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Metformin during pregnancy reduces insulin, insulin resistance, insulin secretion, weight, testosterone and development of gestational diabetes: prospective longitudinal assessment of women with polycystic ovary syndrome from preconception throughout pregnancy

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BACKGROUND: In a prospective observational study of 42 pregnancies in 39 Caucasian women (age 30 ± 4 years) with polycystic ovary syndrome (PCOS), we examined effects of metformin on maternal insulin, insulin resistance (IR), insulin secretion (IS), weight gain, development of gestational diabetes (GD), testosterone and plasminogen activator inhibitor activity. We assessed the hypothesis that diet–metformin (MET) lessens the physiological gestational increase in IR and reduces gestational weight gain, thus reducing GD. METHODS: Preconception, in an out-patient clinical research centre, MET 1.5 (eight pregnancies) to 2.55 g/day (34 pregnancies) was started. Women with body mass index <25 or ≥25 kg/m^2 were given a 2000 or 1500 calorie/day, high-protein (26% of calories), low-carbohydrate (44%) diet. Calorie restrictions were dropped after conception. RESULTS: On MET, GD developed in three out of 42 pregnancies (7.1%). Median entry weight (94.5 kg) fell to 82.7 on MET at the last preconception visit ($P = 0.0001$), fell further to 81.6 during the first trimester, was 83.6 in the second trimester, and 89.1 kg in the third trimester. Median weight gain during pregnancy was 3.5 kg. The median percentage reduction in serum insulin was 40% on MET at the last preconception visit; insulin did not increase in the first or second trimesters ($P > 0.05$), and rose 10% in the third trimester. The median percentage reduction in HOMA IR was 46% on MET at the last preconception visit; IR did not increase ($P > 0.05$) in the first, second or third trimesters. HOMA insulin secretion fell 45% on MET at the last preconception visit, did not increase in the first trimester, rose 24% in the second trimester, and rose 109% in the third trimester. Testosterone fell 30% on MET at the last preconception visit ($P = 0.01$) and then rose 74, 61 and 95% during trimesters 1, 2 and 3; median testosterone during the third trimester did not differ from pre-treatment levels. CONCLUSIONS: By reducing preconception weight, insulin, IR, insulin secretion and testosterone, and by maintaining these insulin-sensitizing effects throughout pregnancy, MET–diet reduces the likelihood of developing GD, and prevents androgen excess for the fetus.

Key words: gestational diabetes/insulin/metformin/polycystic ovary syndrome (PCOS)/pregnancy

Introduction

Pregnancy increases requirements for insulin secretion while increasing insulin resistance (IR), upping demands on pancreatic β cells (Homko *et al*., 2001) and promoting development of gestational diabetes (GD) (Butte, 2000; Glueck *et al*., 2002a, 2002b). Women with GD have 67% impairment in pancreatic β cell compensation for insulin resistance compared with normal women (Xiang *et al*., 1999). In women with a history of GD and impaired glucose tolerance, the predominant response of β cells to troglitazone-induced improved insulin sensitivity was a reduction in insulin release (Buchanan *et al*., 2000). The physiological increase of insulin resistance during pregnancy promotes shifting metabolic fuel supplies from mother to fetus (Buchanan *et al*., 1990; Catalano *et al*., 1999, 2003; Kirwan *et al*., 2001). Women with polycystic ovary syndrome (PCOS), 46% of whom develop GD (Lanzone *et al*., 1996), enter pregnancy with higher IR than normal women (Lanzone *et al*., 1996; Legro *et al*., 1998; Paradisi *et al*., 1998; Lewy *et al*., 2001; Mikola *et al*., 2001; Vrbikova *et al*., 2002; Schachter *et al*., 2003). Women with PCOS probably develop GD when pancreatic β cells cannot overcome the superimposition of the physiological IR of pregnancy on their high preconception IR (Lanzone *et al*, 1995, 1996; Paradisi *et al*, 1998; Glueck *et al*., 2002a,b,c,d).

Obesity, characterizing 60–80% of PCOS patients, has a deleterious additive effect on carbohydrate homeostasis and increases IR during gestation (Galtier-Dereure *et al*., 2000). High maternal insulin in early pregnancy in normal women promotes gestational weight gain and weight retention postpartum, increasing risk of GD and, later, type 2 diabetes mellitus (DM) (Scholl and Chen, 2002). Body mass index (BMI) >25 kg/m^2 is a major predictor of GD (Turhan *et al*., 2003).

During gestation in normal women, the increment of insulin area under the curve during oral glucose tolerance testing is 3- to 3.5-fold higher than pre-pregnancy (Sepe *et al*., 1985; Catalano, 1994; Catalano *et al*., 1999). In normal pregnancies without development of GD, insulin sensitivity falls with advancing gestation, but falls much more sharply in those who develop GD (Catalano *et al*., 1999). Buchanan *et al*. (1990) reported that insulin sensitivity in normal pregnant women was reduced to only one-third that of non-pregnant women. This marked IR during pregnancy in normal women was compensated by a reciprocal enhancement of the first and second phase insulin responses to i.v. glucose, which were increased 3-fold in comparison with non-pregnant women (Buchanan *et al*., 1990). Buchanan *et al*. (1990) reported that during the third trimester, mild GD was characterized by an impairment of β cell function rather than exaggeration of normal IR of late pregnancy.

Lanzone *et al*. (1996) studied 15 women with PCOS who had conceived via pharmacological induction of ovulation. All women with preconception hyperinsulinaemia who became pregnant had impairment of glucose metabolism during pregnancy. PCOS patients were reported to be at higher risk of developing carbohydrate abnormalities during pregnancy than a normal population of similar reproductive age. Lanzone *et al*. (1996) concluded that women with PCOS who had abnormal insulin secretion at the pre-gestational stage might develop impaired gestational glucose tolerance or GD during pregnancy.

Lewy *et al*. (2001) studied 12 obese girls with PCOS, average age 12 years. Compared with normals, the obese girls with PCOS had a 50% reduction in peripheral tissue insulin sensitivity, with hepatic insulin resistance and compensatory hyperinsulinaemia. Lewy *et al*. (2001) concluded that reduced peripheral insulin sensitivity predicts an increased risk for type 2 DM in obese adolescents with PCOS.

Paradisi *et al*. (1998) hypothesized that metformin improves the 'natural' IR changes during gestation in hyperinsulinaemic PCOS women, studying 13 PCOS patients, five of whom developed GD. Those women with PCOS who developed GD had higher insulin area under the curve during oral glucose tolerance testing as well as lower insulin sensitivity in the first trimester. Paradisi *et al*. (1998) proposed that early alteration of insulin sensitivity and secretion constitutes specific risk factors in PCOS for development of GD.

In addition to the contributions of IR to GD, Schachter *et al*. (2003) have reported that IR-hyperinsulinaemia in patients with PCOS is associated with elevated plasma homocysteine, regardless of body weight. We speculate that hyperinsulinaemia-driven homocysteinaemia (Schachter *et al*., 2003) might contribute to the high first trimester miscarriage rate in women with PCOS without metformin therapy (Glueck *et al*., 2002c), and that metformin, by ameliorating hyperinsulinaemia (Velazquez *et al*., 1994, 1997) may, in part, reduce first trimester miscarriage (Glueck *et al*., 2001, 2002c,d) by reducing homocysteine levels.

In a prospective observational study of 42 pregnancies in 39 women (age 30 ± 4 years) with PCOS, we examined effects of metformin on maternal insulin, IR, insulin secretion, weight gain, development of gestational diabetes, testosterone and plasminogen activator inhibitor activity (PAI-Fx). We assessed the hypothesis that diet–metformin lessens the physiological gestational increase in IR and reduces gestational weight gain, thus reducing GD.

Materials and methods

Cases and controls

We used a protocol approved by the Jewish Hospital Institutional Review Board. All patients gave signed informed consent. Procedures followed were in accordance with the ethical standards for human experimentation established by the Declaration of Helsinki.

The diagnosis of PCOS was made on the basis of chronic oligoamenorrhoea and clinical hyperandrogenism [hirsutism; Ferriman–Gallwey (FG) score ⩾7 (Ferriman and Gallwey, 1961); severe acne] and/or biochemical hyperandrogenism [high total or free testosterone, androstenedione or dehydroepiandrosterone sulphate (DHEA-S)] (Table I) (Kawadzki and Dunaif, 1992; Glueck *et al*., 2002c,d). Exclusion criteria included serum creatinine >1.5 mg/dl, type 1 DM, type 2 DM on pharmacological therapy, pituitary insufficiency, persistent hyperprolactinaemia and congenital adrenal hyperplasia (Glueck *et al*., 1999, 2001, 2002c,d). Having selected the cohort on the basis of previous PCOS diagnostic criteria (Kawadzki and Dunaif, 1992; Glueck *et al*., 2002c,d), we also examined the diagnosis of PCOS in the subset of 34 women having polycystic ovaries documented by pelvic ultrasound and/or laparotomy (Table I, bottom 4 rows), using criteria of the very recent (July 2003) ESHRE/ASRM consensus conference on PCOS (Laven *et al*., 2002; Fauser *et al*., 2003). By these new criteria, the diagnosis of PCOS (Table I, bottom 4 rows) requires both the presence of polycystic ovaries and elevated free androgen index (FAI). We used a cut-off point for high FAI of ⩾7.3, the 97.5th percentile in a general female Caucasian population (Sowers *et al*., 2003). We used the definitions of Laven *et al*. (2002) for oligoamenorrhoea (bleeding intervals between 35 days and 6 months), or amenorrhea (bleeding interval >6 months) (Table I).

Since 100% of our prospectively studied patients with PCOS conceived on metformin, we did not have access to a second, potentially informative control group, i.e. women with PCOS who conceived without metformin and, while following the same protein enriched, carbohydrate-restricted diet, did not take metformin during pregnancy. We also did not have access to a prospectively studied control group of normal pregnant women, matched to those with PCOS by age, parity and preconception BMI, followed longitudinally on the same high-protein, low-carbohydrate diet throughout pregnancy.

Study protocol

Non-pregnant women with well-documented (Table I) PCOS entered our study of the efficacy and safety of metformin preconception (Glueck *et al*., 1999), were consecutively enrolled in the current

Table I. Diagnostic characteristics of the 39 PCOS patients at preconception, pre-treatment study entry

	Number of menses in previous year				
	0 *n* = 15 (38%)	1–3 *n* = 10 (26%)	4–6 *n* = 10 (26%)	7–10 *n* = 4 (10%)	All *n* = 39
Ferriman–Gallwey (FG) scores ⩾7	14 (93%)	9 (90%)	9 (90%)	4 (100%)	36 (92%)
Severe acne	8 (53%)	6 (60%)	6 (60%)	3 (75%)	23 (59%)
Clinical hyperandrogenism (FG ⩾7 and/or severe acne)	15 (100%)	10 (100%)	10 (100%)	4 (100%)	39 (100%)
Total testosterone >70 ng/dl	5 (33%)	2 (20%)	1 (10%)	2 (50%)	10 (26%)
Free testosterone >6.8 pg/ml	2 (13%)	3 (30%)	0 (0%)	0 (0%)	5 (13%)
Androstenedione >270 ng/dl	6 (40%)	4 (40%)	2 (20%)	1 (25%)	13 (33%)
DHEA-S >270 μg/dl	4 (27%)	1 (10%)	3 (33%)	0 (0%)	8 (21%)
⩾1 high androgen	7 (47%)	5 (50%)	5 (50%)	2 (50%)	19 (49%)
⩾1 high ⩾1 high androgen and/or FG ⩾7	15 (100%)	9 (90%)	10 (100%)	4 (100%)	38 (97%)
⩾1 high ⩾1 high androgen and/or FG ⩾7 and/or severe acne	15 (100%)	10 (100%)	10 (100%)	4 (100%)	39 (100%)
Polycystic ovaries confirmed (PO[+])	13 (87%)	9 (90%)	9 (90%)	3 (75%)	34[a] (87%)
FAI ⩾7.3[b]	6 (20%)	5 (50%)	7 (70%)	2 (50%)	20 (51%)
PO [+] and FAI ⩾7.3	5/13 (38%)	4/9 (44%)	6/9 (67%)	2/3 (67%)	17/34 (50%)
PO [+] and FAI <7.3	8/13 (62%)	5/9 (56%)	3/9 (33%)	1/3 (33%)	17/34 (50%)

[a]Pelvic ultrasound-laparotomy not done in the other five women.
[b]97.5th percentile for FAI in a general Caucasian population (Sowers *et al*., 2003).

pregnancy follow-up study after conception on metformin, and were prospectively followed throughout pregnancy under our direct supervision (Glueck *et al*., 2002c,d). There was no selection bias related to outcome(s) of previous pregnancies without metformin. All pregnancies (historical, current) were conceived with the same partners. After conception, it was suggested that metformin be continued at the preconception dose level.

Preconception, PCOS women with BMI <25 or ⩾25 kg/m^2 were instructed, respectively, in a 2000 or 1500 calorie/day, high-protein (26% of calories), low-carbohydrate (44%) diet (42% of carbohydrate was complex), with 30% of the calories as fat and a polyunsaturate/saturate ratio of 2/1 (Glueck *et al*., 2002c,d). After conception, calorie restrictions were dropped, but continued adherence to the low-carbohydrate, high-protein diet was encouraged. Before and throughout pregnancy, folic acid (1 mg/day) was given.

During pregnancy, women with PCOS made monthly follow-up visits to our centre with measurement of weight and, after an overnight fast, serum insulin, glucose, testosterone, estradiol, progesterone and PAI-Fx (Glueck *et al*., 1999, 2001, 2002c,d). At each monthly visit during pregnancy, after a 5 min resting period, seated blood pressure was obtained by a single observer and recorded, diet was reviewed, as was adherence to metformin and metformin dose. All medical aspects of the patients' pregnancies were directly managed by the investigators. At gestation weeks 26–28, in collaboration with the patients' obstetricians, evaluation for GD was done (American Diabetes Association, 1986; O'Sullivan *et al*., 1973).

The HOMA model for IR and β cell function was used as per Haffner *et al*. (1996). Upper normal limits for preconception, pre-treatment systolic and diastolic blood pressure, high-density lipoprotein (HDL)-cholesterol and triglycerides were taken from the recent Adult Treatment Panel III guidelines (National Institutes of Health, 2001).

Statistical methods

Paired Wilcoxon tests (SAS/STAT software, 2002) were used to compare pre-treatment, preconception baseline levels against the last preconception levels on metformin (Figures 1–6). Paired Wilcoxon tests were also used to compare these last preconception levels against levels during the first, second and third trimesters (Figures 1–6). The single point (Figures 1–6) shown for the cohort at each trimester was obtained as follows. First, mean values were obtained for each variable in each woman in each of the three trimesters (0–13, 14–26 and 27–40 gestational weeks). Then, the median level of all women's mean values, separately for each of the three trimesters, was exhibited as a single point for each trimester in the upper panels of Figures 1–6. Significant differences between levels during the five measurement periods (pre-treatment baseline, last preconception visit on metformin, values during the first, second and third trimesters on metformin) are displayed, along with differences between pre-treatment baseline and levels during the third trimester, and between the last preconception levels on metformin and levels during the third trimester (upper panels of Figures 1–6). Similarly, paired Wilcoxon tests were used to compare percentage changes from pre-treatment, preconception levels to the last preconception visit on metformin and percentage changes from the last preconception visit on metformin to levels during the first, second and third trimesters (lower panels of Figures 1–6).

Stepwise regression (SAS/STAT software, 2002) was carried out examining correlates of changes from preconception, pre-treatment baseline to the last preconception visit on metformin, and changes from the last preconception visit to levels during the first, second and third trimesters (Table II).

With our sample size of 42, we determined the power of the current study at the 5% level to detect univariate correlations (Sokal and Rohlf, 1998).

Results

Universe of PCOS patients

Over a 6.8-year time period (April 30, 1996 to March 2, 2003), among 936 women (age >12 and <62 years) referred for a study of efficacy and safety of metformin in PCOS (Glueck *et al*., 1999), 115 (ages 21.0–46.8 years) subsequently became pregnant while taking metformin (1.5–2.55 g/day). At the time of this report, these 115 women had 134 pregnancies with 138 fetuses, 105 live births (76%), 13 ongoing pregnancies ⩾13 weeks, one ongoing pregnancy <13 weeks and 19 spontaneous first trimester abortions (14%). In 42 of the 105

Table II. Significant explanatory variables for changes (Δ), increase (↑) or decrease (↓), in 42 pregnancies of 39 women with PCOS on metformin, by stepwise regression

Model	From baseline (not on MET) to last preconception visit on MET	From last preconception visit to levels during first trimester on MET	From last preconception visit to levels during second trimester on MET	From last pre-conception visit to levels during third trimester on MET
Δ Body weight = age + infant gender + baseline BMI + baseline IR + Δ IR	Age older, ↓ more (R^2 = 11%, P = 0.038)	Age older, ↓ less (R^2 = 17%, P = 0.013)		Baseline BMI higher, ↑ less (R^2 = 21%, P = 0.0069)
Δ Insulin = age + infant gender + baseline insulin + baseline BMI + Δ weight	Baseline insulin higher, ↓ more (partial R^2 = 66%, P < 0.0001)		Baseline insulin higher, ↓ more (R^2 = 14%, P = 0.028)	
Δ IR = age + infant gender + baseline IR + baseline BMI + Δ weight	Baseline BMI higher, ↓ less (partial R^2 = 4%, P = 0.036)			
	Baseline IR higher, ↓ more (partial R^2 = 82%, P < 0.0001)			
	Baseline BMI higher, ↓ less (partial R^2 = 3%, P = 0.0083)			
Δ IS = age + infant gender + baseline IS + baseline BMI + Δ weight	Baseline IS higher, ↓ more (R^2 = 85%, P < 0.0001)			
Δ Testosterone = age + infant gender + baseline testosterone + baseline BMI + Δ weight + Δ insulin + Δ IR	Baseline testosterone higher, ↓more (partial R^2 = 83%, P < 0.0001)	Baseline BMI higher, ↑ more (R^2 = 12%, P = 0.045)	Baseline testosterone higher, ↑ more (R^2 = 21%, P = 0.0094)	Insulin ↑ more (↓ more, partial R^2 =15%, P = 0.035)
	Age older, ↓ more (partial R^2 = 2%, P = 0.026)			Baseline testosterone higher, ↑ more (partial R^2 = 14%, P = 0.027)
	IR ↓ more (↓ more, partial R^2 = 2%, P = 0.037)			
Δ PAI-Fx = age + infant gender + baseline PAI-Fx + baseline BMI + Δ weight + Δ insulin + Δ IR	Baseline PAI-Fx higher, ↓ more (partial R^2 = 29%, P = 0.0015)	Baseline PAI-Fx higher, ↓ more (R^2 = 16%, P = 0.046)	Age older, ↑ more (R^2 = 17%, P = 0.039)	
	Age older, ↓ more (partial R^2 = 9%, P = 0.049)			

live birth pregnancies (39 women), there was complete data for five periods including baseline pre-metformin preconception, last preconception on metformin, and first, second and third trimesters with serial measures of weight, fasting insulin and glucose, HOMA IR, HOMA insulin secretion, testosterone and PAI-Fx (Figures 1–6).

During the 6.8-year time period, 821 other women with PCOS started participation in the metformin–diet programme and did not conceive. Of these 821 women, 284 were married, nine had previous hysterectomy and 154 used contraceptive methods (barrier, IUD, tubal ligation) and did not wish to become pregnant. Besides the 115 women who conceived, there were 216 women (of 821) who wished to conceive but have not conceived to date.

The 115 women who conceived were relatively thinner than the 216 women who have, to date, failed to conceive (BMI 33.6 ± 7.9 versus 38.0 ± 8.6 kg/m^2, P < 0.0001), and younger (30 ± 5 versus 33 ± 6 years, P = 0.0003). The 115 women who conceived did not differ from the 216 who failed to conceive for (mean ± SD) pre-treatment fasting serum insulin (22 ± 16 versus 27 ± 27 uU/ml, P = 0.066), HOMA IR (5.29 ± 5.63 versus 6.95 ± 8.27, P = 0.068) and HOMA insulin secretion (309 ± 322 versus 363 ± 362, P = 0.13).

The 39 women (42 pregnancies) who had complete data preconception and throughout pregnancy on metformin were relatively thinner than the 216 women who have failed to conceive (BMI 34.0 ± 8.2 versus 38.0 ± 8.6 kg/m^2, P = 0.007) and younger (30 ± 4 versus 33 ± 6 years, P = 0.005), but did not differ for pre-treatment fasting serum insulin (24 ± 17 versus 27 ± 27, P = 0.83), HOMA IR (5.62 ± 4.81 versus 6.95 ± 8.27, P = 0.64) and insulin secretion (413 ± 510 versus 363 ± 362, P = 0.98).

Our current study focused on these 42 pregnancies in 39 women (three women with two pregnancies) who had complete data preconception and throughout pregnancy on metformin, with 34 pregnancies (80%) on 2.55 g of metformin per day, and eight pregnancies on 1.5 g of metformin per day.

On metformin, 33 of 42 (79%) pregnancies in the 39 women were conceived spontaneously, one (2%) with artificial insemination, six (14%) with 50 mg of clomid, and two (5%) with IVF.

Characteristics of PCOS in 39 women with longitudinal measurements preconception and throughout 42 pregnancies while taking metformin

At study entry, in the cohort of 39 Caucasian women, mean ± SD age was 30 ± 4 years, weight 98 ± 24 kg, BMI 34.0 ± 8.2 kg/m^2. Of the 39 women, 15% had BMI <25 (normal weight), 21% were overweight (BMI 25–30), 36% were obese (BMI 30–40) and 28% had extreme obesity (BMI ⩾40) (Flegal *et al*., 2002). One woman had pre-gestational DM, controlled by diet alone. Systolic blood pressure was high (⩾130 mmHg) in 19%, diastolic blood pressure was high (⩾85 mmHg) in 11%, triglycerides were high (⩾150 mg/dl) in 36%, HDL-cholesterol was low (<50 mg/dl) in 72%, fasting serum insulin was high (⩾17 uU/ml) in 54%, and fasting serum glucose was high (>110 mg/dl) in 5%.

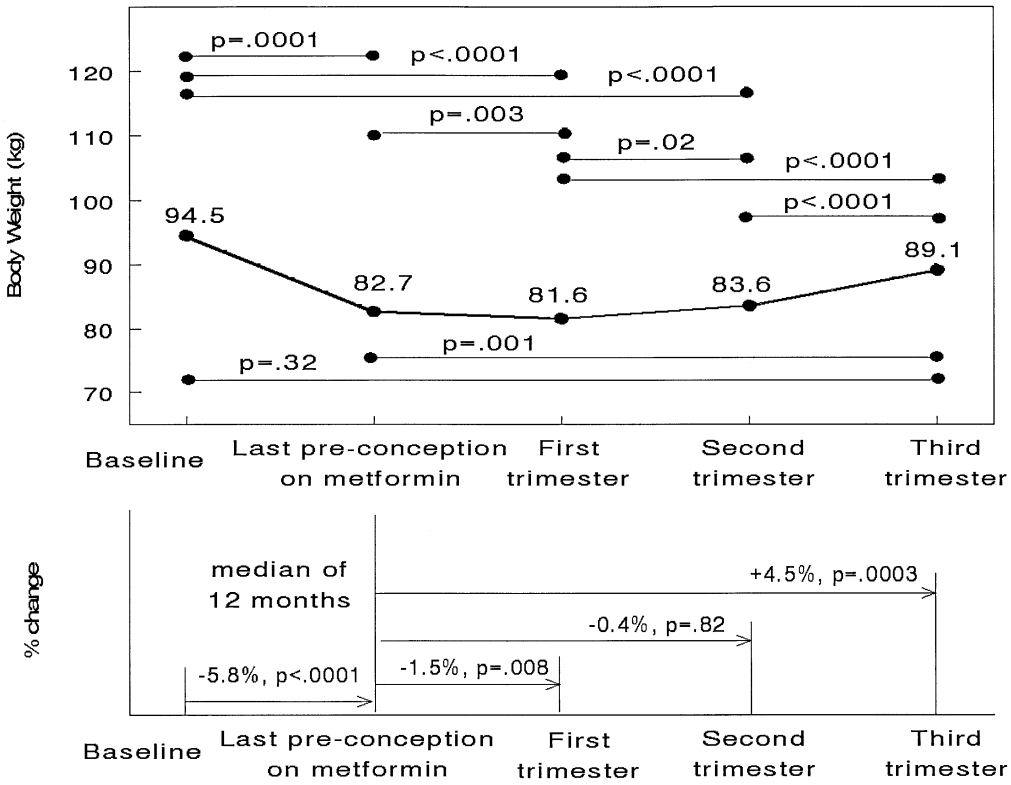

Figure 1. Median body weight (kg) at pre-treatment, preconception baseline, at the last preconception visit on metformin, and during the first, second and third trimesters. Paired Wilcoxon tests of difference. Median percentage change in body weight from pre-treatment, preconception baseline to the last preconception visit on metformin. Median percentage change in body weight from the last preconception visit on metformin to levels during the first, second and third trimesters. Paired Wilcoxon tests of difference.

As displayed in Table I, of the 39 women studied, by selection, all had oligoamenorrhoea, and all also had clinical hyperandrogenism (Table I, top 3 rows). Of the 39 women, 36 (92%) had FG scores ⩾7, and 59% had severe acne. Of the 39 women, 19 (49%) had ⩾1 high androgen level (Table I). All 39 women (100%) met the criteria for the diagnosis of PCOS, oligoamenorrhoea, and clinical and/or biochemical hyperandrogenism (Kawadzki and Dunaif, 1992; Glueck *et al.*, 2002c,d) (Table I, row 10).

Of the 39 women, 34 had pelvic ultrasound and/or laparotomy that documented polycystic ovaries (Table I). Of the 34 women with polycystic ovaries, 17 (50%) had a high FAI [⩾97.5th percentile for a general Caucasian population (Sowers *et al.*, 2003)], and would have been identified as having PCOS by the recent ESHRE/ASRM consensus criteria (Fauser *et al.*, 2003) (Table I, penultimate row).

Gestational diabetes

Of the 42 pregnancies, GD was diagnosed in three (7.1%); one of the 39 patients had pre-gestational type 2 DM controlled by diet alone, but had normal glucose tolerance testing during pregnancy on metformin.

Longitudinal changes in weight, insulin, HOMA IR, HOMA insulin secretion, testosterone and PAI-Fx

Weight. As displayed in Figure 1, the median time from study entry to the last preconception visit on metformin was 12 months. During this 12-month period, the median percentage reduction in weight was 5.8% ($P < 0.0001$) (Figure 1). Median weight at preconception, pre-treatment baseline was 94.5 kg, falling on metformin at the last preconception visit to 82.7 kg ($P < 0.0001$), falling further to 81.6 kg during the first trimester, being 83.6 in the second trimester and 89.1 in the third trimester (Figure 1). From the last preconception visit on metformin through the first trimester, the median percentage reduction in weight was 1.5% ($P = 0.008$), and was 0.4% ($P = 0.82$) during the second trimester (Figure 1). Compared with the last preconception visit on metformin, the median percentage increase in weight during the third trimester was 4.5% ($P = 0.0003$) (Figure 1). From the last preconception visit on metformin, median weight gain during pregnancy was 3.5 kg. After categorizing by preconception, pre-treatment BMI <25, 25–30, 30–40 and ⩾40 kg/m^2, median (range) weight gain during pregnancy from the last preconception visit on metformin was, by group, respectively 8.4 (3.5–14.2), 5.5 (–2.5 to 15.5), 5.4 (–2 to 13.2) and –1.6 (–12.7 to 5.9) kg.

Insulin. The median percentage reduction in insulin from pre-treatment baseline to the last preconception visit on metformin was 40% ($P < 0.0001$) (Figure 2). Median fasting serum insulin fell from 18.9 uU/ml at pre-treatment baseline to 10.2 on metformin at the last preconception visit (Figure 2), remained stable at 10.7 during the first trimester and 10.6 in the second trimester, and then rose to 12.8 in the third trimester, no higher ($P = 0.06$) than the last preconception visit on

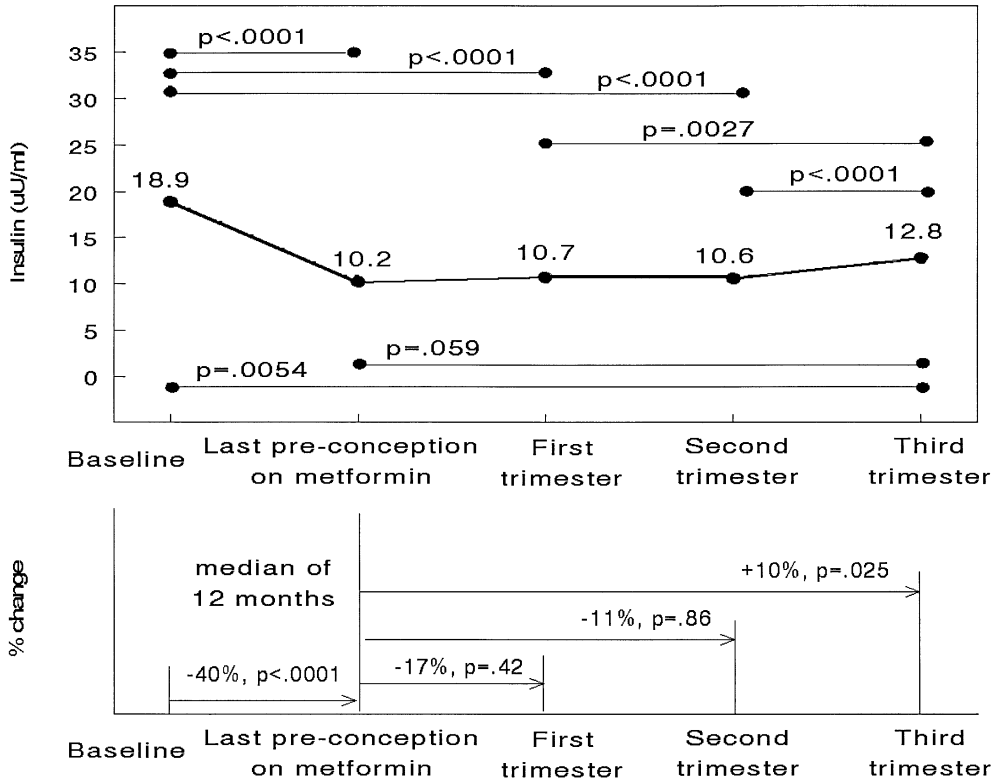

Figure 2. Median fasting serum insulin (uU/ml) at pre-treatment, preconception baseline, at the last pre-conception visit on metformin, and during the first, second and third trimesters. Paired Wilcoxon tests of difference. Median percentage change in fasting serum insulin from pre-treatment, preconception baseline to the last pre-conception visit on metformin. Median percentage change in fasting serum insulin from the last preconception visit on metformin to levels during the first, second and third trimesters. Paired Wilcoxon tests of difference.

metformin and lower than pre-treatment baseline ($P = 0.0054$) (Figure 2). From the last preconception level on metformin, insulin did not rise in the first trimester (–17%, $P = 0.42$), was stable in the second trimester (–11%, $P = 0.86$), and then rose by a median 10% ($P = 0.025$) in the third trimester (Figure 2).

HOMA IR. The median percentage reduction in HOMA IR from pre-treatment baseline to the last preconception visit on metformin was 46%, $P < 0.0001$ (Figure 3). IR fell from 3.95 at baseline to 2.12 on metformin at the last preconception visit ($P < 0.0001$) (Figure 3). Median IR was 2.05 during the first trimester, 1.96 in the second trimester and 2.36 in the third trimester (Figure 3). Median IR at the last preconception visit (2.12) did not differ from that during the third trimester (2.36; $P = 0.13$) (Figure 3). Moreover, during the third trimester, median IR (2.36) was lower than at pre-treatment baseline (3.95; $P = 0.017$) (Figure 3). There were no significant percentage changes in IR from the last preconception visit through the first trimester (–18%, $P = 0.42$), through the second trimester (–19%, $P = 0.53$), or through the third trimester (+11%, $P = 0.12$) (Figure 3).

HOMA insulin secretion. Median HOMA insulin secretion was 292 at baseline and fell to 174 ($P < 0.0001$) at the last preconception visit on metformin (Figure 4). The median percentage reduction in insulin secretion on metformin from baseline to the last preconception visit was 45% ($P = 0.0004$). HOMA insulin secretion was 182 in the first trimester, 258 in the second trimester, and rose to 354 in the third trimester, not different from pre-treatment baseline ($P = 0.092$), and higher than the last preconception level ($P < 0.0001$) (Figure 4).

From the last preconception visit on metformin, there were no significant percentage increments in insulin secretion during the first trimester, with a median 24% increase in the second trimester ($P = 0.0007$), and a 109% increase in the third trimester, $P < 0.0001$ (Figure 4).

Testosterone. On metformin, testosterone fell 30%, going from pre-treatment baseline (56.0 ng/dl) to the last preconception visit (34.0 ng/dl, $P = 0.0002$) (Figure 5). From the last preconception visit on metformin, median testosterone rose during the first trimester to 56.5 ng/dl, was 54.3 during the second trimester, and then rose to 63.8 ng/dl during the third trimester (Figure 5). Compared with the last preconception visit on metformin, during pregnancy, on metformin, the median percentage increases in testosterone were 74, 61 and 95% during the first, second and third trimesters (Figure 5). However, median testosterone in the third trimester of pregnancy did not differ from levels at pre-treatment baseline, $P = 0.078$ (Figure 5).

PAI-Fx. Median PAI-Fx, 15.0 U/ml at baseline, fell to 13.8 at the last preconception visit on metformin, fell further to 8.5 in the first trimester, was 11.9 in the second trimester, and then

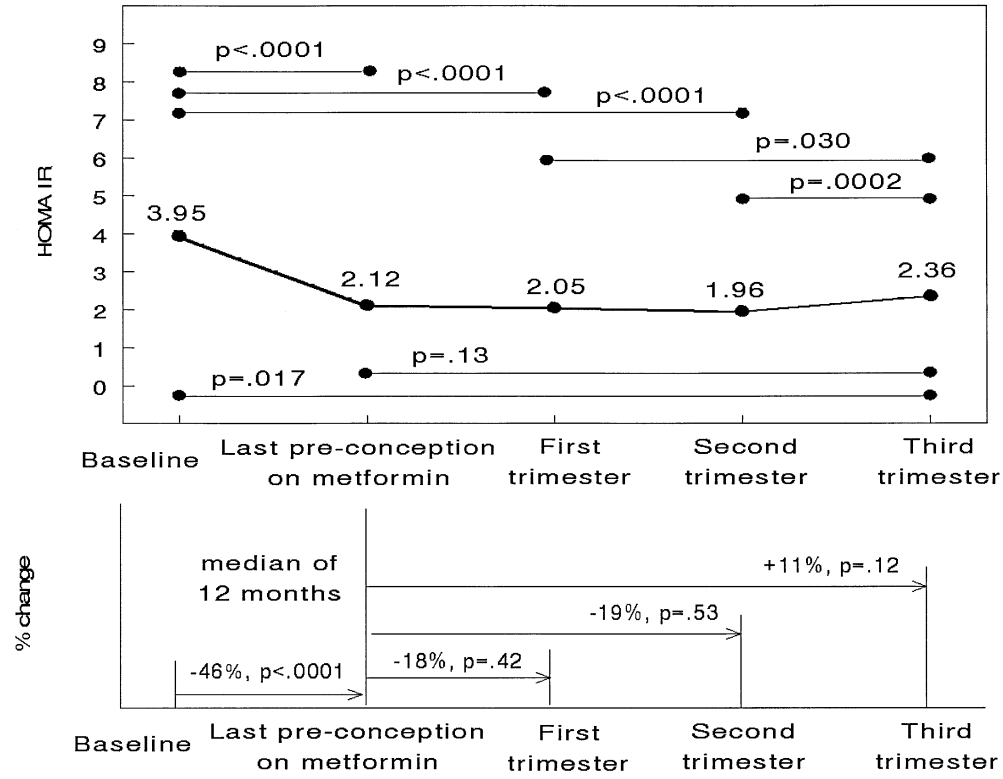

Figure 3. Median HOMA insulin resistance (IR) at pre-treatment, preconception baseline, at the last preconception visit on metformin, and during the first, second and third trimesters. Paired Wilcoxon tests of difference. Median percentage change in HOMA IR from pre-treatment, preconception baseline to the last pre-conception visit on metformin. Median percentage change in HOMA IR from the last pre-conception visit on metformin to levels during the first, second and third trimesters. Paired Wilcoxon tests of difference.

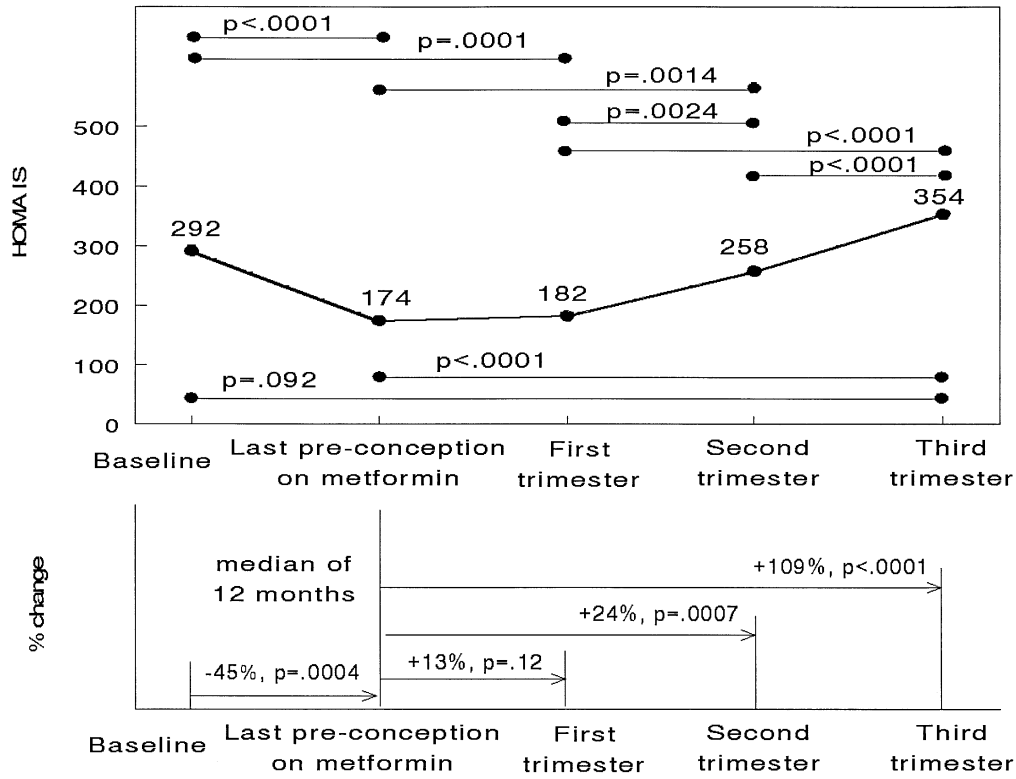

Figure 4. Median HOMA insulin secretion (IS) at pre-treatment, preconception baseline, at the last preconception visit on metformin, and during the first, second and third trimesters. Paired Wilcoxon tests of difference. Median percentage change in HOMA IS from pre-treatment, preconception baseline to the last pre-conception visit on metformin. Median percentage change in HOMA IS from the last pre-conception visit on metformin to the first, second and third trimesters. Paired Wilcoxon tests of difference.

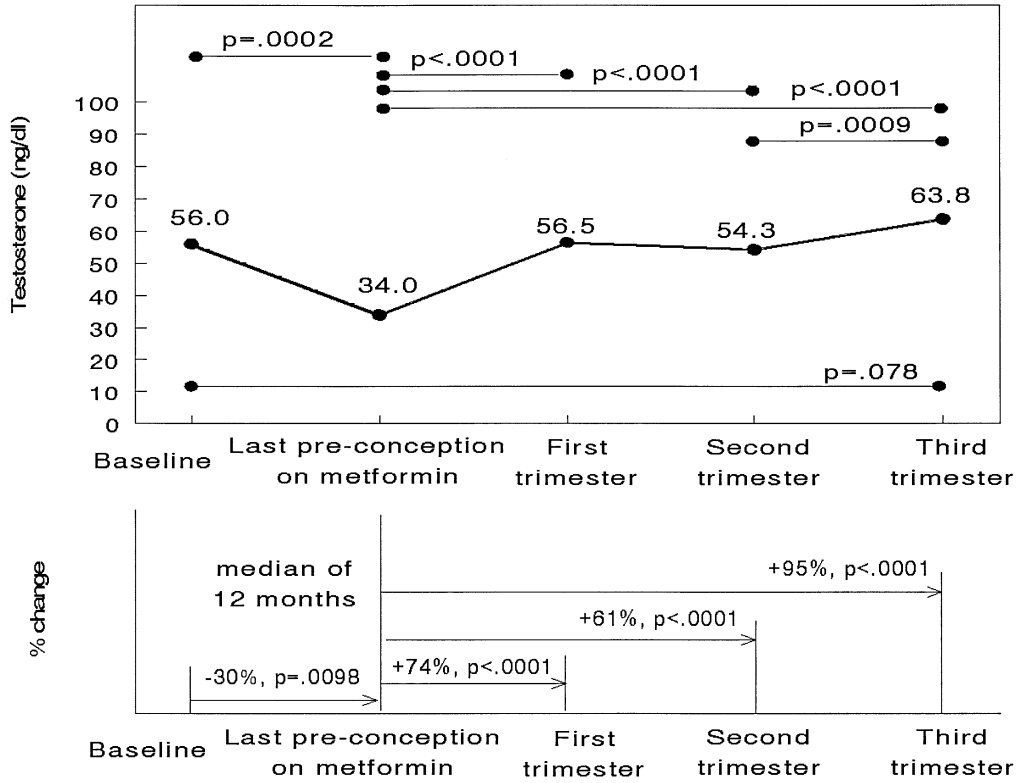

Figure 5. Median testosterone (ng/dl) at pre-treatment, preconception baseline, at the last preconception visit on metformin, and during the first, second and third trimesters. Paired Wilcoxon tests of difference. Median percentage change in testosterone from pre-treatment, preconception baseline to the last preconception visit on metformin. Median percentage change in testosterone from the last preconception visit on metformin to levels during the first, second and third trimesters. Paired Wilcoxon tests of difference.

rose to 20.3 in the third trimester (Figure 6). There was no significant difference between median PAI-Fx in the third trimester and at pre-treatment baseline ($P = 0.35$) (Figure 6). However, median PAI-Fx in the third trimester was higher than at the last preconception level on metformin, 20.3 versus 13.8 U/ml, $P = 0.0029$ (Figure 6).

There was no significant percentage reduction in median PAI-Fx from pre-treatment baseline to the last preconception visit on metformin (Figure 6). PAI-Fx did not rise significantly during pregnancy in the first and second trimesters compared with preconception levels on metformin (Figure 6). The median percentage increase in PAI-Fx was 51% in the third trimester compared with the last preconception levels on metformin, $P = 0.0008$ (Figure 6).

Multivariate determinants of change in body weight, insulin, IR, insulin secretion, PAI-Fx and testosterone

Reduction in weight from baseline, pre-treatment to the last preconception visit on metformin was more in older women, but reduction in weight from the last preconception visit through the first trimester was less in older women (Table II). The increase in weight from preconception through the third trimester was less marked in those women with higher baseline BMI (Table II).

The reduction in serum insulin from pre-treatment, preconception baseline to the last preconception visit (on metformin) was associated with pre-treatment insulin and baseline BMI; the higher the pre-treatment insulin, the greater the reduction on metformin, the higher the pre-treatment BMI, the less the reduction on metformin (Table II). Also during the second trimester, the higher the baseline insulin, the more the reduction in insulin (Table II).

In a similar fashion, the higher the pre-treatment, preconception HOMA IR, the greater the reduction in IR on metformin before conception (Table II). The higher the pre-treatment BMI, the less the reduction in IR on metformin before conception (Table II). The higher the insulin secretion at pre-treatment baseline, the greater the reduction on metformin before conception (Table II). During pregnancy, there were no significant correlates of change in IR or insulin secretion (Table II).

The higher the baseline testosterone, the greater the fall in testosterone from baseline to the last preconception visit on metformin (Table II). However, the higher the baseline testosterone, the greater the increase in testosterone from the last preconception visit to the second and third trimesters (Table II). From pre-treatment baseline to the last preconception visit on metformin, the reduction in testosterone was greater in older women, and the greater reduction in IR, the greater the reduction in testosterone. From last preconception to the third trimester, the greater the increment in insulin, the greater the increment in testosterone (Table II).

The higher the pre-treatment baseline PAI-Fx, the greater the decrease in PAI-Fx on metformin at the last preconception visit

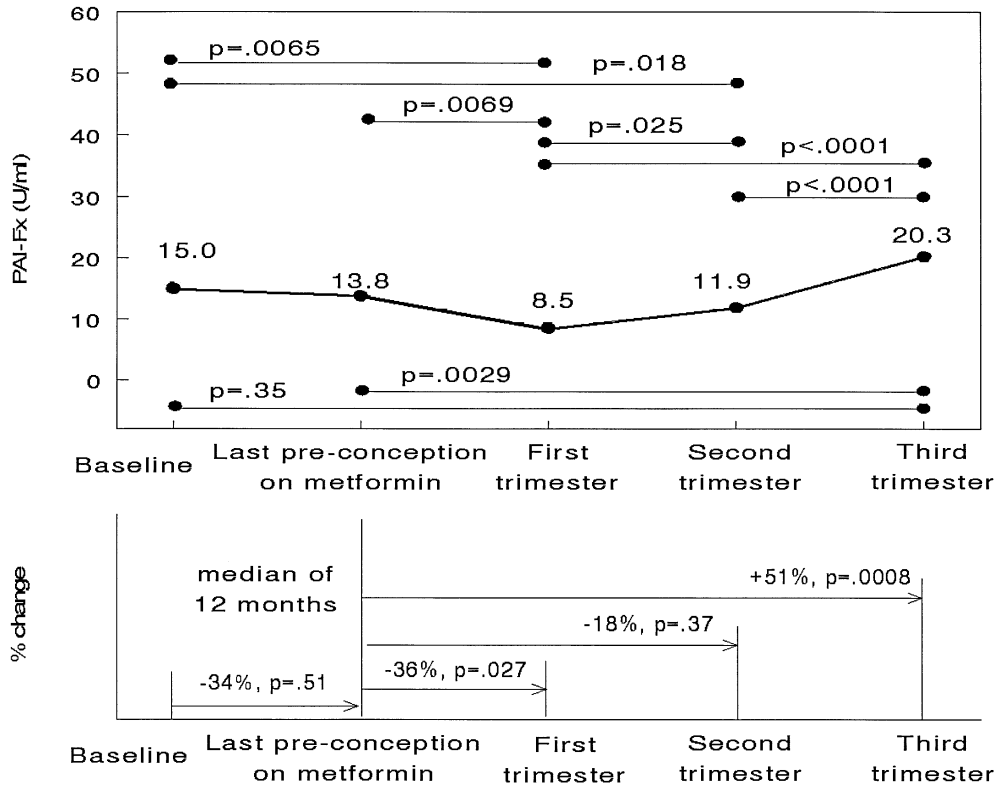

Figure 6. Median plasminogen activator inhibitor activity [PAI-Fx (U/ml)] at pre-treatment, preconception baseline, at the last preconception visit on metformin, and during the first, second and third trimesters. Paired Wilcoxon tests of difference. Median percentage change in PAI-Fx from pre-treatment, preconception baseline to the last preconception visit on metformin. Median percentage change in PAI-Fx from the last preconception visit on metformin to levels during the first, second and third trimesters. Paired Wilcoxon tests of difference.

(Table II). The higher the pre-treatment baseline PAI-Fx, the greater the reduction in PAI-Fx from the last preconception visit through the first trimester (Table II). Maternal age was associated with change in PAI-Fx during the second trimester, with a greater increase in PAI-Fx in older women (Table II).

With sample size $n = 42$, our data had ⩾99% power at the 5% level to detect a univariate correlation of baseline insulin with change in insulin on metformin ($r = 0.81$, $R^2 = 66\%$), ⩾99% power for correlation of baseline HOMA IR with change in IR ($r = 0.90$, $R^2 = 82\%$), ⩾99% power for correlation of baseline insulin secretion with change in insulin secretion ($r = 0.92$, $R^2 = 85\%$), and 95% power for correlation of baseline PAI-Fx with change in PAI-Fx ($r = 0.54$, $R^2 = 29\%$) (Table II).

Discussion

Unless metformin can be shown to be safe, effective and non-teratogenic during pregnancy, there should be no serious consideration of its use in prevention or treatment of GD, hyperinsulinaemia and hyperandrogenemia of pregnancy in women with PCOS. Metformin during pregnancy in women with PCOS safely reduces first trimester spontaneous abortion from 73 to 10%, $P < 0.0001$ (Glueck *et al*., 2001), and from 62 to 26%, $P < 0.0001$, (Glueck *et al*., 2002c), and is not apparently teratogenic (Coetzee and Jackson, 1979a,b, 1980, 1984, 1985; Jackson and Coetzee, 1979; Glueck *et al*., 2001, 2002c,d; Heard *et al*., 2002; Jakubowicz *et al*., 2002). Metformin does not adversely affect infants' birth weight or length (Glueck *et al*., 2002c), and does not alter children's normal growth, motor and social development in the first year of life. On metformin, the development of pre-eclampsia and GD does not differ between women with PCOS and normal controls (Glueck *et al*., 2003).

In whole-embryo culture, metformin produces no alterations in embryonic growth and no major malformations (Denno and Sadler, 1994).

The current observational study documents the preconception and through pregnancy metabolic benefits of metformin–diet. Because there were no placebo–diet or metformin–*ad libitum* diet control groups of women with PCOS, the independent contributions of metformin and diet to the current study's outcomes cannot be determined. A second limitation of the current report was our inability to recruit an equally obese, non-diabetic control group of women without PCOS who could be matched to the PCOS cases by preconception BMI, age and parity, and could then be followed longitudinally on diet throughout pregnancy.

Diet may have some benefit in reducing GD and, presumably, reducing IR during pregnancy. In Chilean women with PCOS who desired pregnancy, Sir-Peterman *et al*. (2002) provided a 6-month diet and exercise programme which may have had some beneficial impact on their low prevalence of GD and pre-eclampsia. However, in the James Bay Cree of Canada who have one of the highest recorded rates of GD among

aboriginal people, and who have a high prevalence of pregravid obesity (Rodrigues *et al*., 1999), regular individual diet counselling and physical activity sessions failed to reduce either the rate of weight gain in the second half of the pregnancy or plasma glucose levels between 24 and 30 weeks gestation (Gray-Donald *et al*., 2000). Like Paradisi *et al*., (1998), we speculate that metformin accounts for a major portion of the improvement in the 'natural' IR changes during gestation in hyperinsulinaemic PCOS women.

In the current study, over 12 months preconception, the median percentage reductions on metformin–diet were 5.8% in body weight ($P < 0.0001$), 40% in insulin ($P < 0.0001$), 46% in HOMA IR ($P < 0.0001$), 45% in HOMA insulin secretion ($P = 0.0004$), 30% in testosterone ($P = 0.01$) and 34% in PAI-Fx ($P = 0.51$). Moreover, preconception, metformin had the most benefit in those women who had the most severe IR and its associated endocrinopathy. The higher the baseline insulin, HOMA IR, HOMA insulin secretion, testosterone and PAI-Fx, the greater the reduction on metformin at the last preconception visit ($P < 0.001$ for all). The benefits of metformin–diet throughout pregnancy included holding median weight gain during pregnancy to 3.5 kg, reducing pregnancy-associated increments in fasting serum insulin, HOMA IR and HOMA insulin secretion, reducing development of GD, and blunting within-pregnancy increments in PAI-Fx and testosterone.

One major benefit of continuing metformin–diet through pregnancy is avoidance of major weight gain in women with PCOS who, at conception, are commonly obese or extremely obese (Glueck *et al*., 1999, 2002c,d; Galtier-Dereure *et al*., 2000; Sir-Peterman *et al*., 2002). In the current report, pre-metformin, preconception, only 15% of women with PCOS had 'normal' BMI (<25 kg/m^2), 21% were overweight (BMI 25–30), 36% were obese (30–40) and 28% were extremely obese ($\geqslant$40) (Flegal *et al*., 2002). In a study by Sir-Peterman *et al*., (2002) of 20 Chilean women with PCOS, median (range) preconception BMI was 29.4 (25.1–34.2), comparable with our Caucasian cohort where median (range) BMI was 32.2 (19.7–54.1). Sir-Peterman *et al*. (2002) reported that median (range) weight gain during pregnancy in untreated Chilean women with PCOS was 14.4 kg (11.2–21 kg), four times higher than in the 39 women in our study on metformin whose median within-pregnancy weight gain was 3.5 kg. Median pregnancy weight gain of 3.5 kg (–12.7 to 15.5 kg) in women with PCOS on metformin was also 3.6 times less than the 12.6 kg (6.9–18.3) gain reported by Sir-Peterman *et al*. (2002) in 26 normal Chilean women, and was about half of the average 6 kg weight gain reported by Halmesmaki *et al*. (1987) in 20 normal pregnant women, and about one third the mean weight gain of 10.7 kg in 1145 pregnant women reported by Dawes and Grudzinskas (1991).

The Institute of Medicine's 1990 recommendations were that normal weight women (preconception BMI 19.8–26.0) gain 11.4–15.9 kg during pregnancy, and that overweight women (preconception BMI 26.1–29.0) gain 6.8–11.4 kg. Considering the weight of the fetus, placenta and amniotic fluid, the median weight gain of 3.5 kg during pregnancy for our full cohort of 39 women whose median entry weight was 94.5 kg, and median entry BMI was 32.2 kg/m^2, reflects a continuing weight reduction effect throughout pregnancy on metformin. In the subset of 15% of women in the current study with normal pre-treatment, preconception BMI (<25), median pregnancy weight gain was 8.5 kg. Conversely, in the subset of women with pre-treatment, preconception extreme obesity (BMI $\geqslant$40), median weight change during pregnancy was –1.6 kg, range –12.7 to 5.9 kg. Obesity, characterizing 60–80% of PCOS patients, has a deleterious additive effect on carbohydrate homeostasis and increases IR during gestation (Galtier-Dereure *et al*., 2000). Metformin's weight maintenance, weight loss action throughout pregnancy in women with PCOS, particularly marked in women with BMI 30–40 and $\geqslant$40 kg/m^2, probably contributes to its reduction in development of GD (Glueck *et al*., 2002a,b,c,d). Without metformin, women with PCOS are at an increased risk of glucose intolerance and pre-eclampsia during pregnancy (Radon *et al*., 1999), while on metformin, the frequency of GD and pre-eclampsia in women with PCOS does not differ from normal pregnant controls (Glueck *et al*., 2003). The limited weight gain on metformin in the current study is all the more striking, since high maternal insulin in early pregnancy in normal women is associated with increased gestational weight gain, post-partum weight retention, increasing risk of GD and, later, type 2 DM (Scholl and Chen, 2002). Within this frame of reference (Galtier-Dereure *et al*., 2000; Glueck *et al*., 2002a,b,c,d; Scholl and Chen, 2002), metformin during pregnancy in PCOS may, speculatively, play a role in primary prevention of subsequent type 2 DM.

A second benefit of metformin during pregnancy in women with PCOS is its testosterone-lowering effect, which, like prevention of weight gain, is probably mediated through its insulin-sensitizing action (Velazquez *et al*., 1994, 1997). Sir-Peterman *et al*., (2002) assessed serum androgen concentrations in 20 PCOS and 26 normal Chilean women during singleton pregnancies. During gestational weeks 10–16, testosterone levels tended to be higher in the PCOS group than in normals, and these differences became significant in gestational weeks 22–28, as did 2-h post-glucose load insulin levels (Sir-Peterman *et al*., 2002). In the current study, median testosterone levels during the first and second trimesters in women with PCOS on metformin [0.57 (range 0.24–2.38 ng/ml) and 0.54 (0.24–1.74 ng/ml)] were much lower than levels reported by Sir-Peterman *et al*.(2002) in 20 untreated Chilean women with PCOS during the first and second trimesters [1.38 (0.75–2.14) and 1.66 (0.58–3.82 ng/ml)], or in 26 normal Chilean women [0.92 (0.52–2.19) and 1.14 (0.62–1.51 ng/ml)]. Moreover, on metformin, in the current study, median testosterone during the first and second trimesters did not differ ($P > 0.05$), in comparison with the findings of Sir-Peterman *et al*., (2002) where median testosterone uniformly rose during the second trimester in the 20 women with PCOS. Sir-Peterman *et al*. (2002) proposed that high androgen levels during pregnancy in untreated women with PCOS 'could provide a potential source of androgen excess for the fetus, without leading to fetal virilization'. Metformin throughout pregnancy in PCOS should, as in the current study, reduce any putative risk of fetal virilization conferred through androgen excess.

A third metabolic effect of metformin during pregnancy is its modulation of the 'natural' augmentation of IR changes during gestation. On metformin in the current study, there was no difference in fasting serum insulin between the third trimester and at the last preconception visit on metformin ($P = 0.06$), with insulin levels also lower in the third trimester than at pre-treatment baseline ($P = 0.005$). Insulin levels during pregnancy did not rise significantly during the first and second trimesters compared with the last preconception visit on metformin, and rose only 10% through the third trimester. Similarly, HOMA IR was lower in the third trimester than at pre-treatment baseline, and did not increase significantly from the last preconception visit on metformin to levels in trimesters 1, 2 and 3. Thus, the expected changes during pregnancy, a substantial increase in insulin and IR (Buchanan *et al*., 1990; Catalano, 1994, Catalano *et al*., 1999; Lanzone *et al*., 1996; Paradisi *et al*., 1998), were abated and blunted, respectively, by metformin. Our results were similar to those reported by Paradisi *et al*. (1998) who hypothesized that metformin improves the 'natural' IR changes during gestation in hyperinsulinaemic PCOS women.

Buchanan *et al*. (1990) reported that insulin sensitivity in normal pregnant women was reduced to only one-third of that of non-pregnant women. They reported that during the third trimester, GD was characterized by an impairment of β cell function rather than exaggeration of normal IR of late pregnancy. In the current study, metformin reduced HOMA insulin secretion 45% at the last preconception visit compared wih pre-treatment baseline, and increments in HOMA insulin secretion were not significant in the first trimester, were 24% in the second trimester, and 109% in the third trimester. Xie *et al*. (2000) have reported that women who develop GD, compared with women having normal glucose tolerance, have impaired insulin secretion and abnormally increased IR. In the current study, metformin reduced IR, and reduced insulin secretion as a measure of reduced IR, thus promoting β cell reserve (Buchanan *et al*., 2000) that may be overwhelmed in those women with increased IR who develop GD when the IR of pregnancy is superimposed on their preconception IR. To the extent that metformin reduces insulin, IR and insulin secretion during pregnancy, while blocking weight gain, and reducing GD, it should play a role in primary prevention of type 2 DM.

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