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DECREASES IN OVARIAN CYTOCHROME P450c17 α ACTIVITY AND SERUM FREE TESTOSTERONE AFTER REDUCTION OF INSULIN SECRETION IN POLYCYSTIC OVARY SYNDROME

JOHN E. NESTLER, M.D., AND DANIELA J. JAKUBOWICZ, M.D.

ABSTRACT

Background Insulin resistance and increased ovarian cytochrome P450c17 α activity are both features of the polycystic ovary syndrome. P450c17 α , which is involved in androgen biosynthesis, has both 17 α -hydroxylase and 17,20-lyase activities. Increased activity of this enzyme results in exaggerated conversion of progesterone to 17 α -hydroxyprogesterone in response to stimulation by gonadotropin. We hypothesized that hyperinsulinemia stimulates ovarian P450c17 α activity.

Methods We measured serum steroid concentrations during fasting and the response of serum 17 α -hydroxyprogesterone to leuprolide, a gonadotropin-releasing hormone agonist, and performed oral glucose-tolerance tests before and after oral administration of either metformin (500 mg three times daily) or placebo for four to eight weeks in 24 obese women with the polycystic ovary syndrome.

Results In the 11 women given metformin, the mean (\pm SE) area under the serum insulin curve after oral glucose administration decreased from 9303 ± 1603 to 4982 ± 911 μ U per milliliter per minute (56 ± 10 to 30 ± 6 nmol per liter per minute) ($P=0.004$). This decrease was associated with a reduction in the basal serum 17 α -hydroxyprogesterone concentration from 135 ± 21 to 66 ± 7 ng per deciliter (4.1 ± 0.6 to 2.0 ± 0.2 nmol per liter) ($P=0.01$) and a reduction in the leuprolide-stimulated peak serum 17 α -hydroxyprogesterone concentration from 455 ± 54 to 281 ± 52 ng per deciliter (13.7 ± 1.6 to 8.5 ± 1.6 nmol per liter) ($P=0.01$). The serum 17 α -hydroxyprogesterone values increased slightly in the placebo group. In the metformin group, the basal serum luteinizing hormone concentration decreased from 8.5 ± 2.2 to 2.8 ± 0.5 mIU per milliliter ($P=0.01$), the serum free testosterone concentration decreased from 0.34 ± 0.07 to 0.19 ± 0.05 ng per deciliter (12 ± 3 to 7 ± 2 pmol per liter) ($P=0.009$), and the serum sex hormone-binding globulin concentration increased from 0.8 ± 0.2 to 2.3 ± 0.6 μ g per deciliter (29 ± 7 to 80 ± 21 nmol per liter) ($P<0.001$). None of these values changed significantly in the placebo group.

Conclusions In obese women with the polycystic ovary syndrome, decreasing serum insulin concentrations with metformin reduces ovarian cytochrome P450c17 α activity and ameliorates hyperandrogenism. (N Engl J Med 1996;335:617-23.)

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THE polycystic ovary syndrome is characterized by anovulation and hyperandrogenism. It affects approximately 6 percent of women of reproductive age.¹ Insulin resistance accompanied by compensatory hyperinsulinemia is a common feature of the syndrome, and both obese and nonobese women with the syndrome are more insulin-resistant and hyperinsulinemic than age- and weight-matched normal women.²⁻⁹

Hyperinsulinemia may play a pathogenetic part in hyperandrogenism in women with the polycystic ovary syndrome by increasing ovarian androgen production and decreasing the serum sex hormone-binding globulin concentration.¹⁰⁻¹⁷ Serum free testosterone concentrations decline in women with the polycystic ovary syndrome when their insulin secretion is reduced by the administration of diazoxide¹⁵ or metformin¹⁸ or by diet.^{19,20} Furthermore, the observation that adolescent girls with hyperandrogenism have insulin resistance⁹ suggests that hyperinsulinemia may play an early and central part in the pathogenesis of the polycystic ovary syndrome.

Cytochrome P450c17 α is a bifunctional enzyme that has both 17 α -hydroxylase and 17,20-lyase activities, and it is a key enzyme in the biosynthesis of ovarian androgens. In ovarian theca cells, P450c17 α converts progesterone to 17 α -hydroxyprogesterone through its 17 α -hydroxylase activity, and then converts 17 α -hydroxyprogesterone to androstenedione through its 17,20-lyase activity. Androstenedione is then converted to testosterone by the enzyme 17 β -reductase (Fig. 1).

Many women with the polycystic ovary syndrome have increased ovarian cytochrome P450c17 α activity,^{21,22} as evidenced by increased 17 α -hydroxylase and, to a lesser extent, 17,20-lyase activity, resulting in excessive ovarian androgen production. In these women, a hallmark of increased ovarian P450c17 α activity is an exaggerated serum 17 α -hydroxyprogesterone response to stimulation by gonadotropin-

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releasing hormone agonists, such as nafarelin,²¹⁻²³ buserelin,²⁴ and leuprolide.²⁵ Whether the increased ovarian P450c17 α activity in women with the polycystic ovary syndrome is an inherited or an acquired phenomenon is not known.

We hypothesized that hyperinsulinemia stimulates ovarian cytochrome P450c17 α activity in women with the polycystic ovary syndrome (Fig. 1) and that amelioration of insulin resistance in these women would return the activity of the enzyme toward normal. To test this hypothesis, we measured the basal serum 17 α -hydroxyprogesterone concentration and the serum 17 α -hydroxyprogesterone response to the administration of leuprolide in obese women with the polycystic ovary syndrome before and after the administration of metformin, which inhibits the production of hepatic glucose and enhances the sensitivity of peripheral tissue to insulin, thereby decreasing insulin secretion.^{26,27}

METHODS

Subjects

We enrolled 25 women who were 18 to 35 years old, 24 of whom completed the study. All the women had the polycystic ovary syndrome, as defined by oligomenorrhea (fewer than six menstrual periods in the previous year) and hyperandrogenemia (elevated serum free testosterone concentrations), and were obese

(body-mass index [weight in kilograms divided by the square of the height in meters], ≥ 27.5). All had hirsutism, and 15 had acanthosis nigricans. Two women had each delivered two children, five women had each delivered one child, and the rest were childless. All had normal serum prolactin concentrations and normal results on thyroid-function tests. Late-onset congenital adrenal hyperplasia was ruled out by a morning serum 17 α -hydroxyprogesterone concentration of less than 200 ng per deciliter (6 nmol per liter). All the women had findings on ultrasonography of the ovaries that were consistent with the diagnosis of the polycystic ovary syndrome.²⁸ None had taken any medications for at least two months, and none had diabetes mellitus. Twelve women were randomly assigned to receive metformin (Glaformil, North Medicamenta, Caracas, Venezuela) and 13 women to receive placebo. The study was approved by the institutional review board of the Hospital de Clinicas Caracas, and each woman gave informed consent.

Study Protocol

The women were evaluated during the follicular phase of the menstrual cycle, as determined by a serum progesterone concentration of less than 2 ng per milliliter (6.4 nmol per liter). On day 1 the women came to the hospital after a 12-hour overnight fast, and their weight, height, waist-to-hip ratio, and blood pressure while supine were measured. Blood samples were drawn at 8:30, 8:45, and 9 a.m., and equal volumes of serum were pooled for the measurement of insulin, glucose, steroids, and sex hormone-binding globulin. At 9 a.m., 75 g of dextrose (Glycolab, Relab Laboratory, Caracas, Venezuela) was given orally. Blood samples were collected for determinations of serum glucose and insulin concentrations at 60 and 120 minutes.

On day 2 the women ate breakfast at 9 a.m. and then fasted until 2 p.m., when a leuprolide stimulation test was performed.

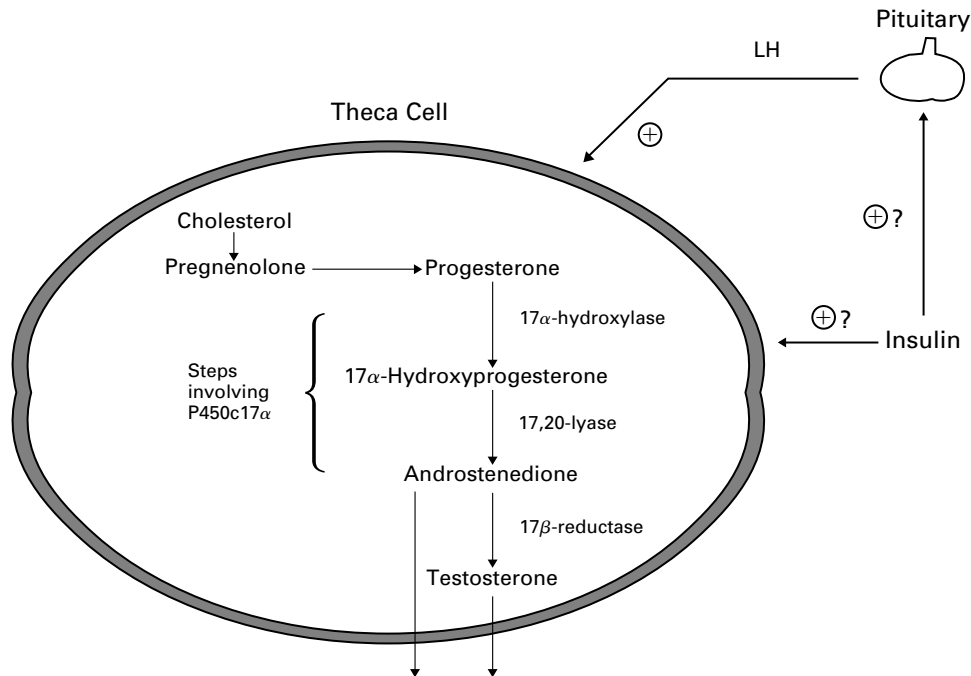


Figure 1. Possible Mechanisms of Insulin Stimulation of Ovarian Cytochrome P450c17 α Activity and Androgen Production. In theca cells, insulin may directly stimulate (plus signs) ovarian cytochrome P450c17 α , resulting in increased 17 α -hydroxylase and, to a lesser extent, 17,20-lyase activity. This would lead to increased production of androstenedione, which is then converted to testosterone by the enzyme 17 β -reductase. Alternatively or in conjunction with this, insulin may stimulate ovarian androgen production indirectly by enhancing the amplitude of serum luteinizing hormone (LH) pulses, and luteinizing hormone may then stimulate ovarian cytochrome P450c17 α activity.

After this test the women took 500 mg of metformin or placebo orally three times daily. They were instructed not to alter their usual eating habits, physical activity, or lifestyle during the study.

The women returned for studies four to eight weeks later, after a low serum progesterone value had confirmed that they were in the follicular phase of the menstrual cycle. Five women had serum progesterone values in the postovulatory range after taking metformin for four weeks. One of them became pregnant despite long-standing infertility; she was dropped from the study and her results were omitted from the analysis. The remaining four women continued to take metformin and were studied four weeks later when their serum progesterone values were low. In the placebo group, one woman had a serum progesterone value in the postovulatory range after four weeks; she was studied again two weeks later.

Leuprolide Stimulation Test

After base-line blood samples had been obtained at 2 p.m. on day 2, leuprolide (10 μ g per kilogram of body weight; Lupron, Abbott Laboratories, Takeda, Japan) was administered subcutaneously. Blood samples for the measurement of serum luteinizing hormone were collected immediately before and 0.5, 1, 16, 20, and 24 hours after leuprolide was administered. Blood samples for the measurement of serum 17 α -hydroxyprogesterone were collected immediately before and 16, 20, and 24 hours after leuprolide was administered. The women ate an evening meal on day 2 but fasted thereafter until the completion of the test. The early response of serum luteinizing hormone was determined from pooled equal volumes of serum taken at 0.5 and 1 hour, and the late serum luteinizing hormone response from pooled equal volumes of serum taken at 16, 20, and 24 hours. The serum concentration of 17 α -hydroxyprogesterone measured immediately before the administration of leuprolide was considered the basal value, and the highest serum concentration of 17 α -hydroxyprogesterone that was measured after the administration of leuprolide was considered the peak value.

Assays

The blood samples were centrifuged immediately, and the serum was stored at -20°C until it was assayed. The serum free testosterone concentration was determined by radioimmunoassay (Diagnostic Products, Los Angeles). All other hormones and sex hormone-binding globulin (measured as protein) were assayed as previously described.^{15,17,29} To avoid variation among assays, all samples were analyzed in duplicate in a single assay for each hormone. The intraassay coefficients of variation for the insulin and luteinizing hormone assays were 5.5 and 1.6 percent, respectively, and they were less than 10 percent for all the steroid hormone assays.

Statistical Analysis

The results are reported as means \pm SE. Within a group, we compared the results before treatment 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 with Student's two-tailed unpaired t-test or the Mann-Whitney rank-sum test.

We analyzed the responses of serum glucose and insulin to the oral administration of glucose and the responses of serum luteinizing hormone and 17 α -hydroxyprogesterone to the administration of leuprolide by calculating the areas under the response curves by the trapezoidal rule using absolute values.

RESULTS

Base-Line Characteristics

The women in the metformin and placebo groups did not differ significantly in age, body-mass index, waist-to-hip ratio, blood pressure, or serum concen-

trations of sex steroids or sex hormone-binding globulin at base line (Table 1). They also did not differ at base line in serum insulin or glucose values measured during fasting, insulin or glucose responses after oral glucose administration, or basal or leuprolide-stimulated serum 17 α -hydroxyprogesterone values (Table 1 and Fig. 2). The base-line serum luteinizing hormone concentration was higher in the metformin group than in the placebo group (8.5 ± 2.2 vs. 3.7 ± 0.7 mIU per milliliter; $P=0.04$) (Fig. 3).

Anthropometric Variables

The body-mass index did not change significantly during the study in either group. The waist-to-hip ratio decreased slightly in the metformin group ($P=0.02$) but did not change substantially in the placebo group. There was no significant change in diastolic or systolic blood pressure in either group.

Serum Insulin and Glucose Profiles

In the metformin group, the mean serum insulin concentration measured during fasting decreased from 17 ± 3 to 9 ± 2 μ U per milliliter (102 ± 18 to 54 ± 12 pmol per liter) ($P=0.03$), and the area under the serum insulin curve decreased from 9303 ± 1603 to 4982 ± 911 μ U per milliliter per minute (56 ± 10 to 30 ± 6 nmol per liter per minute) ($P=0.004$) (Table 1). Neither of these values changed significantly in the placebo group. The serum glucose concentration in fasting women did not change significantly in either group. The area under the serum glucose curve increased in the placebo group ($P=0.03$) but did not change substantially in the metformin group.

Responses of Serum Luteinizing Hormone to Leuprolide

The basal serum luteinizing hormone concentration decreased from 8.5 ± 2.2 to 2.8 ± 0.5 mIU per milliliter ($P=0.01$) in the metformin group but did not change significantly in the placebo group (Fig. 3). The early serum luteinizing hormone responses to leuprolide were lower after the administration of metformin than at base line (17.0 ± 2.5 vs. 40.8 ± 11.9 mIU per milliliter, $P=0.01$). The late serum luteinizing hormone responses were slightly but not significantly lower after the administration of metformin ($P=0.26$). In contrast, in the placebo group the basal serum luteinizing hormone concentrations and the early and late serum luteinizing hormone responses to leuprolide were virtually identical at base line and after the administration of placebo (Fig. 3).

Serum 17 α -Hydroxyprogesterone Responses

In the metformin group, the mean basal serum 17 α -hydroxyprogesterone concentration decreased by 51 percent, from 135 ± 21 to 66 ± 7 ng per deciliter (4.1 ± 0.6 to 2.0 ± 0.2 nmol per liter) ($P=0.01$), but it did not change significantly in the placebo

TABLE 1. CHARACTERISTICS OF WOMEN WITH THE POLYCYSTIC OVARY SYNDROME AT BASE LINE AND AFTER THE ADMINISTRATION OF METFORMIN OR PLACEBO FOR FOUR TO EIGHT WEEKS.*

CHARACTERISTIC	METFORMIN GROUP (N=11)		PLACEBO GROUP (N=13)	
	BASE LINE	AFTER METFORMIN	BASE LINE	AFTER PLACEBO
Age (yr)	29±1	—	29±2	—
Body-mass index	34.1±1.5	34.1±1.3	35.2±1.9	35.2±1.9
Waist-to-hip ratio	0.86±0.01	0.85±0.02†	0.86±0.01	0.87±0.01
Blood pressure (mm Hg)				
Systolic	128±2	127±2	132±4	134±3
Diastolic	87±1	83±1	88±2	88±2
Fasting serum insulin (μU/ml)	17±3	9±2‡	28±5	31±5
AUC for insulin (μU/ml/min)§	9,303±1603	4,982±911¶	9,022±1046	9,970±1119
Fasting serum glucose (mg/dl)	79±2	76±2	83±4	86±3
AUC for glucose (mg/dl/min)§	11,382±406	11,189±679	12,224±524	13,448±526‡
Serum progesterone (ng/ml)	0.8±0.1	0.8±0.1	1.0±0.2	0.8±0.1
Serum testosterone (ng/dl)	90±17	68±14	69±14	81±11
Serum free testosterone (ng/dl)	0.34±0.07	0.19±0.05	0.21±0.05	0.27±0.06
Serum androstenedione (ng/dl)	324±29	289±25	259±20	272±18
Serum 17β-estradiol (ng/dl)	10.6±1.3	9.0±1.1	8.0±0.9	8.9±0.7
Serum dehydroepiandrosterone sulfate (μg/dl)	176±22	196±21	170±19	180±18
Serum sex hormone-binding globulin (μg/dl)	0.8±0.2	2.3±0.6**	0.8±0.1	0.9±0.1

*The mean (±SE) length of administration was 42±4 days in the metformin group and 32±2 days in the placebo group. Plus-minus values are means ±SE. To convert values for insulin to picomoles per liter, multiply by 6.0; to convert values for glucose to millimoles per liter, multiply by 0.056; to convert values for progesterone to nanomoles per liter, multiply by 3.2; to convert values for testosterone to picomoles per liter, multiply by 34.7; to convert values for androstenedione to picomoles per liter, multiply by 34.9; to convert values for 17β-estradiol to picomoles per liter, multiply by 36.7; to convert values for dehydroepiandrosterone sulfate to micromoles per liter, multiply by 0.027; and to convert values for sex hormone-binding globulin to nanomoles per liter, multiply by 34.7. The normal ranges for ovulatory women are as follows: insulin, 5 to 20 μU per milliliter; progesterone, <2.0 ng per milliliter during the follicular phase; testosterone, 22 to 70 ng per deciliter; free testosterone, 0.06 to 0.19 ng per deciliter; androstenedione, 66 to 300 ng per deciliter; 17β-estradiol, 1 to 20 ng per deciliter (early follicular to midfollicular phase); dehydroepiandrosterone sulfate, 35 to 430 μg per deciliter; and sex hormone-binding globulin, 0.6 to 4.0 μg per deciliter.

†P=0.02 for the comparison with base line.

‡P=0.03 for the comparison with base line.

§Values are for the area under the curve (AUC) during an oral glucose-tolerance test.

¶P=0.004 for the comparison with base line.

||P=0.009 for the comparison with base line.

**P<0.001 for the comparison with base line.

group (Fig. 2). Similarly, in the metformin group the peak serum 17α-hydroxyprogesterone concentration after leuprolide administration decreased from 455±54 to 281±52 ng per deciliter (13.7±1.6 to 8.5±1.6 nmol per liter) (P=0.01), and the area under the serum 17α-hydroxyprogesterone curve decreased from 7848±945 to 4592±766 ng per deciliter per hour (237±29 to 139±23 nmol per liter per hour) (P=0.004), whereas these values increased slightly in the placebo group (Fig. 2). The change in the area under the serum 17α-hydroxyprogesterone curve in the metformin group differed significantly from that in the placebo group (−3256±180 vs. 912±105 ng per deciliter per hour [−98±27 vs. 28±10 nmol per liter per hour]) (P<0.001), and the area under the serum 17α-hydroxyprogesterone curve was significantly less after metformin administration than after placebo administration (4592±766 vs. 6949±685 ng per deciliter per hour

[139±21 vs. 210±21 nmol per liter per hour]) (P=0.02).

Serum Sex Steroids

The administration of metformin was associated with a 44 percent decrease in serum free testosterone concentrations, from 0.34±0.07 to 0.19±0.05 ng per deciliter (12±2 to 7±2 pmol per liter) (P=0.009), and a threefold increase in serum sex hormone-binding globulin concentrations, from 0.8±0.2 to 2.3±0.6 μg per deciliter (29±7 to 80±21 nmol per liter) (P<0.001) (Table 1). These values did not change significantly in the placebo group. The serum concentrations of the other measured steroids did not change substantially in either group.

DISCUSSION

In these women with the polycystic ovary syndrome, the administration of metformin reduced the

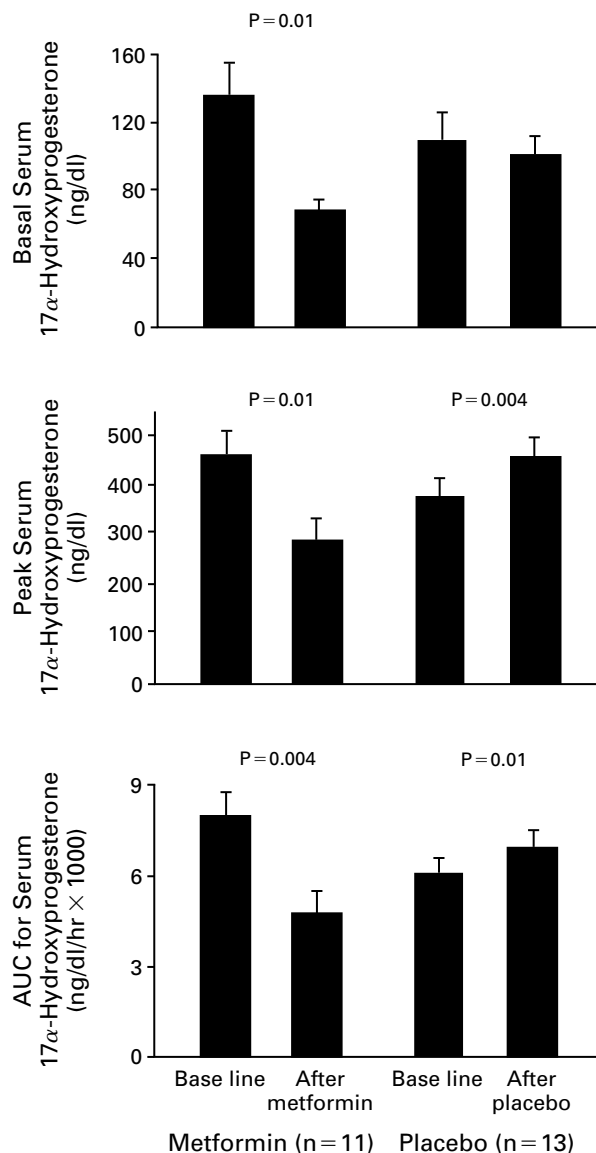


Figure 2. Mean (\pm SE) Serum 17 α -Hydroxyprogesterone Concentrations in Women with the Polycystic Ovary Syndrome at Base Line and after the Administration of Metformin or Placebo for Four to Eight Weeks.

Metformin was administered for a mean (\pm SE) of 42 \pm 4 days, and placebo for 32 \pm 2 days. The women were studied before and after the administration of leuprolide (10 μ g per kilogram). To convert values for 17 α -hydroxyprogesterone to nanomoles per liter, multiply by 0.03. AUC denotes area under the curve.

serum insulin concentration during fasting and the insulin response to oral glucose administration. Concomitantly, ovarian cytochrome P450c17 α activity decreased, as demonstrated by a substantial reduction in the response of serum 17 α -hydroxyprogesterone to the administration of leuprolide (to increase luteinizing hormone secretion). The reduction in P450c17 α activity was accompanied by a decline in

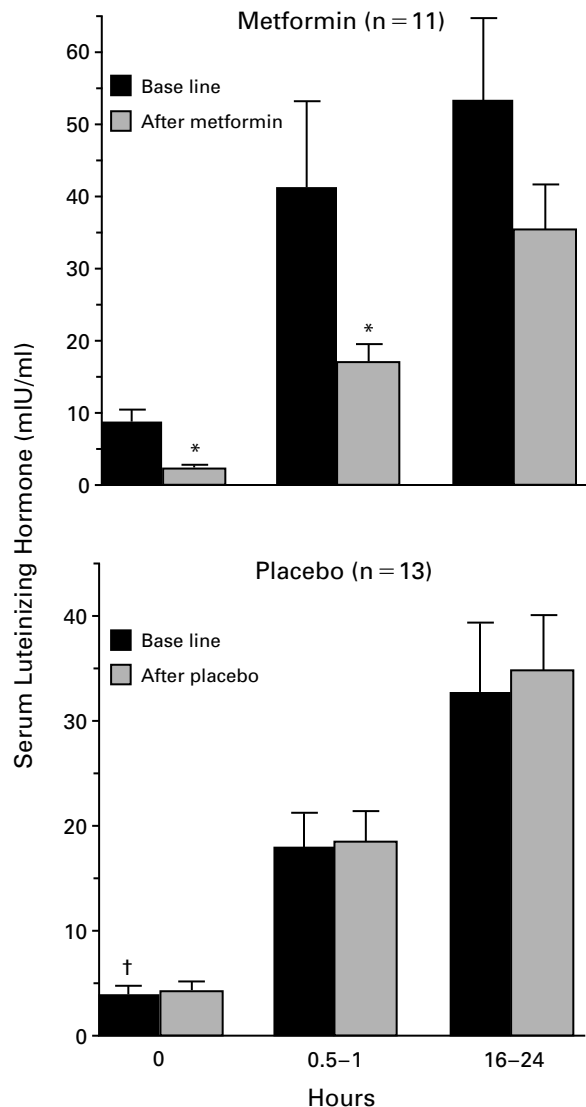


Figure 3. Mean (\pm SE) Serum Luteinizing Hormone Concentrations in Women with the Polycystic Ovary Syndrome at Base Line and after the Administration of Metformin or Placebo for Four to Eight Weeks.

Metformin was administered for a mean (\pm SE) of 42 \pm 4 days, and placebo for 32 \pm 2 days. The women were studied before and after administration of leuprolide (10 μ g per kilogram). The asterisks indicate P=0.01 for the comparison with the base-line value in same group, and the dagger indicates P=0.04 for the comparison with the base-line value in the metformin group.

the serum free testosterone concentration. These findings suggest that increased ovarian cytochrome P450c17 α activity in women with the polycystic ovary syndrome is due to stimulation by insulin (Fig. 1) and can be reversed by reducing the secretion of insulin. We intentionally did not screen the women for the presence of insulin resistance or increased P450c17 α activity so that our results would be ap-

plicable to unselected women with the polycystic ovary syndrome.

We cannot exclude the possibility that the decrease in ovarian P450c17 α activity resulted from the reduction in serum free testosterone or a direct action of metformin, but these possibilities seem remote. Hyperandrogenism is a consequence of increased ovarian P450c17 α activity and is therefore unlikely to be the cause of the stimulated enzyme activity. Hyperandrogenism in women with the polycystic ovary syndrome is ameliorated by diazoxide¹⁵ — a drug structurally unrelated to metformin that suppresses insulin release and worsens glucose tolerance — and by diet.^{19,20} The common factor among these diverse therapies appears to be the reduction in serum insulin concentrations. Because diazoxide is not known to alter insulin sensitivity yet lowers serum testosterone concentrations in women with the polycystic ovary syndrome,¹⁵ hyperandrogenism in such women appears to be related to hyperinsulinemia itself and not to insulin resistance; moreover, insulin stimulates ovarian androgen production *in vitro*.¹¹⁻¹⁴ The recent report by Moghetti et al.³⁰ that hyperinsulinemia may stimulate cytochrome P450c17 α activity in another steroidogenic tissue of women with the polycystic ovary syndrome — namely, the adrenal glands — further supports our findings.

The early and late serum luteinizing hormone responses to leuprolide after the administration of placebo were almost identical to those at base line. In contrast, the administration of metformin was associated with decreased basal and leuprolide-stimulated serum luteinizing hormone concentrations. These observations raise the possibility that insulin enhances both the endogenous (basal) and the exogenous (leuprolide-stimulated) release of luteinizing hormone mediated by gonadotropin-releasing hormone and that increased ovarian cytochrome P450c17 α activity in women with the polycystic ovary syndrome may be related to an insulin-induced abnormality in the dynamics of gonadotropin secretion rather than (wholly or partially) to direct stimulation of ovarian steroidogenesis by insulin (Fig. 1). Insulin receptors have been identified in human pituitary tissue,³¹ and insulin augments the release of luteinizing hormone by cultured rat pituitary cells.³²

The secretion of luteinizing hormone is often increased in women with the polycystic ovary syndrome,³³ and the diurnal changes in the serum concentrations of luteinizing hormone and insulin in these women follow a similar time course.³⁴ Preliminary studies suggest that insulin enhances the amplitude of serum luteinizing hormone pulses but not their frequency in obese women with the polycystic ovary syndrome (unpublished data). An alternative possibility is that the reduction in luteinizing hormone secretion in the women we studied was due to

a decrease in the concentration of circulating androgens. However, raising serum androgen concentrations by parenteral administration in normal women³⁵ or women with the polycystic ovary syndrome³⁶ does not stimulate the secretion of luteinizing hormone. Finally, some of the women in our study who received metformin ovulated, and ovulation itself may influence the dynamics of gonadotropin secretion.³⁷ However, in our study the results in the women who had ovulated and those who had not were similar.

The metformin-induced reduction in insulin secretion was associated with substantial decreases in serum free testosterone concentrations and increases in serum sex hormone-binding globulin concentrations. In women with the polycystic ovary syndrome, insulin stimulates ovarian androgen production¹¹⁻¹⁵ and lowers serum sex hormone-binding globulin concentrations.^{16,17} Our findings, and those of an uncontrolled trial¹⁸ of metformin in women with the polycystic ovary syndrome, support these observations. In contrast, Crave et al. found that neither serum testosterone nor sex hormone-binding globulin concentrations changed in women with the polycystic ovary syndrome who were treated with a hypocaloric diet and metformin for four months.³⁸ The reasons for the discrepancies among these studies are unknown.

In summary, our findings suggest that two features of the polycystic ovary syndrome — hyperinsulinemic insulin resistance and increased ovarian cytochrome P450c17 α activity — are pathogenetically linked, and that hyperinsulinemia stimulates this enzyme either directly or indirectly by increasing gonadotropin secretion (Fig. 1). The ability of insulin to stimulate ovarian cytochrome P450c17 α is probably limited to women with the polycystic ovary syndrome and may be a heritable abnormality, since many other obese women who also are hyperinsulinemic have neither hyperandrogenism nor hyperresponsiveness to gonadotropin-releasing hormone.²² The clinical implication of these results is that therapeutic measures directed at lowering insulin secretion in women with the polycystic ovary syndrome should ameliorate their hyperandrogenism.

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