Serum leptin levels in normal-weight and overweight women with polycystic ovary syndrome

D. Panidis, D. Rousso, A. Kourtis, V. Tsimas, K. Papathanasiou, G. Makedos
Division of Endocrinology and Human Reproduction of the Second Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, Thessaloniki (Greece)

Summary
The aim of this study was to evaluate serum leptin levels in women with polycystic ovary syndrome (PCOS) and in controls with normal or abnormal body mass index (BMI), since the literature data are contradictory. The study population comprised 34 women with PCOS and 30 regularly cycling women, with normal or abnormal BMI. PCOS was defined by clinical, ultrasonographic, and hormonal findings. The women were divided into four groups according to the diagnosis of PCOS and their BMI values. In all women serum levels of FSH, LH, prolactin, testosterone, androstenedione, DHEA-S, 17α-OH progesterone, SHBG, insulin, glucose and leptin were determined. It was found that: (a) there was a significant interaction between BMI and PCOS in increasing serum leptin levels; (b) the dominant factor for serum leptin levels in women of all Groups was BMI, followed by insulin; (c) once we corrected for BMI, it was found that there was a significant correlation between serum leptin levels and insulin values, as well as between serum leptin levels and testosterone concentrations; and (d) the QUICKI IR formula presented the most significant correlation with serum leptin levels than the other measures of insulin sensitivity. Our results showed that serum leptin levels in a subgroup of overweight women with PCOS and insulin resistance were higher than those expected for their BMI, and therefore leptin might interfere in the pathogenesis of this syndrome.

Key words: PCOS; Leptin; Insulin sensitivity; Testosterone; BMI.

Introduction
The detection of leptin, whose molecule was identified in 1995, has been an outstanding event in endocrinology in recent years [1-3]. Leptin is a 167-aminoacid protein with a molecular weight of 16 kDa [4, 5]; its name is derived from the Greek word "leptos", meaning thin. Leptin attracted not only scientific, but also mass media interest, as it was considered to be the definite way of dealing with obesity [6]. It is known that women have higher leptin levels than men [7, 8]. This sexual dimorphism is thought to be due to the higher levels of androgens in men.

Polycystic ovary syndrome (PCOS) is the most frequent endocrinologic disorder causing infertility due to anovulation [9-11]. Hypersecretion of androgens is a typical biochemical feature of PCOS. Moreover, women with PCOS often present with LH hypersecretion and insulin resistance [11-14]. Therefore, these women comprise a useful "model" for evaluating the androgen-leptin and insulin-leptin interactions.

The results of studies, concerning the evaluation of leptin levels in women with PCOS, are contradictory [9, 10, 15-23]. Several authors stated that serum leptin levels in women with PCOS show no difference than those in controls with a similar body mass index (BMI) [24-28]. On the contrary, others claimed that serum leptin levels in a subgroup of women with PCOS were higher than expected for their BMI, and that those higher hormone levels possibly had a negative effect on their ovarian function [29, 30]. This might imply that disorders in the transmission of leptin messages to the reproductive system of these women are possibly involved in the pathogenesis of the polycystic ovary syndrome.

This study was designed in order to evaluate serum leptin concentrations in women with PCOS and in regularly cycling women, with normal or abnormal BMI, since the literature data are contradictory and include only a small number of cases.

Materials and Methods
Sixty-four women, aged 16-34 years, were included in the study. All women were recruited from the Outpatient Fertility and Family Planning Department of a University Clinic. These women were free from any disease, besides PCOS, that could influence the hypothalamic-pituitary-ovarian axis. In addition, no medication was given that could influence the function of the hypothalamus-hypophys-ovary axis within the last six months. These 64 women were divided into four groups, according to the diagnosis of PCOS and BMI values. The first group (Group I) included 19 women, aged 20-29 years mean ± SD 24.0 ± 3.6 years), with PCOS, without insulin resistance and with normal BMI, ranging from 19.1 to 24.9 kg/m² (21.5 ± 1.5). The second group (Group II) comprised 15 women, aged 16-30 years (24.6 ± 3.2 years), with PCOS, high fasting insulin levels (23.2 ± 2.9 µU/ml) and abnormal BMI, ranging from 25.3 to 39.5 kg/m² (31.6 ± 3.8). The third group (Group III) consisted of 15 women, aged 18-34 years (21.9 ± 3.7 years), with normal ovulatory cycles (28 ± 2 days) and normal BMI, ranging from 20.3 to 24.8 kg/m² (22.0 ± 1.5). The fourth group (Group IV) included 15 women, aged 20-32 years (22.9 ± 3.5 years), with

Revised manuscript accepted for publication April 15, 2003
normal ovulatory cycles (28 ± 2 days) and abnormal BMI, ranging from 25.4 to 36.2 kg/m² (29.9 ± 3.4). The local ethical committee approved the study protocol; all subjects provided written informed consent.

The diagnosis of PCOS was considered unequivocal when a woman met the following findings: (a) oligomenorrhea or amenorrhoea; (b) at least one ovary with a volume of > 9 ml and with > 10 cysts of 2-8 mm in diameter in the periphery of the ovary demonstrated on pelvic ultrasound; (c) perimenarcheal onset of hirsutism, obesity or menstrual disturbance; (d) a high concentration of total or free testosterone and/or androstenedione [31]. Insulin sensitivity was evaluated in our study by calculating the ratio fasting glucose to insulin levels [32], by the homeostatic model assessment formula (HOMA IR formula) [33], or the Quantitative Insulin Sensitivity Check Index (QUICKI IR formula) [34, 35].

In groups I, II, III, and IV blood samples were collected once at 9 a.m., after an overnight fast, and between the third and seventh day of spontaneous or induced menstrual cycle for the determination of serum leptin, gonadotropins (FSH, LH), prolactin (PRL), testosterone (T), androstenedione (ΔA, D), dehydroepiandrosterone sulphate (DHEA-S), 17α-hydroxyprogesterone (17α-OHP), sex hormone binding globulin (SHBG) and insulin levels.

Serum leptin concentrations were determined by immunoradiometric assay (IRMA), using commercially available kits (Human leptin: Diagnostic System Laboratories, Inc., Texas, TX, USA). FSH, LH, PRL, T, ΔA, 17α-OHP, DHEA-S and SHBG were measured by radio-immunoassay using commercial kits: FSH: Radioisotopic Kit, Nichols Institute Diagnostics, San Juan Capistrano, CA, USA; LH: Radioisotopic Kit, Nichols Institute Diagnostics, San Juan Capistrano, CA, USA; PRL: Radioisotopic Kit, Nichols Institute Diagnostics, San Juan Capistrano, CA, USA; T: Direct Radio-immunoassay Kit, Sorin, Biomedica; ΔA: Gamma Coat [125I] Radio-immunoassay Kit, Incstar Corp. DHEA-S: Direct Radio-immunoassay Solid Phase Coated Tubes, Zer Science Based Industries Ltd; 17α-OHP: ImmuChem Double Antibody [125I] Radio-immunoassay Kit, ICN Pharmaceuticals, Inc.; SHBG: [125I] Immunoradiometric Assay Kit, Orion Diagnostics; Insulin: Coat-A-count Insulin, Diagnostic Products Corp.).

Two-factor ANOVA, one-way ANOVA, post-hoc multiple comparison test and multiple regression analysis were used for statistical analysis of data. A p value of < 0.05 was considered statistically significant. Data were entered in the spreadsheet package EXCEL (version 9.0, Microsoft Corp., 1999) and biostatistical analysis was performed using SPSS for Windows (release 10.0, SPSS Inc., 2001).

Results

The mean values and the standard deviation (SD) of the age and body mass index (BMI) of women in each group are presented in Table 1. There were no statistically significant differences between the ages of women in the four groups. Moreover, there were no statistically significant differences between BMI values of women in Groups I and III, or between BMI values of women in Groups II and IV.

Table 2 shows serum leptin levels, as well as serum values of FSH, LH, PRL, T, ΔA, DHEA-S, 17α-OHP, SHBG and insulin of women in all groups. The twofactor ANOVA showed that overweight women (Groups II and IV) presented significantly higher serum leptin concentrations than those with normal BMI (Groups I and III) (p < 0.0001). Similarly, women with PCOS (Groups I and II) showed significantly higher serum leptin levels than women without PCOS (Groups III and IV) (p < 0.005). It should be noted that there was a significant interaction between these two factors (BMI and PCOS) (p < 0.0001).

Afterwards, the one-way ANOVA procedure detected that there was an overall intergroup difference (p < 0.0001). The post hoc multiple-comparison test showed that women with normal ovulatory cycles and normal BMI (Group III) did not present any significant differences in serum leptin concentrations than those of women with PCOS and normal BMI (Group I). On the contrary, women with PCOS and high BMI values (Group II) showed significantly higher serum leptin levels than those of controls with high BMI values (Group IV) (p < 0.0001). It should be noted that all women with PCOS and high BMI (Group II) showed high serum leptin levels, and in six out of those 15 women serum leptin concentrations were higher than 40 ng/ml; this value was much higher than the highest value of controls with high BMI values (Group IV), which was 31.1 ng/ml.

There were no statistically significant differences in FSH levels among women in all groups. Women in Groups III and IV presented lower LH values (p < 0.05).

Table 1. — Clinical data (mean ± SD) of women in Groups I, II, III, and IV.

<table>
<thead>
<tr>
<th>Clinical data</th>
<th>I</th>
<th>II</th>
<th>Groups</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of women (n)</td>
<td>19</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCOS, without insulin resistance</td>
<td>24.0 ± 3.6</td>
<td>24.6 ± 3.2</td>
<td>21.9 ± 3.7</td>
<td>22.9 ± 3.5</td>
<td></td>
</tr>
<tr>
<td>and normal BMI</td>
<td>21.5 ± 1.5</td>
<td>31.6 ± 3.8</td>
<td>22.0 ± 1.5</td>
<td>29.9 ± 3.4</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. — Serum hormone levels (mean ± SD) of women in Groups I, II, III and IV.

<table>
<thead>
<tr>
<th>Hormones</th>
<th>I</th>
<th>II</th>
<th>Groups</th>
<th>III</th>
<th>IV</th>
<th>Normal values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin (ng/ml)</td>
<td>10.7 ± 6.3</td>
<td>33.6 ± 13.0</td>
<td>15.2 ± 5.7</td>
<td>18.4 ± 7.5</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>5.3 ± 2.2</td>
<td>5.6 ± 2.3</td>
<td>5.2 ± 1.8</td>
<td>5.4 ± 2.1</td>
<td>3.6-13.7</td>
<td></td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>8.6 ± 3.5</td>
<td>8.8 ± 3.4</td>
<td>3.9 ± 1.7</td>
<td>4.4 ± 2.1</td>
<td>1.9-11.9</td>
<td></td>
</tr>
<tr>
<td>PRL (ng/ml)</td>
<td>13.3 ± 2.8</td>
<td>14.8 ± 3.1</td>
<td>12.1 ± 2.4</td>
<td>14.4 ± 3.5</td>
<td>3.0-23.2</td>
<td></td>
</tr>
<tr>
<td>T (ng/ml)</td>
<td>0.79 ± 0.27</td>
<td>1.1 ± 0.1</td>
<td>0.36 ± 0.08</td>
<td>0.29 ± 0.07</td>
<td>0.2-0.8</td>
<td></td>
</tr>
<tr>
<td>ΔA (ng/ml)</td>
<td>4.71 ± 0.73</td>
<td>3.92 ± 0.19</td>
<td>1.62 ± 0.45</td>
<td>1.58 ± 0.44</td>
<td>0.80-3.01</td>
<td></td>
</tr>
<tr>
<td>DHEA-S (ng/ml)</td>
<td>2.88 ± 1.25</td>
<td>3.04 ± 0.63</td>
<td>1.66 ± 0.37</td>
<td>1.79 ± 0.18</td>
<td>1.2-3.4</td>
<td></td>
</tr>
<tr>
<td>17α-OHP (ng/ml)</td>
<td>0.84 ± 0.17</td>
<td>0.81 ± 0.15</td>
<td>0.79 ± 0.13</td>
<td>0.64 ± 0.11</td>
<td>0.2-1.0</td>
<td></td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>40.0 ± 11.6</td>
<td>28.4 ± 6.1</td>
<td>67.0 ± 12.3</td>
<td>63.0 ± 8.6</td>
<td>16.0-120.0</td>
<td></td>
</tr>
<tr>
<td>Insulin (μU/ml)</td>
<td>7.2 ± 1.8</td>
<td>23.2 ± 2.9</td>
<td>5.4 ± 1.3</td>
<td>9.0 ± 1.4</td>
<td>2.0-20.0</td>
<td></td>
</tr>
</tbody>
</table>
Serum leptin levels in normal-weight and overweight women with polycystic ovary syndrome

0.0001) than those of women in Groups I and II. Moreover, overweight women (Groups II and IV), regardless of PCOS presence, presented higher prolactin values (p < 0.05) than those of normal-weight women (Groups I and III). No statistically significant differences were found between DHEA-S, 17α-OHP and SHBG levels among women in Group I and II, although SHBG levels of women in Group II were much lower (p < 0.001) than those of women in Group I. It should be noted that DHEA-S and 17α-OHP levels in PCOS women (Groups I and II) were significantly higher than those of women without PCOS (Groups III and IV) (p < 0.0001 and p < 0.05 respectively). On the contrary, SHBG levels of women in Groups I and II were significantly lower (p < 0.0001) than those in Groups III and IV. Testosterone levels were significantly higher in women in Groups I and II than those in women in Groups III and IV (p < 0.0001); similarly, testosterone levels were significantly higher in women in Groups II and IV (p < 0.05) than those in Groups I and III. Androstenedione levels were significantly higher in women in Group I (p < 0.05) than those in Group II; similarly, androstenedione levels were significantly higher in women in Groups I and II than those in Groups III and IV (p < 0.0001).

Insulin levels were significantly higher in women in Group II (p < 0.001) than those in Group I and in women in Group IV (p < 0.0001) than those in Group III. Finally, women in Groups I and II presented significantly higher insulin levels than women in Groups III and IV (p < 0.0001).

Multiple regression analysis showed that the dominant factor for serum leptin levels in women in all groups is BMI (p < 0.0001), followed by insulin (p < 0.005). Once we corrected for BMI, it was found that there was a significant correlation between serum leptin levels and insulin values (p < 0.0001), as well as between serum leptin levels and testosterone concentrations (p < 0.005).

We then analyzed the four measures of insulin sensitivity (fasting insulin levels, fasting glucose to insulin levels, HOMA IR formula and QUICKI IR formula) in order to estimate their correlation with leptin levels in women. It was found that QUICKI IR formula has a significant correlation with serum leptin levels (p < 0.05).

Discussion

Our results showed that there were no statistically significant differences in BMI values between normal weight women with PCOS and normal weight controls (Group I vs Group III), as well as between overweight women with PCOS and overweight controls (Group II vs Group IV). It is known that BMI is the most important regulatory factor of leptin secretion [2, 4, 5, 25, 26, 36-40]. Our results lend further support to the above aspect, since the two-factor ANOVA showed that overweight women (Groups II and IV) presented significantly higher serum leptin concentrations than those with normal BMI (Groups I and III) (p < 0.0001). Moreover, there was no statistically significant difference between the ages of women in the four groups, although it is known that age after the second stage of puberty does not influence serum leptin levels [41].

The two-factor ANOVA showed that overweight women (Groups II and IV) presented significantly higher serum leptin concentrations than those with normal BMI (Groups I and III) (p < 0.0001). Similarly, women with PCOS (Groups I and II) showed significantly higher serum leptin levels than women without PCOS (Groups III and IV) (p < 0.005). It should be noted that there was a significant interaction between these two factors (BMI and PCOS) (p < 0.0001). It should also be noted that BMI is a more potent factor in increasing serum leptin levels than PCOS.

Since there was a significant interaction between BMI and PCOS in increasing serum leptin levels, the one-way ANOVA procedure detected that there was an overall intergroup difference (p < 0.0001). A post-hoc multiple-comparison test showed that women with normal ovulatory cycles and normal BMI (Group III) did not present any significant differences in serum leptin concentrations than women with PCOS and normal BMI (Group I). On the contrary, women with PCOS and high BMI values (Group II) showed significantly higher serum leptin levels than those of controls with high BMI values (Group IV) (p < 0.0001).

Multiple regression analysis showed that the dominant factor for serum leptin levels of women in all groups is BMI (p < 0.0001), followed by insulin (p < 0.005). Once we corrected for BMI, it was found that there was a significant correlation between serum leptin levels and insulin values (p < 0.0001), as well as between serum leptin levels and testosterone concentrations (p < 0.005). It should be noted that insulin is a more potent factor in increasing serum leptin levels than testosterone.

All women with PCOS and high BMI (Group II) showed high serum leptin levels, and in six out of those 15 women serum leptin concentrations were higher than 40 ng/ml; this value was much higher than the highest value of controls with high BMI values (Group IV), which was 31.1 ng/ml. Our finding is in accordance with the results of other authors, who have observed that in a subgroup of obese women with PCOS (30%), serum leptin concentrations were higher than those expected for their BMI [30]. These authors have also stated that the particularly high serum leptin levels in this subgroup of women with PCOS may have a negative effect on ovarian function, and that the disorders in the transmission of leptin messages to the reproductive system of these women are possibly involved in the pathogenesis of the PCOS. The last claim can further be made since insulin and testosterone levels remained correlated with leptin after correction for BMI.

We analyzed with multiple regression analysis the four measures of insulin sensitivity (fasting insulin levels, fasting glucose to insulin levels, HOMA IR formula and QUICKI IR formula) in order to estimate their correlation with leptin levels in women. It was found that QUICKI IR formula presented the most significant correlation with serum leptin levels than the other measures of insulin sensitivity. When a multiple regression analysis
with BMI was performed. QUICKI IR formula was the only measure of insulin sensitivity that correlated with serum leptin levels (p < 0.05).

References


Address reprint requests to:
D. ROUSSO, M.D.
46-48, Mitropoleos Str.
546 23 Thesisalloni (Greece)