Acute insulin response to intravenous glucagon in polycystic ovary syndrome

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In order to evaluate the acute insulin response after i.v. injection of glucagon in polycystic ovary syndrome (PCOS), 35 women affected by PCOS and 11 normo-ovulatory controls underwent a 75 g oral glucose tolerance test (OGTT) and, 2 days later, a glucagon test (1 mg i.v.). Patients were analysed according to their degree of obesity; the insulin release after glucagon injection for lean PCOS subjects and control women was not statistically significantly different. Conversely obese PCOS patients had higher insulin secretion after both i.v. glucagon and OGTT when compared to the other groups. Moreover the insulin secretory patterns were heterogeneously represented in lean and obese PCOS women. When the patients were analysed according to their insulinogenic response to OGTT, normoinsulinaemic PCOS women and control subjects had a similar insulin response to i.v. glucagon whereas the hyperinsulinaemic PCOS group had a higher insulin response (P < 0.0001). Moreover, a highly significant relationship was found between the insulin response to OGTT and to glucagon administration in the PCOS population (P < 0.0001; r = 0.73), which was maintained also after controlling for obesity. Our results are consistent with the hypothesis that PCOS patients could have an insulin hyperresponse to glucagon administration, that is partially independent from obesity and related to their insulinogenic status. Moreover, the glucagon test could represent an effective alternative to OGTT in screening insulin disorders of PCOS patients (at least in the absence of other risk factors), due to its reliability, simplicity, and speed of performance.

Key words: glucagon/insulin/obesity/polycystic ovary syndrome

Introduction

Hyperinsulinaemia 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 levels have been reported in PCOS patients, both in the fasting state and in response to an oral glucose tolerance test (OGTT) (Barbieri and Ryan, 1983; Duniaf et al., 1987; Lanzone et al., 1990), the underlying mechanisms have not been clearly defined.

A hyperinsulinaemic response to OGTT was exhibited by ~70% of obese, but also by 20–40% of lean, PCOS subjects (Lanzone et al., 1990), suggesting that hyperinsulinaemia could affect the PCOS population independently from obesity (Lanzone et al., 1996).

Moreover the recent findings in patients with PCOS of an increased early response to i.v. glucose load independent of body mass index (BMI), together with an impairment of insulin sensitivity only in the presence of obesity (Holte et al., 1994), suggests that hyperinsulinaemia and insulin sensitivity are two distinct features of the insulin disorder of PCOS. Our recent data may suggest that insulin resistance could reflect the presence of obesity, while hyperinsulinaemia may be a primary feature of PCOS (Ciampelli et al., 1997).

It is well known that the normal insulin response to i.v. glucose is biphasic, consisting of an initial burst (first-phase) of insulin followed by a slowly increasing phase (second-phase) of insulin secretion (Widén et al., 1992). Glucagon bolus administration is able to directly stimulate insulin release by β-cells before any change in arterial glucose concentration, with a peak reached within 10 min (Samols et al., 1966); moreover both insulin response to glucose and glucagon are related (Viallettes et al., 1993), thus allowing the evaluation of first-phase insulin release with a direct secretagogue stimulus.

In an attempt to clarify the nature of insulin disorder present in PCOS, the aim of this study was to analyse the acute insulin response after i.v. injection of glucagon in patients with PCOS, and to correlate the first-phase insulin release to the insulin secretion after administration of an oral glucose load.

Materials and methods

Thirty-five women affected by PCOS, aged 21–36 years, were recruited for the study. All the women were healthy, euthyroid, and no patient had taken any medication known to affect carbohydrate metabolism for at least 3 months before the study. 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 nmol/l; testosterone 0.6–2.0 nmol/l), and bilaterally normal or enlarged ovaries with the presence of at least 7–10 microcysts (<5 mm diameter) at the time of ultrasonography and laparoscopy. A normal follicle stimulating hormone (FSH)/luteinizing
hormone (LH) ratio was not considered an exclusion criterion (Lanzone et al., 1995). Eleven normo-ovulatory women aged 28–30 years, with a clinical history of infertility due to tubal pathology or uterine leiomyomata, served as controls, the length of the menstrual cycle in these subjects being 29.3 ± 1.1 days. Ovulatory cycles were previously confirmed by mid-luteal progesterone plasma concentrations of at least 25 nmol/l for three consecutive cycles.

Obesity was defined as a body weight >120% of ideal body weight (IBW) calculated according to Lorentz’s (1929) criteria. 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 progesterin-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 OGTT and basal hormone analysis. Two days later each woman underwent a glucagon test. 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.

Concerning the glucagon test, 30 min after the insertion of the needle an i.v. bolus injection of glucagon (Novo, Rome, Italy) at a dose of 1 mg was administered within 1 min to each patient (time 0'); blood samples pending insulin assay were collected 3, 6, 9 and 12 min after injection.

Samples for glucose were assayed immediately, whereas samples for insulin determinations were promptly centrifuged and the plasma was stored at −20°C until assayed. LH, FSH, oestradiol, 17-hydroxyprogesterone, testosterone, dihydroepiandrosterone sulphate (DHEA-S), androstenedione, sex hormone binding globulin (SHBG) and cortisol plasma concentrations were also determined in basal conditions.

All hormone concentrations were determined by commercial radioimmunoassay kits (Radim, Pomezia, Italy). Gonadotrophins and insulin were assayed by double antibody technique; all steroids by radioimmunoassay kits (Radim, Pomezia, Italy). Gonadotrophins and insulin were assayed by double antibody technique; all steroids by radioimmunoassay kits (Radim, Pomezia, Italy). All results were expressed as means ± SEM. Insulin and glucose plasma concentrations were also expressed as area under the curve (AUC) after the secretagogue stimuli, calculated by the trapezoidal rule.

The patients were classified as normo- and hyperinsulinaemic according to their insulin response to the OGTT, considering a cut-off value of 107 625 pmol/l×240 min for the AUC. This cut-off value was calculated by the mean + 2SD of ~100 OGTT performed in control subjects and was confirmed by a cluster analysis (Ciampelli et al., 1997).

Statistical analysis was performed using one-way analysis of variance. 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. Analysis of covariance was used with the purpose of evaluating the impact of obesity on the studied variables. Partial correlation was used to describe the relationship between two variables while adjusting for the effect of one or more variables. Differences were considered to be significant at a level of P < 0.05.

Results

Based on the glycemic response to OGTT four PCOS patients were excluded from the study due to the presence of abnormalities of carbohydrate tolerance; in fact, this condition is known to affect the insulin response to glucagon (Bardet et al., 1991; Vialettes et al., 1993). The remaining 31 patients were divided in 17 hyperinsulinaemic (14 obese and three lean patients) and 14 normoinsulinaemic (six obese and eight lean patients). All control subjects (four of them were obese; %IBW 125 ± 2.21) had a normoinsulinaemic response to glucose load (lean 46 101 ± 6771 versus obese 47 400 ± 6421 pmol/l×240 min).

Table I shows the endocrine and metabolic parameters of PCOS patients as analysed according to their insulin secretion. All PCOS patients showed higher androgen concentrations than controls, whereas no significant differences were found between normo- and hyperinsulinaemic PCOS patients. Fastig, as well as stimulated, glycaemic values were superimposable among the groups, whereas fasting insulin plasma values were higher in hyperinsulinaemic PCOS patients compared to either the normoinsulinaemic group or controls.

Figure 1A shows the insulinemic response to glucose load and glucagon test, expressed as AUC, in PCOS patients and in the control group in relation to their body weight and insulin secretion. No difference was found for the insulin AUC after glucagon administration between lean and obese control subjects (lean 3422.5 ± 588.35 versus obese 3372.2 ± 667.3 pmol/l×12 min). Also the lean PCOS group did not show any statistically significant difference when compared to controls. Conversely obese PCOS patients showed higher insulin secretion after both glucagon and glucose stimuli when compared to the other groups. As shown, the insulin secretory patterns are heterogeneously represented in lean and obese PCOS women.

When the data were analysed in relation to the insulinemic status (Figure 1B), despite having similar glycaemic values, PCOS hyperinsulinaemic patients showed significantly higher first-phase insulin release after glucagon stimulation when compared to both the normoinsulinaemic PCOS patients and the control subjects.

Such increased insulin release seemed to be partially independent from obesity, since the statistically significant difference between hyper- and normoinsulinaemic PCOS groups remained after adjusting for the %IBW, both during i.v. glucagon injection (P < 0.0005) or OGTT (P < 0.0001).

Figure 2 shows the highly significant relationship between the insulin response to OGTT and glucagon in the PCOS population. Concerning the insulin release after glucagon injection, we have also defined a cut-off value on the basis of the mean + 2SD of the insulin response in control subjects; this cut-off value was 6200 pmol/l×12 min. When we analysed the agreement between the insulin response to OGTT and glucagon test, we found that three of 17 (17.6%) hyperinsulinaemic PCOS patients showed a normal insulinemic response after glucagon stimulation (two of them should be considered as borderline and one blunted response), and three of 14 (21.4%) normoinsulinaemic PCOS subjects had a hyperresponse to the direct pancreatic stimulus (one borderline and two exaggerated responses).
Insulin response to glucagon test in PCOS

Figure 1. (A) Insulin area under curve (AUC) after oral glucose load (left) and after glucagon i.v. injection (right) in normoinsulinaemic ( ), hyperinsulinaemic ( ), and all patients classified as lean or obese, compared to controls ( ). (B) Insulin area under curve after oral glucose load (left) and after glucagon i.v. injection ( ) in normoinsulinaemic polycystic ovary syndrome (PCOS) (Normo-I ), hyperinsulinaemic PCOS (Hyper-I ), and controls ( ). Data are expressed as mean ± SEM. Significance: *P < 0.05 versus hyperinsulinaemic group; **P < 0.05 versus controls. For more details see Results section.

Table I. Clinical and endocrine features of the patient groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (11 patients)</th>
<th>PCOS hyperinsulinaemic (17 patients)</th>
<th>PCOS normoinsulinaemic (14 patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBW (%)</td>
<td>119 ± 3.69</td>
<td>133.8 ± 3.78**</td>
<td>125.14 ± 8.31†</td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)</td>
<td>64.11 ± 7.37</td>
<td>148.1 ± 22.83**</td>
<td>64.85 ± 8.41§</td>
</tr>
<tr>
<td>Fasting glucose (nmol/l)</td>
<td>4.67 ± 0.19</td>
<td>4.57 ± 0.35</td>
<td>4.65 ± 0.38</td>
</tr>
<tr>
<td>Glucose AUC (nmol/l x 240 min)</td>
<td>1307.1 ± 95.7</td>
<td>1509.7 ± 103.3</td>
<td>1377.3 ± 77.7</td>
</tr>
<tr>
<td>Androstenedione (nmol/l)</td>
<td>3.7 ± 0.6</td>
<td>5.39 ± 0.57*</td>
<td>5.53 ± 0.65*</td>
</tr>
<tr>
<td>DHEA-S (µmol/l)</td>
<td>2.45 ± 0.41</td>
<td>6.03 ± 0.57**</td>
<td>5.87 ± 0.39*</td>
</tr>
<tr>
<td>Oestradiol (pmol/l)</td>
<td>147.9 ± 23.7</td>
<td>130.5 ± 25.35</td>
<td>123.9 ± 26.74</td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>8.4 ± 0.9</td>
<td>6.72 ± 0.53</td>
<td>6.87 ± 0.73</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>6.9 ± 1.31</td>
<td>9.51 ± 1.51</td>
<td>9.27 ± 2.75</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td>1.3 ± 0.3</td>
<td>2.13 ± 0.21**</td>
<td>1.87 ± 0.08*</td>
</tr>
<tr>
<td>Cortisol (nmol/l)</td>
<td>361.7 ± 37.9</td>
<td>357.1 ± 34.5</td>
<td>347.3 ± 43.7</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>31.3 ± 5.23</td>
<td>24.1 ± 3.87</td>
<td>33.5 ± 5.7</td>
</tr>
</tbody>
</table>

*P < 0.05 versus controls; **P < 0.01 versus controls.
†P < 0.01 versus PCOS hyperinsulinaemic.
Data are expressed as mean ± SEM.

Table II shows the relationship between all the analysed parameters in PCOS and control subjects. In the PCOS group the acute insulin release after glucagon was found to be significantly related to the OGTT-induced insulin area, as well as to the fasting insulin levels or obesity, whereas no relationship was found in the control group. When the data were examined using the partial correlation there was a significant partial correlation (P < 0.0001) between insulin response to i.v. glucagon and OGTT, controlling for %IBW. Conversely the relation between insulin AUC after glucagon administration and %IBW was no longer significant (P < 0.06) after controlling for the insulinaemic status.
insulin resistance and hyperinsulinaemia. Recently we showed that, when PCOS subjects were classified into four groups according to their degree of obesity and their insulin secretion, the insulin resistance evaluated with euglycaemic–hyperinsulinaemic clamp and the β-cells’ hyper-response to oral glucose load were heterogeneously represented within the patients studied and were only weakly causally related (Ciampelli et al., 1997).

The enhanced early insulin response to i.v. glucose in women with PCOS and normal glucose tolerance, present also after weight reduction, suggests a primary abnormality of insulin secretion in PCOS (Holte et al., 1995). On the other hand data on insulin resistant subjects suggest that, even though present at the same time, impaired glucose uptake and impaired first-phase insulin secretion are separate defects without a causal relationship (Widén et al., 1992).

In the light of these concerns we attempted further characterization of the insulin β-cell secretion in the PCOS subjects by separately analysing a direct secretagogue stimulus, exemplified by a glucagon test, and a more prolonged one, of which OGTT is an example. Our data demonstrated that PCOS patients showed a more marked β-cell sensitivity to glucagon when analysed in relation to their insulin response to OGTT.

These results are in disagreement with those of Weber et al. (1993), who observed that stimulation of insulin by glucagon did not lead to an exaggerated response in women with PCOS. These different results could be due to the fact that we analysed hyper- and normoinsulinaemic PCOS patients, as well as lean and obese ones, whereas Weber analysed the same patients only in relation to their BMI. Moreover we have excluded from our study population the patients who exhibited abnormal glycaemic response to OGTT, while Weber did not consider this as an excluding criterion. Finally, another explanation for the discrepancy may be the different time intervals of sampling; in fact, even if there are not guidelines on this concern (Snorgaard et al., 1988; Bardet et al., 1991; Vialettes et al., 1993), it could be postulated that an interval time of 5 min between the bolus injection and the first blood sample is too long when estimating the insulin peak response.

The possible relationship between glucagon and insulin secretion in PCOS was studied by Golland (1990), who showed that fasting and glucose-stimulated plasma concentrations of glucagon were similar in PCOS and in control women, despite increased insulin values seen in obese PCOS women. Moreover,
glucagon values decreased in response to glucose in women with PCOS as in the case of lean controls, thus suggesting a normal reactivity of pancreatic $\alpha$-cells to insulin in women with PCOS, despite associated insulin resistance.

In the light of our results it is possible that PCOS subjects could have a greater sensitivity of $\beta$-cells to glucagon, which in turn could explain the presence of similar glucagon values found in different studied groups despite the different insulin plasma concentrations (Golland et al., 1990).

Our data also showed that the exaggerated insulin response to i.v. glucagon in hyperinsulinaemic PCOS women was partially independent of obesity; in fact significant differences between normo- and hyperinsulinemic patients remained after adjusting for body weight. On the other hand there were heterogeneous patterns of insulin response to both evaluated stimuli within lean and obese PCOS patients. There was a strong correlation between the insulin response to i.v. glucagon and OGTT; only six cases showed a different behaviour, of which three cases could be considered as borderline non-coincident responses.

The lack of differences in the insulin response to glucagon as well as OGTT between lean and obese control subjects may be due to the small sample size. Moreover, the obese control subjects were only moderately obese.

A hypothesis to explain the presence of a normal response to i.v. glucagon in hyperinsulinaemic PCOS patients is that a major portion of the insulin disorders in these patients is ascribable to a reduced hepatic removal of insulin, as suggested by recent data (Buffington and Kitabchi, 1994; Fulghesu et al., 1995); thus it is possible that the effect of alteration of liver metabolism could be more pronounced during a test prolonged for 4 h.

In conclusion, our data are consistent with the hypothesis that PCOS patients may have an insulin hyper-response to glucagon administration that is partially independent of obesity, and strictly related to the insulin secretion after oral glucose load. Moreover the glucagon test may provide an effective alternative to OGTT in screening insulin disorders of PCOS population (at least in absence of other risk factors), due to its reliability, simplicity and speed of performance.

References

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