Heterogeneity in β cell activity, hepatic insulin clearance and peripheral insulin sensitivity in women with polycystic ovary syndrome

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The aim of this study was to evaluate the impact of reduced peripheral insulin sensitivity, β cell hypersecretion and reduced hepatic insulin clearance in the hyper-insulinaemia of lean and obese PCOS patients. A total of 35 women with polycystic ovary syndrome (PCOS) and 10 lean normo-ovulatory controls underwent an oral glucose tolerance test and an euglycaemic–hyper-insulinaemic clamp study. PCOS patients were classified into four groups according to their BMI and insulin secretion (normo-lean; normo-obese; hyper-lean; hyper-obese), and results were compared between groups and with the controls. All the PCOS groups showed significantly higher insulin secretion than controls; there were no differences in insulin response to glucose load between lean and obese normo- and hyper-insulinaemic patients. Secretion of c-peptide was greater in PCOS groups than controls. All the hyper-insulinaemic PCOS patients had lower values of hepatic insulin clearance, independent of BMI, when compared either with controls (P < 0.001) or with PCOS normo-insulinaemic women (P < 0.01). Normo- and hyper-insulinaemic obese patients had similar total body glucose utilization (M value), which was lower than in lean PCOS subjects and controls. Our results suggest that evaluation of insulin resistance alone does not fully characterize the PCOS population; differences in liver metabolism of insulin are present in obese insulin resistant subjects and in lean patients with normal insulin sensitivity when divided into normo- and hyper-insulinaemic subgroups. Insulin resistance and hyper-insulinaemia may represent two distinct features of the insulin disorder in PCOS: the former appear to reflect the presence of obesity, while the latter may be a primary feature of PCOS.

Key words: hepatic clearance/insulin/insulin resistance/obesity/polycystic ovary syndrome

Introduction

Hyper-insulinaemia secondary to a poorly characterized disorder of insulin action is a feature of polycystic ovary syndrome (PCOS) (Barbieri and Ryan, 1983). Although elevated serum insulin concentrations have been reported in PCOS patients both in the fasting state and in response to an oral glucose load (Barbieri and Ryan, 1983; Dunaif et al., 1987; Lanzone et al., 1990), the underlying mechanisms have not been clearly defined. Hyperinsulinaemia may reflect an adaptive response of β cells to the accompanying peripheral insulin resistance (Dunaif et al., 1989). However, before reaching the periphery, insulin must transverse the liver, where a variable extraction takes place (Polonsky and Rubenstein, 1984); this suggests that an abnormal hepatic insulin metabolism may contribute to hyper-insulinaemia.

Obesity is a well recognized cause of hyper-insulinaemia and insulin resistance in normal subjects (Campbell and Gerich, 1990). Thus there is controversy as to whether insulin resistance results from PCOS itself or from the obesity which is frequently associated with the syndrome. Although recent reports of a similar peripheral insulin sensitivity between PCOS patients and control subjects (Despres et al., 1989; Ovesen et al., 1993) have suggested that insulin resistance is not necessarily a primary feature of this syndrome, there seems to be little doubt that the majority of obese PCOS patients are insulin resistant (Dunaif et al., 1989).

Moreover, an exaggerated insulin secretion after an oral glucose tolerance test was exhibited by ~70% of obese, but also by 20–40% of lean, PCOS patients (Lanzone et al., 1990), suggesting that hyper-insulinaemia may affect the PCOS population independently from an accompanying insulin resistance as well as from obesity.

The aim of our study was to evaluate the impact of reduced peripheral insulin sensitivity, β cell hypersecretion and reduced hepatic insulin clearance in determining the hyper-insulinaemia of lean and obese PCOS patients.

Materials and methods

The study population comprised 35 consecutive women affected by PCOS, aged 17–31 years. All were healthy, euthyroid, and had a normal renal function, as demonstrated by normal creatinine clearance values. All patients had spontaneous onset of puberty and normal sexual development, and all had been affected by oligomenorrhea with chronic anovulation since puberty. No patient showed evidence of acanthosis nigricans. Polycystic ovary syndrome was diagnosed by clinical findings (presence of amenorrhea or oligomenorrhea and hirsutism), plasma androgen values at the upper limit of the normal range (androstenedione 2.0–5.6 nM; testosterone 0.6–2 nM), and bilaterally normal or enlarged ovaries with the presence of at least 7–10 microcysts (<5 mm diameter) at the time of ultrasonography and/ or laparoscopy. A normal luteinizing hormone/follicle-stimulating hormone (LH/FSH) ratio was not considered an exclusion criterion (Lanzone et al., 1995).

No patient had received any medication known to affect carbo-
hydrate metabolism for at least 3 months before the study. Obesity was defined as a body mass index (BMI) >25 (normal range 19–25), calculated according to the formula body weight/height squared (kg/m²).

Ten lean normo-ovulatory volunteer women served as controls; the length of the menstrual cycle in these subjects was 29.6 ± 1.07 days. Ovulatory cycles were previously confirmed by mid-luteal progesterone plasma values of at least 25 nM for three consecutive cycles.

Informed consent was obtained from each patient. This study protocol was previously approved by our Ethical Institutional Board. All studies were performed in the follicular phase, 5–8 days after spontaneous or progestin-induced menses. The patients were hospitalized; after following a standard carbohydrate diet (300 g/day) for 3 days and fasting overnight for 10–12 h they underwent an oral glucose tolerance test (OGTT) and basal hormone assay. The following day, after another overnight fast, a euglycaemic–hyper-insulinemic clamp was performed.

The OGTT was performed as follows. At 08.00 h an indwelling catheter was inserted in the antecubital vein of one arm. Blood samples were collected basally and, after ingestion of 75 g glucose in 150 ml water within 5 min, at 30, 60, 90, 120, 180 and 240 min. Samples for glucose measurement were assayed immediately, whereas samples for the other determinations were promptly centrifuged and the plasma was stored at −20°C until assay. Insulin, glucose and c-peptide concentrations were measured in all blood samples. Furthermore LH, FSH, oestradiol, 17-hydroxy-progesterone, testosterone, dihydroepiandrosterone sulphate (DHEAS), androstenedione, sex hormone binding globulin (SHBG) and cortisol plasma concentrations were also determined in basal conditions.

The euglycaemic–hyper-insulinemic clamp was performed as follows. A retrograde intravenous catheter was inserted into a hand or forearm vein for blood sampling and kept in a warming device at >60°C to arterialize the venous blood samples. Another indwelling catheter was inserted in a contralateral forearm vein for the infusions. Insulin (Actrapid HM, Novo Nordisk, Denmark) was administered in a dose of 40 mU/m²/min (De Fronzo et al., 1979). The steady state velocity of the insulin infusion was reached within 10 min. In order to achieve steady state insulin values of ~717 pM during the clamp (range 574–897 pM), a variable infusion of 20% glucose was performed via a separate infusion pump, the rate being adjusted according to plasma glucose determinations taken every 5 min to maintain the plasma glucose concentrations between 4.44 and 4.99 mM. Total body glucose utilization (M) was determined between 90 and 150 min of the glucose clamp, and expressed as mg/(kgBW×min). We prefer to use this index for the measurement of insulin sensitivity because the M/I ratio fails to narrow the range of individual sensitivity values (Bergman et al., 1985).

All hormone concentrations were determined by commercial radioimmunoassay kits (Radim, Pomezia, Italy). Gonadotrophins, insulin and c-peptide were assayed by double-antibody technique; all steroids were assayed by dextran-charcoal technique. Plasma glucose was determined by the glucose-oxidase technique with a glucose analyzer (Beckman Instruments, Palo Alto, CA, USA). For each determination all samples from the same patient were assayed simultaneously. The intra- and inter-assay coefficients of variation were <8 and 15% respectively, for all determinations.

A normal glycaemic response to OGTT was defined according to the criteria of the National Diabetes Data Group (NDDG) (1979).

All results were expressed as mean ± SEM. Insulin, glucose and c-peptide plasma concentrations were expressed as area under curve (AUC) after the glucose ingestion, calculated by the trapezoidal rule. The patients were classified as normo- and hyper-insulinemic according to their insulin response to the OGTT, using a cut-off value of 107.625 PM/240 min for the AUC. This cut-off value was calculated from the mean + 2 SD of ~100 OGTT performed in control subjects and confirmed by a cluster analysis.

Hepatic insulin extraction was estimated from the c-peptide/insulin molar ratio in the fasting state and after glucose loading and the difference between the incremental area of c-peptide and insulin divided by the incremental area of c-peptide, as proposed by other authors (Faber et al., 1981; Bonora et al., 1984). The incremental area was calculated by the difference between AUC and basal AUC (basal AUC = area of the curve due to the basal unstimulated secretion and calculated assuming a constant value during a 4 h period).

Statistical analysis was performed using one-way ANOVA. Log transformation of the data was performed when necessary to achieve homogeneity of variance. Pearson’s correlation coefficient was used to study the relationship between variables. Differences were considered to be significant at a level of P < 0.05.

Results

Based on the insulinaemic response to OGTT, 22 PCOS patients (63%) were classified as hyper-insulinemic (15 obese, PCO-HO; seven lean, PCO-HL), while the remaining 13 (37%) constituted the normo-insulinemic group (five obese, PCO-NO; eight lean, PCO-NL). All control subjects were normo-insulinemic.

Table I shows the endocrine and metabolic parameters of the four groups of PCOS patients together with the controls. All PCOS subgroups had higher androgen concentrations than controls, whereas no significant differences were found between normo- and hyper-insulinemic PCOS patients.

Concerning the glycaemic response to OGTT, four obese hyper-insulinemic PCOS patients had abnormalities of carbohydrate tolerance as shown in their glycaemic response to OGTT.

Fasting insulin plasma concentrations were superimposable in PCO-NL and control groups, while the other PCOS patients had values significantly greater than controls. Conversely no differences were found in the fasting c-peptide concentrations among the five groups; both normo- and hyper-insulinemic obese PCOS patients had slightly elevated values compared to the controls, but these were not significant.

The basal hepatic insulin clearance, as defined by the c-peptide/insulin molar ratio in the fasting state, was significantly reduced in the obese PCOS patients compared to controls, independent of their insulinaemic pattern.

Figure 1 shows the insulin and c-peptide response to OGTT expressed as the area under curve (AUC) of the groups studied as well as the insulin hepatic extraction, evaluated according to the criteria of Faber and coworkers (1981). All the PCOS groups had significantly higher insulin-AUC values than control subjects; moreover similar AUC-insulin values were found between PCO-NO and PCO-NL, and between PCO-HO and PCO-HL.

All PCOS patients had higher c-peptide-AUC when compared to controls. However, no differences were found within the PCOS subgroups except for the PCO-NO patients, who had higher c-peptide-AUC than PCO-NL subjects.

When the hepatic insulin clearance after glucose load was
Table I. Metabolic and endocrine features of the polycystic ovary syndrome (PCOS) patients and control subjects studied

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>PCO-NL</th>
<th>PCO-NO</th>
<th>PCO-HL</th>
<th>PCO-HO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>10</td>
<td>8</td>
<td>5</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.66 ± 0.55</td>
<td>23.17 ± 0.54</td>
<td>28.1 ± 0.76&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>22.42 ± 0.72&lt;sup&gt;c&lt;/sup&gt;</td>
<td>30.2 ± 0.78&lt;sup&gt;abcd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fasting insulin (pM)</td>
<td>44.48 ± 0.07</td>
<td>54.53 ± 6.6</td>
<td>147.01 ± 35.66&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>111.2 ± 37.67&lt;sup&gt;d&lt;/sup&gt;</td>
<td>142.56 ± 24.66&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fasting c-peptide (pM)</td>
<td>287.9 ± 26.5</td>
<td>281.3 ± 46.34</td>
<td>496.5 ± 132.4</td>
<td>294.6 ± 95.9</td>
<td>430.3 ± 62.9</td>
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<tr>
<td>Glucose area under curve (mM/240 min)</td>
<td>1417.38 ± 107.5</td>
<td>1346.72 ± 65.03</td>
<td>1394.2 ± 118.8</td>
<td>1487 ± 141.27</td>
<td>1585 ± 102.1</td>
</tr>
<tr>
<td>Fasting c-peptide/insulin molar ratio</td>
<td>6.49 ± 0.63</td>
<td>5.4 ± 1.11</td>
<td>3.37 ± 0.96&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.09 ± 1.79</td>
<td>3.7 ± 0.69&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sex hormone binding globulin (nM)</td>
<td>53.7 ± 5.52</td>
<td>34.5 ± 4.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20.55 ± 4.07&lt;sup&gt;e&lt;/sup&gt;</td>
<td>22.2 ± 4.96&lt;sup&gt;d&lt;/sup&gt;</td>
<td>18.29 ± 2.38&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>7.3 ± 1.33</td>
<td>6.08 ± 0.45</td>
<td>5.05 ± 0.47</td>
<td>5.4 ± 0.47</td>
<td>6.9 ± 0.62</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>1.86 ± 1.58</td>
<td>11.48 ± 2.28</td>
<td>7.5 ± 1.07</td>
<td>11.45 ± 1.23</td>
<td>8.87 ± 0.78</td>
</tr>
<tr>
<td>Oestradiol (pM)</td>
<td>204.6 ± 31.79</td>
<td>149.3 ± 16.7</td>
<td>194.9 ± 44.09</td>
<td>143.2 ± 17.8</td>
<td>150.5 ± 20.3</td>
</tr>
<tr>
<td>Cortisol (nM)</td>
<td>293.56 ± 35.9</td>
<td>381.56 ± 58.4</td>
<td>395.9 ± 18.3</td>
<td>386.2 ± 37.02</td>
<td>380.9 ± 29.41</td>
</tr>
<tr>
<td>Testosterone (nM)</td>
<td>1.04 ± 0.17</td>
<td>1.94 ± 0.21&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.25 ± 0.31&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.08 ± 0.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.91 ± 0.18&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>Androstenedione (nM)</td>
<td>3.66 ± 0.49</td>
<td>5.55 ± 0.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.56 ± 0.38&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.49 ± 1.29&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.35 ± 0.38&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>17(OH)-progesterone (nM)</td>
<td>0.91 ± 0.09</td>
<td>1.57 ± 0.18&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.81 ± 0.18&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.11 ± 0.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.84 ± 0.12&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>DHEA-sulphate (µM)</td>
<td>3.76 ± 0.51</td>
<td>4.88 ± 0.51&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.42 ± 0.71&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.98 ± 0.53&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.92 ± 0.38&lt;sup&gt;c&lt;/sup&gt;</td>
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</table>

Data are expressed as mean ± SEM; FSH = follicle stimulating hormone; H = luteinizing hormone; DHEA = dihydroepiandrosterone.

<sup>a</sup>p < 0.01 versus controls.
<sup>b</sup>p < 0.01 versus PCO-NL.
<sup>c</sup>p < 0.01 versus PCO-NO.
<sup>d</sup>p < 0.01 versus PCO-HL.
<sup>e</sup>p < 0.05 versus controls.
<sup>f</sup>p < 0.05 versus PCO-NL.

examined, all the hyper-insulinaemic PCOS patients had the lowest values, regardless of their BMI, when compared either with controls or with PCOS normo-insulinaemic patients.

Figure 2 shows the total body glucose utilization (M value) in each of the five groups. Both normo- and hyper-insulinaemic obese patients had lower M values when compared to those of lean PCOS subjects and controls. No significant differences were found among PCO-HO and PCO-NO women, or between lean PCOS and control subjects. However, PCO-HL patients showed a total body glucose utilization significantly lower than PCO-NL patients (P < 0.008).

In PCOS patients total body glucose utilization was inversely related to both insulin response to OGTT (R = –0.6; P < 0.0012) and BMI (R = –0.6; P < 0.001). Hepatic insulin clearance was inversely related to stimulated insulinemia (R = –0.49; P < 0.011) but not to BMI (R = –0.19; P = NS).

Discussion

The presence of insulin resistance in PCOS was initially established by Burghen and coworkers (1980), who demonstrated a correlation between plasma values of insulin and testosterone. The cause of insulin resistance in these patients is poorly understood. In clamp studies it was found that the reduced response of glucose transport to a given concentration of insulin was greater in obese than in non-obese PCOS patients (Dunaif et al., 1988). Obese PCOS patients with acanthosis or hirsutism had greater insulin resistance than their control matched groups (Stuart et al., 1986); furthermore, non obese PCOS had M values similar to those of non obese control subjects (Ovesen et al., 1993).

However, the exaggerated insulin secretion found after intravenous (i.v.) or oral GTT was not necessarily comparable to data obtained by clamp technique. A recent report describes the presence of enhanced insulin response to i.v. GTT, which is not fully explained by insulin resistance in lean PCOS patients (Holte et al., 1994), thus suggesting that PCOS patients may erroneously be classified as insulin resistant on the basis of the insulin response to a glucose load, and that there may be a discrepancy between insulin resistance and hyper-insulinemia. For this reason we have studied PCOS patients in relation to their BMI and insulin secretion. To our knowledge this is the first study addressing this specific point.

In our study normo- and hyper-insulinaemic obese PCOS patients had the lowest values of insulin sensitivity. In lean subjects the hyper-insulinaemic group had significantly lower M values compared to the corresponding normo-insulinaemic group, whereas no differences were found between the lean patients and control subjects.

These data agree with those of Ovesen et al. (1993), who showed normal basal and insulin-stimulated substrate metabolism in lean PCOS patients, similar to those of control subjects. Ehrmann et al. (1995) also found no differences in the degree of insulin resistance of PCOS and women with normal ovaries matched for BMI.

On the other hand, Dunaif et al. (1989) demonstrated that lean PCOS subjects had intermediate M values, higher than those of obese PCOS, but similar to those of obese control subjects. They hypothesized that a peculiar derangement of insulin sensitivity existed in PCOS. However the authors did not distinguish between normo- and hyper-insulinemic PCOS patients. Other authors also reported results consistent with the hypothesis that insulin resistance is a feature of PCOS which is independent of obesity (O’Meara et al., 1993; Dunaif and Finegood, 1996; Chang et al., 1983). Morales and coworkers (1996) recently attempted to explain these disparate results on the basis of the different ethnicity between the European and American studies.

In view of our data it may be reasonable to speculate that in lean PCOS subjects hyper-insulinism and insulin resistance are weakly causally related.

The exclusive evaluation of the level of insulin resistance
Figure 2. Total body glucose utilization (M value) in the PCOS groups and controls. All data are expressed as mean ± SEM. For further details see materials and methods section. Significance: *P < 0.05 versus controls; **P < 0.001 versus controls; §§P < 0.01 versus PCO-NL group; §§§ P < 0.001 versus PCO-NL group; ††P < 0.01 versus PCO-HL group.

patients, in that, when compared to controls, they showed a higher β cell secretion of insulin with a compensatory increase in insulin clearance following a glucose load. Considering that PCO-NO and PCO-HO showed no difference in peripheral insulin sensitivity or β cell secretion, it may be hypothesized that some obese normo-insulinaemic PCOS patients can abnormally increase their insulin levels if a reduction in hepatic insulin metabolism occurs progressively.

Our data suggest that insulin resistance and hyper-insulinaemia may represent two distinct features of the insulin disorder in PCOS: the former seems to be more dependent on obesity, while the latter seems to be a primary feature of PCOS. These data are comparable with those of Holte and coworkers (1994, 1995), who found in PCOS an enhanced insulin increment after i.v. glucose administration, which was not fully explained by insulin resistance and was observed also at low-normal BMI levels; conversely, insulin resistance was found to be present only at higher BMI levels. Another indication of the dissociation between hyper-insulinaemia and insulin resistance is the possibility of improving insulin sensitivity in severely insulin resistant women with PCOS by weight reduction, to levels similar to those of BMI-matched women with normal ovaries, while the exaggerated early insulin response to i.v. GTT remains unchanged (Holte et al., 1995).

On the other hand the same authors (Holte et al., 1995) suggested that in PCOS subjects a major portion of the increased insulin response is due to an increased β cell...
secretion, since a decreased elimination of insulin did not take place in lean PCOS women. This apparent disagreement with our data may be explained by the fact that, in the present study, normo- and hyper-insulinemic lean PCOS patients were separately examined; moreover we also examined the parameters following a glucose load and not only in fasting conditions.

Our data also demonstrated the existence of hyper-insulinemic women with PCOS who are not hypo-glycaemic despite the absence of insulin resistance. A possible explanation of this apparent discrepancy is that PCOS patients have an increased β cell mass, or an enhanced sensitivity to glucose. This is suggested by the findings of the presence of decreased glycated haemoglobin in PCOS (Holte et al., 1994; Golland et al., 1989). Furthermore both obese and lean women with PCOS were found to have a low-postprandial glucose increase (Prelevic et al., 1992). Another hypothesis could ascribe a role to the actions of the hormones counteracting insulin. Finally, other factors may also be involved; many relationships exist between insulin and metabolic parameters, as suggested, for example, by recent data showing a positive relationship between insulin fasting values and serum uric acid concentrations in PCOS (Anttila et al., 1996).

In conclusion our data show that exaggerated insulin secretion and insulin resistance may coexist in PCOS in a heterogeneous manner partially independent of BMI. Obese patients with hyper-insulinism may have exaggerated pancreatic insulin secretion, reduced hepatic clearance of insulin and insulin resistance at the same time. In hyper-insulinemic lean subjects the contribution to hyper-insulinemia may be due mainly to both increased pancreatic secretion and reduced hepatic clearance. Moreover hyper-insulinism may influence hepatic clearance more than obesity in PCOS patients.

Therefore in PCOS the hyper-insulinism–obesity correlation appears to be complex in nature. It may be speculated that hyper-insulinemia represents a primary feature of PCOS, while insulin sensitivity is more influenced by the degree of obesity.

References


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Insulin resistance and PCOS

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