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Combined lifestyle modification and metformin in obese patients with polycystic ovary syndrome. A randomized, placebo-controlled, double-blind multicentre study

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BACKGROUND: It has been reported that women with polycystic ovary syndrome (PCOS) benefit from metformin therapy. METHODS: A randomized, placebo-controlled, double-blind study of obese (body mass index >30 kg/m^2), oligo-/amenorrhoeic women with PCOS. Metformin (850 mg) twice daily was compared with placebo over 6 months. All received the same advice from a dietitian. The primary outcome measures were: (i) change in menstrual cycle; (ii) change in arthropometric measurements; and (iii) changes in the endocrine parameters, insulin sensitivity and lipid profile. RESULTS: A total of 143 subjects was randomized [metformin (MET) = 69; placebo (PL) = 74]. Both groups showed significant improvements in menstrual frequency [median increase (MET = 1, *P* < 0.001; PL = 1, *P* < 0.001)] and weight loss [mean (kg) (MET = 2.84; *P* < 0.001 and PL = 1.46; *P* = 0.011)]. However, there were no significant differences between the groups. Logistic regression analysis was used to analyse the independent variables (metformin, percentage of weight loss, initial BMI and age) in order to predict the improvement of menses. Only the percentage weight loss correlated with an improvement in menses (regression coefficient = 0.199, *P* = 0.047, odds ratio = 1.126, 95% CI 1.001, 1.266). There were no significant changes in insulin sensitivity or lipid profiles in either of the groups. Those who received metformin achieved a significant reduction in waist circumference and free androgen index. CONCLUSIONS: Metformin does not improve weight loss or menstrual frequency in obese patients with PCOS. Weight loss alone through lifestyle changes improves menstrual frequency.

Key words: menstrual frequency/metformin/obese/polycystic ovary syndrome/weight loss

Introduction

The polycystic ovary syndrome (PCOS) is the commonest endocrine disturbance in women (Balen and Michelmore, 2002) and the commonest cause of anovulatory infertility. PCOS is a heterogeneous disorder with features including hyperandrogenism, menstrual irregularity and obesity (Balen *et al.*, 1999; Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). The association between insulin resistance, compensatory hyperinsulinaemia and hyperandrogenism have provided insight into the pathogenesis of PCOS (Tsilchorozidou *et al.*, 2004). Insulin resistance occurs in both slim and overweight women with PCOS, although there is debate on the proportion of women with PCOS with reduced insulin sensitivity (Cibula *et al.*, 2004). At least 40% of women with PCOS are obese (Balen *et al.*, 1995) and they are more insulin resistant than weight-matched individuals with normal ovaries (Dunaif *et al.*, 1995; Morales *et al.*, 1996).

Obesity and particularly abdominal obesity as indicated by an increased waist:hip ratio is correlated with reduced fecundity (Zaadstra *et al.*, 1993; Kirchengast and Huber, 2004; Pasquali *et al.*, 2003), menstrual disorders and hyperinsulinaemia (Conway *et al.*, 1990; Lord and Wille, 2002). Obesity correlates with an increased rate of menstrual cycle disturbance and infertility (Kiddy *et al.*, 1990; Balen *et al.*, 1995). Weight loss improves the endocrine profile, the menstrual cyclicity, the likelihood of ovulation and of a healthy pregnancy (Pasquali *et al.*, 1989; Kiddy *et al.*, 1992; Huber-Buchholz *et al.*, 1999). Studies by Clark *et al.* (1995, 1998), demonstrated that weight loss achieved by an exercise schedule, combined with a hypocaloric diet over a 6 month period, improved insulin sensitivity, endocrine parameters, menstrual regularity, the frequency of spontaneous ovulation and the chance of pregnancy.

Even a modest weight loss of 2–5% of total body weight can restore ovulation in overweight women with PCOS as well as achieving a reduction of central fat and an improvement in insulin sensitivity (Huber-Buchholz *et al.*, 1999). Rather than absolute weight, it is the distribution of fat that is important with android (central) obesity being more of a risk factor than gynaecoid obesity (Despres *et al.*, 2001; Lord and Wille,

2002). Visceral adipose tissue is more metabolically active than subcutaneous fat and the amount of visceral fat correlates with insulin resistance and hyperinsulinaemia. Weight reduction of 5–10% may result in ~30% loss of visceral adipose tissue (Despres *et al.*, 2001) and this may explain why a modest weight loss can significantly improve metabolic and reproductive function. Waist circumference has been shown to correlate better with visceral fat than waist:hip ratio (WHR) (Lord and Wille, 2002), and a waist circumference in women >88 cm is indicative of an increased metabolic risk (Despres *et al.*, 2001).

Lifestyle modification is a key component for the improvement of reproductive function for overweight, anovulatory women with PCOS (Norman *et al.*, 2002, 2004; Pasquali *et al.*, 2003). Weight loss should therefore be encouraged prior to ovulation induction treatments, since these are less effective when the body mass index (BMI) is >28–30 kg/m^2 (Hamilton-Fairley *et al.*, 1992). Monitoring treatment is also harder in the obese as visualization of the ovaries is more difficult which raises the risk of multiple ovulation and multiple pregnancy. Furthermore, pregnancy carries greater risks in the obese, for example: miscarriage, gestational diabetes, hypertension and problems with delivery (Gjonnaess *et al.*, 1989; Sebire *et al.*, 2001; Cedergren, 2004; Linné, 2004).

It is logical to assume that therapy that achieves a fall in serum insulin concentrations should improve the symptoms of PCOS (Norman *et al.*, 2004). The biguanide metformin both inhibits the production of hepatic glucose, thereby decreasing insulin secretion, and enhances insulin sensitivity at the cellular level (Matthaei *et al.*, 2000). The efficacy of metformin in PCOS was first described by Velazquez *et al.* (1994) and a number of small, and often short duration, observational studies followed which showed variable outcomes. Most of the randomized studies have involved only a small number of participants. Indeed in a systematic review by Costello and Eden (2003), nine out 12 published studies on the effects of metformin alone on the menstrual cycle in women with PCOS had a sample size of <30 women. Lord *et al.* (2003) published a systematic review in the Cochrane Database which concluded that metformin has a beneficial effect for women with PCOS, by reducing serum insulin concentrations and thereby lowering androgen levels and improving reproductive outcomes. Back in 1997 we conceived what we anticipated to be an appropriately powered, prospective randomized, double-blind, placebo-controlled multicentre study to evaluate the combined effects of lifestyle modification and metformin on obese anovulatory women (BMI >30 kg/m^2) with PCOS. The study has taken a considerable time to complete and here we present our findings.

Materials and methods

Women were recruited from infertility clinics with anovulatory PCOS and a BMI of >30 kg/m^2, aged between 18 and 39 years inclusive and a desire to conceive. Anovulation was defined as the presence of amenorrhoea or oligomenorrhoea (cycle length >35 days) (Munster *et al.*, 1993; Berek *et al.*, 1996) and the absence of ovarian follicular activity on serial ultrasound scans. The patients had not received ovulation induction therapy from the fertility clinic as the usual criterion for any form of ovulation induction (clomiphene citrate or gonadotropin therapy) was a BMI of <30 kg/m^2.

PCOS was defined as the presence of polycystic ovaries on transvaginal scan, >10 cysts, 2–8 mm in diameter, usually combined with increased ovarian volume >10 cm^3, and an echo-dense stroma (after the transabdominal ultrasound criteria of Adams *et al.*, 1985), together with either oligomenorrhoea or amenorrhoea. Many patients also had clinical or biochemical hyperandrogenism, although this was not an entry criterion for the study. All patients had a baseline androgen profile, including measurement of testosterone and androstenedione. If either were significantly elevated, additional tests were performed to exclude hypercortisolism and congenital adrenal hyperplasia (CAH) (full steroid profile, 24 h urinary cortisol and adrenocorticotrophin hormone stimulation test). When the study was devised the Rotterdam consensus definitions of PCOS (2004) and of the polycystic ovary (Balen *et al.*, 2003) had not been defined, although all of our patients would have been classified as having PCOS by those criteria.

Pretreatment inclusion criteria also included the presence at least one patent Fallopian tube and a normal semen analysis from the male partner. All participants had normal serum prolactin concentrations, thyroid, renal and liver function and haematological indices, including serum B_{12} concentrations.

Exclusion criteria included concurrent hormone therapy within the previous 6 weeks, any chronic disease that could interfere with the absorption, distribution, metabolism or excretion of metformin, and renal or liver disease. Patients with significant systemic disease or diabetes (Type 1 or 2) were excluded. Patients with irregular menstrual bleeding were thoroughly assessed to exclude pathology of the genital tract other than PCOS and a negative pregnancy test was a prerequisite for commencing treatment.

Protocol

A multicentre research ethics committee approval (MREC 1999/8/12) and the local research ethics committee approval of each participating centre were obtained. After obtaining written consent, a full physical examination was performed including assessment of BMI, waist and hip circumference and blood pressure. A baseline transvaginal ultrasound scan was performed to assess ovarian morphology, uterine size and endometrial thickness. A standardized 75 g oral glucose tolerance test (OGTT) was performed with measurement of fasting insulin concentration and glucose at 0 and 120 min. Baseline serum endocrinology included the measurement of FSH, LH, testosterone, sex hormone-binding globulin (SHBG), total cholesterol and triglycerides.

The subjects were randomized to receive either metformin or placebo. The randomization process was carried out by the clinical trials office in the pharmacy department and blinded to patients and investigators. A block-of-four randomization technique was performed using random tables from Linder *et al.* (1970). The code was kept in the trial office until the last patient completed the study. Placebo tablets for metformin were identical in appearance (size and colour) to metformin and were supplied by Penn Pharmaceuticals Ltd (Tredegar, Gwent). One tablet (metformin 850 mg or placebo) was prescribed to be taken 12 hourly for a period of 6 months.

Patients in each group received standardized dietary advice from a research dietitian. Each subject was assessed by the dietitian and an individualized diet [high in carbohydrate (50%) and low in fat (10%)] was given with the aim of a reduction in daily intake by 500 kcal. Written information was given on PCOS and appropriate information on a balanced weight-reducing diet. The patients were also encouraged to increase daily exercise (such as walking, using stairs) by 15 min, although this was not formally assessed. The participants received further encouragement to adhere to the regime at the monthly review visits.

Each participant was assessed monthly with a re-evaluation of anthropometric measurements, endocrine and biochemical parameters together with an ultrasound scan and record of the patient's menstrual

cycle. Side-effects of the treatment and reason for any withdrawals from the study were recorded. The assessment was performed by the same person in each centre (usually the research nurse). All nursing and medical personnel were blind to the treatment arm, with the research pharmacy in Leeds being the only place where this information was held for the duration of the study. Compliance was assessed by the return of empty drug containers.

Outcome measures

The primary outcome measures were: (i) change in menstrual cycle; (ii) change in arthropometric measurements; and (iii) changes in the endocrine parameters, insulin sensitivity and lipid profile. The main secondary outcome measure was pregnancy rate.

Power calculation

In the study by Velazquez *et al.* (1997a), metformin alone improved menstrual regularity in 21/40 (53%) of subjects. If we anticipate an overall 83% improvement with a combination of diet and metformin (i.e. a further 30% improvement compared with metformin alone), the standardized difference (d) would be 0.64. The chosen power in the study was 90% with a type I error of 0.05. From the power table (Machin and Campbell, 1987), when d = 0.64 and the power = 0.90, the projected sample size was 110, with 55 subjects in each arm of the study. When the study was designed the literature from which to calculate power was limited, if we were to consider the more recently published Cochrane meta-analysis by Lord *et al.* (2003), which reported an overall improvement in ovulation rates in 71/156 and 37/154 subjects in the metformin group and the control group respectively with an odds ratio of 3.88 (95% CI 2.25, 6.69) versus placebo for rate of ovulation in favour of metformin. Based on these recent values, the standardized difference is 0.57 with the projected sample size of 130. At the end of the study period, the actual recruitment exceeded this value (see below).

Biochemical assays

All the samples were stored at −20°C and were analysed in the biochemistry department of the coordinating centre. The analyses were as previously described (Wijeyaratne *et al.*, 2002). Plasma glucose was measured using an enzymatic colorimetric assay (Hitachi, Roche) with intra-assay coefficients of variation (CV) of 1.9% at 20.2 mmol/l and 30% at 2.4 mmol/l. A time-resolved fluoroimmunoassay (AutoDELFIA; Perkin Elmer) was used to measure insulin and serum SHBG concentrations, with plasma insulin intra-assay CV of 1.7% at 180.96 pmol/l and 2.4% at 33.9 pmol/l and inter-assay CV of 3.5% at 180.96 pmol/l and 2.3% at 33.9 pmol/l. The serum SHBG intra-assay CV was 6% at 103.36 nmol/l and 7% at 14.88 nmol/l; and the inter-assay CV was 1% at 103.36 nmol/l and 1% at 14.88 nmol/l. Serum testosterone was measured after organic extraction using an in-house radioimmunoassay with an inter-assay CV of 7.7% at 2.20 nmol/l. Free androgen index (FAI) was derived from the ratio of the total testosterone concentration (nmol/l) to the concentration of SHBG (nmol/l) × 100.

Data analysis and statistics

The insulin sensitivity (IS) was calculated from the Quantitative Insulin Sensitivity Check Index (QUICKI), described by Katz *et al.* (2000). QUICKI = $1/[\log(I_0) + \log(G_0)]$, with I_0 = fasting insulin concentrations in mIU/ml (conversion from pmol/l to mIU/ml: multiplied by a factor of 0.144) and G_0 = fasting glucose concentrations in mg/dl (conversion from mmol/l to mg/l: multiplied by a factor of 18.0).

The hyperinsulinaemic–euglycaemic glucose clamp technique is the 'gold standard' for quantifying insulin sensitivity *in vivo* because it directly measures the effects of insulin to promote glucose utilization under steady state conditions; an alternative reference method is the intravenous glucose tolerance test (IV-GTT). Both require sophisticated investigation centres, are labour intensive, expensive and cannot really be performed for large scale studies. In routine clinical practice an OGTT or simple ratios of fasting glucose/insulin are fairly sensitive (Moran and Norman, 2004). More accurate indices of insulin sensitivity and secretion derived from fasting plasma insulin and blood glucose concentrations are reasonable substitutes for the euglycaemic clamp and IV-GTT, these include the HOMA and QUICKI methods (Hanson *et al.*, 2000).

The homeostatic model assessment (HOMA) is a computer-generated model consisting of a series of non-linear empirical equations solved numerically to predict glucose, insulin and C-peptide concentrations in the fasting state for the assessment of pancreatic beta cell function and insulin sensitivity (Matthews *et al.*, 1985). The use of HOMA correlates well with the euglycaemic clamp method and the IV-GTT but cannot be compared between different studies unless the insulin assay is standardized (Bonara *et al.*, 2000). The estimation of the QUICKI provides a robust and reproducible estimate of insulin sensitivity that shows excellent linear correlation with the gold standard clamp estimation with similar variability and discrimination power (Katz *et al.*, 2000). The relative advantages of QUICKI over HOMA include the fact that the data derived from a single blood sample performs just as well as an average of multiple sampling, and the simplicity of the mathematical model. Furthermore, therapeutic changes in insulin sensitivity have been as readily demonstrated with this simple method as with the euglycaemic clamp (Mathur *et al.*, 2001). A recent review on the determination of insulin sensitivity in PCOS has highlighted the good correlation of QUICKI with the clamp technique (Kauffman and Castracane, 2003).

Data were analysed on the basis of intention to treat. All the subjects who withdrew within the first 4 months of the study period, excluding those who conceived, were classified as non-responders. This is because we wished to include only those who completed ≥4 months, and preferably 6 months of the trial. For parametric data, the assumption of normal distribution was assessed by a normal plot and the Kolmogorov–Smirnov test. The assumption of the two groups having the same variances was tested by using the *F*-test. Paired *t*-test or two-sample *t*-test was applied as indicated. When the data did not meet the above assumptions, a $\log_{10}$ transformation of the data was carried out. If the transformed data was still not meeting the assumptions, non-parametric methods, Wilcoxon signed rank test or Mann–Whitney test were applied. $P < 0.05$ was considered to be statistically significant. The *Z*-test was used to analyse the two proportions with Yates' correction.

In the multiple linear regression analysis, the same normality test was used as in the *t*-test and the test for constant variance was computed by using the Spearman rank correlation between the absolute values of the residuals and the observed value of the dependent variable. When the criteria of normality or constant variance were not met, a $\log_{10}$ transformation of the data was performed. Durbin–Watson statistic was used to test residuals for their independence of each other.

In the logistic regression analysis, the regression coefficients computed by minimizing the sum of squared residuals in multiple logistic regression are also the maximum likelihood estimates. *P* is the *P*-value calculated for the Wald statistic, which is the regression coefficient divided by the SE. All the statistical analyses were performed using SigmaStat, version 2.

Recruitment progress

During a 4 year period, between 1999 and 2003, a total of eight centres took part in the recruitment process. A total of 183 women were screened for inclusion in the study. Of these, 40 women were

excluded due to previously undiagnosed tubal disease or co-existing male factor infertility. As a result, a total of 143 subjects were randomized to receive metformin (n = 69) or to receive placebo (n = 74) (Figure 1). In the metformin arm, 13 subjects withdrew within the first 4 months of the trial (11 due to side-effects and two due to spontaneous pregnancies). Eight women withdrew from the placebo arm (six due to 'side-effects' and two due to spontaneous pregnancies, within the first 2 months of the study). The difference in the drop-out rates, excluding because of pregnancy (metformin, 15.9% versus placebo, 8.0%) was not significant (P = 0.229, 95% CI –2.69 to 18.5). At the end of the study, the numbers of patients who completed the trial in the metformin and placebo arms were 56 and 66 respectively. Compliance was high and the drop-out rate relatively low as these were patients motivated by a desire to conceive and the knowledge that they needed to attain a BMI of <30 kg/m^2 to qualify for ovulation induction.

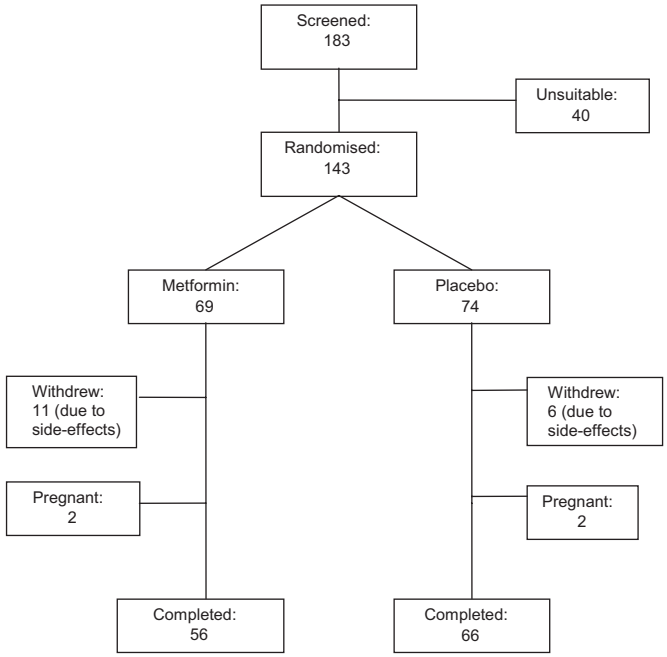

Figure 1. The progress of the subjects through the study.

The total number of patients per centre, with those who withdrew in parentheses, were: Leeds 65 (6), St Mary's Hospital, London 41 (1), MRC Reproductive Medicine Centre, Edinburgh 3 (1), Royal Shrewsbury Hospital, Shrewsbury 12 (4), Royal Free Hospital, London 6 (0), St Bartholomew's Hospital, London 4 (3), Hope Hospital, Salford 4 (0) and The Jessop Hospital for Women, Sheffield 8 (2).

Results

Demographic data

There were no significant differences in the baseline characteristics of the subjects between the two groups (Table I). In the metformin and placebo groups respectively, the mean BMI (37.6 versus 38.9 kg/m^2), the median number of menstrual cycles in the preceding 6 months (2 versus 2), the mean waist circumference (111.9 versus 108.8 cm) and waist:hip ratio (WHR) (0.907 versus 0.900) were similar. The anthropometric measurements of the subjects who withdrew prematurely were also not significantly different from those who completed the study (data not shown).

Table I. The baseline characteristics of the subjects in metformin and placebo groups

	Metformin (n = 69)		Placebo (n = 74)		P
	Mean	SD	Mean	SD	
Age (years)	29.7	3.7	29.8	3.8	0.824
Menses in the preceding 6 months[a]	2		2		0.971
Weight (kg)	101.7	14.5	101.7	17.9	0.996
Body mass index (kg/m^2)	37.6	5.0	38.9	9.5	0.283
Waist circumference (cm)	111.9	13.7	108.8	18.2	0.273
Waist:hip ratio	0.907	0.100	0.900	0.142	0.755
Proportion of subjects who withdrew, excluding pregnancy (%)	15.9		8		0.229
Systolic pressure	125.5	13.5	124.0	15.6	0.538
Diastolic pressure	79.1	11.0	79.0	10.3	0.926
Proportion of smokers (%)	15.9		20.3		0.643
Proportion of nulliparity (%)	53.6		56.8		0.829
LH (IU/l)	9.4	7.2	10.8	5.2	0.221[b]
FSH (IU/l)	5.1	2.0	5.0	1.9	0.867
Testosterone (nmol/l)	2.2	0.60	2.5	0.64	0.089
Sex hormone-binding globulin (nmol/l)	22.8	10.5	24.8	14.1	0.742[b]
Free androgen index	11.3	5.5	13.7	10.3	0.394[b]
Fasting glucose (mmol/l)	5.24	1.23	5.03	0.67	0.628[b]
Fasting insulin (pmol/l)	97.7	76.0	104.3	117.4	0.862[b]
Insulin sensitivity (QUICKI)[c]	0.343	0.05	0.340	0.045	0.890[b]
Total cholesterol (mmol/l)	4.99	1.21	4.85	1.29	0.601
Triglycerides (mmol/l)	2.03	1.2	1.91	1.22	0.580[b]

[a]Mann–Whitney rank sum test was used to analyse the difference, and the median instead of the mean is reported.
[b]Log transformation was carried out on the data before the analysis with two sample t-test.
[c]Quantitative Insulin Sensitivity Check Index (QUICKI) method = $1/[\log(I_0) + \log(G_0)]$.
I_0 = fasting insulin levels in mIU/ml (conversion from pmol/l to mIU/ml: multiplied by a factor of 0.144).
G_0 = fasting glucose levels in mg/dl (conversion from mmol/l to mg/l: multiplied by a factor of 18.0).

As expected, there was a positive correlation between insulin sensitivity and serum SHBG concentrations (log insulin sensitivity = –0.593 + 0.093 × (log SHBG), adjusted R^2 = 0.11, P = 0.001). Additionally, there was a negative correlation between insulin sensitivity and serum triglyceride concentrations and BMI (log insulin sensitivity = –0.454 – 0.061 × (log triglycerides), adjusted R^2 = 0.060, P = 0.011), even after adjustment for age, waist circumference and serum testosterone concentration. Surprisingly, no association between waist circumference and serum insulin concentration and insulin sensitivity was observed.

The mean duration of infertility was similar in each group [MET 4.5 (SD 2.9) years versus PLA 4.9 (3.0) years, P = 0.624]. There was no difference in the percentage of primary infertility (MET 69% versus PLA 73%, P = 0.851) or subjects who had previously been prescribed clomiphene citrate, usually by their primary care physician and not in the context of the fertility clinic, where body mass would have precluded treatment (MET 43% versus PLA 49%, P = 0.718).

Menstrual frequency

At the end of the study period, the menstrual cycles over the time-course of the study increased significantly with a median of improvement of one menstrual cycle per 6 months in both groups (Tables II and III). However, there was no difference between the groups (P = 0.580). Patients who menstruated <4 weeks from starting treatment were not considered to have ovulated in response to the study. A number of studies have used menstrual frequency as an assessment of reproductive function women with PCOS and an improvement in menstrual regularity is considered to be a good surrogate for ovarian function and ovulatory frequency in women with PCOS (Morin-Papunen *et al.*, 1998; Fleming *et al.*, 2002; Haas *et al.*, 2003). Furthermore, Kolstad *et al.* (1999) studied the relationship between menstrual cycle pattern and fertility. Thus the observed improvement in menstrual frequency can be viewed as an indication of improvement of ovulation rate and potential fecundity.

On the basis of intention to treat (ITT), 36 women (52.2%) in the metformin group and 43 women (58.1%) in the placebo group experienced improvement in menses. However, the difference between the two groups was not significant (P = 0.589, 95% CI –10.4, 22.2).

Anthropometric measurements

Significant reductions in body weight and BMI were observed in both groups (Tables II and III). However, the changes in the means between the groups were not significant (–1.02 versus –0.46, 95% CI –1.15, 0.03, P = 0.063). The study was not powered to determine a difference in weight even though the metformin group lost twice as much weight as the placebo group. There was a significant reduction of waist circumference in the metformin group (before 113.5 cm, after 111.1 cm, P = 0.002) (Table II) but not in the placebo group (before 108.5 cm, after 109.1 cm, P = 0.764) (Table III). The difference in the changes of the mean values between the two groups was not statistically significant (–2.34 versus +0.58, 95% CI –7.14, 1.30, P = 0.173). Similarly, the changes in the mean of both systolic and diastolic blood pressure were not significantly different between the two groups.

Endocrine parameters and lipid profiles

Both the fasting insulin and glucose data were skewed and therefore logarithmic transformations were performed on the data before analysis. The geometric means of the fasting insulin concentrations in the metformin group did not change significantly over the course of the study (baseline 72.8 pmol/l, final 80.7 pmol/l, ratio of means 1.11, P = 0.524, Table II). Similarly, no significant changes in the geometric means of the

Table II. The outcomes in the metformin group (n = 56)

	Before (b)		After (a)		Difference		
	Mean	SD	Mean	SD	(a – b)	*P*	95% CI
Menses in 6 months[a]	2		3		1	< 0.001	
Weight (kg)	102.7	15.0	99.9	15.0	–2.84	< 0.001	–3.87, –1.80
Body mass index (kg/m^2)	38.1	5.08	37.1	5.04	–1.02	< 0.001	–1.43, –0.62
Waist circumference (cm)	113.5	13.2	111.1	12.3	–2.34	0.002	–3.75, 0.93
Waist:hip ratio	0.906	0.094	0.911	0.098	0.005	NS	–0.007, 0.017
Systolic pressure	125.0	13.7	121.7	12.5	–3.30	NS	–7.09, 0.481
Diastolic pressure	79.0	11.4	77.2	10.0	–1.82	NS	–5.39, 1.76
Testosterone (nmol/l)	2.2	0.6	1.9	0.6	–0.3	0.008	–0.08, –0.47
SHBG (nmol/l)[b]	20.4		22.1		1.09	NS	0.99, 1.19
Free androgen index[b]	10.5		8.8		0.84	0.013	0.73, 0.96
Fasting glucose (mmol/l)[b]	4.93		4.83		0.971	NS	0.90, 1.05
Fasting insulin (pmol/l)[b]	72.7		80.7		1.11	NS	0.80, 1.52
Insulin sensitivity (QUICKI)[c]	0.402	0.075	0.398	0.072	–0.004	NS	–0.028, 0.020
Total cholesterol (mmol/l)	5.11	1.23	5.14	1.03	0.03	NS	–0.21, 0.28
Triglycerides (mmol/l)	2.07	1.19	2.04	1.01	–0.03	NS	–0.35, 0.29

[a]Wilcoxon signed rank test was used to analyse the difference and the median instead of the mean is reported.
[b]Log transformation was carried out on the data before the analysis. Geometric means, mean ratio (a/b) and the corresponding 95% CI were reported after the results were back-transformed.
[c]Quantitative insulin sensitivity check index (QUICKI) method = 1/[log(I_0) + log(G_0)].
CI = confidence interval; NS = not significant; SHBG = sex hormone-binding globulin.

Table III. The outcomes in the placebo group (n = 66)

	Before (b)		After (a)		Difference		
	Mean	SD	Mean	SD	(a – b)	*P*	95% CI
Menses in 6 months[a]	2		3		1	< 0.001	
Weight (kg)	100.7	17.9	99.2	17.3	–1.46	0.011	–2.57, –0.34
Body mass index (kg/m^2)	37.9	6.5	37.4	6.3	–0.46	0.034	–0.89, –0.03
Waist circumference (cm)	108.5	18.7	109.1	13.4	0.58	NS	–3.28, 4.44
Waist:hip ratio	0.894	0.150	0.899	0.097	0.005	NS	–0.026, 0.035
Systolic pressure	124.1	16.1	121.4	12.1	–2.73	NS	–6.89, 1.42
Diastolic pressure	79.1	10.5	75.5	9.5	–3.6	0.014	–6.45, –0.75
Testosterone (nmol/l)	2.4	0.6	2.3	0.7	0.1	NS	–0.28, 0.15
SHBG (nmol/l)[b]	21.4		21.1		0.98	NS	0.89, 1.08
Free androgen index[b]	11.0		10.9		0.98	NS	0.87, 1.10
Fasting glucose (mmol/l)[b]	4.91		4.88		0.99	NS	0.94, 1.05
Fasting insulin (pmol/l)[b]	74.1		81.8		1.10	NS	0.86, 1.42
Insulin sensitivity (QUICKI)[c]	0.399	0.066	0.392	0.056	–0.007	NS	–0.027, 0.013
Total cholesterol (mmol/l)	4.99	1.18	4.88	1.15	–0.11	NS	–0.33, 0.11
Triglycerides (mmol/l)	1.97	1.19	1.78	1.21	–0.18	NS	–0.43, 0.07

[a]Wilcoxon signed rank test was used to analyse the difference, and the median instead of the mean is reported.
[b]Log transformation was carried out on the data before the analysis. Geometric means, mean ratio (a/b) and the corresponding 95% CI were reported after the results were back-transformed.
[c]Quantitative insulin sensitivity check index (QUICKI) method = $1/[\log(I_0) + \log(G_0)]$.
CI = confidence interval; NS = not significant; SHBG = sex hormone-binding globulin.

fasting insulin concentrations in the placebo group occurred (baseline 74.1 pmol/l, final 81.8 pmol/l, ratio of means 1.10, P = 0.438, Table III). The difference between the changes between the two treatment arms was also not significantly different (1.11 versus 1.10, 95% CI 0.672–1.49, P = 0.985). Similarly, there were no significant changes in fasting glucose concentrations within and between groups (Tables II and III). Improvements in insulin sensitivity were not observed in either the metformin group or the placebo group (Tables II and III). The changes of means in insulin sensitivity were also not different between the two groups (data not shown).

There were no significant changes in the geometric mean SHBG concentrations in either the metformin or placebo arms (Tables II and III), neither was there a difference between the groups (data not shown). There was, however, a significant reduction in the FAI in the metformin arm of the study, with a mean ratio (final:baseline) of 0.84 (95% CI 0.73, 0.96, P = 0.013) and this was because of a significant fall in total testosterone of –0.3 nmol/l (95% CI –0.08, –0.47, P = 0.008) (Table II). This was confirmed by multiple linear regression analysis after adjustment for baseline BMI, change in insulin sensitivity and the percentage of weight change (P = 0.046, Table IV).

At the end of the study period, both the total cholesterol and triglyceride concentrations remained unchanged (Tables II and III) with no between-group differences (data not shown).

Pregnancy rates

There were two pregnancies in each arm of the study within 2 months of commencing and a further four pregnancies in the metformin arm in the 5th and 6th months of the study. The total numbers of conceptions in the metformin (8.7%) and the placebo (2.7%) groups were not significantly different (P = 0.233, 95% CI –1.5, 13.5). Based on our findings, the standardized difference is 0.26 and the required sample size to assess a difference in pregnancy rates would be 600 subjects.

Table IV. Multiple linear regression analysis of the change of free androgen index (log end of study levels – log baseline levels) on the percentage of weight change, the use of metformin, the change of insulin sensitivity and the initial body mass index (BMI)

	Coefficient	SE	*t*	*P*
Constant	0.0585	0.143	0.409	NS
Metformin	–0.080	0.039	–2.035	0.046
Initial BMI	–0.002	0.0037	–1.369	NS
Change of insulin sensitivity	–0.372	0.271	–1.369	NS
% of weight change[a]	–0.002	0.005	–0.465	NS

[a]Percentage of weight change = 100%×(baseline-end of study weight)/baseline weight.
Adjusted R^2 = 0.0442.
The analysis of variance for the regression: F = 1.855, P = 0.128, residual SD = 0.166.
NS = not significant.

Subgroup analysis of those who lost weight

On the basis of intention to treat (ITT), 36 women (52.2%) in the metformin group and 43 women (58.1%) in the placebo group experienced improvement in menses. However, the difference between the two groups was not significant (P = 0.589, 95% CI –10.4, 22.2). If these data are analysed by completion of protocol, the difference is still not significant (P = 0.94, 95% CI –18, 16).

Forty-two subjects (60.8%) in the metformin group and 35 subjects (47.3%) in the placebo group managed to lose weight. The difference between the two groups was not significant (P = 0.147, 95% CI –28.5, 29.9). When we calculated the actual percentage weight change (PWC) [100% × (baseline weight – end of study weight)/baseline weight] among only those women who managed

Table V. Multiple logistic regression analysis of the improvement in menses on the percentage of weight change and initial body mass index (BMI)

	Regression coefficient	SE	Wald statistic	*P*	Odds ratio	95% CI
Constant	–3.185	1.546	4.246	NS	0.041	0.002, 0.856
% of weight change[a]	0.127	0.054	5.462	0.019	1.135	1.021, 1.263
Initial BMI	0.098	0.042	5.556	0.018	1.103	1.017, 1.196

[a]Percentage of weight change = 100%×(baseline – end of study weight)/baseline weight.
Pearson χ^2 statistic: 116.7 ($P = 0.411$).
Likelihood ratio test statistic: 14.0 ($P < 0.001$).
Hosmer–Lemeshow statistic: 10.1 ($P = 0.261$).
NS = not significant.

to lose weight, we showed that the mean percentage of weight loss in the metformin and placebo groups was 3.98 and 4.41% respectively. The difference was not significant ($P = 0.554$, 95% CI –1.88, 1.02).

By using multiple logistic regression analyses of the improvement in menses on the PWC, the use of metformin, the baseline BMI and age, we were able to demonstrate that weight loss (a positive value of PWC) had a significantly positive effect on improvement in menses ($P = 0.047$, regression coefficient = 0.199, odds ratio 1.126, 95% CI 1.00, 1.27). The use of metformin had no influence on menstrual frequency in our study population.

The best model to predict the improvement in menses is $0.127 \times (\text{PWC}) + 0.098 \times (\text{initial BMI}) - 3.185$ (see Table V). This implies that the greater the BMI the more likely it was that improvement in menses would have been experienced through weight loss.

Analysis of those with the metabolic syndrome

The metabolic syndrome is defined as requiring three out of the following five criteria: waist circumference >88 cm, elevated triglycerides ≥1.7 mmol/l, lowered high-density lipoprotein cholesterol <1.3 mmol/l, elevated blood pressure (≥130/85 mmHg) and impaired glucose tolerance test. Twenty-six of those in the metformin arm and 23 in the placebo arm had the metabolic syndrome. There was no difference in outcome between the metformin group and placebo group respectively in the median change of menstrual frequency (1 versus 1, $P = 0.916$), percentage weight loss (3.14 versus 2.65% $P = 0.79$), change in waist circumference (–1.5 versus –0.93 cm, $P = 0.692$), change in serum testosterone concentration (0.889 versus 0.968 nmol/l, $P = 0.408$), change in FAI (0.891 versus 0.995, $P = 0.435$), change in insulin sensitivity (–0.003 versus 0.000, $P = 0.914$) or either cholesterol or triglyceride concentrations.

Discussion

We report a large randomized controlled trial (RCT) to investigate the effects of metformin on very obese patients with anovulatory PCOS. The duration of the study period (6 months) and the dose of metformin used (850 mg, twice daily) were the longest and the highest of the RCT reported in the Cochrane database (Lord *et al.*, 2003). We were unable to demonstrate that metformin had an additional benefit on the improvement of menstrual frequency over weight loss through lifestyle modification and, furthermore, in the study population metformin did not induce weight loss. After adjustment for baseline BMI and age, only weight loss, but not the use of metformin, was associated with a significant improvement in menstrual cyclicity. In addition, the higher the BMI, the more likely women with PCOS were to benefit from weight loss with respect to improvement of menstrual frequency.

The entry criteria required BMI to be >30 kg/m^2, yet the mean BMI was ~38 kg/m^2 and comprised typical central obesity. These were patients who would not be suitable for ovulation induction for anovulatory infertility because of their obesity and so had not yet been enrolled in the ovulation induction programme, although some had previously received clomiphene citrate from their primary care physician before referral to the fertility clinic. The rate of withdrawal in the metformin group was not significantly different from the placebo group and was lower than that reported by Fleming *et al.* (2002) in their large trial in which 42% dropped out of the metformin arm compared with 17% of the placebo arm. This may be explained by the fact that all of our patients had a wish to conceive and may therefore have had a greater incentive to adhere to the protocol.

A surprise finding was the lack of change in insulin sensitivity in either the metformin or placebo groups. This is probably explained by the extreme obesity of our patients and the relatively small amount of weight lost. It has been demonstrated that insulin sensitivity and androgen concentrations are unlikely to improve in patients who lose <5% of their initial weight (Kiddy *et al.*, 1992). Furthermore, the effect of metformin in women with PCOS is reduced by increasing obesity (Crave *et al.*, 1995; Fleming *et al.*, 2002; Maciel *et al.*, 2004). Our findings were similar to the study of Ehrmann *et al.* (1997) in which the average BMI was 39 kg/m^2. Furthermore, the dose of metformin (850 mg twice daily) may be insufficient in this group of patients and we are currently performing a dose-finding study, using different doses at different body weights.

Metformin, however, did improve the FAI, secondary to a significant fall in total testosterone without a change in the insulin sensitivity or SHBG. This observation suggests that metformin may have a direct effect on ovarian steroidogenesis without effecting a change in circulating insulin concentrations (Pirwany *et al.*, 1999; la Marca *et al.*, 2002; Mansfield *et al.*, 2003). There is a consensus that metformin has an additive effect in achieving ovulation and pregnancy when combined with drugs to induce ovulation (mainly clomiphene citrate) (Costello and Eden, 2003; Lord *et al.*, 2003). The effect may be quick and this too supports the possibility of a direct effect on the ovary rather than a systemic effect on metabolism.

The use of metformin and other insulin-lowering or -sensitizing agents has excited much interest in the management of PCOS. The literature is replete with studies of varying design, using varying regimens and assessing different outcomes. A relatively small number of these studies (a total of 13) have been of appropriate design to be included in the Cochrane systematic review (Lord *et al.*, 2003). This included seven studies

comparing metformin with placebo in a total of 310 patients, which showed that metformin was beneficial for ovulation (odds ratio 3.88, 95% CI 2.25, 6.69, $P < 0.0001$). The largest study to be included in this series was of 92 patients (Fleming *et al.*, 2002). The meta-analysis also demonstrated that metformin was effective in reducing fasting insulin and total testosterone concentrations but had no effect on BMI or waist circumference (Lord *et al.*, 2003).

Costello and Eden (2003) in their systematic review reached similar conclusions and again a wide range of entry criteria were reported. In particular the average 'mean BMI' of those studies that compared metformin with placebo was 31.3 kg/m^2 (range 21.4–39.8 kg/m^2). There were variable effects reported, with not all studies demonstrating an improvement in insulin sensitivity or fall in testosterone levels (Costello and Eden, 2003). As with our study, neither of the two RCT that reported an improvement in menstrual cyclicity showed a fall in BMI; both reported a fall in testosterone concentrations and only one an improvement in fasting insulin (Moghetti *et al.*, 2000; Pasquali *et al.*, 2000).

Pasquali *et al.* (2000) studied 20 obese women with PCOS with a control group of 20 obese women without PCOS who were comparable for age and pattern of body fat distribution. All were given a low-calorie diet (1200–1400 kcal/day) for 1 month, after which they were randomized to receive metformin (850 mg twice daily) or placebo for 6 months. Metformin treatment reduced body weight and BMI significantly more than placebo in both PCOS and control women. Fasting insulin decreased significantly in both PCOS women and controls and testosterone concentrations decreased only in PCOS women treated with metformin. SHBG concentrations remained unchanged in all PCOS women, although in the control group, they significantly increased after both metformin and placebo (Pasquali *et al.*, 2000). Thus once again the effects of metformin appear to vary in different study populations.

Women with anovulatory PCOS who lose weight experience an improvement in ovarian function, ovulation and anthropometric indices (Clark *et al.*, 1995; Crosignani *et al.*, 2003). The key component of diet should be calorie restriction, rather than the composition of the diet itself (Moran *et al.*, 2003; Stamets *et al.*, 2004). A recent study randomized 38 women with a mean BMI of >39 kg/m^2 to receive either advice on lifestyle modification (aiming for 500–1000 calorie deficit per day combined with exercise) or no advice with either metformin (850 mg twice daily) or placebo (Hoeger *et al.*, 2004). The greatest effect was in the combination group with respect both to reduction of weight and hyperandrogenism. Yet irrespective of treatment group the greatest improvement in rate of ovulation was achieved by those who lost weight (Hoeger *et al.*, 2004).

There remain a number of unanswered questions concerning the use of metformin in women with PCOS, including which parameters may best predict a response and the appropriate dose for a given body mass. Metformin therapy certainly appears beneficial in certain circumstances and may alone improve menstrual cyclicity, ovulation and hyperandrogenism in some women (Costello and Eden, 2003; Lord *et al.*, 2003). Furthermore metformin may amplify the effects of ovulation-inducing drugs (Costello and Eden, 2003; Lord *et al.*, 2003) or androgen-lowering medication (Gambineri *et al.*, 2004). We have found, however, that in very obese women with anovulatory PCOS, metformin, at a dose of 850 mg twice daily, had no effect on menstrual frequency, body weight or insulin sensitivity, despite a fall in total testosterone and waist circumference. Furthermore a modest reduction in weight through lifestyle modification was the most significant predictor for an improvement in menstrual cyclicity.

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