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The Insulin-Sensitizing Agent Troglitazone Improves Metabolic and Reproductive Abnormalities in the Polycystic Ovary Syndrome*

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ABSTRACT

We performed this study to investigate the hypothesis that insulin resistance plays a role in the pathogenesis of reproductive abnormalities in women with the polycystic ovary syndrome (PCOS). Twenty-five women with PCOS were enrolled in a double-blind randomized 3-month trial of two doses of the insulin-sensitizing agent, troglitazone, 21 of whom completed the study: 200 mg, n = 10; 400 mg, n = 11. Baseline hormonal parameters and glucose tolerance were compared with 12 age- and weight-matched ovulatory control women. There were no significant changes in body mass index during the study. Fasting ($P < 0.01$) and 2-h post-75-g glucose load insulin levels ($P < 0.05$), as well as integrated insulin responses to the glucose load, decreased ($P < 0.05$), and insulin sensitivity assessed by a frequently sampled iv glucose tolerance test increased significantly ($P < 0.001$) during troglitazone treatment. This was accompanied by significant decreases in the levels of nonsex hormone-binding globulin-bound testosterone ($P < 0.01$), dehydroepiandrosterone sulfate ($P < 0.001$), estradiol ($P < 0.01$), and estrone ($P < 0.001$). Stepwise regression analysis indicated that decreases in nonsex hormone-binding globulin testosterone levels were significantly correlated with decreases in integrated insulin responses to the glucose load (r^2 0.44, $P < 0.01$). The only significant changes at the 200-mg troglitazone dose were an increase in insulin sensitivity ($P < 0.05$) and decreases in dehydroepiandrosterone sulfate ($P < 0.01$) and estrone ($P < 0.05$) levels. At the 400-mg dose, in addition to the changes noted in the entire troglitazone treatment group, increases in the disposition index (the product of insulin sensitivity and secretion) achieved significance, as did decreases in androstenedione ($P < 0.01$) and LH ($P < 0.05$) levels and increases in sex hormone-binding globulin levels ($P < 0.01$). Two PCOS women had ovulatory menses.

We conclude that 1) troglitazone improves total body insulin action in PCOS, resulting in lower circulating insulin levels; 2) insulin resistance, probably via hyperinsulinemia, results in a general augmentation of steroidogenesis and LH release in PCOS; and 3) insulin-sensitizing agents, such as troglitazone, may provide a novel therapy for PCOS. (*J Clin Endocrinol Metab* **81:** 3299–3306, 1996)

THE POLYCYSTIC ovary syndrome (PCOS) is among the most common disorders of premenopausal women, affecting 5–10% of this population (1–3). It is a syndrome of unknown etiology characterized by hyperandrogenism and chronic anovulation (3, 4). Women with PCOS frequently are insulin resistant and at increased risk to develop glucose intolerance or noninsulin-dependent diabetes mellitus (NIDDM) in the third and fourth decades of life (3–7). Hyperandrogenism also is a feature of a variety of diverse insulin-resistant states, from the type A syndrome, through leprechaunism and lipoatrophic diabetes, to the type B syndrome, when these conditions occur in premenopausal women (4). Hyperinsulinemia is common to all of these insulin-resistant states, and it has been proposed that hyperinsulinemia *per se* causes hyperandrogenism (4, 5). Consistent with this hypothesis, in short-term studies in PCOS women, insulin infusions have increased androgen levels, whereas decreasing circulating-insulin levels with diazoxide or somatostatin analog has decreased them (8–10).

There are agents available that decrease circulating-insulin levels by improving insulin sensitivity (11, 12). Such agents are particularly attractive for use in insulin-resistant individuals, because they may improve several associated metabolic parameters of the so-called "insulin resistance syndrome" such as hypertension and dyslipidemia, in addition to hyperinsulinemia (11–13). The biguanide, metformin, does improve insulin sensitivity, but its major action is to reduce gluconeogenesis, resulting in decreased hepatic glucose production (11, 14, 15). Metformin administration has resulted in decreased androgen levels in PCOS women (16), but this seems to be the result of weight loss (which can decrease androgen levels and cause ovulation in PCOS (17, 18) induced by this agent (19). Another class of insulin-sensitizing agents, the thiazolidinediones, produce marked improvements in muscle insulin sensitivity without weight changes (12, 20, 21). We have used one of these agents, troglitazone, to determine whether it could improve insulin sensitivity (thereby decreasing hyperinsulinemia) and result in decreases in circulating-androgen levels in PCOS women. We document that improvements in insulin sensitivity are accompanied by decreases in androgen excess in PCOS women.

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Materials and Methods

Subjects

Twenty-five obese (body mass index [BMI] ≥ 27 kg/m^2) PCOS women were recruited from our PCOS population and 21 completed the study. Four women did not complete the study (3 for personal reasons and 1 because she became pregnant in the first month of the study). Twelve age- and weight-matched normal women served as controls for baseline studies of reproductive hormones and carbohydrate tolerance; these studies were performed without regard for the phase of the menstrual cycle. All studies were approved by the Institutional Review Board of the Pennsylvania State University College of Medicine, and written informed consent was obtained from each subject before study. All of the women were in good health, between 18–40 yr old, euthyroid, and, for at least 3 months before study, not taking any medication (including oral contraceptive agents) known to affect carbohydrate or sex hormone metabolism. None of the women engaged in regular aerobic activity, nor did they have a history of diabetes mellitus or hypertension. There was no history of diabetes mellitus in the first degree relatives of the control women. Normal women had regular menses every 27–32 days; no hirsutism or elevated plasma androgen levels were present.

PCOS was diagnosed by an elevation of total or nonsex hormone-binding globulin (non-SHBG) testosterone (T) levels associated with chronic oligo- (6 or fewer menses per year) or amenorrhea (6, 7). Nonclassical 21-hydroxylase deficiency was excluded by a 1-h ACTH-stimulation test (22). No woman had an elevated plasma PRL level. Ovarian morphology was not assessed, and the presence of acanthosis nigricans was not used to stratify subjects (1, 22, 23). Waist-to-hip girth ratios (WHR) were determined and a 2-h 75-g oral glucose tolerance test (OGTT, glucose and insulin levels q 30 min) was performed as reported (24). PCOS women with diabetes mellitus were excluded from the study. All of the control women had normal glucose tolerance by WHO criteria (25).

Study protocol (Fig. 1)

After the baseline OGTT, PCOS women had a modified, frequently sampled iv glucose tolerance test (FSIGT) performed (visit 1). Therapy with troglitazone then was initiated in a randomized double-blind trial of 200 mg or 400 mg daily. Each woman took either two 200-mg troglitazone tablets or a 200-mg troglitazone tablet and an identical placebo tablet orally each morning with breakfast. Women then returned monthly. At baseline and at each monthly visit, an iv catheter was inserted into a forearm or antecubital fossa vein between 0800–0900 h and three blood samples were obtained 10 min apart. The serum was separated from each sample, and equal aliquots of serum were pooled for the assay of T, non-SHBG-bound T (uT), androstenedione (A), dehydroepiandrosterone sulfate (DHEAS), SHBG, estrone (E_1), estradiol (E_2), LH, and FSH levels. Progesterone (P) levels were determined in the serum samples from visits 1 and 4 as well as in a blood sample obtained 21 days after menses to assess whether ovulation had occurred. Blood also was sampled at visits 2 and 3, basally and 2 h after a 75-g glucose load, for plasma glucose and insulin levels. A single blood pressure (BP) determination was performed in the sitting position at each visit. At visit 4, day 1, a 75-g glucose OGTT identical to baseline was performed and on day 2, an FSIGT identical to visit 1. Fasting C-peptide levels were determined at visits 1 and 4.

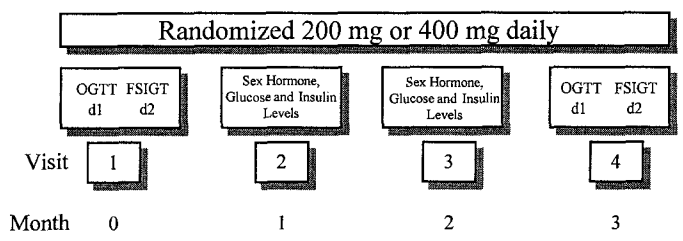

FIG. 1. PCOS women received either 200 mg or 400 mg troglitazone in a single daily dose with breakfast in a randomized double-blind trial. Tests were performed as indicated: 75-g OGTT, modified FSIGT, and hormone levels (C-peptide, sex hormone, SHBG, and gonadotropin).

Before all visits, subjects ate a 300-g carbohydrate diet for 3 days and fasted overnight before testing. Pill bottles were returned at each visit, any remaining pills counted, and pills for the next month dispensed. At each visit, a urine pregnancy test was performed using an Abbot Testpack plus hCG urine kit (Abbot Park, IL). As a monitor of general drug safety, a complete blood count with differential, hepatic, and renal chemistries and an electrocardiogram were performed at baseline and month 3.

Modified FSIGT

The FSIGTs were performed after a standard overnight fasting period of 10 h. Women had two iv catheters inserted, one in each arm, and then were allowed to rest for 30 min. At time zero, glucose (0.3 g/kg) was injected over 1 min, and at 20 min, 500 mg tolbutamide (Upjohn Co, Kalamazoo, MI) was injected over 20 sec. Blood samples were drawn at −15, −10, −5, −1, 0, 2, 3, 4, 5, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 90, 100, 120, 140, 160, and 180 min. The insulin-sensitivity index (S_I) and glucose effectiveness (S_G) (MINMOD computer program version NUDEMM1, Richard Bergman, Los Angeles, CA) and the acute insulin response to glucose (AIRg) were calculated as reported (26, 27).

Assays

Assays for T, uT, A, DHEAS, SHBG, E_1, E_2, P, glucose, LH, and FSH were performed as reported (7, 24, 28, 29). Plasma insulin and C-peptide levels were measured using Diagnostic Products Corporation kits (Los Angeles, CA).

Data analysis

Comparisons between baseline data in control and PCOS women were made by unpaired *t* tests. During the troglitazone treatment, each PCOS woman served as her own control, and comparisons between baseline and treatment were made by paired *t* test or signed rank test, depending on the normality of the data. The integrated glucose (area under the curve glucose [AUC_G]) and insulin (AUC_I) responses during the OGTT were determined using the trapezoidal rule. Repeated measures of ANOVA with Bonferroni correction was performed on the data from each visit (*i.e.* visits 1–4) to determine the time course of hormonal changes. Stepwise regression analysis was performed with: 1) the sex hormone levels that changed significantly during treatment as dependent variables and insulin levels, S_I, AIRg, SHBG, prehormones (*i.e.* T for E_2, A for E_1), and/or LH as independent variables; and 2) S_I or AUC_G as dependent variables and insulin levels, AIRg, S_I, and/or AUC_G as independent variables. Sample size calculations were performed for a power of 80% and a P less than 0.05, assuming that the observed mean and SD reflected the true mean and SD of the population. Statistical analyses were performed using the SAS-PC computer program (SAS Institute, Cary, NC). Differences were considered to be significant ($P \leq 0.05$), and data are reported as the mean ± SE.

Results

Clinical features, baseline data, and drug safety

The clinical features and baseline parameters of glucose tolerance are summarized in Table 1, and baseline sex steroid levels are shown in Table 2. The PCOS women were massively obese, with a mean BMI of 42.9 ± 1.2 kg/m^2; this was not significantly different from the control women by design. At baseline, 2 PCOS women had a diastolic BP of 90 mm Hg, and no woman had a systolic BP more than 140 mm Hg. Fasting glucose levels did not differ, but 2-h post-75-g glucose values were significantly increased ($P < 0.05$) in the PCOS women, and 8 PCOS women fulfilled WHO criteria for impaired glucose tolerance (25). Fasting and 2-h post-75-g

TABLE 1. Clinical features and glucose tolerance

PCOS	Age yrs	BMI kg/m^2	WHR	Glucose		Insulin	
				0 min mmol/L	120 min mmol/L	0 min pmol/L	120 min pmol/L
1	29	30.6	0.87	4.9	6.9	150	972
2	28	49.1	0.97	5.7	8.2	138	924
3	18	52.0	0.86	4.5	6.9	60	432
4	29	38.2	0.74	4.8	9.0	108	804
5	29	42.7	0.93	5.4	7.2	318	1578
6	31	49.4	0.84	5.2	6.7	318	1938
7	22	47.4	0.86	4.7	6.7	204	1362
8	32	43.0	0.92	4.1	7.0	108	876
9	36	45.0	0.76	5.3	8.4	114	2418
10	22	37.4	0.72	5.4	5.0	126	270
11	34	39.8	0.82	4.9	6.0	90	762
12	28	42.6	0.88	4.6	6.6	132	1512
13	35	39.9	0.85	5.7	10.6	126	972
14	24	49.2	0.83	5.1	7.2	144	780
15	37	52.3	0.84	4.4	7.3	90	594
16	33	43.6	0.86	5.6	10.7	228	3858
17	28	42.2	0.78	5.6	6.7	150	918
18	22	42.5	0.87	5.6	7.9	180	1080
19	38	34.9	0.79	4.8	10.1	132	2124
20	24	37.0	0.82	4.2	8.3	72	1446
21	28	41.9	0.75	4.8	5.9	54	240
Mean ± SE	29 ± 1	42.9 ± 1.2	0.84 ± 0.01	5.0 ± 0.3	7.6 ± 0.3	145 ± 15	1231 ± 170
Control n = 12							
Mean ± SE	31 ± 2	40.9 ± 1.9	0.80 ± 0.01	4.9 ± 0.1	6.6 ± 0.3	92 ± 18	679 ± 150
P	NS	NS	NS	NS	<0.05	<0.05	<0.05

TABLE 2. Baseline sex hormone and sex hormone binding globulin levels

PCOS	T nmol/L	uT nmol/L	A nmol/L	DHEAS μmol/L	SHBG nmol/L	E_1 pmol/L	E_2 pmol/L
1	1.5	0.5	8.1	5.4	20	263	191
2	3.4	0.7	9.3	3.2	23	374	246
3	3.2	1.8	15.2	22.1	13	444	323
4	2.1	1.2	11.4	10.0	8	473	228
5	3.0	0.8	11.0	5.3	17	473	264
6	6.6	1.0	15.6	5.1	40	429	301
7	3.4	2.0	10.1	6.0	8	433	253
8	7.8	1.2	10.8	3.9	35	707	330
9	3.5	0.9	9.9	6.1	22	392	239
10	3.2	2.2	13.7	10.2	8	429	382
11	2.3	0.8	12.9	9.7	28	462	290
12	2.7	1.1	9.9	3.8	16	396	374
13	2.9	0.9	13.4	5.2	18	718	349
14	2.0	0.8	8.1	5.5	16	485	206
15	5.7	1.0	8.3	1.9	57	547	191
16	5.7	1.6	13.7	2.5	32	596	268
17	3.4	1.0	12.6	5.3	38	451	198
18	1.0	0.5	4.5	1.7	19	300	132
19	1.7	0.8	12.6	2.2	11	507	246
20	1.8	1.0	8.9	9.5	11	348	217
21	2.6	0.6	8.1	5.2	54	455	261
Mean ± SE	3.3 ± 0.41	1.1 ± 0.1	10.9 ± 0.6	6.2 ± 1.0	23 ± 3	461 ± 25	261 ± 14
Control n = 12							
Mean ± SE	1.3 ± 0.1	0.3 ± 0.1	6.6 ± 0.6	4.3 ± 0.5	32 ± 3	431 ± 95	211 ± 38
P	<0.001	<0.001	<0.001	NS	NS	NS	NS

glucose load insulin levels also were significantly increased in the PCOS women (both $P < 0.05$). As expected (3, 6, 7), levels of T, uT, and A were significantly and substantially increased in the PCOS (compared with the control) women (Table 2). Levels of E_2, E_1, SHBG, and DHEAS did not differ in PCOS (compared with control) women. Serum P levels were in the anovulatory range (<6 nmol/L) in the PCOS women. Ten PCOS women were randomized to the 200-mg dose and 11 PCOS women to the 400-mg dose of troglitazone. There was no difference between the 2 treatment groups at baseline, except for A levels, which were significantly higher in the group receiving 400 mg (9.3 ± 0.7 *vs.* 12.3 ± 0.7 nmol/L, $P < 0.01$). There were no reported adverse effects of the medication, and no abnormalities were detected in

hepatic or renal chemistries, in complete blood counts or in electrocardiograms.

Changes in clinical parameters and in insulin action with troglitazone

There were no significant changes in BMI or blood pressure during drug treatment. Fasting and 2-h post-glucose load glucose levels did not change significantly on troglitazone; however, fasting (144 ± 18 *vs.* 102 ± 18 pmol/L, $P < 0.01$) and 2-h post-75-g glucose load ($P < 0.05$) insulin levels (Fig. 2) did decrease significantly in the entire troglitazone group. C-peptide levels decreased, but this was not significant. The integrated glucose response to the glucose load, AUC_G, decreased (15.7 ± 0.6 *vs.* 14.5 ± 0.7 mmol/L/h, P = NS), but the change was not significant, whereas the decrease in integrated insulin response, AUC_I (2,046 ± 234 *vs.* 1,392 ± 168 pmol/L/h, $P < 0.05$), was. Fasting insulin levels tended to decrease by visit 3 ($P = 0.07$), whereas 2-h post-75-g glucose load levels decreased significantly by visit 2 (Fig. 2). In subject 14, there were insufficient data for minimal model analysis of the treatment FSIGT because of problems with venous access; thus, her FSIGT results were not included in the analyses. S_I increased significantly in the entire troglitazone group ($P < 0.001$), and S_I values increased in each subject except for subjects 4, 13, and 20. The empiric measure of β-cell response, AIRg, and the disposition index (S_I*AIRg) did not change significantly (Fig. 3). Changes in S_I were not significantly correlated with changes in other parameters of insulin action. There were no significant changes in S_G. Five of the eight women with impaired glucose tolerance reverted to normal glucose tolerance. However, three women with normal glucose tolerance developed impaired glucose tolerance despite an increase in S_I with troglitazone (200 mg, n = 1; 400 mg n = 2). Thus, eight PCOS women had glucose intolerance at baseline, and six PCOS women had glucose intolerance at the end of the study. Changes in AUC_G were significantly correlated only with changes in insulin levels (AUC_I r^2 0.56, $P < 0.001$; fasting insulin level r^2 0.13, $P < 0.05$).

When the data were analyzed by troglitazone dose, only S_I changed significantly at the 200-mg troglitazone dose ($P < 0.05$) (Fig. 3). Changes in S_I*AIRg and fasting C-peptide levels (both $P < 0.05$) achieved statistical significance at the 400-mg troglitazone dose (Fig. 3). Fasting insulin levels decreased by visit 3 ($P < 0.05$) and 2-h post-75-g glucose load levels decreased by visit 2 (Fig. 2) at the 400-mg dose. Decreases in diastolic blood pressure almost achieved statistical significance at the 400-mg troglitazone dose (77 ± 2 *vs.* 71 ± 3 mm

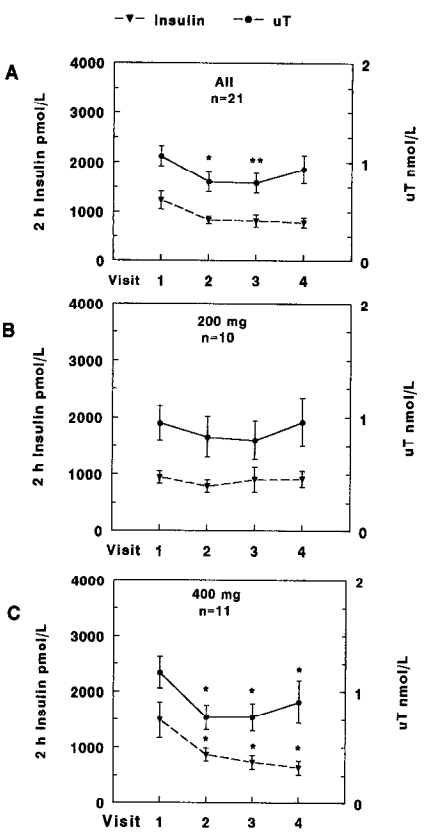

FIG. 2. Biologically available testosterone levels (uT) and insulin levels 2-h post-75-g glucose load are shown at each visit for the entire troglitazone group (A, *top panel*) and for those women receiving 200 mg (B, *middle panel*) and those receiving 400 mg (C, *bottom panel*) daily. *, $P < 0.05$; **, $P < 0.01$ by repeated measures of ANOVA with Bonferonni correction.

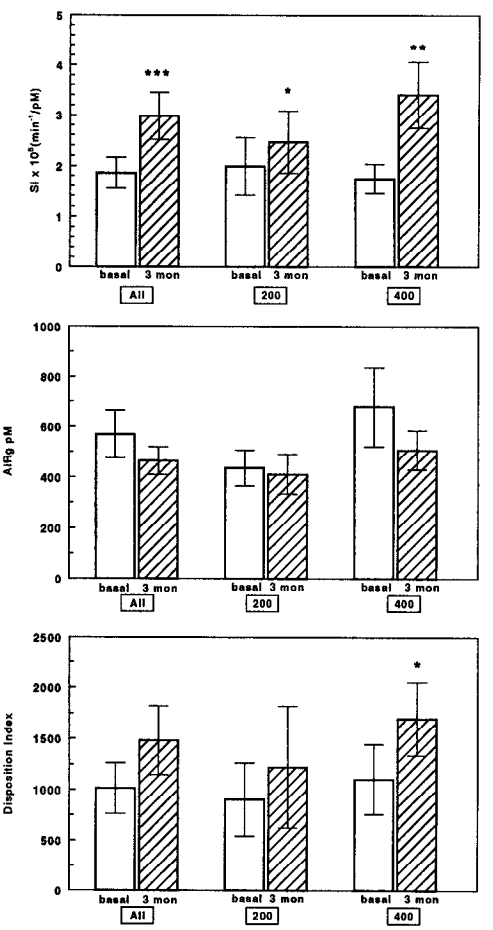

FIG. 3. The insulin S_I (*top*), AIRg (*middle*), and S_I*AIRg (*bottom*) are shown basally (*open bars*) and at the end of 3 months (*hatched bars*) for the entire troglitazone treatment group and for those women receiving 200 mg and those receiving 400 mg daily. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Hg, $P = 0.07$). The improvements in glucose tolerance were accounted for by changes at the 400-mg troglitazone dose; sample size estimates indicated that ~40 women would need to be studied in order for these changes to become statistically significant.

Sex hormone levels and menses on troglitazone

Levels of P were in the anovulatory range at visits 1 and 4 in all PCOS women. There were significant decreases in the levels of uT ($P < 0.01$), DHEAS ($P < 0.001$), E_2 ($P < 0.01$), and E_1 ($P < 0.001$) in the entire troglitazone treatment group (Figs. 2, 4 and 5). The T:E_2 ratio also increased significantly ($P < 0.05$) (data not shown). Levels of FSH and A:E_1 ratios did not change significantly (data not shown). The decreases in uT ($P < 0.001$) were more striking and significant in the group that received 400 mg daily than in the group that received 200 mg daily (Figs. 2, 4 and 5). Levels of uT remained virtually unchanged at the-200 mg troglitazone dose (Figs. 2 and 4). Decreases in A ($P < 0.01$) and LH ($P < 0.05$) levels and increases in SHBG levels ($P < 0.01$) achieved statistical significance in the group that received the 400-mg dose of troglitazone (Figs. 4 and 5). At the 200-mg daily dose, only changes in DHEAS ($P < 0.01$) and E_1 ($P < 0.05$) levels (Figs. 4 and 5) were significant. These changes in sex hormone and gonadotropin levels occurred by visit 2 (Fig. 2) except for E_2, where changes did not become significant until visit 3. There was a significant correlation between decreases in uT levels and in AUC_I (r^2 0.44, $P < 0.01$). Decreases in DHEAS, E_2, and E_1 levels were not significantly correlated with insulin levels, S_I, A, or T levels. Six PCOS women had P levels obtained 21 days after one episode (n = 5) or two episodes (n = 1) of menstrual bleeding. Two PCOS women (subjects 9 and 11) had an ovulatory menstrual cycle documented by appropriately elevated P levels (29 and 27 nmol/L, respectively).

Discussion

Treatment with troglitazone resulted in significant improvements in insulin action in PCOS women. Increases in the insulin S_I, were significant at both doses of troglitazone but were much more marked at the 400-mg (by 95%) than at the 200-mg troglitazone dose (by 24%). This was accompanied by decreases in circulating insulin levels, both basally and after a glucose load, that were accounted for almost entirely by changes at the 400-mg troglitazone dose. Plasma levels of the best biological index of hyperandrogenism (30, 31), uT, decreased significantly in the entire troglitazone treatment group, but this change was completely accounted for by the decreases (by ~25%) in the PCOS women receiving the 400-mg troglitazone dose. Levels of the adrenal androgen, DHEAS, and the estrogens, E_1 and E_2, also decreased significantly during troglitazone therapy. This is the first report that demonstrates that improving insulin sensitivity *per se*, independent of weight loss, can ameliorate hyperandrogenism in insulin-resistant hyperandrogenic women. These observations are consistent with the hypothesis that hyperinsulinemia directly contributes to hyperandrogenism in PCOS (4, 5, 8–10). Two PCOS women had ovulatory menses consistent with the hypothesis that insulin resistance plays an important role in the pathogenesis of anovulation in PCOS (6, 32), but the study was too short to statistically assess changes in ovulation. Because there was no placebo control group, we cannot exclude the possibility that the observed changes represented spontaneous changes in hormonal profiles that can occur in PCOS (22). However, the apparent dose-related effects suggest that these changes were troglitazone-mediated.

Previous small short-term studies in which insulin levels have been lowered with agents that decrease insulin secretion have suggested that hyperinsulinemia plays a direct role in producing hyperandrogenism in PCOS (9, 10). Long-term therapy with these agents, diazoxide and somatostatin analog, is not advisable because they also caused decompensation in glucose tolerance in insulin-resistant PCOS women (9, 10). Metformin has been used to improve insulin sensitivity in PCOS (16, 19). However, its major action on glucose homeostasis is suppression of hepatic glucose output (14),

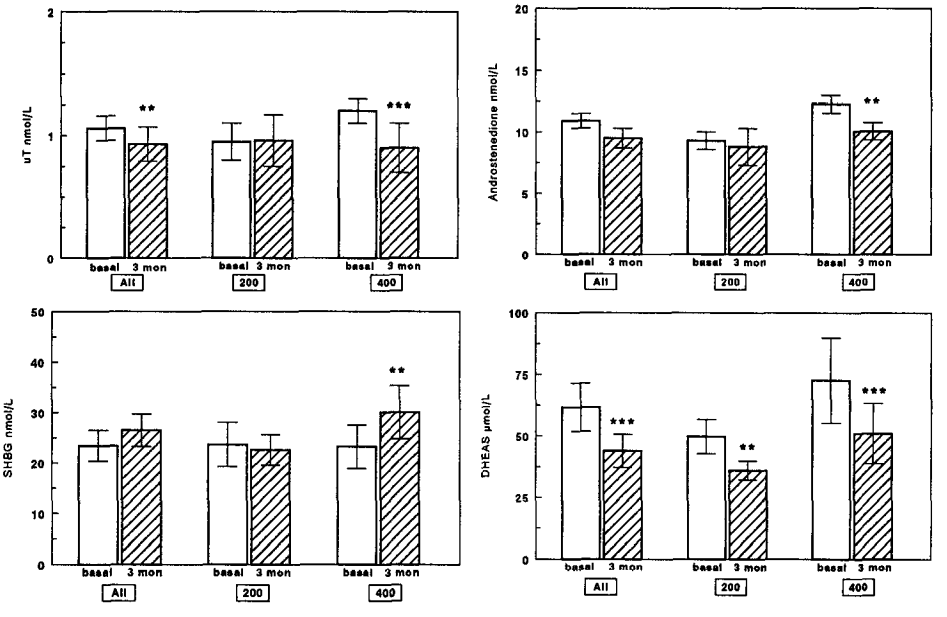

FIG. 4. Biologically available testosterone (uT) (*top left*), A (*top right*), DHEAS (*lower right*), and SHBG levels (*lower left*) are shown at baseline (*open bars*) and at the end of 3 months (*hatched bars*) for the entire treatment group and for those women receiving 200 mg and 400 mg daily. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ by paired analysis.

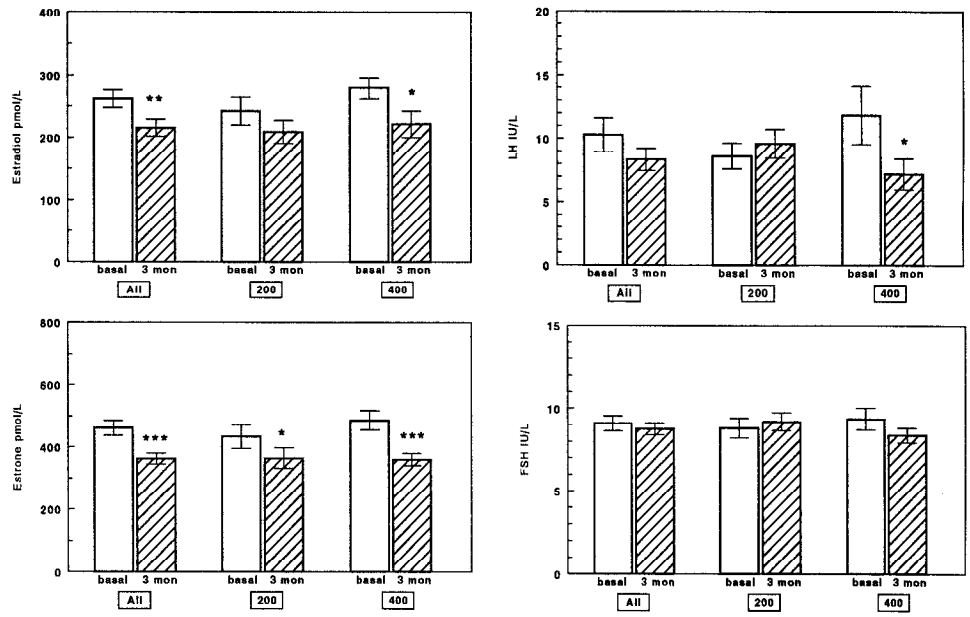

FIG. 5. Levels of the estrogens, E_2 (*top left*) and E_1 (*bottom left*) as well as the gonadotropins, LH (*top right*) and FSH (*bottom right*) are shown basally (*open bars*) and at the end of 3 months (*hatched bars*) in the entire study group and in those women receiving 200 mg and 400 mg troglitazone daily. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ by paired analysis.

and its insulin-sensitizing effects seem to be mediated mainly by weight reduction (15). Consistent with this, metformin with weight loss was no better than weight loss alone at improving hyperandrogenism in PCOS women (19). In another study reporting decreases in androgen levels during metformin treatment, significant weight loss also occurred (16). It is well documented that as little as a 7% decrease in body weight can improve reproductive abnormalities in PCOS (18). In our study, body mass remained constant, and improvements were documented in insulin sensitivity, as well as in hyperinsulinemia. The magnitude of the decrease in biologically available T levels was similar, ~25–30%, with weight loss (16, 18) agents that decrease insulin secretion (9, 10) or with the insulin-sensitizing agent (at the 400-mg dose) used in the present study. This suggests that it is the lowering of insulin levels achieved by these different modalities that is responsible for the decreases in uT levels. This is supported by the stepwise regression analysis, showing a significant correlation between decreases in uT levels and in insulin responses during the OGTT. It is noteworthy that the PCOS women in our study were more obese (BMI ~43 *vs.* ~35 kg/m^2) and had substantially higher uT levels than PCOS women previously studied by us (7, 8, 23, 24) and by others (9, 10, 17, 18). These PCOS women might not improve clinically to the same extent, despite similar percentage decreases in androgen levels. Insulin-sensitizing agents may be more effective in typical PCOS women with moderate obesity and hyperandrogenism.

Levels of A decreased significantly at the 400-mg troglitazone dose; previous studies have suggested that insulin infusions can directly increase A levels in PCOS and in normal women (4, 8). Decreases in LH levels at the 400-mg troglitazone dose may have contributed to decreases in A levels. The decreases in uT levels probably were not the result of decreased LH levels, because total T levels did not change significantly at either drug dose. Rather, the increase in SHBG levels that achieved significance at the 400-mg troglitazone dose probably accounted for the decrease in uT levels. These changes in SHBG levels are consistent with a large body of experimental and epidemiologic data suggesting that insulin is a direct negative regulator of hepatic SHBG production (33–35).

Previous studies have reported that insulin infusions acutely decrease DHEAS levels (36, 37). In chronic studies, improving insulin sensitivity causes increased DHEAS levels in men (38). In women, improving insulin sensitivity has been associated with no change (38), decreases (16), or increases in DHEAS levels (19). In our study, DHEAS levels decreased significantly at both troglitazone doses. This finding suggests a direct role for insulin in augmenting adrenal steroidogenesis in PCOS women, consistent with recent studies in hyperandrogenic women, where insulin infusions have been shown to increase adrenal sensitivity to ACTH (39). The circulating levels of the estrogens, E_2 and E_1, also decreased significantly. This was probably a result of decreased glandular secretion of E_2 as well as decreased conversion of androgen to estrogen (*i.e.* aromatization), because insulin stimulates aromatization *in vitro* (40) and *in vivo* (8).

At the 400-mg troglitazone dose, LH levels decreased significantly. This could be explained either by a direct hypothalamic-pituitary effect of insulin to augment LH release, as has been shown previously in rat pituitary cells *in vitro* (41), or by a loss of positive estrogen feedback because of the decrease in E_2 levels (42). Previous human studies have not shown changes in pulsatile LH secretion during acute insulin infusions (8). Chronic studies of insulin lowering have had conflicting results: lowering insulin levels for ~1 week with diazoxide did not alter LH secretion (9), whereas lowering these levels with somatostatin analog decreased LH levels (10). Decreases in LH levels seen during somatostatin analog, however, may have been related to direct effects of this agent on the hypothalamic-pituitary axis (10). In summary, circulating levels of androgens, estrogens, and LH fell during troglitazone treatment, suggesting that hyperinsulinemia caused a general augmentation of steroidogenesis and gonadotropin release. It remains possible that improving in-

sulin sensitivity altered circulating hormone levels by some other mechanism. Alternatively, troglitazone itself may have had direct effects on steroid metabolism. In any event, the lack of correlation between changes in estrogen or DHEAS levels with insulin levels or sensitivity suggests that the mechanisms of these changes are complex.

AIRg tended to decrease at both troglitazone doses, suggesting that there was less β-cell secretory demand because of improved insulin sensitivity. This resulted in significantly improved total body insulin action as determined by the disposition index, the product of peripheral insulin sensitivity, S_I, and the empiric measure of insulin secretion, AIRg. Thus, troglitazone was effective at ameliorating PCOS-related insulin resistance, which seems to result from a distinctive defect in insulin receptor signaling (43). The reversion from impaired to normal glucose tolerance in five of eight glucose-intolerant PCOS women was the clinical outcome. This is similar to the reported effects of troglitazone, 400 mg daily, in subjects with impaired glucose tolerance *per se* (12). However, three additional PCOS women who had normal glucose tolerance developed impaired glucose tolerance during troglitazone treatment, despite improved insulin sensitivity. This probably reflects the fact that, although improvements in glucose tolerance (AUC_G) were significantly correlated with decreases in insulin levels, 2-h post-75-g glucose load glucose levels did not change significantly and stayed close to the threshold for impaired glucose tolerance (25). This study also was not designed to look at changes in glucose tolerance as an endpoint, and a larger sample of PCOS women would need to be studied for the improvements in glucose tolerance to achieve statistical significance.

In conclusion, troglitazone therapy resulted in marked improvements in insulin sensitivity in PCOS women. This was accompanied by a significant fall in the best biochemical marker of hyperandrogenism, uT levels (30, 31). Circulating levels of the adrenal androgen, DHEAS, and the estrogens, E_2 and E_1, fell in the entire study group. Many of the troglitazone actions were evident only at the 400-mg dose, suggesting that this dose should be used in PCOS. The anticipated clinical consequences of these changes would be an improvement in signs of androgen excess (*e.g.* hirsutism) and a decrease in unopposed estrogen effects (*e.g.* endometrial hyperplasia) (3). Two women had ovulatory menses. These results are consistent with the hypothesis that hyperinsulinemia and insulin resistance are important in the pathogenesis of PCOS. Moreover, PCOS women are at increased risk to develop NIDDM (6), and improving insulin sensitivity may also decrease the likelihood of this outcome (12). Insulin-sensitizing agents thus may provide a novel therapy for both the reproductive and metabolic consequences of this common disorder.

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