Serum levels of insulin-like growth factor-1, IGF binding protein-1 and insulin and the response to human menopausal gonadotrophins in women with polycystic ovary syndrome

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In order to determine which factors influence the large variations in sensitivity to gonadotrophins witnessed in women with polycystic ovary syndrome (PCOS), a prospective study was conducted of the correlation between basal clinical and endocrinological features and gonadotrophin requirements of 20 women with clomiphene-resistant PCOS undergoing ovulation induction. Baseline evaluation of serum concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone, fasting insulin, insulin-like growth factor-1 (IGF-1), IGF binding protein-1 (IGFBP-1) and sex hormone-binding globulin (SHBG) were performed before administering gonadotrophin-releasing hormone agonist (GnRHa). Two weeks later, human menopausal gonadotrophin (HMG) was given in a standard individualized protocol according to ovarian response, until human chorionic gonadotrophin (HCG) was given. Serum concentrations of insulin, IGF-1, and IGFBP-1 were unaffected by GnRHa. The BMI correlated positively with insulin and inversely with IGFBP-1 serum concentrations and insulin and IGFBP-1 were inversely correlated. The amount of HMG required correlated positively with BMI and insulin concentrations and inversely with IGFBP-1 in the whole group and these correlations were maintained in the sub-group of lean women. No correlation was observed between HMG requirements and IGF-1 or other hormones. Women with hyperinsulinaemia and low IGFBP-1 concentrations required significantly more HMG. Multiple regression analysis revealed that insulin concentration is the most significant determinant of HMG requirement even when dissociated from BMI. We concluded that requirement of HMG in PCOS is not merely determined by obesity but by a cardinal role of insulin concentrations which, when high, induce, hypothetically, a hyperandrogenic intrafollicular milieu.

Key words: gonadotrophins/insulin/insulin-like growth factor-1/insulin-like growth factor binding protein-1/polycystic ovary syndrome

Introduction

Gonadotrophin therapy is effective in inducing ovulation in women with clomiphene citrate (CC)-resistant polycystic ovary syndrome (PCOS). In general, the polycystic ovary is very sensitive to gonadotrophin stimulation and combination therapy with gonadotrophin releasing-hormone agonist (GnRHa) does not dampen this heightened sensitivity. However, there are wide variations of response and follicle stimulating hormone (FSH) threshold concentrations within this large group of women with PCOS as compared with a constant FSH threshold concentration in hypogonadotrophic patients with normal ovaries (van Weissenbruch et al., 1993).

As it is now well established that growth factors and their binding proteins are involved in a physiological intra-ovarian regulating mechanism (Adashi et al., 1985; Chegini and Williams, 1992; Giudice, 1992) and that this mechanism is probably disturbed in PCOS (Insler and Lunenfeld, 1991; Cataldo and Giudice, 1992; Homburg et al., 1992), the reason for the wide variations in response may lie within this system.

Hamilton-Fairley et al. (1992), observed that obese women with PCOS require a larger daily and total dose of gonadotrophins than their lean counterparts but an indication that obesity may not be the sole determinant of ovarian sensitivity was provided by Tiitinen et al. (1993), who found an association between the response to CC and serum concentrations of insulin-like growth factor binding protein-1 (IGFBP-1) in lean women with PCOS.

We undertook the present prospective study to determine which factors influence the variations in the sensitivity of the polycystic ovary to gonadotrophin stimulation.

Materials and methods

Definition

Women were diagnosed as having PCOS if they had the typical ultrasonic appearance of polycystic ovaries, i.e. a hyperechogenic central stroma occupying at least 25% of an ovarian volume >9 ml and at least 10 follicles of <9 mm diameter (Adams et al., 1985), anovulatory infertility associated with oligomenorrhea and a clinical manifestation of hyperandrogenism, such as hirsutism or acne.

Design and participants

Twenty consecutive patients with CC-resistant PCOS treated in our infertility unit were recruited to participate in the study. The age range was 22–37 years (mean 29.4 ± 4.8). Pretreatment evaluation included measurements of body mass index (BMI) and the determination of the mean of two baseline serum concentrations of IGF-1, IGFBP-1, fasting insulin, sex hormone-binding globulin (SHBG), luteinizing hormone (LH), FSH and testosterone on day 3 or 4 of a spontaneous or artificial menstruation. Blood samples for fasting insulin were taken at 8.00 a.m. following at least 10 h of overnight fasting.

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Following these baseline clinical and endocrinological evaluations, all women were pretreated with long-acting GnRHa (DTRP6, Decapeptyl, 3.75 mg, i.m.: Ferring, Malmo, Sweden) 2–3 weeks later. When adequate pituitary suppression and ovarian quiescence were observed on examination 2 weeks later (no follicles >9 mm and serum oestradiol < 75 pmol/l), a further serum sample was taken and the patients started ovarian stimulation using one ampoule/day of HMG (Pergonal; Teva, Petah Tikva, Israel). Gonadotrophin dosage was adjusted individually, every 5–7 days, using a step-up approach with increments of one ampoule, according to oestradiol concentrations and vaginal ultrasound measurements of follicular diameter, obtained every 2–3 days. Human chorionic gonadotrophin (HCG) (Chorigon: Teva, Petah Tikva, Israel) 10 000 IU, i.m., was administered in the presence of less than three leading follicles ≥17 mm diameter and a compatible oestradiol concentration.

**Laboratory methods**

IGF-1 was determined using a double antibody disequilibrium radioimmunoassay after extraction and separation from its binding protein by plastic columns containing octadecasilsilica (Immuno Nuclear Corp., Stillwater, MN, USA). The sensitivity of the assay was 1 nmol/l and the intra- and inter assay coefficients of variation were 10 and 15% respectively. IGFBP-1 was measured by radioimmunoassay and had a sensitivity of 9.6 μg/l and intra- and inter-assay coefficients of variation of 8–10% and 10–18% respectively (Suikkari et al., 1989a). Insulin concentrations were measured using a one-step sandwich ELISA assay (Boehringer–Mannheim, Germany).

LH was determined using a double antibody radioimmunoassay kit (Amerlex m/m, Amersham, UK) and World Health Organization (WHO, 1988) second international reference preparation (IRP) 80/552, giving sensitivity of 2 IU/l and intra- and inter-assay coefficients of variation of 7 and 8% respectively. FSH was determined using the double antibody radioimmunoassay kit as above and WHO (1980) second IRP 78/549, giving sensitivity of 2 IU/l and intra- and inter-assay coefficients of variation 9 and 8% respectively.

Testosterone was estimated using a coded tube method (Diagnostic Products Corporation, Los Angeles, CA, USA), sensitivity 0.2 ng/ml, intra- and inter-assay coefficients of variation 6 and 10% respectively.

**Statistical methods**

The data were analysed with the Student’s t-test, Mann–Whitney signed rank test and Pearson’s correlation coefficient where appropriate. Multiple regression analysis was used to determine independent relationships of the variables.

**Results**

The basal clinical and laboratory data of the PCOS patients involved in this study are shown in Table I.

<table>
<thead>
<tr>
<th>Mean (range)</th>
<th>HMG in polycystic ovary syndrome</th>
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</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>29.4 (22–37)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.7 (21–35)</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>10.7 (3.4–20)</td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>6.6 (3.8–8.9)</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>0.8 (0.3–1.9)</td>
</tr>
<tr>
<td>Fasting insulin (μU/ml)</td>
<td>25 (6–54)</td>
</tr>
<tr>
<td>IGFBP-1 (nmol/l)</td>
<td>25.1 (15–38)</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>34.4 (9.5–138)</td>
</tr>
</tbody>
</table>

BMI = body mass index; LH = luteinizing hormone; FSH = follicle stimulating hormone; IGF-1 = insulin-like growth factor-1; IGFBP-1 = IGF binding protein-1; SHBG = sex hormone-binding globulin.

The number of ampoules required during ovulation induction correlated positively with BMI (Figure 1) and fasting insulin (Figure 2) concentrations (r = 0.6, P < 0.004; r = 0.6, P < 0.003 respectively); and an inverse significant correlation was observed between the number of ampoules used and IGFBP-1 (r = −0.5, P < 0.02) (Figure 3). No correlation was observed between HMG requirements and IGF-1, SHBG, FSH, LH or testosterone.

Obese women (BMI > 25; n = 8) required more HMG than lean women (BMI < 25; n = 12) (mean 47.9 versus 24.8 ampoules, P < 0.02) and hyperinsulinaemics (insulin >25 μU/ml; n = 10) more than those with normal insulin concentrations (insulin <25 μU/ml; n = 10) (45.9 versus 22.2 ampoules, P = 0.03). Within the group of lean women, those who were...
Figure 3. Correlation between the required amount of human menopausal gonadotrophin (HMG) (Amp) and insulin-like growth factor binding protein-1 (IGFBP-1) concentrations.

hyperinsulinaemic (n = 5) required more HMG than those with normal insulin concentrations (n = 7) (33.6 versus 18.6 ampoules, P < 0.003) and the HMG requirement was negatively associated with IGFBP-1 concentrations (r = −0.6, P < 0.03). The group of low responders to HMG (>30 ampoules; n = 10), compared with the high responders (<30 ampoules; n = 10), had significantly higher mean BMI (28.7 versus 22.8, P = 0.003) and insulin concentrations (35.2 versus 14.8 μU/ml, P < 0.01) and significantly lower concentrations of IGFBP-1 (13.6 versus 44.6 ng/ml, P = 0.001).

Multiple regression analysis was employed to determine the significance of independent determinants of HMG requirement. The total explained variance involving insulin, IGFBP-1 and BMI was 45.6%, of which 39% was attributed to insulin (standardized regression coefficient 0.62, P < 0.05). The contributions of BMI (5.8%) and IGFBP-1 (1.8%) as independent determinants of HMG requirement were not statistically significant.

In order to answer the question of whether insulin and/or IGFBP-1 are independently associated with HMG requirements and not with the range of BMI, the partial correlation coefficients were examined controlling for BMI and therefore statistically removing BMI as a factor. The correlation between HMG requirement and insulin tended to hold (r = 0.35, P = 0.057) whereas that with IGFBP-1 was lost indicating insulin, but not IGFBP-1, as a factor in HMG requirements independent of BMI.

Discussion

Previous observations (Hamilton-Fairley et al., 1992) that obese women with PCOS are less sensitive to HMG have been confirmed but, clearly, this increased requirement of HMG with increasing BMI in PCOS is not merely determined by obesity alone but primarily by insulin and IGFBP-1. When the effect of obesity was neutralized by examining lean patients only, women with hyperinsulinaemia and low concentrations of IGFBP-1 required more ovarian stimulation to achieve ovulation compared with their counterparts whose insulin concentrations were normal. Additionally, the amount of HMG required to induce ovulation in lean patients was positively correlated with insulin concentrations and negatively associated with IGFBP-1 concentrations.

The importance of insulin to ovarian sensitivity to gonadotrophins was singled out by the application of multiple regression analysis which revealed that insulin was the most significant independent determinant and that the contributions of BMI and IGFBP-1 were marginal. Furthermore, although serum insulin concentrations are positively associated with BMI, when the BMI factor is statistically removed, the positive correlation between insulin and HMG requirement is maintained, again indicating that insulin is independently the significant determinant of ovarian sensitivity.

In accordance with previous observations (Suikkari et al., 1989a; Conway et al., 1990; Homburg et al., 1992), we have confirmed that serum insulin concentrations in women with PCOS are inversely associated with serum concentrations of IGFBP-1. Since BMI was found to correlate positively with insulin concentrations, it may be argued that the decrease in serum IGFBP-1 concentrations is secondary to obesity. However, the insulin resistance and hyperinsulinaemia which have been clearly demonstrated in non-obese women with PCOS in the present and previous studies (Conway et al., 1990; Homburg et al., 1992), rule out the possibility that mere obesity is responsible for the decreased IGFBP-1 concentrations but rather a direct inhibiting effect of insulin on IGFBP-1 production by hepatic cells (Suikkari et al., 1989b).

Similar to the observation by Hatasake et al. (1994), we also demonstrated that adequate pituitary suppression, using GnRHa, does not influence serum IGF-1, IGFBP-1 and insulin concentrations. This observation may indicate that the GH/IGF-1 axis is not influenced by hormones of ovarian origin during this short-term pituitary suppression and therefore GnRHa did not influence our findings by modulating the ovarian growth factor profile.

We found an inverse correlation between the total amount of HMG ampoules used during ovulation induction and serum IGFBP-1 concentration. Tiitinen et al. (1993) found that low serum IGFBP-1 concentration is associated with CC unresponsiveness in lean PCOS patients. Their findings may be seen as complementary to our results obtained using the amount of HMG as a measure of ovarian sensitivity. These observations may be explained by various mechanisms. In concert with LH, chronic hyperinsulinaemia is capable of producing a hyperandrogenic intrafollicular milieu effected through its own or IGF ovarian receptors (Barbieri et al., 1986; Cara et al., 1988). Additionally, an androgen dominated milieu may be putatively acquired by an increase in concentrations of biologically active IGF-1 released by the insulin-induced decrease in IGFBP-1 concentrations (Rosenfield et al., 1990). Furthermore, IGFBP-1 has been shown to inhibit the local actions of IGF-1 in human granulosa-luteal cells and a reduced inhibition of the androgen producing effects of IGF-1 by decreased concentrations of IGFBP-1 has been proposed (Angervo et al., 1991). Enhanced ovarian androgen production, theoretically and clinically, increases gonadotrophin requirements for induction of ovulation. Whatever the exact mechanism may be, coupled with the fact that low IGFBP-1 concentrations have been associated with CC unresponsiveness, our findings suggest that primarily insulin and secondarily...
IGFBP-1 are the main determinants of the variations in response of the polycystic ovary to gonadotrophins.

Since the production of IGF-1 is known to be growth hormone (GH) dependent, Homburg et al. (1988) have used GH in vivo, in an attempt to facilitate ovulation induction by HMG. While many studies have showed an improved response to cotreatment with GH and HMG in hypopituitarism (Homburg et al., 1990) or young poor responders (Volpe et al., 1989), others failed to confirm this observation in normally cycling and perimenopausal patients (Owen et al., 1991; Shaker et al., 1992). It might be suggested that these confusing diverse responses to cotreatment may be the result of the intricate interactions between intraovarian growth factors. Further investigations of the baseline growth factor profile in PCOS patients may lead to a better understanding and detection of the different levels of response to ovulation induction in patients with PCOS, and to the use of the most appropriate and efficient ovulation induction regimen.

References


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