Insulin resistance in patients with polycystic ovaries: its relationship to body weight and androgen levels

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Abstract. Using a combined infusion of somatostatin, insulin and glucose, insulin resistance was assessed in vivo in two groups of females with polycystic ovaries (PCO), obese (OB-PCO) and normal weight (NO-PCO) and in two groups of matched (for age, sex and body mass index) controls (OB and NO). A steady state plasma glucose (SSPG) and insulin (SSI) was attained after 90 min. OB-PCO and NO-PCO showed higher SSPG with respect to matched controls. The SSPG levels were related to body mass index (r = 0.69; P < 0.001). The SSPG values were significantly correlated with the fasting insulin levels (r = 0.47; P < 0.003). Gonadotrophin and steroid peripheral blood concentrations were evaluated in the PCO females. A significant correlation was found between the SSPG values and the dehydroepiandrosterone sulphate levels (r = 0.46; P < 0.05) and between the fasting insulin levels and the androstenedione concentrations (r = 0.64; P < 0.01). Moreover, significant correlation coefficients were found between the glucose to insulin ratio and the A (r = -0.59; P < 0.01) and the DHEA-S (r = -0.50; P < 0.05) plasma levels. Finally, no relationship between body mass index and A or DHEA-S levels was found in PCO females considered as a group. We conclude that insulin resistance is present in females with PCO and it is mainly due to the presence of obesity, but other factors such as androgen levels, probably of adrenal sources, must be considered as a cause.

Patients with polycystic ovaries (PCO) and obesity, commonly present high blood insulin levels and a condition of insulin resistance (Burghen et al. 1980; Pasquali et al. 1982). Moreover, in a previous study, we found that PCO obese patients had higher glucose-stimulated C-peptide levels when compared to a matched group of obese females, suggesting a more increased activity of the β-cell after glucose stimulation, than that found in obesity itself (Pasquali et al. 1982). Possible mechanisms of hyperinsulinism in these patients have not yet been elucidated. Obesity, when present, is probably the major factor (Burghen et al. 1980; Pasquali et al. 1982). On the other hand, it has been speculated that hyperandrogenism could be related to the hyperinsulinaemic state (Burghen et al. 1980), but support for this hypothesis needs further consideration.

The aim of our study was to: 1) evaluate the sensitivity to exogenous insulin in PCO patients and its relationship to body weight in obese and normal weight females with PCO; 2) evaluate the relationships between androgen peripheral concentrations and insulin, C-peptide levels and the degree of insulin resistance. Our results confirm that in PCO patients, hyperinsulinism and insulin resistance are not due entirely to the presence of obesity and may be related to the degree of hyperandrogenism, mainly of adrenal sources.

Materials and Methods

A group of females affected by PCO were studied. All were inpatients of the Department of Reproductive Physiology and Pathology. The diagnostic criterion used for the purpose of this study was based only on laparoscopic examination during which the diagnosis of polycystic ovaries was confirmed. The study protocol was approved by the local ethical committee and each subject gave informed consent.
roscopy and histology of the ovaries. Laparoscopically, all females presented bilaterally enlarged ovaries (twice the size or more of those usually found in normal fertile women), uniformly globose, with white or pearly surface. Biopsies were taken during laparoscopy in all patients; according to Givens (1977), typical histological findings combined in various degrees of histological pattern were evaluated: increased surface collagenization, multiple follicular and atretic cysts lined by hyperplastic theca cells and stromal hyperplasia. Clinical data were also used in the diagnosis.

Patients were divided into two groups, according to body weight: 8 obese (OB-PCO) and 8 normal-weight (NO-PCO) females were selected. Respectively, their mean age was 25.1 ± 1.1 and 28.0 ± 2.2 years; body weight was 79.9 ± 3.9 and 56.2 ± 1.9 kg and body mass index (body weight (kg):height\(^2\) (m)) (BMI) was 31.2 ± 1.9 and 21.5 ± 0.7. Two groups of obese (OB) and normal-weight (NO) females, without clinical or laboratory evidence of endocrine illnesses – except obesity for OB – matched for age (26.5 ± 0.8 and 27.6 ± 1.7 years, respectively), body weight (77.9 ± 3.3 and 55.6 ± 1.4 kg, respectively) and BMI (30.1 ± 1.3 and 21.3 ± 0.7, respectively) were used as control subjects. General data of the patients are summarised in Table 1. No control subject had acanthosis nigricans, hirsutism or menstrual abnormalities. All subjects underwent protocol study after informed consent was obtained. After 3 days of high carbohydrate weight-maintenance diet (almost 300 g/day), tests were performed in the morning, beginning at 08.00–09.00 after an overnight fast. Basal blood samples were obtained from an antecubital vein, kept patent with normal saline. Then, a constant infusion of glucose (6 mg/kg/min), insulin (Actrapid MC, Novo Industries) (0.77 µU/kg/min) and somatostatin (GIF-Bio-data, Rome, Italy) (400 µg/h) was made at constant rate for 150 min in an antecubital vein of the contralateral arm. Gelatin was added to the solution in order to avoid the absorption of insulin by the bottle wall (Haemagel - 0.35% – Hoechst, Germany). This procedure was a modified form of that proposed by Harano et al. (1977). Blood samples were drawn at -15, 0, 30, 60, 75, 90, 105, 120, 135 and 150 min for glucose, insulin and C-peptide determinations. The average values of glucose and insulin concentrations at 90, 105, 120, 135 and 150 min were used to define the 'steady state plasma glucose' (SSPG) and the 'steady state plasma insulin' (SSPI). At the end of the test, spontaneous nutritional intake was evaluated in each subject by means of questionnaires and from memory by an expert dietitian of our staff. Blood samples for gonadotrophin and steroid determination were also obtained in the same period during which all patients (PCO) were inpatients in the hospital. For these hormonal determinations, blood samples were obtained during the early follicular phase in females with oligomenorrhea and in a period chosen at random in females with amenorrhea lasting more than 6 months. In the two matched groups (OB and NO), only testosterone and gonadotrophins were measured, according to the methods described below; all determinations were made in the follicular phase; individual data showed a pattern distribution similar to that normally observed in healthy non-obese females in our laboratory. Blood glucose was determined immediately after the test using the glucose-oxidase method, while hormone assays (RIA's) were performed on serum or plasma samples stored at -20°C until analysis. Insulin (IRI) was determined by bovine anti-insulin antiserum fixed on glass particles, with a kit supplied by Corning Medical Diagnostic (Medfield, USA) and C-peptide (CPR) by the method of Kaneko et al.

Table 1.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age (years)</th>
<th>Body weight (kg)</th>
<th>BMI</th>
<th>Plasma glucose mmol/l</th>
<th>Serum IRI pmol/ml</th>
<th>Serum CPR pmol/ml</th>
<th>SSPG mmol/l</th>
<th>SSPI pmol/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>OB-PCO</td>
<td>25.1 ± 1.1</td>
<td>79.9 ± 3.9</td>
<td>31.2 ± 1.9</td>
<td>4.6 ± 1.4</td>
<td>0.173 ± 0.025</td>
<td>0.52 ± 0.08</td>
<td>10.2 ± 0.6</td>
<td>0.34 ± 0.02</td>
</tr>
<tr>
<td>NO-PCO</td>
<td>28.0 ± 2.2</td>
<td>56.2 ± 1.9</td>
<td>21.5 ± 0.7</td>
<td>4.7 ± 0.2</td>
<td>0.123 ± 0.021</td>
<td>0.30 ± 0.03</td>
<td>6.5 ± 0.7</td>
<td>0.34 ± 0.01</td>
</tr>
<tr>
<td>OB-matched</td>
<td>26.5 ± 0.8</td>
<td>77.9 ± 3.3</td>
<td>30.1 ± 1.3</td>
<td>4.9 ± 0.2</td>
<td>0.170 ± 0.043</td>
<td>0.48 ± 0.06</td>
<td>8.0 ± 1.0</td>
<td>0.37 ± 0.03</td>
</tr>
<tr>
<td>NO-matched</td>
<td>27.6 ± 1.7</td>
<td>55.6 ± 1.4</td>
<td>21.3 ± 0.7</td>
<td>4.9 ± 0.7</td>
<td>0.098 ± 0.006</td>
<td>0.32 ± 0.06</td>
<td>4.9 ± 0.6</td>
<td>0.35 ± 0.01</td>
</tr>
</tbody>
</table>

Statistics are reported in the text. OB-PCO: females with obesity and polycystic ovaries; OB: obese matched group; NO-PCO: normal-weight females with polycystic ovaries; NO: normal-weight matched group.
(1974) using a kit supplied by Byk-Mallinckrodt (Milan, Italy). Gonadotrophin (FSH and LH) plasma levels were determined by rapid double antibody (kits purchased from Biodata, Rome, Italy); cortisol by solid phase separation (kits by Corning Medical Diagnostic, Medfield, USA); 17α-hydroxy-progesterone (17-P) (Jasonni et al. 1976) and progesterone (P) (Youssefnegadian et al. 1972) by chromatographic separation on Sephadex LH-20 columns; dehydroepiandrosterone (DHEA) by plasma extraction with ethyl ether (De Peretti & Forest 1976), dehydroepiandrosterone sulphate (DHEA-S) directly on diluted plasma (Buster & Abraham 1972); testosterone (T), 5α-dihydrotestosterone (DHT), androstenedione (A), oestrone (E1), oestradiol-17β (E2) by TLC on silica gel 60F254 (Paradisi et al. 1980), using antisera made in our laboratory (Cacciarri et al. 1974).

Statistical evaluation of the results was performed by using the Wilcoxon rank sum test and Pearson’s regression analysis. Results are expressed in mean ± standard error mean (SEM).

All procedures followed in this study were in accordance with the Helsinki Declaration of 1975.

Results

All subjects tolerated the infusion test well, except one who presented a febrile reaction 2 h after the end of infusion, rapidly recovering with betamethasone treatment. Spontaneous caloric intake evaluated in each subject did not present significant differences from group to group. As reported in Table 1, fasting glucose values were also similar in all groups; OB-PCO and OB females did not present different values of fasting IRI, nor did they have higher values than those of NO-PCO, while they did have higher values than NO females (P < 0.007 and < 0.05, respectively). CPR in OB-PCO was higher than in NO-PCO (P < 0.01) and NO (P < 0.01) but not different to that in OB; similarly, the OB group showed higher values of CPR when compared to NO-PCO (P < 0.005) and NO subjects (P < 0.04) while no differences were present between NO-PCO and NO group (see Table 1). During the infusion test, the CPR serum levels were markedly and constantly reduced in all groups (OB-PCO: −75%; OB: −78%; NO-PCO: −76% and NO: −73%), indicating that endogenous insulin secretion was suppressed despite the hyperglycaemia. The SSPG obtained in all groups is depicted in Fig. 1. Briefly, OB-PCO and NO-PCO showed significantly higher values when compared to the matched controls (OB and NO, respectively). Moreover, no statistical difference was found between NO-PCO and OB; obviously OB-PCO and OB presented significantly higher values than those found in the NO group. SSP1 values in OB-PCO and OB were similar, as well as in NO-PCO and NO. Moreover, no differences were found between obese and non-obese groups.

As depicted in Fig. 2, a significant correlation was found between SSPG levels and fasting insulin values (r = 0.47; P < 0.003). Moreover, a good correlation between the SSPG values and the BMI (r = 0.69; P < 0.001) was evident when all groups were considered together (Fig. 3). A significant correlation between BMI and SSPG values was also present when obese females (OB-PCO and OB) were considered separately (r = 0.56; P < 0.01).

For the purpose of this study, only gonadotrophin and steroid blood levels in PCO patients will be discussed. LH plasma concentrations were significantly higher (P < 0.04) in NO-PCO (38.5 ± 6.3 mU/ml) with respect to values found in OB-PCO (24.4 ± 3.6 mU/ml) (in our laboratory, normal range in the follicular phase is 5.1−13.8 mU/ml). No differences were found in FSH plasma levels (OB-PCO: 9.6 ± 1.0 mU/ml; NO-PCO: 9.5 ± 1.0 mU/ml; P = ns). Values of steroid measurements are reported in Table 2. No differences were found between the two groups, except for E1, 17-P and P plasma concentrations and for the E2:E1 ratio values (OB-PCO: 0.67 ± 0.13; NO-PCO: 1.24 ±
Correlation coefficient between SSPG and BMI in the four groups of females evaluated (OB-PCO: patients with polycystic ovaries and obesity; OB: matched obese group; NO-PCO: normal weight patients with polycystic ovaries; NO: matched normal weight group).

Fig. 2. Relationship between steady state plasma glucose (SSPG) and fasting insulin (IRI) serum levels in the four groups of females evaluated (OB-PCO: patients with polycystic ovaries and obesity; OB: matched obese group; NO-PCO: normal weight patients with polycystic ovaries; NO: matched normal weight group).

**Table 2.**
Steroids blood concentrations in females with polycystic ovaries, obese (OB-PCO) and normal weight (NO-PCO).
(Symbols used for hormones are explained in the test).

<table>
<thead>
<tr>
<th>Patients</th>
<th>Cortisol (ng/ml)</th>
<th>E₂ (pg/ml)</th>
<th>E₁ (pg/ml)</th>
<th>T (ng/dl)</th>
<th>A (ng/dl)</th>
<th>DHT (ng/dl)</th>
<th>DHEA-S (μg/dl)</th>
<th>DHEA (ng/ml)</th>
<th>17-P (ng/ml)</th>
<th>P (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OB-PCO</td>
<td>136.6 ± 21.6</td>
<td>35.5 ± 5.2</td>
<td>58.6 ± 8.0</td>
<td>56.4 ± 5.5</td>
<td>293.7 ± 42.0</td>
<td>19.9 ± 1.4</td>
<td>3.5 ± 0.2</td>
<td>14.5 ± 2.3</td>
<td>1.26 ± 0.16</td>
<td>0.51 ± 0.05</td>
</tr>
<tr>
<td>NO-PCO</td>
<td>171.0 ± 20.6</td>
<td>49.7 ± 14.0</td>
<td>38.2 ± 7.8</td>
<td>58.2 ± 5.0</td>
<td>251.6 ± 26.8</td>
<td>23.1 ± 2.4</td>
<td>2.7 ± 0.7</td>
<td>9.3 ± 1.8</td>
<td>0.86 ± 0.14</td>
<td>0.25 ± 0.006</td>
</tr>
<tr>
<td>sign.</td>
<td>ns</td>
<td>ns</td>
<td>0.03</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Normal range*</td>
<td>92–184</td>
<td>22.4–43.1</td>
<td>20.6–45.0</td>
<td>20.0–33.2</td>
<td>112–164</td>
<td>10.0–24.3</td>
<td>1.49–3.49</td>
<td>3.8–8.0</td>
<td>0.41–0.61</td>
<td>0.13–0.31</td>
</tr>
</tbody>
</table>

* Normal range was evaluated in 19 healthy normal-weight fertile women, in the follicular phase.
androgens and lutated 0.15; between significant 0.01) BMI, insulin ratio 0.01), correlated values (r A
DHEA-S < 0.05), correlated ratios; Coefficients A:Ej DHEA-S
< 0.01) - 0.50; NO-PCO. 0.05)

Finally, a significant correlation between the SSPG and the DHEA-S levels was found (r = 0.46; P < 0.05) (Fig. 4).

Discussion
At present, several methods are available to assess in vivo insulin resistance (Shen et al. 1970; Harano et al. 1978; Nagulesparan et al. 1979; Greenfield et al. 1981). In this study, we used a modified form of the protocol first proposed by Harano et al. (1977) by which endogenous insulin secretion is suppressed by the infusion of somatostatin, and insulin sensitivity for glucose utilization is evaluated by the contemporary administration of glucose and insulin. Differently from Harano et al. (1977), we infused 400 µg of somatostatin per hour, since we had previously found (personal observations) that these doses were sufficient to achieve a marked reduction of the C-peptide serum levels, which are
the best indicators of the β-cell insulin secretion. In fact, a marked suppression in C-peptide serum values was obtained in our study, similar to or higher than that described by others (Harano et al. 1977, 1978; Nagulesparan et al. 1979). Though different amounts of glucose were infused in normal and obese subjects, similar levels of SSP1 were achieved in all groups, similar to what is reported in other published studies (Harano et al. 1978; Nagulesparan et al. 1979).

Therefore, levels of SSPG can be considered as a conservative index of the ability of the whole body to dispose of glucose under conditions in which similar serum insulin levels were maintained. Based on our results, it can be stated that obese subjects with or without PCO present various degrees of insulin insensitivity, the OB-PCO patients having significantly greater insulin resistance than the OB matched group. Moreover, a close correlation between BMI and SSPG levels was found when all the groups (PCO females and matched controls) were considered together, as well as when obese patients (OB-PCO and OB) were evaluated separately. Therefore, the degree of overweight can be considered as the principal cause of insulin resistance, both in patients with simple obesity and in obese females with PCO.

One of the more interesting findings of our study was that some degree of insulin resistance was present also in NO-PCO patients. On the other hand, no relationship was found in non-obese females (NO-PCO and NO) between SSPG levels and BMI, suggesting that other factors are involved in determining high SSPG in NO-PCO patients, probably linked to the presence of PCO itself. Hyperandrogenism has been suggested as a possible factor of insulin resistance in PCO patients, and a relationship between serum testosterone and androstenedione levels and fasting or glucose stimulated insulin levels have been found (Burghen et al. 1980). Moreover, an impairment of glucose tolerance and an increase in insulin levels after chronic treatment with a synthetic androgen compound, oxymetholone, has been demonstrated in patients with aplastic anaemia (Woodard et al. 1981). Our results confirm that in PCO females, hyperinsulinism and insulin resistance may be related to androgen levels, mainly of adrenal origin. In fact, a significant correlation between fasting IRI and A levels was found in PCO females.

Moreover, both indices of insulin resistance, the fasting glucose:insulin ratio and the SSPG levels were correlated with the DHEA-S plasma concentrations. In our study, we failed to demonstrate a significant correlation between DHEA-S, A and BMI. On the other hand, in patients with simple obesity, an increased production rate of DHEA and DHEA-S (Feber & Halmy 1975; Genazzani et al. 1978) has been found, but in neither study was a correlation between these androgen blood levels and obesity indexes described. Similarly, a lack of correlation between fatty mass and A plasma concentrations was found in post-menopausal women selected from a wide range of body weight (Vermeulen & Verdonk 1978). Moreover, in a small group of obese and non-obese hirsute women, Givens et al. (1980) failed to demonstrate a correlation between body weight and DHEA-S levels. Taken together, these results may be consistent with the hypothesis that the relationship between hyperinsulinism, insulin resistance and hyperandrogenism in PCO females are dependent on the presence of increased body fat, coupled with an increased adrenal function, resulting in increased circulating androgen levels.

Sources of hyperandrogenism in the PCO females are not yet completely established (Yen 1980). On the other hand, circulating levels of DHEA and its sulphate in these patients have been shown to be principally secreted by the adrenal gland, while A levels are probably of combined ovarian and adrenal sources (Yen 1980; Abraham 1975). Moreover, in patients with PCO, the presence of mild adrenal hyperplasia has been established (Yen 1980; Kandeel et al. 1980). The effect of increased body weight may obviously be of importance, particularly with regard to DHEA and DHEA-S dynamic. Obesity may also account for an increased conversion of A to E₁ (Sittiër & Mac Donald 1973; Edman & MacDonald 1978), in addition to PCO itself (Yen 1980). This fact could explain why OB-PCO females had higher values of E₁ levels when compared to NO-PCO subjects. In fact, in normal weight and obese PCO patients, we found that the E₂:E₁ ratio was closely related to BMI, as well as to the parameters of β-cell activity. These findings suggest that obesity per se, independent of the presence of PCO, is probably the major factor in the control of peripheral oestrogen production.

Further studies are needed to establish whether these hormonal conditions are of physio-pathological importance in the development of PCO, especially in young obese girls.
References


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