CLINICAL STUDY

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

Susanne Tan, Susanne Hahn¹, Sven Benson², Tiina Dietz, Harald Lahner, Lars C Moeller, Markus Schmidt³, Sigrid Elsenbruch², Rainer Kimmig³, Klaus Mann and Onno E Janssen⁴

Division of Endocrinology, Department of Medicine, University Hospital of Essen Medical School, Hufelandstr. 55, 45122 Essen, Germany,

¹Center for Endocrine and Metabolic Diseases, Wuppertal, Germany, ²Institute of Medical Psychology and Behavioral Immunobiology and ³Department of Gynecology and Obstetrics, University Hospital of Essen Medical School, Essen, Germany and ⁴Division of Endocrinology, Metabolism and Molecular Medicine, Department of Medicine Feinberg School of Medicine, Northwestern University, Chicago, Illinois, USA

(Correspondence should be addressed to S Tan; Email: susanne.tan@uk-essen.de)

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/m², overweight: BMI 25–29 kg/m², and obese: BMI ≥ 30 kg/m²). 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 ± 1.0 vs 1.1 ± 0.7 $\mu\text{mol/l} \times \text{mmol/l}^2$) and fasting insulin levels (7.7 ± 4.2 vs 5.4 ± 3.9 mU/l), in addition to testosterone levels (2.6 ± 0.9 vs 1.8 ± 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.

European Journal of Endocrinology 157 669–676

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 ² 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 ± 1.65 kg/m ²	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 ± 4.2 kg/m ²	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 ≥ 100 kg or BMI ≥ 30 kg/m²: 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.pco-syndrom.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 µg/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 µg/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 weight/(height)² in (kg/m²). Parameters of IR and β -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- α -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/m², overweight: BMI 25–29 kg/m², obese: BMI ≥ 30 kg/m²). 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, α levels were corrected for inflation of α error by multiple testing by applying the conservative Bonferroni method, which divides the α 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 α -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 α -correction as described above. For the dichotomous variables, χ^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 ± 1.6), *N*=42 were in the

overweight group (mean BMI: 26.8 ± 1.5), and *N*=102 were in the obese group (mean BMI: 38.1 ± 6.0) (Table 2). Out of the total of *N*=102 healthy women, *N*=66 were in the lean group (mean BMI: 21.5 ± 1.6), *N*=24 in the overweight group (mean BMI: 26.7 ± 1.4), and *N*=12 were in the obese group (mean BMI: 34.4 ± 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 ²			BMI 25–29.9 kg/m ²			BMI ≥ 30 kg/m ²			Two-factorial ANOVA ^a
	Lean PCOS		Lean controls	Overweight PCOS		Overweight controls	Obese PCOS		Obese controls	
	($n=44$)	($n=66$)	($n=24$)	($n=42$)	($n=24$)	($n=12$)	($n=102$)	($n=12$)	($n=102$)	
Fasting glucose (mg/dl)	84.6 ± 8.6	83.8 ± 6.9	85.8 ± 7.6	88.8 ± 5.8	99.1 ± 48.0	93.1 ± 5.8	23.4 ± 13.7*	11.8 ± 9.0	n.s.	Diagnosis $F=17.6$, $P<0.001$
Fasting insulin (mU/l)	7.7 ± 4.2	7.44 ± 3.3	14.2 ± 7.6	10.6 ± 6.9	2.7 ± 0.9*	2.83 ± 3.2	5.5 ± 3.4*	2.44 ± 2.2	BMI: $F=13.1$, $P<0.001$	Diagnosis $F=13.1$, $P<0.001$
HOMA-IR ($\mu\text{mol/l} \times \text{mmol/l}^2$)	1.7 ± 1.0	1.4 ± 0.7	3.0 ± 1.7	2.1 ± 1.6	10.9 ± 6.7*	2.83 ± 3.2	5.5 ± 3.4*	2.44 ± 2.2	Diag × BMI: $F=5.3$, $P<0.01$	Diagnosis $F=18.3$, $P<0.001$
AUCI ($\text{mU} \times \text{h/l}$)	142.1 ± 89.1	(Not measured)	265.2 ± 194.1	(Not measured)	389.6 ± 215.9	(Not measured)	389.6 ± 215.9	(Not measured)	Diag × BMI: $F=5.8$, $P<0.01$	Diagnosis $F=17.7$, $P<0.001$
Testosterone (nmol/l)	2.6 ± 0.9*	1.35 ± 0.4	2.5 ± 0.9*	1.4 ± 0.3	2.7 ± 0.9*	1.67 ± 0.4	2.7 ± 0.9*	1.67 ± 0.4	Diagnosis $F=95.2$, $P<0.01$	Diagnosis $F=95.2$, $P<0.01$
Hirsutism score (FG)	6.7 ± 4.3*	1.6 ± 2.0	9.2 ± 6.8*	2.7 ± 3.3	10.9 ± 6.7*	2.83 ± 3.2	10.9 ± 6.7*	2.83 ± 3.2	Diagnosis $F=75.5$, $P<0.001$	Diagnosis $F=75.5$, $P<0.001$

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., non-significant (no significant ANOVA effects). * α -corrected, post hoc comparisons of means comparing PCOS and controls within each BMI group (all $P<0.017$).
^aANOVA 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 BMI < 25 kg/m ² (n = 44)		Overweight PCOS BMI 25–29.9 kg/m ² (n = 42)		Obese PCOS BMI ≥ 30 kg/m ² (n = 102)		Two-factorial ANOVA or McNemar Test ^a
	Baseline	After 6 months of metformin	Baseline	After 6 months of metformin	Baseline	After 6 months of 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 BMI <i>F</i> = 155.3, <i>P</i> < 0.001 Time × BMI <i>F</i> = 4.1, <i>P</i> < 0.05
BMI (kg/m ²)	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 BMI <i>F</i> = 178.4, <i>P</i> < 0.001 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 BMI <i>F</i> = 33.8, <i>P</i> < 0.001 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 Time × BMI <i>F</i> = 4.0, <i>P</i> < 0.05
HOMA-IR (μmol/l × mmol/l ²)	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 BMI <i>F</i> = 28.9, <i>P</i> < 0.001 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 Time = 4.9, <i>P</i> < 0.05 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	McNemar time <i>P</i> < 0.001
Normal menstrual cycles (%)	2.3	59.5*	7.1	46.2*	3.9	50.0*	McNemar n.s.
Acne (%)	31.8	11.6*	53.7	22.0*	42.2	27.8*	
Alopecia (%)	18.2	18.2	22.5	24.4	35.3	25.8	

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).

^aANOVA 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 BMI <25 kg/m ²		Overweight groups BMI 25–29.9 kg/m ²		Obese groups BMI ≥30 kg/m ²		Two-factorial ANOVA or χ^2 test ^a
	Treated PCOS (n=44)	Controls (n=66)	Treated PCOS (n=42)	Controls (n=24)	Treated PCOS (n=102)	Controls (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
HOMA-IR ($\mu\text{mol/l} \times \text{mmol/l}^2$)	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 × Diagn F=3.6, 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=50.2, 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 × Diagn F=9.6, P<0.05
Normal cycles (%)	59.5*	100.0	46.2*	100.0	50.0*	100.0	BMI F=3.5, P<0.05
Acne (%)	11.6*	0	22.0*	0	27.8*	0	BMI × Diagn F=24.5, P<0.001
Alopecia (%)	18.2*	0	24.4*	0	25.8*	0	BMI F=5.1, P<0.01
							Diagnosis F=69.1, 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-Gallwey score. * α -corrected, post hoc comparisons of means comparing PCOS and controls within each BMI group (all P<0.017).

^aANOVA 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^2 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.

References

- Asuncion M, Calvo RM, San Millan JL, Sancho J, Avila S & Escobar-Morreale HE. A prospective study of the prevalence of the polycystic ovary syndrome in unselected Caucasian women from Spain. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 2434–2438.
- Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES & Yildiz BO. The prevalence and features of the polycystic ovary syndrome in an unselected population. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 2745–2749.
- Carmina E & Lobo RA. Polycystic ovary syndrome (PCOS): arguably the most common endocrinopathy is associated with significant morbidity in women. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 1897–1899.
- Diamanti-Kandarakis E, Kouli CR, Bergiele AT, Filandra FA, Tsianateli TC, Spina GG, Zapanti ED & Bartzis MI. A survey of the polycystic ovary syndrome in the Greek island of Lesbos: hormonal and metabolic profile. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 4006–4011.
- ESHRE/ASRM. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Human Reproduction* 2004 **19** 41–47.
- Dunaif A. Insulin action in the polycystic ovary syndrome. *Endocrinology and Metabolism Clinics of North America* 1999 **28** 341–359.
- Dunaif A & Finegood DT. Beta-cell dysfunction independent of obesity and glucose intolerance in the polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* 1996 **81** 942–947.
- Dunaif A, Segal KR, Futterweit W & Dobrjansky A. Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. *Diabetes* 1989 **38** 1165–1174.
- Evans DJ, Hoffmann RG, Kalkhoff RK & Kissebah AH. Relationship of androgenic activity to body fat topography, fat cell morphology, and metabolic aberrations in premenopausal women. *Journal of Clinical Endocrinology and Metabolism* 1983 **57** 304–310.
- Glueck CJ, Morrison JA, Friedman LA, Goldenberg N, Stroop DM & Wang P. Obesity, free testosterone, and cardiovascular risk factors in adolescents with polycystic ovary syndrome and regularly cycling adolescents. *Metabolism* 2006 **55** 508–514.
- Hahn S, Quadbeck B, Elsenbruch S, Gartner R, Finke R, Mann K & Janssen OE. Metformin, an efficacious drug in the treatment of polycystic ovary syndrome. *Deutsche Medizinische Wochenschrift* 2004 **129** 1059–1064.
- Lord JM, Flight IH & Norman RJ. Insulin-sensitising drugs (metformin, troglitazone, rosiglitazone, pioglitazone, D-chiro-inositol) for polycystic ovary syndrome. *Cochrane Database of Systematic Reviews* 2003 **3** CD003053.
- Palomba S, Orto F, Falbo A, Manguso F, Russo T, Cascella T, Tolino A, Carmina E, Colao A & Zullo F. Prospective parallel randomized, double-blind, double-dummy controlled clinical trial comparing clomiphene citrate and metformin as the first-line

- treatment for ovulation induction in nonobese anovulatory women with polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 4068–4074.
- 14 Mansfield R, Galea R, Brincat M, Hole D & Mason H. Metformin has direct effects on human ovarian steroidogenesis. *Fertility and Sterility* 2003 **79** 956–962.
 - 15 Misugi T, Ozaki K, El Beltagy K, Tokuyama O, Honda K & Ishiko O. Insulin-lowering agents inhibit synthesis of testosterone in ovaries of DHEA-induced PCOS rats. *Gynecologic and Obstetric Investigation* 2006 **61** 208–215.
 - 16 Tosca L, Solnais P, Ferre P, Foulle F & Dupont J. Metformin-induced stimulation of adenosine 5' monophosphate-activated protein kinase (PRKA) impairs progesterone secretion in rat granulosa cells. *Biology of Reproduction* 2006 **75** 342–351.
 - 17 Harborne LR, Sattar N, Norman JE & Fleming R. Metformin and weight loss in obese women with polycystic ovary syndrome: comparison of doses. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 4593–4598.
 - 18 Ferriman D & Gallwey JD. Clinical assessment of body hair growth in women. *Journal of Clinical Endocrinology and Metabolism* 1961 **21** 1440–1447.
 - 19 Ludwig E. Classification of the types of androgenetic alopecia (common baldness) occurring in the female sex. *British Journal of Dermatology* 1977 **97** 247–254.
 - 20 Hermans MP, Levy JC, Morris RJ & Turner RC. Comparison of insulin sensitivity tests across a range of glucose tolerance from normal to diabetes. *Diabetologia* 1999 **42** 678–687.
 - 21 Hermans MP, Levy JC, Morris RJ & Turner RC. Comparison of tests of beta-cell function across a range of glucose tolerance from normal to diabetes. *Diabetes* 1999 **48** 1779–1786.
 - 22 Phillips DI, Clark PM, Hales CN & Osmond C. Understanding oral glucose tolerance: comparison of glucose or insulin measurements during the oral glucose tolerance test with specific measurements of insulin resistance and insulin secretion. *Diabetic Medicine* 1994 **11** 286–292.
 - 23 Palomba S, Orio F, Nardo LG, Falbo A, Russo T, Corea D, Doldo P, Lombardi G, Tolino A, Colao A & Zullo F. Metformin administration versus laparoscopic ovarian diathermy in clomiphene citrate-resistant women with polycystic ovary syndrome: a prospective parallel randomized double-blind placebo-controlled trial. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 4801–4809.
 - 24 Fleming R, Hopkinson ZE, Wallace AM, Greer IA & Sattar N. Ovarian function and metabolic factors in women with oligomenorrhea treated with metformin in a randomized double blind placebo-controlled trial. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 569–574.
 - 25 Morin-Papunen L, Rautio K, Ruokonen A, Hedberg P, Puukka M & Tapanainen JS. Metformin reduces serum C-reactive protein levels in women with polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 4649–4654.
 - 26 Pasquali R, Gambineri A, Biscotti D, Vicennati V, Gagliardi L, Colitta D, Fiorini S, Cognigni GE, Filicori M & Morselli-Labate AM. Effect of long-term treatment with metformin added to hypocaloric diet on body composition, fat distribution, and androgen and insulin levels in abdominally obese women with and without the polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 2767–2774.
 - 27 Vandermolen DT, Ratts VS, Evans WS, Stovall DW, Kauma SW & Nestler JE. Metformin increases the ovulatory rate and pregnancy rate from clomiphene citrate in patients with polycystic ovary syndrome who are resistant to clomiphene citrate alone. *Fertility and Sterility* 2001 **75** 310–315.
 - 28 Nestler JE & Jakubowicz DJ. Decreases in ovarian cytochrome P450c17 alpha activity and serum free testosterone after reduction of insulin secretion in polycystic ovary syndrome. *New England Journal of Medicine* 1996 **335** 617–623.
 - 29 Maciel GA, Soares Junior JM, Alves da Motta EL, Abi Haidar M, de Lima GR & Baracat EC. Nonobese women with polycystic ovary syndrome respond better than obese women to treatment with metformin. *Fertility and Sterility* 2004 **81** 355–360.
 - 30 Kumari AS, Haq A, Jayasundaram R, Abdel-Wareth LO, Al Haija SA & Alvares M. Metformin monotherapy in lean women with polycystic ovary syndrome. *Reproductive Biomedicine Online* 2005 **10** 100–104.
 - 31 Goldenberg N, Glueck CJ, Loftspring M, Sherman A & Wang P. Metformin-diet benefits in women with polycystic ovary syndrome in the bottom and top quintiles for insulin resistance. *Metabolism* 2005 **54** 113–121.
 - 32 Yilmaz M, Biri A, Karakoc A, Toruner F, Bingol B, Cakir N, Tiras B, Ayvaz G & Arslan M. The effects of rosiglitazone and metformin on insulin resistance and serum androgen levels in obese and lean patients with polycystic ovary syndrome. *Journal of Endocrinological Investigation* 2005 **28** 1003–1008.
 - 33 Marcondes JA, Yamashita SA, Maciel GA, Baracat EC & Halpern A. Metformin in normal-weight hirsute women with polycystic ovary syndrome with normal insulin sensitivity. *Gynecological Endocrinology* 2007 **23** 273–278.
 - 34 Orio F, Manguso F, Di Biase S, Falbo A, Giallauria F, Labella D, Tolino A, Lombardi G, Colao A & Palomba S. Metformin administration improves leukocyte count in women with polycystic ovary syndrome: a 6-month prospective study. *European Journal of Endocrinology* 2007 **157** 69–73.

Received 19 July 2007

Accepted 3 September 2007