Heterogeneity in the responsiveness to long-term lifestyle intervention and predictability in obese women with polycystic ovary syndrome

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Abstract

Background: Treatment of obesity improves all features of the polycystic ovary syndrome (PCOS). There is, however, a heterogeneous response to weight loss, and predictive factors are unknown.

Objective: This follow-up study aimed to investigate obese women with PCOS treated with a long-term lifestyle program to evaluate responsiveness and predictability.

Methods: One hundred PCOS women meeting the criteria for selection were invited to participate and 65 of them agreed. Lifestyle intervention had consisted of a 1200–1400 kcal/day diet for 6 months, followed by mild calorie restriction and physical activity. The protocol, which was similar at baseline and follow-up, included anthropometry, clinical evaluation, pelvic ultrasound, and laboratory investigations. The mean follow-up period was 20.4 ± 12.5 months.

Results: After the follow-up period, women were reclassified into three groups according to the persistence (group 1, 15.4%), partial (group 2, 47.7%), or complete (group 3, 36.9%) disappearance of the categorical features of PCOS (hyperandrogenism, menses, and ovulatory dysfunctions). Duration of the follow-up and extent of weight loss were similar among the three groups, as were fasting and glucose-stimulated insulin and indices of insulin resistance. Baseline waist circumference, waist to hip ratio (WHR), and androstenedione blood levels were negatively correlated with a better outcome in the univariate analysis. However, only basal androstenedione values persisted to a highly significant extent (P < 0.001) in the multivariate analysis.

Conclusions: Responsiveness to weight loss in overweight/obese PCOS women varies considerably and more than one third of women may achieve full recovery. These findings add new perspectives to the impact of obesity on the pathophysiology of PCOS.

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Introduction

The polycystic ovary syndrome (PCOS) is a hyperandrogenic disorder associated with chronic oligo-anovulation and polycystic ovaries (1, 2). Most women with PCOS are also overweight or obese (3, 4), and are characterized by a list of metabolic derangements, including insulin resistance, impaired glucose tolerance, and a cluster of additional cardiovascular risk factors (5–8). Although obesity is not considered the triggering event in the development of PCOS, it nevertheless has an impact on its pathophysiology and on the insulin resistance state (5).

Treatment of obesity improves PCOS to varying extents. A number of interventional studies with lifestyle modification (9) demonstrate fairly uniform improvements in many key features of PCOS, even with modest weight loss (5–10%), most showing a decrease or a normalization of androgens, menses, and ovulation, as well as of metabolic abnormalities. However, a great interindividual variability in the response to weight loss has been reported, and predictive factors have not been investigated. Whether treatment of obesity should be encouraged in all obese women with PCOS is, therefore, currently unknown.

In this follow-up study, we reinvestigated a number of obese women with PCOS treated for a long time with a lifestyle intervention program and achieving stable weight loss, with the aim of evaluating the extent of changes in the phenotype, of defining an individual range of improvement in clinical, hormonal, and metabolic abnormalities, and, finally, of investigating potential predictive factors of responsiveness.
Subjects and methods

Subjects

Women with PCOS participating in this retrospective study were recruited from a larger group (n=484) including all those with excess weight (body mass index (BMI) ranging from 25.0 to 29.9 kg/m²) or obesity (BMI ≥ 30 kg/m²), consecutively attending the Division of Endocrinology of the St Orsola-Malpighi Hospital in Bologna from 2003 to 2008. At the first examination, the diagnosis of PCOS was made according to the National Institute of Health (NIH) (10) criteria, including hyper-androgenemia (total testosterone ≥ 0.7 ng/ml) and/or hirsutism (the Ferriman–Gallwey (F–G) (11) score ≥ 8), plus chronic oligo-menorrhoea and chronic anovulation, after exclusion of other causes of hyperandrogenism, such as Cushing’s syndrome, nonclassic adrenal hyperplasia, androgen-secreting tumors, drug-induced hyperandrogenism, and hyperprolactinemia, using standardized diagnostic procedures. After diagnosis had been made, all these women were invited to participate in a long-term follow-up study and were included in a specific database. Most of them were lost during the follow up for many reasons, particularly short-term treatment, poor compliance to the lifestyle program, or because they lived some distance away. For the purpose of this study, all women who met the following criteria were included: i) they did not receive any pharmacological treatment, such as antiobesity drugs and/or metformin; ii) they had followed the lifestyle intervention program for a long period of time (minimum 6 months); iii) they had achieved weight loss > 5% initial body weight in the follow-up; iv) they regularly attended the follow-up during the treatment and they were still in follow-up when re-examined; and v) they had maintained a stable weight for a minimum of 3 months.

Lifestyle intervention consisted of a hypocaloric diet (1200–1400 kcal/day, based on individual needs) for 6 months, followed by a mildly restricted dietary intake (a 500 kcal/day energy deficit with respect to theoretical energy expenditure) plus physical activity (a daily walk for 30 min, 5 times weekly) with careful reinforcement during periodical checkups at our institution.

Of the original group, 100 met the defined criteria and were asked personally or by phone to participate in the 1-day post-follow-up examination that was performed from October 2008 to April 2009. Of these 100 women, only 65 agreed to participate in the study, whereas 35 could not participate for different reasons, including residence at some distance or the impossibility to leave their work. No significant differences were found in the following parameters between patients who participated in the study versus those who could not: baseline age, body weight and BMI, and duration of the follow-up. Those who did not participate in the final follow-up examination did not perform the protocol described below.

On the day of the post-follow-up examination, the 65 women participating in the study underwent a full study protocol aimed at re-evaluating the diagnostic criteria for PCOS, always according to the NIH criteria (10). The mean follow-up period was 20.4 ± 12.5 months (range of 10–67 months). All participating women gave their informed consent to the study protocol.

Study protocol performed at baseline and at the end of the follow-up period

At their first attendance and at the time of the final re-evaluation, all 65 women with PCOS underwent the same evaluation, as described below.

Anthropometry and clinical evaluation

Body weight, waist and hip circumferences, and systolic and diastolic blood pressure and pulse rate were carefully measured, according to standardized procedures (12). The ratio between waist and hip circumference (WHR) was used as an index of body fat distribution. Hirsutism was scored as previously described (11). The number of menses that had occurred in the previous 6 months had been carefully recorded at baseline and was again recorded in the 6 months prior to the follow-up examination. Ovulation was checked in the presence of oligomenorrhea (at both baseline and at final evaluation) or normal menses (at final evaluation) by measuring progesterone levels (> 8 ng/ml) in the luteal phase (days 22–23 of the last cycle). Fertility history was also recorded, including pregnancies and any abortions, at baseline and in those with long-term follow-up.

Laboratory evaluations

In women with mild (at least one cycle every 35–40 days in the last 6 months) or moderate (at least three cycles in the last 6 months) oligomenorrhea, hormonal and metabolic evaluation was performed starting from day 2 or 3 of the last menstrual cycle in order to complete it on day 8 or 9, and randomly in women presenting with severe oligomenorrhea (less than three cycles in the last 6 months) or amenorrhea (no cycles in the last 6 months). Blood samples were drawn in all women from 0800 to 0900 h after a 12 h overnight fast. Laboratory evaluations included hormonal (testosterone, androstenedione, DHEA sulfate (DHEA-S), 17-hydroxy-progesterone (17OHP), estradiol (E2), LH, FSH, and sex-hormone-binding globulin (SHBG)) and metabolic parameters (glucose, insulin, total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides). An oral glucose tolerance test (OGTT) (75 g Curvosio, Sclavo, Cinisello Balsamo, Italy) was then performed, taking blood samples after 60, 90, and 120 min for glucose and insulin determinations. Samples for hormone measurement were immediately chilled on ice, centrifuged, and serum aliquots were collected and frozen at −80 °C until assayed.
**Ovarian morphology at ultrasound** Pelvic ultrasound (US) was performed in 59/65 women at baseline: all showing PCO morphology according to previously published criteria (2). Although not initially used as a criterion to diagnose PCOS, all women were invited to perform a second pelvic US at re-examination and 44 (70%) agreed. The procedure was performed by the same experienced doctors both at baseline and the end of the follow-up period, all in the follicular phase of a spontaneous cycle in women with mild-to-moderate oligomenorrhea or randomly in women with severe oligomenorrhea or amenorrhea.

**Assays**

Plasma glucose levels were determined by the glucose-oxidase technique immediately after blood drawing. The assays used for hormonal and biochemical measurements have been reported elsewhere (12). The free androgen index (FAI) was calculated as the ratio between total testosterone and SHBG (13). Insulin resistance and sensitivity were estimated using the homeostasis model assessment (HOMA-IR) (14), the quantitative insulin sensitivity check index (QUICKI) (15), and the insulin sensitivity index during the OGTT (ISI_composite) (16).

**Statistical analysis**

Data are shown as means ± S.D. Normal distribution and homoscedasticity of continuous variables were tested by means of the Kolmogorov–Smirnov and the Levene tests, and those that did not fulfill these tests were log-transformed before analysis. The response of glucose and insulin to the OGTT was analyzed by calculating the area under the curve (AUC) by the trapezoidal method. The data were evaluated by repeated measure two-way ANOVA for trend, and the linear contrast was applied to analyze the effect of the persistence or the partial or complete disappearance of PCOS. Univariate and multivariate logistic regressions were used to estimate the association of a list of independent variables including only those that were different (or borderline different) at baseline among the three groups described in the ‘Results’ section (including BMI, waist, WHR, hirsutism score, and androstenedione) with the status of full recovery from PCOS defined at the end of the follow-up. Statistical analyses were performed by running the SPSS/PC+ version 8 (Chicago, IL, USA) software package. Two-tailed P values < 0.05 were considered statistically significant.

**Results**

**Groups**

After the follow-up period, women were re-classified into three groups according to the persistence or the partial or complete disappearance of the diagnostic criteria used to define PCOS at baseline: i) Group 1 included 10/65 (15.4%) women with persisting PCOS (no significant changes in total testosterone (all still had values ≥ 0.7 ng/ml) or partial improvement of FAI, with values still ≥ 2, a modest improvement of hirsutism but values of the F–G score still ≥ 8, plus oligomenorrhea and anovulation); ii) Group 2 included 31/65 (47.7%) women who had achieved partial recovery but were still hirsute (the F–G score still ≥ 8 with some significant improvement of total testosterone and FAI values; menses recovered in most women, but not in all, and only in 35% of them was ovulation detected during the last menstrual cycles, whereas in the remainder the last cycle was anovulatory). Group 2 therefore included an ‘intermediate’ phenotype, including PCOS women who were still hirsute with incomplete recovery of androgens, menses, and/or ovulation, with some heterogeneity in the individual behavior; and iii) Group 3 included 24/65 (36.9%) women who had fully recovered from PCOS (with total testosterone and FAI in the normal reference range and in any case below 0.7 ng/ml and 2 respectively with a complete normalization of hirsutism (their F–G score was < 8 in all), and with normal menses and ovulation verified in the last menstrual cycle). In all women, ovulation was defined by progesterone values higher than 6 ng/ml on days 22–23 of the last cycle, or randomly in those with severe oligomenorrhea or amenorrhea.

Two women became pregnant and delivered normal babies and none aborted during the follow-up study.

**Baseline characteristics and duration of the follow-up**

Duration of the follow-up was similar in all groups (Table 1). At baseline, there were no significant differences among the groups in age, weight and BMI, hirsutism score, and number of menses in the previous 6 months, whereas waist circumference (P < 0.001) and WHR (P < 0.001) values were progressively and significantly lower from group 1 to group 3 (Group 1 > Group 2 > Group 3). Similarly, no significant differences were detected in the hormonal, metabolic, and ovarian parameters, except androstenedione levels that were significantly and progressively lower from group 1 to group 3.

Values of each parameter after the follow-up and changes after the treatment (expressed as Δ after-baseline) in each group are reported in Tables 1 and 2 respectively.

**Changes in anthropometric parameters and ovulation after weight loss**

Weight loss, often exceeding 10% of basal values, was not significantly different among the three groups (Table 2). Accordingly, values of waist circumference improved significantly and similarly in all groups (P < 0.001 for all), with a persistent significant
Table 1: Clinical, hormonal, and metabolic parameters and ovarian morphology of obese women with PCOS before and after diet-induced weight loss divided according to the outcome (Group 1: still PCOS; Group 2: partial recovery; Group 3: full recovery).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1 (n=10)</th>
<th>Group 2 (n=31)</th>
<th>Group 3 (n=24)</th>
<th>ANOVA</th>
<th>Reference valuesa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follow-up (months)</td>
<td></td>
<td></td>
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<tr>
<td>Age (years)</td>
<td>18.5 ± 14.6</td>
<td>19.9 ± 14.1</td>
<td>21.8 ± 15.6</td>
<td>NS</td>
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<tr>
<td>Weight (kg)</td>
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<tr>
<td>92.1 ± 15.1</td>
<td>80.4 ± 21.8</td>
<td>95.2 ± 13.0</td>
<td>79.9 ± 12.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>34.9 ± 5.0</td>
<td>28.6 ± 6.6a</td>
<td>36.2 ± 4.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>107.9 ± 17.9</td>
<td>94.0 ± 16.1</td>
<td>103.0 ± 11.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>115.0 ± 12.1</td>
<td>106.1 ± 13.2</td>
<td>116.0 ± 7.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.93 ± 0.09</td>
<td>0.89 ± 0.01†</td>
<td>0.89 ± 0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hirsutism score (F-G)</td>
<td>11.8 ± 7.2</td>
<td>7.0 ± 4.4‡</td>
<td>14.7 ± 7.4</td>
<td></td>
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</tr>
<tr>
<td>Menses (number of previous 6 months)</td>
<td>2.40 ± 1.71</td>
<td>4.20 ± 0.92†</td>
<td>2.48 ± 2.01</td>
<td></td>
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<tr>
<td>Metabolism</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>177 ± 38</td>
<td>161 ± 24</td>
<td>179 ± 47</td>
<td>179 ± 31</td>
<td>165 ± 33a</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>47.7 ± 11.2</td>
<td>53.7 ± 13.4†</td>
<td>47.7 ± 11.0</td>
<td>49.2 ± 12.0</td>
<td>52.1 ± 12.2a</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>109.1 ± 47.2</td>
<td>73.6 ± 22.5a</td>
<td>118.8 ± 48.1</td>
<td>108.0 ± 45.8</td>
<td>90.6 ± 45.4a</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>88.6 ± 10.5</td>
<td>90.1 ± 7.3</td>
<td>87.8 ± 11.6</td>
<td>87.0 ± 8.7</td>
<td>82.7 ± 8.5†</td>
</tr>
<tr>
<td>Fasting insulin (mU/ml)</td>
<td>17.3 ± 10.3</td>
<td>11.8 ± 8.6a</td>
<td>18.3 ± 12.0</td>
<td>15.5 ± 7.1</td>
<td>10.3 ± 7.3</td>
</tr>
<tr>
<td>Glucose_{AUC} (mg/ml per min)</td>
<td>14.575 ± 31.77</td>
<td>13.146 ± 22.64</td>
<td>14.792 ± 2781</td>
<td>13.581 ± 2306</td>
<td>13.417 ± 3064</td>
</tr>
<tr>
<td>Insulin_{AUC} (mU/ml per min)</td>
<td>12.458 ± 8274</td>
<td>6964 ± 4709*</td>
<td>13.895 ± 9779</td>
<td>1204 ± 7023</td>
<td>7567 ± 6443*</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.36 ± 2.89</td>
<td>2.53 ± 2.01†</td>
<td>4.08 ± 2.90</td>
<td>3.31 ± 1.50</td>
<td>2.03 ± 1.09*</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.32 ± 0.00</td>
<td>0.35 ± 0.01†</td>
<td>0.32 ± 0.00</td>
<td>0.33 ± 0.00</td>
<td>0.35 ± 0.00</td>
</tr>
<tr>
<td>ISI_{composite}</td>
<td>3.63 ± 2.06</td>
<td>7.38 ± 5.75†</td>
<td>3.37 ± 1.87</td>
<td>4.02 ± 2.22</td>
<td>6.56 ± 3.97†</td>
</tr>
<tr>
<td>Ovarian morphology at US</td>
<td></td>
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</tr>
<tr>
<td>Ovarian volume (mm³)</td>
<td>11.4 ± 3.7</td>
<td>8.4 ± 3.5</td>
<td>12.9 ± 4.9</td>
<td>12.3 ± 3.7</td>
<td>8.4 ± 3.2*</td>
</tr>
<tr>
<td>Follicle number</td>
<td>10.1 ± 2.4</td>
<td>8.7 ± 4.3</td>
<td>9.7 ± 3.5</td>
<td>6.5 ± 2.1</td>
<td>4.6 ± 1.9†</td>
</tr>
</tbody>
</table>

Statistics (within each group: after versus baseline): *P<0.05; †P<0.01; ‡P<0.001. NA, not available.

*Reference values have been obtained from Ref. (12).
difference (P=0.003) among the groups after the follow-up. Therefore, the greater the amount of abdominal fat at baseline the lower the probability of improving the PCOS phenotype after lifestyle management and weight loss.

As reported above, although hirsutism was improved, it did not completely recover in any woman in Group 2, whereas a complete recovery was observed in all in Group 3. In addition, ovulation during the last menstrual cycle was detected in no women in Group 1, in 35% of women in Group 2, and in all in Group 3.

**Changes in hormones and metabolic parameters after weight loss**

Blood androstenedione levels showed a significant and similar decrease in all groups (P=0.154 for trend effect; Table 2). Therefore, the differences among the three groups shown at baseline still persisted after the follow-up (Group 1 > Group 2 > Group 3; P=0.01; Table 1).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1 (n=10)</th>
<th>Group 2 (n=31)</th>
<th>Group 3 (n=24)</th>
<th>Trend effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>1.9±7.9</td>
<td>1.1±7.2</td>
<td>1.7±6.0</td>
<td>0.963</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>−12.7±7.3</td>
<td>−15.3±8.6</td>
<td>−14.1±7.01</td>
<td>0.835</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>−4.8±2.9</td>
<td>−5.8±3.1</td>
<td>−5.4±2.5</td>
<td>0.824</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>−14.1±12.2</td>
<td>−9.3±11.5</td>
<td>−9.9±6.0</td>
<td>0.390</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>−9.7±8.6</td>
<td>−10.2±12.5</td>
<td>−10.1±7.9</td>
<td>0.942</td>
</tr>
<tr>
<td>WHR</td>
<td>−0.05±0.09</td>
<td>−0.01±0.08</td>
<td>0.02±0.05</td>
<td>0.476</td>
</tr>
<tr>
<td>Hirsutism score (F–G)</td>
<td>−4.8±4.7</td>
<td>−5.5±4.7</td>
<td>−4.6±3.8</td>
<td>0.745</td>
</tr>
<tr>
<td>Menses (number of previous 6 months)</td>
<td>1.8±2.0</td>
<td>3.1±2.1</td>
<td>3.3±1.8*</td>
<td>0.092</td>
</tr>
</tbody>
</table>

Hormones

- Testosterone (ng/ml) 0.05±0.16, −0.11±0.23* −0.27±0.24† <0.001
- SHBG (nmol/l) 9.6±7.9, 3.8±9.6 16.5±43.7 0.295
- FAI 1.5±1.3, 1.0±1.4 2.1±2.9 0.239
- Androstenedione (ng/ml) −134±240, −76±120 −40±190 0.154
- DHEA-S (µg/ml) −0.09±0.54, 0.16±1.34 −0.34±0.82 0.313
- 17OHP (ng/ml) 3±197, −20±171 16±118 0.693
- LH (mU/ml) 1.5±6.0, −1.9±5.8 −4.2±6.0* 0.014
- FSH (mU/ml) 0.3±1.7, −0.4±2.3 1.6±2.2 0.099
- LH/FSH ratio 0.3±1.2, −0.4±1.7 −0.8±1.4* 0.205
- Estradiol (pg/ml) 13.5±25.4, 12.3±60.4 −14.0±56.6 0.205

Metabolism

- Total cholesterol (mg/dl) −5.8±20.2, −14.6±41.2 −15.4±35.1 0.548
- HDL cholesterol (mg/dl) 9.2±6.9, 4.8±9.9 3.5±11.2 0.163
- LDL cholesterol (mg/dl) −9.7±16.6, −18.3±34.1 −17.0±29.1 0.627
- Triglycerides (mg/dl) −26.7±34.8, −20.3±44.5 −15.7±47.2 0.504
- Fasting glucose (mg/dl) −4.6±7.9, −5.5±12.0 −6.0±11.1 0.750
- Fasting insulin (µU/ml) −7.5±10.0, −7.5±12.0 −5.3±5.8 0.502
- Glucose_{AUC} (mg/dl per min) −1477±1372, −1851±2912 −583±3935 0.349
- Insulin_{AUC} (µU/ml per min) −5871±5732, −6200±7870 −3419±5527 0.250
- HOMA-IR −1.8±2.6, −1.8±3.0 −1.3±1.2 0.436
- QUICKI 0.03±0.04, 0.03±0.04 0.03±0.03 0.650
- ISI_{composite} 5.1±4.9, 2.3±2.6 2.3±2.5 0.079

Ovarian morphology at US

- Ovarian volume (ml³) −1.9±3.8, −5.4±4.8 −6.5±4.5 0.119
- Follicle number −0.7±4.6, −4.4±2.9 −5.8±4.8 0.050

No major change occurred in DHEA-S, 17OHP, and E2 values in any group, although a modest but significant increase was observed in 17OHP values in Group 3. LH concentrations significantly decreased in Group 3 only, without any change in the other groups (Table 2). By contrast, FSH values and LH/FSH ratio did not significantly vary in any group. To summarize, although it improved, most women in Group 2 still had some degree of androgen excess, whereas all those in Group 3 had androgens in the normal range.

Total cholesterol did not change in Group 1, whereas it significantly decreased in the other groups; HDL cholesterol significantly increased in all groups and triglycerides tended to decrease in all groups. Fasting glucose decreased significantly in Groups 2 and 3 but not in Group 1, and glucose_{AUC} decreased only in Group 2. After the treatment, fasting glucose values in Groups 2 and 3 were significantly lower than in Group 1, whereas no difference was present between the groups in glucose_{AUC}. At variance, fasting insulin and
inclusion, the duration of the follow-up, and the rigorous definition of the three groups at follow-up.

The possibility that sustained weight loss may lead to complete recovery from the PCOS phenotype is, however, not completely new. This has already been described in another study (17), which found that considerable weight loss achieved after bariatric surgery in massively obese women with PCOS led to almost complete resolution of the PCOS phenotype. A relatively new finding is that we found potentially reliable and predictive markers of the response to weight loss treatment in overweight and obese women with PCOS. At baseline, the only differences between the three groups investigated in this study were the prevalence of abdominal obesity and particularly androstenedione blood levels, which were inversely related to patient responsiveness to the treatment. Interestingly, an inverse relationship between androstenedione values at baseline and an improvement in menses was also described in a previous study in women with PCOS following a 6-month metformin treatment (18).

Circulating blood concentrations of androstenedione may be high or relatively normal in women with PCOS, often independent of testosterone values, which may reflect a different origin, production rate, or metabolic processes. High androstenedione concentrations in women who still presented PCOS after weight loss may imply an excess production rate of androgens from both the ovarian theca cells and/or the adrenals (19), which may be relatively independent of the presence of excess weight or obesity and therefore may predict the classic hyperandrogenic PCOS status (20). It may also refer to a subset of PCOS women with a primary ovarian functional hyperandrogenism, in whom the coexistence

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td>Basal BMI</td>
<td>0.886</td>
<td>0.338</td>
</tr>
<tr>
<td>Basal waist circumference</td>
<td>0.949 (0.901–0.999)</td>
<td>0.033</td>
</tr>
<tr>
<td>Basal WHR</td>
<td>0.001 (0.000–0.2947)</td>
<td>0.011</td>
</tr>
<tr>
<td>Basal hirsutism (F–G score)</td>
<td>0.834</td>
<td>0.653</td>
</tr>
<tr>
<td>Basal androstenedione</td>
<td>0.993 (0.987–0.998)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Changes in ovarian morphology after weight loss**

At follow-up, ovarian US was performed in 60% of women in Group 1, in 64% of women in Group 2, and in 67% of women in Group 3. Ovarian volume was significantly reduced only in Group 3, and the number of small follicles decreased in Groups 2 and 3, but not in Group 1 (trend analysis: Z = 0.119 and 0.050 respectively) (Table 2). All women in Group 1 still had PCO, whereas all those in Group 3 had normal ovarian morphology; at variance, in Group 2, ten women still had PCO, whereas 12 had normal ovarian morphology.

**Factors predicting outcome**

In order to investigate the factors predicting the responsiveness to treatment, univariate and multivariate logistic regression analysis was also applied in the whole cohort. In the univariate analysis, baseline higher waist circumference (P = 0.033), WHR (P = 0.011), and androstenedione (P < 0.001) values were significantly and negatively associated with a better outcome. However, only basal androstenedione values were still significantly associated (coefficient: 0.9923 [confidence interval (CI): 0.987–0.998], P < 0.001 in the multivariate logistic regression analysis (Table 3).

**Discussion**

Treatment of obesity with lifestyle intervention in women with PCOS is commonly associated with a series of benefits in clinical, hormonal, metabolic, and reproductive functions, although a clear identification of potential predictors may be difficult, due to the lack of long-term controlled trials. This study shows that individual responsiveness to weight loss by lifestyle intervention may vary widely among women with PCOS. Here, we report that 48% of women had partial but significant recovery and, most importantly, that 37% had full recovery from PCOS, as defined by both the NIH (10) and Rotterdam criteria (2). Therefore, whereas the diagnosis of PCOS could not be sustained in more than a third of women after treatment, a smaller group still presented PCOS by definition after treatment, in spite of a similar degree of weight loss, and a modest but significant improvement of androgens (but not total testosterone), hirsutism, and menses, but not ovulation.
of both ovarian and adrenal overproduction of androstenedione has been demonstrated (12). However, this finding may also disclose a specific subset of women with PCOS characterized by decreased aromatase activity in the ovarian tissues, as suggested by previous studies (21) and recent genetic findings on the activity of this enzyme in PCOS (22).

As reported above, women who still had PCOS after treatment were characterized by both high androstenedione levels and marked abdominal adiposity, in agreement with previous findings (23). It is well known that androgen excess may expand visceral adipose tissue, which may play an important role in the modulation of preadipocyte proliferation and/or differentiation, as well as lipid synthesis and/or lipolysis in mature adipocytes (24). It could be suggested that in these women obesity added only marginal negative effects to the classic hyperandrogenic PCOS phenotype and that enlarged abdominal (visceral) fat may represent a genuine effect of long-term androgen action. By contrast, obesity may have a primary important impact on androgen generation in those who recover after weight loss, which in turn implies a potential causative role of obesity per se on the pathophysiological development of the PCOS. This is further emphasized by the unexpected finding that the metabolic improvement in all the groups was comparable and partially independent of changes in androgens, menses, and ovulatory dysfunction. This implies that amelioration of the dysmetabolic milieu was not sufficient to improve hyperandrogenism and reproductive abnormalities in all PCOS women. In some ways, our data agree with another study (25) reporting that PCOS women who resumed normal menses and ovulation after diet-induced weight loss did not differ in the changes in metabolic parameters and indices of insulin resistance with respect to those who did not achieve this goal. Insulin sensitizers, which also reduce hyperinsulinemia and insulin resistance, have been shown to favor normal menses and ovulation in many (30–60%) but not all obese PCOS patients (26). There is evidence that insulin drives theca-cell androgen production and possibly theca-cell hypertrophy (27–29). Accordingly, most studies have documented that both androgen and insulin blood levels may decline concurrently following weight loss (9); however, neither response appears to forecast subsequent ovulation in women with PCOS. The change in androgenic milieu is thus not likely to be the sole factor responsible for reproductive improvement in PCOS, and our findings imply the need to distinguish the true impact of obesity per se on the accompanying metabolic derangements from the effects on androgens and ovarian dysfunction (30).

In conclusion, the present study shows that overweight and obese women with PCOS may respond disparately to weight loss regimens, ranging from the persistence of the PCOS phenotype to partial or even complete recovery from it, and that these effects are totally independent of the degree of weight loss, treatment duration, and improvement in insulin resistance. Pretreatment extent of the abdominal (visceral) adiposity and, particularly, androstenedione blood levels appear to be the most important predictive factors of outcomes. Our data therefore add a new perspective to the impact of obesity on the pathophysiology of PCOS, and suggest that obesity may play a causative role in this disorder in susceptible individuals, through still undefined mechanisms.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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