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Lean Women with Polycystic Ovary Syndrome Respond to Insulin Reduction with Decreases in Ovarian P450c17α Activity and Serum Androgens*

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ABSTRACT

It is unknown whether hyperinsulinemia plays a role in the pathogenesis of polycystic ovary syndrome (PCOS) in normal weight or thin women. Evidence indicates that these women are insulin resistant and hyperinsulinemic, and this study was conducted to test the hypothesis that hyperinsulinemia stimulates ovarian cytochrome P450c17α activity in nonobese women with PCOS, thereby increasing serum androgen concentrations.

We assessed ovarian P450c17α activity (by measuring the response of 17α-hydroxyprogesterone to a GnRH agonist), fasting serum steroids, and oral glucose tolerance before and after oral administration of either metformin (500 mg) or placebo three times daily for 4–6 weeks in 31 nonobese women with PCOS.

In the 19 women given metformin, the mean (±SE) area under the serum insulin curve after oral glucose administration decreased from 44 ± 5 to 24 ± 3 nmol/L·min ($P = 0.003$). Basal serum 17α-hydroxyprogesterone decreased from 3.4 ± 0.3 to 2.5 ± 0.4 nmol/L ($P = 0.05$), and GnRH-stimulated peak serum 17α-hydroxyprogesterone decreased from 12.2 ± 1.6 to 7.5 ± 0.7 nmol/L ($P = 0.005$). Serum 17α-hydroxyprogesterone values did not change in the placebo group. In the metformin group, serum free testosterone decreased by 70% from 18.2 ± 3.1 to 5.5 ± 0.7 pmol/L ($P < 0.001$), and serum sex hormone-binding globulin increased from 84 ± 6 to 134 ± 15 nmol/L ($P = 0.002$). None of these values changed in the placebo group.

These findings suggest that hyperinsulinemia stimulates ovarian P450c17α activity in nonobese women with PCOS. They also indicate that decreasing serum insulin with metformin reduces ovarian cytochrome P450c17α activity and ameliorates the hyperandrogenism of these women. (*J Clin Endocrinol Metab* **82:** 4075–4079, 1997)

THE POLYCYSTIC ovary syndrome (PCOS) is defined by chronic anovulation and hyperandrogenism and affects approximately 6% of women of reproductive age (1). The majority of women with PCOS are obese and, consequently, insulin resistant and hyperinsulinemic (2–9). In these obese women hyperinsulinemia plays a central role in the pathogenesis of the PCOS (10) by both stimulating ovarian androgen production (11–18) and decreasing the serum sex hormone-binding globulin (SHBG) concentration (19, 20). Obese women with PCOS also demonstrate increased ovarian P450c17α activity, a key enzyme in the biosynthesis of androgens, as demonstrated by an increased response of serum 17α-hydroxyprogesterone to stimulation by GnRH agonists (21–23). P450c17α appears to be stimulated by insulin in PCOS, and reducing insulin release with metformin (16) or weight loss (18) decreases ovarian P450c17α activity and serum free testosterone concentrations in obese women with the disorder.

However, not all women with PCOS are obese. Between 20–50% of women with PCOS are normal weight or thin, and the pathophysiology of the disorder in these women may differ from that in obese women. It has been suggested that PCOS develops in nonobese women because of a hypothalamic-pituitary defect that results in increased release of LH, and that insulin plays no role in the disorder (24–27).

This concept ignores the fact that nonobese women with PCOS demonstrate an intrinsic form of insulin resistance that is unique to the disorder (2, 3, 28) and are hyperinsulinemic compared to their healthy counterparts (29). Nonobese women with PCOS also exhibit increased ovarian P450c17α activity (21, 22, 30). As hyperinsulinemia stimulates P450c17α activity in obese women with PCOS (16, 18), it seems likely that it should do so in nonobese affected women as well.

We hypothesized that hyperinsulinemia stimulates ovarian cytochrome P450c17α activity in normal weight and thin women with PCOS, and that amelioration of insulin resistance in these women should return the activity of the enzyme toward normal. To test this hypothesis, we measured the basal serum 17α-hydroxyprogesterone concentration and the serum 17α-hydroxyprogesterone response to administration of a GnRH agonist in nonobese women with PCOS, who ranged in body weight from normal to thin, before and after the administration of metformin. Metformin is a biguanide that inhibits hepatic glucose production and enhances peripheral tissue sensitivity to insulin, and thereby decreases insulin secretion (31, 32).

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Subjects and Methods

Subjects

We enrolled 31 women with PCOS, aged 18–35 yr, into the study. All women were either normal weight or thin (body mass index, 18.0–23.7 kg/m^2). Women with PCOS had oligomenorrhea (<6 menstrual periods in the last year) and hyperandrogenemia (elevated serum free testosterone concentration). All women had normal serum PRL and normal thyroid function tests. Late-onset adrenal hyperplasia was excluded by a morning serum 17α-hydroxyprogesterone level below 6 nmol/L. All women had ovarian ultrasonic findings consistent with the diagnosis of PCOS (33). None had taken any medications for at least 2 months, and none had diabetes mellitus. The study was approved by the institutional review board of the Hospital de Clinicas Caracas, and each woman gave informed consent.

Initially, 11 women were randomly assigned to receive metformin (Glafornil, North Medicamenta, Caracas, Venezuela), and 12 women received placebo. An analysis of the results revealed a borderline significant decrease in fasting serum insulin in the metformin group (P = 0.06 with low power of 0.36), whereas in the placebo group it increased (see *Results*). Therefore, it was decided to study in nonrandomized fashion an additional 8 women taking metformin to increase the ability of the study to detect a significant decrease in this variable. All other changes in the metformin group remained qualitatively similar with the addition of these women, although the degree of statistical significance increased.

Experimental protocol

The women were studied during the follicular phase of the cycle, as documented by a serum progesterone level below 6.4 nmol/L. On day 1, the women came to the hospital after a 12-h overnight fast, where their weight, height, and waist to hip ratio were measured. Blood samples were drawn at 0830, 0845, and 0900 h, and equal volumes of serum were pooled for measurement of baseline insulin, glucose, steroid, and SHBG concentrations. At 0900 h, 75 g dextrose (Glucolab, Laboratory Relab, Caracas, Venezuela) were given orally. Blood samples were collected for determination of serum concentrations of glucose and insulin at 60 and 120 min.

On day 2, the women ate breakfast at 0900 h and fasted until 1400 h, when a leuprolide stimulation test was performed (see below). After this test, the women were assigned to receive either metformin (500 mg) or placebo orally three times daily. The women ate *ad libitum* while outpatients and were instructed not to alter eating habits, activity level, or lifestyle during the study.

The women returned for the second study after 4–6 weeks, after they were confirmed to be in the follicular phase of the menstrual cycle by a low serum progesterone value. Eight women in the metformin group and two women in the placebo group had serum progesterone values in the postovulatory range after 4 weeks of treatment. These women were continued on their respective medications and were studied 2 weeks later when their serum progesterone values were low. All studies performed at baseline were repeated.

Leuprolide test

After baseline blood samples had been obtained at 1400 h on day 2, the GnRH agonist leuprolide (10 μg/kg) was administered sc. Blood samples were collected immediately before and 0.5, 1.0, 16, 20, and 24 h after leuprolide administration for determination of serum LH concentrations and before and after 16, 20, and 24 h for serum 17α-hydroxyprogesterone concentrations. The women ate supper on day 2, but fasted thereafter until completion of the test. Equal volumes of serum from 0.5 and 1.0 h were pooled for measurement of the early serum LH response, and sera from 16, 20, and 24 h were pooled for the late serum LH response. The 0 h serum 17α-hydroxyprogesterone level was the basal value, and the highest serum 17α-hydroxyprogesterone concentration after leuprolide administration was considered the peak value.

Assays

Blood samples were centrifuged immediately, and serum was stored at −20 C until assayed. Serum hormones and SHBG (measured as protein) were assayed as previously described by us (16). To avoid interassay variation, all samples were analyzed in duplicate in a single assay for each hormone. Intraassay coefficients of variation for the insulin and LH assays were 5.5% and 1.6%, respectively, and were less than 10% for all steroid hormone assays.

Statistical analysis

Results are reported as the mean ± SE. Within a group, results before treatment were compared with those after treatment by testing for normality with the Wilk-Shapiro test and using Student's two-tailed paired t test or the Wilcoxon signed rank test. Comparisons between groups were made by Student's two-tailed unpaired t test or the Mann-Whitney rank sum test.

Serum glucose and insulin profiles during the oral glucose tolerance tests and serum 17α-hydroxyprogesterone profiles during the leuprolide tests were analyzed by transforming data into area under the curve by the trapezoidal rule, using absolute values.

Results

Baseline characteristics

Women with PCOS in the metformin and placebo groups did not differ significantly with respect to age, body mass index, waist to hip ratio, or sex steroid and SHBG concentrations at baseline (Table 1). They also did not differ with respect to fasting serum insulin and glucose values, glucose responses after oral glucose administration, basal or leuprolide-stimulated LH values, or basal or leuprolide-stimulated 17α-hydroxyprogesterone values (Table 1 and Fig. 1). The area under the serum insulin curve was greater in the metformin group than in the placebo group (44 ± 5 *vs.* 31 ± 2 nmol/L/min, respectively; P = 0.05)

Anthropometric variables (Table 1)

Body mass index did not change with treatment in either group of women with PCOS. The waist to hip ratio decreased in the metformin group, but did not change in the placebo group.

Insulin and glucose profiles

The mean fasting serum insulin concentration decreased from 138 ± 24 to 60 ± 6 pmol/L (P = 0.001), and the area under the serum insulin curve decreased from 44 ± 5 to 24 ± 3 nmol/L·min (P = 0.003) in the metformin group, whereas in the placebo group these values increased (Table 1). Fasting serum glucose and the area under the serum glucose curve did not change in the metformin group, but both values increased in the placebo group (Table 1).

Serum LH responses to leuprolide

Basal serum LH decreased from 4.3 ± 0.6 to 2.9 ± 0.9 mIU/mL (P = 0.04) in the metformin group, but did not change in the placebo group (Fig. 2). The early serum LH responses to leuprolide were lower after metformin treatment than at baseline (12.5 ± 2.4 *vs.* 24.1 ± 3.9 mIU/mL, respectively; P = 0.03; Fig. 2), as were the late serum LH responses (30.6 ± 5.0 *vs.* 60.2 ± 7.8 mIU/mL, respectively; P = 0.003; Fig. 2). In contrast, in the placebo group, basal serum LH and the early and late serum LH responses to leuprolide were similar at baseline and after treatment (Fig. 2).

TABLE 1. Clinical characteristics and serum hormone concentrations in lean women with PCOS at baseline and after administration of metformin or placebo for 4–6 weeks

	Metformin group (n = 19)		Placebo group (n = 12)	
	Baseline	After metformin	Baseline	After placebo
Age (yr)	26 ± 1		27 ± 2	
Body mass index (kg/m^2)	21.7 ± 0.3	21.7 ± 0.3	21.4 ± 0.5	21.3 ± 0.5
Waist to hip ratio	0.80 ± 0.01	0.78 ± 0.01[a]	0.83 ± 0.02	0.84 ± 0.02
Fasting serum insulin (pmol/L)	138 ± 24	60 ± 6[b]	90 ± 18	138 ± 24[c]
$AUC_{INSULIN}$ (nmol/L·min)[d]	44 ± 5	24 ± 3[e]	31 ± 2[f]	38 ± 2[a]
Fasting serum glucose (mmol/L)	4.4 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	4.6 ± 0.1[g]
$AUC_{GLUCOSE}$ (mmol/L·min)[d]	645 ± 32	589 ± 27	615 ± 23	641 ± 19[h]
Serum progesterone (nmol/L)	3.1 ± 0.3	2.2 ± 0.2	3.5 ± 0.4	3.7 ± 0.4
Serum testosterone (nmol/L)	2.5 ± 0.4	1.3 ± 0.2[i]	2.1 ± 0.3	2.2 ± 0.3
Serum free testosterone (pmol/L)	18.2 ± 3.1	5.5 ± 0.7[a]	12.2 ± 2.0	11.5 ± 1.6
Serum androstenedione (nmol/L)	9.8 ± 0.8	6.3 ± 0.4[a]	8.3 ± 0.5	8.3 ± 0.4
Serum 17β-estradiol (pmol/L)	327 ± 47	188 ± 33[a]	225 ± 40	196 ± 25
Serum DHEAS (μmol/L)	6.5 ± 0.9	3.7 ± 0.5[a]	4.5 ± 0.3	4.7 ± 0.4
Serum SHBG (nmol/L)	84 ± 6	134 ± 15[j]	90 ± 5	82 ± 6

Values are the mean ± SE. Normal ranges for ovulatory women: insulin, 30–120 pmol/L; progesterone, less than 6.4 nmol/L during the follicular phase; testosterone, 0.8–2.4 nmol/L; free testosterone, 2.1–6.6 pmol/L; androstenedione, 2.3–10.5 nmol/L; 17β-estradiol, 37–734 pmol/L (early to midfollicular range); DHEAS, 0.9–11.7 μmol/L; and SHBG, 20–139 nmol/L.

[a] $P < 0.001$ *vs.* baseline in the same group.
[b] $P = 0.001$ *vs.* baseline in the same group.
[c] $P = 0.03$ *vs.* baseline in the same group.
[d] Area under the curve responses during an oral glucose tolerance test.
[e] $P = 0.003$ *vs.* baseline in the same group.
[f] $P = 0.05$ compared with baseline in the metformin group.
[g] $P = 0.009$ *vs.* baseline in the same group.
[h] $P = 0.05$ *vs.* baseline in the same group.
[i] $P = 0.004$ *vs.* baseline in the same group.
[j] $P = 0.002$ *vs.* baseline in the same group.

17α-Hydroxyprogesterone responses

In the metformin group, mean basal serum 17α-hydroxyprogesterone decreased from 3.4 ± 0.3 to 2.5 ± 0.4 nmol/L ($P = 0.05$), but did not change in the placebo group (Fig. 1). Similarly, in the metformin group, the peak serum 17α-hydroxyprogesterone level after leuprolide administration decreased by 39% from 12.2 ± 1.6 to 7.5 ± 0.7 nmol/L ($P = 0.005$), and the area under the serum 17α-hydroxyprogesterone curve decreased from 194 ± 22 to 118 ± 11 nmol/L·h ($P = 0.002$). These values did not change in the placebo group (Fig. 1). The area under the serum 17α-hydroxyprogesterone curve after metformin treatment was significantly less than that after placebo treatment (118 ± 11 *vs.* 176 ± 12 nmol/L·h, respectively; $P = 0.001$).

Serum sex steroids

Metformin administration was associated with a decrease in serum total testosterone from 2.5 ± 0.4 to 1.3 ± 0.2 nmol/L ($P = 0.004$) and an increase in serum SHBG from 84 ± 6 to 134 ± 15 nmol/L ($P = 0.002$). Consequently, serum free testosterone decreased by 70% from 18.2 ± 3.1 to 5.5 ± 0.7 pmol/L ($P < 0.001$). Serum androstenedione concentrations decreased from 9.8 ± 0.8 to 6.3 ± 0.4 nmol/L ($P < 0.001$). Serum dehydroepiandrosterone sulfate and estradiol concentrations also decreased in the metformin group (Table 1). None of these values changed in the placebo group (Table 1).

Discussion

The aim of this study was to test the hypothesis that hyperinsulinemia stimulates ovarian androgen production in nonobese women with PCOS. Metformin was administered to normal weight and thin women with the disorder to improve insulin sensitivity and reduce insulin secretion, and the fasting serum insulin levels and serum insulin response to an oral glucose challenge decreased significantly. The reduction in insulin secretion was accompanied by decreased ovarian P450c17α activity, as evidenced by decreased serum 17α-hydroxyprogesterone responses to stimulation by the GnRH agonist leuprolide (to stimulate LH release). Furthermore, women with PCOS treated with metformin experienced marked reductions in serum ovarian androgens, namely total testosterone, free testosterone, and androstenedione. In contrast, the serum insulin status did not improve, and serum androgens did not change in women with PCOS treated with placebo.

We have also had the opportunity to administer metformin to nine nonobese normal women for 4 weeks (Nestler, J. E., and D. J. Jakubowicz, unpublished results; data available upon request). At a dose of 1500 mg daily, the first two women studied developed fasting hypoglycemia (probably as a result of suppressed hepatic glucose output), reflecting the normal insulin sensitivity of these women and requiring a decrease in dosage to 1000 mg daily. Metformin treatment decreased the serum insulin response to a glucose challenge in the normal women, but did not affect serum androgens. This indicates that the decrease in serum androgens observed in women with PCOS treated with metformin was not due to the drug itself and is consistent with the idea that the ability of insulin to stimulate ovarian cytochrome P450c17α may be an heritable abnormality limited to women with PCOS (10).

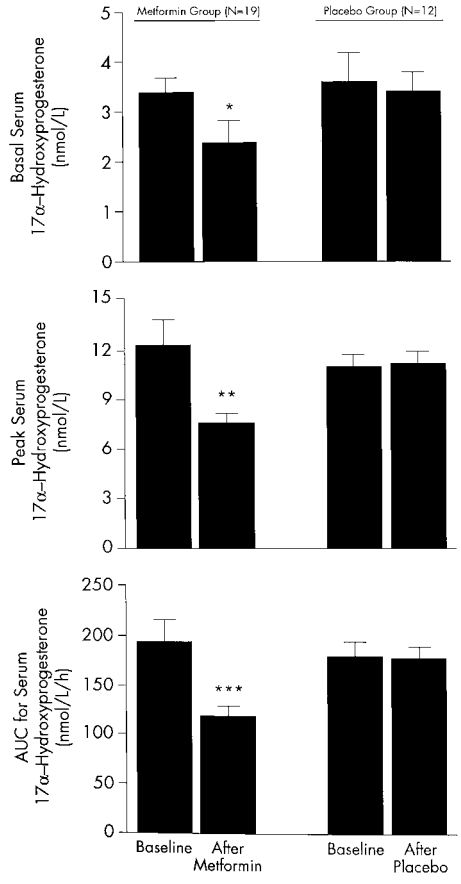

FIG. 1. Serum 17α-hydroxyprogesterone concentrations in nonobese women with PCOS at baseline and after administration of metformin or placebo for 4–6 weeks. The women were studied before and after stimulation with leuprolide (10 μg/kg). Values are the mean ± SE. *, $P = 0.05$; **, $P = 0.005$; ***, $P = 0.002$ (compared with the baseline value in same group).

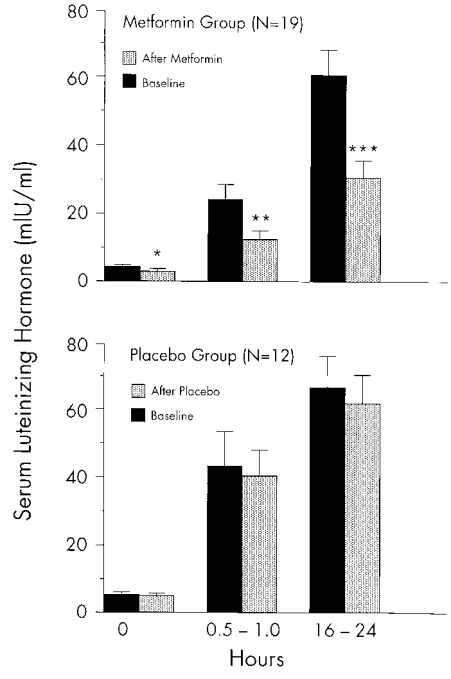

FIG. 2. Serum LH concentrations in nonobese women with PCOS at baseline and after administration of metformin or placebo for 4–6 weeks. The women were studied before and after stimulation with leuprolide (10 μg/kg). Values are the mean ± SE. *, $P = 0.04$; **, $P = 0.03$; ***, $P = 0.003$ (compared with baseline value in same group).

The results of this study do not clarify whether hyperinsulinemia increases ovarian androgen production directly by stimulating the ovaries, indirectly by enhancing LH release, or by a combination of these processes. The women with PCOS treated with metformin experienced decreases in both basal and leuprolide-stimulated LH release. This is consistent with a postulated action of insulin to increase LH pulse amplitude (16, 34–36). Of note, *in vitro* studies suggest that insulin can also directly stimulate testosterone production by human ovarian thecal cells and does so by activating its own receptor and using inositol glycan second messengers as the signal transduction system (37). Alternatively, the reduced secretion of LH may also be related to the observed decrease in serum estradiol with metformin administration.

It is known that insulin suppresses serum SHBG levels in women with PCOS (19, 20), and serum concentrations of this binding protein rose by 60% in the women with PCOS treated with metformin. Serum levels of the adrenal androgen dehydroepiandrosterone sulfate decreased in the women with PCOS treated with metformin, suggesting that hyperinsulinemia may also stimulate the adrenal P450c17α activity of some affected women (38). Finally, women with PCOS treated with metformin experienced a reduction in serum estradiol levels. This may have been due to decreased availability of substrate (*i.e.* thecal androgens) for conversion to estrogens. Alternatively, studies suggest that insulin stimulates the aromatase activity of human granulosa cells (39, 40), and the decrease in serum estradiol may have been related to decreased ovarian aromatase activity.

To our knowledge, drugs to improve insulin sensitivity or reduce insulin release have not been administered previously to nonobese women with PCOS. Our findings demonstrate that women with PCOS who are normal weight or thin respond to a reduction in insulin release with decreases in ovarian P450c17α activity and serum ovarian and adrenal androgens. This is consistent with the observation that although these women are not obese, they nonetheless tend to have an increased waist to hip ratio (41, 42) and are insulin resistant and hyperinsulinemic compared to their normal counterparts (2, 3, 28, 29). Moreover, contrary to the postulate that the pathophysiology of PCOS differs between obese and nonobese women (24–27), these findings support the idea that the pathophysiology is similar in both groups.

Weight loss is first-line therapy for obese women with PCOS, but is not a therapeutic option for nonobese women with the disorder. The clinical importance of our findings is that they suggest that even normal weight and thin women with PCOS should respond to pharmacological measures to improve insulin sensitivity, such as administration of agents like metformin, with decreases in ovarian androgen production and serum androgens.

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