0013-7227/02/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 87(2):569–574

Ovarian Function and Metabolic Factors in Women with Oligomenorrhea Treated with Metformin in a Randomized Double Blind Placebo-Controlled Trial

RICHARD FLEMING, ZOE E. HOPKINSON, A. MICHAEL WALLACE, IAN A. GREER, AND NAVEED SATTAR

University Departments of Obstetrics and Gynecology (R.F., Z.E.H., I.A.G.) and Pathological Biochemistry (A.M.W., N.S.), Royal Infirmary, Glasgow, G31 2ER, United Kingdom

Women with oligomenorrhea and polycystic ovaries show a high incidence of ovulation failure perhaps linked to insulin resistance and related metabolic features. A number of reports show that the biguanide metformin improves ovarian function. However, in these trials the quality of evidence supporting ovulation is suboptimal, and few studies have been placebo-controlled. The aim of our study was to use a double-blind, placebo-controlled approach with detailed assessment of ovarian activity (two blood samples per week) to assess the validity of this therapeutic approach in this group of women.

Of the 94 patients randomized, 2 withdrew before treatment commenced, 47 received placebo, and 45 received metformin (850 mg, twice a day). The numbers discontinuing the study prematurely were higher in the treatment group (n = 15) than the placebo group (n = 5; $P < 0.05$). The ovulation frequency assessed by the ratio of luteal phase weeks to observation weeks was significantly ($P < 0.01$) higher in the treated group (23%) compared with the placebo (13%), and the time to first ovulation was significantly ($P < 0.05$) shorter [23.6 d; 95% confidence interval (CI), 17, 30; compared with 41.8 d; 95% CI, 28, 56]. The proportion of patients failing to ovulate during the placebo-treatment period was higher ($P < 0.05$) in the placebo group, and the majority of ovulations were characterized by normal progesterone concentrations in both groups. The effect of metformin on follicular maturation was rapid, because the E2 circulating concentration increased over the first week of treatment only in the metformin group. Significant ($P < 0.01$) weight loss (and leptin reduction) was recorded in the metformin group, whereas the placebo group actually increased weight ($P < 0.05$). A significant increase in circulating high-density lipoprotein was observed only in the metformin-treated group. Metabolic risk factor benefits of metformin treatment were not observed in the morbidly obese subgroup of patients (body mass index > 37). No change in fasting glucose concentrations, fasting insulin, or insulin responses to glucose challenge was recorded after 14-wk metformin or placebo therapy. There was an inverse relationship between body mass and treatment efficacy.

We show in a large randomized placebo-controlled trial that metformin treatment improves ovulation frequency in women with abnormal ovarian function and polycystic ovaries significantly but to a modest degree, and protracted treatment improves cardiovascular risk factors. These data support a beneficial effect of metformin in improving ovarian function in women with oligomenorrhea and polycystic ovaries. (*J Clin Endocrinol Metab* 87: 569–574, 2002)

POLYCYSTIC OVARY SYNDROME (PCOS) is characterized by disturbed ovarian function, usually manifest as oligomenorrhea, and infertility is one of the principal clinical sequelae. It is now accepted that insulin resistance (1, 2) and/or abnormal insulin responses to glucose stimulus (3) are principal underlying etiologic factors of PCOS. The combination of symptoms has led to a link between PCOS and the metabolic syndrome (2). Women with PCOS exhibit most of the features of metabolic syndrome X, in particular dysipidemia (4, 5) and glucose intolerance (6). In turn, this association has led to treatment of women with PCOS with insulin sensitizing agents such as troglitazone (7) and metformin (8). A number of small randomized and nonrandomized cohort studies have shown that women with PCOS respond to this therapy with increases in ovarian activity and menstrual frequency. The relationships between treatment outcome, anthropometric changes, glycemic, metabolic, and lipid profile adjustments, however, are less comprehensively studied and remain disputed.

Some of the differences in published results may derive from patient selection, because patient profiles can differ between infertility and endocrinology clinics and perhaps also in racial and socioeconomic makeup. Furthermore, a minority of the published studies using metformin are double blind, placebo-controlled in design, with the majority being small cohort studies (<50 patients). In particular, direct assessment of follicular development or ovulation or progesterone elevations has been far from comprehensive. The latter point is relevant because a number of the ovulations in women with PCOS demonstrate subnormal progesterone concentrations (9), which may represent suboptimal follicular maturation and ovulation.

The aim of this study was to investigate the effects of metformin on detailed ovarian function in women with oligomenorrhea and polycystic ovaries (PCOs) who were treated using a randomized, double blind placebo-controlled trial of 16-wk treatment duration. Changes in anthropometric criteria, glycemic indices, leptin and lipid profile were also examined in relation to evolution of ovarian function.

Abbreviations: AUC, Area under curve; BMI, body mass index; CI, confidence interval; GTT, glucose tolerance test; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PCO, polycystic ovary; PCOS, PCO syndrome; VLDL, very LDL; WHR, waist to hip ratio.

Patients and Methods

Patients

Women with oligomenorrhea (cycle length ≥ 41d; <8 cycles per year) or amenorrhea and PCOs, aged less than 35 yr, were recruited from gynecology, endocrine, and infertility outpatient clinics. Patients with significant hyperprolactinemia, abnormal thyroid function tests, and congenital adrenal hyperplasia were excluded. Transvaginal ultrasound scans, effected by a single observer (Z.E.H.) were undertaken to assess ovarian appearance, and ovaries were described as polycystic (PCOs) according to the criteria of Adams *et al.* (10). None of the patients was taking medications likely to influence hormonal profiles. This diagnosis was used on the understanding that the overwhelming majority of patients defined on this basis would demonstrate elevated androgen activity, symptoms of hyperandrogenism, or both (11).

Protocol

Ovarian activity was investigated before and throughout the study, using two blood samples per week for assessment of reproductive hormone concentrations. Before randomization, all patients underwent a 4-wk period of investigation to confirm abnormal ovarian function. The same assessment schedule was maintained through a subsequent 16-wk treatment period after randomization to metformin (Merk, West Drayton, UK) or matching placebo. Anthropometric, endocrine, and ovarian ultrasound assessments were effected before and after 14-wk treatment (effectively between 12–16 wk). The latter time window was used so that the measurements could be taken outside a luteal phase. The tests were performed only after confirmation that the circulating progesterone concentration was less than 6 nmol/liter.

Metformin was administered at a dosage of 850 mg twice daily, except for the first week of treatment when 850 mg was given only once per day. The dosage was graduated in an attempt to reduce the incidence and severity of known gastrointestinal side effects.

Women were advised to use barrier contraception if fertility was not desired. Monitoring of the circulating steroid hormone profiles allowed assessment of possible pregnancy, and plasma concentrations of human chorionic gonadotrophin were used to allow discontinuation of treatment if pregnancy occurred.

Randomization and study power

Randomization was effected in a double blind fashion; patients received either metformin or placebo according to the code provided (computer-generated randomization in blocks of four) by the pharmacy department of the Royal Infirmary (Glasgow). A copy of the code was stored in the hospital pharmacy for emergency situations. The randomization code was not broken until after the last patient completed all observations.

The study power was based upon predicted changes in the ovulation rate and circulating lipoprotein concentrations, using data derived from the literature and our own pilot study (12). The calculation was adapted to account for the fact that 70–80% of the cases would have classical PCOS, a significant dropout rate (15%), and a failure to attain normal menstrual frequency in another 15% of cases. It was estimated that 38 patients in each arm would detect changes in high-density lipoprotein (HDL) cholesterol with more than 90% power with a type 1 error (a) = 0.05. It was predicted that the study required 55 cases in each arm to achieve the stated aim.

Study parameters: endocrinology

Before randomization and during the ovarian function assessment, all patients were evaluated for endocrine factors while outside the luteal phase (progesterone concentration, <6 nmol/liter) when they attended the hospital after an overnight fast. Blood samples were taken for assays of E2, T, androstenedione, LH, FSH, triglycerides, cholesterol, low-density lipoprotein (LDL) cholesterol, and HDL cholesterol. Then, a standardized 75-g oral glucose tolerance test (GTT) was undertaken with blood samples collected at 0, 60, and 120 min for determination of serum glucose and insulin concentrations. This process was repeated at the 14-wk assessment point.

Ovarian activity ovulation and the luteal ratio

Ovarian activity was monitored using serum E2 rapid (same day) measurements; where follicular activity was diagnosed (E2 > 300 pmol/liter), progesterone and LH concentrations were determined to diagnose ovulation and the luteal phase. Ovulation frequency was calculated using the ratio of luteal phase weeks to observation weeks (the luteal ratio), such that an individual with normal menstrual rhythm would show two luteal weeks in four observation weeks, yielding a ratio of 0.5, expressed as a luteal ratio of 50%.

One patient conceived within a week of the end of her treatment schedule, and her data were included in the completed trial analyses, because all samples and tests had been undertaken for the treatment period. Ovarian hormone (E2, T, and inhibin-B) changes during the first week of treatment were assessed to determine short-term effects of the lower dose of treatment (850 mg/d).

Anthropometric and lifestyle parameters

Anthropometric data were collected (height, weight, waist, and hip measurements) before and at the 14th week of treatment or placebo by a single trained observer (Z.E.H.) using standardized techniques (13). The body mass index (BMI) was calculated using the standard formula (kilograms per square meter).

Each volunteer completed a questionnaire of medical and social history (desiring pregnancy, smoking habits), from which subjective information about menstrual patterns, skin oiliness, acne, and hirsutism were recorded. Ovarian ultrasound assessments were also effected before treatment and at 14 wk by the same observer.

Assay methods

The reproductive hormones, E2 and progesterone, were assayed routinely using the semiautomated Immulite technology (Diagnostic Products, Los Angeles, CA). The analytes T, LH, FSH, and β human chorionic gonadotrophin were assayed retrospectively in batches using the same system. Inhibin-B was measured using the specific two-site immunoassay (Serotec Ltd., Oxford, UK). Plasma glucose was measured using the glucose oxidase method (Glucose Reagent Kit, Bayer, Newbury, UK), whereas insulin was measured using a competitive RIA (Coat-A-Count I, Diagnostic Products). Plasma total cholesterol, triglyceride, HDL cholesterol, and LDL cholesterol measurements were performed by a modification of the standard Lipid Research Clinics protocol (14). Serum leptin concentrations were measured by a validated in-house RIA (15). The intra- and interassay coefficients of variation were less than 7 and 10%, respectively, over the sample concentration range. The detection limit of the assay was 0.5 ng/ml.

Data analyses and statistics

Data were analyzed on the basis of intention to treat and also on completed treatment parameters where relevant. Fasting and postglucose insulin [area under curve (AUC)], SHBG, waist to hip ratio (WHR), triglyceride, and the ovulatory function were compared between treatment and placebo groups after log transformation if the distributions were not normal. Hormone and comparative data were presented with confidence limits at 95%. Statistical information was prepared using the SPSS for Windows software (SPSS, Inc., Chicago, IL). Hormone data were compared using *t* test after log transformation if distributions were normalized. Incidences were compared using contingency table (χ^2) analyses.

Ethical approval

Ethical committee approval was obtained before the study, and written informed consent was given by each patient.

Results

Recruitment, randomization, and pretreatment assessments

A total of 136 women were interviewed for inclusion in the study. Of these, two women were diagnosed with late onset congenital adrenal hyperplasia, and there were two new

cases of type 2 diabetes mellitus. Another 38 women did not have true oligomenorrhea on further assessment or declined to proceed for personal reasons. Thus, a total of 94 patients proceeded to randomization, receiving either metformin or placebo. Figure 1 shows the progress of these patients through the program. The reasons for presentation were varied, and infertility was a complaint in only about half of the patients in each group. There was no difference in the proportions of infertile women within the groups (Table 1). Although patient selection was based on the more wide-ranging definition often used in Europe (*i.e.* ultrasound-diagnosed PCOs and oligomenorrhea), 90% had biochemical or clinical evidence of hyperandrogenism. Table 1 also shows that the metformin and placebo groups were matched for menstrual frequency in the preceding year, age, BMI, T, SHBG, fasting glucose, hemoglobin A1c, and circulating lipid fractions before treatment. The proportions of patients seeking fertility treatment were also similar in each group. All women showed a classical picture of PCOs on vaginal ultrasound scan.

Treatment compliance

The difference in the dropout rates (excluding pregnancies) between the placebo (n = 5) and treatment (n = 15) groups was significant ($P < 0.05$) and was due mainly to unacceptable gastrointestinal side effects associated with metformin. In the placebo group, the dropouts occurred after 1, 1, 5, 6, and 7 wk. In the metformin-treated patients, the discontinuations also occurred early, with seven cases in the first 3 wk and the remainder between 6–10 wk. Two of this latter group were for reasons unrelated to the study.

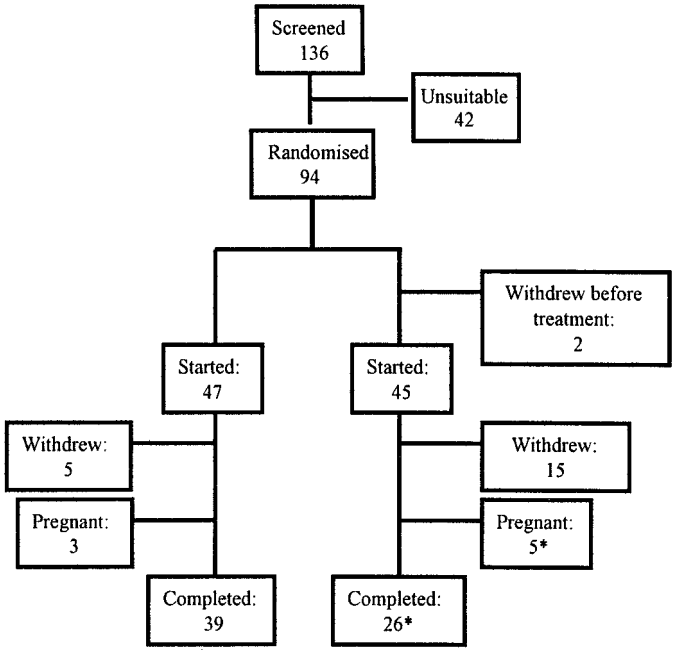

FIG. 1. Chart of patients who were randomized progressing through the trial of metformin or placebo treatment. *, One patient became pregnant at the end of the trial, after the 14-wk assessment, so her data are included in the completed group.

Conception during treatment

There were eight conceptions in eight patients during the study, and one miscarried in the first trimester. However, only 42 of the patients declared before the study that they wished to conceive. Of these, the distribution of pregnancies was: placebo, 1 of 19 patients; and metformin, 4 of 23 patients. These figures are not significantly different ($P = 0.23$).

Ovarian function: ovulation

An intention to treat analysis revealed that 8 of 45 metformin-treated patients failed to ovulate during treatment, compared with 17 of 47 placebo-treated. This difference was statistically significant (Fisher's exact test; $P = 0.04$; odds ratio, 0.38).

Table 2 shows the data from all cases in which ovulation data (over any length of time) were available. The metformin-treated group had a significantly increased frequency of ovulation compared with the placebo group, defined by the luteal ratio.

Figure 2 shows the proportions of both groups exhibiting zero to four ovulations during the 16-wk treatment period. The distributions show that the placebo group was dominant at low ovulation rate (zero and one ovulations), whereas the metformin group was dominant in the high ovulation rate (two to four ovulations). Contingency table analysis (Fisher's exact) showed these distributions to be marginally different as follows: zero and one ovulation, $P = 0.059$; two to four ovulations, $P = 0.059$.

TABLE 1. Characteristics of the patients randomized to receive metformin or placebo treatment

	Placebo		Metformin	
	Mean	CIs	Mean	CIs
Age (yr)	29.2	27.5–30.7	28.6	26.9–30.3
Menses per year	4.0	3.1–4.9	4.6	3.5–5.6
BMI (kg/m^2)	35.0	32.6–37.3	34.2	31.7–36.7
WHR	0.88	0.86–0.90	0.88	0.86–0.90
LH (IU/liter)	10.1	8.3–11.9	8.3	6.9–9.7
T (nmol/liter)	3.8	3.3–4.2	3.0	2.6–3.5
SHBG (nmol/liter)	28.1	22.6–33.6	29.2	24.3–34.1
Free androgen index	13.7	10.7–16.8	10.3	8.6–12.1
Fasting insulin (mIU/liter)	18.4	14.5–22.3	16.7	13.0–20.4
Insulin AUC (GTT)	228	177–280	191	155–227
Fasting glucose (nmol/liter)	4.93	4.81–5.05	5.05	4.87–5.23
Leptin (ng/ml)	40.7	33.8–47.7	40.0	32.1–48.0
Inhibin-B (pg/ml)	82	67–97	101	88–115

No. of patients: placebo-treated, 47 (infertile, 19; hirsutism, 22); metformin-treated, 45 (infertile, 23; hirsutism, 13). *P* values are NS.
CIs, Confidence intervals (95%).

TABLE 2. Details of ovulations during placebo and metformin treatment

	Placebo	Metformin	*P*
Observation weeks	503	345	
Luteal weeks [luteal ratio (%)]	66 (13)	78 (23)	<0.001
Luteal phases with Pmax <7 ng/ml (%)	5 (13)	2 (8)	NS
Days to first ovulation, mean	41.8	23.6	0.02
(CIs, 95%)	(28, 56)	(17, 30)	

Pmax, Maximum progesterone concentration.

Table 2 also shows the frequency of ovulations with deficient luteal phases assessed by the maximum progesterone concentration less than 7 ng/ml. There was no significant difference between the incidences of this phenomenon in either group. In fact, the concentrations of progesterone recorded during monitoring of ovarian function indicated that most of the ovulations showed normal endocrine profiles during both metformin and placebo treatment.

All patients started treatment outside the luteal phase, and the delay to the first ovulation after starting the program (Table 2) was significantly shorter in the metformin-treated group.

Initial responses to treatment: follicular development

Inhibin-B is a marker of early follicular granulosa cell activity, and circulating E2 represents follicular maturation. Table 3 shows the E2, inhibin-B, and T concentrations on the first and eighth days of treatment, showing that the metformin-treated group had a significant ($P < 0.03$, paired data) increase in mean E2, whereas the control group showed no change. There was no change in the circulating inhibin-B or T concentrations. These profiles suggest that although improved follicular maturation was detected, there appeared to be no change in the remainder of the ovarian metabolism (total immature granulosa cell activity and stromal androgen biosynthesis).

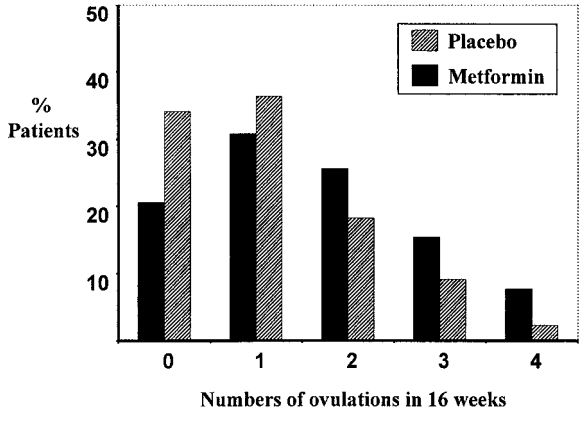

FIG. 2. Distributions of ovulations among the metformin- and placebo-treated patients. Ovulations are presented as percentages of patients completing treatment with zero to four ovulations during the 16-wk monitoring period.

TABLE 3. The reproductive hormone changes over the first week of metformin treatment

	Day 1		Day 8		*P*
	Mean	CIs	Mean	CIs	
Placebo					
E2 (pmol/liter)	164	110–217	183	127–240	NS
Inhibin-B (pg/ml)	82	67–97	88	71–105	NS
T (nmol/liter)	3.8	3.4–4.5	4.2	3.5–4.9	NS
Metformin					
E2 (pmol/liter)	142	123–161	226	150–302	<0.03
Inhibin-B (pg/ml)	101	88–114	96	83–108	NS
T (nmol/liter)	3.1	2.4–3.8	3.5	2.8–4.2	NS

Metabolic and anthropometric assessments

Table 4 shows that after 14-wk treatment, the BMI decreased significantly in the metformin group, whereas it increased in the placebo group. There was no change seen in the WHR in either group. The circulating leptin concentration declined in the metformin-treated group, in contrast to the control group, but there was no change recorded in the fasting glucose, fasting insulin, or insulin AUC in response to the glucose challenge in either group. Circulating very LDL (VLDL) showed little change during the treatment period, but the LDL showed a trend toward reduction, and HDL increased significantly in the metformin group. It is possible that the reduction in HDL was related to the weight loss achieved in the metformin-treated patients, although the ANOVA ($r = 0.34$; $P = 0.07$) did not reach conventional levels of significance.

Subgroup analyses

Characteristics of the group that responded to metformin with normal ovulation frequency. A total of 11 patients who responded to metformin by establishing normal ovulation frequency ($n = 6$) and/or pregnancy ($n = 5$) were compared with those patients who did not respond with establishment of normal ovarian function (less than three ovulations in 16 wk; $n = 19$). The two groups showed similar BMI, WHR, and circulating E2 and inhibin-B concentrations. However, responders to metformin treatment showed significantly lower T (2.5 nmol/liter *vs.* 3.5 nmol/liter; 95% CI = 0.07 and 2.1, respectively; $P = 0.04$), higher SHBG (36.5 nmol/liter *vs.* 26.3 nmol/liter; 95% CI, 20.6 and 0.13; $P = 0.05$), and thus lower free androgen index (7.2 *vs.* 11.9; 95% CI, 1.2 and 8.1; $P = 0.01$). Fasting insulin or glucose concentrations or responses to the GTT were not significantly different.

Metabolic responses and obesity. It was observed that morbidly obese women (BMI > 37; $n = 11$) showed a similar number of ovulations (mean, 1.6) during 16-wk metformin treatment to the leaner women (mean, 2.1), but they showed no indication of changes in either BMI (pretreatment, 42.5 kg/m^2; week 14, 42.3 kg/m^2) or HDL cholesterol (pretreatment, 0.95 mmol/liter; week 14, 0.95 mmol/liter). The leaner women (BMI < 37 kg/m^2) showed distinct changes during treatment as follows: BMI, pretreatment, 29.4 kg/m^2; week 14, 28.5 kg/m^2 ($P < 0.01$); or HDL cholesterol, pretreatment, 1.21 mmol/liter; week 14, 1.32 mmol/liter ($P < 0.02$).

Discussion

This study is the first to present a comprehensive, detailed endocrinological assessment of ovarian function in the context of a large randomized placebo-controlled trial of metformin in women with abnormal ovarian function. Our data show clear beneficial effect of metformin treatment upon ovarian function, anthropometric measures, and lipid profiles in women with oligomenorrhea and PCOS. We observed that more than 30% of the patients established normal ovarian rhythm (three or more ovulations) through the 16-wk treatment period. This contrasted with 18% for the placebo group. The ovulations showed normal progesterone concentration profiles in a high frequency of the cycles, in-

TABLE 4. Changes in metabolic parameters during placebo or metformin treatment

	Placebo (n = 39 pairs)			Metformin (n = 26 pairs)		
	Pretreatment	14 wk	*P*	Pretreatment	14 wk	*P*
BMI (SD)	35.3	35.6	0.04	35.2	34.6	0.03
WHR	0.88	0.88	NS	0.88	0.88	NS
Leptin (ng/ml) (SD)	40.6	38.5	NS	41.1	37.3	0.05
Fasting insulin (mIU/liter)	18.4	17.5	NS	16.8	16.4	NS
GTT insulin AUC	221	221	NS	188	204	NS
Fasting glucose (nmol/liter)	4.9	5.0	NS	5.0	5.0	NS
Total cholesterol (nmol/liter)	4.93	4.90	NS	4.61	4.50	NS
Triglycerides (mmol/liter)	1.40	1.44	NS	1.62	1.63	NS
VLDL cholesterol (mmol/liter)	0.42	0.54	NS	0.52	0.54	NS
LDL cholesterol (mmol/liter)	3.41	3.27	NS	3.01	2.81	0.09
HDL cholesterol (mmol/liter)	1.13	1.13	NS	1.08	1.14	0.03

Statistical probability by *t* test for paired data.

dicating that these were fertile cycles. The mean time until the first ovulation was significantly shorter in the metformin-treated group (24 d) than in the placebo-treated group (42 d). This suggests a relatively rapid effect of treatment upon ovarian function, which is further supported by the significant increase in E2 concentrations during the first week of treatment when the metformin dosage was only 850 mg/d. At week 14 assessment, the metformin patients showed significant reductions in weight, in contrast to patients in the placebo group who actually increased their BMI. Associated with the weight loss were significant reductions in circulating leptin and increased HDL cholesterol concentrations in the metformin-treated group. LDL cholesterol showed a trend toward reduction, and overall the LDL cholesterol to HDL cholesterol ratio improved significantly in the metformin group (data not shown). Despite increased ovulation frequency, there were no changes in circulating androgen concentrations, glycemic indices, basal or provoked insulin levels, or circulating VLDL cholesterol concentrations. Our data on HDL cholesterol are important, because only one previous study (16) has addressed this important issue.

Subgroup analyses comparing those patients who showed a high ovulation rate during metformin treatment with those who were resistant to it indicated that the least androgenic patients were more likely to respond with establishment of normal menstrual rhythm. Furthermore, the morbidly obese patients (BMI $>$ 37) showed no cardiovascular risk factor (BMI and HDL cholesterol) benefit. Taken together, these data suggest that either higher doses of metformin may prove to be more beneficial in the morbidly obese patient or such patients may be resistant to this form of therapy. These assertions remain to be tested in future studies.

A number of reports have indicated that insulin sensitizing agents improve ovulation rates in women with PCOS, and they have shown conflicting results with respect to changes in ovulation rate and also changes in endocrinology during metformin treatment. Our results are consistent with two studies (17, 18) that also show that metformin treatment failed to bring about changes in hyperandrogenism or hyperinsulinemia. On the other hand, a number of studies have shown decreases in hyperandrogenism and markers of insulin resistance with metformin in PCOS (19–22). A recent comprehensive multicenter, multidose study using the peroxisome proliferator-activated receptor agonist troglitazone (7) showed improvements in hyperandrogenism, mediated through circulating free androgens rather than total androgen concentrations, and also in glycemic indices. These changes were dose-related, as were improvements in ovulation rates. It is possible that patient selection criteria may have an impact on the potential for beneficial effects of metformin on surrogate markers of insulin resistance and hyperandrogenism. The principal inclusion criteria in our study was disturbances of ovarian function, whereas in other studies the emphasis may have been on more profound metabolic derangements, including clinical manifestations of hyperandrogenism.

It is noteworthy that the higher doses of troglitazone treatment (300 and 600 mg) were associated with weight increase in women who were generally overweight at the time of starting (7). Weight loss achieved in the metformin-treated patients would be considered a beneficial side effect of treatment, and indeed, in our study women in the active arm lost significant weight during treatment, whereas those on placebo gained weight over the 4-month period.

The increase in ovulation rate seen in the metformin-treated patients appeared to take place rapidly, as evidenced by significant increases in circulating E2 concentrations, representing follicular maturation, within the first 8 d of treatment and also the shorter mean time to first ovulation. This effect is likely to have taken place before significant weight loss or changes in the lipid profiles, and also in the absence of changes in glycemic indices. This leads to the possibility of direct gonadal effects of metformin as has been demonstrated for the peroxisome proliferator-activated receptor agonist troglitazone (23). Reduced total ovarian steroid 17α hydroxylase activity has been reported during metformin treatment (18), which may be either a direct effect upon insulin metabolism at the ovarian level or a consequence of rearranged follicular profiles secondary to follicular maturation and ovulation.

A recent editorial (24) proposed that metformin should become front line therapy for infertility, with or without clomiphene citrate therapy. Our randomized study provides some support for this proposal, although it should be noted that ovulation was only modestly improved in the metformin group. Indeed, only a third of the treated cases established normal ovulation frequency with immediate effect. Thus, before treatment with metformin becomes established prac-

tice in this circumstance, larger and longer controlled studies should be undertaken. These should be dose-determining and aimed to define patient characteristics that best predict beneficial response to metformin treatment. Furthermore, we also suggest that the problems of maternal obesity be carefully considered with such treatment, and that weight loss may be the better approach (25) in many circumstances.

Finally, the high dropout rate in the metformin arm (more than 30%) is notable. Clinically, this observation is important and indicates that significant side effects on the dosage regime we used are common. Most of the discontinuation cases occurred at the early part of treatment, suggesting that women prescribed metformin should be adequately counseled and perhaps actively supported through this stage. Care should be taken in the introduction of this treatment, starting perhaps with 500-mg tablets initially, rather than 850-mg tablets used in this study.

In conclusion, using a comprehensive, detailed endocrinological assessment of ovarian function, we have shown that metformin treatment increases ovulation rates by a significant but modest degree in women with oligomenorrhea and PCOS. Continued treatment also resulted in significant weight loss (and leptin reduction) and an associated change in HDL cholesterol. These beneficial effects of metformin support a future therapeutic role in women with PCOS. Further large randomized studies are needed to determine appropriate dosages and duration of treatment.

Acknowledgments

This work was sponsored by the Scottish Office [K/MRS/50/C2584]. Thanks are due to Dr. Harry Howlett (Merk, West Drayton, UK) for supplying the metformin and placebo tablets.

Received August 16, 2001. Accepted November 3, 2001.

Address all correspondence and requests for reprints to: Dr. Richard Fleming, University Department of Obstetrics and Gynecology, Level 3, Queen Elizabeth Building, Royal Infirmary, Glasgow, G31 2ER, United Kingdom. E-mail: gqta13@udcf.gla.ac.uk.

References

1. **Dunaif A** 1997 Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. Endocr Rev 18:774–800
2. **Hopkinson ZE, Sattar N, Fleming R, Greer IA** 1998 Polycystic ovary syndrome: the metabolic syndrome comes to gynaecology. Br Med J 317:329–332
3. **Holte J, Bergh T, Berne C, Berglund L, Lithell H** 1994 Enhanced early insulin response to glucose in relation to insulin resistance in women with polycystic ovary syndrome and normal glucose tolerance. J Clin Endocrinol Metab 78: 1052–1058
4. **Robinson S, Henderson AD, Gelding SV, Kiddy D, Niththyananthan R, Bush A, Richmond W, Johnston DG, Franks S** 1996 Dyslipidaemia is associated with insulin resistance in women with polycystic ovaries. Clin Endocrinol (Oxf) 44:277–284
5. **Pirwany IR, Fleming R, Greer IA, Packard CJ, Sattar N** 2001 Lipids and lipoprotein subfractions in women with PCOS: relationship to metabolic and endocrine parameters. Clin Endocrinol (Oxf) 54:1–8
6. **Ehrmann DA, Barnes RB, Rosenfield RL, Cavaghan MK, Imperial J** 1999 Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. Diabetes Care 22:141–146
7. **Azziz R, Ehrmann D, Legro RS, Whitcomb RW, Hanley R, Fereshetian AG, O'Keefe M, Ghazzi MN, PCOS/Troglitazone Study Group** 2001 Troglitazone improves ovulation and hirsutism in the polycystic ovary syndrome: a multicenter, double blind, placebo-controlled trial. J Clin Endocrinol Metab 86: 1626–1632
8. **Nestler JE, Jakubowicz DJ, Evanc WS, Pasquali R** 1998 Effects of metformin on spontaneous and clomiphene-induced ovulation in the polycystic ovary syndrome. N Engl J Med 338:1876–1880
9. **Fleming R, McQueen D, Yates RWS, Coutts JRT** 1995 Spontaneous follicular and luteal function in infertile women with oligomenorrhoea: role of luteinizing hormone. Clin Endocrinol (Oxf) 43:735–739
10. **Adams J, Polson JW, Franks S** 1986 Prevalence of polycystic ovaries in women with anovulation and idiopathic hirsutism. Br Med J 293:355–359
11. **White DM, Polson DW, Kiddy D, Sagle P, Watson H, Gilling-Smith C, Hamilton-Fairley D, Franks S** 1996 Induction of ovulation with low-dose gonadotropins in polycystic ovary syndrome: an analysis of 109 pregnancies in 225 women. J Clin Endocrinol Metab 81:3821–3824
12. **Pirwany IR, Yates RW, Cameron IT, Fleming R** 1999 Effects of the insulin sensitizing drug metformin on ovarian function, follicular growth and ovulation rate in obese women with oligomenorrhoea. Hum Reprod 14:2963–2968
13. **World Health Organization** 1989 Measuring obesity: classification and distribution of anthropometric data. Copenhagen: WHO (Nutr UD, EUR/ICP/NUT 125)
14. **Lipid Research Clinics Program** 1975 Lipid and lipoprotein analysis, manual of laboratory operations, 1. Bethesda, MD: National Institutes of Health, DHEW Publications; 75
15. **McConway MG, Johnson D, Kelly A, Griffin D, Smith J, Wallace AM** 2000 Differences in circulating concentrations of total, free and bound leptin relate to gender and body composition in adult humans. Ann Clin Biochem 37: 717–723
16. **Moghetti P, Castello R, Negri C, Tosi F, Perrone F, Caputo M, Zanolin E, Muggeo M** 2000 Metformin effects on clinical features, endocrine and metabolic profiles, and insulin sensitivity in polycystic ovary syndrome: a randomized, double-blind, placebo-controlled 6-month trial, followed by open, long-term clinical evaluation. J Clin Endocrinol Metab 85:139–146
17. **Ehrmann DA, Cavaghan MK, Imperial J, Sturis J, Rosenfield RL, Polonsky KS** 1997 Effects of metformin on insulin secretion, insulin action, and ovarian steroidogenesis in women with polycystic ovary syndrome. J Clin Endocrinol Metab 82:524–530
18. **Morin-Papunen LC, Koivunen RM, Ruokonen A, Martikainen HK** 1998 Metformin therapy improves the menstrual pattern with minimal endocrine and metabolic effects in women with polycystic ovary syndrome. Fertil Steril 69:691–696
19. **Nestler JE, Jakubowicz DJ** 1997 Lean women with polycystic ovary syndrome respond to insulin reduction with decreases in ovarian P450c17α hydroxylase activity and serum androgens. J Clin Endocrinol Metab 82:4075–4079
20. **la Marca A, Morgante G, Paglia T, Ciotta L, Ciani A, De Leo V** 2000 Effects of metformin on adrenal steroidogenesis in women with polycystic ovary syndrome. Fertil Steril 72:985–989
21. **Saleh A, Morris D, Tan SL, Tulandi T** 2001 Effects of laparoscopic ovarian drilling on adrenal steroids in polycystic ovary syndrome patients with and without hyperinsulinaemia. Fertil Steril 75:501–504
22. **Kolodziejczyk B, Duleba AJ, Spaczynski RZ, Pawelczyk L** 2000 Metformin therapy decreases hyperandrogenism and hyperinsulinaemia in women with polycystic ovary syndrome. Fertil Steril 73:1149–1154
23. **Gasic A, Bodenburg Y, Nagami M, Green A, Urban RJ** 1998 Troglitazone inhibits progesterone production in porcine granulosa cells. Endocrinology 139:4962–4966
24. **Kim L, Taylor AE, Barbieri RL** 2000 Insulin sensitizers and polycystic ovary syndrome: can a diabetes medication treat infertility? Fertil Steril 73:1097–1099
25. **Clark AM, Thornley B, Tomlinson L, Galletley C, Norman RJ** 1998 Weight loss in obese infertile women results in improvement in the reproductive outcome for all forms of fertility treatment. Hum Reprod 13:1502–1505