

## CLINICAL STUDY

# Metformin improves polycystic ovary syndrome symptoms irrespective of pre-treatment insulin resistance

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## Abstract

**Objective:** Insulin resistance (IR) and obesity are common features of the polycystic ovary syndrome (PCOS). Insulin-sensitizing agents have been shown to improve both reproductive and metabolic aspects of PCOS, but it remains unclear whether it is also beneficial in lean patients without pre-treatment IR. The aim of this study was to determine the influence of metformin on the clinical and biochemical parameters of PCOS irrespective of the presence of basal obesity and IR.

**Design:** The effect of 6 months of metformin treatment was prospectively assessed in 188 PCOS patients, divided into three groups according to body mass index (BMI; lean: BMI < 25 kg/m<sup>2</sup>, overweight: BMI 25–29 kg/m<sup>2</sup>, and obese: BMI ≥ 30 kg/m<sup>2</sup>). Outcome parameters, which were also assessed in 102 healthy controls, included body weight, homeostasis model assessment for IR (HOMA-IR), fasting glucose and insulin levels, area under the curve of insulin response (AUCI), hyperandrogenism, and menstrual irregularities.

**Results:** In comparison with the respective BMI-appropriate control groups, only obese but not lean and overweight PCOS patients showed differences in fasting insulin and HOMA-IR. Metformin therapy significantly improved all outcome parameters except fasting glucose levels. Subgroup analyses revealed that in the group of lean PCOS patients without pre-treatment IR, metformin significantly improved HOMA-IR ( $1.7 \pm 1.0$  vs  $1.1 \pm 0.7$   $\mu\text{mol/l} \times \text{mmol/l}^2$ ) and fasting insulin levels ( $7.7 \pm 4.2$  vs  $5.4 \pm 3.9$  mU/l), in addition to testosterone levels ( $2.6 \pm 0.9$  vs  $1.8 \pm 0.7$  nmol/l), anovulation rate (2.3 vs 59.5%), and acne (31.8 vs 11.6%; all  $P < 0.017$ ). In the overweight and obese PCOS groups, metformin also showed the expected beneficial effects.

**Conclusion:** Metformin improves parameters of IR, hyperandrogenemia, anovulation, and acne in PCOS irrespective of pre-treatment IR or obesity.

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## Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting 6% of women of reproductive age (1–4). The current Rotterdam consensus defines PCOS as the presence of at least two out of the three criteria chronic anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovaries (PCO), when other disorders of the pituitary, adrenals, or ovary have been excluded (5). Its clinical manifestations are oligomenorrhea or amenorrhea paired with infertility, hirsutism, acne, and alopecia. Many PCOS-affected women also suffer from insulin resistance (IR) (6–8) and obesity (9). Given the well-documented association between PCOS and IR, insulin-sensitizing agents have been used in the therapy of PCOS. To date, over 50 intervention studies have demonstrated a positive effect of metformin on both reproductive and metabolic aspects of PCOS (10–13). Nevertheless, the

mechanisms underlying the beneficial effects of metformin in the treatment of PCOS remain incompletely understood. Improvement of IR by metformin may not only result from the well-known reduction of hepatic glucose production and increase of peripheral glucose utilization but also from a direct effect on ovarian steroidogenesis, as demonstrated by *in vitro* studies (14–16).

Since not all PCOS patients are obese or insulin resistant, it is not clear whether PCOS patients without IR also benefit from a therapy with insulin sensitizers. Several smaller studies have suggested positive effects of metformin irrespective of pre-treatment weight and/or presence of IR (Table 1). For example, Goldenberg *et al.* (31) demonstrated an equal improvement of menstrual irregularities both in insulin-resistant and insulin-sensitive PCOS patients. To further address this important clinical question, the aim of this study was to determine whether metformin treatment improves

**Table 1** Published studies on metformin treatment in lean polycystic ovary syndrome (PCOS) women.

| Reference                     | Characterisation   | Duration of treatment dose of metformin  | Outcome parameters  |
|-------------------------------|--|--|---|
| Maciel <i>et al.</i> (29)     | $n=29$ lean versus obese PCOS patients   | 6 months of 500 mg TID   | Significant reduction of parameters of insulin resistance (fasting insulin, AUCI), androgen levels (total and free testosterone, androstenedione) in lean PCOS patients. In obese PCOS patients only improvement of free testosterone levels, while all other parameters did not change                               |
| Kumari <i>et al.</i> (30)     | $n=17$ lean versus 17 obese PCOS patients BMI 24 vs 36 kg/m <sup>2</sup> fasting insulin 12 vs 21 mU/l | 12 weeks of 500 mg TID   | Greater improvement of ovulation rate (88 vs 29%) and pregnancy rate (65 vs 18%) in the lean PCOS group   |
| Goldenberg <i>et al.</i> (31) | $n=32$ top versus 35 bottom quintile HOMA-IR PCOS patients   | 12 months of 850 mg TID in addition: diet with 6300–8400 J/day, 26% protein, 44% carbohydrates | Significant reduction of body weight, parameters of insulin resistance (fasting insulin, HOMA-IR, insulin secretion), and ovulation rate in both groups with smaller but significant reduction in the bottom insulin-resistant group concerning insulin resistance and without group effect concerning ovulation rate |
| Yilmaz <i>et al.</i> (32)     | $n=20$ lean versus 20 obese each in the metformin and rosiglitazone group                              | 12 weeks of metformin: 850 mg BID rosiglitazone: 4 mg/day                                      | Significant reduction in parameters of insulin resistance (HOMA-IR, AUCI, fasting insulin, C-peptide) as well as improvement of ovulation rate in all four groups. No effect of metformin on androgens in lean or obese PCOS patients (testosterone, androstenedione, DHEAS)  |
| Marcondes <i>et al.</i> (33)  | $n=12$ normal weight PCOS patients BMI $21.5 \pm 1.65$ kg/m <sup>2</sup>                               | 850 mg TID for 4 months  | No effect on BMI or hirsutism score, significant improvement of ovulation rate, testosterone levels, and insulin resistance parameters (fasting insulin, AUCI, HOMA-IR) as well as LH decrease and FSH increase   |
| Orio <i>et al.</i> (34)       | $n=50$ normal weight PCOS patients BMI $25.6 \pm 4.2$ kg/m <sup>2</sup>                                | 850 mg BID for 6 months  | Improvement of free androgen index, fasting insulin, HOMA-IR, AUCI, and white blood cell count. Normalisation of menstrual cycles by 91.1%. No significant change in hirsutism score, testosterone, and androstenedione levels  |

clinical and biochemical parameters of PCOS irrespective of the presence of obesity and IR prior to treatment.

## Materials and methods

### Study design and outcome parameters

PCOS patients were evaluated at baseline and following treatment with metformin in a weight-adapted dose for 6 months (body weight <60 kg: 500 mg metformin twice a day (BID), 60–100 kg: 850 mg BID, and  $\geq 100$  kg or  $\text{BMI} \geq 30 \text{ kg/m}^2$ : 1000 mg BID). Outcome parameters included ovulation rate, presence of acne, alopecia, testosterone levels, and hirsutism, in addition to changes in weight as well as in parameters of IR.

### Recruitment of participants, inclusion, and exclusion criteria

PCOS patients ( $n=188$ ) were recruited from the outpatient clinics of the Division of Endocrinology, Department of Medicine, and the Department of Gynecology at the University of Duisburg-Essen. Some patients were also attracted by the clinic's PCOS homepage ([www.pcosyndrom.de](http://www.pcosyndrom.de)). PCOS was defined according to the 2003 Rotterdam criteria (5). Age-matched healthy controls ( $n=102$ ) were recruited from a mandatory screening program for employees instituted at the University of Duisburg-Essen. Exclusion criteria in controls included any known medical condition or disease. In addition, care was taken to exclude all NIH criteria in controls. Specifically, control women were required to have a normal menstrual cycle (i.e. shorter than 35 days), testosterone levels lower than 2.0 nmol/l and/or dehydroepiandrosterone sulfate (DHEAS) lower than 270  $\mu\text{g/dl}$  and/or androstenedione lower than 3.3 ng/ml, and no hirsutism, acne, or alopecia. The study protocol was approved by the Ethics Committee of the University of Duisburg-Essen. All participants gave written informed consent before entering the study. All study participants were not taking any medication at least 3 months before entering the study.

### Data collection

All women underwent a personal interview with a physician, including an evaluation of demographic characteristics, previous medication, physical examination, and blood sampling. Based on the criteria derived from the 2003 Rotterdam consensus conference (5), diagnosis of PCOS was established when two of the following criteria were present: oligomenorrhea (cycles lasting longer than 35 days) or amenorrhea (less than two menstrual cycles in the past 6 months), clinical or biochemical signs of hyperandrogenism (hirsutism) with a Ferriman–Gallwey score (FG) of more than 7 (18) or obvious acne or alopecia (19) or an elevated total

testosterone (normal range <2.0 nmol/l) and/or DHEAS (normal range 45–270  $\mu\text{g/dl}$ ) and/or androstenedione (normal range 0.2–3.3 ng/ml) and PCO (at least one ovary with at least 12 follicles of a diameter of 2–9 mm or a volume >10 ml), and other pituitary, adrenal, or ovarian diseases could be excluded. Therefore, laboratory analysis of luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, prolactin, cortisol, adrenocorticotrophin (ACTH), thyrotrophin (TSH), insulin-like growth factor, androstenedione, and dehydroepiandrosterone sulfate was completed. In addition, an ACTH test with measurement of 17-hydroxyprogesterone was accomplished. When the stimulated value (after 60 min) was >10 ng/ml, a genetic analysis (21-hydroxylase deficiency) was added.

Hirsutism was routinely evaluated independently by two physicians using the common modified FG. This method to assess hirsutism requires the visual scoring of the extent of terminal hairs in nine body areas, named 1) upper lip, 2) chin, 3) chest, 4) upper abdomen, 5) lower abdomen, 6) upper back, 7) lower back, 8) thighs, and 9) upper arms. The lower arms and lower legs were not included in the hair assessment. Each area was scored from 0 to 4, resulting in a possible maximum score of 36. Hirsutism was diagnosed when a score above 7 was evaluated. FG scores never differed by more than 2, and whenever ratings were not identical, the patient was re-evaluated by a third physician and the median value was then used.

Body mass index (BMI) was calculated as  $\text{weight}/(\text{height})^2$  in ( $\text{kg}/\text{m}^2$ ). Parameters of IR and  $\beta$ -cell function were evaluated using a 3-h oral glucose tolerance test. After an overnight fast of 12 h patients ingested 75 g glucose and had their glucose and insulin levels determined at baseline and at 30, 60, 90, 120, and 180 min. IR was defined by the homeostasis model assessment (HOMA) model (20, 21) and hyperinsulinemia by calculating the area under the insulin response curve (AUCI) (22). IR in control women was evaluated by fasting glucose, fasting insulin, and the HOMA model.

### Biochemical analyses

Automated chemiluminescence immunoassay systems were used for the determination of LH, FSH, ACTH, TSH, testosterone, estradiol, cortisol, free thyroxine, blood glucose (ADVIA Centaur, Bayer Vital, Fernwald, Germany), dehydroepiandrosterone sulfate, androstenedione, sex hormone-binding globulin, insulin (IMMULITE 2000, DPC Biermann, Bad Nauheim, Germany), and insulin-like growth factor (Nichols Advantage, Nichols Institute Diagnostics, Bad Vilbel, Germany). 17-Hydroxyprogesterone was measured by the Biosource 17- $\alpha$ -OH-RIA-CT kit (Biosource International, Camarillo, CA, USA; analytical sensitivity 0.02 ng/ml) provided by IBL Hamburg (IBL, Gesellschaft für Immunchemie und Immunbiologie, Hamburg, Germany). Intraassay variation was <5% and inter-assay variation was <8% for all parameters.

## Statistical analyses

PCOS patients and controls were each divided into three subgroups according to BMI (lean: BMI <25 kg/m<sup>2</sup>, overweight: BMI 25–29 kg/m<sup>2</sup>, obese: BMI ≥ 30 kg/m<sup>2</sup>). Statistical analyses were completed in three consecutive steps.

- 1) The goal of the first analysis was to establish differences between untreated PCOS and healthy controls, which was particularly relevant for the lean PCOS group. Therefore, in a first step, pre-treatment values of PCOS patients were compared with controls using two-factorial ANOVA to assess the effects of diagnosis and BMI-group. Subsequently, within each weight group, means of patients were compared with controls using *post hoc* comparisons of means. In doing so,  $\alpha$  levels were corrected for inflation of  $\alpha$  error by multiple testing by applying the conservative Bonferroni method, which divides the  $\alpha$  level of 0.05 by the number of tests for each variable (in this case given three groups:  $0.05/3=0.017$ ). Hence, in evaluating the results of the *post hoc* comparisons, only comparisons with a *P* level that was smaller than 0.017 were considered statistically significant.
- 2) The aim of the second set of analyses was to establish treatment effects of metformin based on pre-treatment weight and IR in PCOS. Therefore, the effects of metformin in PCOS were addressed using two-factorial ANOVA with the factors time (pre-treatment, post-treatment) and BMI group, again followed by  $\alpha$ -corrected *post hoc* comparisons of means within each weight group. For repeated assessment of frequency distributions given dichotomous variables (e.g. acne, alopecia, menstrual disturbances), no statistical method that is equivalent to the ANOVA exists; therefore, McNemar tests for repeated measurements of dichotomous variables in a sample were applied to address the effects of treatment. The *P* levels were again corrected here using the Bonferroni method described above.
- 3) Finally, post-treatment values in PCOS were compared with controls to establish whether or not metformin achieved a complete normalization. This was again accomplished with two-factorial ANOVA with the factors diagnosis and weight group, followed by *post hoc* comparisons of patient versus control means with  $\alpha$ -correction as described above. For the dichotomous variables,  $\chi^2$  tests were applied. Data are presented as mean  $\pm$  s.d. or as number and percent affected.

## Results

### Baseline parameters

Of the total of *N*=188 PCOS patients, *N*=44 were in the lean group (mean BMI:  $22.0 \pm 1.6$ ), *N*=42 were in the

overweight group (mean BMI:  $26.8 \pm 1.5$ ), and *N*=102 were in the obese group (mean BMI:  $38.1 \pm 6.0$ ) (Table 2). Out of the total of *N*=102 healthy women, *N*=66 were in the lean group (mean BMI:  $21.5 \pm 1.6$ ), *N*=24 in the overweight group (mean BMI:  $26.7 \pm 1.4$ ), and *N*=12 were in the obese group (mean BMI:  $34.4 \pm 5.1$ ). In comparison with the respective BMI-appropriate control group, lean and overweight PCOS patients showed no differences in fasting insulin, HOMA-IR, and fasting glucose levels. On the other hand, obese PCOS patients had similar fasting glucose but significant higher fasting insulin and HOMA-IR levels compared with obese controls (Table 2). As expected, when compared with controls, all PCOS patient groups demonstrated significantly higher testosterone levels and FG scores.

### Effects of metformin treatment

Overall, 6 months of metformin therapy significantly improved all outcome parameters, indicated by significant ANOVA time effects (see Table 3), with the exception of fasting glucose levels. Interestingly, subgroup analyses revealed that in the group of lean PCOS patients without pre-treatment IR (see above), metformin significantly improved HOMA-IR and fasting insulin levels, in addition to testosterone levels, menstrual irregularities, and acne (all *P*<0.017, see Table 3). In the overweight and obese PCOS groups, metformin also showed the expected beneficial effects, including significant improvements in weight, HOMA-IR, AUCI, fasting insulin as well as testosterone (all *P*<0.017) (Tables 3 and 4).

A comparison of treated PCOS patients with the appropriate BMI control groups revealed that treatment normalized parameters of IR in all PCOS BMI groups, and even improved fasting insulin levels beyond normal values in lean PCOS (Table 4). On the other hand, hyperandrogenism and/or hirsutism, as well as the prevalence of menstrual cycle irregularities, acne, and alopecia remained significantly more pronounced in patients even following treatment (Table 4).

## Discussion

PCOS is one of the most common endocrine disorders of women of reproductive age. Since the role of IR in the pathogenesis of PCOS has been established, many interventional studies have demonstrated a positive effect of insulin-sensitizing agents in the treatment of PCOS. For example, Hahn *et al.* (11) pointed out a positive effect of metformin on hyperandrogenism, chronic anovulation, and IR in a German PCOS cohort. Palomba *et al.* (13, 23) reported a significant improvement of ovulation, pregnancy and ovulation rate in comparison with clomiphene and ovarian drilling. Many metformin intervention studies from Italy, Finland, UK, and the United States demonstrated a positive effect of metformin on body weight and/or BMI (17, 24–27). An improvement of body weight, low density

**Table 2** Baseline parameters of insulin resistance and hyperandrogenism in polycystic ovary syndrome (PCOS) patients (n=188) and controls (n=102).

| Variable                                | BMI < 25 kg/m <sup>2</sup> |    |                      | BMI 25–29.9 kg/m <sup>2</sup> |               |                            | BMI ≥ 30 kg/m <sup>2</sup> |    |                       | Two-factorial ANOVA <sup>a</sup> |   |
|---|----------------------------|----|----------------------|-------------------------------|---------------|----------------------------|----------------------------|----|-----------------------|----------------------------------|---|
|   | Lean PCOS (n=44)           |    | Lean controls (n=66) | Overweight PCOS (n=42)        |               | Overweight controls (n=24) | Obese PCOS (n=102)         |    | Obese controls (n=12) |                                  |   |
|   | Mean                       | SD | Mean                 | SD                            | Mean          | SD                         | Mean                       | SD | Mean                  |                                  | SD  |
| Fasting glucose (mg/dl)                 | 84.6 ± 8.6                 |    | 83.8 ± 6.9           |                               | 85.8 ± 7.6    |                            | 88.8 ± 5.8                 |    | 99.1 ± 48.0           |                                  | n.s.  |
| Fasting insulin (mU/l)                  | 7.7 ± 4.2                  |    | 7.44 ± 3.3           |                               | 14.2 ± 7.6    |                            | 10.6 ± 6.9                 |    | 23.4 ± 13.7*          |                                  | Diagnosis F=17.6, P<0.001<br>BMI: F=13.1, P<0.001   |
| HOMA-IR (μmol/l × mmol/l <sup>2</sup> ) | 1.7 ± 1.0                  |    | 1.4 ± 0.7            |                               | 3.0 ± 1.7     |                            | 2.1 ± 1.6                  |    | 5.5 ± 3.4*            |                                  | Diag × BMI: F=5.3, P<0.01<br>Diagnosis F=18.3, P<0.001<br>BMI: F=17.7, P<0.001<br>Diag × BMI: F=5.8, P<0.01 |
| AUCI (mU × h/l)                         | 142.1 ± 89.1               |    | (Not measured)       |                               | 265.2 ± 194.1 |                            | (Not measured)             |    | 389.6 ± 215.9         |                                  | Diagnosis F=95.2, P<0.01<br>BMI: F=75.5, P<0.001<br>Diag × BMI: F=4.7, P<0.01                               |
| Testosterone (nmol/l)                   | 2.6 ± 0.9*                 |    | 1.35 ± 0.4           |                               | 2.5 ± 0.9*    |                            | 1.4 ± 0.3                  |    | 2.7 ± 0.9*            |                                  |   |
| Hirsutism score (FG)                    | 6.7 ± 4.3*                 |    | 1.6 ± 2.0            |                               | 9.2 ± 6.8*    |                            | 2.7 ± 3.3                  |    | 10.9 ± 6.7*           |                                  |   |

Values are shown as mean ± s.d. or percent affected. BMI, body mass index; AUCI, area under the curve of insulin response; HOMA-IR, homeostasis model assessment for insulin resistance; FG, Ferriman-Gallway score; n.s., non-significant (no significant ANOVA effects). \**z*-corrected, post hoc comparisons of means comparing PCOS and controls within each BMI group (all P<0.017).  
<sup>a</sup>ANOVA with the two factors diagnosis (PCOS, controls) and BMI group. Shown are F and P values for significant main effects.

lipoprotein (LDL)-cholesterol and systolic blood pressure has been shown by an analysis of 13 randomized interventional studies summarized in a Cochrane review (12). In accordance with these studies, our results show that PCOS patients benefit from metformin treatment with regard to hyperandrogenism, menstrual disturbances, acne, and IR. Similar to the significant BMI reduction reported in a British (24) and an American study (28) metformin caused a significant weight reduction in our PCOS cohort of overweight and obese patients.

Since not all PCOS patients are obese or insulin resistant, it has thus far remained unclear whether PCOS patients without evidence of IR would also benefit from a therapy with insulin sensitizers. Indeed, our sample of lean PCOS patients did not differ from lean controls in BMI or levels of fasting insulin and HOMA-IR. Interestingly, in response to metformin treatment, this group of lean PCOS patients clearly showed significant improvements not only in androgen levels, acne, and menstrual irregularities, but also in HOMA-IR, AUC-I, and fasting insulin levels. Hence, even in patients who do not show basal elevation in parameters of IR and present with normal BMI, there is a clear benefit of metformin. This finding is in accordance with several smaller previous studies, which however did not include healthy controls (Table 1). For example, in a Brazilian study with 29 PCOS patients hyperandrogenemia was reduced by treatment with 500 mg three times daily (TID) metformin (29). In addition, lean PCOS patients benefitted from metformin through a reduction of AUCI and fasting insulin. Maciel *et al.* (29) concluded that lean PCOS patients respond even better to treatment with metformin than obese patients. Kumari *et al.* (30) demonstrated in a study including 17 lean and 17 obese PCOS patients treated with 1500 mg metformin daily that ovulation and pregnancy rates were higher in the lean PCOS group. These studies are in agreement with our study data, showing a positive effect of metformin therapy on endocrine and metabolic variables even in lean and insulin-sensitive women. Harborne *et al.* (17) demonstrated a dose-dependent effect of metformin, the divergent effect concerning the outcome of our overweight and obese patients may result from the higher dose applied to our patients. Goldenberg *et al.* (31) divided their PCOS population into quintiles according to HOMA-IR as a parameter for IR and compared the bottom and top quintile after 1 year of intervention with metformin and diet. They demonstrated an improvement of menstrual cyclicity in the bottom quintile that did not differ from that of the top quintile. Our study confirms the independence from IR of metformin action on menstrual cyclicity in PCOS patients.

Several *in vitro* studies have revealed some aspects of the mechanisms presumably underlying metformin action. Using cultured ovarian cells from women undergoing bilateral salpingoophorectomy, an inhibition of progesterone and estradiol in granulosa cells and androstenedione in theca cells by metformin has been

**Table 3** Metformin treatment effects in polycystic ovary syndrome (PCOS).

| Variable                              | Lean PCOS<br>BMI < 25 kg/m <sup>2</sup> (n=44) |                                | Overweight PCOS<br>BMI 25–29.9 kg/m <sup>2</sup> (n=42) |                                | Obese PCOS<br>BMI ≥ 30 kg/m <sup>2</sup> (n=102) |                                | Two-factorial ANOVA or<br>McNemar Test <sup>a</sup>  |
|---------------------------------------|--|--------------------------------|---|--------------------------------|--|--------------------------------|--|
|                                       | Baseline                                       | After 6 months of<br>metformin | Baseline  | After 6 months of<br>metformin | Baseline   | After 6 months of<br>metformin |  |
| Body weight (kg)                      | 62.9±8.3                                       | 61.6±7.8                       | 74.2±6.6  | 70.9±8.4*                      | 105.7±18.7                                       | 99.2±19.0*                     | Time <i>F</i> =19.1, <i>P</i> <0.001<br>BMI <i>F</i> =155.3, <i>P</i> <0.001<br>Time×BMI <i>F</i> =4.1, <i>P</i> <0.05 |
| BMI (kg/m <sup>2</sup> )              | 22.0±1.6                                       | 21.6±1.6                       | 26.8±1.5  | 25.8±2.3*                      | 38.1±6.0   | 36.7±8.9                       | Time <i>F</i> =4.5, <i>P</i> <0.05<br>BMI <i>F</i> =178.4, <i>P</i> <0.001<br>BMI <i>F</i> =3.3, <i>P</i> <0.05        |
| Fasting glucose (mg/dl)               | 84.6±8.6                                       | 82.6±8.2                       | 85.8±7.6  | 83.4±7.4                       | 99.1±48.0  | 90.0±18.0                      | Time <i>F</i> =34.6, <i>P</i> <0.001<br>BMI <i>F</i> =33.8, <i>P</i> <0.001<br>Time <i>F</i> =38.3, <i>P</i> <0.001    |
| Fasting insulin (mU/l)                | 7.7±4.2  | 5.4±3.9*                       | 14.2±7.6  | 9.8±5.2*                       | 23.4±13.7  | 16.9±9.8*                      | BMI <i>F</i> =42.3, <i>P</i> <0.001<br>Time×BMI <i>F</i> =4.0, <i>P</i> <0.05  |
| HOMA-IR (μmol/l×mmol/l <sup>2</sup> ) | 1.7±1.0  | 1.1±0.7*                       | 3.0±1.7   | 2.0±1.2*                       | 5.5±3.4  | 3.7±2.2*                       | Time <i>F</i> =17.3, <i>P</i> <0.001<br>BMI <i>F</i> =28.9, <i>P</i> <0.001<br>Time×BMI <i>F</i> =4.1, <i>P</i> <0.05  |
| AUCI (mU×h/l)                         | 142.1±89.1                                     | 136.3±69.4                     | 265.2±194.1   | 197.2±106.5*                   | 389.6±215.9                                      | 293.5±153.7*                   | Time <i>F</i> =78.2, <i>P</i> <0.001<br>Time=4.9, <i>P</i> <0.05<br>BMI <i>F</i> =7.7, <i>P</i> <0.001                 |
| Testosterone (nmol/l)                 | 2.6±0.9  | 1.8±0.7*                       | 2.5±0.9   | 1.9±0.6*                       | 2.7±0.9  | 2.1±0.8*                       | McNemar time <i>P</i> <0.001   |
| Hirsutism score (FG)                  | 6.7±4.3  | 6.2±4.6                        | 9.2±6.8   | 8.3±5.4                        | 10.9±6.7   | 10.6±6.8                       | Time=4.9, <i>P</i> <0.05<br>BMI <i>F</i> =7.7, <i>P</i> <0.001   |
| Normal menstrual cycles (%)           | 2.3  | 59.5*                          | 7.1   | 46.2*                          | 3.9  | 50.0*                          | McNemar time <i>P</i> <0.001   |
| Acne (%)                              | 31.8   | 11.6*                          | 53.7  | 22.0*                          | 42.2   | 27.8*                          | McNemar time <i>P</i> <0.001   |
| Alopecia (%)                          | 18.2   | 18.2                           | 22.5  | 24.4                           | 35.3   | 25.8                           | McNemar n.s.   |

Values are shown as mean ± s.d. or percent affected. BMI, body mass index; AUCI, area under the curve of insulin response; HOMA-IR, homeostasis model assessment for insulin resistance; FG, Ferriman–Gallwey score; n.s., no significant effects. \**α*-corrected, *post hoc* comparisons of means comparing PCOS pre-treatment versus 6-month post-treatment within each BMI group (all *P*<0.017).

<sup>a</sup>ANOVA with the two factors time (pre-treatment, 6-month post-treatment) and BMI group. Shown are *F* and *P* values for significant main effects as well as for significant interactions between treatment effects and BMI group (Time×BMI). For dichotomous variables, the McNemar test was applied to address treatment effects (Time).

**Table 4** Comparison of treated polycystic ovary syndrome (PCOS) patients and controls.

| Variable                              | Lean groups<br>BMI <25 kg/m <sup>2</sup> |                    | Overweight groups<br>BMI 25–29.9 kg/m <sup>2</sup> |                    | Obese groups<br>BMI ≥30 kg/m <sup>2</sup> |                    | Two-factorial ANOVA or<br>χ <sup>2</sup> test <sup>a</sup> |
|---------------------------------------|--|--------------------|--|--------------------|---|--------------------|--|
|                                       | Treated PCOS<br>(n=44)                   | Controls<br>(n=66) | Treated PCOS<br>(n=42)                             | Controls<br>(n=24) | Treated PCOS<br>(n=102)                   | Controls<br>(n=12) |  |
| Fasting glucose (mg/dl)               | 82.6±8.2                                 | 83.8±6.9           | 83.4±7.4   | 88.8±5.8           | 90.0±18.0                                 | 93.1±5.8           | BMI F=6.9, P<0.001   |
| Fasting insulin (mU/l)                | 5.4±3.9*                                 | 7.44±3.3           | 9.8±5.2  | 10.6±6.9           | 16.9±9.8                                  | 11.8±9.0           | BMI F=17.9, P<0.001<br>BMI×Diagn F=3.6, P<0.05             |
| HOMA-IR (μmol/l×mmol/l <sup>2</sup> ) | 1.1±0.7                                  | 1.4±0.7            | 2.0±1.2  | 2.1±1.6            | 3.7±2.2                                   | 2.44±2.2           | BMI F=50.2, P<0.001<br>BMI×Diagn F=9.6, P<0.05             |
| Testosterone (nmol/l)                 | 1.8±0.7*                                 | 1.35±0.4           | 1.9±0.6*   | 1.4±0.3            | 2.1±0.8                                   | 1.67±0.4           | BMI F=3.5, P<0.05<br>BMI×Diagn F=24.5, P<0.001             |
| Hirsutism score (FG)                  | 6.2±4.6*                                 | 1.6±2.0            | 8.3±5.4*   | 2.7±3.3            | 10.6±6.8*                                 | 2.83±3.2           | BMI F=5.1, P<0.01<br>Diagnosis F=69.1, P<0.001             |
| Normal cycles (%)                     | 59.5*                                    | 100.0              | 46.2*  | 100.0              | 50.0*                                     | 100.0              | P<0.001  |
| Acne (%)                              | 11.6*                                    | 0                  | 22.0*  | 0                  | 27.8*                                     | 0                  | P<0.001  |
| Alopecia (%)                          | 18.2*                                    | 0                  | 24.4*  | 0                  | 25.8*                                     | 0                  | P<0.001  |

Values are shown as mean ± s.d. or percent affected. BMI, body mass index; HOMA-IR, homeostasis model assessment for insulin resistance; FG, Ferriman-Gallway score. \**t*-corrected, *post hoc* comparisons of means comparing PCOS and controls within each BMI group (all *P*<0.017).  
<sup>a</sup>ANOVA with the two factors diagnosis (PCOS, controls) and BMI group. Shown are *F* and *P* values for significant main effects or significant interactions between BMI group and diagnosis (BMI×Diagn). For dichotomous variables, χ<sup>2</sup> tests were carried out.

demonstrated (14). Furthermore, ovarian steroidogenesis in bovine granulosa cells was reportedly inhibited by decreased phosphorylation of MAPK3/MAPK14 by metformin (16). In Wister rats with androgen-induced PCOS, an inhibition of DHEA-induced testosterone concentration by metformin application has also been shown (15). Together, these studies suggest a direct inhibitory effect of metformin on ovarian steroidogenesis. While the exact mechanism of metformin action is still unclear, it appears that besides reducing IR and thus hyperinsulinemia, metformin has significant effects that are independent from pre-treatment IR.

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