EFFECTS OF METFORMIN ON SPONTANEOUS AND CLOMIPHENE-INDUCED OVULATION IN THE POLYCYSTIC OVARY SYNDROME

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ABSTRACT

Background Obese women with the polycystic ovary syndrome are relatively unresponsive to the induction of ovulation by clomiphene. We hypothesized that reducing insulin secretion by administering metformin would increase the ovulatory response to clomiphene.

Methods We performed oral glucose-tolerance tests before and after the administration of 500 mg of metformin or placebo three times daily for 35 days in 61 obese women with the polycystic ovary syndrome. Women who did not ovulate spontaneously were then given 50 mg of clomiphene daily for five days while continuing to take metformin or placebo. Serum progesterone was measured on days 14, 28, 35, 44, and 53, and ovulation was presumed to have occurred if the concentration exceeded 8 ng per milliliter (26 nmol per liter) on any of these days.

Results Twenty-one women in the metformin group and 25 women in the placebo group were given clomiphene because they did not ovulate spontaneously during the first phase of the study. Among the 21 women given metformin plus clomiphene, the mean (±SE) area under the serum insulin curve after oral glucose administration decreased from 6745±2021 to 3479±455 µU per milliliter per minute (40.5±12.1 to 20.9±2.7 nmol per liter per minute, P=0.03), but it did not change significantly in the 25 women given placebo plus clomiphene. Nineteen of the 21 women (90 percent) who received metformin plus clomiphene ovulated (mean peak serum progesterone concentration, 23.8±3.4 ng per milliliter [7.6±10.9 nmol per liter]). Two of the 25 women (8 percent) who received placebo plus clomiphene ovulated (P<0.001). Overall, 31 of the 35 women (89 percent) treated with metformin ovulated spontaneously or in response to clomiphene, as compared with 3 of the 26 women (12 percent) treated with placebo.

Conclusions The ovulatory response to clomiphene can be increased in obese women with the polycystic ovary syndrome by decreasing insulin secretion with metformin. (N Engl J Med 1998;338:1876-80.)
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P OLYCYSTIC ovary syndrome, which affects approximately 6 percent of women of reproductive age and is characterized by chronic anovulation and hyperandrogenism,[1] is the most common cause of infertility in women in the United States. Insulin resistance with compensatory hyperinsulinemia is a prominent feature of the syndrome[2-5] and appears to have a pathophysiologic role in the hyperandrogenism of the disorder. Ovarian androgen production and serum free testosterone concentrations decrease in women with polycystic ovary syndrome when insulin secretion is reduced by drugs such as diazoxide,[6] metformin,[7-10] and troglitazone.[11,12] However, whether such therapy improves ovulatory function is not known.

Clomiphene citrate, an antiestrogenic drug, is the primary therapy used for ovulation induction in women with the polycystic ovary syndrome.[13-15] However, obese women with the syndrome often require multiple courses and high doses of clomiphene, and there is a positive correlation between obesity and the dose of clomiphene required to induce ovulation.[15] Since increasing obesity is associated with increasing hyperinsulinemia,[16] the high degree of hyperinsulinemia in obese women with the polycystic ovary syndrome may account for their poor responsiveness to clomiphene.[13] Hyperinsulinemia could adversely affect folliculogenesis and ovulation by increasing intraovarian androgen production,[6,8,17-19] altering gonadotropin secretion,[8,9,20-22] or directly affecting follicular development.

The aim of this study was to determine whether reducing hyperinsulinemia with metformin would increase the ovulatory response to clomiphene in obese women with the polycystic ovary syndrome.

METHODS

Subjects

We studied 61 obese women (body-mass index [the weight in kilograms divided by the square of the height in meters], >28) with polycystic ovary syndrome in the United States, Venezuela, and Italy. All had oligomenorrhea (fewer than six menstrual periods in the preceding year) and hyperandrogenemia (elevated serum free testosterone concentrations, determined at local clinics), and none had diabetes mellitus. All had normal serum prolactin concentrations and normal thyroid-function tests. Late-onset adrenal hyperplasia was ruled out by the finding of a morning serum 17α-hydroxyprogesterone concentration below 200 ng per deciliter (6 nmol per liter). All the women had findings on ultrasonography of the ovaries consistent with the diagnosis of the polycystic ovary syndrome.[23] Thirty-one women had received clomiphene previously, but none had taken it or any other medica-

tion for at least two months before the study. The study was approved by the institutional review board at each study site, and each woman gave informed consent.

Experimental Protocol

At the time of entry into the study, all the women were in the equivalent of the follicular phase of the menstrual cycle, as documented by a serum progesterone concentration below 2 ng per milliliter (6.4 nmol per liter), and their last episode of menstrual bleeding had been at least two months earlier.

On day 0, the women came to the hospital after a 12-hour overnight fast, at which time their weight, height, and waist-to-hip ratio 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, steroid hormones, and sex hormone–binding globulin. At 9 a.m. 75 g of glucose was given orally, and blood samples were collected after 60 and 120 minutes for the determination of serum glucose and insulin concentrations.

Findings from a previous study[8] suggested that metformin might increase spontaneous ovulation. Given that a goal of the study was to have an equal number of women in the metformin and placebo groups during the administration of clomiphene, more women were randomly assigned to receive metformin. Thirty-five women were assigned to take 500 mg of metformin (Glucophage, Bristol-Myers Squibb, Princeton, N.J.; Glafornil, North Medicamenta, Caracas, Venezuela; or Metforal, Laboratori Guidotti, Pisa, Italy) orally three times daily, and the remaining 26 women were assigned to receive placebo. The women were asked to abstain from sexual intercourse or to use a barrier method of contraception during the study.

The women took metformin or placebo alone on days 1 to 34 to allow sufficient time for metformin to exert its putative insulin-sensitizing effects. Serum progesterone was measured on days 14, 28, and 35, and ovulation was presumed to have occurred if the value exceeded 8 ng per milliliter (25.6 nmol per liter) on any of these days. All tests performed at base line (day 0) were repeated on day 35.

The 21 women in the metformin group and the 25 women in the placebo group who did not ovulate and had serum progesterone concentrations below 2 ng per milliliter between days 0 and 35 were given 50 mg of clomiphene citrate (Serophene, Teva Pharmaceuticals, Jerusalem, Israel) daily for five days (days 35 to 39), while continuing to receive metformin or placebo through day 53. Serum progesterone was measured on days 44 and 53 to assess the ovulatory response to clomiphene.

Assays

Blood samples were centrifuged as soon as they were obtained, and the serum was stored at −20°C until assayed. Serum hormones and sex hormone–binding globulin (measured as protein) were assayed as previously described,[6,24,25] except for serum free testosterone, the concentration of which was calculated according to the method of Sodergard et al.[26] and was predicated on the assumption of a constant serum albumin concentration of 4.0 g per deciliter. To avoid variation between assays, all samples from an individual woman were analyzed in duplicate in a single assay for each hormone. The intraassay coefficient of variation was 5.5 percent for the insulin assay and less than 10 percent for all steroid hormone assays.

Statistical Analysis

We analyzed the serum glucose and insulin responses to oral glucose administration by calculating the areas under the response curves by the trapezoidal rule using absolute values. Fisher's exact test was used to analyze the differences in ovulation rates between the metformin and placebo groups. For other variables, we compared results within a group by testing for normality with the Wilk–Shapiro test and then using Student's two-

tailed paired t-test or the Wilcoxon signed-rank test, and we compared results between groups with Student's two-tailed unpaired t-test or the Mann–Whitney rank-sum test.

RESULTS

Treatment with Metformin or Placebo Alone

On entry into the study (day 0), the metformin and placebo groups did not differ with respect to history of clomiphene treatment, anthropometric variables, and biochemical values (Table 1).

In the metformin group, the mean (±SE) area under the serum insulin curve during the oral glucose-tolerance test decreased from 6598±1267 to 3764±317 μU per milliliter per minute (39.6±7.6 to 22.6±1.9 nmol per liter per minute, P=0.002), whereas this value did not change significantly in the placebo group (P=0.20). There was also a small but significant decrease in the waist-to-hip ratio during testing (from 0.89±0.01 to 0.88±0.01, P<0.001) in the metformin group, but not in the placebo group (P=0.60). In neither group was there any

TABLE 1. BASE-LINE CHARACTERISTICS OF WOMEN WITH THE POLYCYSTIC OVARY SYNDROME.*

CHARACTERISTIC	METFORMIN GROUP (N=35)	PLACEBO GROUP (N=26)
Age — yr	29±1	28±1
Previous treatment with clomiphene — no. (%)	18 (51)	13 (50)
Body-mass index	32.3±0.8	32.2±1.0
Waist-to-hip ratio	0.89±0.01	0.90±0.01
Serum insulin during fasting — μU/ml	19±2	22±6
Serum glucose during fasting — mg/dl	78±3	75±2
AUC$_{insulin}$ — μU/ml/min†	6598±1267	6558±1030
AUC$_{glucose}$ — mg/dl/min†	11,277±553	10,914±486
Serum progesterone — ng/ml	0.8±0.1	0.6±0.1
Serum testosterone — ng/dl	70±5	63±5
Serum free testosterone — ng/dl	1.0±0.1	0.8±0.1
Serum androstenedione — ng/dl	232±13	215±18
Serum 17β-estradiol — pg/ml	47±6	56±10
Serum dehydroepiandrosterone sulfate — μg/dl	222±23	211±21
Serum sex hormone–binding globulin — μg/dl	2.0±0.2	2.7±0.4

*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 3.67; 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 area under the curve (AUC) of the responses of serum glucose and insulin to oral glucose administration was calculated.

change in the body-mass index, serum insulin or glucose concentrations during fasting, or the area under the serum glucose curve during the oral glucose-tolerance test.

During the first 35 days of treatment, serum total testosterone concentrations did not change significantly in either the metformin group (P=0.84) or the placebo group (P=0.53). Serum sex hormone–binding globulin concentrations increased in both the metformin group (from 2.0±0.2 to 2.7±0.3 μg per deciliter [69±7 to 93±10 nmol per liter], P=0.01) and the placebo group (2.7±0.4 to 3.6±0.5 μg per deciliter [94±13 to 124±17 nmol per liter], P=0.06). As a result, serum free testosterone concentrations decreased in the metformin group, from 1.0±0.1 to 0.8±0.1 ng per deciliter (347±35 to 278±35 pmol per liter, P=0.07), and in the placebo group, from 0.8±0.1 to 0.6±0.1 ng per deciliter (278±35 to 208±35 pmol per liter, P=0.04).

Twelve of the 35 women (34 percent) in the metformin group ovulated spontaneously during treatment with metformin alone, as compared with only 1 of the 26 women (4 percent) in the placebo group (P<0.001). The mean peak serum progesterone concentration in the 12 women in the metformin group who ovulated was 13.0±1.0 ng per milliliter (41.6±3.2 nmol per liter). Two other women in the metformin group had peak serum progesterone concentrations between 4 and 8 ng per milliliter (12.8 to 25.6 nmol per liter) and were not included in the second phase of the study. Figure 1 shows the number of women who had serum progesterone concentrations above 8 ng per milliliter (indicative of ovulation) on days 14, 28, and 35 in the metformin and placebo groups.

Treatment with Clomiphene and Metformin or Placebo

Twenty-one women in the metformin group and 25 women in the placebo group continued into the second phase of the study, during which they received concurrent clomiphene treatment. The two groups had not differed on study day 0 with respect to the area under the serum insulin curve (6745±2021 μU per milliliter per minute [40.5±12.1 nmol per liter per minute] in the group given metformin and clomiphene, as compared with 6574±1072 μU per milliliter per minute [39.4±6.4 nmol per liter per minute] in the group given placebo and clomiphene; P=0.83), but on day 35, before clomiphene treatment was started, this value was significantly lower in the group given metformin and clomiphene (P=0.03) (Table 2). The difference was due to a 48 percent decrease (P=0.03) in the value in the 21 women in the group given metformin and clomiphene between days 0 and 35, from 6745±2021 to 3479±455 μU per milliliter per minute (40.5±12.1 to 20.9±2.7
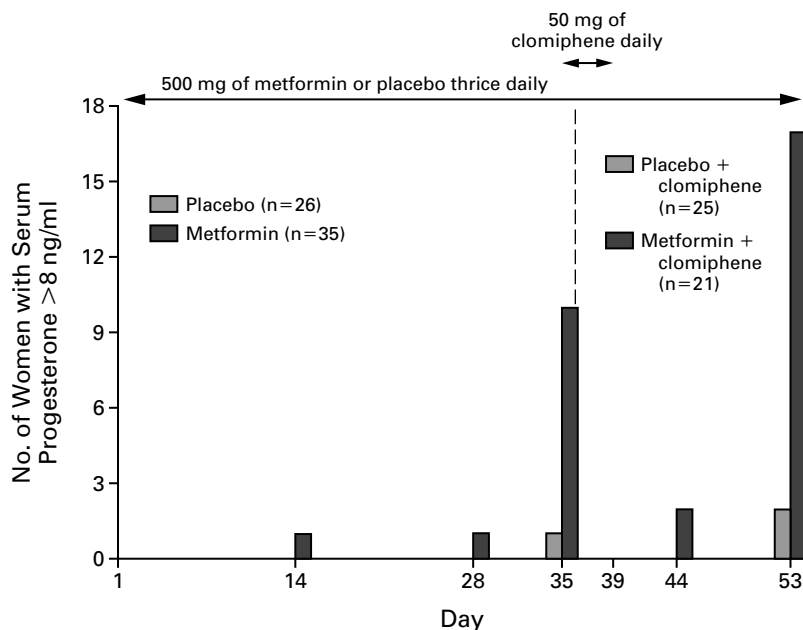
Figure 1. Number of Women with a Serum Progesterone Concentration above 8 ng per Milliliter on Each Day of the Study on Which It Was Measured.

During days 1 to 34 the women were treated with metformin or placebo alone. On day 35, ovulation induction with clomiphene was started while metformin or placebo was continued. Only women whose serum progesterone concentrations were less than 2 ng per milliliter throughout the first phase of the study were included in the ovulation-induction phase of the study.

nmol per liter per minute). On day 35 the two groups did not differ with respect to history of clomiphene treatment, anthropometric variables, or biochemical values (Table 2).

Nineteen of the 21 women (90 percent) who received combined metformin and clomiphene ovulated; the mean peak serum progesterone concentration in these 19 women was 23.8±3.4 ng per milliliter (76.1±10.9 nmol per liter). In contrast, only 2 of the 25 women (8 percent) in the group given placebo and clomiphene ovulated (P<0.001); 2 other women in this group had serum progesterone concentrations between 4 and 8 ng per milliliter. Figure 1 shows the number of women who had serum progesterone concentrations above 8 ng per milliliter on days 44 and 53 in the two groups.

DISCUSSION

We conducted this study to determine whether decreasing insulin secretion in obese women with the polycystic ovary syndrome would facilitate the induction of ovulation by clomiphene. We found that treatment with metformin, but not placebo, significantly decreased the serum insulin response to oral glucose administration. Simultaneously with the reduction in serum insulin, the women given metformin had marked increases in both spontaneous ovulation and clomiphene-induced ovulation, as compared with the women given placebo. Thirty-one of the 35 women treated with metformin (89 percent) ovulated either spontaneously or in response to clomiphene, as compared with only 3 of the 26 women in the placebo group (12 percent). These findings support the idea that hyperinsulinemia impedes ovulation in obese women with the polycystic ovary syndrome and that decreasing insulin secretion facilitates both spontaneous ovulation and the induction of ovulation by clomiphene.

Our findings are consistent with those of previous studies that noted an increased frequency of menstruation or ovulation in women with the polycystic ovary syndrome during treatment with metformin[7-9] or troglitazone.[11,12] However, the specific effect of insulin reduction on ovulation was not assessed in those studies. In a recent study, several previously infertile women with the polycystic ovary syndrome who were treated with metformin for six months became pregnant.[27] However, the study was uncontrolled and was not specifically designed to monitor ovulation, and nearly half the women recruited did not complete the study. None of the studies examined the effect of the reduction in insulin secretion on the induction of ovulation by clomiphene.[7-9,11,12,27]

To assess clomiphene-induced ovulation, we purposefully chose to administer a low daily dose of only 50 mg of clomiphene, because few obese women with the polycystic ovary syndrome would be expected to have a response to this dose.[13,15] For example, in a study of women with the polycystic ovary syndrome who weighed more than 91 kg (200 lb), 50 mg of clomiphene daily for five days resulted in an ovulatory rate of only 20 percent.[15] The rate of clomiphene-induced ovulation was lower in our placebo group, most likely because half the women had not had a response to previous clomiphene therapy.

We chose to use metformin because several previous studies had documented its ability to decrease serum insulin in women with the polycystic ovary syndrome.[7-10,28] It is classified as a category B drug, which means that no teratogenic effects have been demonstrated in vitro. It is commonly given to premenopausal women with type II diabetes mellitus and simply discontinued when they become pregnant. Metformin was administered to a limited number of South African women with diabetes throughout their pregnancies,[28] and no teratogenic effects

TABLE 2. CHARACTERISTICS OF WOMEN WITH THE POLYCYSTIC OVARY SYNDROME ON INITIATION OF OVULATION INDUCTION WITH CLOMIPHENE AFTER THE ADMINISTRATION OF METFORMIN OR PLACEBO FOR 35 DAYS.*

CHARACTERISTIC	METFORMIN PLUS CLOMIPHENE (N=21)	PLACEBO PLUS CLOMIPHENE (N=25)
Age — yr	30±1	28±1
Previous treatment with clomiphene — no. (%)	11 (52)	13 (52)
Body-mass index	34.0±1.0	32.2±1.1
Waist-to-hip ratio	0.88±0.01	0.90±0.01
Serum insulin during fasting — μU/ml	14±2	13±3
Serum glucose during fasting — mg/dl	81±3	76±2
AUC$_{insulin}$ — μU/ml/min†	3479±455‡	5100±55
AUC$_{glucose}$ — mg/dl/min†	11,089±587	10,839±500
Serum progesterone — ng/ml	0.8±0.1	0.6±0.1
Serum testosterone — ng/dl	66±5	59±6
Serum free testosterone — ng/dl	0.8±0.1	0.6±0.1
Serum androstenedione — ng/dl	210±18	200±20
Serum 17β-estradiol — pg/ml	68±13	54±9
Serum dehydroepiandrosterone sulfate — μg/dl	201±20	155±14
Serum sex hormone–binding globulin — μg/dl	2.7±0.4	3.6±0.5

*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 3.67; 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 area under the curve (AUC) of the responses of serum glucose and insulin to oral glucose administration was calculated.

‡P=0.03 for the comparison with the group given placebo plus clomiphene.

were reported. It is not known to influence hypothalamic, pituitary, or ovarian function independently, but this possibility cannot be ruled out.

There are two main limitations to our study. First, pregnancy was not an outcome measure, and we do not know whether the increased frequency of ovulation would be accompanied by an increased pregnancy rate or birth rate. Second, we do not know whether the improved ovulatory function in the women given metformin was due to a decrease in intraovarian androgen production, normalization of spontaneous or clomiphene-induced gonadotropin secretion, diminution of the potential direct effects of insulin on ovarian folliculogenesis, or a combination of these processes.

In summary, the frequency of spontaneous ovulation and ovulation induced by clomiphene can be increased in obese women with the polycystic ovary syndrome by decreasing serum insulin concentrations with metformin.

REFERENCES

1. Franks S. Polycystic ovary syndrome. N Engl J Med 1995;333:853-61. [Erratum, N Engl J Med 1995;333:1435.]
2. Dunaif A, Segal KR, Futterweit W, Dobrjansky A. Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. Diabetes 1989;38:1165-74.
3. Carmina E, Koyama T, Chang L, Stanczyk FZ, Lobo RA. Does ethnicity influence the prevalence of adrenal hyperandrogenism and insulin resistance in polycystic ovary syndrome? Am J Obstet Gynecol 1992;167:1807-12.
4. Ehrmann DA, Sturis J, Byrne MM, Karrison T, Rosenfield RL, Polonsky KS. Insulin secretory defects in polycystic ovary syndrome: relationship to insulin sensitivity and family history of non-insulin-dependent diabetes mellitus. J Clin Invest 1995;96:520-7.
5. Apter D, Butzow T, Laughlin GA, Yen SS. Metabolic features of polycystic ovary syndrome are found in adolescent girls with hyperandrogenism. J Clin Endocrinol Metab 1995;80:2966-73.
6. Nestler JE, Barlascini CO, Matt DW, et al. Suppression of serum insulin by diazoxide reduces serum testosterone levels in obese women with polycystic ovary syndrome. J Clin Endocrinol Metab 1989;68:1027-32.
7. Velazquez EM, Mendoza S, Hamer T, Sosa F, Glueck CJ. Metformin therapy in polycystic ovary syndrome reduces hyperinsulinemia, insulin resistance, hyperandrogenemia, and systolic blood pressure, while facilitating normal menses and pregnancy. Metabolism 1994;43:647-54.
8. Nestler JE, Jakubowicz DJ. Decreases in ovarian cytochrome P450c17α activity and serum free testosterone after reduction in insulin secretion in polycystic ovary syndrome. N Engl J Med 1996;335:617-23.
9. *Idem.* Lean women with polycystic ovary syndrome respond to insulin reduction with decreases in ovarian P450c17α activity and serum androgens. J Clin Endocrinol Metab 1997;82:4075-9.
10. Velazquez EM, Mendoza SG, Wang P, Glueck CJ. Metformin therapy is associated with a decrease in plasma plasminogen activator inhibitor-1, lipoprotein(a), and immunoreactive insulin levels in patients with the polycystic ovary syndrome. Metabolism 1997;46:454-7.
11. Ehrmann DA, Schneider DJ, Sobel BE, et al. Troglitazone improves defects in insulin action, insulin secretion, ovarian steroidogenesis, and fibrinolysis in women with polycystic ovary syndrome. J Clin Endocrinol Metab 1997;82:2108-16.
12. Dunaif A, Scott D, Finegood D, Quintana B, Whitcomb R. The insulin-sensitizing agent troglitazone improves metabolic and reproductive abnormalities in the polycystic ovary syndrome. J Clin Endocrinol Metab 1996;81:3299-306.
13. Shepard MK, Balmaceda JP, Leija CG. Relationship of weight to successful induction of ovulation with clomiphene citrate. Fertil Steril 1979;32:641-5.
14. O'Herlihy C, Pepperell RJ, Brown JB, Smith MA, Sandri L, McBain JC. Incremental clomiphene therapy: a new method for treating persistent anovulation. Obstet Gynecol 1981;58:535-42.
15. Lobo RA, Gysler M, March CM, Goebelsmann U, Mishell DR Jr. Clinical and laboratory predictors of clomiphene response. Fertil Steril 1982;37:168-74.
16. DeFronzo RA, Ferrannini E. Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 1991;14:173-94.
17. Barbieri RL, Makris A, Randall RW, Daniels G, Kistner RW, Ryan KJ. Insulin stimulates androgen accumulation in incubations of ovarian stroma obtained from women with hyperandrogenism. J Clin Endocrinol Metab 1986;62:904-10.
18. Bergh C, Carlsson B, Olsson JH, Selleskog U, Hillensjo T. Regulation of androgen production in cultured human thecal cells by insulin-like growth factor I and insulin. Fertil Steril 1993;59:323-31.
19. Nahum R, Thong KJ, Hillier SG. Metabolic regulation of androgen production by human thecal cells in vitro. Hum Reprod 1995;10:75-81.
20. Prelevic GM, Wurzburger MI, Balint-Peric L. LH pulsatility and response to a single s.c. injection of buserelin in polycystic ovary syndrome. Gynecol Endocrinol 1990;4:1-13.
21. Adashi EY, Hsueh AJW, Yen SSC. Insulin enhancement of luteinizing hormone and follicle-stimulating hormone release by cultured pituitary cells. Endocrinology 1981;108:1441-9.
22. Yen SS, Laughlin GA, Morales AJ. Interface between extra- and intraovarian factors in polycystic ovarian syndrome. Ann N Y Acad Sci 1993;687:98-111.
23. Yeh HC, Futterweit W, Thornton JC. Polycystic ovarian disease: US features in 104 patients. Radiology 1987;163:111-6.
24. Nestler JE, Powers LP, Matt DW, et al. A direct effect of hyperinsulinemia on serum sex hormone-binding globulin levels in obese women with the polycystic ovary syndrome. J Clin Endocrinol Metab 1991;72:83-9.
25. Nestler JE, Beer NA, Jakubowicz DJ, Beer RM. Effects of a reduction in circulating insulin by metformin on serum dehydroepiandrosterone sulfate in nondiabetic men. J Clin Endocrinol Metab 1994;78:549-54.
26. Sodergard R, Backstrom T, Shanbhag V, Carstensen H. Calculation of free and bound fractions of testosterone and estradiol-17β to human plasma proteins at body temperature. J Steroid Biochem 1982;16:801-10.
27. Velazquez E, Acosta A, Mendoza SG. Menstrual cyclicity after metformin therapy in polycystic ovary syndrome. Obstet Gynecol 1997;90:392-5.
28. Coetzee EJ, Jackson WPU. Metformin in management of pregnant insulin-independent diabetics. Diabetologia 1979;16:241-5.