Article

Metformin monotherapy in lean women with polycystic ovary syndrome

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Abstract

This study was carried out to compare ovulation and pregnancy rates in response to metformin therapy in lean and obese women with polycystic ovary syndrome (PCOS). A total of 34 (17 lean and 17 obese) women with PCOS were treated with 500 mg metformin 3 times daily for 12 weeks. In the lean and obese groups, the mean body mass index was 24 and 36, and the mean fasting insulin concentrations were 12 and 21 mIU/l respectively. There was no difference between the two groups as regarding age, DHEA-S, androstenedione, 17-OH progesterone and LH concentrations. In the lean and obese groups 15/17 women (88%) and 5/17 women (29%) ovulated while 11/17 women (65%) and 3/17 women (18%) conceived respectively. Comparison between the groups was found to be statistically significant. Metformin monotherapy is very effective in improving ovulation and pregnancy rates in lean women with PCOS as compared with obese women.

Keywords: *body mass index, lean women, metformin, obese women, PCOS*

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder that affects women of reproductive age, and is characterized by oligomenorrhoea, hyperandrogenism, anovulation, and more often than not obesity. Although the exact aetiology of PCOS is unknown, current research supports insulin resistance and hyperinsulinaemia as playing a pivotal role in the pathogenesis of this disease (Burghen *et al*., 1980; Nestler and Strauss, 1991).

Insulin is thought to possess true gonadotrophic activity, directly increasing androgen production by the theca cells of the ovary (Gambineri, 2002). Insulin has also been shown to augment the activity of p450-c 17α, the rate-limiting enzyme in the production of testosterone from ovarian thecal cells (Iuorno and Nestler, 2001). It has also been shown to increase LH-mediated androgen synthesis from the ovary. Effects of insulin on the hypothalamic–pituitary axis in increasing LH concentrations and on ovary, causing premature follicular atresia, have been suggested, but remain to be proven (Franks, 1995).

At least 50–60% of PCOS patients are obese or overweight, and hyperinsulinaemia is the usual accompaniment in these women. The pathogenesis in the remaining 25–30% who are lean has been suggested to be due to a defect in the hypothalamo–pituitary axis, resulting in increased LH production, and insulin appears to play no role in the disease process in this subset of PCOS patients (Grulet *et al*., 1993). This theory has been substantiated by the finding of near-normal insulin values in these women (Date *et al*., 1992). However, subsequent research has demonstrated that these women, although they have lower insulin concentrations when compared with obese PCOS patients, are definitely hyperinsulinaemic and insulin resistant when compared with their healthy counterparts (Dunaif *et al*., 1992). Insulin sensitizers are being tested extensively in the treatment of PCOS women. Metformin (MTF) is the most widely employed insulin-sensitizing agent used in ovulation induction regimes, and also to improve outcome in 'coasted' patients with PCOS undergoing IVF (Stadtmauer *et al*., 2002). It improves insulin sensitivity in the liver, reducing gluconeogenesis, and improves uptake and utilization of glucose in the peripheral

tissue, thereby reducing insulin concentrations. Thirteen studies to date have evaluated the efficacy of MTF monotherapy on restoration of regular menstrual cycles. However, almost all of these studies were performed in obese PCOS women with an average body mass index (BMI) of 31.3 (Costello and Eden, 2003). Only one study included lean adolescent PCOS patients with BMI of <25; menstrual cyclicity was restored in all 18 girls after 6 months of MTF treatment (Ibanez *et al*., 2001).

During initial efforts to normalize insulin concentrations using MTF alone in PCOS women, some lean women became pregnant before initiating clomiphene therapy. With this experience, a prospective study was conducted to determine the differences in response as regards restoration of normal cycles and pregnancy rates between lean and obese PCOS women. A meta-analysis (Lord *et al*., 2003) of 13 randomized controlled trials (RCT) evaluating the efficacy of MTF monotherapy, comprising 428 participants, showed a 4-fold increase in ovulation rate, with the number needed to treat being 4.4. Based on these numbers, it was calculated that a sample size of 34 women (17 in each group) would have sufficient power to address this question.

Materials and methods

Thirty-four consecutive new PCOS patients attending outpatient clinics at the Al-Mafraq Hospital, Abu Dhabi, United Arab Emirates were the study subjects. A diagnosis of PCOS was made if either of the following two criteria was met: oligomenorrhoea (fewer than six cycles during the last year) or amenorrhoea, clinical features of androgen excess such as hirsutism with Ferriman–Gallwey score >7, acne and alopecia or biochemical evidence of androgen excess such as elevated free testosterone, androstenedione and dehydroepiandrosterone sulphate (DHEAS). Congenital adrenal hyperplasia, Cushing's syndrome, thyroid disorders and hyperprolactinaemia were excluded by appropriate tests. The presence of multiple subcapsular follicles on days 1–3 of a spontaneous cycle was considered suggestive of PCOS. All these women had infertility of at least 2 years duration. None of them had been on any medication during the past 3 months preceding the study. Male factor and tubo–uterine factors were excluded by semen analysis and hysterosalpingogram or laparoscopy. Diabetes with a fasting blood sugar (FBS) of <120 mg/dl was excluded. Seventeen women had a BMI <25 and the other 17 belonged to the obese group with BMI >30.

All women were recruited during the follicular phase of the cycle. They presented to the hospital after 12 h of overnight fasting. Their height and weight were recorded and blood samples were drawn for FSH, LH, insulin, fasting glucose and androgen profile (testosterone, androstenedione, DHEA-S and 17-hydroxyprogesterone). The protocol was to administer MTF alone at a dose of 500 mg, three times daily, to all PCOS women for a period of 3 months. At the end of 10–12 weeks of MTF treatment or immediately after the missed period (whichever was first), blood samples were taken and hormonal profile and fasting glucose were repeated. If conception did not occur within 3 months, clomiphene was added to MTF. After the commencement of MTF, all these women were tested by ultrasound for evidence of ovulation. Follicular rupture, presence of free fluid in the pouch of Douglas and endometrial thickness of >7.5 were considered as evidence of ovulation.

Laboratory measurements

Fasting blood samples were collected from the subjects. The samples were centrifuged at 200 *g* for 10 min and sera were separated and stored at –20°C until measurement. FSH, LH, total testosterone and prolactin concentrations were quantitatively measured by the Abbott ARCHITECT Immuno-analyser (Abbott Laboratories; from GmbH Diagnostics, Wiesbaden-Delkenheim, Germany). Hormonal measurements were also carried out in all patients in the lean and obese groups after MTF therapy. The sensitivity of the ARCHITECT System was calculated to be better than 0.07 mIU/ml for LH and 0.05 mIU/ml for FSH. The functional sensitivity of the ARCHITECT testosterone assay was calculated to be 0.14 ng/ml (95% confidence interval of 0.11–0.17 ng/ml). Concentrations of DHEA-S and insulin were measured by electrochemiluminescence immunoassay on Roche Elecsys 2010 (Roche Diagnostics; GmbH, Mannheim, Germany). The lower detection limits of DHEA–S and insulin immunoassay were 0.0003 µmol/l and 0.20 µIU/ml respectively. 17-Hydroxy-progesterone and androstenedione were measured by RIA using Coat-a-Count kits supplied by Diagnostic Products Corporation (Los Angeles, CA, USA). Analytical sensitivities of radioimmunoassay for the detection of androstenedione and 17-hydroxyprogesterone were 0.04 and 0.07 ng/ml respectively. The normal values for 17-OH progesterone measured by RIA with the Coat-a-Count kits supplied by Diagnostic Products Corporation ranged between 0.27 and 4.9 ng/ml.

Glucose concentrations were measured by the glucose oxidase method (Boehringer Ingleheim GmbH, Ingleheim, Germany).

Intra- and interassay coefficients of variation (CV) for hormonal measurements were as follows: androstenedione (3.2 and 5.6%); DHEA-S (2.4 and 4.7%); testosterone (4.5 and 8%); 17-hydroxy progesterone (7.1 and 7.3%); LH (3 and 3.6%); FSH (3.3 and 4.2%); insulin (1.9 and 2.8%).

Data analysis

Statistical analysis of the data was performed using Microsoft Excel. Inter-group differences were compared by Student's *t*-test and $P < 0.05$ was considered as statistically significant.

Results

The baseline characters of 17 lean women and 17 obese women are shown in **Table 1**. The mean age in both groups was 25.9 and 24.3 respectively. The mean BMI of the obese group was 36, which was significantly higher ($P < 0.001$) as compared with the lean group (BMI = 24). None had an FBS above 120 mg%. Three women in the obese group and none in the lean group had impaired glucose tolerance on a 75 g oral GTT. The common side effects noted with MTF were nausea and dizziness, but not severe enough to stop treatment. **Table 2** demonstrates the differences in response between lean and obese PCOS women to MTF therapy. Resumption of menstrual cyclicity, ovulation and pregnancy rates were significantly higher in the lean PCOS group as compared with the obese group. In one out of 11 women in the lean group, and in all three in the obese group who conceived on MTF, pregnancy ended in a first trimester miscarriage.

Results of hormonal measurements pre- and post-MTF therapy are shown in **Table 3**. Significant reductions were noted in serum

Table 1. Mean age, BMI, glucose and hormone concentrations in lean and obese women with polycystic ovary syndrome.

Parameters	*Lean women (mean ± SD)*	*Obese women (mean ± SD)*	P-*value*
Age (years)	24.31 ± 4.3	25.93 ± 5.3	NS
BMI (kg/m^2)	24.3 ± 4.3	35.9 ± 5.3	<0.001
Androstenedione (ng/ml)	4.8 ± 1.5	5.5 ± 2.5	NS
DHEA-S (µmol/l)	6.6 ± 2.7	6.4 ± 3.4	NS
Testosterone (nmol/l)	2.5 ± 1.0	3 ± 1.8	NS
17-Hydroxyprogesterone(ng/ml)	2.5 ± 1.0	2.6 ± 1.7	NS
LH (mIU/ml)	16.2 ± 6.9	13.7 ± 8.1	NS
FSH (mIU/ml)	6.3 ± 1.62	5.5 ± 1.89	NS
FBS (mg/dl)	94 ± 7.2	97.7 ± 10.3	NS
Insulin (µIU/ml)	12 ± 3.2	20.5 ± 10.2	0.001

NS = not significant.

Table 2. Metformin efficacy in lean and obese women. Figures in parentheses are percentages.

Efficacy of metformin	*Lean women (n = 17)*	*Obese women (n = 17)*	P-*value*
Menstrual cyclicity	17 (100)	7 (41.1)	<0.001
Ultrasound evidence of ovulation	15 (88.2)	5 (29.4)	<0.001
Pregnancy	11 (64.7)	3 (17.6)	0.001

Table 3. Mean BMI, glucose and hormone concentrations, in lean and obese women with PCOS, pre- and post-metformin therapy.

Parameters	*Lean women (mean ± SD)*			*Obese women (mean ± SD)*		
	Before	*After*	P-*value*	*Before*	*After*	P-*value*
BMI (kg/m^2)	24.3 ± 4.3	22.1 ± 3.2	NS	35.9 ± 5.3	34.2 ± 4.2	NS
Androstenedione (ng/ml)	4.8 ± 1.5	4.3 ± 0.9	NS	5.5 ± 2.5	4.3 ± 2.0	NS
DHEA-S (µmol/l)	6.6 ± 2.7	4.9 ± 2	0.046	6.4 ± 3.4	4.3 ± 2	0.046
Testosterone (nmol/l)	2.5 ± 1.0	1.4 ± 0.8	< 0.01	3 ± 1.8	1.2 ± 0.9	0.001
17-Hydroxyprogesterone (ng/ml)	2.5 ± 1.0	2.4 ± 1.2	NS	2.6 ± 1.7	2.3 ± 0.9	NS
LH (mIU/ml)	16.2 ± 6.9	11.2 ± 1.2	0.01	13.7 ± 8.1	9.2 ± 5.2	0.05
FSH (mIU/ml)	6.3 ± 1.62	5.2 ± 1.6	0.046	5.5 ± 1.89	5.1 ± 1.9	NS
FBS (mg/dl)	94 ± 7.2	90 ± 4.2	0.046	97.7 ± 10.3	96.2 ± 11	NS
Insulin (µIU/ml)	12 ± 3.2	9.1 ± 1.2	0.001	20.5 ± 10.2	14.2 ± 7.1	0.046

NS = not significant.

DHEAS ($P = 0.046$), testosterone ($P = 0.001$), LH ($P = 0.05$) and fasting insulin ($P = 0.046$) concentrations after 10–12 weeks of MTF treatment, although the magnitude of reduction was greater in the obese group as compared with the lean patients. No reduction was noted in BMI and blood glucose concentrations after MTF treatment.

Discussion and conclusions

Insulin resistance is defined as an impaired action of insulin in the uptake and metabolism of glucose. The exact molecular mechanisms involved in insulin resistance are not known, but current research is focused on the events that occur after receptor binding by insulin. Insulin resistance occurs in obese individuals usually at the post-receptor site where there is an apparent failure to activate post-receptor tyrosine kinase (Dunaif and Thomas, 2001). Therefore, obesity-related hyperinsulinaemia plays a key role in hyperandrogenism in these women. Other factors such as increased oestrogen production, high lipid intake and decreased sex hormone-binding globulin (SHBG) production may be additional mechanisms by which obesity leads to hyperandrogenaemia in PCOS women (Gambineri, 2002). Non-obese women with PCOS also demonstrate an intrinsic form of insulin resistance (Dunaif *et al*., 1989) and have higher insulin concentrations than their counterparts (Morales *et al*., 1996). It has been shown that lean women treated with MTF for 4–6 weeks demonstrated a reduction in p450–17 alpha activity, a decrease in fasting and glucose stimulated insulin concentrations, basal and gonadotrophin-releasing hormone (GnRH) stimulated LH release, free and total testosterone concentration and increase in SHBG similar to obese women with PCOS (Nestler *et al*., 1997). This study was the first of its kind to argue for a common aetiopathogenesis in both obese and lean PCOS women. However, the degree of insulin resistance and hyperandrogenism related clinical features is more pronounced in obese compared with lean PCOS women. Amelioration of insulin resistance by MTF has been shown to decrease basal and GnRH stimulated LH release and increase serum SHBG concentrations by 60%, thereby lowering free testosterone concentrations by 44% (Nestler and Jakubowicz, 1996). These improvements occurred without any change in body weight among the treated women. Among 12 studies evaluating the efficacy of MTF monotherapy on restoration of menstrual cyclicity, nine uncontrolled studies performed on predominantly obese women with BMI of 31 and fasting insulin concentration of 22 demonstrated 62% improvement in menstrual cyclicity (Costello and Eden, 2003). In the present study, 41% of obese subjects and 100% of lean ones resumed menstrual cyclicity after 3 months of MTF treatment. The corresponding ovulation and pregnancy rates in obese and lean women were 29.4 versus 89.2% ($P < 0.001$) and 17.6 versus 64.7% ($P < 0.001$) respectively. From these results, it is not unreasonable to conclude that lean PCOS women with milder degrees of insulin resistance resume menstrual cyclicity and ovulation faster than their obese counterparts. In one placebo controlled trial, an 8-fold increase was reported in ovulation rates in moderately obese PCOS women during their pretreatment period with MTF (Nestler *et al*., 1998). The combined data of five uncontrolled studies that attempted to demonstrate ovulation rates showed that 61% of women with PCOS ovulate on MTF treatment (Costello and Eden, 2003). Two larger RCT showed ovulation rates of 34% after 1 month and 82% after 4 months of treatment (Fleming *et al*., 2002). However, all the women in these studies were unselected for obesity. The rates of resumption of menses and ovulation are higher than those quoted earlier because this study has analysed lean and obese women separately. Several studies have shown the benefit of adding MTF to clomiphene-resistant PCOS in improving ovulation and pregnancy rates (Costello and Eden, 2003). Few studies used MTF as the first line therapy in inducing ovulation (Cheang and Nestler, 2004). In the present study using only MTF as first line therapy for ovulation induction, 11 out of 17 lean and three out of 17 obese PCOS women (64.7 versus 17.6%) ($P < 0.001$) conceived. It is convincing from these results that milder degrees of insulin resistance in lean women can be ameliorated by MTF, but this does not happen in obese women with severe degrees of insulin resistance and hyperinsulinaemia. The three out of 17 women who conceived in the obese group had in fact followed a concurrent strict weight reduction programme that resulted in a loss of 5–7 kg over 3 months. Weight loss is associated with beneficial effects on hormones, metabolism and clinical features. Further clinical and endocrinological improvements can be achieved in these women by adding insulin sensitizers to the weight loss regimens. This study is in agreement with Nestler *et al*. (1998), in that weight loss is the first therapeutic option for obese PCOS women but not for lean PCOS patients. This observation suggests that lean PCOS women have subtle degrees of insulin resistance that respond to insulin sensitizers by reducing androgen production and thereby improving ovulation and pregnancy rates. However, the number of women in this study is small, and these observations need to be confirmed in large RCT.

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