No changes of peripheral insulin resistance in polycystic ovary syndrome after long-term reduction of endogenous androgens with leuprolide

Antonino Lasco, Domenico Cucinotta, Alfonso Gigante, Giulia Denuzzo, Marilena Pedulla, Aldo Trifiletti and Nicola Frisina

Department of Internal Medicine, University of Messina, Messina, Italy; Institute of Medical Physics, University of Messina, Messina, Italy


The aim of this study was to investigate the relationship between plasma insulin levels, peripheral insulin sensitivity and androgen secretion in ten patients with polycystic ovary syndrome and in six obese women as compared with six normal-weight control subjects. During a euglycemic–hyperinsulinemic clamp no significant change of testosterone, androstenedione or dehydroepiandrosterone sulfate plasma levels was observed in the two groups of patients or in the control subjects; insulin sensitivity was clearly reduced and was similar in polycystic ovary patients and in obese women. In spite of the different plasma androgen levels, a long-term (5 months) androgen suppression with the gonadotropin-releasing hormone agonist leuprolide was not able to improve significantly the insulin sensitivity. These results demonstrate that the short-term hyperinsulinemia achieved with the clamp technique does not affect androgen secretion and that insulin resistance, measured with the same technique, is not influenced by long-term suppression of plasma androgen levels in polycystic ovary syndrome.

A Lasco, Via Faustina e Tertullo 19, 98100 Messina, Italy

The association of hyperandrogenism and insulin resistance in polycystic ovary syndrome (PCOS) is well assessed (1) and it is known that serum levels of insulin and androgens are increased and reciprocally related (2), but the pathophysiology of this association has not yet been clarified.

It has been suggested that both hyperinsulinemia and hyperandrogenemia are due to a decreased insulin sensitivity at peripheral levels (2, 3). Moreover, there is evidence that insulin suppression is able to reduce serum testosterone in PCOS (4), although it is uncertain whether insulin administration or increased serum insulin levels are able to enhance androgen production (5–8).

On the other hand, some reports indicate that hyperandrogenism is the primary biochemical abnormality in PCOS, whereas hyperinsulinemia is a consequence of it. This view is based on the experimental observation that circulating insulin levels may be increased or decreased by androgen (9, 10) or antiandrogen (11) administration, respectively.

The aims of the present study are:

(i) to investigate whether changes of androgen plasma levels occur during the euglycemic–hyperinsulinemic clamp in PCOS and in obese non-PCOS patients, as well as in the control group;

(ii) to evaluate in the same patients the peripheral insulin sensitivity with the clamp technique before and after a 5-month ovarian suppression by means of a gonadotropin-releasing hormone agonist.

Subjects and methods

Subjects

Three groups of subjects were enrolled in this study. The first included 10 women with PCOS, in whom diagnosis was made by the observation of polycystic ovaries on ultrasound examination, combined with clinical symptoms (hirsutism and oligo/amenorrhea) and hyperandrogenemia. Their mean age was 28 ± 1.9 years and their body mass index (BMI) was 36.4 ± 2.2 kg/m². Hirsutism was assessed by a global scoring system in which a single observer verified hair growth in 11 body areas on a 0–4 scale (12). The average score in PCOS was 16.8 ± 3.2. The second group included six obese women (mean age 25 ± 1.3, mean BMI 33.5 ± 2.7) with different degrees of hirsutism (average score 14 ± 3.2) but without any evidence of PCOS. The third group (control group) included six normal-weight eumenorroid women (mean age 24 ± 2, mean BMI 21 ± 0.8) without PCOS or hirsutism. Individual
Table 1. Clinical characteristics of the study subjects.

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Control women</th>
<th>Obese non-PCOSa women</th>
<th>PCOS women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age (years)</td>
<td>BMI (kg/m²)</td>
<td>Age (years)</td>
</tr>
<tr>
<td>1</td>
<td>22</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>23</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>20</td>
<td>24</td>
</tr>
<tr>
<td>4</td>
<td>24</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>26</td>
<td>21</td>
<td>26</td>
</tr>
<tr>
<td>6</td>
<td>27</td>
<td>21</td>
<td>27</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a PCOS = polycystic ovary syndrome.
*b Scored according to Ref. 12.

clinical characteristics of the study subjects are reported in Table 1.

Glucose tolerance, assessed by the oral glucose tolerance test and evaluated according to the WHO criteria (13), was always normal in all subjects.

Other endocrine disorders, such as Cushing’s syndrome, androgen-secreting tumors or prolactinomas, were excluded. No medications capable of affecting glucose tolerance were taken for at least 3 months before the study. Each woman gave her informed consent to participate in the study, which has been approved by the local Ethical Committee.

Study design and methods

On day 7 of the menstrual cycle (spontaneous cycle or drug-induced cycle by means of medroxyprogesterone acetate, 10 mg once a day for 7 days), after an overnight fast, basal levels of FSH, LH, testosterone, androstenedione and dehydroepiandrosterone sulfate (DHEAS) were measured on plasma samples from each subject.

Peripheral insulin sensitivity was then assessed by the euglycemic–hyperinsulinemic clamp technique (14), according to previously described methods (15). Briefly, after a priming insulin infusion with 2 mU·min⁻¹·kg⁻¹ over 10 min, subjects were infused for 120 min with 1 mU·min⁻¹·kg⁻¹ insulin while blood glucose was kept at the fasting level by a variable 20% glucose infusion. Arterialized plasma samples were taken periodically for insulin measurements during the last 40 min of the clamp. Assuming that endogenous glucose production was fully suppressed, the average rate of glucose infused during this period was used for calculating the insulin sensitivity (M index), which was expressed as mg glucose·kg⁻¹ body weight·min⁻¹.

At the end of the clamp testosterone, androstenedione and DHEAS plasma levels were evaluated again. The PCOS and non-PCOS obese women were then treated with leuprolide acetate, a synthetic monopeptide analog of the naturally occurring gonadotropin-releasing hormone (GnRH) in its long-acting formulation (enanthate depot, Takeda Italia Farmaceutici, Catania, Italy), which was administered at the fixed dose of 3.75 mg (one vial)im once a month for 5 months. A weight-maintaining diet was also given. At the end of this period the plasma androgen levels and insulin sensitivity were reassessed, as described above.

Assays

Plasma glucose concentration during the clamp was immediately determined by an enzymatic method (glucose analyzer 2, Beckman, Milan, Italy). For hormone determination, aliquots of plasma were stored at −20°C and the assays were performed by commercial kits. Insulin (RIA kit, Diagnostic Product Corporation, Los Angeles, CA) intra- and interassay coefficients of variation (CV) were 4.6 and 7.1%, respectively. The FSH and LH (ELISA kit, Boehringer Mannheim, Milan, Italy) CVs were 4.1–8.2% and 5.1–7.6%, respectively. Testosterone, androstenedione and DHEAS assays, performed by RIA kits (Diagnostic System Laboratories, Webster, TE), showed CVs of 3.8–4.8%, 4.3–6.0% and 6.3–9.9%, respectively. Normal fasting plasma ranges in our laboratory are: insulin, 5–15 mU/l; FSH, 1.7–11 IU/l; LH, 1.7–7.2 IU/l; testosterone, 0.3–3 nmol/l; androstenedione, 1.2–7.9 nmol/l; DHEAS, 1.8–8.6 µmol/l.

Statistical analysis

All results are expressed as means ± sd. Comparisons between groups were performed by Student’s paired and unpaired tests. A p value of < 0.05 was considered statistically significant.
Table 2. Plasma androgen and fasting insulin levels (mean ± so) and insulin sensitivity during a euglycemic–hyperinsulinemic clamp (M index) at basal evaluation and after leuprolide treatment for a 5-month period.

<table>
<thead>
<tr>
<th></th>
<th>LH (IU/l)</th>
<th>FSH (IU/l)</th>
<th>Androstenedione (nmol/l)</th>
<th>Testosterone (nmol/l)</th>
<th>DHEAS* (µmol/l)</th>
<th>Insulin (mU/l)</th>
<th>M index (mg·kg⁻¹·min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Basal evaluation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCOS women (N=10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obese non-PCOS women</td>
<td>3.3 ± 0.6</td>
<td>6.1 ± 1.2</td>
<td>5.2 ± 1.3</td>
<td>1.5 ± 0.5</td>
<td>5.1 ± 1.7</td>
<td>12.6 ± 2.6</td>
<td>2.4 ± 1.2</td>
</tr>
<tr>
<td>Control women (N=6)</td>
<td>3.9 ± 1.1</td>
<td>5.4 ± 1.1</td>
<td>4.8 ± 1.4</td>
<td>1.6 ± 0.7</td>
<td>5.3 ± 1.6</td>
<td>5.4 ± 0.6</td>
<td>5.8 ± 2.4</td>
</tr>
<tr>
<td><strong>After leuprolide</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCOS women (N=10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obese non-PCOS women</td>
<td>0.9 ± 0.2</td>
<td>2.7 ± 0.6</td>
<td>3.1 ± 0.7α</td>
<td>1.6 ± 0.5</td>
<td>6.2 ± 1.1</td>
<td>14.6 ± 2.1</td>
<td>3.2 ± 1.6</td>
</tr>
<tr>
<td>Control women (N=6)</td>
<td>1.1 ± 0.2</td>
<td>3.6 ± 0.8</td>
<td>3.2 ± 0.9α</td>
<td>1.2 ± 0.4</td>
<td>4.8 ± 1.1</td>
<td>13.1 ± 1.5</td>
<td>2.5 ± 1.7</td>
</tr>
</tbody>
</table>

* DHEAS = dehydroepiandrosterone sulfate.
† PCOS = polycystic ovary syndrome.
‡ p < 0.001 vs obese and control women.
§ p < 0.01 vs obese and control women.
∥ p < 0.05 vs obese and control women.
¶ p < 0.001 vs control women.
µ p < 0.05 vs PCOS and obese women.
ν p < 0.001 vs basal evaluation.
ω p < 0.01 vs basal evaluation.

Results

**Basal evaluation**

As expected, plasma levels of LH, androstenedione and testosterone were significantly higher in PCOS women compared with obese women and controls, while no difference was observed between these last two groups. Average fasting plasma insulin was similar in PCOS and in obese women and was significantly higher than in controls (Table 2).

During the euglycemic–hyperinsulinemic clamp, the achieved plasma insulin levels were stable and superimposable in the three groups (Table 3). The average glucose utilization in this period (M index) was similar in PCOS and in non-PCOS obese women and was greatly reduced with respect to the control group (Table 2). At the end of the 120-min hyperinsulinemic clamp period no evident modification of plasma androgen levels was observed in the three groups of women (Table 3).

**After leuprolide treatment**

The 5-month period of leuprolide administration suppressed LH secretion and induced a significant fall of FSH, androstenedione and testosterone levels in the PCOS patients. In the obese non-PCOS women there was a similar reduction of FSH, LH and androstenedione values, while testosterone did not change significantly. Despite the reduced androgen levels, in the PCOS group insulin sensitivity did not improve at the end of this period, as demonstrated by the substantially unmodified glucose utilization during the clamp. Also,

Table 3. Plasma androgen and insulin levels (mean ± so) before (I) and after (II) a 120-min euglycemic–hyperinsulinemic clamp.

<table>
<thead>
<tr>
<th></th>
<th>Androstenedione (nmol/l)</th>
<th>Testosterone (nmol/l)</th>
<th>DHEAS* (µmol/l)</th>
<th>Insulin (mU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>PCOS women (N=10)</td>
<td>8.0 ± 2.2</td>
<td>8.5 ± 2.6</td>
<td>2.9 ± 1.1</td>
<td>2.8 ± 1.0</td>
</tr>
<tr>
<td>Obese non-PCOS women</td>
<td>5.2 ± 1.3</td>
<td>4.9 ± 1.4</td>
<td>1.5 ± 0.5</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>Control women (N=6)</td>
<td>4.8 ± 1.4</td>
<td>4.9 ± 1.2</td>
<td>1.6 ± 0.7</td>
<td>1.8 ± 0.6</td>
</tr>
</tbody>
</table>

* DHEAS = dehydroepiandrosterone sulfate.
† PCOS = polycystic ovary syndrome.
no modification of the M index was observed in the obese non-PCOS women after leuprolide therapy. Fasting insulin levels were similar to those measured before treatment and no significant change of BMI was observed in the two groups of patients (37.8 ± 2.1 and 34.1 ± 2.5, respectively) (Table 2).

Discussion

Our results indicate that in PCOS patients hyperandrogenism is not linked to a short-term increase of plasma insulin or to the degree of insulin resistance, and that its long-term suppression does not improve insulin sensitivity. Concerning the first point, in our study the acute increase of insulinemia elicited by the euglycemic clamp was not accompanied by any change of endogenous androgen secretion in the two groups of patients or in the controls. We have no data about the effect of a long-term increase of insulin levels on androgen production and it is known that insulin can stimulate theca–interstitial cell androgen production, probably through IGF-1 receptor (16). Consequently, we cannot exclude that the lack of effect on androgen levels is due to the short-term duration of hyperinsulinemia.

Insulin resistance did not apparently correlate with hyperandrogenism, because testosterone and androstenedione plasma concentrations at basal evaluation were significantly higher in PCOS than in non-PCOS patients, in spite of the similar degree of insulin sensitivity. The M index was significantly lower in both groups of patients than in controls; nevertheless, the serum androgen pattern was not different in non-PCOS patients or in controls. These data indirectly suggest that there is no evident relationship between insulin resistance and androgen secretion. Moreover, the results of our study confirm, on the basis of a direct measurement of insulin sensitivity, a previous report where unmodified levels of androstenedione and testosterone after an oral glucose tolerance test were found in hyperinsulinemic PCOS women, thus suggesting that androgens do not play a role in sustaining insulin resistance in PCOS (17).

As far as the other point is concerned, no effects of suppressed hyperandrogenism on insulin sensitivity and insulin plasma levels were observed. The long-term significant decrease of both androstenedione and testosterone concentrations that followed leuprolide treatment in PCOS patients was accompanied neither by modification of fasting insulin levels nor by a significant improvement of insulin resistance. Moreover, the baseline M index was similar in PCOS and in non-PCOS obese patients, despite the different testosterone plasma levels exhibited by these two groups. Thus, our data confirm previous observations where suppression of hyperandrogenism in PCOS, obtained with different methods and for different periods, modified neither insulin secretion (17, 18) nor insulin sensitivity (19, 20), suggesting that the insulin resistance observed in PCOS patients is not directly linked to the increased plasma androgen concentration. It has been demonstrated recently that reduced insulin sensitivity in PCOS may have a genetic basis and that defects producing insulin resistance in these patients appear to be located at the early steps of insulin-receptor mediated signalling (21). This genetic background can explain why androgen suppression does not improve insulin resistance in PCOS.

In conclusion, our data indicate that an acute increase of plasma insulin, as observed during the glucose clamp, does not affect androgen secretion and that insulin resistance is not modified by a long-term suppression of plasma androgen concentration in PCOS.

References


Received February 24th, 1995
Accepted July 26th, 1995