FERTILITY AND STERILITY®
VOL. 78, NO. 6, DECEMBER 2002

Published by Elsevier Science Inc.
Printed on acid-free paper in U.S.A.

Insulin-lowering treatment reduces aromatase activity in response to follicle-stimulating hormone in women with polycystic ovary syndrome

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Objective: To investigate the effects of reduction of insulin resistance and hyperinsulinemia associated with the polycystic ovary syndrome (PCOS) on FSH-stimulated ovarian aromatase activity.

Design: Prospective study.

Setting: Academic health center, Siena, Italy.

Patient(s): Twenty women 18 to 26 years of age in whom PCOS was diagnosed on the basis of oligomenorrhea or amenorrhea and hyperandrogenemia.

Intervention(s): Recombinant FSH was administered. The next day, therapy with metformin (500 mg t.i.d.) was begun. After 35 to 40 days of treatment, the pretreatment protocol was repeated.

Main Outcome Measure(s): Plasma levels of estradiol (E_2), androstenedione (A), and testosterone (T). The ratios of basal levels and areas under the curve (AUCs) of products and substrates were compared before and after metformin administration to detect differences in aromatase activity.

Result(s): Metformin treatment was associated with significant reduction in basal free testosterone plasma levels, insulin plasma levels, and insulin response to oral glucose tolerance testing. Administration of FSH was followed by a significantly lesser E_2 response after metformin therapy than before this therapy. The ratios of AUC_{E2} to AUC_A and to AUC_T, indicative of aromatase activity in response to FSH, were significantly lower after metformin therapy than before.

Conclusion(s): Metformin therapy in women with PCOS is associated with a reduction in aromatase activity in response to FSH. Insulin affects production of both androgen and estrogen. Insulin therefore plays a central role in regulating the activity of thecal and granulosa cells. (Fertil Steril® 2002;78:1234–9. ©2002 by American Society for Reproductive Medicine.)

Key Words: Aromatase, insulin, metformin, polycystic ovary syndrome, steroids

Received September 25, 2001; revised and accepted April 14, 2002.

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0015-0282/02/$22.00
PII S0015-0282(02)04346-7

The polycystic ovary syndrome (PCOS) is a common endocrine disorder whose pathogenesis is still unclear. In the past 20 years, research into the causes of PCOS has focused on the hypothalamopituitary axis (1), the ovaries (2), the adrenals (3), and insulin resistance (4).

Insulin resistance seems to be a central element in the pathogenesis of PCOS. Hyperinsulinemia may explain the high plasma levels of LH and the altered ovarian and adrenal androgen synthesis typical of this disorder. This hypothesis is supported by the demonstration that insulin-sensitizing drugs reduce plasma levels of LH as well as synthesis of ovarian (5) and adrenal (6) androgens.

Insulin is proposed to directly stimulate activity of cytochrome P450c17, an enzyme involved in ovarian androgen synthesis that is found in thecal cells (5, 7, 8). Hyperinsulinemia may therefore be the cause of hyperandrogenism associated with PCOS through hyperstimulation of androgen synthesis.

Another key enzyme in ovarian steroidogenesis is aromatase, which is found in granulosa cells and participates in conversion of androgens to estrogens (9). Most studies have concluded that aromatase is present at higher functional levels in women with PCOS than in healthy women. This may be why hyperestrogenism is often observed in patients with PCOS (10–12).

Insulin stimulates aromatase activity and increases the effects of FSH (13). When cultured granulosa cells were treated with insulin, the conversion of testosterone to estradiol was stimulated by 60% to 70% of that elicited by FSH under the same conditions. Combined treatment with FSH and insulin produced an E_2 level that equaled the amount produced by each hormone alone (13). These observations suggest that in PCOS, hyperinsulinemia may cause the overproduction of E_2 in response to FSH observed in vivo (14, 15) and in cultured granulosa cells from patients with PCOS (16).

We sought to evaluate the effect of the reduction of insulin resistance and hyperinsulinemia associated with PCOS on FSH stimulated ovarian aromatase activity.

MATERIALS AND METHODS

We recruited 20 women 18 to 26 years of age (mean age [± SD], 22 ± 4 years). In all women, PCOS was diagnosed on the basis of oligomenorrhea (fewer than six menstrual periods in the previous year) (n = 16) or amenorrhea (n = 4) and concurrent hyperandrogenemia.

Fourteen women had a body mass index greater than 27 kg/m^2. Eighty-three percent of patients had a Ferriman–Gallwey score greater than 7. None of the women had hypothyroidism, hyperthyroidism, the Cushing syndrome, adrenal hyperplasia, or hyperprolactinemia. All patients had transvaginal ultrasonographic evidence of polycystic ovaries. Ultrasonographic diagnosis of polycystic ovaries was based on the presence of 10 or more follicles 2 to 10 mm in diameter in both ovaries. No patient was taking drugs or had any significant findings on pathologic examination.

All women had fasting insulin levels greater than 10 μU/mL and accumulated insulin levels (area under the insulin curve during a 2-hour, 75 g, oral glucose test [$AUC_{insulin}$]) greater than 8,000 μU/mL. No woman had type 2 diabetes mellitus.

The study was approved by the institutional review board of the University of Siena. All patients gave written informed consent.

Study Protocol

The women were studied in follicular phase of the menstrual cycle (days 5 to 7), as determined by a plasma progesterone concentration less than 2 ng/mL. All patients were studied at 8 A.M. after overnight bed rest and fasting.

Recombinant FSH (Gonal-F, 75 U; Serono, Rome, Italy) was administered s.c., and blood samples were obtained 0, 4, 8, 12, 16, 20, and 24 hours thereafter for measurement of plasma levels of estradiol (E_2), androstenedione (A) and testosterone (T). The next day, therapy with metformin (Metforal; Guidotti, Pisa, Italy) was begun (500 mg t.i.d.). After 35 to 40 days of therapy, the pretreatment protocol was repeated.

Spontaneous ovulation was checked by analyzing blood samples obtained on days 20, 27, and 34 of metformin administration. Five women had serum progesterone values in the postovulatory range and were excluded from the study.

Assays

Plasma concentrations of LH, FSH, E_2, P, T, free T, and A were measured by performing double-antibody recombinant immunoassay: Radim (Rome, Italy) kits for LH, FSH and A, DSL, Webster (Texas) kits for free T, and Sorin (Saluggia, Italy) kits for T, P, and E_2. The samples were assayed in duplicate at two dilutions. All samples from each participant were assayed together. Quality control pools at low, normal and high LH, FSH, E_2, P, A, T and free testosterone concentrations were present in each assay.

The detection limits of the assays were 0.20 mIU/mL for LH, 0.18 mIU/mL for FSH, 5 pg/mL (18 pmol/L) for E_2, 50 pg/mL (0.16 nmol/L) for P, 30 pg/mL for A, 80 pg/mL for T, and 0.15 pg/mL for free testosterone. Intraassay and interassay variations were 7.8% and 8.2% for LH, 6.2% and 6.5% for FSH, 4.2% and 4.9% for E_2, 8.5% and 10.8% for P, 5.6% and 6.4% for A, 3.4% and 4.6% for T, and 3.2% and 3.4% for free T, respectively.

Statistical Analysis

After assessing the normal distribution of the data, the response before and after metformin therapy, basal hormone concentrations, peak response, and areas under the curve (AUC) (cumulative increase above baseline) were compared using analysis of variance and the Student two-tailed *t*-test. Areas under the curve (AUC) were calculated by using the trapezium method and are expressed as μU/mL/time and pg/mL/time as appropriate. Ratios of basal levels (time 0) and AUC of products to substrates before and after metformin administration were compared for differences in aromatase activity (E_2:A and E_2:T for basal aromatase activity and AUC_{E2}:AUC_A and AUC_{E2}:AUC_T for FSH-stimulated aromatase activity). $P<.05$ was considered statistically significant.

RESULTS

Complete results were obtained for 15 of 20 women; 5 women ovulated during metformin therapy and were excluded. Metformin therapy was followed by a significant decrease in plasma concentrations of free testosterone (12.9 ± 1.9 pg/mL vs. 10.2 ± 1.9 pg/mL; $P<.05$), fasting insulin (16.2 ± 3.3 vs. 11.3 ± 2.6 μU/mL; $P<.05$), and $AUC_{insulin}$ (11,310 ± 2,509 vs. 6,520 ± 1,308 μU/mL/time; $P<.05$). Levels of other sex steroids, fasting glucose concentrations, and body mass index did not change significantly (Table 1).

Administration of FSH was followed by a lower E_2 response after metformin therapy than before therapy. Both the E_2 peak and AUC were significantly smaller after therapy (112 ± 21 pg/mL vs. 85 ± 18 pg/mL [$P<.05$] and 350 ± 80 pg/mL/time

TABLE 1

Clinical data and hormone profiles of 15 women with PCOS before and after metformin treatment.

Characteristic	Normal values	Basal values (n = 15)	After metformin therapy (n = 15)
Age (y)		22 ± 4	
Body mass index (kg/m^2)	<25	29.2 ± 3.2 (80)	29.1 ± 3.3 (80)
LH level (mIU/mL)	1.1–11.6	9.8 ± 1.7 (45)	10.1 ± 1.9 (47)
FSH level (mIU/mL)	3–12	5.7 ± 0.9 (0)	5.9 ± 1.1 (0)
E_2 level (pg/mL)	20–100	80.7 ± 18.7 (14)	70.6 ± 15 (16)
Free T level (pg/mL)	0.6–10	12.9 ± 1.9 (42)	10.2 ± 1.9 (27)[a]
T level (pg/mL)	100–500	320 ± 49 (18)	294 ± 51 (12)
A level (pg/mL)	210–3,100	1,500 ± 580 (37)	1,350 ± 420 (31)
Fasting glucose level (mg/dL)	<110	82.1 ± 3.5 (8)	77 ± 2.5 (8)
Fasting insulin level (μU/mL)	<10	16.2 ± 3.3 (100)	11.3 ± 2.6 (89)[a]
$AUC_{insulin}$ (μU/mL/time)	<8,000	11,310 ± 2,509 (100)	6,520 ± 1,308 (79)[a]

Note: Values are means ± SD. Numbers in parentheses are the percentage of patients with abnormal values. To convert LH values and FSH values to U/L, multiply by 1; to convert E_2 values to pmol/L, multiply by 3.67; to convert free and total T values to pmol/L, multiply by 3.47; to convert A values to pmol/L, multiply by 3.49; to convert glucose values to mmol/L, multiply by 0.056; to convert insulin values to pmol/L, multiply by 6.

[a] $P<.05$ vs. basal values.

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vs. 220 ± 60 pg/mL/time [$P<.05$] respectively) (Fig. 1). No significant correlations were observed between reduced estradiol production and body mass index. A significant positive correlation was found between the reduction in $AUC_{insulin}$ after oral glucose tolerance testing and the reduction in estradiol response to FSH (Fig. 2). The responses of A and T to FSH (both peak and AUC) did not significantly differ after metformin therapy.

Aromatase activity was evaluated by the ratios E_2:A (time 0) and E_2:T (time 0), which did not significantly differ after metformin therapy (Table 2). However the ratios of AUC_{E2} to AUC_A and AUC_{E2} to AUC_T, which indicate aromatase activity after FSH, were significantly lower (Table 2), with mean reductions of 48% and 36%, respectively.

DISCUSSION

Several recent, mostly uncontrolled studies have assessed the effects of metformin on ovarian steroidogenesis, with varying and contrasting results (5, 8, 17–22). In general, metformin has been demonstrated to improve insulin sensitivity, decrease androgen levels, and improve frequency of menstrual cycle and ovulation.

In our study, 25% of patients ovulated after only 35 to 40 days of metformin treatment. Discrepancies in the reduction

FIGURE 1

Response of E_2 to FSH administration before (*circles*) and after (*squares*) metformin therapy in women with the polycystic ovary syndrome. AUC = area under the curve.

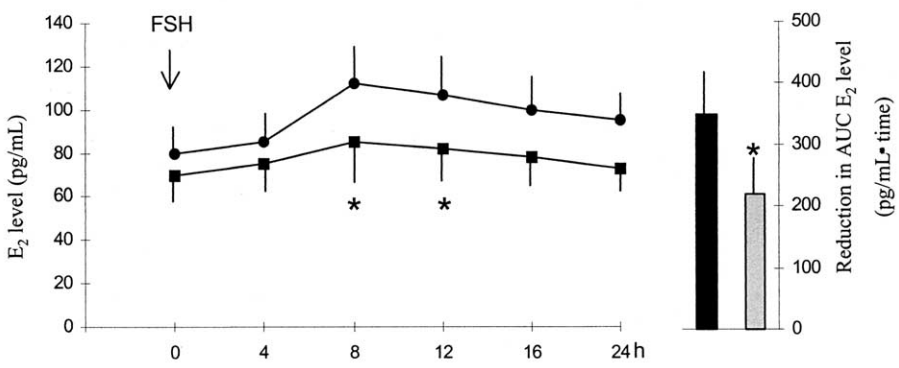

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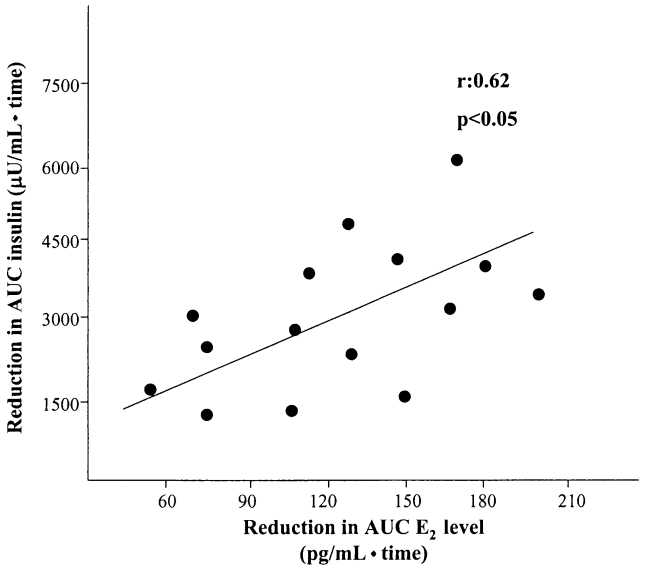
FIGURE 2

Insulin resistance and E_2 response to FSH administration after metformin therapy. A significant positive correlation was found between the reduction in insulin resistance and the reduction in E_2 response. AUC = area under the curve.



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in androgen plasma levels after metformin therapy are probably due to differences in study samples, drug dosages, and duration of therapy. A recent randomized, double-blind, placebo-controlled trial (23) showed that metformin administration was associated with significant reduction in basal plasma levels of free testosterone but no significant changes in DHEAS and androstenedione levels, which agrees with our results.

The polycystic ovary produces more androgens than the normal ovary, owing to dysregulation of thecal cell cytP450 (24). Monolayer cultures of dispersed thecal interstitial tissue from women with PCOS produce considerably more androstenedione per cell than does tissue from normal ovaries (25). Theca cells from PCOS express increased side-chain cleavage enzyme and 17α-hydroxylase/17,20 desmolase messenger RNA and increased 17α-hydroxylase/17,20 desmolase, 3β-hydroxysteroid dehydrogenase, and 17β-hydroxysteroid dehydrogenase enzyme activity. Consequently, increased production of T, progesterone, and 17-hydroxyprogesterone are intrinsic properties of PCOS theca cells (26). Besides altered theca cell steroidogenesis, sufficient evidence suggests increased aromatase activity in granulosa cells from women with PCOS (10, 11, 16). Women with PCOS are reported to have a significantly higher response of E_2 to a single dose of GnRH agonist than do healthy women (12).

TABLE 2

Ratios of product (E_2) to substrates (A and T) at time 0 and in response to FSH before and after metformin treatment.

Time point	E_2:$A_{(T0)}$	AUC_{E2}:AUC_A	E_2:$T_{(T0)}$	AUC_{E2}:AUC_T
Before therapy	0.05 ± 0.01	0.44 ± 0.08	0.25 ± 0.03	1.1 ± 0.2
After therapy	0.05 ± 0.01	0.23 ± 0.05[a]	0.24 ± 0.03	0.7 ± 0.15[a]

Note: AUC = area under the curve.

[a] $P<.05$ vs. before therapy.

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In the polycystic ovary, thecal cells synthesize more androgens than normal and granulosa cells have higher-than-normal aromatase activity, which converts a large quantity of substrate into estrogens. The high aromatase activity explains recent observations of normal quantities of androgens in follicular fluid of polycystic ovaries (25).

It was recently demonstrated that the reduction in hyperinsulinemia induced by metformin is associated with a reduction in the response of 17-hydroxyprogesterone to GnRH (5) and hCG (8). This finding suggests that insulin stimulates ovarian P450c17.

Our results demonstrate that metformin therapy in women with PCOS is associated with a reduction in aromatase activity in response to FSH and that insulin affects not only androgen production but also estrogen production. Insulin therefore plays a central role in regulating the activity of the two ovarian compartments: theca cells and granulosa cells.

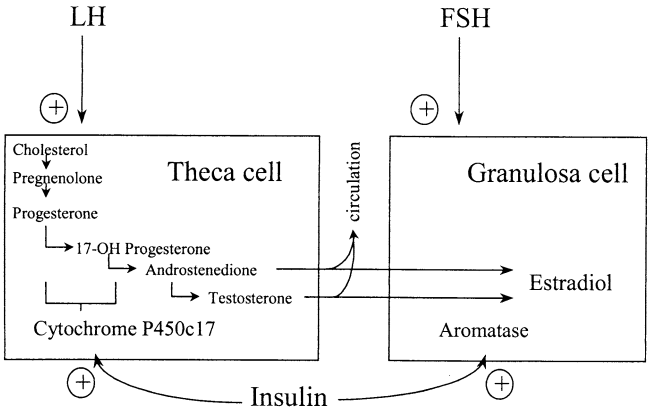
Vribikova et al. first observed modifications in ovarian steroidogenesis after metformin therapy (27). They evaluated ovarian enzyme activities in response to GnRH before and after metformin administration and found a significant decrease in 3β-hydroxysteroid dehydrogenase, 17-20 lyase in 17β-hydroxysteroid dehydrogenase, and aromatase.

Granulosa cells from normal and polycystic ovaries respond to physiologic doses of insulin with an increase in E_2 production and FSH-induced E_2 production (28). This effect is not due to an increase in the number of cells in culture (13) but rather seems to result from direct stimulation of aromatase by insulin. This stimulation may be direct or mediated by receptors of insulin-like growth factors. It has been shown that physiological doses of insulinlike growth factor-1 strongly stimulate E_2 production and are as effective or more effective than FSH in doing so (29).

In a recent study, we demonstrated that metformin therapy is associated with a significant increase in plasma concentrations of insulinlike growth factor binding protein-1 and therefore with a reduction in the ratio of insulinlike growth factor-1 to insulinlike growth factor binding protein-1, indicating a reduction in levels of free insulinlike

FIGURE 3

Role of insulin in synthesis of ovarian steroids. Luteinizing hormone stimulates androgen formation in thecal cells. Follicle-stimulating hormone stimulates biosynthesis of E_2 from androgen by granulosa cells. Insulin enhances cytochrome P450c17 and aromatase activity, leading to increased androgen levels in circulation and increased conversion to E_2 in granulosa cells.



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growth factor-1 (22). Hence, the reduction in hyperinsulinemia and plasma concentrations of free IGF-1 may explain the reduction in FSH-stimulated aromatase activity after metformin therapy.

A specific feature of women with PCOS is increased ovarian sensitivity to exogenous gonadotropins. The response of the polycystic ovary to gonadotropin stimulation is associated with a significant risk for ovarian hyperstimulation and cysts (30). It was shown that during ovarian stimulation, E_2 production and the E_2-to-A ratio are higher in patients with PCOS who have elevated insulin levels than in normoinsulinemic women (14). Increased insulin levels thus involve greater ovarian endocrine and morphologic responses to FSH-induced ovulation, which predispose to the ovarian hyperstimulation syndrome. It therefore seems that the typical response of the polycystic ovary to exogenous gonadotropin therapy is related to increased plasma concentrations of insulin.

In confirmation of this hypothesis, our group recently demonstrated that metformin therapy reduces the number of follicles larger than 15 mm in diameter on the day of hCG administration and decreases the percentage of cycles with hCG that are withheld because of excessive follicular development when exogenous FSH is administered in patients with PCOS. Pretreatment with metformin decreased the number of follicles larger than 15 mm in diameter in controlled ovarian hyperstimulation cycles, cycles in which hCG was withheld, and E_2 levels on the day of hCG administration. These findings indicate a lower incidence of ovarian hyperstimulation and support the hypothesis that insulin plays a role in the endocrine and paracrine control of the ovaries (15).

Hyperinsulinemia has been increasingly shown to have a central role in the complex pathogenesis of PCOS. Insulin stimulates steroidogenesis in thecal cells and conversion of androgens to estrogens in granulosa cells (Fig. 3). Hyperinsulinemia increases estrogen synthesis and the sensitivity of the granulosa cells to FSH and LH (28). This, together with high levels of LH in PCOS, may lead to arrested differentiation of granulosa cells and, thus, ovulation failure. This observation supports the hypothesis that hyperinsulinemia is a central element in the pathogenesis of PCOS.

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