Comparative study of plasma ghrelin levels in women with polycystic ovary syndrome, in hyperandrogenic women and in normal controls

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BACKGROUND: Ghrelin is a novel peptide associated with energy balance, obesity, and perhaps gonadal function. The present study was designed in order: (i) to compare plasma ghrelin levels between women with PCOS, women who presented only with hyperandrogenaemia and healthy controls; and (ii) to investigate the relationship between circulating ghrelin and the heterogeneity of clinical and biochemical manifestations of PCOS. METHODS: Two hundred and fifty-nine women with PCOS, 25 women who had only hyperandrogenaemia and 46 controls, were studied. Women with PCOS were further divided, based on the presence of chronic anovulation, biochemical hyperandrogenaemia, clinical hyperandrogenism, and polycystic ovary morphology on ultrasound evaluation. In all women, the basal levels of gonadotrophins, androgens, 17-OH-progesterone, sex hormone-binding globulin, glucose, insulin and ghrelin were measured. RESULTS: Women with PCOS had lower ghrelin levels, compared to both women with hyperandrogenaemia and controls; women with hyperandrogenaemia had lower ghrelin levels, compared to controls, but not significantly so. While PCOS-associated hyperandrogenaemia was inversely related to ghrelin levels, anovulation and polycystic ovary morphology were associated with higher concentrations. Ghrelin levels were negatively correlated with 17-OH-progesterone levels. CONCLUSIONS: In PCOS, circulating ghrelin and androgens are inversely related and it is possible that this peptide is involved in steroidal synthesis and/or action. It is also likely that different clinical and biochemical manifestations of the syndrome are also associated with different ghrelin concentrations.

Key words: ghrelin/17OH-progesterone/polycystic ovary syndrome

Introduction

Ghrelin is a 28 amino acid peptide, which is primarily produced by the stomach (Kojima et al., 1999). This hormone is also found in the intestine (Date et al., 2000), kidney (Mori et al., 2000), lung (Volante et al., 2002), pancreas (Wierup et al., 2002), testis (Tanaka et al., 2001; Tena-Sempere et al., 2002), placenta (Gualillo et al., 2001), ovary (Gaytan et al., 2003), thyroid (Volante et al., 2001), pituitary (Korbonits et al., 2001) and in cells of the immune system (Hattori et al., 2001).

Ghrelin is a strong secretagogue of growth hormone (GH), exerting its action on the hypothalamic–pituitary system via the GHSR-1A (growth hormone secretagogue receptor-1A) (Howard et al., 1996). GHSR have also been detected in a variety of peripheral tissues (Muccioli et al., 1998, Muccioli et al., 2000; Gnanapavan et al., 2002; Katugampola et al., 2002), indicating a pleiotrophic action of ghrelin. Indeed, besides its GH-secretagogue properties, ghrelin has also been found to: (i) induce the hypothalamic secretion of prolactin and adrenocorticotrophic hormone (Arvat et al., 1997); (ii) exert a negative effect on the hypothalamic–pituitary–ovarian axis (Van Der Lely et al., 2004); (iii) stimulate food intake and positive energy balance (Horvath et al., 2001); and (iv) interact with insulin, most probably in a negative feedback circuit, affecting glucose homeostasis (Wierup et al., 2002). Intriguingly, decreased ghrelin levels have been associated with obesity and states of insulin resistance (IR) (Tschop et al., 2001; Shiiya et al., 2002).

The polycystic ovary syndrome (PCOS), probably the most common endocrine disorder in women of reproductive age, is traditionally characterized by chronic anovulation, functional hyperandrogenism with or without elevated total androgen levels and polycystic ovaries on ultrasound examination (Zawadski and Dunaif, 1992; Rotterdam Human Reproduction Vol.20, No.8 pp. 2127–2132, 2005
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ESHRE/ASRM-sponsored PCOS Consensus Workshop Group, 2004). Obesity of the central type and IR are highly associated with the syndrome, predisposing women with PCOS to the development of glucose intolerance and, ultimately, type 2 diabetes mellitus (Goodarzi and Korenman, 2003). IR also plays a major role in PCOS-associated functional hyperandrogenism (Adashi et al., 1985; Nestler et al., 1991). It should be noted that PCOS is definitely a heterogeneous disorder, and few women with the syndrome present with the classic triad.

Data on ghrelin levels in women with PCOS are rather conflicting: both decreased (Pagotto et al., 2002; Schofl et al., 2002; Moran et al., 2004) and elevated (Wasko et al., 2004) concentrations have been reported, while others (Orio et al., 2003) have found no significant differences between women with the syndrome and normal ovulatory women. Negative associations of ghrelin with body mass index (BMI) (Orio et al., 2003; Wasko et al., 2004), as well as with indices of IR have been observed (Schofl et al., 2002; Wasko et al., 2004), while a negative correlation between circulating ghrelin and androgen levels has also been reported (Pagotto et al., 2002; Gambineri et al., 2003). It should be noted that the number of women involved in the above studies was relatively small.

In summary, ghrelin is a novel peptide associated with energy balance, obesity, IR and, probably, gonadal function. Given the discrepancy of results considering ghrelin levels in PCOS, a state of increased IR characterized by metabolic and reproductive disorders, the present study was designed in order to: (i) compare plasma ghrelin levels between a sizeable cohort of women with PCOS, women who had hyperandrogenaemia but normal menses and no signs of polycystic ovary morphology on ultrasound examination and ovulatory women with normal androgen levels and ovarian morphology; (ii) investigate the relationship between circulating ghrelin and the heterogeneity of clinical and biochemical manifestations of PCOS.

Materials and methods

Subjects
Two hundred and eighty-four women, aged 13–38 years old, were divided into two groups: women with PCOS (n = 259) and women with isolated hyperandrogenaemia (n = 25) but without the syndrome. Diagnosis of PCOS was based on the presence of two out of the following three features: chronic anovulation (fewer than six spontaneous bleeding episodes per year), biochemical hyperandrogenaemia or clinical hyperandrogenism (assessed by calculation of the Ferrimann–Gallwey index) and polycystic ovary morphology (≥12 follicles) on ultrasound. Other common causes of hyperandrogenism (prolactinoma, congenital adrenal hyperplasia, Cushing syndrome and virilizing ovarian or adrenal tumours) were excluded, in accord with the criteria proposed in 2003 by the Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group, 2004.

All women with PCOS demonstrated clinical hyperandrogenism. Therefore, they were divided into those with biochemical hyperandrogenaemia (n = 226) and those with signs of hyperandrogenism (Ferrimann–Gallwey index ≥8) but normal androgen levels (n = 33). Women with PCOS were also divided into three groups: a group which comprised women who demonstrated chronic anovulation and polycystic ovary morphology (n = 112); another group with chronic anovulation, but no polycystic ovary morphology (n = 134); and a group with polycystic ovaries on ultrasound, but normal menses (n = 13).

Forty-six women, aged 18–38 years old, volunteered as controls. Women with hyperandrogenaemia who were not diagnosed with PCOS and controls had normal ovulating cycles (mean ± SD) 28 ± 2 days, blood progesterone levels >10 ng/ml in two consecutive cycles and normal ovarian morphology on ultrasound. Controls had no signs of hyperandrogenism and their levels of circulating androgens were within normal limits. None of the women studied had galactorrhoea, nor any systemic disease that could possibly affect their reproductive physiology. Furthermore, no woman reported use of any medication that could interfere with the normal function of the hypothalamic–pituitary–gonadal axis, during the last semester. Informed consent was obtained from all 330 women and the study was approved by the Ethical Committee of the Institution.

Hormonal and biochemical measurements and calculations
Blood samples were collected between the 3rd and 6th days of a menstrual cycle of healthy controls and of women who presented only with hyperandrogenaemia and a spontaneous bleeding episode of the PCOS group, at 09:00, after an overnight fast. On the same day, transvaginal ultrasound examination was performed. The basal serum levels of FSH, LH, PRL, testosterone, Δ4-androstenedione and dehydroepiandrosterone sulphate (DHEA-S), 17-OH-progesterone, sex hormone-binding globulin (SHBG), glucose and insulin were measured, as previously described (Panidis et al., 2003, 2004). Ghrelin levels were measured with a commercial enzyme-linked immunoassorbent assay (ELISA) kit (Phoenix Pharmaceuticals Inc., Belmond, CA, USA). Ghrelin concentration was estimated by enzyme-linked immunoassay, using a commercial kit (Phoenix Pharmaceuticals). A minimum detectable concentration of 0.08 ng/ml, an intra-assay variation of 5% and an inter-assay variation of 14% are reported in the accompanying information sheet of the kit. Free androgen index (FAI) was calculated according to the equation: testosterone (nmol/l) × 100/SHBG (nmol/l). HoMA-IR was derived from the equation: [glucose (mmol/l) × insulin (µIU/ml)]/22.5. The glucose:insulin ratio was also calculated.

Statistical analysis
The Kolmogorov–Smirnov test was used to test the normality of distribution and values that did not fit the Gaussian normal distribution and values that did not fit the Gaussian normal distribution were log-transformed. Means were compared with analysis of variance (ANOVA) and post hoc analyses for multiple (>3) comparisons were performed with Tamhane’s T2 test. To avoid confounding variables bias, adjustments and comparisons were also performed by means of general linear model (GLM)-based analysis of covariance (ANCOVA), where indicated. Bivariate correlation analysis (calculation of the Pearson coefficient) was used to assess the correlation of ghrelin levels with other parameters. All analyses were performed by SPSS software (v.11.5 SPSS, Inc., Chicago, IL, USA). P < 0.05 was considered statistically significant.
Table I. Anthropometric features and basal hormonal levels of women with polycystic ovary syndrome (PCOS), ovulatory women who had isolated hyperandrogenaemia and healthy controls (mean ± SD)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PCOS (n = 259)</th>
<th>Isolated hyperandrogenaemia (n = 25)</th>
<th>Controls (n = 46)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>23.04 ± 5.12</td>
<td>27.40 ± 5.36*</td>
<td>28.33 ± 5.94*</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.84 ± 6.88</td>
<td>26.07 ± 5.68</td>
<td>23.70 ± 4.24*</td>
</tr>
<tr>
<td>Waist:hip ratio</td>
<td>0.78 ± 0.07</td>
<td>0.80 ± 0.12</td>
<td>0.77 ± 0.05</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>5.59 ± 1.62</td>
<td>5.68 ± 1.01</td>
<td>6.84 ± 2.37*</td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>8.02 ± 6.21</td>
<td>5.69 ± 2.81</td>
<td>5.98 ± 2.68*</td>
</tr>
<tr>
<td>Prolactin (ng/ml)</td>
<td>14.91 ± 8.50</td>
<td>16.09 ± 8.69</td>
<td>14.64 ± 10.88</td>
</tr>
<tr>
<td>Testosterone (ng/dl)</td>
<td>80.51 ± 28.55</td>
<td>68.28 ± 14.64</td>
<td>42.77 ± 9.25*</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>9.91 ± 7.34</td>
<td>6.67 ± 2.53</td>
<td>2.67 ± 1.03*</td>
</tr>
<tr>
<td>Free androgen index</td>
<td>2.75 ± 1.07</td>
<td>2.10 ± 0.54*</td>
<td>1.56 ± 0.39*</td>
</tr>
<tr>
<td>Δ4-Androstenedione (ng/ml)</td>
<td>2.79 ± 1.11</td>
<td>2.51 ± 0.85</td>
<td>1.72 ± 0.57*</td>
</tr>
<tr>
<td>DHEA-S (µg/ml)</td>
<td>1.17 ± 0.56</td>
<td>0.98 ± 0.50</td>
<td>0.67 ± 0.29*</td>
</tr>
<tr>
<td>17-OH-Progesterone (ng/ml)</td>
<td>36.63 ± 16.40</td>
<td>41.14 ± 20.49</td>
<td>62.73 ± 23.07*</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>93.77 ± 13.26</td>
<td>96.64 ± 13.41</td>
<td>91.44 ± 12.97</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>11.38 ± 9.53</td>
<td>10.36 ± 5.85</td>
<td>8.12 ± 4.50*</td>
</tr>
<tr>
<td>Insulin (µIU/ml)b</td>
<td>11.48 ± 6.03</td>
<td>11.70 ± 5.31</td>
<td>13.96 ± 6.19*</td>
</tr>
<tr>
<td>Glucose:insulin ratiob</td>
<td>2.69 ± 2.33</td>
<td>2.54 ± 1.64</td>
<td>1.86 ± 1.13*</td>
</tr>
<tr>
<td>HoMA-IRb</td>
<td>513.58 ± 294.96</td>
<td>525.00 ± 295.56</td>
<td>563.80 ± 226.71</td>
</tr>
</tbody>
</table>

*Women with PCOS versus women with isolated hyperandrogenaemia or controls, P < 0.05.
*Women with isolated hyperandrogenaemia versus controls, P < 0.05.
*The difference was body mass index (BMI)-dependent. HoMA-IR was derived from the equation: [glucose (mmol/l) × insulin (µIU/ml)]/22.5. DHEA-S = dehydroepiandrosterone sulphate; SHBG = sex hormone-binding globulin.

Results

The anthropometric and basal hormonal features of the three groups are summarized in Table I. A significant difference in age was observed; therefore, all values were adjusted for age before comparisons between the three groups. BMI values were significantly higher in women with PCOS, compared to controls, therefore comparisons between women with PCOS and controls were performed after additional adjustment for BMI.

LH, testosterone, Δ4-androstenedione, DHEA-S and 17OH-progesterone levels and FAI values were significantly higher and FSH levels significantly lower in women with PCOS, compared to controls. No significant difference in the above parameters was observed between women with PCOS and hyperandrogenic women without PCOS, except for Δ4-androstenedione levels, which were significantly higher in women with the syndrome. SHBG levels were significantly lower in women with PCOS, compared to controls, while no difference was observed between women with PCOS and hyperandrogenic women without the syndrome. Fasting insulin levels and HoMA-IR values were significantly higher, and glucose:insulin values significantly lower in women with PCOS, compared to the control group. However, differences in insulin levels, HoMA-IR and the glucose:insulin ratio were blunted, after adjustment for BMI. Plasma ghrelin levels were lower in women with PCOS, compared to both the other two groups, but not significantly so (Figure 1).

Testosterone, Δ4-androstenedione, DHEA-S and 17-OH-progesterone levels, FAI and HoMA-IR values were significantly higher in hyperandrogenic women without PCOS, compared to controls. Plasma ghrelin levels were lower in hyperandrogenic women without PCOS, compared to controls, but this difference was not statistically significant, either (Figure 1).

Since all women with PCOS demonstrated some form of increased androgen activity, they were further divided into those with normal or elevated androgen levels. Women with PCOS were also divided, based on the presence of chronic anovulation and/or polycystic ovary morphology. In two-way ANOVA, neither classification had a significant effect on ghrelin levels. However, after post hoc analysis, both controls and hyperandrogenic women with PCOS and normal androgen levels had significantly higher concentrations of ghrelin than women with PCOS and biochemical hyperandrogenaemia (post hoc P < 0.05) (Figure 2).

A significant difference was seen between women with chronic anovulation and the control group (P = 0.03). It should also be noted that women with PCOS and both chronic anovulation and polycystic ovary morphology had higher ghrelin concentrations compared to women with the syndrome and only one of these two characteristics.
However, these differences were not statistically significant (Figure 3).

No significant correlation between ghrelin levels and BMI, HoMA-IR and glucose:insulin values was observed. However, ghrelin levels were significantly correlated with 17-OH-progesterone levels \( (r = 0.274, P = 0.022) \).

**Discussion**

Ghrelin is a novel peptide associated with energy balance and insulin resistance, while a possible involvement of this peptide in hypothalamic–pituitary–gonadal function has also been implicated (Van Der Lely *et al.*, 2004). Results on plasma ghrelin levels in women with the PCOS are rather conflicting and, in most studies, the number of women involved was relatively small (Pagotto *et al.*, 2002; Schofl *et al.*, 2002; Gambineri *et al.*, 2003; Orio *et al.*, 2003; Moran *et al.*, 2004; Wasko *et al.*, 2004).

The present study was designed in order to assess the possible correlations of ghrelin to the metabolic and hormonal features of PCOS, in a sizeable number of women with the syndrome. The significant number of women studied made it also possible to further divide women with the syndrome, based on the presence of chronic anovulation, biochemical hyperandrogenaemia, clinical hyperandrogenism, and polycystic ovary morphology on ultrasound evaluation. Furthermore, ghrelin levels were also measured in women who had only hyperandrogenaemia but no other features of PCOS and in normal controls. Our results concerning the basic hormonal profile of women with PCOS are in accord with well-established evidence on the fundamental characteristics of the syndrome (Kandarakis *et al.*, 1996; Panidis *et al.*, 2003, 2004).

In women with PCOS, decreased ghrelin concentrations have been reported in most (Pagotto *et al.*, 2002; Schofl *et al.*, 2002; Moran *et al.*, 2004), but not all (Orio *et al.*, 2003; Wasko *et al.*, 2004) previous studies. In the present study, women with PCOS had lower ghrelin levels than women with isolated hyperandrogenaemia and controls. Moreover, women with increased androgen levels but without the syndrome had lower ghrelin levels than controls. However, none of these differences were statistically significant.

Nevertheless, women with PCOS and elevated circulating androgens had significantly lower plasma ghrelin levels than controls and hyperandrogenic women with the syndrome, but normal androgen concentrations (Figure 2). Ghrelin levels were lower in women with isolated hyperandrogenaemia compared to controls, but not significantly so. We postulate, therefore, that PCOS-associated hyperandrogenaemia results in reduced ghrelin concentrations. This finding is in agreement with previously reported negative correlations between ghrelin and androgens in rodents (Tena-Sempere *et al.*, 2002) and in women with PCOS (Pagotto *et al.*, 2002; Gambineri *et al.*, 2003).

In the present study, a significant negative correlation between ghrelin and 17-OH-progesterone levels was observed. This finding further supports the hypothesis that ghrelin is involved in ovarian steroid biosynthesis and/or action (Pagotto *et al.*, 2002; Gambineri *et al.*, 2003; Gaytan *et al.*, 2003). It should also be noted that progesterone-induced menstruation implemented in some studies (Orio *et al.*, 2003) might interfere with measurements of ghrelin plasma concentration.

It is interesting that anovulatory women with the syndrome but normal ovarian morphology had significantly lower ghrelin levels compared to the control group. Ghrelin levels were higher in anovulatory women with polycystic ovary morphology, compared to anovulatory women with the syndrome but normal ovarian morphology, as well as to those with...
polycystic ovaries on ultrasound but normal menses (Figure 3), although these differences were non-significant. Since ghrelin has been shown to interfere with hypothalamic–pituitary function (Van Der Lely et al., 2004), it is possible that androgen-independent mechanisms of chronic anovulation combined with polycystic ovaries in the syndrome tend to attenuate the negative association between ghrelin and hyperandrogenaemia. Given the small number of women involved in most studies so far, the discrepancy of previous results could be attributed to the heterogeneity of the samples studied, regarding the presence of chronic anovulation, biochemical hyperandrogenaemia, clinical hyperandrogenism and polycystic ovary morphology.

In conclusion, the present results support previous evidence that, in women with PCOS, circulating ghrelin and androgens are negatively associated. Furthermore, it is likely that mechanisms that result in different clinical and biochemical manifestations of the syndrome might also affect ghrelin concentrations. Therefore, we propose that a more detailed research classification system should be used when the role of novel peptides is investigated in women with PCOS.

References


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