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

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Sixteen nondiabetic women with polycystic ovary syndrome (PCOS) aged 18 to 33 years were studied before and after 8 weeks on metformin (1.5 g/d) therapy to assess whether reducing hyperinsulinemia would reduce the levels of the major inhibitor of fibrinolysis, antigenic plasminogen activator inhibitor type 1 (PAI-1). Compared with six normal control women, PCOS women had a higher body mass index (BMI), waist to hip ratio, fasting insulin (I_o), insulin area under the curve during oral glucose tolerance testing (IA), glucose area under the curve during oral glucose tolerance testing (GA), IA/GA ratio, PAI-1, luteinizing hormone (LH) and ratio of LH to follicle-stimulating hormone (FSH), and free testosterone, and lower high-density lipoprotein (HDL) cholesterol (all $P < .025$). On metformin, BMI decreased 1.3% ($P = .04$), I_o 43% ($P = .002$), IA 31% ($P = .03$), GA 11% ($P = .02$), PAI-1 16% ($P = .01$), lipoprotein(a) [Lp(a)] 42% ($P = .004$), free testosterone 46% ($P = .0006$), LH 44% ($P = .004$), and the LH/FSH ratio 41% ($P = .0001$). On metformin, absolute and percent reductions in I_o correlated with absolute and percent reductions in PAI-1 ($r = .60$, $P = .015$ and $r = .64$, $P = .008$). On metformin, by stepwise multiple regression, the absolute reduction in I_o was a significant determinant of the absolute reduction in PAI-1 (partial $R^2 = 35\%$, $P = .02$), and the percent reduction in I_o was a significant determinant of the percent reduction in PAI-1 (partial $R^2 = 52\%$, $P = .003$). Metformin decreases I_o in hyperinsulinemic PCOS patients, reverses the hyperinsulinemia-driven endocrinopathy, decreases PAI-1, and decreases Lp(a), and should thus reduce the increased risk of atherothrombosis in PCOS.

PLASMINOGEN ACTIVATOR inhibitor type 1 (PAI-1) is a potent inhibitor of fibrinolysis, binding to and rapidly inactivating both tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator.[1] Plasma PAI-1 levels are high in patients with coronary artery disease[2,3] and ischemic stroke[4] and have prognostic value in predicting the recurrence of myocardial infarction.[5]

PAI-1 correlates with plasma insulin,[6-10] suggesting a possible link between PAI-1, insulin resistance, and hyperinsulinemia. Hyperinsulinemia may be a risk factor for coronary artery disease.[11-13] The biguanide metformin, which reduces insulin resistance and decreases insulin levels,[14] also reduces PAI-1 levels.[15-17] Dahlgren et al[18] reported a positive correlation between fasting insulin and PAI-1 in polycystic ovary syndrome (PCOS). We[19] and others[14,20] have previously reported that metformin significantly reduces insulin levels and reverses the hyperinsulinemia-driven endocrinopathy of PCOS.

Our specific aim in the current study of 16 patients with PCOS before and after 8 weeks on metformin (1.5 g/d) therapy was to determine whether metformin, by decreasing insulin levels, would decrease PAI-1.

SUBJECTS AND METHODS

Patients With PCOS and Controls

This study was performed following a protocol approved by the Universitario de Los Andes Institutional Review Board, and with provision of informed consent. Sixteen nondiabetic women (aged 18 to 33 years) with PCOS were selected in the Endocrinology outpatient clinic at the Hospital Universitario de Los Andes, Merida, Venezuela. The diagnosis was based on clinical features, endocrinologic abnormalities, and ovarian ultrasound, following previously reported diagnostic criteria.[19] Clinical features included oligomenorrhea or amenorrhea. All patients had hirsutism with a Ferriman-Gallwey score greater than 10.[19,21] Endocrine features included a luteinizing hormone (LH) to follicle-stimulating hormone (FSH) ratio of 1.5 or greater on a blood sample taken on days 2 to 5 of spontaneous menstrual bleeding or during amenorrhea. Pelvic ultrasound was performed in all patients, and polycystic ovaries were diagnosed if the ovaries had a thickened abnormal stroma and more than 10 cysts of 2 to 8 mm in diameter arranged peripherally around a dense core of stroma, scattered through an increased amount of stroma, or both.[19] All 16 women had normal fasting glucose levels (<105 mg/dL) and 2-hour glucose levels less than 200 mg/dL during oral glucose tolerance tests. No patients were being treated with hypoglycemic agents. Excluded from the study were patients with diabetes or other endocrinopathies or those who were receiving sex hormones or drugs known to affect lipoprotein metabolism in the last 2 months preceding the study.

Six healthy nondiabetic women aged 18 to 34 years served as controls. None of the controls had hirsutism and/or acne; all had regular cyclic menses, and none were taking oral contraceptives or any other medications. The controls were studied in the early follicular phase of the menstrual cycle.

Experimental Protocol

All subjects had a body mass index ([BMI] kg/m^2) calculated and a waist to hip ratio recorded.[19] Waist circumference was measured midway between the lowest rib margin and the iliac crest in the standing position. Hip circumference was measured over the widest part of the gluteal region.

On the second to fourth day after spontaneous or induced menstrual bleeding, after 10 to 12 hours of overnight fasting, venous samples were obtained. Patients and controls were studied between 8 and 9 AM after an overnight fast. An intravenous sampling catheter was inserted into the forearm. Serum aliquots were stored at −20°C for analysis of LH and FSH (chemiluminescence method) and testosterone and insulin (by radioimmunoassay) using commercial products from Diagnostic Products (Los Angeles, CA). The radioimmunoassay is specific for insulin; cross-reactivity with proinsulin is approximately 40%. Within- and

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Submitted September 23, 1996; accepted October 24, 1996.

Supported by Grant No. M-519-95-07-B from the University of Los Andes, Merida, Venezuela, and by the Medical Research Council and Lipoprotein Research Fund, Jewish Hospital, Cincinnati, OH.

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0026-0495/97/4604-0022$03.00/0

between-day coefficients of variation were 3% to 5% and 8% to 10%, respectively. Free testosterone levels were calculated as previously described.[19]

After a 300-g carbohydrate diet for 3 days and an overnight fast, a 75-g oral glucose tolerance test was performed. Blood glucose and insulin levels were measured at 0, 30, 60, and 120 minutes after the glucose load. Areas under the insulin curve (IA) and glucose curve (GA) were calculated by the trapezoidal method. Glucose, triglyceride, and total cholesterol levels were measured by enzymatic methods with kits from Boehringer (Mannheim, Germany), analyzed with the Abbot Bichromatic ABA-100 (Chicago, IL). High-density lipoprotein (HDL) cholesterol level was measured after precipitation of plasma with 2 mol/L manganese chloride and heparin.[19] The low-density lipoprotein (LDL) cholesterol level was calculated when triglycerides were less than 400 mg/dL. Lipoprotein(a) [Lp(a)] level was measured by enzyme immunoassay (Innogenetics, Antwerp, Belgium).

Measurement of Basal and Stimulated Fibrinolytic Activity

Blood was drawn with a tourniquet for measures of basal fibrinolysis in seated patients at 8 to 9 AM to reduce effects of the circadian rhythm on fibrinolysis.[22] The tourniquet was used for the shortest time possible, usually less than 15 seconds. There was no difficulty in obtaining blood samples. Stimulated fibrinolytic activity was measured after 10 minute's venous occlusion by a blood pressure cuff at 100 mm Hg.[22] Blood samples were drawn in tubes containing sodium citrate (0.13 mol/L) and placed immediately on ice. Plasma was separated by centrifugation at 2,000 × *g* for 10 minutes at 4°C and stored at −70°C until assayed. Antigenic tPA (tPA-Ag),[22,23] a precursor of the major fibrinolytic activity stimulator (tPA-Fx), was determined by an enzyme immunoassay (Innogenetics). Antigenic PAI-1 was assayed by previously reported methods.[22,23] Basal PAI-1 activity[22,23] and stimulated tPA activity[22,23] were not measured, nor was euglobulin lysis time measured.

After baseline (pretreatment) evaluation, all PCOS patients were treated with metformin (Glafornil-Lab North Medicamenta, Laboratorios Palenzona, Caracas, Venezuela) 500 mg three times per day (with food) for 8 weeks. After 8 weeks on metformin, patients were resampled for comparison to pretreatment baseline.

Statistical Methods

Because most of the data were not normally distributed, differences between PCOS patients and controls were measured by Wilcoxon tests, and differences between variables before and after metformin therapy were measured by paired Wilcoxon tests.[24] Correlations between absolute and percent changes in variables were made by Spearman's nonparametric tests.[24] Stepwise multiple regression[24] was used to assess the determinants of changes (on metformin) in PAI-1 or Lp(a) as the dependent variables, with explanatory variables including changes (on metformin) in BMI, free testosterone, LH, I_0, IA, GA, and triglyceride.

RESULTS

Patients

Fasting plasma glucose in PCOS patients (88 ± 10 mg/dL) did not differ ($P > .05$) from the level in controls (78 ± 11 mg/dL). Fasting insulin and IA were 2.4 and 3.6 times higher than in normal controls ($P < .01$ and .001), and GA in PCOS patients was higher than in controls ($P < .01$; Table 1). PCOS patients were heavier than controls, with higher BMI ($P < .025$) and waist to hip ratio ($P < .01$; Table 1). Compared with controls, patients with PCOS had higher LH ($P < .01$), LH/FSH ratio ($P < .001$), and free testosterone ($P < .01$; Table 1). PCOS patients had higher basal antigenic PAI-1 ($P < .01$) and tPA ($P < .05$) and lower HDL cholesterol ($P < .01$) than controls (Table 1).

Table 1. Effects of Metformin Treatment on Anthropometrics, Lipids, Lipoproteins, Insulin, Hormones, and Fibrinolytic Activity in 16 Patients With PCOS (mean ± SD)

Parameter	Before Treatment	After Treatment	Controls (n = 6)
BMI (kg/m²)	26.74 ± 3.6	26.4 ± 3.76*	22.89 ± 1.81¶††
WHR	0.82 ± 0.04	0.82 ± 0.05	0.74 ± .05#§§
TG (mg/dL)	154 ± 85	164 ± 90	105 ± 52
TC (mg/dL)	215 ± 65	206 ± 39	188 ± 27
HDL cholesterol (mg/dL)	45 ± 11	44 ± 12	58 ± 10#‡‡
LDL cholesterol (mg/dL)	137 ± 54	128 ± 36	108 ± 23
Lp(a) (mg/dL)	23 ± 10	13 ± 7‡	17 ± 5
LH (mIU/mL)	9.63 ± 3.6	5.38 ± 2.79‡	5.17 ± 2.32#
FSH (mIU/mL)	5.69 ± 1.78	5.33 ± 2.03	5.50 ± 2.51
LH/FSH ratio	1.74 ± 0.46	1.03 ± 0.47§	0.97 ± 0.19**
Free T (pg/mL)	2.57 ± 0.98	1.40 ± 0.36§	1.18 ± 0.47#
tPA (ng/mL)	7.34 ± 3.05	5.93 ± 2.54	4.07 ± 3.34‖
S-tPA (ng/mL)	12.32 ± 4.2	13.07 ± 4.16	10.90 ± 4.44
PAI-1 (ng/mL)	74.5 ± 13.2	62.6 ± 10.7†	40.3 ± 21.3#††
S-PAI-1 (ng/mL)	82.1 ± 9.13	81.9 ± 15.4	92.00 ± 18.9
Insulin (μU/mL)	18.2 ± 9.9	10.3 ± 4.9‡	7.67 ± 4.3#
IA	272.9 ± 112.6	187.3 ± 87.9*	74.8 ± 31**‖‖
GA	249.9 ± 54.7	223.7 ± 53.3†	167.7 ± 41#††
IA/GA	1.05 ± 0.43	0.91 ± 0.42	0.42 ± 0.18#‡‡

Abbreviations: WHR, waist to hip ratio; TG, triglyceride; TC, total cholesterol; free T, free testosterone; S-tPA, stimulated tissue plasminogen activator antigen; S-PAI-1, stimulated PAI-1.

*$P < .05$, †$P < .025$, ‡$P < .01$, §$P < .001$: before *v* after treatment.

‖$P < .05$, ¶$P < .025$, #$P < .01$, **$P < .001$: PCOS before *v* controls.

††$P < .05$, ‡‡$P < .025$, §§$P < .01$, ‖‖$P < .001$: PCOS after *v* controls.

Effects of 8 Weeks of Metformin Therapy

On metformin therapy, BMI decreased 1.3% ($P = .04$), whereas the waist to hip ratio did not change (Table 1 and Fig 1). Fasting serum insulin decreased 43% (from 18.2 ± 9.9 to 10.3 ± 4.9 μU/mL, $P < .002$). IA decreased 31% (from 273 ± 113 to 187 ± 88 U, $P = .03$). GA decreased 11% ($P = .02$). Basal antigenic PAI-1 decreased 16% (from 74.5 ± 13.2 to 62.6 ± 10.7 ng/mL, $P = .01$). Lp(a) decreased

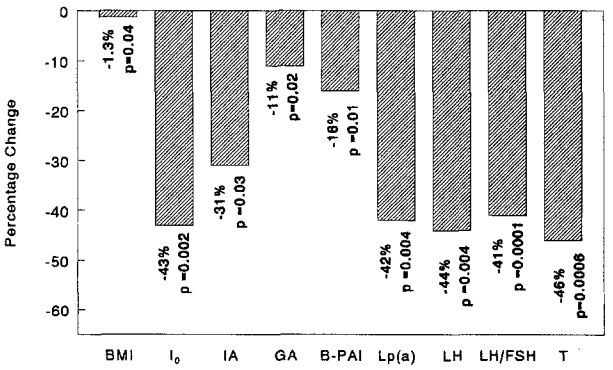

Fig 1. Percentage changes in the mean value for each parameter in 16 patients with PCOS after 8 weeks on metformin 1.5 g/d. B-PAI, basal PAI-1; T, free testosterone.

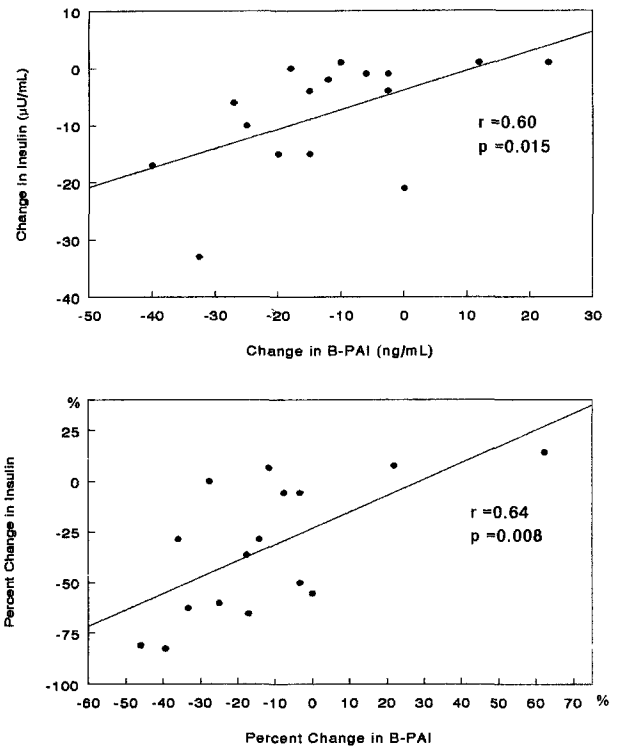

Fig 2. Absolute change and percentage change in fasting insulin on metformin therapy; correlations with absolute and percentage change in B-PAI.

42% (P = .004), from 23 ± 10 to 13 ± 7 mg/dL. LH decreased 44% (P = .004), and the LH/FSH ratio decreased 41% (P = .0001). Free testosterone decreased 46% (from 2.57 ± 0.98 to 1.40 ± 0.36 pg/mL, P = .0006; Table 1 and Fig 1).

After 8 weeks of metformin therapy, PCOS patients retained higher BMIs and waist to hip ratios (P < .05 and <.01), IA (P < .001), GA (P < .05), and IA/GA ratios (P < .025) and lower HDL cholesterol (P < .025) than controls (Table 1). PAI-1 remained higher after metformin in PCOS patients than in controls (P < .05). On metformin, fasting serum insulin in PCOS patients did not differ from the normals levels. On metformin, LH, FSH, LH/FSH ratio, and free testosterone did not differ from levels in normals (Table 1).

Relationships Between Absolute and Percentage Change in I_o and PAI-1 on Metformin

On metformin, absolute and percentage changes (decreases) in fasting serum insulin correlated with absolute and percentage reductions in basal PAI-1 (r = .60, P = .015 and r = .64, P = .008; Fig 2).

By stepwise multiple regression, with the absolute change in PAI-1 on metformin therapy as the dependent variable, the absolute change in fasting insulin was a significant explanatory variable (R^2 = 35%, P = .02). With the percent change in PAI on metformin therapy as the dependent variable, the percent change in fasting insulin was a significant explanatory variable (R^2 = 52%, P = .0025).

There were no significant independent predictors of absolute or percent change in Lp(a) on metformin.

DISCUSSION

As shown in the current study and previous studies,[14,19,20] metformin, by decreasing fasting insulin and the insulin response to glucose in hyperinsulinemic PCOS patients, reduces the hyperinsulinemia-driven hyperandrogenism and can reverse the endocrinopathy, often enough to allow regular menstrual cycles, reversal of infertility, and spontaneous pregnancy.[19] As shown in the current study, when metformin decreases fasting insulin, PAI-1 decreases, and the percent change in fasting insulin is a significant explanatory variable for the percent change in PAI-1 (R^2 = 52%, P = .0025). Although PAI-1 levels decreased significantly after metformin, they remained higher (P < .05) than in normal controls, suggesting that the risk for thrombosis, although reduced, was not normalized.

Unexpectedly, metformin decreased Lp(a). The mechanisms of metformin's effects on Lp(a) are unknown. There were no significant correlations between absolute or percent change in Lp(a) and changes in any of the other measured variables. A metformin-stimulated increase in endogenous estradiol might speculatively decrease Lp(a),[25] but metformin has not been reported to significantly increase estradiol in PCOS patients.[14,19]

Anfosso et al[26] have shown in vitro that insulin increases PAI-1 synthesis by the human hepatoma line, Hep G2. Anfosso et al[27] have shown in vitro that metformin inhibits insulin-mediated PAI-1 synthesis. Proinsulin increases PAI-1 activity in conditioned media of endothelial cells, with increased expression of PAI-1 mRNA.[28] In vivo, fasting insulin is a predominant predictor of PAI-1.[29-32] Dahlgren et al[18] previously reported a positive correlation between fasting insulin and PAI-1 in PCOS. Within this frame of reference, our finding of a metformin-induced reduction in insulin and PAI-1 in PCOS patients was not unexpected.

Since high PAI-1 is a risk factor for myocardial infarction,[1-3,5] stroke,[4] and osteonecrosis,[23,33] a reduction of PAI-1 through reduction of hyperinsulinemia should have considerable preventive medical implications. Women with PCOS, by virtue of hyperandrogenemia, hyperinsulinemia, obesity, hypertriglyceridemia, hypoalphalipoproteinemia, and high PAI-1, are at high risk for premature atherothrombosis.[5,14,19,29-31,34] Metformin, by virtue of ameliorating most, if not all, of these risk factors, should provide considerable protection from atherothrombosis in PCOS.

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