Metformin Improves Pregnancy and Live-Birth Rates in Women with Polycystic Ovary Syndrome (PCOS): A Multicenter, Double-Blind, Placebo-Controlled Randomized Trial

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Background: The role of metformin in the treatment of infertility in women with polycystic ovary syndrome (PCOS) is still controversial.

Objective and Outcomes: We investigated whether metformin decreases the early miscarriage rate and improves the pregnancy rates (PR) and live-birth rates (LBR) in PCOS.

Methods: This was a multicenter, randomized (1:1), double-blind, placebo-controlled study. Three hundred twenty women with PCOS and anovulatory infertility were randomized to metformin (n = 160, Diformin; obese women, 1000 mg two times daily; nonobese subjects, 500 mg + 1000 mg daily) or identical doses of placebo (n = 160). After 3 months' treatment, another appropriate infertility treatment was combined if necessary. If pregnancy occurred, metformin/placebo was continued up to the 12th week.

Results: Miscarriage rates were low and similar in the two groups (metformin 15.2% *vs.* placebo 17.9%, $P = 0.8$). Intent-to-treat analysis showed that metformin significantly improved PR and LBR (*vs.* placebo) in the whole study population (PR: 53.6 *vs.* 40.4%, $P = 0.006$; LBR: 41.9 *vs.* 28.8%, $P = 0.014$) and PR in obese women (49.0 *vs.* 31.4%, $P = 0.04$), and there was a similar trend in nonobese (PR: 58.6 *vs.* 47.6%, $P = 0.09$; LBR: 46.7 *vs.* 34.5%, $P = 0.09$) and in obese women with regard to LBR (35.7 *vs.* 21.9%, $P = 0.07$). Cox regression analysis showed that metformin plus standard infertility treatment increased the chance of pregnancy 1.6 times (hazard rate 1.6, 95% confidence interval 1.13–2.27).

Conclusion: Obese women especially seem to benefit from 3 months' pretreatment with metformin and its combination thereafter with routine ovulation induction in anovulatory infertility. **(*J Clin Endocrinol Metab* 97: 1492–1500, 2012)**

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Abbreviations: AUC_{gluc}, Glucose area under the curve; AUC_{ins}, insulin area under the curve; BMI, body mass index; FAI, free androgen index; hs-CRP, high-sensitivity C-reactive protein; IVF, *in vitro* fertilization; ICSI, intracytoplasmic sperm injection; LBR, live-birth rate; OGTT, oral glucose tolerance test; PCOS, polycystic ovary syndrome; PR, pregnancy rate; RCT, randomized controlled trial; T, testosterone; WHR, waist to hip ratio.

Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting 5–10% of women of reproductive age (1). Anovulation is the cause of infertility in about one third of couples seeking treatment, and PCOS accounts for 90% of these cases. Clinical manifestations of PCOS include irregular menses, hirsutism, and acne. In addition, insulin resistance and hyperinsulinemia play a central role in the pathophysiology of PCOS (1). Early pregnancy loss has also been reported to occur in 30–50% of women with PCOS (2, 3), which is 3-fold higher than in healthy women (4, 5).

During the last decade, use of metformin has been under debate in the treatment of PCOS. Most earlier studies indicated that metformin improved hyperinsulinemia and hyperandrogenemia and restored ovulatory function and that its use, alone or combined with clomiphene citrate, could increase ovulation and pregnancy rates in women with PCOS (6–8). Moreover, nonrandomized prospective studies suggested that metformin may reduce first-trimester spontaneous abortions in women with PCOS (3, 9). Therefore, at the time of planning this study (January 2003), several factors supported the use of metformin in the treatment of PCOS with metabolic and hormonal disturbances and for the prevention of early miscarriages, but large randomized controlled trials (RCT) designed to evaluate the effectiveness of this clinical practice were lacking. However, more recent RCT have shown controversial results, with either a beneficial effect (10–13) or no effect of metformin on fertility in PCOS (14–17). In the present study, the aim was to explore the effectiveness of metformin in a study protocol reflecting routine clinical practice encountered when treating anovulatory infertility associated with PCOS. More importantly, we wanted to test the hypothesis that metformin needs time to exert its beneficial metabolic effects fully (18) by using it alone for at least 3 months before standard infertility treatment.

The primary end point was to see whether metformin decreases early pregnancy loss, and the second one was to clarify whether it improves pregnancy rates (PR) and live-birth rates (LBR) in women with anovulatory infertility and PCOS.

Materials and Methods

Study design and randomization

This was a multicenter randomized (1:1), double-blind, placebo-controlled, parallel-group study conducted in all university hospitals of Finland (five sites). During the study period (January 2003 to December 2009), the women with PCOS referred to the clinics because of anovulatory infertility were asked to participate in the study and assigned to intervention by the main investigators of the different University Hospitals (L.M.-P. in Oulu, L.U.-K., and A.T. in Helsinki, M.H. in Kuopio, A.P. in Turku, and H.T. in Tampere).

Eligible participants were women aged 18–39 yr at entry, with a body mass index (BMI) greater than 19 kg/m^2 and diagnosed with PCOS according to Rotterdam criteria (19). All the subjects had polycystic ovaries at ultrasound according the European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine definition, and a large majority (n = 303, 94.7%) had oligoamenorrhea, 143 (44.7%) had hyperandrogenism [either serum T levels >48.7 ng/dl (2.3 nmol/liter) or hirsutism (Ferriman-Gallwey score >7), or both]. The characteristics of the women did not differ between the two randomization groups (Table 1).

The women had suffered from anovulatory infertility for at least 6 months and a washout period of at least 3 months since the last infertility treatment was required. Exclusion criteria were type 2 diabetes mellitus, active liver disease (alanine aminotransferase >100 IU/liter), history of cardiac or renal failure, hormone medication, alcohol use, and regular smoking.

The study population was divided into obese (BMI ≥27 kg/m^2) and nonobese subjects, based on prior studies indicating increased insulin resistance at BMI of 27 kg/m^2 in PCOS patients (20).

Randomization (after simple randomization procedures) was performed by the hospital pharmacy with 1:1 allocation in random blocks of 10 using two computer-generated lists, one for the nonobese and one for the obese women. Metformin and placebo tablets were provided by Leiras (Turku, Finland) and prepacked in opaque identical containers of 100 tablets and consecutively numbered for each woman according to the randomization schedule. Each woman was assigned a number and received the tablets in the corresponding container. Randomization codes remained blinded until the database lock had taken place. The patients and all study site personnel were blinded to the study drug codes.

Procedures

Metformin (metformin hydrochloride depot tablets, Diformin 500 mg; Leiras) or placebo was initiated at a dose of one tablet once a day for the first week and increased thereafter by

TABLE 1. Characteristics of the subjects of the study

	Metformin (n = 160)	Placebo (n = 160)	All subjects (n = 320)
PCO + OA + HA	59 (36.9%)	69 (43.1%)	128 (40.0%)
PCO + OA	90 (56.2%)	85 (53.1%)	175 (54.7%)
PCO + HA	11 (6.9%)	6 (3.8%)	17 (5.3%)

PCO, Polycystic ovaries at ultrasonography; OA, oligoamenorrhea; HA, hyperandrogenism (serum testosterone >48.7 ng/dl and/or hirsutism with a Ferriman-Gallwey score >7).

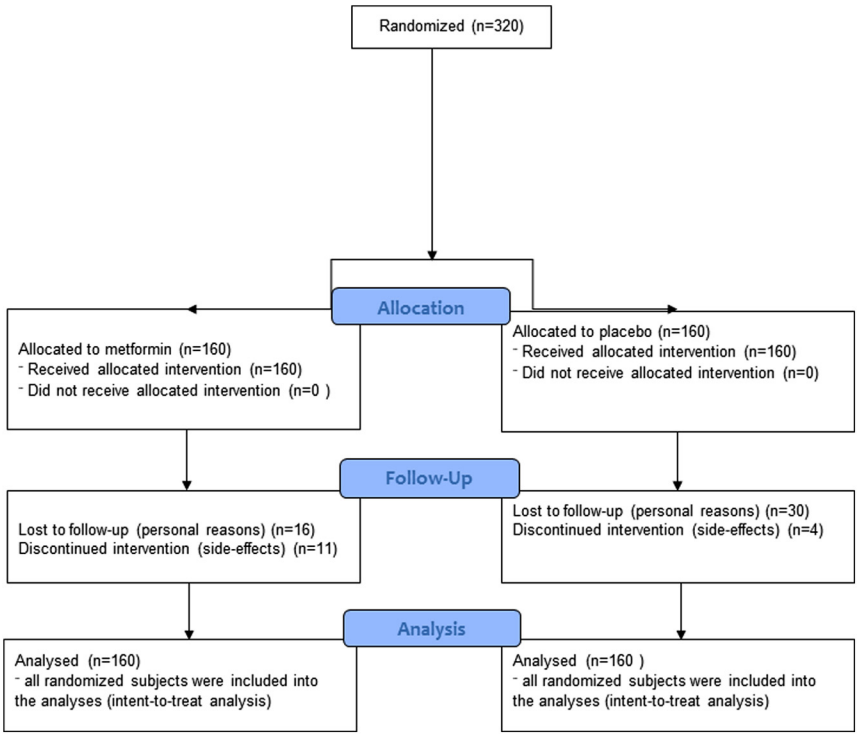


FIG. 1. Flow diagram of the study.

one tablet daily in weekly steps up to three tablets (one + two daily) in nonobese women and to four tablets (two + two daily) in obese women and was continued up to a maximum of 9 months. If pregnancy occurred, metformin/placebo was continued up to the 12th week. Previous studies of ours and others (21, 22) have shown that a dose of 1500 mg is efficient enough to restore ovulation in most of nonobese women with PCOS and to improve significantly hyperandrogenism and insulin sensitivity. The idea of using a smaller dose in nonobese patients was to minimize possible side effects and thereby dropouts. Partners' sperm was analyzed and tubal patency was tested at baseline.

The women used metformin or placebo alone for at least 3 months. If pregnancy did not occur, ovulation induction was commenced: if the woman ovulated after clomiphene, she continued metformin/placebo with the same dose of clomiphene for four to six cycles or until the 12th week of pregnancy. After four to six unsuccessful cycles with metformin/placebo and clomiphene, either gonadotrophins or aromatase inhibitors were used. In cases of male subfertility, either insemination or *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) was performed, according to standard protocols.

Pregnancy was defined as a positive pregnancy test result. First-trimester miscarriage was defined as lack of embryonic heart activity in ultrasonography before 12 completed weeks of gestation after a positive pregnancy test, *i.e.* preclinical spontaneous abortions were included. Birth was defined when the infant was born at a gestation of 22 weeks or longer or weighed 500 g or greater (23).

Clinical, metabolic, and hormonal parameters were assessed 1–7 d after spontaneous menstruation (oligomenorrhoeic subjects) or at any other convenient time (amenorrhoeic subjects) at baseline and at 3 months of treatment with metformin/placebo. Urinary pregnancy tests were performed monthly.

Study population

The women were randomized to metformin (n = 160) or placebo (n = 160). Seventy women in the metformin group and 73 women in the placebo group were obese, and 90 and 87 nonobese, respectively (Fig. 1).

The study was approved by the Ethics Committee of Northern Ostrobothnia Hospital District and the Finnish National Agency of Medicines. Written informed consent was obtained from all participants.

Clinical parameters and assays

Weight and waist and hip circumferences (measured to the nearest centimetre with a soft tape at the narrowest part of the torso and at the widest part of the gluteal region) were assessed at each visit, and the waist to hip ratio (WHR) was calculated.

Oral glucose tolerance tests (OGTT)

After an overnight fast of 10–12 h, all subjects underwent an OGTT (a load of 75 g glucose in 300 ml of water). Venous blood samples for blood glucose and serum insulin assays were drawn at 0, 30, 60, and 120 min. Glucose tolerance was defined according to World Health Organization criteria: impaired fasting glucose level (IFG) was diagnosed when in OGTT the fasting glucose concentrations were 110–125 mg/dl (6.1–6.9 mmol/liter), impaired glucose tolerance (IGT) when glucose levels at 2 h were 140–200 mg/dl (7.8–11.0 mmol/liter) and diabetes when fasting glucose concentrations were greater than 125 mg/dl (6.9 mmol/liter) and/or 2-h concentrations greater than 200 mg/dl (11.0 mmol/liter) (24).

Incremental insulin area under the curve (AUC_{ins}) and glucose area under the curve (AUC_{gluc}) were calculated by the trapezoidal method. To quantify the degree of insulin resistance, the whole-body insulin sensitivity index, *i.e.* the Matsuda index, was calculated (25).

Plasma glucose and alanine aminotransferase were determined by chemical analyzer (Advia 1800; Siemens Healthcare Diagnostics, Tarrytown, NY), serum insulin, SHBG by chemiluminescent enzyme immunoassays (Immulite 2000; Siemens Healthcare, Llanberis, UK), androstenedione by RIA (Siemens Healthcare Diagnostics, Los Angeles, CA), and high-sensitivity C-reactive protein (hs-CRP) (BN ProSpec; Siemens Healthcare Diagnostics, Marburg, Germany) by immunonephelometry. Testosterone (T) was analyzed using Agilent triple-quadrupole 6410 liquid chromatography/mass spectrometry equipment with an electrospray ionization source operating in positive-ion mode (Agilent Technologies, Wilmington, DE). Multiple reaction monitoring was used to quantify T by trideuterated testosterone with the following transitions: m/z 289.2 to 97 and 289.2 to 109 for T and 292.2 to 97, and 292.2 to 109 for d_3-T. Intraassay coefficients of vartiation of the method were 5.3, 1.6, and 1.2% for T at 0.6, 6.6, and 27.7 nmol/liter, respectively. Interassay coefficients of variation were 5.3, 4.2, and 1.0% for the respective concentrations. The free androgen index (FAI) was calculated according to the following equation: (T × 100)/SHBG.

TABLE 2. Clinical parameters at baseline and at 3 months of treatment with metformin/placebo only in the whole study population

	Metformin baseline (n = 142–160)[a]	Metformin 3 months (n = 106–128)[a]	Placebo baseline (n = 145–160)[a]	Placebo 3 months (n = 111–125)[a]
Age (yr)	28.4 ± 3.9		27.9 ± 4.1	
Weight (kg)	74.8 ± 18.7	73.5 ± 18.0[b]	75.1 ± 18.1	76.0 ± 18.0
BMI (kg/m^2)	27.1 ± 6.3	26.9 ± 6.2[b]	27.4 ± 6.2	27.7 ± 6.2
Waist (cm)	84.2 ± 14.5	84.3 ± 15.0	85.8 ± 15.4	86.1 ± 15.2
WHR	0.80 ± 0.1	0.80 ± 0.1	0.81 ± 0.1	0.81 ± 0.1
Hirsutism score	5.4 ± 4.5	5.3 ± 4.9	5.4 ± 4.5	5.2 ± 4.6
Ov vol dx (cm^3)	8.7 ± 4.2	8.8 ± 4.5	10.2 ± 7.5	10.1 ± 10.7
Ov vol sin (cm^3)	9.1 ± 9.1	9.1 ± 6.2	10.1 ± 13.3	8.6 ± 4.9

ov vol, Ovarian volume; dx, dexter; sin, sinister.

[a] Number of patients varies due to lacking data in some subjects.

[b] $P < 0.001$ (change at 3 months in the metformin group).

Sample size and statistical methods

Power analysis indicated that a total number of 120 pregnant women would be needed to reveal a possible decrease in risk of miscarriage from 45 to the 15% observed in the general population (4, 5) ($\alpha = 0.05$ and power [1-β] = 0.9). At the time of onset of the study, most investigators had shown significantly elevated risks of miscarriage (30–50%) in women with PCOS. The rate of 45% was chosen because it was the mean value calculated from the studies published at that time (2, 3). Additionally, it was estimated that at least 120 patients would be needed in each group to demonstrate an increase of 15% (from 35 to 50%) in clinical pregnancy rate in the metformin group ($\alpha = 0.05$ and power [1-β] = 0.8), which can be considered as a clinically meaningful difference. To allow for dropouts, the planned sample size was at least 150 in each group.

For normally distributed variables, independent-sample *t* tests were used for comparisons between PCOS women and controls, and paired *t* tests were applied to evaluate changes between measurements at baseline and after 3 months of treatment. Logarithmic conversions were performed to approximate normal distribution when data were not normally distributed. Mann-Whitney and Wilcoxon tests were performed for variables with persisting skewed distribution after logarithmic transformation.

Pregnancy rates were assessed using intent-to-treat analysis (including all women randomized to treatment) with Kaplan-Meier estimation, using as a variable the time to get pregnant, which was calculated as the number of days from the baseline (when the medication was started) until the day of the positive pregnancy test. Miscarriage rates and LBR were analyzed by percentage calculations and χ^2 tests. Statistical analyses were performed using SPSS for Windows (version 16.0; Chicago, IL). For all analyses, $P < 0.05$ was considered statistically significant. Data are reported as mean ± SD.

Results

Clinical, hormonal, and metabolic parameters

Baseline characteristics of the women did not differ between the metformin and placebo groups (Tables 2 and 3). As expected, obese women were more hirsute, more hyperandrogenic, insulin resistant, and hyperinsulinemic and had a more unfavorable metabolic profile than nonobese women at baseline (Table 4). All women had patent tubes in sonosalpingography. There was slightly more primary infertility in the metformin group ($P = 0.047$; Table 5). There were no significant differences between the two

TABLE 3. Metabolic and hormonal parameters at baseline in the whole study population

	Normal range	Metformin (n = 140–160)[a]	Placebo (n = 136–159)[a]
Fasting glucose (mg/dl)	70–110	91.9 ± 7.2	91.9 ± 9.0
Fasting insulin (μIU/ml)	0–29	11.0 ± 11.2	11.4 ± 11.8
AUC_{gluc}		13720.7 ± 3158.6	13964.0 ± 2877.5
AUC_{ins}		8085.5 ± 6819.4	8730.2 ± 7036.2
Matsuda index	≤4.5	6.7 ± 4.6	6.3 ± 4.2
A (ng/dl)	49–370	510.0 ± 217.8	527.2 ± 196.3
T (ng/dl)	12–66	43.2 ± 17.3	45.8 ± 20.2
SHBG (μg/dl)	0.5–3.5	1.25 ± 0.66	1.29 ± 0.73
FAI		3.8 ± 2.4	3.9 ± 2.6
DHEAS (μg/dl)	29–403	152.7 ± 69.2	155.6 ± 74.9
hs-CRP (mg/liter)	0.2–3	2.9 ± 4.1	2.5 ± 3.6

Conversion factors to SI units: glucose, 0.0555 (mmol/liter); insulin, 6.945 (pmol/liter); androstenedione, 0.0349 (nmol/liter); dehydroepiandrosterone sulphate (DHEAS), 0.0347 (μmol/liter); testosterone, 0.0347 (nmol/liter); SHBG, 40 (nmol/liter).

[a] Number of patients varies due to lacking data in some subjects.

TABLE 4. Clinical and metabolic parameters of the obese and nonobese subjects at baseline

	Obese 0 months (n = 131–143)[a]	Nonobese 0 months (n = 156–177)[a]	*P* value
Age (yr)	28.2 ± 4.4	28.1 ± 3.7	0.75
Weight (kg)	91.0 ± 13.8	61.8 ± 7.7	<0.001
BMI (kg/m^2)	33.1.±4.5	22.6 ± 2.4	<0.001
Waist (cm)	98.6 ± 10.6	74.1 ± 6.7	<0.001
WHR	0.85 ± 0.1	0.77 ± 0.1	<0.001
Hirsutism score	6.1 ± 5.3	4.7 ± 3.5	0.01
Fasting glucose (mg/dl)	93.7 ± 7.2	90.1 ± 7.2	<0.001
Fasting insulin (μIU/ml)	16.5 ± 12.3	7.0 ± 8.7	<0.001
AUC_{gluc}	15333.3 ± 2821.6	12704.5 ± 2661.3	<0.001
AUC_{ins}	12260.6 ± 8238.0	5368.4 ± 3384.0	<0.001
Matsuda index	3.6 ± 2.6	8.6 ± 4.2	<0.001
A (ng/dl)	527.2 ± 212.0	510.0 ± 203.4	0.50
T (ng/dl)	43.2 ± 20.2	46.1 ± 20.2	0.04
SHBG (μg/dl)	0.87 ± 0.4	1.6 ± 0.7	<0.001
FAI	5.0 ± 2.8	3.0 ± 1.8	<0.001
DHEAS (μg/dl)	164.3 ± 74.9	147.0 ± 57.3	0.04
hs-CRP (mg/liter)	4.5 ± 4.6	1.3 ± 2.1	<0.001

Conversion factors to SI units: glucose, 0.0555 (mmol/liter); insulin, 6.945 (pmol/liter); androstenedione, 0.0349 (nmol/liter); dehydroepiandrosterone sulphate (DHEAS), 0.0347 (μmol/liter); T, 0.0347 (nmol/liter); SHBG, 40 (nmol/liter).

[a] Number of patients varies due to lacking data in some subjects.

groups in duration or etiology of infertility, frequency of abnormal sperm (Table 5), or distribution of infertility treatments after the 3-month period with metformin/placebo only (Table 6). At baseline, 13 women (4%) had impaired glucose tolerance and eight women (2.5%) had impaired fasting glucose level. None of the women had diabetes.

During metformin treatment, weight ($P < 0.001$) and BMI ($P < 0.001$ decreased significantly at 3 months (Table 2).

Dropouts and side effects

Dropout rates did not differ between the groups (16.8% in the metformin group *vs.* 21.2% in the placebo group, P = NS; Fig. 1). More women in the metformin group compared with the control group suffered from side effects (34.6 *vs.* 7.1%, $P < 0.001$) and stopped treatment (6.9 *vs.* 2.5%, $P = 0.02$; Fig. 1).

Miscarriage rates

Miscarriage rates did not differ between the two groups (metformin 15.2% *vs.* placebo 17.8%, $P = 0.7$). All miscarriages occurred during the first trimester except for one at the 19th week of gestation in the placebo group. In the whole study population, women who experienced miscarriage did not differ at baseline from the women giving birth (data not shown).

Pregnancy rates

There were 135 pregnancies: 79 in the metformin group (31 in obese and 48 in nonobese women) and 56 in the placebo group (19 and 37, respectively). One woman in the placebo group suffering from early preeclampsia underwent cesarean section at the 24th week of gestation, and the infant died after 3 d. This case was included in the PR and LBR analyses, and omitting it did not change the results.

TABLE 5. Infertility history in the subjects of the study

	Metformin (n = 160) (%)	Placebo (n = 160) (%)
Duration of infertility (months)	23.8 ± 18.8	25.3 ± 22.3
Patent tubes	160 (100)	160 (100)
Abnormal sperm	42 (26.2)	39 (24.4)
Previous pregnancies	37 (23.1)	53 (33.1)[a]
Previous first-trimester miscarriage	16 (10.0)	22 (13.8)
Previous delivery	27 (16.9)	32 (20.0)
First infertility treatment	112 (70)	109 (68.1)

[a] $P = 0.047$ between the metformin and the placebo groups.

TABLE 6. Distribution of the infertility treatments in the metformin and placebo groups

	Metformin (n = 160) (%)	Placebo (n = 160) (%)
Metformin/placebo only	73 (45.6)	68 (42.5)
Clomiphene	53 (33.1)	49 (30.6)
Aromatase inhibitor	2 (1.3)	2 (1.3)
Gonadotrophin ovulation induction	1 (0.6)	3 (1.9)
IUI	7 (4.4)	6 (3.8)
Clomifene four cycles, then IUI	9 (5.6)	10 (6.3)
IVF/ICSI	15 (9.4)	22 (13.8)

IUI, Intrauterine insemination.

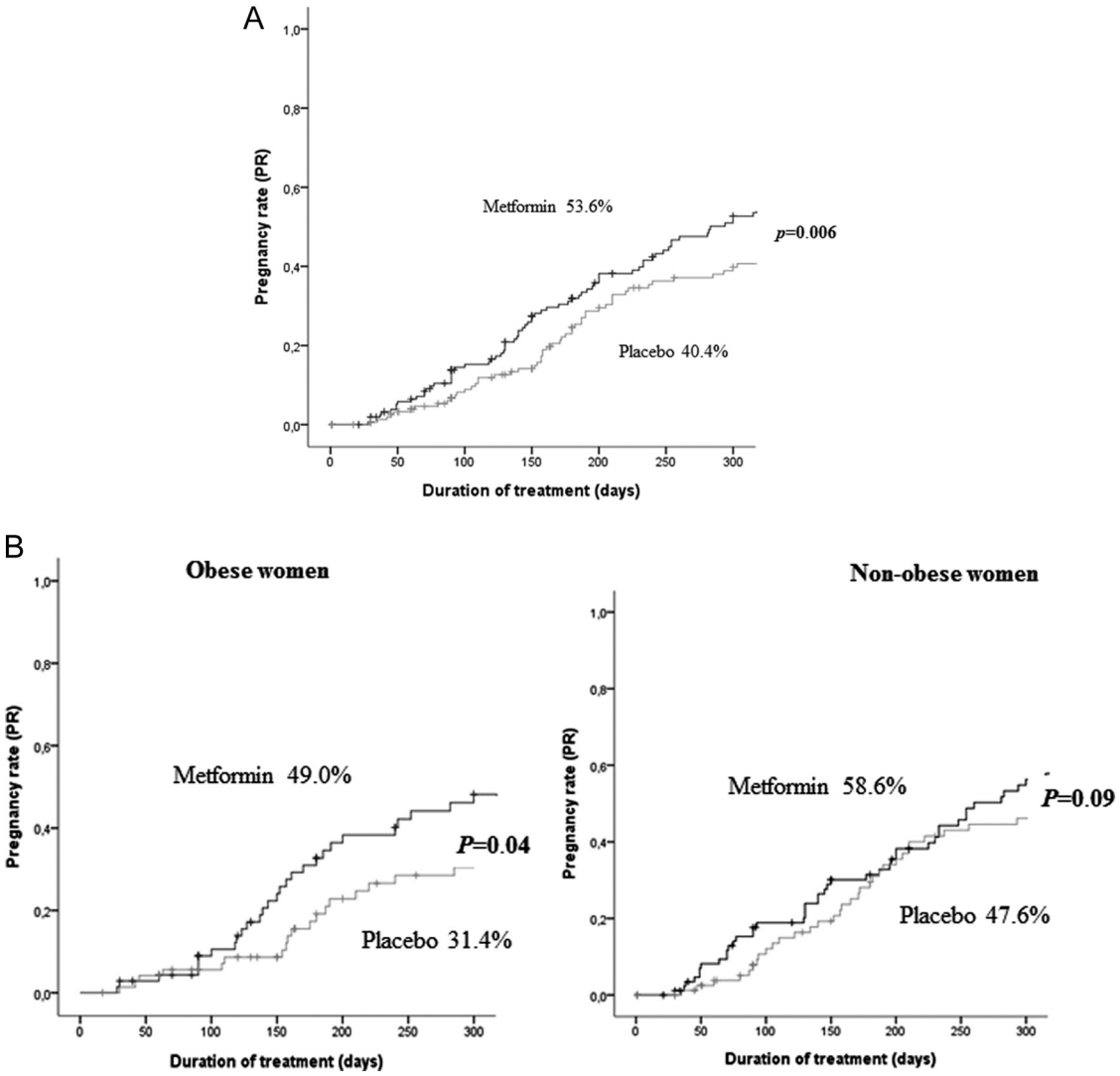


FIG. 2. A, Pregnancy rates in the metformin and placebo groups in the whole population (intent to treat analysis). B, Pregnancy rates in the metformin and placebo groups in the obese and nonobese women (intent to treat analysis).

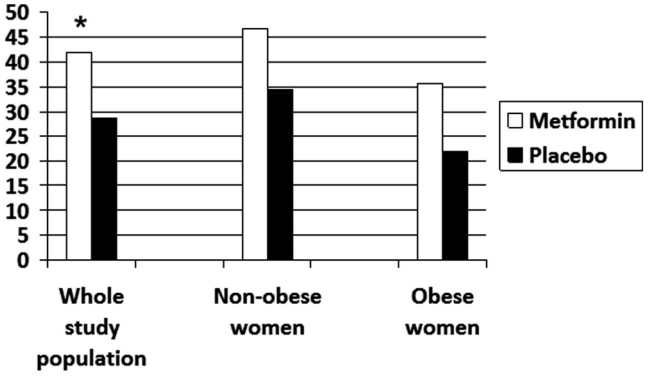
Intent-to-treat analysis showed that metformin significantly improved PR (53.6 *vs.* 40.4%, $P = 0.006$; Fig. 2). In the nonobese women, PR were 58.6% in the metformin group and 47.6% in the placebo group ($P = 0.09$) and in the obese women 49.0 *vs.* 31.4%, respectively ($P = 0.04$; Fig. 2).

Cox regression analysis showed that metformin plus standard infertility treatment increased the chance of pregnancy 1.6 times [crude hazard rate (HR) 1.60, 95% confidence interval 1.13–2.27]. The introduction of the mode of treatment into the Cox regression analysis model did not affect the HR. Duration of infertility was the only variable that tended to decrease PR [$P = 0.058$, HR 0.99 (0.979–1.000)], but after introduction of this variable into the model, HR for pregnancy after metformin remained significant [adjusted HR 1.58 (1.12–2.24)]. The mean time to get pregnant did not differ between the two groups (171 ± 94 d in the metformin group and 172 ± 88 d in the placebo groups, $P = 0.9$).

After excluding women undergoing IVF/ICSI, 58% of the women in the metformin group and 39.4% of the women in the placebo group became pregnant ($P = 0.008$).

Live-birth rates

LBR were significantly higher in the metformin group *vs.* the placebo group in the whole study population (41.9 *vs.* 28.8%, $P = 0.014$) and tended to be higher both in the nonobese (46.7 *vs.* 34.5%, $P = 0.09$) and obese women (35.7 *vs.* 21.9%, $P = 0.07$; Fig. 3). After excluding the women undergoing IVF and/or ICSI, the difference remained significant in the whole study population (metformin group: 41.1% *vs.* placebo group: 28.9%, $P = 0.03$) and tended to be higher also in the nonobese (45.5 *vs.* 34.2%, $P = 0.16$) and obese groups (35.9 *vs.* 22.6%, $P = 0.1$).



*$P = 0.014$ between the metformin and the placebo groups

FIG. 3. Live birth rates in the metformin and placebo groups in the whole population and in the nonobese and obese subjects.

Discussion

The results demonstrate that the miscarriage rate was low in this population of infertile women with PCOS and did not change during metformin therapy. Treatment with metformin alone or combined with other infertility treatments, however, improved PR and LBR in women with PCOS, the most beneficial effects being observed in obese women.

The miscarriage rate among PCOS subjects was similar to the prevalence in the general population (4, 5), although previous studies have indicated a 2- to 5-fold increased risk (2, 3, 26). However, in these studies the numbers of patients were low and the women had had long-lasting infertility and were older and more obese, whereas most of our subjects were relatively young and slim and sought infertility treatment for the first time. In line with our results, later studies in women with PCOS and primary infertility (14), and (in a recent large Finnish cohort study) in women suffering from oligoamenorrhea or hirsutism (27), have not shown increased rates of miscarriage.

Because miscarriage rates were not increased, it was not surprising that metformin did not decrease the risk of spontaneous abortion. Previously the results of small RCT have suggested a beneficial effect of metformin on miscarriage rates (10), but more recent trials (13, 14, 16) and two recent meta-analyses (28, 29) showed that metformin had no effect on early miscarriage rates in cases of PCOS when administered until a positive pregnancy test results.

In the whole study population, however, metformin significantly improved PR. Some recent RCT have also shown significant improvements in PR after metformin *vs.* placebo in subjects with PCOS (10–12) or similar efficacy of metformin and standard ovulation-induction treatments (12, 13), but large recent RCT showed no advantage after the addition of metformin compared with placebo (14, 16, 17).

Most importantly, in the present study, metformin improved LBR compared with placebo. This is an important result because the birth of a healthy infant is the only meaningful outcome for a couple with infertility. In the most recent Cochrane meta-analysis, the addition of metformin to an ovulation-induction agent significantly improved clinical PR, but not LBR (29), and the few RCT having LBR as a primary outcome showed controversial results, with either beneficial (11) or similar effects (13) or no advantage of metformin *vs.* placebo (14, 16, 17). These discrepant results may be explained by differences in study populations: the women exhibited higher T levels (14) or significantly worse obesity and longer duration of infertility, with fewer of them being new to infertility treatment (16), which may have affected the chance of pregnancy because hyperandrogenism, extreme obesity, and long duration of infertility have been associated with more severe ovulation and implantation failure (30). Accordingly, in the present study, nonobese women exhibited better PR and LBR than obese women in both study groups.

The most important difference, however, is the 3-month duration of metformin pretreatment, in contrast to a maximum of 1 month in other RCT (13, 14, 16, 17). Furthermore, metformin was continued until the 12th week of pregnancy, which may have provided a more beneficial milieu for implantation and early fetal development. Previous studies have shown that a beneficial effect on hyperandrogenism (31) appears as early as within a few weeks of treatment with metformin, but several months seem to be required to allow this drug to improve hyperinsulinemia and to exert its beneficial metabolic effects fully and improve fertility outcomes (10, 18). The role of androgens in follicle maturation is complex. Long-lasting (several months) dehydroepiandrosterone supplementation has been shown to improve primordial follicle recruitment and ovarian performance in women with diminished ovarian reserve. The length of androgen exposure seems to be important because androgens, synergistically with FSH, affect follicle maturation at small follicle stages, in which androgen receptors are present in granulosa cells (32). Inversely, in PCOS, the reduction in androgen levels may also take time to exercise an effect because it should primarily affect small growing follicles, which need weeks to months to reach ovulation. All in all, the understanding of the mechanism of metformin action is still incomplete. It activates the AMP-activated protein kinase pathway, a mechanism reducing hepatic glucose production and increasing insulin sensitivity in peripheral tissues (33), and it is not clear whether the beneficial effects on fertility are due to improvement of insulin sensitivity, hyperandrogenism, or both. Additionally, weight loss

could to some extent explain the beneficial effects of metformin.

In line with our data, the results of a multicenter Scandinavian study showed similar improvements (15%) in PR and LBR in nonobese women with PCOS pretreated with metformin for 3 months before IVF/ICSI (34). Altogether our findings suggest reconsideration of the consensus statement by the European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine, which recommends that metformin use in PCOS should be restricted to women with glucose intolerance (35), and they support the use of pretreatment with metformin for several months before standard ovulation induction, as suggested previously (18).

This study has several strengths but also some limitations. A strength is that Finland is a homogeneous country with regard to ethnicity (all patients were Caucasian) and treatment protocols of infertility. Furthermore, the dropout rates were relatively low and similar in both treatment groups. Another strength is the use of a pragmatic clinical approach: the women were treated routinely and after treatment with metformin/placebo only, appropriate additional treatment was chosen. This may also be considered as a limitation of the study because it resulted in different treatment subgroups. In particular, the allocation of some of the couples to IVF/ICSI because of the coexistence of a male factor could have biased the results. This is not probable, however, because the distribution to metformin and placebo groups was random and, after excluding the IVF/ICSI cases, PR and LBR remained significantly higher in the metformin group. This study was not specifically designed to explore the mechanism of action of metformin, and it appears that at least some of its beneficial effect on PR and LBR is associated with the weight loss in the metformin group. To explore this issue, weight loss should have been used as a time-dependent covariate in the intent-to-treat analysis. This would have required weight measurement at frequent weekly intervals, which was not possible. Thus, whether the beneficial effects of metformin are due to its weight-lowering properties or to other reasons cannot be solved on the basis of the results of this study. Another limitation is the possibility that the lower dose of metformin (1500 *vs.* 2000 mg/d in the obese group) in nonobese women may have contributed to the less marked effect in this group. However, previous studies in nonobese women with PCOS have shown that a dose of 1500 mg/d is efficient enough to restore ovulation and significantly improve hyperandrogenism and insulin sensitivity (21, 22). By using a smaller dose in nonobese patients we aimed to minimize side effects and dropouts. Still, the difference in dosage between obese and nonobese patients could be responsible for the differences in the effects of metformin between the two groups, and further studies with similar doses in obese and nonobese women are needed to clarify this important issue.

In conclusion, the miscarriage rate was not elevated and metformin treatment up to the 12th week of pregnancy did not decrease it. Three months of pretreatment with metformin only and its combination with standard infertility treatment thereafter improved PR and LBR in women with PCOS. Obese women especially seem to benefit from metformin in the treatment of anovulatory infertility in cases of PCOS.

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