug withdrawal in 2 of 66 cases. Therefore, major adverse events leading to drug withdrawal were less in the combination group (n = 2) than in the metformin (n = 3) and spironolactone (n = 4) groups, suggesting better compliance in the combination group.

Various grades of glucose intolerance improved in all 3 groups by the end of 6 months. Two subjects with DM and 5 with impaired glucose tolerance (IGT)/impaired fasting glucose (IFG) remained in the combination group at the end of the study, out of the 4 DM and 9 IGT/IFG subjects detected at baseline. The effect was similar in the metformin group (4 DM and 10 IGT/IFG subjects at baseline and 2 DM and 6 IGT/IFG at 6 mo) and the spironolactone group (5 DM and 9 IGT/IFG subjects at baseline and 3 DM and 5 IGT/IFG at 6 mo).

Discussion

This is the first study comparing the efficacy of low-dose spironolactone in combination with metformin in women with PCOS. The study was carried out for a period of 6 months in an open-label manner. The key findings suggest superior efficacy (menstrual cyclicity, FG score, serum total T, insulin sensitivity, and compliance) of low-dose spironolactone and metformin over either drug alone in the management of PCOS, without increasing the adverse event rate.

Metformin, a known insulin sensitizer, is now considered the first-line drug in the management of women with PCOS (15, 33). It is also known to directly reduce androgen synthesis from ovaries (19). Administration of metformin (1g/d) to women with PCOS in the present study increased menstrual cyclicity and hirsutism score, decreased serum total T levels, and improved insulin sensitivity at 3 and 6 months of therapy, which was similar to our earlier observations (10) and findings reported by other researchers (17, 18, 34, 35). No significant benefit on BP, BMI, WHR, LH, and FSH levels was observed in the present study. The observed findings are similar to previous observations (36). However, the dose used in most of the studies was higher. In contrast, other researchers have shown a decrease in body weight, BMI, waist circumference, WHR, and gonadotropins (37).

Spironolactone, an antiandrogen administered at a dose of 50 mg/d in this study, also improved menstrual cyclicity and hirsutism score and decreased serum total T levels and insulin resistance parameters. The beneficial effects of spironolactone in this study are comparable to that of metformin and are in agreement with our previous observations (10). Our study reiterates the beneficial effect of spironolactone in lowering the serum total T levels and improving the hirsutism score as observed by us earlier and by other authors (10, 38). Although the spironolactone scores over metformin for benefits in hirsutism, the efficacy was similar for most other parameters. The data on spironolactone in the management of PCOS is scanty, and only a few studies have analyzed the effect of spironolactone on metabolic abnormalities in these subjects. Zulian et al (39), using 100 mg/d spironolactone, showed improvement in lipid profile and insulin sensitivity in overweight subjects and no negative changes in insulin secretion and sensitivity in lean subjects. Studen et al (11), using a similar dose (100 mg/d) for a period of 6 months, showed improvement in serum cholesterol and endothelial function. Because spironolactone is known to induce menstrual irregularities at higher doses, it is primarily used to treat hirsutism, acne, and androgenic alopecia. Thus, there is limited experience in PCOS as compared to that of metformin. There was no significant benefit on BP, BMI, WHR, LH, and FSH levels as demonstrated by other studies (7). Větr and Sobek (38) showed a fall in serum LH levels with a similar dose of spironolactone. The combined therapy of low-dose spironolactone with metformin or spironolactone alone in the present study, as well as in our previous study (14), demonstrates higher acceptability of the drug in women with PCOS because it did not induce any significant menstrual irregularities to affect the patient compliance.

The purpose of combining antiandrogens to metformin has been to improve the efficacy in various components of PCOS (26–29, 40–42). Ibáñez and de Zegher (29), using the combination of metformin and flutamide, showed the superior benefit of the combination over either drug alone.

However, there are no studies combining metformin with spironolactone, but cyproterone acetate with or without OCPs with metformin has been shown to increase the efficacy on various components of PCOS (25–29, 40, 41). In the present study, the combination of low-dose spironolactone and metformin improved menstrual cyclicity, hirsutism score, serum total T levels, and insulin sensitivity parameters in women with PCOS. Although increased fat mass is known to induce clinical and metabolic abnormalities in women with PCOS, in this study there was no significant difference in various anthropometric parameters at baseline, nor did any change in body weight and BMI manifest during the 6-month study period. The significant decrease in WHR in the combination group after 6 months of follow-up could partly explain the beneficial effect of the combination on menstrual cyclicity or various metabolic aberrations. Moreover, Kebapcilar et al (40) demonstrated the positive benefits of spironolactone plus ethinyl estradiol (EE)/cyproterone acetate on metabolic parameters, insulin sensitivity, and coagulation parameters, although inferior to the metformin-EE/cyproterone acetate combination. Harmanci et al (42) demonstrated in a recent study that a spironolactone plus EE/drospirenone combination improved androgen levels without any adverse effect on obesity, glucose tolerance, and lipids. This combination, however, increased serum high-sensitivity C-reactive protein and homocysteine levels, which can be attributed to the estrogen component of the combination. The combination may therefore be suitable for women not desiring pregnancy or women with increased cardiometabolic risk because, theoretically, the use of OCPs may be responsible for worsening of already existing cardiovascular risk in women with PCOS. The addition of spironolactone to metformin in the present study had no significant effect on weight, BMI, and BP, but there was a small change in WHR.

In our study, the 1- and 2-hour post-OGTT glucose values showed a decreasing trend in all 3 groups, but the effect was significant with the combination as compared to spironolactone for AUC-G. Insulin sensitivity calculated by HOMA-IR, Matsuda index, and AUC-I improved at 6 months in all 3 drug groups, but the benefit was superior in the combination group. This interesting observation of improved insulin sensitivity with spironolactone, although to a lesser extent than metformin, is similar to our earlier observation (10). On the contrary, some authors demonstrated no effect on insulin sensitivity (11, 39). Moreover, combination of low-dose spironolactone and metformin improved insulin sensitivity to a magnitude superior to either drug alone. Previous studies combining metformin with other antiandrogens failed to demonstrate this important finding (28, 43). Because the precise etiology of PCOS is unknown, insulin resistance and hyperandrogenism are 2 major cardinal pathophysiological derangements in these women. In women with PCOS, insulin resistance leading to hyperinsulinemia affects approximately 65–70%, whereas hyperandrogenism is demonstrable in 70–75%, suggesting that both of these principal pathophysiological mechanisms are not universal in all of them (7, 44). Therefore, the use of insulin sensitizers or antiandrogens alone can justify the rationale of monotherapy in most but not all women with PCOS. Whether insulin resistance and hyperandrogenism have a cause-and-effect relationship is also not clear. Accordingly, the beneficial effect of spironolactone on insulin sensitivity raises the possibility of hyperandrogenism as the cause of insulin resistance. Further studies are required to explore the mechanism at the molecular level to understand whether this effect is specific to spironolactone or secondary to the fall in androgens. Based on the study results, it may be postulated that insulin resistance and androgen excess are 2 different components of PCOS, and addressing both components simultaneously translates into superior clinical benefits in women with PCOS not desiring fertility. As a routine practice in our clinics, the diet and exercise counseling was administered to all the subjects before randomization and was reinforced at each visit throughout the study period. Because there was no placebo arm in the present study, it is possible that some of the clinical benefits were due to lifestyle modification. Because the lifestyle modification was similar in all 3 groups, its impact could be similar.

The most important finding of the study is that the combination of small dose spironolactone and metformin improved the efficacy and compliance without any increase in adverse events. The increased efficacy in terms of increased frequency of menstrual cyclicity by spironolactone alone or the combination in these oligo-amenorrheic women can be one of the reasons for better compliance.

To our knowledge, this is the first study demonstrating that, compared to monotherapy, the combination of a low dose of spironolactone with metformin augments the clinical benefits and improves treatment outcomes in women with PCOS without an increase in the adverse event rate.

Acknowledgments

The authors gratefully acknowledge the help of Dr V. Sreenivas for statistical review and Drs Mohammad Mir Muzzafar, Imran Ali, Sanjeed Ahmed, and Dilafroze Bhat for their technical assistance and manuscript editing.

Address all correspondence and requests for reprints to: Dr Mohd Ashraf Ganie, Department of Endocrinology and Metab-

olism, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India. E-mail: ashraf.endo@gmail.com or ashrafendo@rediffmail.com.

Disclosure Summary: The authors have nothing to declare.

References

1. **Goodarzi MO, Dumesic DA, Chazenbalk G, Azziz R.** Polycystic ovary syndrome: etiology, pathogenesis and diagnosis. *Nat Rev Endocrinol*. 2011;7:219–231.
2. **Stein IF, Leventhal ML.** Amenorrhea associated with bilateral polycystic ovaries. *Am J Obstet Gynecol*. 1935;29:181–191.
3. **Teede H, Deeks A, Moran L.** Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. *BMC Med*. 2010; 8:41.
4. **Escobar-Morreale HF.** Polycystic ovary syndrome: treatment strategies and management. *Expert Opin Pharmacother*. 2008;9:2995–3008.
5. **Corvol P, Michaud A, Menard J, Friefeld M, Mahoudean J.** Antiandrogenic effect of spironolactone: mechanism of action. *Endocrinology*. 1975;97:52–58.
6. **Lobo RA, Shoupe D, Serafini P, Brinton D, Horton R.** The effects of two doses of spironolactone on serum androgens and anagen hair in hirsute women. *Fertil Steril*. 1985;43:200–205.
7. **Spritzer PM, Lisboa KO, Mattiello S, Lhullier F.** Spironolactone as a single agent for long-term therapy of hirsute patients. *Clin Endocrinol (Oxf)*. 2000;52:587–594.
8. **Brown J, Farquhar C, Lee O, Toomath R, Jepson RG.** Spironolactone versus placebo or in combination with steroids for hirsutism and/or acne. *Cochrane Database Syst Rev*. 2009;2:CD000194.
9. **Milewicz A, Silber D, Kirschner MA.** Therapeutic effects of spironolactone in polycystic ovary syndrome. *Obstet Gynecol*. 1983;61: 429–432.
10. **Ganie MA, Khurana ML, Eunice M, et al.** Comparison of efficacy of spironolactone with metformin in the management of polycystic ovary syndrome: an open-labeled study. *J Clin Endocrinol Metab*. 2004;89:2756–2762.
11. **Studen KB, Sebestjen M, Pfeifer M, Prezelj J.** Influence of spironolactone treatment on endothelial function in non-obese women with polycystic ovary syndrome. *Eur J Endocrinol*. 2011;164:389–395.
12. **Shaw JC, White LE.** Long-term safety of spironolactone in acne: results of an 8-year followup study. *J Cutan Med Surg*. 2002;6:541–545.
13. **Moghetti P, Tosi F, Tosti A, et al.** Comparison of spironolactone, flutamide, and finasteride efficacy in the treatment of hirsutism: a randomized, double blind, placebo-controlled trial. *J Clin Endocrinol Metab*. 2000;85:89–94.
14. **Keletimur F, Everest H, Unlühizarci K, Bayram F, Sahin Y.** A comparison between spironolactone and spironolactone plus finasteride in the treatment of hirsutism. *Eur J Endocrinol*. 2004;150: 351–354.
15. **Tang T, Lord JM, Norman RJ, Yasmin E, Balen AH.** Insulin-sensitizing drugs (metformin, rosiglitazone, pioglitazone, D-chiro-inositol) for women with polycystic ovary syndrome, oligo amenorrhoea and subfertility. *Cochrane Database Syst Rev*. 2012;5: CD003053.
16. **Azziz R, Ehrmann D, Legro RS, et al.** Troglitazone improves ovulation and hirsutism in the polycystic ovary syndrome: a multicenter, double blind, placebo-controlled trial. *J Clin Endocrinol Metab*. 2001;86:1626–1632.
17. **Diamanti-Kandarakis E, Christakou CD, Kandaraki E, Economou FN.** Metformin: an old medication of new fashion: evolving new molecular mechanisms and clinical implications in polycystic ovary syndrome. *Eur J Endocrinol*. 2010;162:193–212.
18. **Palomba S, Falbo A, Zullo F, Orio F Jr.** Evidence-based and potential benefits of metformin in the polycystic ovary syndrome: a comprehensive review. *Endocr Rev*. 2009;30:1–50.
19. **Viollet B, Guigas B, Sanz Garcia N, Leclerc J, Foretz M, Andreelli F.** Cellular and molecular mechanisms of metformin: an overview. *Clin Sci (Lond)*. 2012;122:253–270.
20. **Kocak M, Caliskan E, Simsir C, Haberal A.** Metformin therapy improves ovulatory rates, cervical scores, and pregnancy rates in clomiphene citrate-resistant women with polycystic ovary syndrome. *Fertil Steril*. 2002;77:101–106.
21. **Tso LO, Costello MF, Albuquerque LE, Andriolo RB, Freitas V.** Metformin treatment before and during IVF or ICSI in women with polycystic ovary syndrome. *Cochrane Database Syst Rev*. 2009;2: CD006105.
22. **Vanky E, Stridsklev S, Heimstad R, et al.** Metformin versus placebo from first trimester to delivery in polycystic ovary syndrome: a randomized, controlled multicenter study. *J Clin Endocrinol Metab*. 2010;95:E448–E455.
23. **Tarkun I, Dikmen E, Cetinarslan B, Cantürk Z.** Impact of treatment with metformin on adipokines in patients with polycystic ovary syndrome. *Eur Cytokine Netw*. 2010;21:272–277.
24. **Ibáñez L, Lopez-Bermejo A, Diaz M, Marcos MV, de Zegher F.** Pubertal metformin therapy to reduce total, visceral, and hepatic adiposity. *J Pediatr*. 2010;156:98–102.e1.
25. **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.
26. **Ibáñez L, de Zegher F.** Ethinylestradiol-drospirenone, flutamide-metformin, or both for adolescents and women with hyperinsulinemic hyperandrogenism: opposite effects on adipocytokines and body adiposity. *J Clin Endocrinol Metab*. 2004;89:1592–1597.
27. **Fruzzetti F, Perini D, Lazzarini V, Parrini D, Gambacciani M, Genazzani AR.** Comparison of effects of 3 mg drospirenone plus 20 μg ethinyl estradiol alone or combined with metformin or cyproterone acetate on classic metabolic cardiovascular risk factors in nonobese women with polycystic ovary syndrome. *Fertil Steril*. 2010;94:1793–1798.
28. **Gambineri A, Patton L, Vaccina A, et al.** Treatment with flutamide, metformin, and their combination added to a hypocaloric diet in overweight-obese women with polycystic ovary syndrome: a randomized, 12-month, placebo-controlled study. *J Clin Endocrinol Metab*. 2006;91:3970–3980.
29. **Ibáñez L, de Zegher F.** Low-dose flutamide-metformin therapy for hyperinsulinemic hyperandrogenism in nonobese adolescents and women. *Fertil Steril*. 2006;86(suppl 1):S24–S25.
30. **Azziz R, Carmina E, Dewailly D, et al.** Positions statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an Androgen Excess Society guideline. *J Clin Endocrinol Metab*. 2006;91:4237–4245.
31. **Balen AH, Laven JS, Tan SL, Dewailly D.** Ultrasound assessment of the polycystic ovary: international consensus definitions. *Hum Reprod Update*. 2003;9:505–514.
32. **Cakir M, Sari R, Tosun O, Saka O, Karayalcin U.** Reproducibility of fasting and OGTT-derived insulin resistance indices in normoglycemic women. *Can J Diabetes*. 2006;30:46–51.
33. **Geller DH, Pacaud D, Gordon CM, Misra M.** State of the art review: emerging therapies: the use of insulin sensitizers in the treatment of adolescents with polycystic ovary syndrome (PCOS). *Int J Pediatr Endocrinol*. 2011;2011:9.
34. **Glueck CJ, Wang P, Fontaine R, Tracy T, Sieve-Smith L.** Metformin-induced resumption of normal menses in 39 of 43 (91%) previously amenorrheic women with the polycystic ovary syndrome. *Metabolism*. 1999;48:511–519.
35. **Morin-Papunen LC, Koivunen RM, Ruokonen A, Martikainen HK.** Metformin therapy improves the menstrual pattern with minimal

endocrine and metabolic effects in women with polycystic ovary syndrome. *Fertil Steril*. 1998;69:691–696.
36. **Baillargeon JP, Jakubowicz DJ, Iuorno MJ, Jakubowicz S, Nestler JE.** Effects of metformin and rosiglitazone, alone and in combination, in nonobese women with polycystic ovary syndrome and normal indices of insulin sensitivity. *Fertil Steril*. 2004;82:893–902.
37. **Ladson G, Dodson WC, Sweet SD, et al.** The effects of metformin with lifestyle therapy in polycystic ovary syndrome: a randomized double-blind study. *Fertil Steril*. 2011;95:1059–1066.e1–e7.
38. **Větr M, Sobek A.** Low dose spironolactone in the treatment of female hyperandrogenemia and hirsutism. *Acta Univ Palacki Olomuc Fac Med*. 1993;135:55–57.
39. **Zulian E, Sartorato P, Benedini S, et al.** Spironolactone in the treatment of polycystic ovary syndrome: effects on clinical features, insulin sensitivity and lipid profile. *J Endocrinol Invest*. 2005;28:49–53.
40. **Kebapcilar L, Bilgir O, Taner CE, et al.** Oral contraceptives alone and with spironolactone increase sCD40 ligand in PCOS patients. *Arch Gynecol Obstet*. 2010;281:539–543.
41. **Luque-Ramírez M, Alvarez-Blasco F, Botella-Carretero JI, Martínez-Bermejo E, Lasunción 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;92:2453–2461.
42. **Harmanci A, Cinar N, Bayraktar M, Yildiz BO.** Oral contraceptive plus antiandrogen therapy and cardiometabolic risk in polycystic ovary syndrome. *Clin Endocrinol (Oxf)*. 2013;78:120–125.
43. **Ibáñez L, Valls C, Ferrer A, Ong K, Dunger DB, De Zegher F.** Additive effects of insulin-sensitizing and anti-androgen treatment in young, nonobese women with hyperinsulinism, hyperandrogenism, dyslipidemia, and anovulation. *J Clin Endocrinol Metab*. 2002;87:2870–2874.
44. **DeUgarte CM, Bartolucci AA, Azziz R.** Prevalence of insulin resistance in the polycystic ovary syndrome using the homeostasis model assessment. *Fertil Steril*. 2005;83:1454–1460.