Metformin treatment reduces ovarian cytochrome P-450c17α response to human chorionic gonadotrophin in women with insulin resistance-related polycystic ovary syndrome

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It has recently been proposed that hyperinsulinaemic insulin resistance and increased ovarian cytochrome P-450c17α activity, two features of the polycystic ovary syndrome (PCOS), are pathogenetically linked. The aim of the present study was to test the hypothesis of the linkage between hyperinsulinaemia and supranormal activity of cytochrome P-450c17α using the human chorionic gonadotrophin (HCG) challenge, which is a more direct ovarian stimulus than gonadotrophin-releasing hormone (GnRH) in detecting modifications in ovarian steroidogenesis. Eleven women with insulin resistance-related PCOS were studied. HCG (10 000 IU) was given i.m., and blood samples were obtained 0, 8, 12, 16 and 24 h thereafter. Next day, metformin was given at a dose of 500 mg three times a day for 30–32 days, at which time the pretreatment study was repeated. Two women ovulated after metformin treatment. The administration of metformin was associated with a decrease in area under the curve for insulin during a 2 h, 75 g oral glucose tolerance test, in plasma free testosterone concentrations and an increase in plasma sex hormone binding globulin concentration. The plasma 17-hydroxyprogesterone response to HCG was significantly lower after metformin treatment. The present study gives a direct demonstration that metformin treatment reduces ovarian cytochrome P-450c17α activity in women with polycystic ovary syndrome.

Key words: androgens/HCG/insulin/metformin/17OHP/PCOS

Introduction

It has been recently proposed that hyperinsulinaemic insulin resistance and increased ovarian cytochrome P-450c17α activity, two features of the polycystic ovary syndrome (PCOS), are pathogenetically linked (Nestler and Jakubowicz, 1996).

Cytochrome P-450c17α is a key enzyme in ovarian androgen steroidogenesis. It has been known for many years that the vast majority of women with PCOS have a supranormal plasma 17-hydroxyprogesterone (17OHP) response and poorer responsiveness of androstenedione to gonadotrophin-releasing hormone (GnRH) administration (Barnes et al., 1989; Sahin and Kelestimir, 1997), indicating increased ovarian cytochrome P-450c17α activity.

Insulin resistance and the resulting raised plasma concentrations of insulin are reported to be responsible for high androgen concentrations in PCOS. Indeed there is evidence that insulin stimulates ovarian oestrogen, androgen and progesterone secretion in vitro (Dunaif, 1992). Insulin inhibits liver secretion of sex hormone binding globulin (SHBG), increasing the availability of androgens (Nestler et al., 1991). Another mechanism of action could be inhibition of liver synthesis of insulin-like growth factor binding protein-1 (IGFBP-1) by insulin, leading to increased availability of insulin-like growth factor-1 (IGF-I) in the ovary (Leroith et al., 1995).

Reduction in hyperinsulinaemia in women with PCOS by diazoxide (Nestler et al., 1989), a somatostatin analogue (Prelevic et al., 1990), and by the insulin sensitizing agent troglitazone (Dunaif et al., 1996; Ehrmann et al., 1997a) resulted in a significant decrease in baseline androgen concentrations. The somatostatin analogue significantly decreased androstenedione, oestradiol and testosterone responses to GnRH agonist administration; however, 17OHP response was not determined (Prelevic et al., 1990).

To test the hypothesis that hyperinsulinaemia stimulates ovarian cytochrome P-450c17α activity in PCOS, the basal 17OHP concentrations and the plasma 17OHP responses to administration of leuprolide in PCOS women have been measured before and after administration of metformin (Nestler and Jakubowicz, 1996).

Metformin, an insulin-sensitizing agent, is normally used to treat non-insulin-dependent diabetes. Its multiple mechanism of action includes inhibition of gluconeogenesis in the liver and stimulation of peripheral uptake of glucose (Bailey, 1992; Bailey and Turner, 1996).

Interestingly, in another study metformin did not improve hyperinsulinaemia nor did it improve dysregulation of ovarian steroidogenesis as determined by the GnRH test (Ehrmann et al., 1997b).

The aim of the present study was to test the hypothesis of a linkage between hyperinsulinaemia and supranormal activity of cytochrome P-450c17α using the HCG challenge, which is a more direct ovarian stimulus than GnRH in detecting modifications in ovarian steroidogenesis.

Materials and methods

Subjects

Eleven women with PCOS were recruited. The clinical diagnosis of PCOS was based on hyperandrogenaemia (plasma free testosterone
Two women ovulated after taking metformin. Complete data from nine of the 11 women were analysed.

**Results**

Complete data from nine of the 11 women were analysed. Two women ovulated after taking metformin. The body mass index did not change significantly during the study. Metformin administration led to a significant reduction in $\text{AUC}_{\text{insulin}}$ (65 850 ± 11 220 versus 45 900 ± 3 11.4 nmol/l). Values are means ± SD. *P < 0.05 versus basal.

**Table I. Clinical and hormonal data from nine women with polycystic ovary syndrome (PCOS) before and after metformin treatment**

<table>
<thead>
<tr>
<th></th>
<th>Basal (n = 9)</th>
<th>After metformin (n = 9)</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>29 ± 4</td>
<td>28.1 ± 3.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.3 ± 3.1</td>
<td>28 ± 3.2</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>11.2 ± 1.4</td>
<td>10.6 ± 1.9</td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>5.3 ± 0.8</td>
<td>5.6 ± 1</td>
</tr>
<tr>
<td>Oestradiol (pmol/l)</td>
<td>185 ± 38</td>
<td>178 ± 45</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td>2.2 ± 0.4</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>17OHP (nmol/l)</td>
<td>4.1 ± 0.5</td>
<td>3.9 ± 0.4</td>
</tr>
<tr>
<td>Free testosterone (pmol/l)</td>
<td>43 ± 4</td>
<td>35 ± 4*</td>
</tr>
<tr>
<td>Androstenedione (nmol/l)</td>
<td>12 ± 3</td>
<td>11.4 ± 2.9</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>64 ± 10</td>
<td>97 ± 15*</td>
</tr>
<tr>
<td>Fasting insulin concentration (pmol/l)</td>
<td>108 ± 24</td>
<td>99 ± 34.8</td>
</tr>
<tr>
<td>$\text{AUC}_{\text{insulin}}$ (pmol/l/min)</td>
<td>65 850 ± 11 220</td>
<td>45 900 ± 3 11.4</td>
</tr>
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</table>

Values are means ± SD.

*P < 0.05 versus basal.

**Hormone assay**

Plasma LH, FSH, oestradiol, testosterone, androstenedione, free testosterone, 17OHP and SHBG were measured by double antibody radioimmunoassay using Radim kits (Rome, Italy) for LH, FSH, Sorin kits (Saluggia, Italy) for androstenedione and testosterone, DPC kits (Los Angeles, CA, USA) for SHBG, free testosterone and 17OHP and Biodata Kits (Rome, Italy) for oestradiol. The samples were assayed in duplicate at two dilutions. All samples from each subject were assayed together. Quality control pools at low, medium and high hormone concentrations were included in each assay. The intra-assay and inter-assay coefficients of variation did not exceed 10 and 15% respectively.

**Statistical analysis**

To compare the response before and after metformin therapy, hormone basal concentrations, maximum increments (the maximum rise above baseline) and the areas under the curve (cumulative rise above baseline) were compared using paired Student’s two-tailed t-test. Areas under the curves above baseline (AUC) were calculated by the trapezoidal method and expressed as nmol/l per unit of time. Differences were considered significant for $P < 0.05$.

**Discussion**

The results of the present study show that metformin administration in women with PCOS leads to a reduction in ovarian cytochrome P-450c17α activity, as demonstrated by
a significant reduction in the responses of 17OHP to the administration of HCG.

Our results confirmed those obtained previously (Nestler and Jakubowicz, 1996). In that report, it was concluded that the metformin-induced insulin reduction was accompanied by a decline in ovarian cytochrome activity. This suggests that increased ovarian cytochrome activity in PCOS women is probably due to stimulation by insulin. This conclusion, however, is supported only in part by their data. The treatment group and the placebo group differed significantly with respect to basal LH plasma concentrations (8.5 ± 2.2 versus 3.7 ± 0.7 IU/l; P < 0.05).

Moreover, metformin administration led to a reduction in LH response to GnRH. The decline in androgen production was attributed to the direct effect of insulin on ovarian enzyme, but it may be also have been due to a reduction in LH response to GnRH.

The HCG test was recently re-evaluated for the diagnosis of ovarian hyperandrogenism (Levrant et al., 1997). HCG is essentially an LH analogue which induces a direct stimulation of thecal cell steroidogenesis. It has been shown that the responses of 17OHP, androstenedione and testosterone to HCG in women with functional ovarian hyperandrogenism (defined by androgen excess and an increased 17OHP response to nafarelin) were significantly greater than in normal women. We used the HCG challenge to test the hypothesis that metformin administration leads to a reduction in cytochrome P-450c17α activity. This avoids problematic statistical corrections with respect to the reduction in LH response to GnRH after metformin administration reported (Nestler and Jakubowicz, 1996).

In our study, metformin administration led to an increase in SHBG and to a consequent reduction in free testosterone; however, there was no reduction in total testosterone plasma concentration. On the other hand, a reduction of total testosterone after metformin treatment has been reported (Velazquez, 1997). Only 18% (2/11) of women in our study ovulated spontaneously after metformin therapy compared with published data where values such as 34% (Nestler et al., 1998) and 95% (Velazquez et al., 1997) are shown. The reasons for these discrepancies are not known, but the results of the HCG stimulation test confirmed those obtained previously (Nestler and Jakubowicz, 1996).

Metformin treatment led to a reduction in 17OHP response to HCG administration.

The present study gives a direct demonstration that metformin leads to a reduction in stimulated ovarian cytochrome P-450c17α activity in women with PCOS.

References


Prelevic, G.M., Wurzburger M.I., Balint-Peric, L. and Nesic, J.S. (1990) Inhibitory effect of sandostatin on secretion of luteinizing hormone and nafarelin) were signiﬁcantly higher than in normal women. We used the HCG challenge to test the hypothesis that metformin administration leads to a reduction in cytochrome $P\text{-}450c17\alpha$ activity. This avoids problematic statistical corrections with respect to the reduction in LH response to GnRH after metformin administration reported (Nestler and Jakubowicz, 1996).

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