ORIGINAL ARTICLES
Clinical Investigations

EFFECT OF SHORT-TERM STANDARD THERAPEUTIC REGIMENS ON NEUROPEPTIDE Y AND ADIPOSE TISSUE HORMONES IN OVERWEIGHT INSULIN-RESISTANT WOMEN WITH POLYCYSTIC OVARY SYNDROME

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ABSTRACT
AIM: The study was aimed at elucidating the influence of a 3-month treatment with routine therapeutic regimens – oral hormonal contraceptives (OHC) with antiandrogenic activity (a standard combination of ethynil estradiol 35 μg plus cyproterone acetate 2 mg) in combination with insulin sensitizing agents – metformin (Group I) and rosiglitazone (Group II) on adipose tissue hormones and hypothalamic neuropeptide Y (NPY) in women with polycystic ovary syndrome.

PATIENTS AND METHODS: The study included 66 overweight insulin resistant women with PCOS according to the recent ESHRE-ASRM criteria randomized into 2 age-matched therapeutic groups.

RESULTS: Significant decrease of leptin (P < 0.01; P = 0.001, resp.), resistin (P < 0.01; P < 0.01, resp.), tumour necrosis factor α (TNFα) (P = 0.001; P < 0.001, resp.), and NPY (P < 0.05; P < 0.001, resp.) was observed in both groups after treatment. These findings were in parallel with a significant decrease in the anthropometric parameters of body weight in the metformin group only. No significant changes in hormonal characteristics of the groups were found except for a significant decrease in androstenedione and DHEA-S (P < 0.05) in the metformin group and in 17-OH-progesterone (P < 0.05) in the rosiglitazone group. HDL-cholesterol rose and diastolic blood pressure fell significantly (P < 0.05) in the metformin group.

CONCLUSION: Our data suggest beneficial effects of the treatment on potential cardiovascular risk in insulin resistant PCOS women.

Key words: neuropeptide Y, adipose tissue hormones, polycystic ovary syndrome, insulin resistance, metformin, rosiglitazone

INTRODUCTION
Polycystic ovary syndrome (PCOS) is one of the most common endocrine metabolic diseases, affecting up to 10% of women of reproductive age.1 Obesity is present in 30–70% of women depending on the setting of the study and the ethничal background of the subjects, and it is characterized by central distribution of fat.2,3 Thus, we found overweight in 28.72% and obesity in 48.94% out of 94 women with PCOS, and in 22%, and 51%, respectively in another cohort of 142 Bulgarian patients.4,5 In women with PCOS, hyperinsulinemia, dyslipidemia, and/or hypertension are highly dependent on obesity which worsens all of the clinical manifestations of PCOS.6,7

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The discovery of leptin in 1994 provoked the interest in the adipose tissue which was no longer considered as an inert tissue storing energy in the form of triglycerides, but as the greatest endocrine organ in human body. It is already known that adipocytes secrete a series of substances with local and distant effects. These are leptin, adiponectin, resistin, TNFα, omentin, visfatin, interleukin 6, etc. These hormones play an important role in metabolism, reproductive processes, cardiovascular function, and immunity. At present, there is an increasing body of evidence of high levels of atherogenic adipocytokines and low levels of adiponectin in women with PCOS that change according to variations of fat mass. Endocrine function of the adipocytes is regulated mainly by nutritional status, and both these factors are integrally interweaved with the energy storing mechanism in the adipose tissue. Neuropeptide Y (NPY) is a potent orexigenic peptide synthesized in the hypothalamus whose secretion is increased in response to metabolic challenges. It is still not clear if there are consistent differences in the levels or in the effects of appetite-regulating hormones as is NPY in PCOS.

Routine therapeutic regimens in women with PCOS include oral hormonal contraceptive (OHC) with antiandrogenic activity alone or in combination with insulin sensitizing agents (metformin and thiazolidinediones) in populations of women with PCOS. Both metformin and rosiglitazone are supposed to counteract unfavorable metabolic consequences of OHC and markedly potentiate its antiandrogenic activity. Few studies have so far directly compared metformin with thiazolidinediones for the treatment of PCOS. In a report involving the comparison between metformin and rosiglitazone, it was demonstrated that they both improved ovarian function and reduced hirsutism, especially the latter drug. The effect of combined treatment on adipose tissue hormones and hypothalamic neuropeptide Y (NPY) in PCOS is under investigation.

**AIM**

The aim of the study was to elucidate the influence of a 3-month treatment with routine therapeutic regimens– OHC with antiandrogenic activity (Diane 35 - a standard combination of ethynil estradiol 35 μg plus cyproterone acetate 2 mg) in combination with the insulin sensitizing agents – metformin and rosiglitazone on adipose tissue hormones and hypothalamic NPY in overweight insulin resistant PCOS women.

**PATIENTS AND METHODS**

The study comprised 66 overweight insulin resistant women with PCOS randomized into 2 age-matched therapeutic groups (mean age 23.58 ± 4.17 yrs; 23.25 ± 4.95 yrs, resp., P > 0.05). Group I (n = 32) was treated with Diane 35 and metformin (1275-1500 mg daily); Group II (n = 34) was treated with Diane 35 and rosiglitazone (4 mg daily) for 3 months. The diagnosis of PCOS was defined according to the consensus criteria for PCOS accepted in May 2003, Rotterdam: oligo- or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovaries. The diagnosis was made in the presence of two out of the three mentioned criteria. None of the subjects had previously been diagnosed as having PCOS or had been receiving hormonal treatment for at least 3 months prior to their evaluation. Homeostasis model of insulin resistance (HOMA-IR) was calculated as immune reactive insulin (IRI) x blood glucose/22.5. The cutoff of 2.5 for HOMA-IR and/or >100 μIU/ml for IRI during a standard oral glucose tolerance test (oGTT) were used for determining insulin resistance.

A comprehensive set of hormonal tests was performed in all study subjects in order to exclude other endocrine pathology such as thyroid dysfunction, prolactinoma, Cushings syndrome, congenital or non-classical adrenal hyperplasia, hormone secreting tumours, diabetes mellitus, and pregnancy. Waist circumference was measured at the smallest part of the waist and hip circumference was measured at the widest level of the buttocks, and the average of two measurements was calculated. The amount and percentage of the adipose tissue, fat free tissues (FFT) and total body water (TBW) were determined by bioimpedance method using Tanita TBF-300M body composition analyzer.

Blood samples were collected from all participants in early morning at fasting stage for determination of biochemical and hormonal parameters. All laboratory tests were performed in the early follicular phase (days 1-5) of spontaneous bleeding or withdrawal bleeding. Blood samples for hormone analysis were taken in the morning at 08:00 h after an overnight fast and centrifuged immediately. The serum was separated and frozen at -77°C until assayed.

The following serum levels were determined at baseline and after 3 months of treatment: leptin, resistin, TNFα, NPY (primary outcomes); total testosterone (T), androstenedione, dehydroepiandrosterone sulphate (DHEAS), 17-OH progesterone, estradiol (E2), FSH, LH (LH/RH ratio was calculated),
pentrin, glucose and IRI during an oGTT, total cholesterol, HDL-cholesterol, triglycerides, ASAT, ALAT, GGTP (secondary outcomes).

Leptin was determined by means of a solid-phase immunoenzyme assay (Human) ELISA (commercial kit of DRG, Germany) with the following characteristics: sensitivity - 0.2 ng/ml; inter assay variation, CV% < 5.4; intra assay variation, CV% < 8.7%; correlation with RIA, r = 0.95. Resistin was determined using a commercial kit of PHOENIX PHARMACEUTICAL INC, USA based on the principle of a “competitive” solid-phase immunoenzyme method (Human) EIA with the following characteristics: sensitivity - 1.16 ng/ml; inter assay variation, CV% < 5.0; intra assay variation, CV% < 14. TNFα was determined using a commercial kit of DRG, Germany by means of a solid-phase immunoenzyme method of greater sensitivity (Human) ELISA with the following characteristics: sensitivity - 3.0 pg/ml; inter assay variation, СV% < 5.2; intra assay variation, СV% < 10.0. NPY was determined using a commercial kit of PHOENIX PHARMACEUTICAL INC, USA based on the principle of a “competitive” solid-phase immunoenzyme method (Human) EIA with the following characteristics: sensitivity - 0.21 ng/ml; inter assay variation, CV% < 5.0; intra assay variation, CV% < 14. Insulin was tested using a commercial kit for quantitative determination of immunoreactive insulin on the basis of microparticul-immunoenzyme analysis (MEIA) by means of AxSYM system (ABBOTT, USA) with the following characteristics: sensitivity ≤ 0.8 μIU/ml; inter assay variation, CV% < 2.9; intra assay variation, CV% < 5.3. The hormonal tests including T, androstenedione, DHEA-S, 17-OH progesterone, E2, LH, FSH, prolactin, were performed on an AxSYM™ System (Abbott Diagnostics, Abbott Park, Il, USA) using commercial kits. Serum levels of glucose were determined by standard GOD-POD method. The area under the curve (AUC) for glucose and insulin during the oGTT was calculated using the trapezoid method. Total cholesterol was determined by ChOD, PAP, triglycerides by GPO, PAP, and HDL-cholesterol by MgSO₄-dextran SO₄ precipitation, Schneiders Analysers; Netherlands test; Delta Kone Autoanalyser. LDL-cholesterol was calculated using Friderwald formula. Liver enzymes (ASAT, ALAT, GGTP) were determined by routine laboratory analyses.

The statistical analysis was performed by SPSS version 12.0.1 (SPSS, Inc., Chicago, IL, USA). The distribution of the data was tested using the Kolmogorov–Smirnov test for normality. Correla-

| Table 1. Clinical characteristics of the groups before and after treatment |
|-------------------------------|-------------|-------|----------------|-----------------|-------------|-------------|--------------|----------------|
| Weight (kg) | BMI (kg/m²) | Waist (cm) | Waist-to-hip ratio | Fats % | Fats (kg) | FFM (kg) | TBW (kg) |
|-----------------|-------------|-------------|-----------------|---------|-------------|-------------|-------------|----------------|
| Group 1 - basal | 78.2±20.14 | 28.45±4.38 | 88.69±7.72 | 0.81±0.07 | 35.56±10.10 | 29.77±15.20 | 48.78±5.51 | 35.73±4.04 |
| - after 3 months | 75.5±18.66 | 27.45±3.73 | 86.63±5.92 | 0.80±0.06 | 33.98±8.77 | 27.23±13.39 | 48.31±6.21 | 35.29±4.65 |
| Group 2 - basal | 82.41±16.17 | 29.27±4.24 | 88.97±8.05 | 0.82±0.08 | 35.55±12.40 | 32.30±17.86 | 50.90±8.10 | 37.51±5.97 |
| - after 3 months | 82.23±15.80 | 29.22±4.27 | 88.24±7.63 | 0.81±0.08 | 34.72±13.09 | 31.33±17.94 | 50.78±7.43 | 37.03±5.09 |

* p < 0.05 – vs basal; ** p < 0.01 – vs basal.

<p>| Table 2. Hormonal parameters before and after treatment |
|----------------|----------------------------|----------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Testosterone (nmol/l)</th>
<th>Androstenedione (nmol/l)</th>
<th>DHEA-S (nmol/l)</th>
<th>17-OH-progesterone (nmol/l)</th>
<th>LH (IU/l)</th>
<th>FSH (IU/l)</th>
<th>LH/FSH</th>
<th>E2 (pmol/l)</th>
<th>Prolactin (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 - basal</td>
<td>4.12±1.53</td>
<td>12.91±6.21</td>
<td>9.81±6.25</td>
<td>2.79±1.88</td>
<td>5.57±3.00</td>
<td>3.70±2.08</td>
<td>3.12±3.66</td>
<td>306.0±150.00</td>
</tr>
<tr>
<td>- after 3 months</td>
<td>3.37±1.49</td>
<td>9.81±6.25</td>
<td>7.73±3.43</td>
<td>1.69±1.13</td>
<td>4.48±2.85</td>
<td>3.24±2.01</td>
<td>2.16±2.51</td>
<td>322.3±110.71</td>
</tr>
<tr>
<td>Group 2 - basal</td>
<td>3.99±1.31</td>
<td>15.61±4.88</td>
<td>10.67±5.00</td>
<td>3.69±1.19</td>
<td>6.47±3.86</td>
<td>3.90±1.88</td>
<td>2.16±1.73</td>
<td>383.17±189.96</td>
</tr>
<tr>
<td>- after 3 months</td>
<td>3.77±1.16</td>
<td>13.92±4.29</td>
<td>9.42±5.17</td>
<td>2.56±1.22</td>
<td>5.45±2.00</td>
<td>3.98±1.69</td>
<td>1.49±0.71</td>
<td>388.67±199.69</td>
</tr>
</tbody>
</table>

* p < 0.05 – vs basal.
tion analyses of Pearson and Spearman were used. Comparison between groups was performed by an unpaired t-test for normally distributed data. The results are presented as mean ± standard deviation (SD). Results were considered significant if P < 0.05. All P-values are two-tailed.

The main baseline characteristics of the groups are presented in Tables 1-3.

**RESULTS**

The two studied groups were age and weight matched that forms a part of the study design. There was no significant difference in the body weight, BMI, waist circumference WHR and parameters of body fat distribution between the two groups of PCOS women (Table 1). No significant differences were found in the baseline hormonal (Table 2, Table 3)

<table>
<thead>
<tr>
<th>Table 3. NPY and adipose tissue hormones before and after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1 (n = 32)</strong></td>
</tr>
<tr>
<td><strong>0 months</strong></td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
</tr>
<tr>
<td>Resistin (ng/ml)</td>
</tr>
<tr>
<td>TNFα (pg/ml)</td>
</tr>
<tr>
<td>NPY (ng/ml)</td>
</tr>
</tbody>
</table>

*p < 0.05 – vs basal; **p < 0.01 – vs basal; ***p = 0.001 – vs basal; ****p < 0.001 – vs basal.

**Table 4. Biochemical parameters before and after treatment**

<table>
<thead>
<tr>
<th>Glucose (mmol/l)</th>
<th>AUC glu</th>
<th>Insulin (μIU/ml)</th>
<th>AUC ins</th>
<th>HOMA-index</th>
<th>Total chol. (mmol/l)</th>
<th>HDL-chol. (mmol/l)</th>
<th>LDL-chol. (mmol/l)</th>
<th>TGl (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1 - basal</strong></td>
<td>4.54±0.48</td>
<td>1072.97±164.18</td>
<td>20.21±13.30</td>
<td>17883.56±15283.40</td>
<td>4.16±2.86</td>
<td>4.34±0.83</td>
<td>1.32±0.24</td>
<td>2.59±0.83</td>
</tr>
<tr>
<td>- after 3 months</td>
<td>4.45±0.52</td>
<td>1084.25±192.21</td>
<td>19.90±11.00</td>
<td>15283.56±9763.26</td>
<td>3.81±2.65</td>
<td>4.80±0.63</td>
<td>1.57±0.47</td>
<td>2.62±0.41</td>
</tr>
<tr>
<td><strong>Group 2 - basal</strong></td>
<td>4.81±0.75</td>
<td>1104.08±269.58</td>
<td>25.18±10.79</td>
<td>19232.84±10404.04</td>
<td>4.71±2.29</td>
<td>4.54±0.97</td>
<td>1.23±0.27</td>
<td>2.69±0.73</td>
</tr>
<tr>
<td>- after 3 months</td>
<td>4.52±0.57</td>
<td>1043.97±166.21</td>
<td>21.64±15.66</td>
<td>16512.18±8302.32</td>
<td>4.08±2.98</td>
<td>4.67±0.82</td>
<td>1.26±0.21</td>
<td>2.78±0.79</td>
</tr>
</tbody>
</table>

*p < 0.05 – vs basal.

**Table 5. Correlation coefficients at baseline**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Leptin</th>
<th>Resistin</th>
<th>TNFα</th>
<th>NPY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin</td>
<td>1.000</td>
<td>-0.108</td>
<td>0.307*</td>
<td>0.286*</td>
</tr>
<tr>
<td>TNFα</td>
<td>0.307</td>
<td>-0.236</td>
<td>1.000</td>
<td>0.459***</td>
</tr>
<tr>
<td>Weight</td>
<td>0.378**</td>
<td>0.060</td>
<td>0.342**</td>
<td>-0.053</td>
</tr>
<tr>
<td>BMI</td>
<td>0.416**</td>
<td>0.035</td>
<td>0.332**</td>
<td>-0.037</td>
</tr>
<tr>
<td>Waist</td>
<td>0.382**</td>
<td>-0.019</td>
<td>0.297*</td>
<td>-0.092</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>0.408**</td>
<td>0.043</td>
<td>0.312*</td>
<td>-0.030</td>
</tr>
<tr>
<td>Fat (kg)</td>
<td>0.386**</td>
<td>0.047</td>
<td>0.352**</td>
<td>-0.041</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>-0.070</td>
<td>-0.021</td>
<td>-0.250</td>
<td>-0.330*</td>
</tr>
<tr>
<td>Glucose (30 min)</td>
<td>0.310*</td>
<td>0.065</td>
<td>0.310*</td>
<td>0.000</td>
</tr>
<tr>
<td>Glucose (60 min)</td>
<td>0.243</td>
<td>-0.038</td>
<td>0.309*</td>
<td>-0.083</td>
</tr>
<tr>
<td>Insulin (0 min)</td>
<td>0.333**</td>
<td>0.080</td>
<td>0.003</td>
<td>-0.163</td>
</tr>
<tr>
<td>Insulin (60 min)</td>
<td>0.157</td>
<td>-0.062</td>
<td>0.046</td>
<td>-0.277*</td>
</tr>
<tr>
<td>Insulin (120 min)</td>
<td>0.072</td>
<td>-0.084</td>
<td>-0.123</td>
<td>-0.372**</td>
</tr>
<tr>
<td>AUC (insulin)</td>
<td>0.157</td>
<td>-0.080</td>
<td>-0.013</td>
<td>-0.316*</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.374**</td>
<td>0.034</td>
<td>0.020</td>
<td>-0.136</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>-0.255*</td>
<td>0.053</td>
<td>-0.260*</td>
<td>0.116</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01, ***p < 0.001.
and biochemical characteristics (Table 4) of the groups.

After the 3-month treatment leptin, resistin, TNFα and NPY significantly decreased in both treatment groups. The changes were more pronounced in the rosiglitazone group (Table 3). The significant correlations of NPY, and adipose tissue hormones with the main clinical, hormonal and biochemical parameters at baseline are shown on Table 5.

Treatment Group I (Diane 35+metformin)

In Group I body weight, BMI, amount of fats and waist circumference decreased significantly (P = 0.009; P = 0.009, P = 0.032, P = 0.019, resp.) after treatment. All androgens tended to decrease but the changes reached significance only for androstenedione and DHEA-S (P = 0.017, P = 0.042, resp.). LH (LH/FSH ratio, resp.) showed a tendency to decrease, while FSH, E2 and prolactin did not change considerably. All parameters of glucose and insulin during oGTT and HOMA-IR did not change. The marked variations in insulin may contribute to the lack of significant dynamics of the associated variables. A positive impact on lipid profile is observed – HDL-cholesterol increased significantly (P = 0.032). Hepatic enzymes did not change significantly (ASAT-19.71 ± 6.85; 17.93 ± 4.48, resp.; ALAT-20.36 ± 6.77; 19.57 ± 6.35, resp. GGTP-22.10 ± 12.49; 22.45 ± 10.53, resp., P > 0.05). Diastolic blood pressure fell significantly from 76.54 ± 10.28 mm Hg to 72.69 ± 8.57 mm Hg (P = 0.035) while systolic blood pressure did not show significant dynamics (119.23 ± 17.06 mm Hg; 116.15 ± 14.46 mm Hg, resp.). In this group the significant correlations between leptin and parameters of body weight remained similar after treatment. In addition, TNFα showed positive correlations with weight, BMI, waist circumference, percentage of fat tissue and fat mass in kg, (r = 0.669, r = 0.629, r = 0.612, r = 0.585, r = 0.671, resp., p < 0.01), total and LDL-cholesterol (r = 0.501, p < 0.05; r = 0.586, resp., p < 0.01), and a negative correlation with HDL-cholesterol (r = -0.516, p < 0.05). Diastolic blood pressure showed negative correlations with leptin and NPY (r = -0.511, r = -0.515, resp., p < 0.05). Interestingly, blood glucose at the 180th min. during the oGTT showed significant positive correlations with leptin, TNFα, and NPY (r = 0.554, r = 0.505, r = 0.487, resp., p < 0.05), and insulin at the 120th min. during the oGTT showed significant negative correlation with NPY (r = -0.478, p < 0.05).

Treatment Group 2 (Diane 35+rosiglitazone)

Body weight, BMI and fat parameters on TANITA analyzer, waist circumference and WHR did not change in the patients from Group II. Among all hormonal parameters only 17-OH progesterone decreased significantly (p < 0.05). As seen in Group I, glucose and insulin parameters during the oGTT did not change significantly. Lipid profile remained similar. No alterations of hepatic enzymes were observed (ASAT-19.69 ± 4.37; 18.59 ± 5.30, resp.; ALAT-19.46 ± 6.48 17.35 ± 4.53, resp., p>0.05), GGTP even fell significantly from 25.42 ± 13.21 to 21.50 ± 12.25, P = 0.009. Neither systolic (119.23 ± 17.06 mmHg; 116.15 ± 14.46 mmHg, resp.), nor diastolic blood pressure showed significant changes, but there was a trend towards decrease for the latter (79.14 ± 9.82 mm Hg; 75.88 ± 9.56 mm Hg, resp.), similarly to the findings in Group I. As in Group I, positive correlation of the anthropometric parameters with leptin persisted. Leptin showed significant positive correlation with basal insulin, HOMA-IR, insulin at the 60-th min, and AUCins (r = 0.698, r = 0.681, resp., p < 0.01; r = 0.451, r = 0.454, resp. p < 0.05), and negative one – with HDL-cholesterol (r = -0.555, p < 0.05). TNFα showed positive correlation with weight, BMI and waist circumference ((r = 0.508, r = 0.513, r = 0.461, resp., p < 0.05), as in Group 1, but also with the WHR (r = 0.448, p < 0.05) and only with the fat mass in kg (r = 0.492, p < 0.05), but not with the percentage of fat. In addition, there was a positive correlation of TNFα with glucose and insulin at the 60-th min. during the oGTT (r = 0.502; r = 0.480, resp., p < 0.05), and a negative one with HDL-cholesterol (r = -0.483, p < 0.05).

Discussion

PCOS is considered to be the most common endocrine disorder in women during their reproductive years. Since 1980, PCOS has been strongly associated with the presence of insulin resistance (IR) and secondary hyperinsulinemia, mainly but not exclusively, in obese women. The prevalence of IR among these women varies according to the sensitivity and specificity of the assays used and the population of women studied. Previous studies have demonstrated that secondary hyperinsulinemia is the key factor responsible for the hyperandro- genism characteristic of the syndrome, which is attributed to an increased stimulation of the activity of the cytochrome P450c 17α in the ovary. Hyperinsulinemia also decreases the circulating
concentrations of SHBG and contributes to greater concentrations of free androgens in the blood.18 Women with PCOS and IR have a greater risk for carbohydrate intolerance and type 2 diabetes mellitus (DM), dyslipidemia, arterial hypertension, coagulation disorders induced by endothelial dysfunction, and cardiovascular disease.5,7,19,20

Because IR is a cardinal feature of PCOS, one therapeutic approach is to use drugs to improve insulin sensitivity and ovarian function.11 Therapies that decrease insulin concentrations can be effective not only in obese but also in normal-weight PCOS women. Metformin, a biguanide class drug, has become the most widely used drug to treat women with IR and PCOS.10-14 Other drugs include thiazolidinediones (TZD) like rosiglitazone and pioglitazone, and most studies using these drugs in women with PCOS did not report serious adverse reactions.11,21,22 Some studies have demonstrated an increase in ovulatory cycles in PCOS women when insulin sensitivity is increased by metformin or a thiazolidinedione.23-25 In short-term (6-month) studies, insulin sensitivity and androgen excess improved in women with PCOS treated either with metformin (2500 mg/day) or pioglitazone.26,27 In a report involving the comparison between metformin and rosiglitazone, it was demonstrated that they both improved ovarian function and reduced hirsutism, especially the latter drug.28

Oral contraceptive pills have been the traditional medical therapy for the long-term treatment of PCOS in order to regularize menses, to improve hirsutism and/or acne by reducing ovarian androgen production and to provide endometrial protection. The most clinically efficacious approach using oral contraceptives in PCOS women can be obtained combining estrogen with cyproterone acetate, which has both progestagen and anti-androgen activity and also induces hepatic metabolism and increases testosterone clearance.29,30 In combination with estrogens, cyproterone acetate is almost as effective as GnRH agonist at suppressing serum LH and T, and the clinical efficacy of the two treatments are equivalent.31 The combination of 35 μg ethynil oestradiol and 2 mg cyproterone acetate (Diane 35) is one of the most widely prescribed OHC in Europe for treatment of PCOS in women who do not desire conception with effective and relatively rapid amelioration of menstrual abnormalities and clinical signs of hyperandrogenism.32 In our study we observed a decrease of all androgens, but the changes reached significance for androstenedione and DHEA-S in Group I, and 17-OH progesterone in Group II. LH and LH/FSH also showed a trend to decrease, but possibly due to the inter-group variations this was not significant. Adding insulin sensitizers to OHC could neutralize possible unfavourable metabolic consequences of monotherapy with the latter and to provide additional beneficial effects on reproductive and hormonal disturbances. No negative influence on carbohydrate and lipids metabolism was observed in our patients, even there was a positive effect on HDL-cholesterol in Group I.

Adipose tissue is now considered as the most abundant endocrine organ in the body. Adiposity is closely linked to insulin resistance, diabetes, and cardiovascular disease through different mechanisms including production and secretion of several hormones, cytokines, and other molecules; defects in insulin signalling; low-grade chronic inflammation; and dysfunctional steroidogenesis. Abdominal adiposity or obesity are present in most women with PCOS, adversely influencing both the metabolic and reproductive phenotype of the syndrome.32,33 There are a series of abnormalities in the adipose tissue hormones in women with PCOS, some of which are due to obesity but others that could be disease specific.33,34 At present there is an increasing body of evidence of high levels of leptin and other atherogenic adipocytokines and low levels of adiponectin in women with PCOS that change according to variations of fat mass8,34, but the data are somewhat controversial. We found a significant decrease in the atherogenic adipocytokines leptin, resistin and TNFα after treatment.

Some reports have shown that women with PCOS have higher circulating concentrations of leptin as compared with BMI-matched controls, in contrast with other studies on both adolescent and adult subjects with PCOS that show that the concentrations of leptin in PCOS are not significantly different from those of controls when BMI is used as covariate.35 However, a report on south Indian women demonstrated that circulating concentrations of leptin were higher in normal-weight and obese subjects with PCOS than in controls.36 The role of hyperleptinemia in women with PCOS is not clear yet, and the possibility exists that women with PCOS are leptin resistant.9,34 Hyperleptinemia seems to be inversely related to the degree of fertility in women with PCOS and elevated leptin levels may contribute to the lack of follicular maturation in PCOS.37

Resistin is a positive marker of coronary atherosclerosis. Its levels were found to be higher in
patients with PCOS than in age- and BMI-matched controls.\(^4\) Given the poor relation between resistin and insulin resistance in humans, however, it seems unlikely that resistin is responsible for the increased insulin resistance in our PCOS patients. Nevertheless, it may play a contributory role in the proinflammatory state\(^5\), associated with metabolic syndrome, and the fact that its levels decreased with treatment is considered beneficial in the terms of potential cardiovascular risk in overweight insulin resistant women with PCOS.

One of the mechanisms of insulin resistance in obesity may be inhibition of insulin signalling by TNF\(\alpha\)\(^6\), furthermore, TNF\(\alpha\) decreases adipocyte secretion of adiponectin and suppresses Glut-4 glucose transporters and insulin receptors\(^7\). TNF\(\alpha\) levels were found elevated in both lean and obese PCOS by Li et al.\(^8\) Similarly to that of resistin, the decrease of TNF\(\alpha\) after treatment should be beneficial in terms of cardiovascular risk.

Increased plasma NPY levels were found in both obese and non-obese women with PCOS, as compared to controls, independent of body weight increase.\(^9\) Gennarelli et al.\(^3\) reported similar NPY levels at rest in PCOS and normo-ovulatory women, but an impaired NPY response to hypoglycemia in PCOS women. Clinical evidence suggests that NPY exerts primarily an inhibitory effect on the hypothalamic-pituitary-ovarian axis.\(^4\)

It is known that NPY stimulates appetite leading to hyperphagia, increase of body fat, decrease of thermogenesis and suppression of sympathetic activity, thus it serves as main regulator of food intake, body weight and energy consumption.\(^4\) So, the decrease observed in our patients after treatment should be beneficial in terms of ameliorating ovarian function and prevention of weight gain.

In our study leptin and TNF\(\alpha\) were in significant positive correlation with weight, BMI and anthropometric parameters. The hyperglycaemic response after glucose load and HDL-cholesterol were also associated with these two adipocytokines. Basal hyperinsulinaemia and markers of insulin resistance in this cohort of PCOS were associated with leptin and the postload hyperinsulinaemia was associated with NPY. No correlations were found between adipose tissue hormones and androgens, there was a negative one between androstenedione and NPY. Resistin did not show significant correlations with any of the clinical and biochemical characteristics of PCOS.

Interventions that influence reproductive and metabolic function in PCOS may also affect levels of the adipose tissue hormones and regulators of appetite, such as NPY. It has been postulated that some of the effects of insulin sensitizing agents in PCOS may be mediated through changes in adipocytokines levels. In our study the serum concentrations of NPY and all investigated adipocytokines decreased significantly after treatment in both groups. The data in the literature about changes of adipocytokines after treatment of PCOS women with OHC in combination with insulin sensitizers are scanty and we could not find studies with similar design to compare our data. Thus, monotherapy with rosiglitazone (8 mg daily for 4 months) had a significant effect on plasma adipocytokines levels, decreasing resistin levels in overweight women with PCOS.\(^4\) But Jakubowska et al. did not find changes in resistin levels in obese PCOS women after 3 and 6 months treatment with rosiglitazone (4 mg daily).\(^4\) Lewandowski et al.\(^4\) reported that there was no correlation between the serum resistin and adiponectin levels, insulin resistance, lipids and serum androgen levels in patients with PCOS which is similar to our data.

The change of NPY and adipocytokines was associated with weight loss only in the metformin group that is an expected effect of the drug and in conformity with other studies. Thus, metformin decreased BMI significantly in the pioneering observational study of Velazquez et al.\(^4\) Recently, a meta-analysis of 14 trials including 649 women and comparing metformin and placebo with or without lifestyle modification showed a statistically significant decrease in BMI after treatment.\(^4\) It is proven that in PCOS weight loss improves insulin sensitivity, diminished insulin levels, decreased androgens (testosterone), and increased ovulation rates.\(^3\) We also observed decrease in androgens – androstenedione and DHEA-S fell significantly and there was a non significant diminution in total testosterone. No significant changes in glucose and insulin levels at fasting state and after oGTT, as well as in HOMA-IR were observed. Our results are similar to those of Elter, et al, and Cibula, et al.\(^4\) A relatively short duration of the treatment is a limitation of the study, conclusions can be drawn after a longer period of follow-up of the patients.

However, recent and contradictory observational evidence has raised the concern that oral contraception may reduce insulin sensitivity and glucose tolerance in PCOS women. A recent review by Costello et al.\(^4\), comparing metformin versus oral contraception in PCOS, did not show significant
difference between the two therapies in reducing fasting glucose or total cholesterol concentrations. Moreover, fasting insulin and triglycerides concentrations did not increase after oral contraception treatment. Thus, the limited evidence to date does not show adverse metabolic risk with the use of oral contraception. We also did not find unfavourable metabolic effects when Diane 35 was used in combination with insulin sensitizers, there was a trend to lower basal and postload insulinaemia and even a rise in HDL-cholesterol in the metformin group. No adverse effects were observed during the whole treatment period.

CONCLUSIONS

In conclusion, the present data suggest that a 3-month treatment with metformin and rosiglitazone, added to OHC (a standard combination of ethinyl oestradiol 35 µg plus cyproterone acetate 2 mg) in overweight insulin resistant women with PCOS resulted in decrease of the serum concentrations of NPY and atherogenic adipocytokines (leptin, resistin, and TNFα) that may have beneficial effects in the future prevention of atherosclerosis and cardiovascular diseases in this risk cohort of young women. HDL-elevation and diastolic blood pressure diminution confirm this suggestion. Having in mind that the decrease in NPY and adipocytokines was not in parallel with changes in body weight and composition in the rosiglitazone group and is associated with only slight and non significant influence on hyperinsulinaemia, resp. insulin resistance, additional direct adipose tissue and/or disease specific effects of the treatment may come into consideration that needs further elucidation.

REFERENCES


ЭФФЕКТ КРАТКОВРЕМЕННОГО ПРИМЕНЕНИЯ СТАНДОРДНЫХ ТЕРАПЕВТИЧЕСКИХ РЕЖИМОВ НА НЕЙРОПЕТИН-Y И НА ГОРМОНЫ ЖИРОВОЙ ТКАНИ У ЖЕНЩИН С ПОЛИКИСТОЗНЫМ СИНДРОМОМ (PCOS) И С ИЗБЫТОЧНОЙ МАССОЙ ТЕЛА

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РЕЗЮМЕ

Цель: Исследование ставит себе цель изучить эффект трехмесячного лечения с помощью стандартных терапевтических режимов (оральные гормональные контрацептивы), обладающих антиандрогеновой активностью (стандартная комбинация - этинил эстрadiол 35 μg + ципроферон ацетат 2 mg в комбинации с метформином - группа I и росиглитазоном - группа II на группы жировой ткани и на нейропептический нейропетин Y (NPY)) у женщин с PCOS.

Пациенты и методы: Исследование охватывает 66 инсулин-резистентных женщин с PCOS и с избыточной массой тела. Диагноз поставлен по критериям ESHRE-ASRM (рандомизация – две терапевтические группы близкие по возрасту).

Результаты: И в обеих группах после лечения отмечено снизится уровень глюкозы (P < 0.01; P = 0.001, респ.), уровень глюкозы (P < 0.01; P < 0.01, респ.), уровень тестостерона и прогестерона (P < 0.05; P < 0.001, респ.). Эти результаты параллельно снизится уровень и антропометрическим параметрам массы тела только в группе женщин, леченной метформином. Не наблюдаются снизится уровень гормональных показателей и в обеих группах за исключением снижается уровень антропометрических параметров массы тела только в группе женщин, леченной метформином. После лечения метформином HDL-холестерол повышается, а диастолическое артериальное давление снижается (P < 0.05).

Выводы: Полученные данные показывают благоприятный эффект лечебных схем на потенциальные сердечно-сосудистые факторы риска у инсулин-резистентных женщин с PCOS.