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Pioglitazone and metformin in obese women with polycystic ovary syndrome not optimally responsive to metformin

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BACKGROUND: In an observational study of 13 women with polycystic ovary syndrome (PCOS) not optimally responsive to metformin diet, we assessed the efficacy and safety of addition of pioglitazone. We also compared these 13 women to 26 women with PCOS, who were responsive to metformin diet, matched by age and by pre-treatment menstrual history and not different by obesity categories. METHODS: Prospectively, as outpatients, with diet constant [1500–2000 calorie (depending on entry body mass index), 26% protein, 44% carbohydrate, 30% fat], metformin (2.55 g/day) was given for 12 months to 39 women, 13 not optimally responsive, 26 responsive to metformin diet, followed by addition of pioglitazone (45 mg/day) for 10 months in the 13 non-responders. Outcome measures included changes in sex hormones, insulin, insulin resistance (IR), insulin secretion, high density lipoprotein cholesterol, weight, and menstrual status. RESULTS: In 13 non-responders, on metformin diet, median serum insulin fell (21 to 16 μIU/ml, $P < 0.05$) and insulin secretion fell from 251 to 200 ($P < 0.01$); weight, dehydroepiandrosterone sulphate (DHEAS), testosterone and IR were unchanged ($P \geqslant 0.07$). Compared with 14% pre-treatment, on metformin diet, expected menses occurred 46% of the time at 3 months ($P = 0.05$), 38% at 6 months ($P = 0.07$), 27% at 9 months, and 24% at 12 months. In 26 responders, on metformin diet, median weight fell (93 to 87 kg), testosterone fell (50 to 32 ng/dl), insulin fell (26 to 16 μIU/ml), IR fell (5.32 to 3.45) and insulin secretion fell (351 to 271) ($P \leqslant 0.017$ for all). The occurrence of expected menses in the 26 responders was 2.5-fold higher than in the 13 non-responders ($P < 0.0001$). In 11 non-responders, on pioglitazone + metformin diet over 10 months versus antecedent metformin diet, DHEAS fell (211 to 171 μg/dl, $P = 0.02$), insulin fell (16 to 10 μIU/ml, $P = 0.001$), IR fell (3.37 to 1.73, $P = 0.002$), insulin secretion fell (217 to 124, $P = 0.004$), sex hormone-binding globulin rose (31 to 43 nmol/l, $P = 0.006$), and HDL cholesterol rose (38 to 42 mg/dl, $P = 0.003$). On pioglitazone + metformin diet, the occurrence of expected menses was 2-fold higher than on metformin diet ($P < 0.0001$). CONCLUSIONS: In women with PCOS who failed to respond optimally to metformin, when pioglitazone was added, insulin, glucose, IR, insulin secretion, and DHEAS fell, HDL cholesterol and sex hormone-binding globulin rose, and menstrual regularity improved, without adverse side-effects.

Key words: insulin resistance/metformin/pioglitazone/polycystic ovary syndrome

Introduction

Polycystic ovary syndrome (PCOS) may be the most common endocrinopathy of women, present in 4–7% of women (Knochenhauer *et al*., 1998). It is characterized by oligoamenorrhoea and clinical and/or biochemical hyperandrogenism, and, commonly but not always, associated with hyperinsulinaemia, gestational diabetes, type 2 diabetes mellitus (DM), frequent first trimester pregnancy loss, and increased risk for cardiovascular disease and endometrial cancer (Glueck *et al*., 2002a; Nestler, 2002; Nestler *et al*., 2002; Homburg, 2002). More than 50% of women with PCOS are obese (Gambineri *et al*., 2002). Historically, treatment has been directed at ovulation induction for infertility, oral contraceptives or progestins for menstrual irregularity, and oral contraceptives and/or antiandrogens for hirsutism (Guzick, 1998). Recently, insulin-sensitizing drug treatment has been directed at a major contributor to PCOS, insulin resistance and hyperinsulinaemia (Velazquez *et al*., 1994; 1997a;b; Fleming *et al*., 2002; Ghazeeri *et al*., 2003). Weight reduction, difficult to achieve in PCOS because of insulin resistance, reduces hyperinsulinaemia and hyperandrogenaemia, and may restore ovulation and fertility (Kiddy *et al*., 1992; Moran *et al*., 2003).

Metformin and other insulin-sensitizing drugs (thiazolidinediones), by reducing insulin resistance and hyperinsulinaemia, reduce insulin-driven ovarian and adrenal hyperandrogenism, usually restoring normal LH and FSH secretion, facilitating normal ovulatory cycles and pregnancy (Glueck *et al*., 2002a;b;c; Nestler, 2002; Nestler *et al*., 2002; Ghazeeri *et al*.,

Table I. Clinical characteristics at study entry in 13 patients with polycystic ovary syndrome, not optimally responsive to metformin plus diet

ID no.	Age (years)	BMI <30 kg/m^2	Blood pressure <130/85 mmHg	Previous no. of menses/year	FG score <7	Acne	Testosterone ⩽70 ng/dl	Free testosterone ⩽6.8 pg/ml	Androstenedione ⩽270 ng/dl	DHEAS ⩽240 μg/dl	Polycystic ovaries by ultrasound	*Acanthosis nigricans*
1	31.8	34.8	142/80	2	10	Yes	33	2.50	133	240	+	N
2	34.6	44.1	148/84	4	13	Yes	47	2.20	126	113	+	Y
3	23.8	35.8	118/76	0	23	No	19	1.10	86	122	+	N
4	39.6	47.5	–	0	16	No	27	1.60	83	198	–	N
5	13.9	40.1	110/74	0	13	Yes	146	5.30	338	93	–	Y
6	22.8	43.0	–	0	4	No	183	30.40	181	155	+	Y
7	23.2	24.3	104/68	6	8	Yes	123	5.20	500	339	–	N
8	37.0	45.4	138/84	0	12	No	30	1.50	86	178	+	N
9	27.3	51.1	136/86	2	16	Yes	80	11.00	300	225	+	N
10	19.1	25.4	118/68	0	23	No	57	2.40	281	155	–	N
11	29.0	35.2	136/84	5	16	Yes	68	4.70	263	253	+	N
12	30.7	48.9	146/90	0	12	Yes	69	2.70	–	–	–	Y
13	19.7	25.8	120/70	3	12	No	46	2.40	223	296	+	N
	27.1 ± 7.6	38.6 ± 9.2	129 ± 15/79 ± 8		12(92)	7(54)	4(31)	2(15)	4(33)	3(25)	8(62)	4(31)

Values in parentheses are percentages.
BMI = body mass index; FG = Ferriman and Gallwey (1961) hirsutism score.
Cutpoints for BMI, blood pressure, FG score, testosterone, free testosterone, androstenedione and DHEAS displayed.

2003). There have been 38 open label and/or placebo-controlled studies which have documented metformin's effectiveness in reducing hyperinsulinaemia and improving the endocrinopathy of PCOS with resultant normalization of menstrual cyclicity and restoration of ovulation in 23–90% of both adults and adolescents (Glueck *et al*., 2002a;b; Nestler, 2002). In the largest randomized controlled trial of metformin in PCOS to date, ovulation frequency was 23% in 45 metformin-treated cases versus 13% in 47 placebo-treated controls ($P < 0.01$), and time to first ovulation was shorter (23.6 days versus 41.8 days, $P < 0.05$) (Fleming *et al*., 2002). Benefits of metformin were not observed in morbidly obese subjects with body mass index (BMI) >37 kg/m^2 (Fleming *et al*., 2002).

In PCOS, insulin-sensitizing drugs as a class increase spontaneous ovulation, enhance induction of ovulation with clomiphene, and increase clinical pregnancy rates (Nestler, 2002; Nestler *et al*., 2002; Ghazeeri *et al*., 2003). Although the peroxisome proliferator activator receptor (PPAR)-gamma binding agent, troglitazone, has been withdrawn because of hepatotoxicity, it was effective in women with PCOS in increasing spontaneous ovulation, and increasing clomiphene induction of ovulation (Azziz *et al*., 2001). In a 2 month, double-blind, placebo-controlled trial, rosiglitazone (8 mg/day another PPAR-gamma binding agent) enhanced both spontaneous and clomiphene-induced ovulation by improving insulin sensitivity and reducing hyperandrogenaemia in overweight and obese women with PCOS, whose mean BMI were 35.5–38.5 kg/m^2 (Ghazeeri *et al*., 2003). To date, no data have been published on the use of a third PPAR-gamma binding agent, pioglitazone, in women with PCOS.

In an observational study of 13 women with PCOS not optimally responsive to metformin and diet, our specific aim was to assess efficacy and safety of pioglitazone when added to antecedent metformin diet, and to compare these 13 women with 26 women with PCOS, responsive to metformin and diet, matched by age and by pre-treatment menstrual history.

Materials and methods

Patients with PCOS

We used a protocol approved by the Jewish Hospital Institutional Review Board, with signed informed patient consent. The diagnosis of PCOS (Kawadzki *et al*., 1992; Glueck *et al*., 2002b) was made in pre-menopausal women on the basis of chronic oligoamenorrhoea and either clinical hyperandrogenism [hirsutism (Ferriman and Gallwey, 1961) score ⩾7, severe acne], or biochemical hyperandrogenism (high levels of total or free testosterone, androstenedione, or DHEAS). Exclusionary criteria included serum creatinine >1.5 mg/dl, Types 1 and 2 DM, pituitary insufficiency, persistent hyperprolactinaemia, and congenital adrenal hyperplasia (Glueck *et al*., 2002b).

Study protocol

This prospective, open label, single centre, consecutive case series study included 39 women from the Midwestern United States who were referred for a 2 year study of the efficacy and safety of metformin in PCOS (Glueck *et al*., 1999a;b;c). They came from a larger cohort of 743 women, referred for the diagnosis and therapy of PCOS from 6/12/97 to 1/23/02. None of the 39 women (13 non-optimal responders to metformin, 26 responders) had previous laparoscopic diathermy. The percentage of women having previously received clomiphene ovulation stimulation did not differ between non-responders (2/13, 15%) and the matched responders (7/26, 27%), Fisher's $P = 0.7$ (non-significant).

At pre-metformin baseline, and after an overnight fast, blood was obtained for measurements of serum insulin and glucose, lipids and lipoprotein cholesterols, sex hormones, and sex hormone-binding globulin (SHBG) using previously reported methods (Glueck *et al*., 2002b). The homeostatic model assessment (HOMA) for insulin resistance (IR) and beta cell function was used as per Haffner *et al*. (1996). A detailed history of menstrual status from menarche to the time of study entry was obtained, with special focus on menstrual frequency in the year before study entry (Table I and Table II). Weight and seated systolic and diastolic blood pressure were recorded.

While receiving metformin 2.55 g/day (850 mg, taken 3 times per day with meals), and while on pioglitazone (Takeda–Lilly) + metformin, the women with PCOS were evaluated every 2 months with serial measurements of weight, blood pressure, insulin, lipids, glucose, IR and beta cell function (Haffner *et al*., 1996), and sex

Table II. Clinical characteristics at study entry in 26 patients with polycystic ovary syndrome, responsive to metformin plus diet

ID no.	Age (years)	BMI <30 kg/m^2	Blood pressure <130/85 mmHg	Previous no. of menses/year	FG score <7	Acne	Testosterone ⩽70 ng/dl	Free testosterone ⩽6.8 pg/ml	Androstenedione ⩽270 ng/dl	DHEAS ⩽240 μg/dl	Polycystic ovaries by ultrasound	*Acanthosis nigricans*
1	17.0	41.3	108/76	1	8	No	38	3.10	137	98	+	No
2	27.0	27.0	118/72	0	10	Yes	111	4.10	469	254	–	Yes
3	35.0	41.4	–	0	0	No	76	6.40	315	119	+	No
4	21.0	30.9	–	0	10	Yes	20	1.00	57	131	+	No
5	20.6	45.7	144/88	0	16	Yes	44	9.50	230	343	–	Yes
6	19.4	35.9	–	6	10	Yes	65	2.80	305	198	–	No
7	38.7	51.1	124/84	0	8	Yes	47	1.10	79	110	+	Yes
8	25.6	40.5	110/70	2	13	No	65	3.40	–	–	–	No
9	32.3	43.2	140/90	5	19	No	18	4.10	120	104	+	Yes
10	28.3	40.1	134/88	5	23	Yes	57	1.70	63	176	+	Yes
11	32.1	39.9	136/88	1	12	No	52	2.50	193	188	+	No
12	36.3	50.1	150/82	4	11	No	63	3.40	214	213	–	Yes
13	31.1	30.7	116/74	2	20	No	70	13.10	280	78	–	No
14	29.3	30.6	130/78	0	11	Yes	30	1.30	139	202	+	Yes
15	24.9	30.6	132/80	5	16	Yes	27	1.00	100	105	–	No
16	24.4	30.9	122/74	2	35	Yes	52	12.90	330	310	–	Yes
17	16.0	28.4	–	0	0	Yes	135	5.50	332	89	–	No
18	34.2	31.9	104/76	0	12	Yes	22	2.10	145	180	–	No
19	19.6	47.9	–	0	7	No	30	1.90	142	138	–	No
20	32.6	28.8	142/84	0	12	No	82	1.40	231	–	+	No
21	37.1	35.3	122/68	0	7	No	31	1.10	78	129	+	No
22	19.0	26.9	100/68	0	12	Yes	33	1.80	130	237	–	No
23	14.3	23.8	120/86	0	0	Yes	20	1.00	49	109	–	Yes
24	36.9	48.2	–	4	27	No	46	6.80	190	87	+	No
25	35.3	35.6	120/72	0	12	Yes	56	1.30	185	110	+	No
26	32.6	29.0	122/78	2	20	Yes	72	2.90	211	174	+	No
	27.7 ± 7.5	36.4 ± 8.0	125 ± 14/79 ± 7		23 (88)	15 (58)	5 (19)	3 (12)	6 (24)	3 (13)	13 (50)	9 (35)

Values in parentheses are percentages.
BMI = body mass index; FG = Ferriman and Gallwey (1961) hirsutism score.
Cutpoints for BMI, blood pressure, FG score, testosterone, free testosterone, androstenedione and DHEAS displayed.

hormones. At each 2 month follow-up visit, menstrual status was recorded including dates of last menstrual periods, length of menses, and frequency (if more than once/month). The regularity of menses was assessed (in 3 month blocks) as a percentage (number of observed menses/number of expected menses).

When using pioglitazone in patients with PCOS, following United States FDA guidelines (Murray and Kelly, 2002), we obtained pre-treatment liver function tests, proceeding with caution if the baseline alanine aminotransferase (ALT) levels were mildly elevated, and not using the drug if baseline ALT was >2.5 times the upper limit of normal. We also rechecked liver function every 2 months during the first year of therapy and stopped pioglitazone if ALT levels increased to >3 times the upper normal limit or if clinical hepatitis developed (Murray and Kelly, 2002).

At study entry, all women with BMI ⩾25, categorized as overweight by Flegal *et al*. (2002), were instructed on a 1500 calorie diet with 26% of the calories as protein, 44% as carbohydrates (42% complex), and 30% of calories as fat (polyunsaturate:saturate ratio 2:1). For women with normal BMI <25 (Flegal *et al*., 2002), a 2000 calorie diet was given. For all women, diet was maintained throughout follow-up, with re-instruction every 4–6 months.

The five women who wished to conceive (nos. 3, 4, 6, 8, 11; Table I) were given folic acid, 1 mg/day, and were instructed to stop pioglitazone as soon as pregnancy was verified (subject no. 6, Table I). hCG was quantified during the pioglitazone + metformin treatment period if menses failed to appear within 6 weeks of the previous menses.

We knew, based on previous studies (Glueck *et al*., 1999b, 2001a;b; 2002;c; Fleming *et al*., 2002), that up to 23% of women with PCOS receiving diet plus metformin (2.55 g/day) would be not optimally responsive to treatment, defined by failure to significantly reduce weight, androstenedione, testosterone, DHEAS, IR, insulin secretion, to increase SHBG and HDL cholesterol, and/or by failure to resume regular menstrual cycles. In 13 women who did not achieve these positive outcomes, after a median of 12 months on metformin plus diet, while maintaining the same diet and metformin 2.55 g/day, pioglitazone (45 mg/day) was added. Provision of concurrent pioglitazone and metformin was based on the hypothesis that addition of a second insulin-sensitizing drug (Smith, 2001) in women non-optimally responsive to metformin diet might lead to more favourable endocrine outcomes. Eleven of the 13 non-optimally responsive women were evaluated every 2 months on pioglitazone + metformin diet for a median of 10 months, and their response compared with metformin diet alone. Two of the 13 women (Nos. 8 and 10), to date, have received pioglitazone + metformin <4.5 months and their responses to combined medication are not included.

In an attempt to characterize any unique features of the 13 women with PCOS who were not optimally responsive to metformin diet, we matched two 'control' women with PCOS by age and by pre-treatment menstrual history to each non-responder (Table II and Table III). These 26 'control' women with PCOS were responsive to metformin diet over the same 12 month treatment period and were recruited into the study at the same time as the 13 non-responders.

Outcome measures

Outcome measures included reduction in weight, blood pressure, androstenedione, DHEAS, testosterone, insulin, IR, beta cell function

Table III. Changes on metformin therapy in women with polycystic ovary syndrome, 13 women not optimally responsive to metformin, and 26 responsive (median values exhibited)

	13 women not optimally responsive to metformin, on metformin for a median of 12 months		26 women responsive to metformin, on metformin for a median of 13 months	
	Baseline	On metformin	Baseline	On metformin
Weight (kg)	114	114	93	87****
Systolic BP (mmHg)	128	119	122	119
Diastolic BP (mmHg)	78	75	78	74*
Androstenedione (ng/dl)	202	259	185	164
DHEAS (μg/dl)	188	201	131	178**
SHBG (nmol/l)	40	31	23	21
Testosterone (ng/dl)	56	51	50	32****
Free testosterone (pg/ml)	2.4	6.1	2.8	2.8
Insulin (μIU/ml)	21.0	16.2*	26.0	16.4****
Glucose (mg/dl)	87	89	87	86
HOMA insulin resistance	4.10	3.37	5.32	3.45****
HOMA beta cell function	251	200**	351	271*
HDL (mg/dl)	44	39	40	41

Changes from baseline to the mean on metformin, by paired Wilcoxon test: *$P \leqslant 0.05$, **$P < 0.01$, ***$P \leqslant 0.001$, ****$P \leqslant 0.0001$.
BP = blood pressure; DHEAS = dehydroepiandrosterone sulphate; SHBG = sex hormone-binding globulin; HOMA = homeostatic model assessment; HDL = high density lipoprotein.

Table IV. Changes on metformin alone and on metformin plus pioglitazone in 11 women with polycystic ovary syndrome, who were not optimally responsive to metformin (median value exhibited)

	On metformin alone for a median of 12 months		On metformin + pioglitazone for a median of 10 months
	Baseline	On metformin	
Weight (kg)	114	118	111
Systolic BP (mmHg)	128	118	121
Diastolic BP (mmHg)	78	75	76
Androstenedione (ng/dl)	202	269	260
DHEAS (μg/dl)	212	211	171†
SHBG (nmol/l)	42	31*	43††
Testosterone(ng/dl)	56	51	40
Free testosterone (pg/ml)	2.5	6.8	8.7
Insulin (μIU/ml)	23.0	16.2*	10.2†††
Glucose (mg/dl)	90	89	86†
HOMA insulin resistance	4.24	3.37	1.73††
HOMA beta cell function	251	217*	124††
HDL (mg/dl)	39	38	42††

Changes from baseline to the mean on metformin, by paired Wilcoxon test: *$P \leqslant 0.05$, **$P < 0.01$, ***$P \leqslant 0.001$.
Changes from the mean on metformin to the mean on metformin + pioglitazone, by paired Wilcoxon test: †$P \leqslant 0.05$, ††$P < 0.01$, †††$P \leqslant 0.001$.
BP = blood pressure; DHEAS = dehydroepiandrosterone sulphate; SHBG = sex hormone-binding globulin; HOMA = homeostatic model assessment; HDL = high density lipoprotein.

(insulin secretion), and glucose, increments in SHBG and HDL cholesterol, and resumption of regular, normal menstrual cycles (Table III and Table IV). Development of any abnormal liver function tests was also monitored on pioglitazone.

Study limitations

An optimal study design of addition of pioglitazone to metformin in women who fail to respond optimally to metformin would be a randomized, placebo-controlled, double-blind clinical trial after a metformin-alone period, metformin + placebo versus metformin + pioglitazone. However, to design and successfully carry out such a trial, efficacy and safety data must first be collected to allow construction of power and sample size estimates (below). Because, to date, no data have previously been published on the efficacy–safety of pioglitazone alone or pioglitazone + metformin in women with PCOS not optimally responsive to metformin, power and sample size estimates for future placebo-controlled, blinded studies cannot be constructed without accrual of non-blinded, open-label pilot data found in this study.

Although there was a broad range of age among the 13 non-responders (two teenagers aged 14 and 19 years, six women aged 20–30, four aged 31–39, and one aged 39.6 years), each non-responder was age-matched with two responders (26 total responders), to reduce the likelihood that age or age range could distort the comparison of response to metformin in the two groups. Similarly, although there was a broad range of pre-treatment BMI in the 13 non-responders (one <25 kg/m^2, two from 25 to <30, 3 from 30 to <40, and 7 ⩾40), neither BMI nor its categorical distribution differed (P = 0.7)

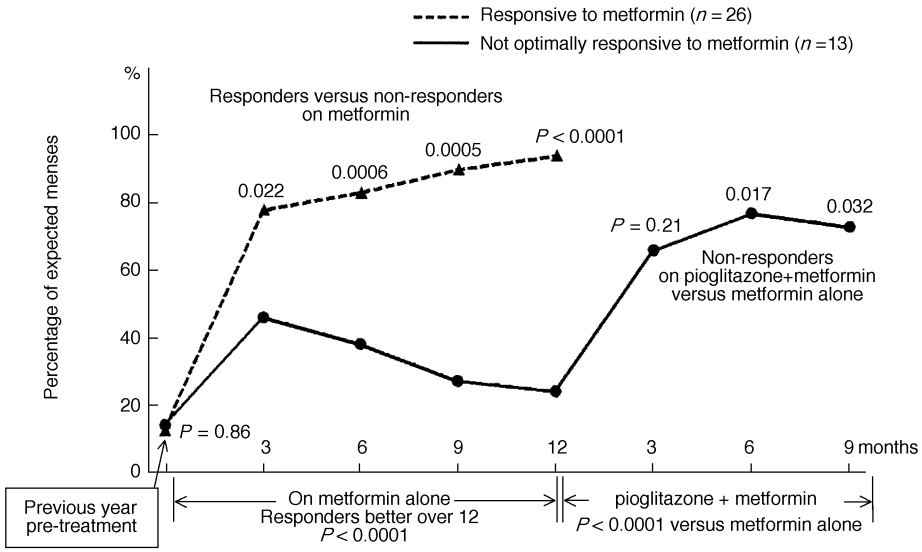

Figure 1. Percentage of expected menses occurring in two cohorts of women with polycystic ovary syndrome (PCOS), 13 non-optimally responsive to metformin therapy, first receiving metformin (2.55 g/day) for a median of 12 months, then metformin (2.55 g/day) and pioglitazone (45 mg/day) for a median of 10 months, and 26 responsive to metformin, matched to the 13 women by age and by pre-treatment menstrual history, receiving metformin (2.55 g/day) for a median of 12 months. Menstrual frequency in responsive versus non-responsive cohorts compared by Wilcoxon tests of difference (left panel). Menstrual frequency in non-responsive cohort on metformin + pioglitazone versus metformin alone compared by paired Wilcoxon tests (right panel).

from the 26 age–menstrual-matched controls, reducing the likelihood that obesity or the range of obesity could distort the comparison of metformin response. In the paired comparison of metabolic and menstrual response to metformin versus metformin + pioglitazone in 13 women, with each subject as her own control, age and weight should not have affected differences in response to the drugs.

Statistical analysis

Pre-treatment, baseline variables in non-responders versus responders were compared by Wilcoxon tests (SAS/STAT, 2002). We also used stepwise logistic regression where group [metformin responders (n = 26) and non-responders (n = 13)] was the dependent variable, and explanatory variables (at pre-treatment baseline) were BMI, insulin, glucose, IR, beta cell secretion, SHBG, androstenedione, DHEAS, and total testosterone. Paired Wilcoxon tests (SAS/STAT, 2002) were used to compare pre-treatment baseline levels against the mean levels of each patient during therapy on metformin diet.

To further assess the contribution of weight to metformin response, the 13 non-responders and 26 responders were pooled, and stepwise regression analyses were done separately with the following variables (separately) as dependent variables: change on metformin in systolic and diastolic blood pressure, androstenedione, DHEAS, SHBG and testosterone (total and free). Explanatory variables in each model were group (responders, non-responders), pre-treatment values for weight, insulin, and glucose, and change on metformin in weight, insulin, and glucose. A separate stepwise regression model was run with change on metformin in insulin as the dependent variable and explanatory variables including group (responders, non-responders), pre-treatment values for weight, insulin, and glucose, as well as change in glucose and weight on metformin.

To judge the success of metformin + pioglitazone, mean values of each patient on metformin were compared with mean values on metformin + pioglitazone using paired Wilcoxon tests, (Table IV).

Menstrual status pre-treatment and the observed/expected percentage of menses on treatment with metformin (13 non-responders versus 26 responders) was compared by Wilcoxon tests, and for metformin versus metformin + pioglitazone, by paired Wilcoxon tests (SAS/STAT, 2002). We tested by trend analysis whether any increase in menstrual frequency and/or regularity was achieved early after starting metformin, and after adding pioglitazone, and persisted thereafter (Figure 1). Our hypothesis was that demonstration of an early increase in menses on metformin, with stability of menstrual status in the final 3–6 months of metformin, followed by a change when pioglitazone was started, would make a stronger case for the increase during pioglitazone treatment being the result from pioglitazone rather than a carryover or continuation of a progressive increase in menses from metformin.

Power and sample size calculations were carried out (Sokol *et al.*, 1995) based on the results of the current study, comparing changes in insulin, IR, and menstrual frequency in sequential 3 month treatment periods on metformin alone versus metformin + pioglitazone. These calculations estimated how many cases and controls would be needed for a placebo-controlled, blinded study of the addition of pioglitazone to metformin in women not optimally responsive to metformin to allow declaration of a difference in the two treatment groups, at a significance level alpha = 0.05, with power of 80%.

Results

Entry, pre-metformin characteristics of the women with PCOS

All 39 women, by selection, had oligoamenorrhoea and either clinical hyperandrogenism and/or biochemical hyperandrogenism (Table I and Table II).

At pre-treatment baseline, the 13 non-responders did not differ from the 26 responders by weight, by BMI category, systolic or diastolic blood pressure, sex hormones, insulin, glucose, IR, or insulin secretion (Wilcoxon $P > 0.07$ for all). By stepwise logistic regression, at pre-treatment baseline, the 13

non-responders did not differ ($P > 0.15$) from the 26 responders for BMI, insulin, glucose, IR, beta cell secretion, DHEAS, or total testosterone, but the 13 non-responders had higher SHBG ($P = 0.016$) and androstenedione ($P = 0.04$) than the 26 responders.

The 13 non-responders and the 26 responders did not differ from the remaining 704 women with PCOS also referred to our centre from December 6, 1997 to January 23, 2002 by BMI category (Flegal *et al*., 2002) (normal <25, overweight 25–30, obese 30–40, severely obese ⩾40) ($\chi^2 = 8.36$, 6 df, $P = 0.21$). The 13 non-responders and 26 responders differed from the remaining 704 women with PCOS by having no women age ⩾ 40 years, versus 15%, $\chi^2 = 17.5$, 6 df, $P = 0.008$. Thus, although there was a broad range of BMI and age in the 13 non-responders and 26 responders, BMI was representative of our PCOS referral study cohort, and age range was tighter.

Outcomes on metformin plus diet

In the non-optimally responsive cohort of 13 women, on metformin plus diet for a median of 12 months, median insulin fell from 21 to 16 μIU/ml ($P = 0.048$), and insulin secretion fell from 251 to 200 ($P < 0.01$) (Table III).

In the responsive cohort of 26 women, on metformin plus diet for a median of 13 months, weight fell, median DHEAS unexpectedly rose ($P < 0.01$) (within the normal range), testosterone fell, insulin fell, IR fell, and insulin secretion fell (all $P \leqslant 0.017$, Table III), and diastolic blood pressure fell ($P = 0.02$). We do not know why DHEAS rose within the normal range in the 26 responders on metformin (Table III).

By stepwise regression with all 39 women pooled, group status was a significant co-variate for change (on metformin) in SHBG which fell more in non-responders ($R^2 = 11\%$, $P = 0.042$), and for change in free testosterone which rose more in non-responders ($R^2 = 20\%$, $P = 0.007$). Pre-treatment weight and weight change on metformin were significant co-variates for change in insulin on metformin. Insulin fell more on metformin when pre-treatment insulin was high (partial $R^2 = 57\%$, $P < 0.0001$), when pre-treatment weight was lower (partial $R^2 = 9.1\%$, $P = 0.004$), and when weight fell more on metformin (partial $R^2 = 7.3\%$, $P = 0.005$).

After 3 months on metformin, 46% of expected menses had occurred in the 13 non-responders, better than 14% at pre-treatment baseline, $P = 0.05$ (Figure 1). At months 6, 9 and 12, 38% ($P = 0.07$), 27% ($P = 0.9$) and 24% ($P = 0.5$) of menses occurred, versus 14% at pre-treatment baseline (Figure 1). Trend analysis did not reveal any significant trend in menses on treatment ($P = 0.9$).

By selection, the pre-treatment menstrual history in the 26 PCOS responders did not differ from the 13 non-responders (13 versus 14%, $P = 0.9$, Figure 1). During the first 3 months on metformin, 78% of expected menses occurred in the responders, versus 46% in the non-responders ($P = 0.022$, Figure 1). At months 6, 9 and 12, 83%, 91% and 94% of menses occurred in the responders, versus 38% ($P = 0.0006$), 27% ($P = 0.0005$) and 24% ($P < 0.0001$) in the non-responders (Figure 1). Over the 12 months, the occurrence on expected menses in the 26 responders was 2.5-fold higher than in the 13 non-responders ($P < 0.0001$, Figure 1). In the responders, trend analysis revealed a significant trend ($P < 0.0001$) towards increased menstrual frequency over time on treatment (Figure 1).

Outcomes on metformin and pioglitazone

In 11 women, non-optimally responsive to metformin diet alone, on pioglitazone + metformin diet over 10 months, compared with their antecedent 12 months on metformin diet, median DHEAS fell (211 to 171 μg/dl, $P = 0.02$), SHBG rose from 31 to 43 nmol/l ($P = 0.006$), insulin fell (16.2 to 10.2 μIU/ml, $P = 0.001$), IR fell from 3.37 to 1.73 ($P = 0.002$), beta cell function fell from 217 to 124 ($P = 0.004$), glucose fell from 89 to 86 mg/dl ($P < 0.05$), and HDL cholesterol rose from 38 to 42 mg/dl ($P = 0.003$) (Table IV). Weight did not change (Table IV).

On pioglitazone + metformin diet, the percentage of menses over 9 months increased 2-fold when compared with that on metformin diet alone (Figure 1), $P < 0.0001$. During the first 3 months on pioglitazone + metformin, 67% of expected menses occurred versus 46% on metformin alone ($P = 0.2$), 77 versus 38% on metformin alone during the second 3 months ($P = 0.017$), and 73 versus 27% on metformin alone during the third 3 months ($P = 0.032$) (Figure 1). Trend was not significant ($P = 0.61$).

None of the women developed abnormal liver function tests while taking metformin + pioglitazone. There was no lactic acidosis, and no hypoglycaemia. In the one subject who conceived on pioglitazone + metformin, pioglitazone was immediately stopped and metformin was continued throughout (no. 6, Table I). Her pregnancy was uneventful, without gestational diabetes, and with delivery of a normal 5 lb 12 oz male infant at gestation week 36.

Power and sample size estimates

To determine whether outcomes on pioglitazone + metformin diet differed from those on metformin diet at $P = 0.05$, the current study's sample size had a power = 90% based on menstrual frequency in the second 3 month treatment period, 80% if based on the third 3 months, and 70% based on changes in insulin.

To perform a successful randomized controlled clinical trial at a significance level of 5% with power of 80%, comparing pioglitazone + metformin versus placebo + metformin, based on the current study's changes in insulin, there would need to be 14 in each group. Based on IR there would need to be 14 in each group, and based on menses in the first 3, 6 and 9 months on therapy, 40, 9 and 10 respectively, in each group.

Discussion

Metformin fails to promote resumption of normal menses in up to 23% of women with PCOS (Velazquez *et al*., 1994; 1997; Glueck *et al*., 1999b; Fleming *et al*., 2002); these women also often fail to normalize androgens or to achieve significant weight loss. The most common apparent cause of metformin treatment failure in PCOS appears to be morbid obesity, common in women with PCOS (Fleming *et al*., 2002; Gambineri *et al*., 2002). In one of the two studies of PCOS which, to date, failed to reveal metabolic benefit from

metformin (Acbay *et al*., 1996; Ehrmann *et al*., 1997), mean pre-treatment BMI was 39 (Ehrmann *et al*., 1997). It is possible that obesity and extreme obesity is more prevalent in women from the USA with PCOS than in women from the rest of the world, as in the current and other USA studies (Ehrmann *et al*., 1997), and it would be valuable to have metformin + pioglitazone studies in PCOS cohorts with less preponderant obesity.

In the current report, the major characteristics which differentiated the 26 responders from the 13 non-responders were greater weight loss on metformin diet, and more marked resumption of regular menses, with progressive improvement over time. Neither pre-treatment BMI nor obesity category by BMI differed between non-responders and responders. With all 39 women pooled, by stepwise regression, on metformin insulin fell more when pre-treatment insulin was high, when pre-treatment weight was less, and when weight fell more on metformin. Since change in insulin on metformin is probably central to its beneficial metabolic and endocrine effects, this finding is consistent with the report of Fleming *et al*. (2002) that metabolic benefits of metformin were not observed in morbidly obese women with PCOS whose BMI was >37.

Based on the current study, in treatment of PCOS with metformin, if menstrual cyclicity does not improve by 3–6 months, and if endocrinopathy is not ameliorated, then consideration should be given to adding a second insulin-sensitizing drug, pioglitazone. We postulated that pioglitazone, with a different locus of insulin sensitization than metformin (Ikeda and Sugiyama, 2001; Smith *et al*., 2001; Pittas and Greenberg, 2002), would further ameliorate hyperinsulinaemia and resolve endocrinopathy, when added to metformin diet in obese women with PCOS who had not responded optimally to metformin diet. In the current study, when pioglitazone was added, there were significant incremental benefits beyond metformin diet; DHEAS, glucose, insulin, IR and insulin secretion fell, and HDL cholesterol and SHBG rose (all $P < 0.01$). On pioglitazone + metformin diet, the occurrence of expected menses was 2-fold higher than on metformin diet ($P < 0.0001$).

As in the current study, the normal response to reduced IR is reduced beta cell insulin output (falling insulin), which is essentially what models measure in relation to fasting glucose (Buchanan *et al*., 2002). In women with prior gestational diabetes, a fall in insulin output (-beta cell function) induced by troglitazone was protective for beta cells and prevented development of type 2 diabetes (Buchanan *et al*., 2002).

We speculate that the apparently safe (no hepatotoxicity, no hypoglycaemia), added benefit of pioglitazone in the current study rose from further lowering of IR (Ikeda and Sugiyama, 2001; Smith *et al*., 2001; Pittas and Greenberg, 2002), with consequent lowering of insulin and insulin secretion, and with subsequent improvement in hyperandrogenaemia manifested by reduction in DHEAS and increments in SHBG. One potential drawback of addition of pioglitazone to metformin diet is cessation of weight loss. Pioglitazone apparently promotes weight gain by inhibition of glycerol kinase, with reduction of circulating fatty acids and subsequent reduction in insulin and retention of the fatty acids as triglycerides in adipose tissue (Guan *et al*., 2002).

Studies with rosiglitazone and pioglitazone suggest that hepatotoxicity is not a class effect of the glitazones, an issue that rose from the hepatotoxicity of troglitazone (Hanefeld and Belcher, 2001; Lebovitz *et al*., 2002; Ghazeeri *et al*, 2003). Overall, despite two case reports of apparent drug-related hepatotoxicity (Maeda, 2001; May *et al*., 2002), clinical experience with pioglitazone has not revealed any trend towards unacceptable hepatotoxicity (Hanefeld *et al*., 2001; Scheen, 2001; Lebovitz *et al*., 2002; Scherbaum *et al*., 2002). In the current report, there were no elevations of ALT >3 times the upper normal limit, and no clinical hepatitis developed.

Rosiglitazone and pioglitazone increase insulin sensitivity by acting on muscle and liver to increase glucose utilization and decrease glucose production (Nolan *et al*., 1994; Iwamoto *et al*., 1996). Rosiglitazone and pioglitazone have both been classified in category 'C'. In animal models, treatment during mid–late gestation was associated with fetal death and growth retardation. There are no case reports or systematic clinical studies of use of thiazolidindeones during pregnancy. In the current study, the single woman who conceived on pioglitazone + metformin stopped pioglitazone at first confirmation of pregnancy and delivered a normal infant at 36 weeks gestation. Given their pregnancy category 'C', neither rosiglitazone (Cataldo *et al*., 2001; Ghazeeri *et al*., 2003) nor pioglitazone should be continued after conception or used in the treatment of gestational diabetes. This differs from metformin which is pregnancy category 'B', appears to be safe during pregnancy for mother and fetus, reduces first trimester miscarriage (Glueck *et al*., 2002c; Jakubowicz, 2002) and reduces gestational diabetes (Glueck *et al*., 2002c).

The current report was an observational, non-blinded pilot study to evaluate whether addition of pioglitazone (45 mg/day) to metformin 2.55 g/day in obese women who failed to optimally respond to metformin would further improve the endocrinopathy of PCOS. A randomized, placebo-controlled, double-blind trial will be required to confirm the improved outcomes on pioglitazone + metformin versus metformin alone in women not optimally responsive to metformin.

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