Impact of overnight dexamethasone suppression on the adrenal androgen response to an oral glucose tolerance test in women with and without polycystic ovary syndrome

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In order to test the hypothesis that adrenocortical overactivity, possibly related to the stress of testing, may impact on the measurement of circulating androgen concentrations during glucose-induced hyperinsulinaemia, we prospectively screened 10 patients with the polycystic ovary syndrome (PCOS) and nine healthy control women with an oral glucose tolerance test (OGTT), before and after the administration of dexamethasone. Blood sampling was performed at 0, 30, 60, 90, and 120 min following the oral ingestion of 75 g of glucose, before and after the administration of 1.0 mg dexamethasone on the evening prior to testing. Total and free testosterone, androstenedione, dehydroepiandrosterone (DHEA), DHEA sulphate (DHEAS), cortisol, glucose and insulin were assessed during the 2 h OGTT. Women with PCOS had increased basal concentrations of free testosterone, total testosterone, androstenedione, and insulin compared to control women. In women with PCOS an acute decline in circulating concentrations of DHEAS occurred during the OGTT. In PCOS women there were no changes in other ovarian or adrenal androgens during OGTT before or following dexamethasone administration. In control women DHEA concentrations declined during the OGTT. Following overnight dexamethasone suppression in control women, circulating concentrations of DHEAs and testosterone also declined. It is concluded that: (i) in PCOS women only the concentration of circulating DHEAS decreased during glucose-induced hyperinsulinaemia and dexamethasone administration did not further alter androgen responses to an OGTT; (ii) it is possible that, in these hyperandrogenic patients, the insulin-related suppression of adrenocortical testosterone and DHEA is negated by their much greater ovarian secretion of these androgens; (iii) in control women DHEA concentrations acutely declined during the OGTT and the administration of dexamethasone resulted in the acute decline of DHEA, DHEAS, and testosterone; (iv) it appears that the stress related to testing impacts on the androgen response to OGTT, at least in healthy women.

Key words: androgens/dexamethasone/glucocorticoids/insulin/polycystic ovary

Introduction

Although we often utilize dynamic testing [e.g. acute ACTH-(1–24) stimulation, insulin tolerance test, long-acting gonadotrophin stimulation, etc.] in the study of women with the polycystic ovary syndrome (PCOS), the impact of the stress of the test on its results has largely been ignored. We have previously observed that adrenal androgen concentrations are higher if the basal samples for an acute ACTH-(1–24) test are obtained within 15 min of the placement of an i.v. catheter, compared to obtaining the samples after a 45 min rest period (R.Azziz, unpublished data). It is possible that the stress of the placement of the i.v. catheter may cause a transient increase in adrenocortical activity, probably mediated through increased ACTH stimulation.

Following experimentally induced increases in insulin, various investigators have demonstrated, in both normal women and patients with PCOS, a decrease in the circulating concentrations of the adrenal androgens, dehydroepiandrosterone (DHEA) and DHEA sulphate (DHEAS), and/or an increase in testosterone and androstenedione (Nestler et al., 1987; Diamond et al., 1991). Alternatively, we (Buyalos et al., 1991) and others (Dunaif and Graf, 1989; Elkind-Hirsch et al., 1991) have not been able to demonstrate these findings. In order to explain these discordant observations, we have postulated that the stress associated with testing may impact on the results of these studies. For example, a transient stress-induced rise in adrenal androgen concentrations may mask their legitimate insulin-related decrease. Alternatively, if this phenomenon is short-lived, it may actually result in a factitious decrease, since the stress-induced elevation in androgen concentrations will resolve during the time of testing. To test this hypothesis, we studied, in a prospective controlled fashion, the adrenal androgen response to a 2 h oral glucose tolerance test (OGTT) before and after overnight dexamethasone suppression. Dexamethasone was administered in an effort to minimize the ACTH-mediated rises in adrenal androgens resulting from the stress of i.v. catheter placement.

Materials and methods

Subjects

Ten patients with PCOS and nine healthy control women were studied. All PCOS subjects reported the perimenarchal onset of oligomenorrhoea and all exhibited at least facial hirsutism. None was virilized or had acanthosis nigricans. None of the PCOS patients had

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**results**

**comparison of basal characteristics of control and PCOS patients**

Patients with PCOS had higher mean values for total and free testosterone, androstenedione (Table I). The groups did not differ in mean age, body mass index (BMI), or DHEA, DHEAS or cortisol. Two patients with PCOS were amenorrhoic (menstrual cycles >90 days), while the remaining were oligo-menorrhoic (menstrual cycles 35–90 days).

**glucose and insulin response to the OGTT, before and after dexamethasone administration**

Following dexamethasone administration there was a significant rise in circulating glucose (86 ± 9 versus 98 ± 7 mg/dl, *P* = 0.03) and insulin (10 ± 5 versus 14 ± 4 µU/ml, *P* = 0.05) at time 0 in control women (Table II). In PCOS women there was a significant rise in glucose (93 ± 10 versus 103 ± 15 mg/dl, *P* = 0.01) but not insulin (24 ± 27 versus 26 ± 19 µU/ml, *P* = 0.69) at time 0 following dexamethasone administration.

Dexamethasone administration resulted in an increase in the glucose AUC for both controls (13 800 ± 1440 versus 16 740 ± 2100 mg·min/dl, *P* = 0.005) and PCOS women (17 580 ± 3120 versus 20 160 ± 2100 mg·min/dl, *P* = 0.02) (Table II). Alternatively, after dexamethasone administration the insulin AUC increased significantly only for controls (6900 ± 1140 versus 11 940 ± 4020 µU·min/ml, *P* = 0.03), although there was a trend toward an increase among PCOS patients (14 940 ± 3300 versus 18 180 ± 4860 µU·min/ml, *P* = 0.15).

**Androgen and cortisol response to the OGTT, before and after dexamethasone administration**

**Baselure values**

As expected, in both PCOS and control women the concentrations (time 0) of androstenedione, DHEA, DHEAS and cortisol decreased significantly at time 0, and free testosterone did not change, following the administration of dexamethasone (Table III). Total testosterone decreased with dexamethasone suppression only in patients with PCOS. Following dexamethasone administration, the value of circulating cortisol at time 0 was at or below the assay detection limit (<1.2 µg/dl) in 18/19 study subjects.

**Androgen response during OGTT**

In control women, from 0 to 120 min during the OGTT, only DHEA decreased (mean change of −24.4%, *P* < 0.04); all other androgens remaining essentially unchanged. Following the overnight administration of dexamethasone in control patients, a significant decrease in not only DHEA, but also in DHEAS and

### Table I. Comparison of Characteristics between Controls (n = 9) and Women with Polycystic Ovary Syndrome (PCOS) (n = 10)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls</th>
<th>PCOS</th>
<th><em>P</em>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28.5 ± 5.2</td>
<td>28.7 ± 5.1</td>
<td>NS</td>
</tr>
<tr>
<td>BMI</td>
<td>27.2 ± 7.8</td>
<td>31.1 ± 7.8</td>
<td>NS</td>
</tr>
<tr>
<td>Hirsutism</td>
<td>0/9</td>
<td>10/10</td>
<td>0.001</td>
</tr>
<tr>
<td>Oligo amenorrhea</td>
<td>0/9</td>
<td>10/10</td>
<td>0.001</td>
</tr>
<tr>
<td>Total testosterone (ng/dl)</td>
<td>29.2 ± 18.6</td>
<td>52.3 ± 20.6</td>
<td>0.02</td>
</tr>
<tr>
<td>Free testosterone (ng/dl)</td>
<td>0.5 ± 0.2</td>
<td>1.0 ± 0.5</td>
<td>0.008</td>
</tr>
<tr>
<td>Androstenedione (ng/ml)</td>
<td>4.6 ± 2.2</td>
<td>10.3 ± 5.2</td>
<td>0.05</td>
</tr>
<tr>
<td>DHEA (ng/ml)</td>
<td>3.7 ± 2.1</td>
<td>4.3 ± 3.2</td>
<td>NS</td>
</tr>
<tr>
<td>DHEAS (ng/ml)</td>
<td>1625 ± 729</td>
<td>2388 ± 1058</td>
<td>NS</td>
</tr>
<tr>
<td>Cortisol (µg/dl)</td>
<td>9 ± 6</td>
<td>8 ± 5</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are shown as mean ± SD.

BMI = body mass index; DHEA = dehydroepiandrosterone; DHEAS = dehydroepiandrosterone sulphate.

Conversion factors to SI units are as follows: testosterone × 0.3467 = nmol/l, androstenedione × 3.49 = nmol/l, DHEA × 3.467 = nmol/l, DHEAS × 0.002714 = µmol/l, cortisol × 27.59 = nmol/l.

NS is non-significant, *P* > 0.05.

**Evidence of an androgen-secreting neoplasm, pituitary adenoma, non-classic adrenal hyperplasia, acromegaly, or Cushings’s syndrome. None of the patients studied had diabetes mellitus as defined by the National Diabetes Data Group (1979) criteria. All control patients had regular menses (27–32 days), and none was hirsute or had acanthosis nigricans. The mean ages were similar between PCOS and control women (Table I). All subjects were ambulatory during the day and slept between 2300 and 0600 h. No subject, PCOS or control, had received hormonal medication within 30 days prior to study. Some of these subjects were reported in a previous study (Buyalos et al., 1991).

**Study protocol**

These studies were approved by the University of California Los Angeles (UCLA) Medical Center Human Subject Protection Committee and all patients gave informed written consent. All OGTT were performed in the UCLA Clinical Research Center beginning between 0800 and 0900 h, after 3 days of >300 g carbohydrate diet and a 10–12 h overnight fast. All control subjects were studied during the mid-follicular phase of the menstrual cycle. For the performance of the OGTT, an indwelling i.v. catheter with heparin lock was placed in an antecubital vein 30 min prior to study. Patency of the catheter was maintained with dilute heparin flushes (100 U/ml). Seventy-five grams of dextrose (Glucola, Miles Laboratories, Elk hart, IN, USA) was then administered orally over a 2 min interval, and blood samples were obtained immediately before, and 30, 60, 90 and 120 min following dextrose ingestion. On a separate admission, a second OGTT was performed after the oral administration of 1.0 mg of dexamethasone at 1100 h on the night preceding study. Plasma for glucose measurements and serum for hormonal determinations were obtained and stored at −20°C until assayed.

**Endocrine assays**

Serum androstenedione, total and free testosterone, DHEA, DHEAS, cortisol, insulin and plasma glucose were measured as previously described (Buyalos et al., 1991, 1993). In our laboratory, the intra-assay coefficients of variation for these assays range between 4 and 6%. All samples from an individual woman were analysed in a single assay.

**Statistical analysis**

Changes in steroid values were computed as the difference from baseline (time 0) to 120 min using one-way analysis of variance (ANOVA) with pair-wise comparisons using the least significant difference method.
total testosterone was observed (mean changes of –8.9, –20.8 and –20.6% respectively, P < 0.03–0.003).

In women with PCOS a decrease in DHEAS was noted during the OGTT (mean change of –9.4%, P < 0.01). In PCOS patients, dexamethasone administration did not alter this result, and DHEAS was the only androgen to decrease during the OGTT (mean change of –5.6%, P < 0.04). Following dexamethasone administration, in both PCOS patients and controls, the value of circulating cortisol was at or below the assay detection limit at the conclusion of the OGTT (time 120 min).

**Discussion**

Data regarding the impact of physiological increases in insulin concentrations on adrenal androgens have been conflicting (Nestler et al., 1987, 1989; Smith et al., 1987; Dunai and Graf, 1989; Buyalos et al., 1991, 1993; Diamond et al., 1991; Elkind-Hirsch et al., 1991; Hubert et al., 1991; Azziz et al., 1995; Norman et al., 1995). In order to explain some of these discrepancies we postulated that ACTH-mediated adrenocortical overactivity resulting from the stress of the test itself may either mask or exacerbate the changes in adrenal androgens. Using overnight dexamethasone to diminish ACTH secretion, our data suggest that the presence of stress may have masked androgen changes in response to the OGTT, at least in control subjects. Cortisol concentrations were significantly reduced during the OGTT, reflecting ACTH inactivity. However, under these conditions, the short-term physiological increases in insulin concentrations during the OGTT were sufficient to suppress circulating total testosterone, DHEA and DHEAS in healthy control women. The decrease in circulating testosterone during the OGTT may reflect the significant adrenocortical component of this androgen (~25%). Alternatively, under non-glucocorticoid suppressed conditions, only the decrease in DHEA was observed.

The changes in basal circulating androgen and cortisol concentrations in women with PCOS and controls following dexamethasone administration were as expected. Interestingly, even the small dose of dexamethasone used in this study (1.0 mg on the evening before ACTH testing) was sufficient significantly to increase basal and stimulated glucose concentrations in both control and PCOS patients. Basal insulin concentrations and AUC insulin increased significantly during OGTT only among control women. The inability to achieve a statistically significant increase in AUC insulin secretion in PCOS subjects following dexamethasone administration (despite a significant rise in AUC glucose concentrations) is probably a reflection of the already markedly increased secretion of insulin associated with this condition. Thus, dynamic tests or therapies that incorporate ‘low-dose’ pre-test glucocorticoid administration may significantly increase the circulating glucose concentrations, possibly through a change in insulin resistance, and consequently alter the androgenic milieu, particularly in normal women.

In PCOS women, the pretest administration of dexamethasone did not alter the androgen response to hyperinsulinaemia induced
by the OGTT. DHEAS concentrations declined significantly during OGTT with and without dexamethasone administration. In women with PCOS, no other ovarian or adrenal androgen changed during OGTT. However, a reduction in adrenal cortical activity was obtained following dexamethasone administration, as demonstrated by the fall in baseline concentrations of testosterone, androstenedione, DHEA, DHEAS and cortisol in these women. In the present study, in contrast to our previous report (Buyalos et al., 1991), we observed a decrease in circulating DHEAS among PCOS women during the OGTT-induced increase in insulin concentrations, regardless of the administration of pre-test glucocorticoid. It is possible that any decreases in the adrenocortical secretion of testosterone and/or DHEA associated with the rise in insulin may be negated by the much greater ovarian secretion of these androgens in the women with PCOS.

In contrast to our previous report (Buyalos et al., 1991), a decline in circulating DHEAS was observed following glucose-induced hyperinsulinaemia. The current investigation included nine patients (five control and four PCOS) who participated in both studies. We compared the DHEAS values at all time points from patients who participated in both studies and observed a high degree of correlation (Pearson’s coefficient of correlation $r = 0.93$, $P < 0.001$) between values assayed in both investigations. In addition, in our previous report only three time points (0, 60, and 120 min) were measured, whereas the present study measured five time points (0, 30, 60, 90 and 120 min), which improves the ability to detect small changes over time. Most reports which have demonstrated a decline in circulating DHEAS values have observed relatively small changes (10–20%), which may be obscured when a small number of subjects is studied, and may overlap with the variability of the radioimmunoassay.

The mechanism(s) whereby glucose-induced hyperinsulinemia promotes a decline in adrenal and/or ovarian androgens remain unclear and cannot be elucidated by this study design. Experimental evidence suggests that hyperinsulinemia is associated with an increase in metabolic clearance rate of adrenal androgens, due in part to increased urinary excretion (Nestler et al., 1987, 1989). It is interesting that dexamethasone-induced suppression of adrenal cortical activity ‘unmasked’ the observed decline in DHEAS and testosterone in the control women. This suggests that the insulin-induced decline in circulating androgen concentrations may be obscured by the stress of testing in normal women, which may result in transiently increased adrenal androgen secretion. It is conceivable that more glucocorticoid is necessary to unmask androgen changes in PCOS women than is needed to suppress the ACTH–cortisol axis. To our knowledge, this is the first report evaluating the impact of insulin-induced androgen changes following glucocorticoid administration in PCOS or normal women. Additional studies incorporating prolonged adrenal suppression may help elucidate the mechanism(s) responsible for alterations in circulating androgen concentrations following acute hyperinsulinemia.

In summary, in PCOS women DHEAS concentrations declined in response to oral glucose-induced hyperinsulinemia independent of the administration of dexamethasone. In control women, DHEA concentrations acutely declined during OGTT, and the administration of dexamethasone resulted in the acute decline of DHEA, DHEAS, and testosterone. In conclusion, it is possible that the stress associated with testing, and particularly i.v. catheter placement, may mask the decrease in adrenal androgen concentrations associated with short-term physiological increases in insulin. This effect is far more evident among healthy control women than those with PCOS, probably due to the greater ovarian androgen contribution to circulating androgen concentrations in these latter patients. Finally, since even a small dose of dexamethasone administered before the test may result in significant increases in glucose and insulin responses, a preferable method of reducing stress-related changes in adrenocortical function may be to allow subjects to rest for 45–60 min after i.v. catheter placement prior to beginning blood sampling.

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**References**


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