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Effect of Long-Term Treatment with Metformin Added to Hypocaloric Diet on Body Composition, Fat Distribution, and Androgen and Insulin Levels in Abdominally Obese Women with and without the Polycystic Ovary Syndrome

RENATO PASQUALI, ALESSANDRA GAMBINERI, DOMENICO BISCOTTI, VALENTINA VICENNATI, LORENZA GAGLIARDI, DONATELLA COLITTA, STEFANIA FIORINI, GRACIELA ESTELA COGNIGNI*, MARCO FILICORI*, AND ANTONIO MARIA MORSELLI-LABATE

Endocrine Unit (R.P., A.G., D.B., V.V., L.G., D.C., S.F., A.M.M.-L.), Department of Internal Medicine and Gastroenterology, S. Orsola-Malpighi Hospital; and Department of Obstetric and Gynecology, Reproductive Endocrinology Center (G.E.C., M.F.), University of Bologna, 40138 Bologna, Italy

ABSTRACT

Abdominal obesity and hyperinsulinemia play a key role in the development of the polycystic ovary syndrome (PCOS). Dietary-induced weight loss and the administration of insulin-lowering drugs, such as metformin, are usually followed by improved hyperandrogenism and related clinical abnormalities. This study was carried out to evaluate the effects of combined hypocaloric diet and metformin on body weight, fat distribution, the glucose-insulin system, and hormones in a group of 20 obese PCOS women [body mass index (BMI) $>$ 28 kg/m^2] with the abdominal phenotype (waist to hip ratio $>$0.80), and an appropriate control group of 20 obese women who were comparable for age and pattern of body fat distribution but without PCOS. At baseline, we measured sex hormone, sex hormone-binding globulin (SHBG), and leptin blood concentrations and performed an oral glucose tolerance test and computerized tomography (CT) at the L4-L5 level, to measure sc adipose tissue area (SAT) and visceral adipose tissue area. All women were then given a low-calorie diet (1200–1400 kcal/day) alone for one month, after which anthropometric parameters and CT scan were newly measured. While continuing dietary treatment, PCOS women and obese controls were subsequently placed, in a random order, on metformin (850 mg/os, twice daily) (12 and 8, respectively) or placebo (8 and 12, respectively), according to a double-blind design, for the following 6 months. Blood tests and the CT scan were performed in each woman at the end of the study while they were still on treatment.

During the treatment period, 3 women of the control group (all treated with placebo) were excluded because of noncompliance; and 2 PCOS women, both treated with metformin, were also excluded because they became pregnant. Therefore, the women cohort available for final statistical analysis included 18 PCOS (10 treated with metformin and 8 with placebo) and 17 control women (8 treated with metformin and 9 with placebo).

The treatment was well tolerated. In the PCOS group, metformin therapy improved hirsutism and menstrual cycles significantly more than placebo. Baseline anthropometric and CT parameters were similar in all groups. Hypocaloric dieting for 1 month similarly reduced BMI values and the waist circumference in both PCOS and control groups, without any significant effect on CT scan parameters. In both PCOS and control women, however, metformin treatment reduced body weight and BMI significantly more than placebo. Changes in the waist-to-hip ratio values were similar in PCOS women and controls, regardless of pharmacological treatment. Metformin treatment significantly decreased SAT values in both PCOS and control groups, although only in the latter group were SAT changes significantly greater than those observed during the placebo treatment. On the contrary, visceral adipose tissue area values significantly decreased during metformin treatment in both PCOS and control groups, but only in the former was the effect of metformin treatment significantly higher than that of placebo.

Fasting insulin significantly decreased in both PCOS women and controls, regardless of treatment, whereas glucose-stimulated insulin significantly decreased only in PCOS women and controls treated with metformin. Neither metformin or placebo significantly modified the levels of LH, FSH, dehydroepiandrosterone sulphate, and progesterone in any group, whereas testosterone concentrations decreased only in PCOS women treated with metformin. SHBG concentrations remained unchanged in all PCOS women; whereas in the control group, they significantly increased after both metformin and placebo. Leptin levels decreased only during metformin treatment in both PCOS and control groups.

In summary, this study shows that, in PCOS women with abdominal obesity, long-term treatment with metformin added to hypocaloric diet induced, in comparison with placebo, a greater reduction of body weight and abdominal fat, particularly the visceral depots, and a more consistent decrease of serum insulin, testosterone, and leptin concentrations. These changes were associated with a more significant improvement of hirsutism and menses abnormalities. Moreover, the effects on body weight, insulin, and leptin were similar to those observed in the group of comparable abdominally obese controls, in whom, however, a more pronounced reduction of sc fat in the abdominal region and an increase of SHBG concentrations were found. These findings, therefore, indicate that hyperinsulinemia and abdominal obesity may have complementary effects in the pathogenesis of PCOS. (*J Clin Endocrinol Metab* **85:** 2767–2774, 2000)

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Address correspondence and requests for reprints to: Renato Pasquali, M.D., Endocrine Unit, Department of Internal Medicine and Gastroenterology, S. Orsola-Malpighi Hospital, Via Massarenti 9, 40138, Bologna, Italy. E-mail: rpasqual@almadns.unibo.it.

THE METABOLIC syndrome is an integral part of the polycystic ovary syndrome (PCOS) in most affected women. In its typical form, it includes insulin resistance and hyperinsulinemia, obesity (predominantly the abdominal phenotype), and altered lipid profile (1–3). Both hyperinsulinemia and obesity may be intimately related to the development and maintenance of hyperandrogenism (1, 4, 5). In

fact, hyperinsulinemia is directly involved in determining increased ovarian androgen secretion (1, 5), through the activation of the cytochrome P450c17 enzyme system (6), and in reducing sex hormone-binding globulin (SHBG) synthesis by the liver (7, 8), which allows greater free androgen fraction availability in peripheral target tissues (9). The role of obesity in the development of hyperandrogenism is still under debate. On the other hand, it is well established that, in women, the abdominal obesity phenotype is associated with a marked decrease of SHBG levels (10–14) and some increase in total and free testosterone (T) (10, 11, 13), which is consistent with a state of relative hyperandrogenism. Numerous clinical and experimental data, in fact, indicate that obese women with PCOS, particularly those with the abdominal body fat distribution, may have a worse clinical condition and higher circulating androgen levels than their normal-weight counterparts (14). However, hyperandrogenism *per se* may favor enlargement of visceral fat in women (15). In fact, androgen administration to postmenopausal women has been shown to increase visceral fat (16). In addition, there are theoretical possibilities that increased androgen levels may directly affect insulin sensitivity in the target tissues, particularly muscles, therefore contributing to the development of the insulin resistance state (15).

Dietary-induced weight loss is usually followed by reduced hyperandrogenism and hyperinsulinemia and improved clinical status (such as fewer menses abnormalities, less hirsutism, and increased fertility rate) in many obese women with PCOS (17–19). On the other hand, with the exception of one study (20), the administration of insulin-lowering drugs, such as diazoxide (21), metformin (6, 22, 23), troglitazone (24), and (more recently) D-chito-inositol (25), has been proved to obtain the same results, regardless of significant changes in body weight, thus emphasizing the role of hyperinsulinemia in the pathophysiology of PCOS. Whether these effects may be mediated, at least in part, by selective reduction of visceral fat is still unknown. In addition, studies performed so far in obese PCOS women failed to investigate the effect of long-term hypocaloric dieting with or without the association of insulin sensitizers on body composition and fat distribution.

Therefore, we carried out this study to evaluate the effects of combined hypocaloric diet and metformin, an insulin-sensitizer agent, on body weight and fat distribution in a group of PCOS women with the abdominal obesity phenotype. The fasting insulin and glucose-stimulated insulin levels and androgen and leptin blood concentrations were also investigated. Moreover, to evaluate whether the effects of such a treatment were specifically conditioned by the presence of PCOS or by the presence of abdominal obesity, a control group of women, comparable for age and pattern of body fat distribution but without PCOS, was also investigated.

Subjects and Methods

Subjects

A group of 20 women with PCOS and a group of 20 controls, comparable for age and weight, were included in the study. They were recruited as outpatients attending the Endocrine Unit of the Department of Internal Medicine and Gastroenterology of the S. Orsola-Malpighi Hospital of Bologna. All PCOS and control women were obese, with body mass index (BMI; kg/m^2) values greater than 28, and had abdominal body fat distribution defined by waist-to-hip ratio (WHR) values greater than 0.80 (26). The diagnosis of PCOS was made according to the presence of oligomenorrhea (less than four cycles in the last 6 months) or amenorrhea (no menses in the last 6 months) and hyperandrogenism, defined by supranormal total and free T concentrations, according to normal reference values in our laboratory (27). All women with PCOS had ovarian ultrasonic findings consistent with the diagnosis (28). None of the PCOS or control women had thyroid dysfunction, type II diabetes, or concomitant cardiovascular, renal, and liver dysfunction, based on clinical examination and routine laboratory findings. Other causes of hyperandrogenisms, such as Cushing syndrome and disease and congenital adrenal hyperplasia, were excluded by normal cortisol suppression after an overnight 1-mg dexamethasone test and normal fasting and stimulated (250 mg Synacthen iv) 17-hydroxyprogesterone concentrations. All PCOS women also had normal PRL levels. None of the PCOS or control women had taken any medication for at least 3 months before the study, nor were they dieting. Women of the control group had regular monthly menses and no clinical or laboratory evidence of androgen excess.

The protocol was approved by the Ethics Committee of S. Orsola-Malpighi Hospital, and all women gave their informed consent.

Anthropometry and measurement of body fat distribution

Body height was measured (without shoes) to the nearest 0.5 cm, and body weight (without clothes). According to the recommendation of the World Health Organization (29), waist circumference was obtained as the minimum value between the iliac crest and the lateral costal margin, whereas hip circumference was determined as the maximum value over the buttocks, using a 1-cm-wide metal measuring tape. Body fat distribution was also defined by a standardized measurement of body fat at the L4-L5 level, by computerized tomography (CT), which was performed on a scanner (Siemens, Erlangen, Germany). Total adipose tissue area (TAT), visceral adipose tissue area (VAT), and sc adipose tissue area (SAT) were calculated as previously described (30). Previous studies (reviewed in Ref. 31) have shown that visceral fat areas from a single scan taken at the level L4-L5 were highly correlated to total visceral fat ($r > 95\%$), measured by multiple CT scans.

Protocol study

At baseline, PCOS women were studied within the first 10 days after the last menstruation if they had mild oligomenorrhea, or randomly if they had severe oligomenorrhea or amenorrhea, whereas all control women were studied during the early follicular phase of the menstrual cycle, except 2 women who were studied during the luteal phase. All women were following their usual diet, providing at least 250–300 g of carbohydrates were ingested. Blood samples for baseline hormone were drawn in the morning, at 0800–0830 h, after an overnight fast. An oral glucose tolerance test (OGTT) (75 g Curvosio, Sclavo, Cinisello Balsamo, Italy) was then performed, and blood samples were collected after 30, 60, 90, 120, and 180 min for glucose determination and after 60, 120, and 180 min for insulin determination. In the afternoon of the same day, the CT scans were performed. The day after, all women were then placed, for a month, on a standardized hypocaloric diet consisting of 1200–1400 kcal daily and containing 50% carbohydrates, 30% total lipids, and 20% proteins. The women returned after 1 month for a checkup, when body weight and body circumferences were newly measured and the CT scan was repeated. Apart from anthropometric and CT scan parameters, the OGTT and sex hormone blood samples were not performed after the first month of dietary therapy. In fact, it is well known that early reduction in body weight may mainly reflect a large loss of body water and that the changes in metabolic and hormonal parameters observed in these conditions may be caused by the effects of undernutrition rather than by changes in body composition (32). While continuing dietary treatment, PCOS women and obese controls were subsequently placed, in a random order, on metformin (Laboratori Guidotti Spa, Pisa, Italy; 850 mg/os, twice daily) (12 PCOS and 8 controls, respectively) or placebo (8 PCOS and 12 controls, respectively), according to a double-blind design, for the following 6 months. The randomization schedule was generated in blocks of 4, and the drug and placebo were packaged and labeled according to subject number. Dietary and pharmacological treatment were maintained for the following 6 months, during which the women

were regularly checked, at monthly intervals, to evaluate compliance with the diet and pharmacological treatment and any side effects. Each woman was given 1 fresh 1-month pack of metformin or placebo at the start of the treatment and again at each monthly visit. Compliance with the treatment was evaluated by counting the number of pills remaining to each woman at each control visit. At the end of the trial, the women returned for the final study, which included the same protocol performed at baseline. In this case, blood testing was performed, regardless of the menstrual cycle, in both the women with PCOS and the controls. During the treatment period, 3 women of the control group (all treated with placebo) were excluded because of noncompliance with the diet. Another 2 PCOS women, both treated with metformin, were also excluded from the trial because they became pregnant while they were on month 1 and 4 of the treatment, respectively. Therefore, the women cohort available for final statistical analysis included 18 PCOS (10 treated with metformin and 8 with placebo) and 17 control women (8 treated with metformin and 9 with placebo).

Assays

Plasma glucose levels were determined by the glucose-oxidase method immediately after blood samples had been obtained. Blood samples for hormones were centrifuged immediately, and serum was stored at −20 C° until assayed. To avoid variation between assays, all the samples from an individual woman were analyzed in duplicate in a single assay for each hormone. Insulin and C-peptide were measured by reagents purchased from Eiken Chemical Corporation (Tokyo, Japan) and Sclavo (Cinisello Balsamo, Italy), respectively. Gonadotropin LH and FSH, T, dehydroepiandrosterone sulphate (DHEA-S), estradiol (E2), progesterone (P), SHBG, and leptin levels were measured as previously described (27, 30, 33). The intraassay coefficient of variation in our laboratory was 3.0% for insulin, 3.7% for C-peptide, 7.0% for T, 5.9% for DHEA-S, 5.6% for E2, 4.1% for P, 6.5% for SHBG, 3.0% for leptin, 4.8% for LH, and 1.9% for FSH.

Statistical analysis

Results are reported as the mean values ± SD, unless otherwise indicated. The response of glucose, insulin, and C-peptide to the OGTT was analyzed by calculating the (AUC) by the trapezoidal method. Normal distribution and homoscedasticity of continuous variables were tested by means of the Kolmogorov-Sminorv (34) and the Levene tests (35). Variables that did not fulfill these tests were log-transformed before analysis. To avoid multiple comparisons, the data at the different times of the study were evaluated by means of two-way ANOVA, applying a within-treatment and group design, while the within-subject ANOVA, with the same design, was used to compare the modifications observed during the course of the study. The scores of clinical parameters were analyzed by means of the Wilcoxon matched-pairs and the Mann-Whitney tests (34). Statistical evaluations were performed by running the SPSS, Inc.(Chicago, IL)/PC+ software package on a personal computer (36). Two-tailed P values less than 0.05 were used to define statistical significance.

Results

Tolerance and side effects

The treatment was well tolerated by all women. No women suspended the therapy because of side effects, although some of them (one PCOS and one control woman, both treated with metformin) experienced transient mild diarrhea and flatulence during the first 2 weeks of treatment.

Clinical parameters

At baseline, 13 PCOS women were hirsute (9 in the metformin group and 4 in the placebo group). During treatment, the Ferriman-Gallway score decreased significantly in those treated with metformin (basal, 14.8 ± 7.5; after, 12.9 ±.7.6; $P < 0.05$) but not in those taking placebo [basal, 11.5 ± 10.7; after, 10.3 ± 10.5; P = NS (not significant)]. None of the control women were hirsute.

At baseline, nine PCOS and six control women had acanthosis nigricans. Although several of them who were included in both treatments improved, no significant difference was found in either group between metformin and placebo.

Both PCOS groups improved the frequency of their menstrual cycles (metformin group: basal, 1.2 ± 1.6; after, 4.7 ± 2.1; $P < 0.01$) (placebo group: basal, 1.3 ± 1.5; after, 3.5 ± 2.3; $P < 0.05$), but the effects of metformin were significantly higher than those of placebo ($P < 0.05$).

Anthropometry and fat distribution

Baseline anthropometric and CT scan parameters and their changes during treatment are reported in Table 1. In basal conditions, there was no difference in any of them between PCOS and controls within each group, between women treated with metformin or placebo. Changes in body weight and BMI during the first month of hypocaloric dieting were similar in PCOS and control women and were not significantly different in subgroups treated with metformin or placebo. However, during the 6-month pharmacological treatment, both PCOS and controls treated with metformin similarly and significantly decreased body weight (PCOS, $P < 0.05$; controls, $P < 0.001$) and BMI (PCOS, $P < 0.05$; controls, $P < 0.01$) more than women treated with placebo. In all groups, there was a significant reduction in waist circumference after the first month of hypocaloric diet. Metformin therapy further significantly reduced waist circumference values during the 6-month treatment in both PCOS and controls, but only in the latter was a significant difference *vs.* placebo treatment found ($P < 0.05$). On the contrary, metformin and placebo induced a similar decrease in hip circumference in both PCOS and control women. Neither grouping nor treatment had a significant effect on WHR values.

TAT and SAT values were not significantly influenced by the first month of hypocaloric diet in any group, whereas those of VAT were weakly (but significantly) reduced only in the PCOS women included in the metformin group, and in the controls included in the placebo group. During the pharmacological treatment, TAT values significantly decreased in both PCOS and controls taking metformin and in the controls taking placebo. However, no differences in TAT changes between metformin and placebo were found in the PCOS groups; whereas, in the control group, they were significantly greater in those taking metformin than placebo ($P < 0.05$). The SAT values significantly decreased only in the PCOS and control metformin-treated groups. However, a significantly greater effect of metformin treatment was evident in controls ($P < 0.05$) but not in PCOS women. The opposite was found in VAT values. In fact, they significantly decreased in both PCOS and control groups treated with metformin, but only in the PCOS women was the effect of metformin significantly higher ($P < 0.05$) than that of placebo. Finally, after 1-month hypocaloric dieting, a significant reduction of the VAT/SAT ratio was found only in the PCOS women included in the metformin group. However,

TABLE 1. Anthropometric parameters (m ± SD) and indices of body fat distribution (measured by CT scan) in PCOS women and control women (Obese) with abdominal obesity at baseline, after 1-month hypocaloric dieting and after 6-month combined treatment with hypocaloric diet plus metformin or placebo

Parameters	Groups	Metformin Time			Placebo Time		
		Baseline	1st Month	7th Month	Baseline	1st Month	7th Month
Age (yr)	PCOS	30.8 ± 7.4			32.3 ± 5.0		
	Obese	31.6 ± 10.3			36.3 ± 9.5		
Weight (Kg)	PCOS	103 ± 18	99 ± 16[a]	94 ± 17[a,b]	102 ± 19	99 ± 19[a]	97 ± 18[c]
	Obese	101 ± 8	97 ± 7[a]	88 ± 7[a,b]	106 ± 13	102 ± 13[a]	100 ± 13[a]
BMI (Kg/m^2)	PCOS	39.8 ± 7.9	38.3 ± 7.4[a]	36.4 ± 7.4[a,b]	39.6 ± 6.9	38.4 ± 6.9[a]	38.0 ± 6.2[d]
	Obese	37.4 ± 3.0	35.8 ± 2.6[a]	32.9 ± 3.4[a,b]	40.1 ± 6.2	38.5 ± 5.9[a]	37.8 ± 5.7[a]
Waist Circ (cm)	PCOS	107 ± 16	103 ± 15[a]	100 ± 15[a,e]	109 ± 19	106 ± 17[c]	104 ± 13[c]
	Obese	102 ± 6	99 ± 6[c]	94 ± 6[a,b]	109 ± 11	105 ± 11[a]	105 ± 12[d]
Hip Circ (cm)	PCOS	122 ± 12	119 ± 13[c]	117 ± 15[a]	122 ± 10	120 ± 10	118 ± 11
	Obese	119 ± 5	117 ± 3	112 ± 5[a,d]	124 ± 11	120 ± 10[c]	118 ± 9[a]
WHR	PCOS	0.87 ± 0.07	0.87 ± 0.06	0.86 ± 0.07	0.91 ± 0.11	0.88 ± 0.08	0.88 ± 0.05
	Obese	0.85 ± 0.04	0.85 ± 0.05	0.84 ± 0.04	0.88 ± 0.07	0.88 ± 0.08	0.90 ± 0.1
TAT (cm^2)	PCOS	685 ± 192	712 ± 191	598 ± 216[a,b]	710 ± 150	704 ± 167	682 ± 137
	Obese	688 ± 84	646 ± 76	562 ± 109[a,b]	733 ± 185	684 ± 204	667 ± 168[d]
SAT (cm^2)	PCOS	535 ± 147	571 ± 142	485 ± 170[f]	589 ± 127	598 ± 133	574 ± 111
	Obese	554 ± 79	524 ± 72	462 ± 81[c,f]	554 ± 118	518 ± 130	508 ± 107
VAT (cm^2)	PCOS	151 ± 91	140 ± 72[c]	113 ± 59[a,f]	121 ± 48	106 ± 41	108 ± 36
	Obese	133 ± 38	121 ± 32	100 ± 37[c,f]	181 ± 94	166 ± 91[d]	159 ± 83[d]
VAT/SAT	PCOS	0.28 ± 0.18	0.24 ± 0.1[d]	0.24 ± 0.09	0.21 ± 0.08	0.18 ± 0.05[g]	0.19 ± 0.05[g]
	Obese	0.24 ± 0.09	0.23 ± 0.07	0.22 ± 0.07	0.32 ± 0.15	0.31 ± 0.14	0.31 ± 0.14

WHR, TAT, SAT, and VAT were all measured by CT scan. Circ, circumference.
[a] $P < 0.001$ for comparison between values at month 1 and month 7 *vs.* baseline within each group.
[b] $P < 0.001$ for comparison between values at month 7 *vs.* month 1 within each group.
[c] $P < 0.01$.
[d] $P < 0.05$.
[e] $P < 0.05$.
[f] $P < 0.01$.
[g] $P < 0.05$ between PCOS and controls (Obese) at the reference times.

TABLE 2. Fasting and glucose-stimulated (as AUC) values (m ± SD) of glucose, insulin, and C-peptide blood concentrations in PCOS women and control women (Obese) with abdominal obesity at baseline and after combined treatment with hypocaloric diet plus metformin or placebo

Parameters	Groups	Metformin Time			Placebo Time		
		Baseline	7th Month	*P*	Baseline	7th Month	*P*
Glucose, fasting (mg/dL)	PCOS	99 ± 29	90 ± 17	<0.05	101 ± 18	95 ± 11	NS
	Obese	89 ± 10	89 ± 13	NS	92 ± 10	93 ± 17	NS
$Glucose_{AUC}$ (mg/mL × min)	PCOS	25726 ± 8887	23481 ± 4713	NS	25955 ± 8798	24740 ± 4173	NS
	Obese	21414 ± 2282	22264 ± 1833	NS	23188 ± 5832	24833 ± 7166	NS
Insulin, fasting (μU/mL)	PCOS	43.0 ± 30.4	21.6 ± 31.2	<0.001	33.5 ± 29.9	19.0 ± 14.4	<0.05
	Obese	30.3 ± 8.2	14.3 ± 8.5	<0.001	20.8 ± 11.1	14.4 ± 10.6	<0.01
$Insulin_{AUC}$ (μU/mL × min)	PCOS	41750 ± 24994	16730 ± 14425	<0.001	24295 ± 18644	15120 ± 6861	NS
	Obese	28277 ± 18059	10684 ± 5086	<0.001	14897 ± 9038	12520 ± 6899	NS
C-peptide, fasting (ng/mL)	PCOS	7.46 ± 3.00	4.18 ± 3.21	<0.001	4.05 ± 1.14	3.88 ± 1.98	NS
	Obese	4.84 ± 1.41	3.94 ± 1.70	NS	4.14 ± 1.28	3.28 ± 1.01	NS
$C\text{-peptide}_{AUC}$ (ng/mL × min)	PCOS	3641 ± 2002	2052 ± 1165	<0.001	2110 ± 442	1787 ± 686	NS
	Obese	2384 ± 448	1733 ± 390	<0.01	1835 ± 646	1589 ± 469	NS

To convert glucose to mmol/L, multiply by 0.056; to convert insulin to pmol/L, multiply by 7.175; to convert C-peptide to pmol/L, multiply by 331. Reference values for fasting values in our laboratory are, respectively: glucose, 86 ± 11 mg/dL; insulin, 6.9 ± 4.1 μU/mL; C-peptide, 2.3 ± 1.1 ng/mL. At baseline, there were no differences in any parameters between PCOS and controls, or between metformin and placebo within each subgroup.

compared with baseline values, no significant changes were found in either PCOS or controls during either treatment; but in placebo-treated groups, values of the VAT/SAT ratio during treatment were significantly higher in controls than in PCOS women.

Glucose, insulin, and C-peptide

Fasting and glucose-stimulated values of glucose, insulin, and C-peptide before and at the end of the study are reported in Table 2. At baseline, there were no differences in any parameters between PCOS and controls or between metformin and placebo within each subgroup.

Fasting glucose levels decreased significantly only in the metformin-treated PCOS group, without any significant changes in the control metformin group and in either PCOS or controls treated with placebo. On the contrary, no significant difference was found in $glucose_{AUC}$ in either PCOS or controls during either treatment.

TABLE 3. Sex hormones and SHBG blood concentrations (m ± SD) in PCOS women and control women (Obese) with abdominal obesity at baseline and after combined treatment with hypocaloric diet plus metformin or placebo

Parameters	Groups	Metformin Time			Placebo Time		
		Baseline	7th Month	*P*	Baseline	7th Month	*P*
LH (mIU/mL)	PCOS	8.45 ± 3.44	7.37 ± 3.87	NS	10.5 ± 2.0	15.4 ± 12.9	NS
	Obese	3.89 ± 1.89[a]	4.80 ± 3.84	NS	4.28 ± 1.29[b]	8.50 ± 7.40	NS
FSH (mIU/mL)	PCOS	4.63 ± 1.15	7.05 ± 8.74	NS	6.93 ± 4.48	7.28 ± 4.96	NS
	Obese	5.61 ± 2.43	4.94 ± 1.28	NS	4.21 ± 1.29	6.64 ± 3.15	NS
T (ng/mL)	PCOS	0.68 ± 0.35	0.49 ± 0.25	<0.01	0.51 ± 0.17	0.47 ± 0.13	NS
	Obese	0.42 ± 0.11[b]	0.36 ± 0.11	NS	0.38 ± 0.12[a]	0.33 ± 0.1	NS
DHEA-S (μg/mL)	PCOS	1.42 ± 0.80	1.66 ± 0.96	NS	0.90 ± 0.27	1.30 ± 0.21	NS
	Obese	1.20 ± 0.76	1.37 ± 0.93	NS	1.47 ± 0.61	1.32 ± 0.75	NS
P (ng/mL)	PCOS	0.59 ± 0.23	3.51 ± 5.08	NS	0.44 ± 0.24	4.30 ± 9.31	NS
	Obese	0.77 ± 0.22	2.41 ± 2.85	NS	2.15 ± 3.23[a]	2.23 ± 4.17	NS
E2 (pg/mL)	PCOS	48.4 ± 16.0	84.7 ± 34.9	<0.05	53.2 ± 10.4	76.5 ± 61.6	NS
	Obese	60.7 ± 21.7	91.6 ± 63.2	NS	95.6 ± 76.1	81.6 ± 59.1	NS
SHBG (nmol/L)	PCOS	18.7 ± 15.0	16.7 ± 8.1	NS	16.0 ± 7.04	13.8 ± 2.1	NS
	Obese	23.4 ± 22.7	28.9 ± 16.5[a]	<0.05	20.2 ± 10.7	28.1 ± 14.7[a]	<0.05

To convert T to nmol/L, multiply by 3.467; to convert DHEA-S to μmol/L, multiply by 2.714, to convert P to nmol/L, multiply by 3.18; to convert E2 to pmol/L, multiply by 3.671. Reference values for fasting values in our laboratory are, respectively: T, 0.41 ± 0.14 ng/mL; DHEA-S, 1.69 ± 0.15 μg/mL; P, 0.31 ± 0.10 ng/mL; E2, 23.7 ± 8.7 pg/mL; SHBG, 61.2 ± 18.3 nmol/mL; LH, 6.7 ± 1.6 mIU/mL; FSH, 6.0 ± 3.5 mIU/mL.

[a] $P < 0.05$.

[b] $P < 0.01$ for comparison between PCOS and control group (Obese) at each time.

At baseline, mean fasting insulin levels and insulin_{AUC} tended to be higher, although not significantly, in PCOS than in controls. In both groups, however, they significantly decreased during the treatment, regardless of therapy. On the contrary, insulin_{AUC} significantly decreased only in the PCOS group and in the controls treated with metformin, whereas no significant variation was found in the placebo-treated groups. As a consequence, changes in insulin_{AUC} after metformin seemed to be higher than those observed after placebo, in both PCOS ($P < 0.06$) and control women ($P < 0.01$).

Fasting C-peptide decreased significantly only in the metformin-treated PCOS group. Conversely, C-peptide_{AUC} significantly decreased in both PCOS and controls treated with metformin, but not in the placebo-treated groups. However, within each group, no significant differences in C-peptide_{AUC} values were found between metformin and placebo treatment.

Sex hormones and SHBG

Baseline and posttreatment sex hormone and SHBG values are reported in Table 3. At baseline, PCOS women had significantly higher LH and T levels than controls, whereas no significant difference was found in FSH, DHEA-S, and E2 values. However, because, in two control women included in the placebo group, baseline blood samples were collected in the luteal phase, this group had significantly higher P levels than the PCOS group treated with placebo.

Neither metformin nor placebo modified blood levels of LH, FSH, DHEA-S, and P in any group. Testosterone levels significantly decreased only in the metformin-treated PCOS group but not in those taking placebo. On the other hand, approximately half of them (five included in the metformin treatment and four in the placebo treatment) still had higher T and insulin [fasting and areas under the response curve (AUC)]rsqb] values than those observed in the control group after treatment.

Treatment did not significantly modify SHBG levels in PCOS groups; whereas, in the controls, SHBG significantly increased after both metformin and placebo. Therefore, posttreatment SHBG levels were significantly higher in controls than in PCOS women, regardless of treatment. E2 concentrations increased significantly only in the metformin-treated PCOS women, without any changes in those taking placebo and in the control groups. However, no significant difference was present in the PCOS group between metformin and placebo treatment.

Leptin

There were no differences in baseline leptin concentrations between PCOS women and controls. Both PCOS women and controls treated with metformin significantly decreased their leptin concentrations, whereas no significant variation after placebo was found in either group (Fig. 1). However, in both PCOS women and controls, mean changes observed during metformin or placebo were not significantly different.

Discussion

Dietary-induced weight loss may represent an appropriate means of improving hyperandrogenism and all parameters of the metabolic syndrome in many obese PCOS women (17–19). The amelioration of hyperinsulinemia and insulin sensitivity may explain these biological effects, together with the concomitant improvement of related clinical manifestations. However, available studies agree in suggesting that, even without changes in body weight, the reduction of insulin levels, which can be achieved by administering insulin-sensitizing agents, may be sufficient, in many cases, to reduce increased androgen levels, even after short-term administration (6, 21–25). These findings clearly emphasize the independent role of hyperinsulinemia as a key factor in the development of hyperandrogenism in PCOS.

What the role of a reduction in adipose tissue, particularly visceral fat, is in determining these modifications is not yet clearly established. Our study was specifically conducted to

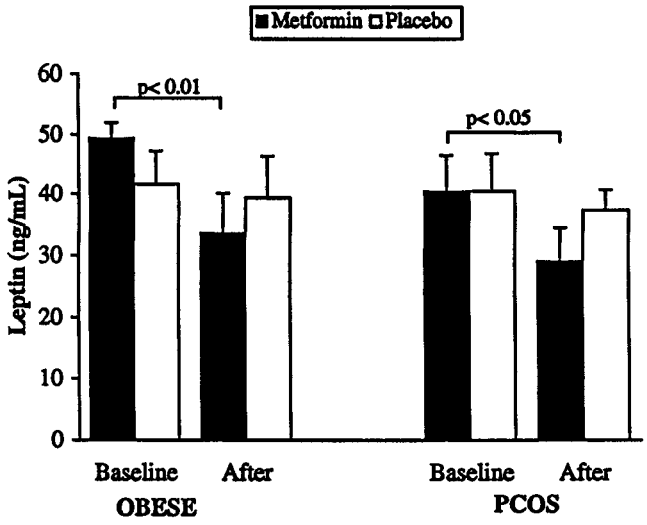

FIG. 1. Leptin serum concentrations (mean ± SEM) in PCOS women and control women (Obese) with abdominal obesity at baseline and after combined treatment with hypocaloric diet plus metformin or placebo. To convert leptin to pmol/L, multiply by 167. Reference values for fasting values in our laboratory are 5.9 ± 4.3 ng/mL.

determine whether the long-term administration of metformin, which can improve insulin resistance and reduce hyperinsulinemia, may have effects that supplement hypocaloric diet in reducing circulating insulin and androgen blood levels in obese PCOS women and to investigate whether these effects may be related, at least in part, to changes in body weight and fat distribution. To avoid confounding factors, only PCOS women with the abdominal obesity phenotype were included in the study, together with an age- and fat distribution-matched group of women with obesity but without PCOS. Both conditions are, in fact, associated with moderate-to-severe hyperinsulinemia and insulin resistance (1, 37). An apparent limitation of the study is that we did perform hormone blood levels and OGTT after 1 month of hypocaloric dieting. On the other hand, this was done to avoid the counterproductive effects of hypocaloric diet on hormones and metabolism. However, as was to be expected, the loss of weight after such a short time was similar in the PCOS women and in controls, regardless of the pharmacological treatment. This makes it unlikely that not having repeated these measurements after 1 month lead-in period could have affected the interpretation of the results. However, even if the above is taken into account, our data indicate that obese PCOS women and obese controls lost more weight while on metformin than on placebo. Compared with other studies, the weight loss in PCOS women and controls treated with metformin seems to have been greater than expected. In effect, our findings are in agreement with those reported by Crave *et al.* (38), who treated a group of obese hirsute PCOS women with hypocaloric diet (1500 kcal/day) and metformin (1500 mg/day) or placebo for 4 months and found a tendency toward greater weight loss in the metformin-treated group than in those receiving placebo. Unfortunately, any further comparison regarding the effects of metformin on weight loss in PCOS is difficult because, in most of the studies carried out (6, 20, 22, 23), metformin was administered without dietary restriction and, therefore, changes in body weight were negligible. It is also important to consider that, in our study, we only included PCOS and control women with abdominal obesity, whereas all other cited studies examined obese PCOS women regardless of their body fat distribution pattern. Because, during hypocaloric dieting, women with the abdominal obesity phenotype respond better than those with the peripheral (or sc) phenotype (39), it could have been expected that, when metformin is combined with a hypocaloric diet, the weight loss could be greater than that observed in the majority of studies carried out in non-selected obese subjects (with or without PCOS). In addition, metformin therapy favored a greater reduction of the waist circumference in both groups, which suggests a significant modification of the pattern of fat distribution, particularly at the abdominal level. However, whereas obese PCOS lost significantly more VAT in the abdomen area during metformin than during placebo treatment, without any significant difference in changes of SAT, the opposite was observed in the control obese group. These findings, therefore, suggest disparate effects of metformin added to the diet on visceral fat in PCOS, with respect to control women, in spite of the fact that they were characterized by similar obesity phenotype. They clearly seem to be related to changes in the hormonal environment that occurred during treatment in PCOS and in controls.

As expected, treatment significantly reduced fasting insulin levels; but, unlike placebo, metformin significantly decreased insulin and C-peptide response to oral glucose administration, which indicates a contemporary improvement of both insulin resistance and β-cell function. The extent of these effects was similar in PCOS and control metformin-treated women, which means that the responsiveness of the insulin-glucose system was not affected by the presence of PCOS *per se*, but rather by the reduction of abdominal obesity.

The pathogenetic role of obesity and body fat distribution in PCOS is still a matter of intensive debate. Available data seem to support the concept that PCOS and obesity may have an additive effect, or a synergistic, negative impact on insulin sensitivity (1). However, in a study performed in a cohort of normal-weight and obese women with and without PCOS and different patterns of body fat distribution, we previously showed that hyperinsulinemia was more consistently correlated with abdominal fat distribution, regardless of the presence of PCOS (40). In addition, others found that women with PCOS may not be insulin-resistant in the absence of increased abdominal fatness, in spite of significant hyperandrogenism (3). In addition, our present and previous intervention data (41) indicate that hyperinsulinemia and insulin resistance may be largely reversible with reduction or normalization of abdominal fat depots. Therefore, it is hypothesized that abdominal (visceral) fatness may have a dominant role in determining these abnormalities in most women with PCOS, regardless of other factors, including genetic predisposition (1).

A reduction in serum T levels occurred only in the metformin-treated PCOS group but not in the control group or in the PCOS taking placebo in addition to hypocaloric diet. Some previous studies had, in fact, shown that weight re-

duction obtained with hypocaloric diet alone was associated with a significant decrease in serum T levels (17–19). At variance, Crave *et al.* (38) found no difference between metformin and placebo added to hypocaloric diet after 4 months treatment in a group of obese hirsute women, most of whom probably had PCOS. The most likely explanation seems to be that all these studies included PCOS women based on the presence of obesity but with a wide range of body fat distribution. Contrary to what is reported by other studies (19, 21, 38), we found no significant variations in SHBG concentrations in PCOS women, whereas they were significantly increased in both metformin- and placebo-treated control women. The lack of SHBG increase in the PCOS group, particularly in those treated with metformin, was an unexpected finding. On the other hand, the fact that SHBG levels increased in the controls (both during metformin and placebo treatment) makes it improbable that the data depend on the size of the sample, unreliable assay systems, or statistical inadequacy. Other factors may probably be involved. In fact, at variance with previous studies, all women included in the study had abdominal obesity, a condition always associated with reduced SHBG concentrations (12). However, among PCOS women, lowered SHBG concentrations can also be found in those with the peripheral obesity phenotype, regardless of whether they may be relatively less hyperandrogenic and hyperinsulinemic, with respect to those with abdominal obesity (14). Moreover, the fact that approximately half the PCOS women were still relatively hyperinsulinemic and hyperandrogenic, although sia mean values of both T and insulin (fasting and glucose-stimulated) were significantly lower during treatment, particularly in the metformin-treated group, may further explain the nonincrease of the SHBG concentrations during treatment. Therefore, further studies are needed in this area, focusing on the effects of weight loss and insulin-lowering drugs in PCOS women according to specific obesity phenotype.

Finally, we found a significant effect of metformin treatment on leptin levels, which was identical in PCOS women and in controls. Because leptin levels are dependent on the amount of total body fat (42), these findings can be explained by the greater weight reduction found in both groups treated with metformin, compared with those treated with placebo. In addition, they may be dependent on the greater reduction of insulin levels induced by the metformin treatment. In fact, metformin administration has been found to decrease serum leptin in obese PCOS women, even in the absence of changes in body weight (43). Therefore, these findings confirm the regulatory role of insulin on leptin synthesis and secretion (44).

In summary, this study shows that, in PCOS women with abdominal obesity, long-term treatment with metformin, added to hypocaloric diet, induced (in comparison with placebo) a greater reduction of body weight and visceral fat and a more consistent decrease of serum insulin, T, and leptin concentrations. These changes were associated with a more significant improvement of menses abnormalities. The effects on body weight, insulin, and leptin were similar to those observed in the group of comparable abdominally obese controls in whom, however, a more pronounced reduction of sc fat in the abdominal region and an increase of SHBG concentrations were found. These findings, therefore, indicate that hyperinsulinemia and abdominal obesity may have complementary effects in the pathogenesis of PCOS.

References

1. **Dunaif A.** 1997 Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. Endocr Rev. 18:774–800.
2. **Pasquali R, Casimirri F.** 1993 The impact of obesity on hyperandrogenism and polycystic ovary syndrome in premenopausal women. Clin Endocrinol (Oxf). 39:1–16.
3. **Holte J.** 1996 Disturbances in insulin secretion and sensitivity in women with the polycystic ovary syndrome. Clin Endocrinol Metab NA. 10:221–247.
4. **Franks S.** 1995 Polycystic ovary syndrome. N Engl J Med. 333:853–861.
5. **Poretsky L.** 1991 On the paradox of insulin-induced hyperandrogenism in insulin-resistant state. Endocr Rev. 12:3–13.
6. **Nestler JE, Jakubowicz DJ.** 1996 Decrease of ovarian cytochrome P45017α activity and serum free testosterone after reduction of insulin secretion in polycystic ovary syndrome. N Engl J Med. 335:617–623.
7. **Plymate SR, Leonard JM, Paulsen CA, et al.** 1983 Sex hormone-binding globulin changes with androgen replacement. J Clin Endocrinol Metab. 57:645–648.
8. **Plymate SR, Matej LA, Jones RE, et al.** 1988 Inhibition of sex hormone-binding globulin production in the human hepatoma (Hep G2) cell line by insulin and prolactin. J Clin Endocrinol Metab. 67:460–464.
9. **Von Schultz B, Carlsrom K.** 1989 On the regulation of sex-hormone-binding globulin: challenge of an old dogma and outlines of an alternative mechanism. J Steroid Biochem. 32:327–334.
10. **Kissebah AH, Peiris AN.** 1988 Biology of regional body fat distribution: relationship to non-insulin dependent diabetes mellitus. Diabetes Metab Rev. 5:83–109.
11. **Evans DJ, Hoffman RG, Kalkoff RK, et al.** 1983 Relationship of androgenic activity to body fat topography, fat cell morphology and metabolic aberrations in premenopausal women. J Clin Endocrinol Metab. 57:304–310.
12. **Pasquali R, Casimirri F, Platè L, et al.** 1990 Characterization of obese women with reduced sex hormone-binding globulin concentrations. Horm Metab Res. 22:303–306.
13. **Leenen R, Van Der Koy K, Seidell JC, Deurenberg P, Kopperschaar HPF.** 1994 Visceral fat accumulation in relation to sex hormones in obese men and women undergoing weight loss therapy. J Clin Endocrinol Metab. 78:1515–1520.
14. **Pasquali R, Casimirri F, Venturoli S, et al.** 1994 Body fat distribution has weight-independent effects on clinical, hormonal, and metabolic features of women with polycystic ovary syndrome. Metabolism. 43:706–713.
15. **Björntorp P.** 1993 Hyperandrogenicity in women-a prediabetic condition? J Intern Med. 234:579–583.
16. **Lovejoy JC, Bray GA, Bourgeois MO, et al.** 1996 Exogenous androgens influence body composition and regional body fat distribution in obese postmenopausal women-a clinical research center study. J Clin Endocrinol Metab. 81:2198–2203.
17. **Pasquali R, Antenucci D, Casimirri F, et al.** 1989 Clinical and hormonal characteristics of obese amenorrheic hyperandrogenic women before and after weight loss. J Clin Endocrinol Metab. 68:173–179.
18. **Kiddy DS, Hamilton-Fairley D, Bush A, et al.** 1992 Improvement in endocrine and ovarian function during dietary treatment of obese women with polycystic ovary syndrome. Clin Endocrinol (Oxf). 36:105–111.
19. **Jakubowicz DJ, Nestler JE.** 1997 17α-hydroxyprogesterone responses to leuprolide and serum androgens in obese women with and without polycystic ovary syndrome after dietary weight loss. J Clin Endocrinol Metab. 82:556–560.
20. **Ehrmann DA, Cavaghan MK, Imperial J, Sturis J, Rosenfield RL, Polonsky KS.** 1997 Effects of metformin on insulin secretion, insulin action, and ovarian steroidogenesis in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 82:524–530.
21. **Nestler JE, Clore JN, Blackard WG.** 1989 Suppression of serum insulin by diazoxide reduces serum testosterone levels in obese women with polycystic ovary syndrome. J Clin Endocrinol Metab. 68:1027–1032.
22. **Velazquez EM, Mendoza S, Hamer T, Sosa F, Glueck CJ.** 1994 Metformin therapy in polycystic ovary syndrome reduces hyperinsulinemia, insulin resistance, hyperandrogenemia, and systolic blood pressure, while facilitating normal menses and pregnancy. Metabolism. 43:647–654.
23. **Moghetti P, Castello R, Negri C, et al.** 2000 Metformin effects on clinical features, endocrine and metabolic profiles, and insulin sensitivity in polycystic ovary syndrome: a randomized, double-blind, placebo-controlled 6-month trial, followed by open, long-term clinical evaluation. J Clin Endocrinol Metab. 85:139–146.
24. **Dunaif A, Scott D, Finegood D, Quintana B, Whitcomb R.** 1996 The insulin-sensitizing agent troglitazone improves metabolic and reproductive abnormalities in the polycystic ovary syndrome. J Clin Endocrinol Metab. 81:3299–3306.

25. **Nestler JE, Jakubowicz DJ, Reamer P, Gunn RD, Allan G.** 1999 Ovulatory and metabolic effects of d-chiro-inositol in the polycystic ovary syndrome. N Engl J Med. 340:1314–1320.
26. **WHO.** 1997 Preventing and managing the global epidemic. Report of a WHO consultation on obesity. Geneva: WHO/NUT/NCD/98.1.
27. **Pasquali R, Cantobelli S, Vicennati V, et al.** 1995 Nitrendipine treatment in women with polycystic ovarian syndrome: evidence for a lack of effects of calcium channel blockers on insulin, androgens, and sex hormone-binding globulin. J Clin Endocrinol Metab. 80:3346–3350.
28. **Orsini LP, Venturoli S, Lorusso R, Pluchinotta V, Paradisi R, Bovicelli L.** 1985 Ultrasonic findings in polycystic ovarian diseasae. Fertil Steril. 43:709–714.
29. **WHO.** 1988 Measuring obesity-classification and description of anthropometric data. Copenhagen, Denmark: WHO Regional Office for Europe; Eur/ICP/NUT 125–0612v.
30. **Vicennati V, Gambineri A, Calzoni L, et al.** 1998 Serum leptin in obese women with polycystic ovary syndrome is correlated with body weight and fat distribution but not with androgen and insulin levels. Metabolism. 47:988–992.
31. **van der Koy E, Seidell JC.** 1993 Techniques for the measurement of visceral fat: a practical guide. Int J Obes. 17:187–196.
32. **Garrow JS.** 1988 Obesity and related diseases. Edinburgh: Churchill Livingstone.
33. **Pasquali R, Gambineri A, Anconetani B, et al.** 1999 The natural history of the metabolic syndrome in young women with the polycystic ovary syndrome and the effect of long-term oestrogen-progestagen treatment. Clin Endocrinol (Oxf). 50:517–527.
34. **Siegel S.** 1956 Non-parametric statistics of the behavioral sciences. New York: McGraw-Hill.
35. **Levene H.** 1960 Robust test for equality of variance. In: Olkin I, ed. Contribution to probability and statistics. Palo Alto, CA: Stanford University Press.
36. **Norusis MJ, SPSS Inc.** 1990 SPSS/PC+ 4.0 base and advanced statistics manuals. Chicago: SPSS Inc.
37. **Caro J.** 1991 Insulin resistance in obese and nonobese man. J Clin Endocrinol Metab. 73:691–695.
38. **Crave JC, Fimbel S, Lejeune H, Cugnardey N, Déchaud H, Pugeat M.** 1995 Effects of diet and metformin administration on sex hormone-binding globulin, androgens, and insulin in hirsute and obese women. J Clin Endocrinol Metab. 80:2057–2062.
39. **Dwyer JT.** 1992 Treatment of obesity: conventional programs and fat diets. In: Bjorntorp P, Brodoff BN, eds. Obesity. Philadelphia: J. B. Lippincott Co.; 662–676.
40. **Pasquali R, Casimirri F, Cantobelli S, et al.** 1993 Insulin and androgen relationships with abdominal body fat distribution in women with and without hyperandrogenism. Horm Res. 39:179–187.
41. **Holte J, Bergh I, Berne C, et al.** 1995 Restored insulin sensitivity but persistently increased early insulin secretion after weight loss in obese women with polycystic ovary syndrome. J Clin Endocrinol Metab. 80:2586–2593.
42. **Considine RV, Sinha MK, Heiman HL, et al.** 1996 Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med. 334:292–295.
43. **Morin-Papunen LC, Koivunen RM, Tomas C, Ruokonen A, Martikainen HK.** 1998 Decreased serum leptin concentrations during metformin therapy in obese women with polycystic ovary syndrome. J Clin Endocrinol Metab. 83:2566–2568.
44. **Coleman RA, Herrman TS.** 1999 Nutritional regulation of leptin in humans. Diabetologia. 42:639–646.