Lack of relationship between 17-hydroxyprogesterone response to buserelin testing and hyperinsulinemia in polycystic ovary syndrome

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Abstract

Objective: To determine whether hyperinsulinism affects cytochrome P450c17α activity by investigating the correlation between 17–hydroxyprogesterone (17-OHP) hyper–responsiveness to the gonadotropin–releasing hormone (GnRH) agonist, buserelin, and the insulin response to oral glucose in polycystic ovary syndrome (PCOS).

Design: Ultrasound, clinical and hormonal parameters were used to define PCOS in this prospective clinical study. We investigated the correlation between the 17–OHP response to buserelin testing and the insulin response to oral glucose in PCOS.

Methods: Twenty-eight women with PCOS and eighteen normal women were included in the study. 17–OHP response to buserelin, and insulin and C–peptide responses to oral glucose were measured.

Results: Twenty–five women with PCOS had an increased 17–OHP response. The PCOS patients showed significantly higher mean post–glucose load insulin and C–peptide levels than controls (P<0.05). No significant correlations were found between basal 17–OHP and fasting insulin or fasting C–peptide, between peak 17–OHP and fasting insulin, peak insulin or peak C–peptide, between 17–OHP area under the curve (AUC) and insulin AUC or C–peptide AUC, and between percent increment in 17–OHP and insulin AUC or C–peptide AUC (P > 0.05).

Conclusions: Lack of a relationship between the 17–OHP response to the GnRH agonist buserelin and hyperinsulinemia suggests that hyperinsulinemia may not play a role in the dysregulation of the cytochrome P450c17α enzyme seen in PCOS.

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Introduction

The women with polycystic ovary syndrome (PCOS) have a high 17–hydroxyprogesterone (17–OHP) response to gonadotropin–releasing hormone (GnRH) agonist testing with nafoxidine (1) or buserelin (2). It has been hypothesized that PCOS may be caused by an increased activity of steroidogenesis through 17–hydroxylation and/or increased but relatively inefficient activity of 17,20–lyase. It has been suggested that PCOS is a form of functional ovarian hyperandrogenism with underlying dysregulation of the cytochrome P450c17α enzyme. This enzyme binds progesterone and converts it to 17–OHP (by 17α–hydroxylase activity) and 17–OHP is converted to androstenedione (by 17,20–lyase activity) (3).

PCOS is also associated with insulin resistance and hyperinsulinemia (4). Hyperinsulinemia and elevated luteinizing hormone (LH), acting synergistically, result in increased ovarian androgen production (5, 6). Insulin resistance may be involved in the pathogenesis of PCOS, but the actual role of hyperinsulinemia in the development of PCOS and the precise relationship between insulin and androgen secretion have not yet been clarified. The nature of dysregulation of the cytochrome P450c17α enzyme is unclear.

The aim of this study was to evaluate in an adequate number of patients the relationship between the 17–OHP response to buserelin testing and hyperinsulinemia, and the role of insulin in the dysregulation of the cytochrome P450c17α enzyme in PCOS.

Patients and methods

The study was approved by the Ethical Committee of Erciyes University Medical School, and informed consent was obtained from each woman. Forty–six women were enrolled in the study between January 1993 and March 1995.

Twenty-eight women with PCOS (aged 18 to 33 years) (mean ± s.e.m., 25.14 ± 0.82) were recruited

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consecutively for the study from our outpatient clinic population. The diagnosis of PCOS was made by the presence of polycystic ovaries on pelvic ultrasound examination combined with three or more of the following criteria: oligo/amenorrhea, hirsutism, hyper-androgenemia, and a serum LH:follicle-stimulating hormone (FSH) ratio > 2. All patients had hyperandrogenemia (serum free testosterone > 11·1 pmol/l, normal range 2·8 to 11·1 pmol/l). Of the patients, 29% were eumenorrheic (intermenstrual interval between 21 and 35 days), 64% were oligomenorrheic (intermenstrual interval > 35 days) and 7% were amenorrheic (no menstrual period in more than 6 months). All patients had hirsutism (Modified Ferriman-Gallway score > 8, mean ± s.e.m. score: 16·8S ± 1·20) (7). Eleven women (39%) had an elevated ratio of serum LH to serum FSH (LH:FSH > 2). Late onset 21-hydroxylase deficiency, Cushing's syndrome and androgen-secerting tumors were excluded by appropriate tests including dexamethasone suppression and adrenocorticotropin (ACTH) stimulation tests. The ACTH stimulation test was performed in subjects by administration of a single i.v. bolus of 0·25 mg synthetic ACTH(1-24) (Synacthen; Ciba, Switzerland) at 0800 h. Venous blood was withdrawn in the midfollicular phase at 0, 30 and 60 min for determination of serum 17-OH-progesterone. Serum 17-OH-progesterone levels > 30 nmol/l were considered as the criteria of late onset 21-hydroxylase deficiency (8). Pelvic ultrasound examinations were performed by the same investigator (Y S) using a 3·5 MHz abdominal transducer in 3 women and a 6·5 MHz vaginal endo¬probe (Hitachi, EUB 450, Japan) in 43 women. The ultrasound diagnosis of polycystic ovary was made by the presence of 10 or more cysts 2-10 mm in diameter, arranged around a dense stroma or scattered throughout an increased amount of stroma (9, 10). All sonograms were obtained early in the cycle.

The control group consisted of 18 normal women similar in age (aged 21 to 32 years) (mean ± s.e.m., 26·94 ± 0·75) and body mass index (BMI) to the subjects with PCOS. All had regular menses every 26 to 32 days, no hyperandrogenemia, and no evidence of hirsutism (mean ± s.e.m. hirsutism score: 3·11 ± 0·27). None had polycystic ovaries on ultrasound examination.

None of the subjects in the PCOS or control group had received any hormonal medication for at least 8 weeks before the study, had a history of diabetes or was receiving medications having an adverse effect on glucose tolerance. A total of 46 women was studied, each in the midfollicular phase (day 5 to 9) of her cycle in controls and in patients with either regular cycles or oligomenorrhea or, in the event that the patient was amenorrheic, when the serum progesterone level was less than 2·5 ng/ml (8·0 nmol/l). Buserelin testing (1 mg s.c.) was carried out as we have previously described (2). After a 3-day 2000 Cal standardized diet (300 g carbohydrate/day) a 100 g, 2 h oral glucose tolerance test (OGTT) was performed on all subjects after an overnight fast, beginning at 0800 h. Blood samples were obtained 0, 30, 60, 90, and 120 min after oral glucose administration to measure serum glucose, insulin and C-peptide levels. The serum samples were stored at −20°C until assayed.

Serum FSH, LH, testosterone, free testosterone, androstenedione, dehydroepiandrosterone sulfate (DHEA-S), 17-OHP, insulin and C-peptide were measured by RIA, using commercial kits (DPC, Los Angeles, CA, USA). Sex hormone–binding globulin (SHBG) (Orion Diagnostica, Finland) and prolactin (PRL) (ICN Biomedicals Inc., Costa Mesa, CA, USA) were measured by immunoradiometric assay. The intra-assay and interassay precision coefficients of variation were, respectively, 6·5% (mean, 8·3 IU/l) and 5·6% (mean, 8·0 IU/l) for FSH; 7·0% (mean, 8·0 IU/l) and 7·9% (mean, 8·0 IU/l) for LH; < 10% and 12·9% (mean, 2·50 nmol/l) for testosterone; 4·3% (mean, 15·9 pmol/l) and 5·5% (mean, 16·29 pmol/l) for free testosterone; 8·3% (mean, 10·8 nmol/l) and 9·2% (mean, 10·3 nmol/l) for androstenedione; 3·9% (mean, 6·05 µmol/l) and 7·0% (mean, 7·14 µmol/l) for DHEA-S; 5·6% (mean, 4·84 nmol/l) and 4·5% (mean, 3·33 nmol/l) for 17-OHP; < 10% and 7·1% (mean, 241·13 pmol/l) for insulin; 3·4% (mean, 0·29 nmol/l) and 1·9% (mean, 0·30 nmol/l) for C-peptide; 4·0% (mean, 45·8 nmol/l) and 5·5% (mean, 53·6 nmol/l) for SHBG; and 4·8% (mean, 16·8 µg/l) and 8·2% (mean, 17·1 µg/l) for PRL. Glucose was measured by the glucose oxidase method with a RAXT Autoanalyzer (USA), using a Biosub GLU kit (Biocon, Burbach, Germany). All samples from the same patients were assayed in the same assay. 17-OHP response to the GnRH agonist buserelin, and insulin and C-peptide responses to OGTT were also expressed as area under the curve (AUC) calculated by the trapezoidal rule and expressed as amount of hormone/l×time (h for 17-OHP, min for insulin and C-peptide). For statistical analysis Student’s paired and unpaired t-tests were used for comparisons within the same group and between the groups respectively. A correlation regression analysis was also carried out. A P value of < 0·05 was regarded as statistically significant.

Results

BMI (kg/m²) was slightly, but not significantly, higher in the PCOS group (27·18 ± 0·66) than in the control (25·40 ± 0·83) subjects. Baseline hormonal characteristics of PCOS and normal women are shown in Table 1. The mean values of serum testosterone (P < 0·01), free testosterone (P < 0·01), androstenedione (P < 0·05), DHEA-S (P < 0·01) and LH (P < 0·05) were significantly higher in the PCOS group than in the controls. The PCOS patients had significantly lower mean SHBG level than the controls (P < 0·05). There were no significant differences in serum FSH and PRL levels between PCOS and normal women (P > 0·05).

The serum 17-OHP levels in 18 normal women and 28 women with PCOS before and after buserelin testing
are shown in Table 2. The mean basal 17-OHP level in 28 women with PCOS was significantly higher (P < 0.05) than in the controls. At 6, 12, 18 and 24 h after buserelin treatment, the mean serum 17-OHP levels had increased significantly in the women with PCOS (P < 0.0005) and also in the control group (P < 0.0005). The serum 17-OHP responses reached peak levels at 18 h in 8 (29%) and 24 h in 20 (71%) of the patients with PCOS, and at 12 h in 4 (22%), at 18 h in 5 (28%) and at 24 h in 9 (50%) of the control women. The mean levels of 17-OHP at 6, 12, 18 and 24 h after buserelin stimulation in the women with PCOS were significantly higher than in the control group (P < 0.001). Also, the increase (percent differences between the peak and the basal values, mean% ± S.E.M.) in 17-OHP level and 17-OHP AUC were significantly higher in PCOS patients than in the control group (P < 0.0005).

Twenty-five out of twenty-eight PCOS women had an increased response of 17-OHP. Their peak serum 17-OHP levels were > 9-29 nmol/l (3-07 ng/ml). Two S.D. (S.D.: 1-42) above the mean peak value in the same 18 normal women has been accepted as the cut-off level of peak value (6-45 + (2 x 1-42) = 9-29 nmol/l).

During the OGTT, although the mean glucose levels at 0, 30, 60 and 120 min were significantly higher in PCOS patients than in controls (P < 0.05), the glucose levels under basal conditions and postprandially in all study subjects were within the normal range. Basal insulin levels were elevated in PCOS patients compared with those in controls, while basal C-peptide levels were similar (P > 0.05). The PCOS patients showed significantly higher mean post–glucose load insulin (Fig. 1) and C-peptide levels (Fig. 2) than controls (P < 0.05). The mean AUC insulin (97 599-69 ± 7890-25 pmol/l x 120 min) and AUC C-peptide (160-09 ± 6-67 nmol/l x 120 min) values in PCOS women were significantly higher than in control subjects (19 549-60 ± 1560-49 pmol/l x 120 min, 118 61 ± 6-24 nmol/l x 120 min respectively) (P < 0.0005).

The correlations between 17-OHP response to the GnRH agonist, and insulin and C-peptide responses to OGTT in 25 PCOS women with 17-OHP hyper-responsiveness to buserelin are given in Table 3. No significant correlations were found between basal 17-OHP and fasting insulin or fasting C-peptide, between peak 17-OHP and fasting insulin, peak insulin or peak C-peptide, between AUC 17-OHP and insulin AUC or C-peptide AUC, and between percent increment in 17-OHP and insulin AUC or C-peptide AUC (P > 0.05). We have also examined the findings of 19 patients with oligo/amenorrhea remaining after excluding eumenorrheic patients. No significant correlations were seen between basal 17-OHP and fasting insulin (r: 0.283) or fasting C-peptide (r: 0.095), between peak 17-OHP and peak insulin (r: 0.042) or peak C-peptide

### Table 1 Basal hormone levels in PCOS and control subjects. Values are means ± S.E.M.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>PCOS group (n = 28)</th>
<th>Control group (n = 18)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone (nmol/l)</td>
<td>2.97 ± 0.16</td>
<td>1.15 ± 0.06</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Free testosterone (pmol/l)</td>
<td>20.58 ± 1.21</td>
<td>7.49 ± 0.45</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>DHEA-S (μmol/l)</td>
<td>7.60 ± 0.62</td>
<td>5.59 ± 0.11</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>37.44 ± 4.21</td>
<td>62.55 ± 4.27</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Androstenedione (nmol/l)</td>
<td>11.60 ± 0.63</td>
<td>9.42 ± 0.42</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>5.38 ± 0.49</td>
<td>4.50 ± 0.44</td>
<td>NS</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>7.88 ± 0.65</td>
<td>3.87 ± 0.41</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>PRL (μg/l)</td>
<td>17.40 ± 2.27</td>
<td>14.40 ± 1.01</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, not significant.

### Table 2 17-OHP levels (nmol/l) before and 6, 12, 18 and 24 h after buserelin testing in PCOS and control subjects. Values are means ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>PCOS group (n = 28)</th>
<th>Control group (n = 18)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>5.42 ± 0.42</td>
<td>3.63 ± 0.24</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>6 h</td>
<td>8.81 ± 0.57*</td>
<td>4.66 ± 0.30*</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>12 h</td>
<td>10.47 ± 0.57*</td>
<td>5.20 ± 0.27*</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>18 h</td>
<td>12.65 ± 0.76*</td>
<td>6.02 ± 0.30*</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>24 h</td>
<td>14.65 ± 0.82*</td>
<td>6.17 ± 0.33*</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Peak</td>
<td>15.49 ± 0.76*</td>
<td>6.45 ± 0.33*</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>% increment*</td>
<td>221.62 ± 24.08</td>
<td>82.56 ± 9.88</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td>AUC (nmol/l x 24 h)</td>
<td>251.98 ± 12.30</td>
<td>125.21 ± 6.13</td>
<td>&lt; 0.0005</td>
</tr>
</tbody>
</table>

* % increment is percentage difference between the peak and the basal values.
* P < 0.0005, compared with the corresponding basal value.
(r: 0.112), between percent increment in 17-OHP and peak insulin (r: 0.139) or peak C-peptide (r: 0.025), and between 17-OHP AUC and insulin AUC (r: 0.097) or C-peptide AUC (r: 0.085) in PCOS patients with oligo/amenorrhea (P > 0.05).

**Discussion**

In several studies, the presence of insulin resistance in both obese and non-obese patients with PCOS (11, 12) and significant positive correlations between insulin and androgen levels have been demonstrated (13). These studies support a primary role of insulin resistance in the pathogenesis of ovarian hyperandrogenism and PCOS. In contrast to these data, some other studies showed no positive correlation between insulin and androgen levels in PCOS women (14–17). Also, studies using hyperinsulminemic euglycemic clamps that permit the maintenance of hyperinsulinemia for several hours did not show a consistent effect of the elevated insulin levels on androgen production in women with PCOS (18, 19). So, the relation between hyperandrogenemia and hyperinsulinemia is still not clear.

Since a large proportion of the insulin released by the β-cell is metabolized by the liver before reaching the systemic circulation (20), peripheral insulin levels are not a good marker of endogenous insulin secretion. The plasma C-peptide assay may be a more reliable parameter of pancreatic secretion of insulin than the immunoreactive insulin assay (21). In the present study the PCOS patients showed significantly higher mean basal insulin and higher mean post–glucose load insulin and C-peptide levels than controls similar to that previously reported in women with PCOS (22, 23).
Basal and AUC insulin values were significantly higher in the PCOS group. AUC C-peptide values were also significantly higher in the PCOS group. The findings of the OGTT suggest that the patients with PCOS have increased pancreatic insulin secretion.

In vitro studies have demonstrated that insulin can enhance the effect of LH on ovarian production of steroid hormones (24). Anttila et al. (5) suggested that insulin release during an OGTT was accompanied by increased androgen production only in women with elevated LH levels. The above studies suggest that insulin and LH may act synergistically to enhance androgen production. However, no significant positive correlation between the peak plasma 17-OHP after nafarelin administration and basal serum LH level was found in hyperandrogenic women (25). A recent study demonstrated that PCOS women with 17-OHP hyperresponsiveness to GnRH agonist testing have a remarkably consistent pattern of steroid responses regardless of whether they have elevated basal LH levels (26). It has been suggested that the stimulation of 17α-hydroxylase/17,20-lyase by LH may be augmented by specific intra-ovarian and hormonal factors such as inhibin, insulin-like growth factor-I and insulin and ovarian hyperandrogenism can result from abnormal modulation of ovarian androgen responsiveness to LH (25).

Hyperinsulinemic patients with PCOS exhibited a significantly greater 17-OHP and androstenedione response to ACTH when compared with normoinsulinemic PCOS subjects (27). After insulin infusion, ACTH stimulation resulted in a significant rise in the serum ratio of 17-OHP to androstenedione in men. The insulin-induced change in this steroid ratio was due to a relative increase in precursor (17-OHP) and decrease in product (androstenedione) responsiveness to ACTH. The findings indicate that insulin selectively inhibits 17,20-lyase activity (28). These studies suggest that insulin may influence the responsiveness of the adrenal to its tropic hormones.

Whether hyperinsulinemia may play a role in abnormal regulation of ovarian P450c17α in women with PCOS has not, to our knowledge, been examined before. For this reason, we have investigated whether there is a relation between 17-OHP response to the GnRH agonist buserelin and hyperinsulinemia in PCOS. In the present study, the GnRH agonist buserelin sequentially stimulated ovarian 17-OHP secretion in PCOS and control subjects. The levels of 17-OHP after buserelin stimulation in the patients with PCOS were significantly higher than in the control women (P < 0.001). We have shown previously that 17-OHP response to buserelin is a useful test in the diagnosis of PCOS (2). We have found similar results of basal and buserelin-stimulated 17-OHP in both studies (peak values were 16.88 ± 1.46 nmol/l in the previous study and 15.49 ± 0.76 nmol/l in the present study). So, this study also confirms the reliability of buserelin testing in PCOS.

With regard to the relationship between hyperinsulinemia and 17-OHP response to buserelin, we have found no significant correlations between basal 17-OHP and fasting insulin or fasting C-peptide, between peak 17-OHP and fasting insulin, peak insulin or peak C-peptide, between 17-OHP AUC and insulin AUC or C-peptide AUC, and between percent increment in 17-OHP and insulin AUC or C-peptide AUC (P>0.05). Also, there was no significant correlation between hyperinsulinemia and 17-OHP response to GnRH agonist in oligo/amenorrheic patients.

The findings indicate that PCOS patients have an increased 17-OHP response to the GnRH agonist buserelin and enhanced insulin secretion in response to OGTT. The regulation of ovarian P450c17α may be multifactorial, with insulin being just one of several modulators. In our study, no apparent correlation existed between indices of hyperinsulinemia and P450c17α activity. This suggests that hyperinsulinism and dysregulation of P450c17α represent distinct and probably unrelated abnormalities in PCOS.

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