The treatment of insulin resistance does not improve adrenal cytochrome P450c17 α enzyme dysregulation in polycystic ovary syndrome

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Abstract

Objective: To determine whether metformin, when given to non-diabetic women with polycystic ovary syndrome (PCOS), results in a reduction of insulin resistance and hyperinsulinemia while body weight is maintained. Also we aimed to see whether the reduction in insulin levels attenuates the activity of adrenal P450c17 α enzyme in patients with PCOS.

Design: We investigated the 17-hydroxyprogesterone (17-OHP) and androstenedione responses to ACTH, insulin responses to an oral glucose tolerance test (OGTT) and glucose disposal rate in an insulin tolerance test before and after metformin therapy (500 mg, orally, twice daily, for 12 weeks).

Methods: The presence of hyperinsulinemia in 15 women with PCOS was demonstrated by an OGTT and results were compared with those of 10 healthy women. Insulin sensitivity was measured by the rate of endogenous glucose disposal after i.v. bolus injection of insulin. 17-OHP and androstenedione responses to ACTH were measured in all the women with PCOS and the normal women.

Results: Women with PCOS were hyperinsulinemic (102.0 ± 13.0 (S.E.M.) vs 46.2 ± 4.4 pmol/l) and hyperandrogenemic (free testosterone 15.3 ± 1.7 vs 7.9 ± 0.6 nmol/l; androstenedione 11.8 ± 0.8 vs 8.2 ± 0.6 nmol/l) and more hirsute (modified Ferriman–Gallwey score, 17.7 ± 1.6 vs 3.0 ± 0.3) than healthy women. In addition, women with PCOS had higher 17-OHP and androstenedione responses to ACTH when compared with healthy women. Metformin therapy resulted in some improvement in insulin sensitivity and reduced the basal and post-glucose load insulin levels. But 17-OHP and androstenedione responses to ACTH were unaltered in response to metformin.

Conclusions: PCOS is characterized by hyperactivity of the adrenal P450c17 α enzyme and insulin resistance. It seems that there is no direct relationship between insulin resistance and adrenal P450c17 α enzyme dysregulation.

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Introduction

Polycystic ovary syndrome (PCOS) is a common and heterogeneous disorder which, in its fullblown state, is characterized by menstrual abnormalities, hirsutism and obesity (1). Hyperinsulinemia is a prominent feature of the syndrome, which clearly is not related to obesity, and a number of data suggest that hyperinsulinemia may play a central role in the development of ovarian hyperandrogenism (2–4). Although it has been well established that women with PCOS have a high 17-hydroxyprogesterone (17-OHP) response to long-acting gonadotropin-releasing hormone agonist testing (5, 6), the role of the adrenal gland in the genesis of PCOS and the relative contribution of the adrenal gland to androgen overproduction is not well known. Ehrman *et al.* (7) showed that 40–60% of hyperandrogenic women have abnormal adrenocorticotropin (ACTH) tests and most of the women do not have an

ACTH response consistent with an adrenal steroidogenic block but demonstrate elevated dehydroepiandrosterone (DHEA) or androstenedione responses to ACTH.

PCOS is thought to be caused by an increased activity of steroidogenesis through 17-hydroxylation and/or increased but relatively inefficient activity of 17,20 lyase. Both 17-hydroxylase and 17,20 lyase activity arise from the action of the same enzyme, cytochrome P450c17 α , which is important for ovarian and adrenal steroid hormone biosynthesis. Overactivity of P450c17 α has been shown in most cases of women with PCOS and a similar kind of abnormal regulation of adrenocortical P450c17 α may contribute to hyperandrogenism (8). Additionally, it is hypothesized that insulin resistance and hyperinsulinemia contribute to ovarian P450c17 α activity in women with PCOS and amelioration of insulin resistance in these women either with metformin (9, 10) or troglitazone (11, 12) would return the

activity of the enzyme towards normal. However, the role and the mechanism responsible for the adrenal androgen excess in women with PCOS is not well understood. The biguanide metformin is a drug commonly used for increasing insulin sensitivity in non-insulin-dependent diabetes mellitus (NIDDM) without producing hypoglycemia. Although the precise mechanisms involved in the action of metformin are incompletely understood, metformin administration is known to increase peripheral glucose uptake with some reduction in basal hepatic glucose production (13). In this study, we aimed to determine the effect of insulin resistance and hyperinsulinemia on adrenal P450c17 α activity before and after metformin therapy in patients with PCOS.

Subjects and methods

The study was approved by the Ethical Committee of Erciyes University Medical School and informed consent was obtained from each woman. Fifteen patients with PCOS (aged 17–36 years, mean $\pm$ S.E.M., 23.3 ± 1.7) and ten healthy women (aged 21–32 years, mean $\pm$ S.E.M., 27.2 ± 1.2) were included in the study. In the women with PCOS, two had a body mass index (BMI) less than 27 kg/m^2 and 13 women had a BMI higher than 27 kg/m^2 . The diagnosis of PCOS was made on the basis of three or more of the following criteria: polycystic ovaries on pelvic ultrasound examination, oligo/amenorrhea, hirsutism, hyperandrogenemia and serum luteinizing hormone (LH)/follicle-stimulating hormone (FSH) >2 . All patients had hyperandrogenemia (serum free testosterone $>11.1 \text{ pmol/l}$, normal range 2.8–11.1). All of the patients had hirsutism (modified Ferriman–Gallwey score >8) (14), and seven (46.6%) women had an elevated serum LH/FSH ratio (LH/FSH >2). Cushing's syndrome and androgen-secreting tumors were excluded by appropriate tests including a dexamethasone suppression test. Pelvic ultrasound examinations were performed by the same investigator (YŞ) using a 3.5 MHz abdominal transducer in six women and a 6.5 MHz vaginal endoprobe (Hitachi, EMB 450, Japan) in nine. The ultrasound diagnosis of polycystic ovary was made by the presence of ten or more cysts, 2–10 mm in diameter, arranged around a dense stroma or scattered throughout an increased amount of stroma (15). The control group consisted of ten healthy women similar in age and BMI to the subjects with PCOS. The normal women served as controls for the insulin levels and ACTH-stimulated steroid plasma concentrations. All had regular menses every 26–32 days, no hyperandrogenemia and no evidence of hirsutism. None of them had polycystic ovaries on ultrasound examination. None of the subjects in the PCOS or control group had received any medication for at least 6 months before the study. The patients and control subjects had normal prolactin and thyroid hormone levels and all were free of other disease.

The patients and healthy women were studied in the midfollicular phase (day 5–9) of their cycle with either regular cycles or oligomenorrhea, or in the event that the patient was amenorrheic when serum progesterone concentration was $<8.0 \text{ nmol/l}$.

After an overnight fast, blood samples were obtained for the determination of fasting blood glucose (FBG), insulin, free testosterone, androstenedione, DHEA sulfate (DHEAS) and sex hormone-binding globulin (SHBG) levels. The patients were given a 3 day 2000 cal standardized diet (300 g carbohydrate/day) before an oral glucose tolerance test (OGTT) which was performed after ingestion of a 75 g glucose load, and blood samples were obtained at 30 min intervals for 2 h for measurement of glucose and insulin. The glucose and insulin responses to OGTT were also expressed as area under the curve (AUC) estimated by the trapezoidal rule. Glucose tolerance was evaluated using the criteria of the WHO. In women with PCOS, insulin sensitivity was assessed by an i.v. insulin tolerance test (ITT) (16). The rate constant for the plasma glucose disappearance rate (K_{ITT}) was calculated from the formula $0.693/t_{1/2}$ (16). The plasma glucose $t_{1/2}$ was calculated from the slope of least square analysis of the plasma glucose concentrations from 3 to 15 min after i.v. insulin injection (16).

The ACTH stimulation test was performed in PCOS and control women by administration of a single i.v. bolus of 0.25 mg synthetic ACTH(1–24) (Synacthen; Ciba-Geigy, Basel, Switzerland) at 0800 h. Venous blood was drawn through an indwelling catheter in the midfollicular phase at 0, 30 and 60 min for determination of serum 17-OHP and androstenedione. 17-OHP and androstenedione responses to ACTH were also expressed as AUCs.

After the initial tests, the women with PCOS took 500 mg metformin orally twice daily. They were instructed not to alter their usual eating habits, physical activity or lifestyle during the study. In control women only basal sampling, OGTT and ACTH testing were performed, as metformin administration was unacceptable and not ethical. Twelve weeks after metformin therapy, the women with PCOS returned for re-evaluation, after a low serum progesterone value had confirmed that they were in the follicular phase of the menstrual cycle.

Serum insulin, androstenedione (DPC, Los Angeles, CA, USA), free testosterone (DSL, TX, USA), 17-OHP, DHEAS (ICN, CA, USA) were measured by RIA and FSH, LH (DSL) and SHBG (Radim, Rome, Italy) were measured by IRMA, using commercial kits. The intra-assay and inter-assay coefficients of variation were: 3.6 and 7.5% for FSH; 6.8 and 7.3% for LH; 3.7 and 7.9% for free testosterone; 3.7 and 4.9% for androstenedione; 9 and 9.5% for DHEAS; 7.8 and 9.8% for 17-OHP; and 4.6 and 5.1% for SHBG. All samples from the same patient were assayed in the same assay.

The results are reported as means $\pm$ S.E.M. In women with PCOS, we compared the results before treatment

Table 1 Clinical and basal hormonal characteristics of PCOS and control subjects. Values are means $\pm$ S.E.M.

	PCOS (n = 15)	Controls (n = 10)	P value
Age (year)	23.3 $\pm$ 1.7	27.2 $\pm$ 1.2	NS
BMI (kg/m ²)	30.2 $\pm$ 1.6	26.8 $\pm$ 0.5	NS
Hirsutism score	17.7 $\pm$ 1.6	3.0 $\pm$ 0.3	<0.0005
FBG (mmol/l)	4.3 $\pm$ 0.2	4.0 $\pm$ 0.1	NS
Free testosterone (nmol/l)	15.3 $\pm$ 1.7	7.9 $\pm$ 0.6	<0.0005
Androstenedione (nmol/l)	11.8 $\pm$ 0.8	8.2 $\pm$ 0.6	<0.05
DHEAS (μ mol/l)	9.4 $\pm$ 1.6	5.9 $\pm$ 0.6	<0.05
LH/FSH	1.6 $\pm$ 0.4	0.76 $\pm$ 0.0	<0.05
SHBG (nmol/l)	29.0 $\pm$ 2.8	60.1 $\pm$ 3.2	<0.0005
Basal insulin (pmol/l)	102.0 $\pm$ 13.0	46.2 $\pm$ 4.4	<0.005
AUC _{insulin} (pmol/l $\times$ 120 min)	67 784.7 $\pm$ 6203.4	13 318.2 $\pm$ 1573.4	<0.0005
AUC _{glucose} (mmol/l $\times$ 120 min)	855.3 $\pm$ 67.1	649.3 $\pm$ 31.9	NS

NS; not significant ($P > 0.05$).

with those after treatment by using the Wilcoxon signed-rank test. Between the PCOS and control subjects the results were compared by using the Mann–Whitney U test. A P value of <0.05 was regarded as statistically significant.

Results

The clinical and basal hormonal characteristics of PCOS and control subjects are shown in Table 1. PCOS women and controls did not differ in mean age, BMI, FBG and AUC_{glucose}. The mean hirsutism score ($P < 0.0005$), free testosterone ($P < 0.0005$), androstenedione ($P < 0.05$), basal insulin ($P < 0.005$) and AUC_{insulin} ($P < 0.0005$) values were significantly higher in PCOS subjects than in controls. Basal serum SHBG concentration was significantly lower in the PCOS group than in controls ($P < 0.0005$).

In PCOS women, there was no significant difference between BMI before (30.2 ± 1.6 kg/m²) and after (30.3 ± 1.5 kg/m²) the metformin therapy. In women with PCOS, the mean basal serum insulin level ($P < 0.005$) and AUC_{insulin} value decreased significantly ($P < 0.05$) after the metformin therapy. However, basal glucose level and AUC_{glucose} did not change significantly after the metformin therapy. Insulin sensitivity (the plasma glucose disappearance rate, K_{ITT}) had improved significantly ($P < 0.05$) after metformin therapy (Table 2).

In PCOS women, peak 17-OHP ($P < 0.005$) and androstenedione ($P < 0.005$) responses to an ACTH test were significantly higher than in control subjects. AUC_{17-OHP} ($P < 0.05$) and AUC_{androstenedione} ($P < 0.05$) values in PCOS women were significantly higher than in control subjects. Net increment (difference between the peak and the basal value) of 17-OHP ($P < 0.005$) and androstenedione ($P < 0.05$) responses to the ACTH test were also significantly higher in the women with PCOS than in controls. Except two, all women with PCOS had higher 17-OHP responses to the ACTH test than normal. Two women had 17-OHP response to ACTH similar to levels obtained in normal women. The mean 17-OHP and androstenedione responses to the ACTH test in PCOS and control subjects are shown in Table 3.

In women with PCOS, peak 17-OHP, net increment of 17-OHP, AUC_{17-OHP} and peak androstenedione, net increment of androstenedione and AUC_{androstenedione} responses to ACTH stimulation were not significantly different before and after metformin therapy (Table 4).

Discussion

PCOS is one of the most common endocrine disorders among premenopausal women. The clinical and biochemical features are heterogeneous and there has been much debate on the pathogenesis of the disease. It has been suggested that PCOS is a form of functional

Table 2 Basal, OGTT-stimulated insulin levels and insulin sensitivity index (K_{ITT} before and after metformin therapy in women with PCOS. Values are means $\pm$ S.E.M.

	Before metformin	After metformin	P value
Basal insulin (pmol/l)	102.0 $\pm$ 13.0	64.5 $\pm$ 9.1	<0.005
AUC _{insulin} (pmol/l $\times$ 120 min)	67 784.7 $\pm$ 6203.4	38 878.7 $\pm$ 6339.1	<0.05
AUC _{glucose} (mmol/l $\times$ 120 min)	855.3 $\pm$ 67.1	870.3 $\pm$ 50.4	NS
K_{ITT} (%/min)	3.3 $\pm$ 0.5	3.8 $\pm$ 0.8	<0.05

NS; not significant ($P > 0.05$).

Table 3 The mean 17-OHP and androstenedione responses to ACTH in PCOS and control subjects. Values are means $\pm$ S.E.M.

	PCOS group (n = 15)	Control group (n = 10)	P value
Peak 17-OHP (nmol/l)	11.9 $\pm$ 0.7	8.9 $\pm$ 0.3	<0.005
Δ 17-OHP (nmol/l)	6.9 $\pm$ 0.8	3.3 $\pm$ 0.6	<0.005
AUC _{17-OHP} (nmol/l $\times$ 60 min)	518.8 $\pm$ 27.6	449.7 $\pm$ 20.2	<0.05
Peak androstenedione (nmol/l)	16.8 $\pm$ 1.2	12.3 $\pm$ 0.4	<0.005
Δ Androstenedione (nmol/l)	5.7 $\pm$ 0.8	3.4 $\pm$ 0.4	<0.05
AUC _{androstenedione} (nmol/l $\times$ 60 min)	873.6 $\pm$ 63.0	636.3 $\pm$ 38.8	<0.05

Δ indicates net increment.

ovarian hyperandrogenism with underlying dysregulation of the cytochrome P450c17 α enzyme (8, 17). On the other hand, numerous studies have found elevated androgen production by the adrenal glands in patients with PCOS and have suggested that the adrenal glands may play a role in the genesis of PCOS (18, 19). Previously, we reported that women with PCOS have an exaggerated 17-OHP response to ACTH stimulation, and we have also previously shown that there is an adrenocortical hyperactivity in hyperandrogenic women (20, 21).

Maximal adrenal stimulation with ACTH has been the principal challenge test for estimating the relative activity of adrenocortical enzymes (22). In the present study, none of the subjects met the criteria, either basally or in response to an ACTH test, to diagnose the non-classical form of congenital adrenal hyperplasia due to 21-hydroxylase or 11 β -hydroxylase deficiency. However, this study confirms that an overactivity of adrenal P450c17 α enzyme exists in women with PCOS.

Several hypotheses have been put forward to explain the association between PCOS and adrenal hyperandrogenism: (i) an enhanced ovarian and adrenal cytochrome P450c17 α enzyme activity as the mechanism of ovarian and adrenal hyperandrogenism in PCOS (17); (ii) an increased activity of enzymes involved in cortisol clearance such as 11 β -hydroxysteroid dehydrogenase (23) could lead to lowering cortisol

concentrations, and consequently to ACTH stimulation; (iii) hyperinsulinemia may enhance both 17-hydroxylase and, to a lesser extent, 17,20 lyase adrenal activities, contributing to adrenal hyperandrogenism in these conditions (24); and (iv) ovarian hyperestrogenism has been implicated in the increased Δ^4 -17,20 lyase activity found in PCOS women with concurrent adrenal hyperandrogenism (25).

Hyperinsulinemia and insulin resistance, independent of obesity, have been recognized in a large number of PCOS subjects (2, 3, 9, 26–28) and have been implicated in the pathogenesis of PCOS. A number of studies have been performed for reducing hyperinsulinemia and insulin resistance either with diet alone (29), metformin (9, 30, 31) or troglitazone (11). In the present study, we used metformin, which improves insulin action and reduces insulin resistance without affecting insulin secretion in patients with NIDDM (32). Two studies (9, 10) have reported optimistically that metformin can reduce the hyperinsulinemia and insulin resistance in patients with PCOS, while two further studies (30, 31) have not confirmed this possibility, leaving the matter unresolved. In this study, metformin decreased fasting serum insulin concentration and also decreased the AUC_{insulin} during OGTT. Additionally, metformin resulted in a significant improvement of insulin resistance, as estimated by an ITT.

In the present study, 17-OHP and androstenedione responses, 30 and 60 min after ACTH stimulation, the

Table 4 17-OHP and androstenedione responses to ACTH before and after metformin therapy in women with PCOS. Values are means $\pm$ S.E.M.

	Before metformin	After metformin	P value
Peak 17-OHP (nmol/l)	11.9 $\pm$ 0.7	10.3 $\pm$ 0.4	NS
Δ 17-OHP (nmol/l)	6.9 $\pm$ 0.8	5.8 $\pm$ 0.6	NS
AUC _{17-OHP} (nmol/l $\times$ 60 min)	518.8 $\pm$ 27.6	477.8 $\pm$ 21.0	NS
Peak androstenedione (nmol/l)	16.8 $\pm$ 1.2	16.2 $\pm$ 1.6	NS
Δ Androstenedione (nmol/l)	5.7 $\pm$ 0.8	5.2 $\pm$ 1.5	NS
AUC _{androstenedione} (nmol/l $\times$ 60 min)	873.6 $\pm$ 63.0	834.9 $\pm$ 61.0	NS

Δ indicates net increment.

NS; not significant ($P > 0.05$).

net increment and the AUC_{17-OHP} and AUC_{androstenedione} were significantly higher in the PCOS group than in healthy women. This indicates an overactivity of adrenal glands and adrenal P450c17 α enzyme activity in patients with PCOS. However, amelioration of hyperinsulinemia and insulin resistance did not significantly alter the 17-OHP and androstenedione responses to ACTH stimulation. This result suggests that hyperinsulinemia has no direct effect on adrenal P450c17 α enzyme activity *per se*. Azziz *et al.* (22) have reported that the measurements of steroidal increment rather than the absolute post-stimulation level after acute adrenal stimulation may more accurately reflect adrenal biosynthesis and they did not find evidence in support of the adrenal secretion of 17-OHP being exaggerated among the patients with non-late onset adrenal hyperplasia. The conflicting results between the studies may be in part due to the difference of populations. However, in the study of Azziz *et al.* (22), only a 30 min post-stimulation response was obtained, which may underestimate the peak value. In a different study of Azziz *et al.* (33), the authors reported that a generalized alteration of adrenocortical steroid biosynthesis, rather than P450c17 α enzyme dysregulation, is observed in hyperandrogenic women. Martikainen *et al.* (18) reported a positive correlation between insulin in the adrenal vein and androgens secreted by the adrenal glands and suggested that insulin stimulates adrenal androgen secretion. Our results partially agree with this suggestion that the women with PCOS are hyperinsulinemic, insulin resistant, hyperandrogenic and hyperresponsive to ACTH stimulation but, although metformin significantly decreased insulin levels and insulin resistance, we could not see any effect on 17-OHP and androstenedione levels. Moghetti *et al.* (34) reported that insulin potentiates ACTH-stimulated steroidogenesis in hyperandrogenic women during a 3 h hyperinsulinemic euglycemic clamp. The discrepancies between our data and those of Moghetti and colleagues (34) are not easily explained. The differences in study populations and the study protocols are the most likely explanations. The study of Moghetti *et al.* (34) was performed in an acutely hyperinsulinemic condition while our study was on the natural chronic hyperinsulinemic condition. We think that insulin resistance and hyperinsulinemia are not directly related to adrenal androgen overproduction but associated with the disease. However, this study does not completely rule out the effect of hyperinsulinemia on adrenal P450c17 α enzyme activity, because metformin decreased the insulin levels significantly but not to the values compatible with those of the control subjects.

In conclusion, PCOS is associated with hyperactivity of the adrenal P450c17 α enzyme, and hyperinsulinemia is an important feature of the disease. However, it seems unlikely that there is a direct relationship between insulin resistance and adrenal P450c17 α

dysregulation. Further studies are required to evaluate the role of insulin resistance in adrenal functions.

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