Metformin-Induced Resumption of Normal Menses in 39 of 43 (91%) Previously Amenorrheic Women With the Polycystic Ovary Syndrome

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In 43 amenorrheic women with polycystic ovary syndrome (PCOS), 31 (74%) with fasting hyperinsulinemia (≥ 20 μU/mL), our aim was to determine whether Metformin (Bristol-Myers Squibb, Princeton, NJ), which reduces hyperinsulinemia, would reverse the endocrinopathy of PCOS, allowing resumption of regular normal menses. A second aim was to assess the effects of weight loss versus other Metformin-induced effects on ovarian function, and to determine if there were different responses to Metformin between those who lost weight and those who did not. A third aim was to assess associations between PCOS, 4G/5G polymorphism in the promoter sequence of the plasminogen activator inhibitor-1 gene (PAI-1 gene), and PAI activity (PAI-Fx). Of the 43 women, 40 (93%) had normal fasting blood glucose and 37 had normal hemoglobin A1C (HgA1C); only three (7%) had type 2 diabetes mellitus. Metformin (1.5 to 2.25 g/d) was given for 6.1 ± 5.1 months (range, 1.5 to 24), to 16 patients for less than 3 months, to 12 for 3 to 6 months, and to 15 for at least 6 months. On Metformin, 39 of 43 patients (91%) resumed normal menses. The percentage of women resuming normal menses did not differ among treatment duration groups ($P < .1$) or among dose groups ($P > .1$). The body mass index (BMI) decreased from 36.4 ± 7 Kg/m^2 at study entry to 35.1 ± 6.7 on Metformin ($P = .0008$). Of 43 patients, 28 (67%) lost weight (1 to 69 pounds), with nine (21%) losing at least 12 pounds. On Metformin, the median fasting serum insulin decreased from 26 μU/mL to 22 ($P = .019$), testosterone decreased from 61 ng/dL to 47 ($P = .003$), and estradiol increased from 41 pg/mL to 71 ($P = .0001$). Metformin-induced improvements in ovarian function were independent of weight loss (testosterone decrease, $P < .002$; estradiol increase, $P < .0004$). The change in response variables on Metformin did not differ ($P > .05$) between those who lost weight and those who did not, excepting Lp(a), which increased 4 mg/dL in those who lost weight and decreased 9 mg/dL in those who did not ($P = .003$). The change in response variables on Metformin did not differ among the five quintiles of weight loss, excepting fasting glucose ($P < .05$), which increased 6 mg/dL in those who lost the least weight on Metformin versus those in the 60th to 80th percentile for weight loss, in whom glucose decreased 33 mg/dL. Although the pretreatment fasting serum insulin was not significantly correlated with testosterone ($r = .24$, $P = .13$) or androstenedione ($r = .27$, $P = .09$), on Metformin, the change in insulin correlated positively with the change in testosterone ($r = .35$, $P = .047$) and with the change in androstenedione ($r = .48$, $P = .01$). Patients were more likely than normal controls (83% *v* 64%, $P = .016$) to be heterozygous or homozygous for 4G polymorphism of the PAI-1 gene and were also more likely to have high PAI-Fx (≥22 U/mL, 28% *v* 3%, $\chi^2 = 10.1$, $P = .001$). Metformin reduces the endocrinopathy of PCOS, allowing resumption of normal menses in most (91%) previously amenorrheic women with PCOS.

POLYCYSTIC OVARY SYNDROME (PCOS) is very common, affecting 5% to 10% of the population, and is characterized by oligomenorrhea-amenorrhea, anovulation, infertility, abnormal gonadotropins, androgen excess, hirsutism, obesity, acne, excessive skin pigmentation, and hyperinsulinemia.[1-15] Insulin resistance with resultant hyperinsulinemia leads to abnormal ovarian and adrenal androgen secretion,[1-15] which in turn leads to abnormal gonadotropins and oligomenorrhea-amenorrhea. During the childbearing years, therapies for PCOS have previously been directed (with only modest success) at the presenting clinical features, amenorrhea, infertility, hirsutism, acne, and morbid obesity.[1] PCOS is associated with type 2 diabetes mellitus, obesity, hyperlipidemia, hyperandrogenemia, hyperinsulinemia, and insulin-induced elevations of plasminogen activator inhibitor-1 (PAI-1), the most potent inhibitor of fibrinolysis.[1,2,4-6] Thus, in adulthood,[1,4-6,8,11 14,15] PCOS is associated with a substantially increased risk of heart attack and stroke.

Nine studies in PCOS using Metformin[2-6,10,11,14,15] (Bristol-Myers Squibb, Princeton, NJ) have shown improvements in insulin sensitivity and a reduction in hyperinsulinemia. Many but not all of these studies have also reported weight loss and often, but not uniformly, normalization of circulating androgens, luteinizing hormone (LH), follicle-stimulating hormone (FSH), sex hormone–binding globulins (SHBGs), and PAI-1.[1-6.10.11.14,15] A recent study by Nestler et al[2] in 61 obese women with PCOS reported an improvement in hyperinsulinemia and ovarian function on Metformin without any significant decrement in the body mass index (BMI). In PCOS patients, small but significant weight loss often occurs on Metformin, in contrast to Rezulin, which also reduces insulin resistance[8,9] but does not reduce weight.

Most importantly, Metformin commonly leads to resumption of normal ovulatory menses and often to reversal of infertility.[2-6 10,11,14,15] In a recent non–placebo-controlled study, 21 of 22 (96%) oligomenorrheic-amenorrheic Mestizo women with PCOS had restoration of menstrual cyclicity; four (19%) became pregnant after 6 to 7 months on Metformin (1,500 mg/d) and delivered healthy infants.[5] Thirteen of 15 women who had regular menses on Metformin had luteal phase serum progesterone levels within the ovulatory range (3.1 to 28 ng/mL).[5] Fasting insulin or the integrated insulin response to glucose decreased ($P < .001$) after 8 weeks on Metformin.[5] This was accompanied by decrements in serum LH ($P < .001$), free testosterone ($P < .001$), the LH/FSH ratio ($P < .001$), and BMI ($P < .001$).[5] The investigators[5] concluded that Metformin im-

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proves menstrual cyclicity and fertility in women with PCOS. Morin-Papunen et al[14] reported that 69% of Finnish PCOS women with menstrual disturbances developed regular menstrual cyclicity on Metformin, 1,500 mg/d for 6 months. Diamanti-Kandarakis et al[15] reported that seven of 16 (44%) Greek women with PCOS resumed normal menstruation (with two pregnancies) on Metformin 1,700 mg/d for 6 months. However, two studies[7,12] using Metformin 850 mg twice per day[7] and 850 mg three times per day for 10 and 12 weeks[12] showed little to no benefit with respect to hyperinsulinemia, hormone concentrations, or lipid variables. Crave et al[13] showed that Metformin therapy had no additional benefit over the effect of a low-calorie diet that induced weight loss. Overall[1-6,8-11,14,15] insulin-sensitizing agents provide a safe, effective, and physiologically rational approach to the treatment of the metabolic and endocrine abnormalities in PCOS women.

In 43 amenorrheic women with PCOS, our aim was to determine whether Metformin would reverse the endocrinopathy of PCOS, allowing resumption of regular normal menses. A second aim was to assess the effects of weight loss versus other Metformin-induced effects on ovarian function, and to determine whether there were differences in the response to Metformin between patients who lost weight and those who did not. A third aim was to assess the associations between PCOS, 4G/5G polymorphism in the promoter sequence of the PAI-1 gene, and PAI activity (PAI-Fx).

SUBJECTS AND METHODS

Patients With PCOS

The study was performed using a protocol approved by the Jewish Hospital Institutional Review Board, with written informed consent. Forty-three caucasian American females (aged 16 to 46 years) with PCOS were enrolled in the study in the consecutive sequence of their referral. They were studied at any time since, by selection, they were amenorrheic for at least 6 months. The diagnosis of PCOS[4,6] was based on the following findings: (1) polycystic ovaries (≥10 subcapsular follicles 2 to 8 mm in diameter)[4,6] diagnosed by pelvic ultrasound or laparoscopy, (2) chronic oligomenorrhea (≤six menses per year and amenorrhea for ≥6 months), (3) hirsutism (Ferriman-Gallwey score ≥7),[16] LH/FSH ratio greater than 1.5, (4) morbid obesity resistant to weight-loss programs, and (5) the presence of acanthosis nigricans.

Patients with serum creatinine greater than 1.5 mg/dL, with other endocrinopathies causing amenorrhea including hyperprolactinemia and pituitary insufficiency, or taking exogenous sex hormones or drugs known to affect endogenous sex hormones or lipoprotein metabolism in the 2 months preceding the study were excluded. The three patients (7%) with type 2 diabetes mellitus were not excluded.

Congenital adrenal hyperplasia might mimic some of the clinical features of PCOS.[4] However, the absence of normal menses in all patients mitigated against congenital adrenal hyperplasia, which is, moreover, a rare disorder.[4] Hence, we did not perform the corticotropin stimulation test to rule out congenital adrenal hyperplasia.[4]

Normal Controls

Studies in healthy normal controls were performed during the same period as the patient studies. Forty normal hospital personnel (17 men and 23 women; mean ± SD age, 37 ± 7 years) served as controls for comparison to the patients' PAI-Fx; 234 healthy normal subjects served as controls for PAI-1 gene polymorphisms.

Study Design and Metformin Therapy

To enter the study, patients had to have well-documented PCOS[4,6] and to be amenorrheic (absence of menstrual cycles for ≥6 months). Because, by selection, the patients were very unlikely to spontaneously resume normal menstrual cycles, we did not include a randomized placebo group. On Metformin therapy, menstrual cyclicity was defined if subjects had two or more sequential regular menstrual cycles encompassing 21 to 35 days.

At the initial visit in the morning after an overnight fast, a medical history and physical examination were obtained with measurement of height, weight, systolic and diastolic blood pressure, fasting blood glucose, hemoglobin A1C (HgA1C), and endocrine, lipid, and coagulation tests (Table 1). We did not measure the waist to hip ratio.

After the initial visit, the first nine patients started on Metformin 850 mg three times per day with meals, and continued on this dose throughout the study. The subsequent 34 patients started on Metformin 500 mg three times per day with meals for 8 weeks. To reduce the diarrhea and/or gastritis, which were commonly experienced in the first week of therapy, these 34 patients were instructed to start on 500 mg Metformin with the evening meal for 2 days, then 500 mg with breakfast and with the evening meal for 2 days, and thereafter 500 mg three times per day with meals. After 8 weeks in all subjects, blood

Table 1. Variables in 43 Patients With PCOS at Study Entry, Pre-Metformin

Variable	No. of Subjects	Mean ± SD	Median	Abnormality Range	Abnormality No.	Abnormality %
Age (yr)	43	30 ± 7	31			
Treatment duration (mo)	43	6.1 ± 5.1	4.9			
Weight (lb)	43	217 ± 42	213			
BMI	43	36.3 ± 6.9	34.4	>27	40	93
Systolic BP (mm Hg)	36	127 ± 13	129	>140	2	5
Diastolic BP (mm Hg)	36	81 ± 8	82	>90	4	11
Insulin (μU/mL)	42	37 ± 44	25	≥20	31	74
Glucose (mg/dL)	43	96 ± 36	86	>126	3	7
HgAIC (%)	41	5.6 ± 1.1	5.3	>6.4	4	10
Estradiol (pg/mL)	43	54 ± 75	42	<22	12	28
				<69	36	84
Testosterone (ng/dL)	43	64 ± 38	59	>83	8	19
Androstenedione (ng/dL)	41	200 ± 117	181	>250	10	24
DHEA (ng/dL)	28	502 ± 234	488	>980	2	7
DHEAS (μg/dL)	12	210 ± 150	171	>308	2	17
LH (mIU/mL)	42	9.8 ± 6.9	9.8	>12	12	29
FSH (mIU/mL)	41	5.2 ± 2.2	5.4	<5	16	39
LH/FSH	41	1.86 ± 1.16	1.62	>1.5	23	56
SHBG (nmol/L)	43	44 ± 38	37	<8	5	12
				>85	8	19
Lp(a) (mg/dL)	42	19 ± 25	14	>35	5	12
Cholesterol (mg/dL)	42	193 ± 34	184	>200	14	33
Triglyceride (mg/dL)	42	144 ± 92	108	>250	4	10
HDLC (mg/dL)	42	47 ± 12	46	<35	4	10
LDLC (mg/dL)	42	117 ± 30	110	>130	14	33
PAI-Fx (U/mL)	43	20 ± 16	16	>22	12	28
4G/5G PAI-1 gene polymorphism*	4G4G (n = 11, 27%), 4G5G (n = 23, 56%), 5G5G (n = 7, 17%)					

*5G5G, wild-type normal; 4G5G, heterozygote; 4G4G, homozygote.

sampling (as above) was repeated. If normal menses did not resume by week 8, Metformin was increased to 850 mg three times per day with meals (n = 11). Follow-up evaluation every 8 weeks for at least 6 months was scheduled with a serial interval history, review of adherence to Metformin, review of menstrual status, assessment of any Metformin-related side effects, brief physical examination, determination of weight and blood pressure, and blood sampling after overnight fasting. Pill counts were not made to quantify adherence. No dietary modification was provided. Because follow-up evaluation occurred every 8 weeks and because this time interval intersected the 21 to 35-day intervals for normal menstrual cycles, we could not systematically schedule blood sampling between days 20 and 24 of the menstrual cycle for serum progesterone measurement as an indicator of corpus luteum function.[5]

Although insulin resistance and hyperinsulinemia may be central to many of the pathophysiologic aspects of PCOS,[1-15] to avoid selection bias, we did not require fasting hyperinsulinemia as a mandatory study inclusion criterium.

Nineteen of 43 patients lived greater than 200 miles from Cincinnati. After the initial visit in Cincinnati, they were evaluated in their home towns in conjunction with their local physicians, following the same treatment and laboratory assessment protocol as the 24 Cincinnati-area patients.

Laboratory Methods

At any time during amenorrhea, after 10 to 12 hours of fasting, venous samples were obtained in tubes containing EDTA and in tubes without anticoagulant. Radioimmunoassay[4-6] was used to measure LH, FSH, estradiol, testosterone, androstenedione, dehydroepiandrosterone (DHEA), DHEA sulfate (DHEAS), progesterone, SHBG, and insulin levels. Due to laboratory problems, DHEA and DHEAS levels were measured only at study entry and not in the full cohort. Gonadotropins (LH and FSH) manifest a high degree of intrasubject variation (day to day and hour to hour).[17] Since we did not obtain multiple determinations of LH and FSH over extended periods or use pooled samples from different times, caution must be exercised in the interpretation of LH and FSH results. Additional measurements included glucose, baseline HgA1C, lipid profile, lipoprotein(a), and PAI-Fx, all by previously established methods.[4,6] PAI-Fx and the 4G/5G polymorphism of the PAI-1 gene in controls were not correlated ($P > .09$) with age, sex, or race, making it unlikely that differences between patients and controls for PAI-Fx or 4G/5G polymorphism of the PAI-1 gene represented age, sex, or race effects.[18] The 4G/5G polymorphism of the PAI-1 gene was assessed by the allelle-specific amplification refractory mutation system (ARMS).[18]

Statistical Analysis

The data were predominantly non–normally distributed (Tables 1 to 4 and Fig 1); thus, paired Wilcoxon nonparametric tests[19] comparing variables at baseline versus the last visit on Metformin were made (Table 2). Separately (data not shown), we used paired Wilcoxon tests comparing baseline levels versus the mean of the last two visits on Metformin, with results comparable to those in Table 2. Chi-square analyses or Fisher's exact test (when cell size was <5)[19] were used to compare patients with normals. Spearman correlations[19] between variables at baseline (Table 3) and between changes on Metformin (Table 4) were performed. Stepwise multiple regression[19] was used to assess determinants of PAI-Fx at study entry and determinants of Metformin-induced changes in estradiol, testosterone, systolic blood pressure, diastolic blood pressure, BMI, and triglyceride.

We used ANOVA[19] to determine whether different doses of Metformin (2.55 g/d throughout, n = 9; 1.5 g/d throughout, n = 23; and 1.5 g/d for 8 weeks increased to 2.55 g/d thereafter, n = 11) lead to different degrees of Metformin-induced change in fasting insulin, testosterone, estradiol, SHBG, triglyceride, BMI, and systolic and diastolic blood pressure. We used covariance adjustment for entry levels of the variables and for the duration of Metformin therapy (as a continuous variable) and compared the covariance-adjusted least-square means[19] of the Metformin-induced changes in the variables versus baseline among the three dose groups. Separately, for the three Metformin therapy duration groups (<3 months, n = 16; 3 to 6 months, n = 12; and ≥6 months, n = 15), we used ANOVA with covariance adjustment for entry levels of the variables and for the Metformin dose, comparing the covariance-adjusted least-square means[19] of the Metformin-induced changes in variables versus baseline among the three duration groups.

To determine if there were differences in the response between 28 patients who lost weight on Metformin and those who did not, we performed the following analyses. We assessed correlations between Metformin-induced changes in BMI and response variables by Spearman correlations (Table 4).[19] We compared the responses to Metformin in patients who lost weight and those who did not (Wilcoxon tests).[19] We also stratified changes in weight on Metformin into quintiles, and compared response variables among quintiles by Newman-Keuls analysis.[19] Separately, we covariance-adjusted[19] the changes in response variables on Metformin by the change in weight to determine whether and to what degree the change in weight determined a change in response variables (Fig 1).

RESULTS

Characteristics of Patients With PCOS

Polycystic ovaries (thickened abnormal stroma, ≥10 cysts 2 to 8 mm in diameter)[4] were diagnosed in 40 (93%) of the patients, by pelvic ultrasound in 28 (65%) and laparoscopy in 12 (28%). The three patients (7%) without pelvic ultrasound or laparoscopy had acanthosis nigricans and a LH/FSH ratio greater than 1.5 (1.82, 2.5, and 2.7). Acanthosis nigricans was present in 11 (26%) patients. All 43 patients had chronic oligomenorrhea and all (by selection) were amenorrheic for at least 6 months. All 43 patients were hirsute with Ferriman-Gallwey Scores greater than 7.[16] At study entry, 23 of 41 (56%) patients had a LH/FSH ratio greater than 1.5 (Table 1).[4]

At study entry, fasting blood glucose and HgA1C were normal (<126 mg/dL and <6.4%) in 40 (93%) and 37 women (two missing HgA1C levels). One woman had normal glucose (88 mg/dL) and marginally high HgA1C (6.5%). Three (7%) women had type 2 diabetes mellitus with fasting blood glucose and HgA1C levels of 234 mg/dL and 11.1%, 146 mg/dL and 8.7%, and 251 mg/dL and 7.2%, respectively.

The cohort was very obese (median weight, 213 pounds); 93% had a BMI greater than 27[4] (Table 1). Most women (72%) were morbidly obese (Quetelet index [$kg/cm^2 \times 1{,}000$] ≤ the Lipid Research Clinics population prevalence age/sex-specific 95th percentile[20]). The cohort was hyperinsulinemic (median fasting serum insulin, 25 μU/mL; normal, <20 μU/mL); 74% had fasting hyperinsulinemia. Systolic and diastolic hypertension were present in 5% and 11% of the patients (Table 1).

Low estradiol was a characteristic finding, below normal follicular levels (<22 pg/mL) in 28% and below normal midcycle levels (<69 pg/mL) in 84%. Nineteen percent of the patients had high testosterone, 24% high androstenedione, 7% high DHEA, and 17% high DHEAS (Table 1).

The median PAI-Fx was high (16 U/mL; 95th and 97.5th percentile in 40 healthy normal adults, 16.4 and 22 U/mL). Women with PCOS were much more likely than the 40 normal controls (28% *v* 3%, $\chi^2 = 10.1$, $P = .001$) to have high PAI-Fx (>22 U/mL) (Table 1).

High LDL cholesterol (>130 mg/dL) was present in 33% of the women, high triglyceride (>250 mg/dL) in 10%, and low HDL cholesterol (<35 mg/dL) in 10% (Table 1).

4G/4G Polymorphism of the PAI-1 Gene

The patients were more likely than the 234 normal controls (83% *v* 64%, $P = .016$) to be heterozygous or homozygous for the 4G polymorphism[18] of the PAI-1 gene. Of 43 patients, 27% were homozygous for the 4G/4G polymorphism of the PAI-1 gene, 56% heterozygous, and 17% wild-type normal, versus 20%, 44%, and 36% of normals[17] ($\chi^2 = 5.82$, $P = .055$). By stepwise multiple regression with PAI-Fx as the dependent variable and insulin, triglyceride, BMI, and PAI gene polymorphism as explanatory variables, 14% of the variance of PAI-Fx could be accounted for by 4G/5G polymorphism of the PAI gene ($R^2 = 14.0\%$, $P = .019$).

Metformin Therapy and Resumption of Normal Menses

Metformin was given for 6.1 ± 5.1 months (median, 4.9; range, 1.5 to 23.6 m). Sixteen patients took Metformin for less than 3 months (five of 16 for <2 months), 12 for 3 to 6 months, and 15 for at least 6 months. On Metformin, 39 of 43 patients (91%) resumed normal menses. The percentage of patients resuming normal menses did not differ among Metformin treatment duration groups ($P > .1$) or among Metformin dose groups ($P > .1$).

Serum progesterone was measured during the follicular phase of the menstrual cycle in 13 menstruating women (0.3, 0.6, 0.6, 0.6, 0.8, 0.8, 1.0, 1,1, 1.3, 1.4, 1.5, 1.9, and 2.0 ng/mL; normal range, 0.2 to 2.1). Serum progesterone was measured during the luteal phase in six women who resumed normal menses (1.1, 2.3, 2.9, 5.7, 6.1, and 10.3 ng/mL; normal range, 2.3 to 25.9). Five of these six women had progesterone at an ovulatory level[5] (≥2.3 ng/mL).

Reduction in BMI and Weight on Metformin Therapy

The median BMI decreased from 35 to 34.4 Kg/m^2 on Metformin ($P = .0008$; Fig 1 and Table 2). The mean weight at study entry, 217 ± 43 pounds, decreased on therapy to 210 ± 42 ($P = .0008$). Weight loss occurred in 28 (67%) patients, from 1 to 69 pounds, with 21% having a weight loss of at least 12 pounds.

Relationship Between Reduction in BMI or Weight and Changes in Response Variables

The change in BMI on Metformin was not correlated ($P > .05$) with the change in fasting glucose, insulin, testosterone, androstenedione, estradiol, LH, FSH, or SHBG. The change in BMI was inversely related to the duration of Metformin therapy ($r = -.41$, $P = .007$), the change in Lp(a) ($r = -.55$, $P = .0035$), and the change in PAI-Fx ($r = -.55$, $P = .004$) (Table 4). The change in response variables on Metformin did not differ between 28 patients who lost weight and those who did not ($P > .05$), excepting Lp(a), which increased 4.3 mg/dL in those who lost weight and decreased 9 mg/dL in those who did not ($P = .003$). The change in response variables on Metformin did not differ among the five quintiles of weight loss, excepting fasting glucose ($P < .05$), which increased 6 mg/dL in those who lost the least weight on Metformin versus those in the 60th to 80th percentile for weight loss, whose mean blood glucose decreased 33 mg/dL. After covariance adjustment for changes in weight on Metformin, Metformin-induced changes in the following response variables remained significant: testosterone decreased ($P = .002$), estradiol increased ($P = .0004$), and systolic blood pressure decreased ($P = .055$) (Fig 1).

Reversal of Endocrinopathy on Metformin

The median testosterone at baseline was 61 ng/dL, decreasing on therapy to 47 ($P = .003$). The median estradiol was 41 pg/mL at study entry, and increased on Metformin to 71 ($P = .0001$). The median LH was 9.5 mIU/mL at study entry, and increased slightly on therapy to 10.7. The median SHBG was 19 nmol/L at baseline and 22 on Metformin (Table 2 and Fig 1).

Reduction in Fasting Serum Insulin on Metformin

The median fasting serum insulin, 26 μU/mL at study entry, decreased on Metformin to 22 ($P = .019$) (Table 2 and Fig 1). Of 31 patients with high entry insulin (≥20 μU/mL), three showed a decrease to less than 20 on therapy.

PCOS Patients With Normal Baseline Fasting Insulin (<20 μU/mL) Versus High Fasting Insulin (≥20 μU/mL): Response to Metformin

When categorizing subjects by high fasting serum insulin at study entry (≥20 [n = 31] *v* normal <20 μU/mL [n = 11]), there was no insulin group difference ($P > .05$) in the changes for any of the other measured variables during Metformin therapy. Patients with fasting insulin less than 20 μU/mL at study entry (n = 11) did not have a significant reduction of insulin on Metformin (median, 0), while those with entry insulin of at least 20 had a significant reduction (median, −6 μU/mL, $P = .011$).

Reduction in Triglycerides on Metformin

The median triglyceride level at study entry was 106 mg/dL, and decreased on therapy to 79 ($P = .012$; Table 2 and Fig 1).

Reduction in Blood Pressure on Metformin

On Metformin, the median systolic blood pressure decreased from 128 to 118 mm Hg ($P = .008$); median diastolic blood pressure decreased from 78 to 74 mm Hg ($P = .03$) (Table 2 and Fig 1).

Response to Metformin Relative to Duration and Dose of Therapy

The three treatment duration groups (<3, 3 to 6, and ≥6 months) did not differ ($P > .05$) with regard to Metformin-induced changes in insulin, testosterone, estradiol, SHBG, triglyceride, systolic blood pressure, or diastolic blood pressure. They did differ in regard to weight reduction, with a mean weight reduction in 15 subjects on Metformin for at least 6 months (16 pounds) greater than that in the group with treatment for less than 3 months (2.4 pounds, $P = .036$, n = 16) and the 3- to 6-month group (2 pounds, $P = .027$, n = 12). The

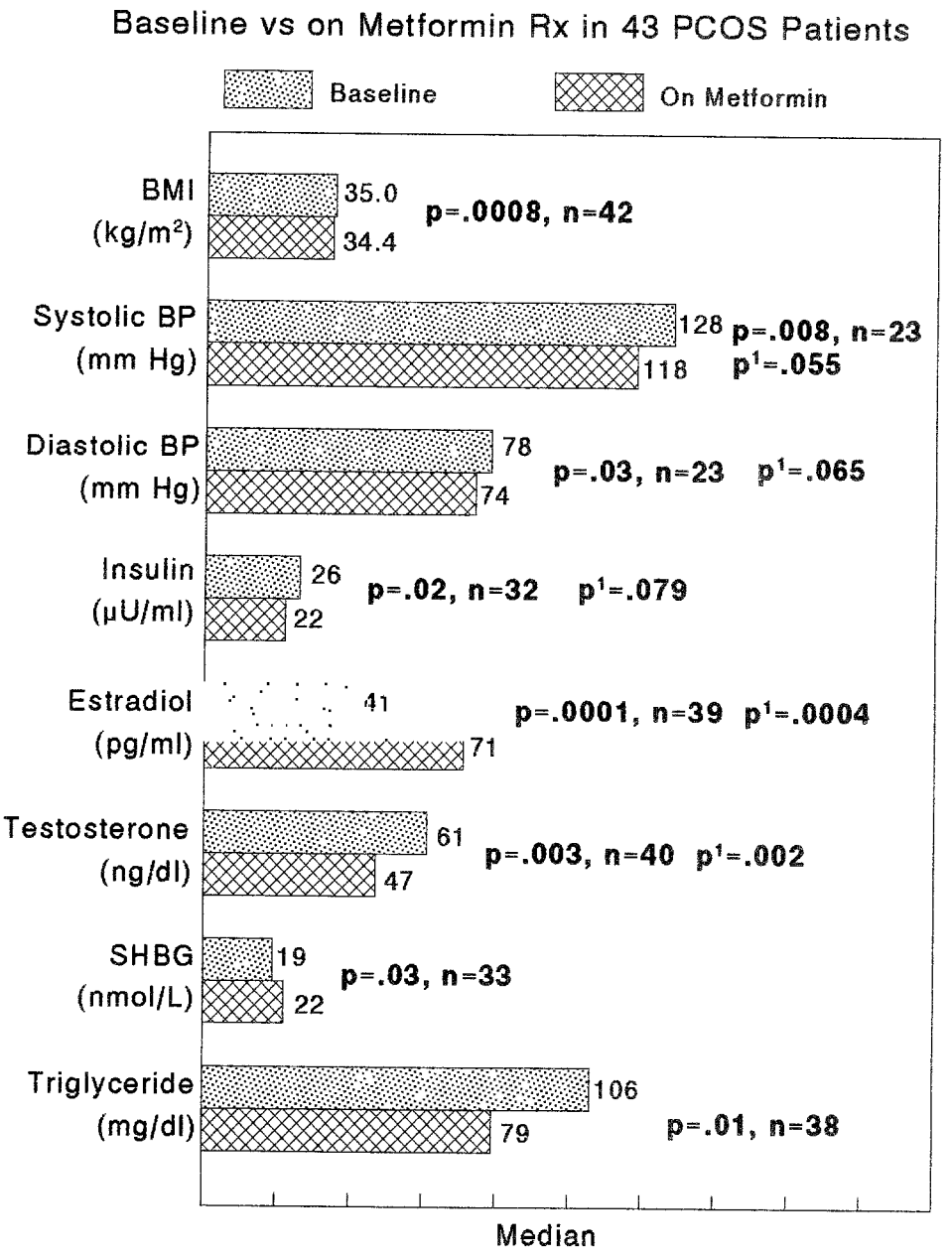

Fig 1. Median BMI, systolic and diastolic blood pressure, fasting serum insulin, estradiol, testosterone, SHBG, and triglyceride levels at study entry and at the last visit on Metformin. *P* values are for paired Wilcoxon[19] tests, and *P*¹ values are for paired tests after covariance adjustment[19] for weight loss.

longer the duration of Metformin therapy, the greater the reduction in BMI ($r = -.41$, $P = .007$). Among the three Metformin dose groups, changes from baseline did not differ ($P > .05$) for insulin, testosterone, estradiol, SHBG, triglyceride, weight, or diastolic blood pressure. Metformin-induced decrements in systolic blood pressure were greater in 11 subjects whose Metformin dosage increased from 1.5 to 2.55 g/d versus nine on 2.55 g/d throughout ($P = .012$).

Correlations Between Variables at Baseline

At baseline, fasting serum insulin was positively correlated with the BMI, glucose, HgA1C, and triglyceride and inversely correlated with SHBG, Lp(a), and HDL cholesterol. At baseline, fasting serum insulin was not significantly correlated with testosterone ($r = .24$, $P = .13$) or androstenedione ($r = .27$, $P = .09$) (Table 3). Fasting glucose was positively correlated with androstenedione and triglyceride and inversely associated with Lp(a) and HDL cholesterol. Testosterone was positively correlated with estradiol, androstenedione, and LH and inversely correlated with SHBG. Androstenedione correlated positively with LH and estradiol and inversely with SHBG (Table 3).

Correlations Between Changes on Metformin

On Metformin, the change in fasting serum insulin correlated positively with the change in testosterone ($r = .35$, $P = .047$) and with androstenedione ($r = .48$, $P = .01$) (Table 4). Changes in testosterone were positively related to changes in androstenedione and inversely related to changes in SHBG. Changes in androstenedione correlated positively with changes in estradiol and LH. Changes in triglycerides were inversely associated with changes in Lp(a). The longer the duration of therapy on

Table 2. Changes on Metformin Therapy in 43 PCOS Patients

Variable	No. of Subjects	Baseline		On Metformin		Change		
		Mean ± SD	Median	Mean ± SD	Median	Mean	Median	P*
Weight (lb)	42	217 ± 43	216	210 ± 42	214	−7	−4	.0008
BMI	42	36.4 ± 7.0	35.0	35.1 ± 6.7	34.4	−0 13	−0.07	.0008
Systolic BP (mm Hg)	23	124 ± 13	128	118 ± 9	118	−7	−6	.008
Diastolic BP (mm Hg)	23	80 ± 8	78	76 ± 6	74	−4	−2	.03
Insulin (μU/mL)	32	41 ± 49	26	25 ± 17	22	−16	−5	.019
Glucose (mg/dL)	33	98 ± 38	86	92 ± 18	87	−5	−1	
Estradiol (pg/mL)	39	44 ± 33	41	91 ± 68	71	+48	+26	.0001
Testosterone (ng/dL)	40	66 ± 38	61	49 ± 26	47	−16	−20	.003
Androstenedione (ng/dL)	29	196 ± 122	171	186 ± 106	178	−10	−1	
LH (mIU/mL)	34	9.3 ± 5.8	9.5	13.9 ± 11.7	10.7	+4.6	+1.8	
FSH (mIU/mL)	36	5.5 ± 2.1	5.6	6.0 ± 2.5	5.5	+0.5	+0.3	
LH/FSH	33	1.70 ± 0.89	1.60	2.22 ± 1.34	2.00	+0.52	+0.15	
SHBG (nmol/L)	33	36 ± 32	19	26 ± 20	22	−10	−1	.03
Lp(a) (mg/dL)	26	20 ± 23	14	19 ± 23	13	−1	0	
Cholesterol (mg/dL)	38	194 ± 35	186	188 ± 44	185	−5	−11	
Triglyceride (mg/dL)	38	141 ± 96	106	115 ± 76	79	−26	−19	.01
HDLC (mg/dL)	38	48 ± 12	47	48 ± 13	45	0	0	
LDLC (mg/dL)	38	117 ± 31	110	118 ± 34	118	0	−5	
PAI-Fx (U/mL)	26	20 ± 16	15	16 ± 11	16	−4	−1	

*Wilcoxon paired test.

Metformin, the smaller the change in HDL cholesterol (Table 4). The change in PAI-Fx was inversely correlated with the change in systolic blood pressure.

By stepwise multiple regression with the change in testosterone as the dependent variable and the change in insulin, BMI, and SHBG as explanatory variables, the change in SHBG was a negative determinant of change in testosterone ($R^2 = 17.8\%$, $P = .03$). By stepwise multiple regression with the change in triglyceride as the dependent variable and the change in insulin, BMI, estradiol, and testosterone as explanatory variables, the change in insulin was a positive determinant of the change in triglyceride ($R^2 = 23\%$, $P = .011$). By stepwise multiple regression, there were no significant explanatory variables for the Metformin-induced change in systolic blood pressure, diastolic blood pressure, BMI, or estradiol. By stepwise multiple regression as above, the change in BMI was never a significant independent variable for any of the other changes on Metformin.

Table 3. Significant Correlations Between Variables at Study Entry in 43 PCOS Patients

Variable	r	P
Insulin		
BMI	.31	.049
Glucose	.38	.013
HgA1C	.34	.034
Triglyceride	.52	.0005
SHBG	−.46	.002
Lp(a)	−.42	.007
HDLC	−.44	.004
Glucose		
Androstenedione	.39	.012
Triglyceride	.41	.008
HDLC	−.32	.04
Lp(a)	−.43	.004
Testosterone		
Estradiol	.70	.0001
Androstenedione	.74	.0001
LH	.40	.008
SHBG	−.41	.006
Androstenedione		
LH	.58	.0001
Estradiol	.59	.0001
SHBG	−.53	.0003

Metformin Side Effects

Most patients experienced intermittent diarrhea and/or gastritis during the first week of therapy, which then resolved for the most part. Three patients targeted for 2.55 g/d Metformin could not receive it because of diarrhea, taking 2.0, 1.5, and 1 g/d, respectively, during the study. No patients developed lactic acidosis, and none became hypoglycemic. Type 2 diabetes mellitus was well controlled (HgA1C < 7%) in the three diabetic patients.

Table 4. Significant Correlations Between Changes on Metformin in 43 PCOS Patients

Variable	No. of Subjects	r	P
ΔBMI/duration on Rx	42	−.41	.007
ΔBMI/ΔLp(a)	26	−.55	.004
ΔBMI/ΔPAI-Fx	26	−.55	.004
ΔInsulin/Δtestosterone	32	.35	.047
ΔInsulin/Δandrostenedione	27	.48	.012
ΔTestosterone/Δandrostenedione	29	.45	.013
ΔTestosterone/ΔSHBG	33	−.48	.005
ΔAndrostenedione/Δestradiol	28	.41	.033
ΔAndrostenedione/ΔLH	24	.70	.0001
ΔTriglyceride/ΔLp(a)	25	−.53	.006
ΔHDLC/duration on Rx	38	−.40	.014
ΔHDLC/ΔFSH	36	−.36	.033
ΔPAI-Fx/Δsystolic BP	19	−.53	.02

Abbreviation: Δ, change.

DISCUSSION

Nine previous studies[2-6,10,11,14,15] using Metformin (≥1.5 g/d) in obese women with PCOS (mean BMI, 27.7,[5] 29.1,[4] 26.7,[6] 31.5,[14] 32.2 to 32.3,[2] 33.6,[15] and 34.1[3]) have shown a significant improvement in insulin sensitivity, a reduction of hyperinsulinemia, and, to varying degrees, weight loss and reductions in hyperandrogenemia and PAI-1. The women in our study had even greater relative ponderosity,[2-6,14,15] with a mean BMI of 36.4. Importantly, these nine previous studies[2-6,10,11,14,15] have, to varying degrees, shown improvements in ovulatory function, development of normal menses, and restoration of fertility. As many as 21 of 22 (96%) previously oligomenorrheic-amenorrheic obese (mean, BMI 27.7) Mestizo women with PCOS had a restoration of menstrual cyclicity on Metformin (1,500 mg/d) for at least 6 months.[5] In a recent study of 20 obese Finnish women with PCOS, Morin-Papunen et al[14] reported that 11 (69% of those with menstrual disturbances) experienced more regular cycles during therapy with Metformin 1,500 mg/d for 6 months. On Metformin, fasting serum insulin decreased, testosterone decreased at 2 months but returned close to starting levels at 6 months, and free testosterone decreased.[14] There were no significant changes in hirsutism, BMI, or blood pressure.[14] Diamanti-Kandarakis et al[15] reported resumption of normal menses in 44% of 16 Greek women with PCOS on Metformin 1,700 mg/d for 6 months, accompanied by increased glucose utilization ($P = .0001$) measured by the two-step euglycemic-hyperinsulinemic clamp. After 35 days on Metformin 1,500 mg/d in 35 obese women with PCOS, Nestler et al[2] reported that 12 (34%) ovulated, versus one of 26 (4%) given placebo ($P < .001$). Subsequently, 21 women continued on Metformin and 25 on placebo, with both groups given 50 mg Clomiphene for 5 days.[2] Of 21 women on Metformin and Clomiphene, 19 (90%) ovulated, versus two of 25 (8%) on placebo and Clomiphene ($P < .001$).[2] In the Metformin group, the area under the serum insulin curve during the oral glucose tolerance test decreased ($P = .002$), but it did not change in the placebo group.[2] The BMI did not change on Metformin or placebo; the waist to hip ratio decreased on Metformin ($P < .001$) but not on placebo.[2]

However, the response to Metformin in PCOS patients has not been entirely uniform.[7,12] Ehrmann et al,[12] in very obese women with PCOS (mean BMI, 39.0), used 2.55 g Metformin/d for 12 weeks and reported no changes in insulin, hormones, BMI, or fat distribution. Similarly, Acbay et al[7] gave 1,700 mg Metformin/d for 10 weeks to obese women with PCOS (mean BMI, 29.8) and noted no changes in insulin metabolism, hormones, BMI, or waist to hip ratio.

A second insulin-sensitizing agent, Rezulin (200/400 mg/d for 12 weeks[8] or 400 mg/d for 12 weeks[9]), has been reported to be efficacious in two studies of very obese women with PCOS (mean BMI, 42[8] and 39.9[9]). In both studies,[8,9] there were improvements in insulin metabolism, androgens, SHBG, and PAI-1,[9] without changes in the BMI. In one study,[8] two women resumed ovulatory menses.

In aggregate, hyperinsulinism and resultant hyperandrogenism chronically alter gonadotropin secretion, increasing LH,[1-15,21,22] disrupting the normal pituitary-ovarian axis, and leading to oligomenorrhea-amenorrhea and infertility. Hyperinsulinism, in conjunction with hyperandrogenemia, also leads to morbid obesity, hirsutism, acne, frequent hypertension, hyperlipidemia, and increased levels of hypofibrinolytic PAI-1, which together increase the risk for myocardial infarction and stroke later in life.[1-14,21-24] Hyperinsulinemia, independent of other risk factors for coronary heart disease (CHD), is a major CHD risk factor.[23,24] Insulin resistance-hyperinsulinemia is also a risk factor for type 2 diabetes mellitus,[25] common in PCOS. Despite their predominant obesity and fasting hyperinsulinemia, in the current study, only three of 43 (7%) patients had type 2 diabetes mellitus.

In patients with PCOS, we have previously shown that the decrement in fasting serum insulin on Metformin was a significant independent positive determinant of the reduction in total and free serum testosterone and the free androgen index.[4] The metformin-induced improvement in ovarian function was independent of the weight loss and reduction in the waist to hip ratio.[4] The significant associations between the decrements in fasting serum insulin and in testosterone and androstenedione in the current study are entirely congruent with previous data suggesting that when Metformin reduces serum insulin, the reductions in serum insulin lead directly to consequent decrements in androgens with unblocking of the normal pituitary-ovarian feedback system.[2-6,10,11,14,15,22]

The association of fasting and postglucose challenge plasma insulin response areas with fasting testosterone and androstenedione has been known for nearly two decades.[26] In the current study, although pretreatment fasting serum insulin was not significantly correlated with testosterone ($r = .24$, $P = .13$) or androstenedione ($r = .27$, $P = .09$), on Metformin, and congruent with previous reports,[4,6] the change in insulin correlated positively with the change in testosterone ($r = .35$, $P = .047$) and with the change in androstenedione ($r = .48$, $P = .01$). The lack of a significant correlation between baseline fasting insulin, testosterone, and androstenedione in the current study is consistent with the findings of Toscano et al[27] but different from the findings of Burghen et al.[26] We speculate that differences in the relative ponderosity, itself strongly correlated with fasting serum insulin, as in the current study, account in part for the differences among insulin, testosterone, and androstenedione correlations.[12,14,26,27]

Metformin[6] and Rezulin,[8,9] by reducing PAI-1 and (for Metformin) by reducing weight, triglycerides, and blood pressure, should also reduce the increased risk for atherothrombosis that characterizes PCOS patients in later adulthood.[1,3-6,14,15,21,22] In the current study, Metformin therapy was associated with reductions in fasting serum insulin and testosterone, increments in estradiol, and reductions in the BMI, systolic and diastolic blood pressure, and triglycerides, and should thus be cardioprotective.[4-6,14,15,21-25]

Insulin resistance and consequent hyperinsulinemia and hyperandrogenemia are probably central in the pathogenesis of PCOS.[1-16,21-25] In the current study, congruent with nine previous reports,[1-6,10,11,14,15] on Metformin, 39 of 43 (91%) previously amenorrheic women resumed normal menstrual cyclicity. Five of six women who resumed normal menses and who were sampled during the luteal phase between cycle days 20 and 24

had serum progesterone at ovulatory levels.[5] Fasting hyperinsulinemia (≥20 μU/mL) was not a prerequisite for the response to Metformin. By restoring normal menstrual cycles,[2,5] Metformin may also reduce the likelihood of endometrial hyperplasia and carcinoma associated with PCOS.[28]

In the current study, Metformin-induced ovarian function improvements (reduction in testosterone and increment in estradiol) were independent of weight loss. After covariance adjustment for changes in weight on Metformin, decrements in testosterone and increments in estradiol remained significant. On Metformin, the change in BMI was not correlated ($P > .05$) with the change in fasting glucose, insulin, testosterone, androstenedione, estradiol, LH, FSH, or SHBG. The change in response variables on Metformin did not differ between patients who lost weight and those who did not ($P > .05$), excepting Lp(a), which increased 4.3 mg/dL in those who lost weight and decreased 9 mg/dL in those who did not ($P = .003$). The change in response variables did not differ among the five quintiles of weight loss, excepting fasting glucose, which increased 6 mg/dL in those who lost the least weight and decreased 33 mg/dL in those in the 60th to 80th percentile for weight loss on Metformin. Although weight loss did not appear to play a significant independent role in the reversal of endocrinopathy or amenorrhea in the current study, ovarian dysfunction in obese oligomenorrheic women with PCOS can be improved by weight loss alone.[13,29] However, weight loss (through caloric restriction and increased exercise) in PCOS patients is difficult to achieve and maintain, due to the countervailing anabolic effects of high insulin, androstenedione, testosterone, and DHEAS.

In this study, the 4G/5G polymorphism of the PAI-1 gene[18] independently ($P = .019$) accounted for 14% of the variance of PAI-Fx. Since women with PCOS were more likely than normals to have the 4G/5G polymorphism, it is not surprising that women with PCOS had high PAI-Fx.

There are several limitations to our study. It was not randomized or placebo-controlled and had no crossover from placebo to Metformin and vice versa. We did not attempt a weight maintenance program where caloric intake would have been adjusted to avoid weight loss, so that the effects of Metformin could be studied independently of weight loss. Such a study probably cannot successfully be performed in outpatients, requiring months in a clinical research center where energy intake and output are minutely controlled. Some of the findings in the current study were unexpected and are unexplained, in contrast to our previous studies[4,6] but not the previous studies by Morin-Papunen et al.[14] Despite an improvement in ovarian function on Metformin, LH did not decrease as shown in some[4,6] but not all[14] studies, and SHBG failed to increase substantially as in earlier studies.[4,6] Systematic measurement of DHEAS and the testosterone/DHEAS ratio in future extensions of the current study will be important in potentially providing further insight into the mechanism of improved ovarian function with Metformin.

Further confirmation of the beneficial effects of Metformin[1-6,10,11,14,15] and Rezulin[8,9] on the endocrinopathy of PCOS may, either alone or coupled with Clomiphene,[2] revolutionize its treatment, and should also reduce the increased risk of atherothrombosis in patients with PCOS long-term.

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