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Insulin Reduction with Metformin Increases Luteal Phase Serum Glycodelin and Insulin-Like Growth Factor-Binding Protein 1 Concentrations and Enhances Uterine Vascularity and Blood Flow in the Polycystic Ovary Syndrome*

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ABSTRACT

We hypothesized that hyperinsulinemia contributes to early pregnancy loss in the polycystic ovary syndrome by adversely affecting endometrial function and environment. Serum glycodelin, a putative biomarker of endometrial function, is decreased in women with early pregnancy loss. Insulin-like growth factor-binding protein-1 may also play an important role in pregnancy by facilitating adhesion processes at the feto-maternal interface.

We studied 48 women with polycystic ovary syndrome before and after 4 weeks of administration of 500 mg metformin (n = 26) or placebo (n = 22) 3 times daily. Oral glucose tolerance tests were performed, and serum glycodelin and insulin-like growth factor-binding protein-1 were measured during the follicular and clomiphene-induced luteal phases of menses.

In the metformin group, the mean (±SE) area under the serum insulin curve after glucose administration decreased from 62 ± 6 to 19 ± 2 nmol/L·min ($P < 0.001$). Follicular phase serum glycodelin concentrations increased 20-fold from 150 ± 46 to 2813 ± 1192 pmol/L ($P < 0.001$), and serum insulin-like-growth factor-binding protein-1 concentrations increased from 936 ± 152 to 2396 ± 300 pmol/L ($P < 0.001$). Similarly, luteal phase serum glycodelin concentrations increased 3-fold from 3434 ± 1299 to 10624 ± 1803 pmol/L ($P < 0.001$), and serum insulin-like growth factor-binding protein-1 concentrations increased from 1220 ± 136 to 4916 ± 596 pmol/L ($P < 0.001$). Uterine vascular penetration also increased in the metformin group, as did blood flow of spiral arteries, as demonstrated by a 20% decrease in the resistance index from 0.71 ± 0.02 to 0.57 ± 0.03 ($P < 0.001$). These variables did not change in the placebo group.

We conclude that insulin reduction with metformin increases follicular and luteal phase serum glycodelin and insulin-like growth factor-binding protein-1 concentrations and enhances luteal phase uterine vascularity and blood flow in the polycystic ovary syndrome. These changes may reflect an improved endometrial milieu for the establishment and maintenance of pregnancy. (*J Clin Endocrinol Metab* **86:** 1126–1133, 2001)

THE POLYCYSTIC ovary syndrome is characterized by chronic anovulation and hyperandrogenism, affects 6–10% of women of childbearing age, and is the most common cause of female infertility in the United States (1). The polycystic ovary syndrome is also associated with an increased rate of early pregnancy loss of 30–40% (2–6). As many as 82% of women with early pregnancy loss have the polycystic ovary syndrome (6). In most cases no apparent cause can be identified (7), but, in addition to defects in the developing embryo, adverse alterations in endometrial function may play a role.

A key feature of the polycystic ovary syndrome is insulin resistance and compensatory hyperinsulinemia (8–12). Hyperinsulinemia contributes to the hyperandrogenism of the polycystic ovary syndrome by stimulating ovarian androgen production (13–15) and decreasing serum sex hormone-binding globulin concentrations (16). Hyperinsulinemia appears to impede ovulation in the polycystic ovary syndrome, and reducing insulin release with insulin-sensitizing drugs increases the frequency of spontaneous ovulation (17–20), enhances ovulation induction with clomiphene (19), and allows for more controlled ovulation induction with gonadotropins (21).

We hypothesized that hyperinsulinemia contributes to the increased rate of early pregnancy loss in the polycystic ovary syndrome by adversely affecting endometrial function and the periimplantation environment. To test this hypothesis, we conducted a double blind, placebo-controlled study using metformin to decrease serum insulin concentrations before a clomiphene-induced ovulatory cycle. We monitored serum glycodelin and insulin-like growth factor-binding protein-1 concentrations, serum androgens, and other hormones. We

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also monitored endometrial thickness, vascularization, and blood flow by Doppler ultrasonography, because these correlate with endometrial receptivity (22–24), which is decreased in the polycystic ovary syndrome (25, 26).

Glycodelin is a protein whose circulating concentration may reflect endometrial function (27). Glycodelin is a major secretory product synthesized by secretory/decidualized endometrial glands (28, 29) and may inhibit the endometrial immune response to the embryo (30, 31). Both early pregnancy loss and retarded endometrial development are associated with decreased secretion of glycodelin from secretory endometrium (32, 33). Insulin-like growth factor-binding protein-1 is a protein that appears to facilitate adhesion processes at the feto-maternal interface (34, 35) and may play an important role in the periimplantation period. Serum insulin-like growth factor-binding protein-1 concentrations are decreased in the polycystic ovary syndrome (36). By monitoring these parameters, we were able to noninvasively assess endometrial function and risk for early pregnancy loss *in vivo*.

Subjects and Methods

Subjects

Seventy-four women with polycystic ovary syndrome entered the study. Twenty-six women failed to ovulate during both arms of the study (see *Results*) and were discontinued, leaving 48 women who completed the study. All had oligomenorrhea (8 or fewer menstrual periods in the last year) and hyperandrogenemia (elevated serum free testosterone concentration), and none had diabetes mellitus. All had normal serum PRL concentrations and thyroid function tests. Late-onset adrenal hyperplasia was excluded by a morning serum 17α-hydroxyprogesterone concentration below 6 nmol/L. All women had findings on ultrasonography of the ovaries consistent with the polycystic ovary syndrome (37). The women were recruited from the practice of Dr. Daniela Jakubowicz, and 30 women were Caucasian of European descent, 15 were Hispanic, 2 were Arabic, and 1 was South American Indian. The study was approved by the institutional review board of the Hospital Clinicas de Caracas, and each woman gave informed consent.

Protocol

The women came to the hospital between 0700–1100 h after a 12-h overnight fast, and weight, height, and waist to hip ratio were measured. Blood samples were drawn at −30, −15, and 0 min, and equal volumes of serum were pooled for measurements of glycodelin, insulin-like growth factor-binding protein-1, insulin, glucose, steroid hormones, and sex hormone-binding globulin. At 0 min, 75 g glucose were given orally, and blood samples were collected for determination of serum glucose and insulin at 60 and 120 min.

All women then received clomiphene citrate (150 mg daily) for 5 days to induce a baseline ovulatory cycle, and it was assumed that the clomiphene was administered on the equivalent of days 5–9 of the menstrual cycle. Only women in whom ovulation was documented by both an elevated serum progesterone level (>12.7 pmol/L) and ultrasound criteria were continued in the study.

Fourteen days after the last dose of clomiphene administration (*i.e.* during the luteal phase and the equivalent of day 23 of the cycle), morning (0700–1100 h) blood samples were drawn for determination of glycodelin, insulin-like growth factor-binding protein-1, sex steroids, and sex hormone-binding globulin. In a subset of 16 women in the metformin group and 13 women in the placebo group, transvaginal color pulsed Doppler ultrasonography was performed, as described below.

Four weeks after the administration of clomiphene, the women were randomized to receive either 500 mg metformin (Glafornil, North Medicamenta, Caracas, Venezuela) or matched placebo tablet (provided by North Medicamenta) orally three times daily. The study was double blind, and investigators were not aware of drug assignment until completion of the study.

After 4 weeks of drug treatment, the blood sampling and oral glucose tolerance test were repeated, and another ovulatory cycle was induced by administering clomiphene citrate (150 mg daily) for 5 days. Fourteen days after the last dose of the second clomiphene administration (*i.e.* on the equivalent of day 23 of the cycle), the blood sampling and Doppler ultrasound assessments performed at baseline were repeated. The women were asked to either abstain from sexual intercourse or use a barrier method of contraception for the first (baseline) clomiphene-induced ovulation, but were allowed to attempt pregnancy after the second clomiphene-induced ovulation.

Doppler ultrasonography

Endometrial thickness, vascular penetration, and blood flow of spiral arteries were evaluated using a computerized vaginal sonographic ultrasound instrument with integrated pulsed Doppler (Voluson 530D, Kretz Technik, Zipf, Austria). Vascular penetration was classified as described by Zaidi *et al.* (24): zone 1, subendometrial zone; zone 2, outer hyperechogenic zone; zone 3, inner hypoechogenic zone; and zone 4, layer surrounding the uterine cavity. The position of uterine spiral arteries was identified in real time, blood flow patterns were recorded, and the resistance index was calculated as previously described (24, 38). The resistance index was determined by the equation: resistance index = (peak systolic velocity − end diastolic velocity)/peak systolic velocity.

Assays

Blood samples were centrifuged, and serum was stored at −20 C until assayed. Serum glycodelin and insulin-like growth factor-binding protein-1 were assayed in the laboratory of Dr. Seppälä; remaining assays were performed in the laboratory of Dr. Nestler. Serum glycodelin and insulin-like growth factor-binding protein-1 were measured by sandwich-type immunofluorometry, as reported previously (39, 40). Serum hormones and sex hormone-binding globulin (measured as protein) were assayed as previously described (19, 20), except for serum free testosterone, which was calculated by the Sodergard method, assuming a serum albumin level of 4.0 g/dL (41). All samples from an individual woman were analyzed in duplicate in a single assay for each hormone. Intraassay coefficients of variation were 8% for glycodelin and insulin-like growth factor-binding protein-1, 5.5% for insulin, and less than 10% for all steroid hormones.

Statistical analysis

Results were analyzed by comparing the change from baseline to the end of the study in the metformin group to the corresponding change in the placebo group. Changes in each group were tested for normality with the Wilk-Shapiro test and were compared using Student's two-tailed unpaired *t* test or the Mann-Whitney rank sum test. For intergroup comparisons of borderline significance, we report the within-group comparison, which was analyzed by Student's two-tailed paired *t* test or the Wilcoxon signed rank test. Serum glucose and insulin responses to oral glucose administration were analyzed by calculating the areas under the response curves by the trapezoidal rule.

Results

Ovulation responses of all study subjects

Of the 74 women who entered the study, 18 failed to ovulate in response to the first clomiphene induction. This left 56 eligible women, 28 of whom were randomized to metformin, and 28 to placebo. Of the 28 women randomized to metformin, 2 failed to respond to the second clomiphene induction. Of the 28 women randomized to placebo, 6 failed to respond to the second clomiphene induction. Therefore, 26 women in the metformin group and 22 women in the placebo group completed the study. The results reported below are from only those women who completed the study.

None of the women in the placebo arm ovulated during the 4-week treatment period. However, eight of the women

in the metformin group ovulated spontaneously during treatment, as documented by ultrasound and an elevated serum progesterone concentration. In these eight women initiation of the second clomiphene induction was delayed until 5 days after the onset of menstruation; therefore, these eight women were treated for approximately 5 weeks before the second clomiphene induction.

In the metformin group, menstrual bleeding occurred 19.8 ± 0.3 days (range, 18–23 days) after the last dose of the baseline clomiphene induction and 19.3 ± 0.2 days (range, 17–22 days) after the last dose of the postmetformin clomiphene induction. In the placebo group, menstrual bleeding occurred 20.1 ± 0.3 days (range, 18–24) after the last dose of the baseline clomiphene induction and 20.3 ± 0.3 days (range, 17–23 days) after the last dose of the postplacebo clomiphene induction. The time to menstruation did not differ between groups at baseline ($P = 0.46$), nor did it change significantly with treatment in either the metformin ($P = 0.12$) or the placebo ($P = 0.71$) group.

TABLE 1. Baseline anthropometric and follicular phase serum hormone concentrations in women with the polycystic ovary syndrome before administration of either metformin or placebo

	Metformin group (n = 26)	Placebo group (n = 22)	*P* value
Age (yr)	27 ± 1	27 ± 1	0.89
History of EPL [no. (%)]	9/26 (34)	9/22 (41)	0.77
BMI (kg/m^2)	31.8 ± 0.3	31.7 ± 0.3	0.91
Waist to hip ratio	0.84 ± 0.01	0.85 ± 0.01	0.86
Fasting insulin (pmol/L)†	206 ± 27	328 ± 52	0.33
AUC insulin (nmol/L·min)‡	62 ± 6	64 ± 6	0.63
Fasting glucose (mmol/L)	4.9 ± 0.1	5.2 ± 0.2	0.27
AUC glucose (mmol/L/min)	712 ± 20	693 ± 23	0.54
17β-Estradiol (pmol/L)	360 ± 51	349 ± 55	0.89
Progesterone (pmol/L)	5.1 ± 0.6	7.6 ± 2.9	0.97
Testosterone (nmol/L)	339 ± 35	382 ± 57	0.95
Free testosterone (pmol/L)	28.7 ± 3.1	34.9 ± 6.0	0.76
Androstenedione (nmol/L)	6.3 ± 0.3	6.6 ± 0.3	0.50
DHEA sulfate (μmol/L)	11.3 ± 1.0	10.0 ± 0.8	0.35
SHBG (nmol/L)	130 ± 11	127 ± 11	0.74
Glycodelin (pmol/L)	150 ± 46	107 ± 29	0.93
IGFBP-1 (pmol/L)	936 ± 152	800 ± 100	0.79

Values are the mean ± SE.

Baseline characteristics

At baseline, the women in the metformin (n = 26) and placebo (n = 22) groups did not differ significantly with respect to age, history of early pregnancy loss, body mass index, waist to hip ratio, fasting serum insulin and glucose values, serum insulin and glucose responses after oral glucose administration, serum sex steroid and sex hormone-binding globulin concentrations, or serum glycodelin and insulin-like growth factor-binding protein-1 concentrations (Table 1).

Anthropometric variables

Body mass index did not change with treatment in the metformin (31.8 ± 0.3 *vs.* 31.8 ± 0.3 kg/m^2; $P = 0.39$) or placebo (31.7 ± 0.3 *vs.* 31.7 ± 0.3 kg/m^2; $P = 0.64$) group. Waist to hip ratio also did not change in the metformin (0.84 ± 0.1 *vs.* 0.84 ± 0.2; $P = 0.09$) or placebo (0.85 ± 0.01 *vs.* 0.85 ± 0.01; $P = 0.10$) group.

Follicular phase values

Insulin and glucose. Follicular phase fasting serum insulin concentrations decreased significantly in the metformin group from 206 ± 27 to 79 ± 14 pmol/L ($P < 0.001$), but did not change in the placebo group ($P = 0.25$; Table 2). The change in this value in the metformin group did not differ significantly from that in the placebo group ($P = 0.06$). In the metformin group, the 69% decrease in the area under the serum insulin curve from 62 ± 6 to 19 ± 2 nmol/L·min differed significantly from the lack of change in the placebo group ($P < 0.001$; Table 2).

Fasting serum glucose concentrations decreased in the metformin group from 4.9 ± 0.1 to 4.3 ± 0.2 mmol/L ($P = 0.004$), but did not change in the placebo group ($P = 0.10$; Table 2). The change in this value in the metformin group did not differ significantly from that in the placebo group ($P = 0.09$). The area under the serum glucose curve decreased significantly in both the metformin ($P = 0.01$) and placebo ($P = 0.03$) groups (Table 2), but the change differed significantly between groups ($P = 0.01$).

Glycodelin and insulin-like growth factor-binding protein-1. Substantial variability in baseline follicular phase glycodelin and insulin-like growth factor-binding protein-1 levels was observed in both the metformin and placebo groups. For example, follicular phase serum glycodelin varied over a 65-fold range from 18–1180 pmol/L, and follicular phase serum insulin-like growth factor-binding protein-1 varied over a 25-fold range from 100-2520 pmol/L.

Follicular phase serum glycodelin concentrations increased almost 20-fold in the metformin group from 150 ± 46 to 2813 ± 1192 pmol/L ($P = 0.001$), but did not change in the placebo group (107 ± 29 *vs.* 121 ± 29 pmol/L; $P = 0.44$; Fig. 1). The change in this value in the metformin group differed significantly from the lack of change in the placebo group ($P < 0.001$).

Similarly, follicular phase serum insulin-like growth factor-binding protein-1 concentrations increased greater than 2-fold in the metformin group from 936 ± 152 to 2396 ± 300 pmol/L ($P < 0.001$), but did not change in the placebo group (800 ± 100 *vs.* 740 ± 88 pmol/L; $P = 0.19$; Fig. 1). The change in this value in the metformin group differed significantly from the lack of change in the placebo group ($P < 0.001$).

Sex steroids (Table 2). The decrease in the follicular phase serum total testosterone concentration in the metformin group from 339 ± 35 to 133 ± 16 nmol/L differed significantly ($P < 0.001$) from the lack of change in the placebo group. The increase in the serum sex hormone-binding globulin concentration in the metformin group from 131 ± 11 to 196 ± 13 nmol/L differed significantly ($P < 0.001$) from the lack of change in the placebo group. These changes resulted in a 73% decrease in the calculated serum free testosterone concentration from 28.7 ± 3.1 to 7.8 ± 0.9 pmol/L in the metformin group, which differed significantly ($P < 0.001$) from the lack of change in the placebo group.

Decreases in serum androstenedione and dehydroepi-

TABLE 2. Follicular phase serum hormone concentrations in women with the polycystic ovary syndrome who were treated with either metformin or placebo for 4 weeks

	Metformin group (n = 26)		Placebo group (n = 22)	
	Baseline	Week 4	Baseline	Week 4
Fasting insulin (pmol/L)	206 ± 27	79 ± 14	328 ± 52	276 ± 39
AUC insulin (nmol/L/min)	62 ± 6	19 ± 2[a]	64 ± 6	60 ± 8
Fasting glucose (mmol/L)	4.9 ± 0.1	4.3 ± 0.2	5.2 ± 0.2	5.0 ± 0.2
AUC glucose (mmol/L·min)	712 ± 20	592 ± 21[b]	693 ± 23	659 ± 26
Testosterone (nmol/L)	339 ± 35	133 ± 16[a]	382 ± 57	373 ± 41
17β-Estradiol (pmol/L)	360 ± 51	294 ± 37	349 ± 55	374 ± 37
Free testosterone (pmol/L)	28.7 ± 3.1	7.8 ± 0.9[a]	34.9 ± 6.0	34.3 ± 5.0
Androstenedione (nmol/L)	6.3 ± 0.3	4.5 ± 0.3[a]	6.6 ± 0.3	6.6 ± 0.3
DHEA sulfate (μmol/L)	11.3 ± 1.0	7.3 ± 0.7[c]	10.0 ± 0.8	10.3 ± 0.7
SHBG (nmol/L)	131 ± 11	196 ± 13[a]	127 ± 11	120 ± 9
Glycodelin (pmol/L)	150 ± 46	2813 ± 1192[a]	107 ± 29	121 ± 29
IGFBP-1 (pmol/L)	936 ± 152	2396 ± 300[a]	800 ± 100	740 ± 88

Values are the mean ± SE.
[a] $P < 0.001$ for change in metformin group compared with change in placebo group.
[b] $P = 0.01$ for change in metformin group compared with change in placebo group.
[c] $P = 0.002$ for change in metformin group compared with change in placebo group.

androsterone sulfate concentrations in the metformin group differed significantly from the lack of changes in these values in the placebo group ($P < 0.001$ and $P = 0.002$, respectively). Serum estradiol concentrations did not change in either group.

Luteal phase values after ovulation induction with clomiphene

Glycodelin and insulin-like growth factor-binding protein-1. Substantial variability in baseline luteal phase glycodelin and insulin-like growth factor-binding protein-1 levels was observed. For example, luteal phase serum glycodelin varied over a 300-fold range from 107–32,500 pmol/L, and luteal phase serum insulin-like growth factor-binding protein-1 varied over a 20-fold range from 160-3280 pmol/L.

Baseline luteal phase serum glycodelin concentrations were higher in the placebo group than in the metformin group (6519 ± 1189 *vs.* 3434 ± 1299 pmol/L; $P = 0.009$). After drug treatment, luteal phase serum glycodelin concentrations were almost 3-fold greater in the metformin group than in the placebo group (10,624 ± 1,803 *vs.* 3,659 ± 1,289 pmol/L, respectively; $P < 0.001$), and the increase in this value in the metformin group differed significantly from the decrease in the placebo group ($P < 0.001$; Fig. 2).

Baseline luteal phase serum insulin-like growth factor-binding protein-1 concentrations did not differ between the metformin and placebo groups (1220 ± 136 *vs.* 1468 ± 180 pmol/L; $P = 0.99$). After drug treatment, luteal phase serum insulin-like growth factor-binding protein-1 concentrations were almost 4-fold greater in the metformin group than in the placebo group (4916 ± 596 *vs.* 1324 ± 200 pmol/L; $P < 0.001$), and the increase in this value in the metformin group differed significantly from the lack of change in the placebo group ($P < 0.001$; Fig. 2).

Sex steroids (Table 3). In the metformin group, the increase in luteal phase serum progesterone concentrations from 70.3 ± 5.4 to 123.7 ± 12.4 pmol/L differed significantly from the lack of change in the placebo group ($P = 0.001$). The marked increase in luteal phase serum estradiol concentrations in the

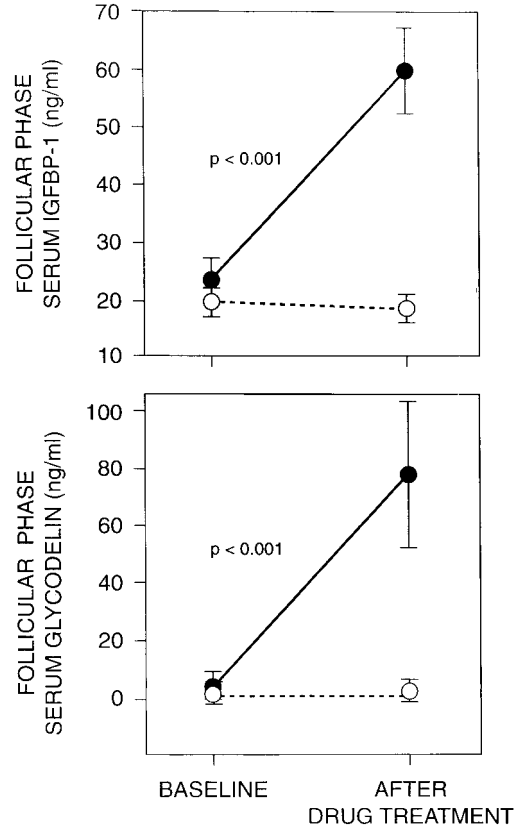

FIG. 1. Follicular phase serum glycodelin and insulin-like growth factor-binding protein-1 (IGFBP-1) concentrations in women with the polycystic ovary syndrome at baseline and after administration of either metformin or placebo for 4 weeks. For both glycodelin and IGFBP-1, the change from baseline to after treatment in the metformin group (●; n = 26) differed significantly ($P < 0.001$) from the corresponding change in the placebo group (○; n = 22). In normal women, serum glycodelin concentrations have been reported to be 100–150 pmol/L during the follicular phase and 1400–2900 pmol/L during the luteal phase (48). To convert glycodelin from nanograms per mL to picomoles per L, multiply by 35.7, and to convert IGFBP-1 from nanograms per mL to picomoles per L, multiply by 40. Values are the mean ± SE.

metformin group from 675 ± 88 to 5220 ± 1065 pmol/L differed significantly from the lack of change in the placebo group ($P = 0.001$).

Luteal phase serum total testosterone concentrations did not change in either group. The increase in luteal phase serum sex hormone-binding globulin concentrations in the metformin group from 137 ± 12 to 238 ± 17 nmol/L differed significantly from the lack of change in the placebo group ($P = 0.001$). As a result, the luteal phase calculated serum free testosterone concentration decreased by 37% in the metformin group from 18.5 ± 2.4 to 11.7 ± 1.3 pmol/L ($P < 0.001$), but this change did not differ significantly ($P = 0.23$) from that in the placebo group.

Neither luteal phase serum androstenedione nor dehydroepiandrosterone sulfate changed in either group.

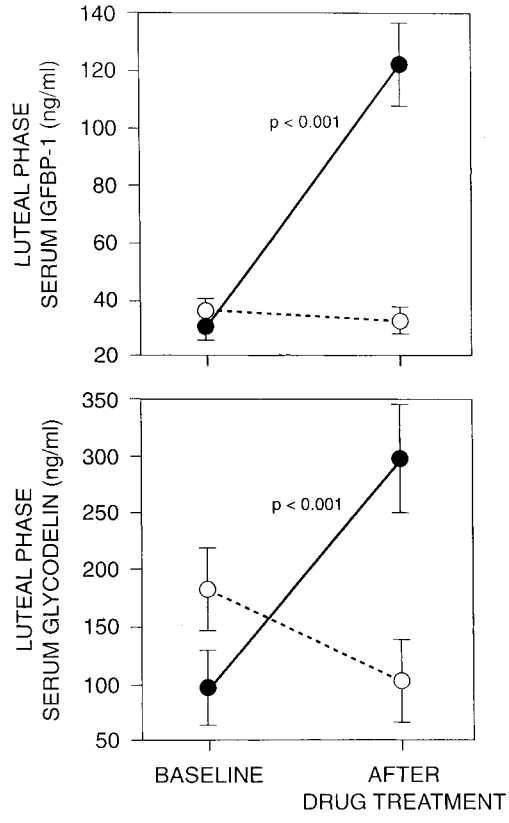

FIG. 2. Clomiphene-induced luteal phase serum glycodelin and insulin-like growth factor-binding protein-1 (IGFBP-1) concentrations in women with the polycystic ovary syndrome at baseline and after administration of either metformin or placebo for 4 weeks. For both glycodelin and IGFBP-1, the change from baseline to after treatment in the metformin group (●; n = 26) differed significantly ($P < 0.001$) from the corresponding change in the placebo group (○; n = 22). In normal women, serum glycodelin concentrations have been reported to be 100–150 pmol/L during the follicular phase and 1400–2900 pmol/L during the luteal phase (48). To convert glycodelin from nanograms per mL to picomoles per L, multiply by 35.7, and to convert IGFBP-1 from nanograms per mL to picomoles per L, multiply by 40. Values are the mean ± SE.

Doppler ultrasonography

Endometrial thickness during the luteal phase did not change with treatment in either the metformin (7.7 ± 0.4 *vs.* 8.5 ± 0.5 mm; $P = 0.13$) or placebo (7.6 ± 0.5 *vs.* 7.4 ± 0.7 mm; $P = 0.74$) group.

In the metformin group, endometrial vascular penetration into zone 3 or 4 was observed in 3 of 16 women (19%) at baseline and increased to 11 of 16 women (69%) after treatment ($P = 0.01$, by Fisher's exact test). In the placebo group, endometrial vascular penetration into zone 3 or 4 was observed in 5 of 13 women (38%) at baseline and in 4 of 13 women (31%) after treatment ($P = 1.00$).

The resistance index of uterine spiral arteries decreased significantly in the metformin group from 0.71 ± 0.02 to 0.57 ± 0.03 ($P < 0.001$), but tended to increase in the placebo group (0.63 ± 0.03 *vs.* 0.67 ± 0.03; $P = 0.053$). The change in the metformin group differed significantly ($P < 0.001$) from that in the placebo group.

Discussion

Our fundamental hypothesis is that hyperinsulinemia contributes to the increased rate of early pregnancy loss in the polycystic ovary syndrome (2–6). The present study tested the specific hypothesis that treatment with metformin to reduce serum insulin would improve luteal phase endometrial function and the periimplantation environment in the polycystic ovary syndrome.

Metformin treatment significantly decreased both serum insulin and glucose concentrations and simultaneously decreased serum androgens and increased serum sex hormone-binding globulin concentrations. No effect on these parameters was observed in women who received placebo. These effects were consistent with those previously reported by our group (14, 42) and by others (43–45). Although not determined directly, metformin probably improved glucose tol-

TABLE 3. Clomiphene-induced luteal phase serum hormone concentrations in women with the polycystic ovary syndrome who underwent ovulation induction with clomiphene at baseline and then again after having received either metformin or placebo for 4 weeks

	Metformin group (n = 26)		Placebo group (n = 22)	
	Baseline	Week 4	Baseline	Week 4
Progesterone (pmol/L)	70.3 ± 5.4	123.7 ± 12.4[a]	62.6 ± 4.8	68.4 ± 6.0
17β-Estradiol (pmol/L)	675 ± 88	5220 ± 1065[a]	943 ± 143	712 ± 84
Testosterone (nmol/L)	187 ± 17	218 ± 15	220 ± 12	218 ± 11
Free testosterone (pmol/L)	18.5 ± 2.4	11.7 ± 1.3	19.5 ± 3.6	14.8 ± 1.5
Androstenedione (nmol/L)	6.3 ± 0.3	6.6 ± 0.3	7.3 ± 0.3	7.7 ± 0.3
DHEA sulfate (μmol/L)	11.7 ± 0.8	10.8 ± 0.8	11.7 ± 1.2	11.2 ± 0.5
SHBG (nmol/L)	137 ± 12	238 ± 17[a]	194 ± 17	179 ± 14

Values are the mean ± SE.
[a] $P = 0.001$ for change in metformin group compared with change in placebo group.

erance by suppressing hepatic glucose production (46, 47) and improving whole body insulin sensitivity (43, 44).

Metformin treatment was also associated with marked increases in circulating glycodelin and insulin-like growth factor-binding protein-1 concentrations, both during the follicular phase of menses and during a postovulatory luteal phase induced with clomiphene. Embryo implantation occurs during the luteal phase, and women treated with metformin demonstrated a 3-fold increase in luteal phase serum glycodelin concentrations and a 4-fold increase in serum insulin-like growth factor-binding protein-1 concentrations. However, it should be noted that luteal phase serum glycodelin concentrations were not low at baseline in either the metformin or placebo group compared with existing normative values (48), and that metformin treatment raised luteal phase serum glycodelin concentrations into the range observed during early pregnancy (49).

Serum glycodelin concentrations are decreased in early pregnancy loss (32, 33), most likely because of decreased endometrial production (28, 29). A specific glycoform of glycodelin, isolated from amniotic fluid and probably produced by decidualized endometrium, has been named glycodelin A because of its unique carbohydrate structure and inhibitory action on sperm-egg binding (50). Glycodelin inhibits mixed lymphocyte reaction and natural killer cell activity (30, 31) and may be important for protection of the embryo from the maternal immune response during the implantation period. Glycodelin appears in the endometrium at the time when the embryo enters the uterine cavity (51). It is possible that deficient production of endometrial glycodelin results in a locally hostile immunological environment.

Insulin-like growth factor-binding protein-1 is produced by several tissues, including liver, ovaries, endometrium, and corpus luteum (29, 52–54). Secretion of insulin-like growth factor-binding protein-1 is regulated by insulin (55). In early pregnancy loss, decreased serum insulin-like growth factor-binding protein-1 concentrations are probably due to decreased hepatic production (56), although decreased production by endometrium and corpus luteum may also be present (57, 58). Insulin-like growth factor-binding protein-1 exerts paracrine actions and is correlated with adhesion processes at the feto-maternal interface (34, 35). Presumably a decrease in endometrial insulin-like growth factor-binding protein-1 would adversely affect embryo implantation. The increase in serum insulin-like growth factor-binding protein-1 concentrations with metformin treatment was probably due to increased hepatic production, although increased production by the endometrium and/or corpus luteum is possible.

The above observations suggest that insulin resistance adversely affects endometrial function, as evidenced by suppressed circulating glycodelin and may also increase the risk of early pregnancy loss by decreasing insulin-like growth factor-binding protein-1 production. Further evidence for this arises from the findings on Doppler ultrasonography. Failure of implantation is associated with the absence of vascular penetration into zones 3 or 4 of the uterus (24) and also with a resistance index of uterine spiral arteries greater than 0.5 (23, 26). With metformin administration, the number of women with uterine vascular penetration into zone 3 or 4 increased significantly from 19% to 69%, and the resistance index of uterine spiral arteries decreased significantly by 20%. Both changes should favor embryonic implantation and maintenance of pregnancy (23, 24, 26) and suggest that insulin resistance adversely affects uterine vascularity and blood flow.

There are limitations to this study. First, endometrial tissue could not be obtained for direct assessment of glycodelin expression for ethical reasons, as many women were actively attempting conception. Glycodelin is produced by several extrauterine sources, including bone marrow (59), sweat glands (60), breast tissue (61), and ovary (62), and it is possible that insulin regulates glycodelin expression by these tissues. However, although immunoreactive forms of glycodelin and its messenger ribonucleic acid have been found in several tissues, the major contribution to circulating glycodelin in women is probably from secretory endometrium. Evidence for this arises from the observation that hormone replacement therapy caused a much greater cyclical elevation in serum glycodelin concentrations in women with intact uteri than in hysterectomized women receiving similar treatment (27). Specifically, serum glycodelin concentrations were low at baseline in postmenopausal women with intact uteri and increased by 48% during replacement therapy with estrogen and progestogen, whereas the incremental change in serum glycodelin was only 7% in hysterectomized postmenopausal women (27).

Second, the study cannot distinguish whether the increases in serum glycodelin and insulin-like growth factor-binding protein-1 concentrations were due to the reduction in serum insulin or to the simultaneous reduction in serum androgens. An association between elevated serum androgens and early pregnancy loss has been reported (63, 64). Androgens are not known to alter serum glycodelin or insulin-like growth factor-binding protein-1 concentrations *in vivo*.

Despite these limitations, the present study is noteworthy in several respects. First, the findings demonstrate for the first time *in vivo* regulation of glycodelin metabolism by insulin, although it remains unknown whether this represents a direct or indirect action of insulin. Second, the findings suggest that hyperinsulinemia may contribute to early pregnancy loss by 1) retarding endometrial function, as reflected by decreased circulating glycodelin, and 2) decreasing circulating insulin-like growth factor-binding protein-1. Finally, the findings indicate that metformin treatment increases serum glycodelin and insulin-like growth factor-binding protein-1 concentrations in the polycystic ovary syndrome and suggest that studies of metformin to prevent early pregnancy loss in this disorder are warranted.

Finally, it is notable that luteal phase serum estradiol concentrations were nearly 8-fold higher in women who received metformin. Insulin influences aromatase activity in cultured human ovarian cells (65, 66) and placental cytotrophoblasts (67, 68). Aromatase activity in ovarian cells obtained from women with polycystic ovary syndrome is decreased (65), and insulin resistance may contribute to this. Increased luteal phase serum estradiol concentrations may have acted in concert with decreased circulating insulin (16)

to increase luteal phase serum sex hormone-binding globulin concentrations in these women.

In summary, insulin resistance and hyperinsulinemia not only appear to play a role in the infertility of the polycystic ovary syndrome, but may contribute through impaired endometrial function and adverse effects on the periimplantation environment to the increased rate of early pregnancy loss of the disorder as well.

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