Midpregnancy Doppler Ultrasound of the Uterine Artery in Metformin- Versus Placebo-Treated PCOS Women: A Randomized Trial

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Context: Metformin is used to reduce pregnancy complications in women with polycystic ovary syndrome (PCOS), although it is not approved for this indication and solid evidence is lacking. Midpregnancy Doppler ultrasound is one of the best methods for prediction of adverse pregnancy outcome.

Objective: The objectives of the study were to investigate the following: 1) whether metformin treatment influenced the midpregnancy pulsatility index (PI) of the uterine artery; 2) whether metabolic or endocrine factors affect the PI of the uterine artery of PCOS women; and 3) whether PI predicted adverse pregnancy outcome in PCOS woman.

Design: This is a substudy of a randomized, placebo-controlled, double-blind, multicenter study conducted at 11 secondary care centers. We randomly assigned 273 pregnancies to receive metformin or placebo, from the first trimester of pregnancy to delivery. In the present substudy, 231 pregnancies are included, ie, those who completed the ultrasound examinations.

Main Outcome Measures: Midpregnancy PI in the uterine artery related to metformin use, androgen levels, an oral glucose tolerance test, and insulin levels was measured. We found no difference in the PI between the metformin and placebo groups. In multivariate analyses, fasting serum glucose of the first and second trimester correlated positively to the midpregnancy PI. Only in univariate analyses a weak correlation between androstenedione and PI was seen.

Conclusions: Metformin treatment did not affect uterine artery blood flow, measured by PI. High fasting blood glucose correlated inversely to uterine artery blood flow. The midpregnancy PI correlated positively to preeclampsia, hypertension, and gestational diabetes mellitus in PCOS pregnancies. Androgen levels correlated only to PI in univariate analyses. **(*J Clin Endocrinol Metab* 99: 972–977, 2014)**

Polycystic ovary syndrome (PCOS) has implications both for fertility and pregnancy outcome. An increasing number of reports suggest a high prevalence of pregnancy complications (1–5). Hyperinsulinemia and hyperandrogenism have been suggested as pathogenic factors in both PCOS and pregnancy complications (6, 7). Recent studies report that the prevalence of PCOS according to the National Institutes of Health criteria is 6%–9% and according to the Rotterdam criteria is 12%–20% (8–11).

Metformin is an insulin-sensitizing drug used in the treatment of diabetes mellitus type 2 for decades. It re-

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Abbreviations: BMI, body mass index; BP, blood pressure; DHEAS, dehydroepiandrosterone sulfate; FTI, free T index; GDM, gestational diabetes mellitus; OGTT, oral glucose tolerance test; PCOS, olycystic ovary syndrome; PI, pulsatility index; PregMet, Metformin Treatment in Pregnant PCOS Women.

duces fasting insulin and T levels in nonobese, nonpregnant PCOS women (12). During the last decade, a number of retrospective and nonrandomized studies in PCOS women have reported beneficial effects of metformin on pregnancy loss and pregnancy complications, in particular gestational diabetes mellitus (2, 12–17). Our randomized pilot study of 40 patients indicated an overall reduction in pregnancy complications, and there was a greater mean bilateral uterine artery pulsatility index (PI) at 12 weeks ($P = .02$) and a greater reduction in mean PI from 12 to 19 weeks ($P = .03$) for metformin-treated women (18, 19). Midpregnancy Doppler ultrasound is one of the best methods to predict adverse pregnancy outcome, preeclampsia, and growth restriction in particular (20–26). We wanted to see whether metformin treatment affected midpregnancy uterine artery blood flow, measured as PI. We also wanted to investigate whether androgen, glucose, and insulin levels and 2-hour glucose levels during an oral glucose tolerance test (OGTT) correlated to PI.

Materials and Methods

Study design

The present study is a substudy of the Metformin Treatment in Pregnant PCOS Women (PregMet) study, which was a prospective, randomized, double-blind, multicenter trial, comparing 2000 mg metformin daily with placebo (27). Women included in the PregMet study in whom Doppler of the uterine artery flow was examined at gestational week 19 comprises the participants of this substudy.

Inclusion criteria were as follows: 1) PCOS diagnosed according to the Rotterdam Criteria (28), 2) age 18–45 years, 3) gestational age between 5 and 12 weeks, and 4) a singleton viable fetus. Exclusion criteria were alanine aminotransferase greater than 90 IU/L, serum creatinine concentration greater than 130 μmol/L, known alcohol abuse, previously diagnosed diabetes mellitus or fasting serum glucose 7.0 mmol/L or greater at the time point of inclusion, treatment with oral glucocorticoids, or the use of drugs known to interfere with metformin.

The diagnosis of PCOS was based on documentation before the actual pregnancy, all diagnosed by a gynecologist. The participants were included at 11 study centers. Before inclusion, women who met the PCOS criteria were screened with vaginal ultrasound to confirm a single viable fetus and gestational age. Fasting plasma glucose concentrations, creatinine, and alanine aminotransferase were determined to exclude overt diabetes mellitus or kidney or liver disease before inclusion in the study. Three hundred forty-eight PCOS women, with a total of 364 pregnancies were informed about the study; 32 did not meet inclusion criteria and 58 declined to participate. Two hundred seventy-four pregnancies were included and randomized (16 women participated twice). In one patient a partial 21-hydroxylase deficiency had been overlooked, and she was excluded after randomization. Of these 273 remaining pregnancies, eight women dropped out immediately after inclusion, three had a miscarriage, and in 31 women Doppler ultrasound was not performed at gestational week 19 due to a lack of resources.

The present substudy comprises 231 women

At inclusion, vaginal ultrasonography, drawing of fasting blood samples, and a 75-g OGTT were performed before randomization. All participants received written and individual verbal counseling on diet and lifestyle at inclusion. Thereafter treatment with metformin 500 mg (metformin hydrochloride, metformin; Weifa AS) or identical placebo capsules was initiated. The participants were instructed to take one capsule twice daily during the first week and two capsules twice daily for the rest of the study period. To counteract a possible metformin action on vitamin B levels, patients were advised to take 0.8 mg of folate daily and one daily multivitamin tablet.

Biometric variables, including height, weight, blood pressure (BP), and heart rate, were recorded at inclusion and at each prescheduled visit at gestational weeks 19, 24, 32, and 36. Fasting blood samples were directly analyzed at each study center for plasma glucose. An OGTT (75 g glucose) was performed at inclusion and gestational weeks 19 and 32.

Due to the time elapse between information about the study, clinical examination, and inclusion, 18 women (11 in the metformin and seven in the placebo group) were included later in pregnancy than intended, ie, between gestational weeks 13 and 15.

Participants were asked about their intake of study medication at prescheduled visits in gestational weeks 19, 24, 32, and 36 and at delivery. Dose reductions or intermittent stops in study medication were recorded.

The aim of this substudy is to study midpregnancy PI

The Committee for Medical Research Ethics of Health Region IV (Norway) and The Norwegian Medicines Agency approved the study. Written informed consent was obtained from each patient before inclusion and the Declaration of Helsinki was followed throughout the study. The study was conducted according to principles of good clinical practice.

Measurements

Venous blood samples were drawn from an antecubital vein between 8:00 and 11:00 AM after an overnight fast. Blood samples were collected and processed and plasma glucose measured at each study site in accordance with local standardized manual of operation. A 75-g OGTT was performed according to the World Health Organization (1998) recommendations with the definition for gestational diabetes mellitus (GDM) as follows; fasting plasma glucose of 7.0 mmol/L or greater and/or 2-hour plasma glucose of 7.8 mmol/L or greater (29, 30).

Dehydroepiandrosterone sulfate (DHEAS) and SHBG were analyzed by the ELISA technique with the reagents and calibrators supplied by the manufacturer (DRG Instruments GmbH). We used organic solvent extraction (dichloromethane for T and ethyl ether for androstenedione) prior to quantification to analyze serum T and androstenedione. For quantification we used the ELISA technique for T (DRG Instruments GmbH) and Coat-A-Count RIA kits (Diagnostic Products Corporation) for androstenedione. The intra- and interassay coefficients of variation were 6.6% and 4.0% for DHEAS, 5.3% and 2.8% for androstenedione, 11.9% and 9.1% for T, and 12.0% and 2.0% for SHBG, respectively. The free T index (FTI) was calculated: (T/SHBG) $\times$ 100. Insulin was analyzed by the ELISA technique with

Table 1. Baseline Characteristics of the Participants

	Metformin (n = 115)	Placebo (n = 116)
Age, y	29.9 ± 4.3	29.6 ± 4.4
Weight, kg	82.4 ± 20.4	79.5 ± 18.3
BMI, kg/m^2	29.5 ± 7.2	28.6 ± 7.4
Systolic BP, mm Hg	119 ± 12	118 ± 11
Diastolic BP, mm Hg	74 ± 9	73 ± 10
Heart rate, beats/min	75 ± 10	74 ± 10
Fasting glucose, mmol/L	4.6 ± 0.5	4.6 ± 0.6
Two-hour glucose, mmol/L	5.4 ± 1.5	5.4 ± 1.6
Gestational length at examination, d	132 ± 6	132 ± 6
Smoking, %	10 (8.7)	5 (4.3)
Caucasian, %	112 (97)	115 (99)

Values are given in mean ±SD or total number (percentage) as appropriate. There was no significant difference between the groups

the reagents and calibrators supplied by the manufacturer (DRG Instruments GmbH).

BP and heart rate were measured while the patient was in the sitting position after at least 10 minutes of comfortable rest in a chair. The BP and heart rate were measured three times, 2 minutes apart, with digital devices. The mean of the second and third measurements was calculated. Body weight was recorded with light clothes on and without shoes. Gestational age was determined by examination with vaginal ultrasonography, measuring crown-rump length and/or biparietal diameter of the fetus.

Midpregnancy Doppler ultrasound

The midpregnancy Doppler ultrasound was performed in gestational week 19 (±1 week). Experienced doctors or midwifes at the 11 study centers examined the patients. Up-to-date ultrasound machines in daily practice, from different manufacturers, were used. We chose to examine the uterine artery because it is easy to access, standardize, and reproduce the measurements. Doppler of the uterine artery is a widely used examination in routine clinic evaluations.

Doppler ultrasound was measured by transabdominal approach. The point at which the uterine artery crosses the internal iliac artery was identified, and the gate was placed within 1 cm on each side of this point. The angle was kept below 30 degrees. PI was measured on the right and left side three times. The lowest PI for each side was used for future references. Presence of a diastolic notch was registered. All Doppler examinations were performed in the morning after an overnight fast.

Data management

All data entry, data management, and data analyses were performed at the Department of Laboratory Medicine, Children's and Women's Health and the Unit for Applied Clinical Research, Department for Cancer Research and Molecular Medicine, both at the Norwegian University of Science and Technology. The first participant was included in February 2005 and the last in January 2009. The last patient gave birth in August 2009. Twelve women dropped out, ie, discontinued medication and did not turn up at scheduled visits. They gave consent that data from their hospital records about pregnancy, delivery, and postpartum period could be included in the analyses. Three women in the placebo group had miscarriages before gestational week 19.

Statistical analyses

The data were analyzed according to the intention-to-treat principle using IBM SPSS Statistics software version 20.0 for Windows. Student's *t* tests were used to examine differences in patient characteristics between the groups. Due to uneven distribution, a linear regression on the natural logarithm of PI was then used to examine the association between the different patient characteristics, pregnancy complications, and PI at gestational week 19. In the multivariate analysis, we included only variables with a value of $P \leq .10$ in the univariate analyses.

Results

There was no difference between baseline characteristics in the metformin and placebo groups (Table 1). During the study, eight women dropped out immediately after inclusion (three in the metformin group and five in the placebo group) and four (one in the metformin group and three in the placebo group) after gestational week 24.

There was no difference in PI at gestational week 19 between the metformin and placebo groups (Table 2). PI correlated positively to fasting glucose at gestational week 19 in both univariate ($P < .001$) and multivariate ($P = .004$) regression analyses (Table 3). Insulin, androstene-

Table 2. Patient Characteristics and PI at Gestational Week 19

	Metformin (n = 115)	Placebo (n = 116)	*P* Value
BMI, kg/m^2	30.0 ± 7	28.9 ± 6	.22
Systolic BP, mm Hg	116 ± 12	115 ± 12	.43
Diastolic BP, mm Hg	70 ± 10	71 ± 10	.94
Fasting glucose, mmol/L	4.3 ± 0.5	4.4 ± 0.4	.03
Two-hour glucose, mmol/L	5.9 ± 1.6	5.5 ± 1.4	.08
Insulin, pmol/L	97 ± 61	110 ± 71	.14
Gestational length, d	132 ± 6	132 ± 6	.50
LPI right uterine artery	0.84 ± 0.36	0.88 ± 0.41	.38
LPI left uterine artery	0.87 ± 0.45	0.89 ± 0.39	.60
Mean LPI	0.85 ± 0.34	0.89 ± 0.33	.40

Abbreviation: LPI, lowest pulsatility index. Values are given as means ± SD.

Table 3. Association Between PI at Week 19 and Glucose, Insulin, and Androgen Levels at Inclusion and Week 19

	Univariate			Multivariate		
	r	B (95% CI)	*P* Value	r	B (95% CI)	*P* Value
Inclusion						
Fasting glucose, mmol/L	0.15	0.09 (0.01–0.17)	.02	0.13	0.08 (−0.00 to 0.16)	.055
Two-hour glucose, mmol/L	0.10	0.02 (−0.01 to 0.05)	.14			
Insulin, pmol/L	0.11	0.001 (0.00–0.001)	.10	0.07	0.00 (0.00 to 0.00)	.28
SHBG, nmol/L	−0.03	0.00 (−0.00 to 0.00)	.62			
Androstendione, nmol/L	−0.13	−0.01 (−0.01 to 0.00)	.045	−0.09	−0.00 (−0.01 to 0.00)	.17
T, nmol/L	−0.07	−0.10 (−0.03 to 0.01)	.33			.94
DHEAS, μmol/L	−0.08	−0.01 (−0.03 to 0.01)	.23			.29
FTI	−0.05	−0.12 (−0.43 to 0.20)	.47			.64
Week 19						
Fasting glucose, mmol/L	0.23	0.16 (0.07–0.25)	.00	0.19	0.14 (0.05–0.23)	.004
Two-hour glucose, mmol/L	0.11	0.02 (−0.01 to 0.05)	0.12			
Insulin, pmol/L	0.12	0.001 (0.00–0.001)	0.08	0.01	7.522E-005 (−0.00 to 0.00)	.84
SHBG, nmol/L	−0.13	0.00 (−0.00 to 0.00)	0.06	−0.05	0.00 (−0.00 to 0.00)	.46
Androstendione, nmol/L	−0.11	−0.01 (−0.01 to 0.00)	0.10	−0.57	−0.01 (−0.01 to 0.00)	.38
T, nmol/L	−0.06	−0.01 (−0.03 to 0.01)	0.37			
DHEAS, μmol/L	−0.06	−0.01 (−0.03 to 0.01)	0.39			
FTI	0.02	0.09 (−0.46 to 0.63)	0.76			

Abbreviation: CI, confidence interval. The linear regression was performed on lnpulsatility index. In the multivariate analyses, adjustment for BMI, parity, and randomization in the multivariate analyses did not essentially change the results.

dione, and SHBG tended to correlate to PI in univariate but not in multivariate analyses. PI at gestational week 19 correlated positively to fasting glucose also at the first trimester ($P = .02$). In week 32 ($P = .08$), the association was weak and in week 36 the association was lost ($P = .77$).

High PI correlated positively to gestational diabetes, preeclampsia, and hypertensive disorders of pregnancy (Table 4). These correlations persisted when adjusting for possible confounding factors such as age, body mass index (BMI), randomization and parity.

Discussion

The main findings of this substudy are that metformin treatment had no effect on midpregnancy uterine blood flow in PCOS women as measured by PI and that midpregnancy PI correlated positively to fasting glucose in both the first and second trimesters.

Contrary to our hypothesis and observations from our small pilot study, metformin did not affect midpregnancy PI or pregnancy complications (19). To our knowledge, this is the first report addressing the effect of metformin on PI in pregnancy. This has not been reported in women with PCOS, GDM, or type 2 diabetes mellitus. It should be noted that the present findings differ from studies on nonpregnant PCOS women, in whom metformin improved coronary microvascular function (31) and improved blood flow to the ovaries (32) and the endometrium (33). However, these were all relatively small studies, possibly hampered by the same limitations as our pilot study, which indicated an effect of metformin on PI in pregnant PCOS women.

Metformin has been reported to increase skeletal muscle insulin sensitivity and to relax arterial smooth muscles, resulting in increased blood flow, particularly in the microcirculation (31). If metformin treatment had any major impact on the uterine artery blood flow or placental per-

Table 4. Associations Between PI at Gestational Week 19 and Adverse Pregnancy Outcome

		Univariate			Adjusted[a]		
	n	r	B (95% CI)	*P* Value	r	B (95% CI)	*P* Value
Preterm birth	15	0.04	−0.05 (−0.21 to 0.12)	.60	−0.02	−0.02 (−0.19 to 0.14)	.78
Gestational diabetes	66	0.16	0.14 (0.03–0.25)	.02	0.15	0.13 (0.02–0.24)	.02
Preeclampsia	16	0.14	0.22 (0.02–0.41)	.03	0.14	0.20 (0.01–0.40)	.04
Hypertensive disorder in pregnancy	52	0.15	0.05 (0.01–0.08)	.02	0.04	0.04 (0.003–0.08)	.04

Abbreviation: CI, confidence interval.

[a] Adjusted for randomization (metformin/placebo), parity, BMI, and age.

fusion, we would have expected to find a difference in PI between the groups at gestational week 19, which we did not. Accordingly, if metformin has any impact on pregnancy complications and/or pregnancy outcome, it is most probably not mediated by altering blood flow to or in the placenta.

The uterine artery blood flow increases during pregnancy to accommodate to the needs of the growing fetus. We found that midpregnancy PI correlated with fasting blood glucose in both the first and the second trimesters. The correlation was, however, strongest at the time of the ultrasound examination (second trimester). The higher the fasting glucose, the lower was the blood flow to the uterus.

Elevated maternal blood glucose results in increased glucose flux to the fetus and thereby increased fetal insulin levels. Insulin promotes fetal growth and large fetuses require more oxygen. In theory, we therefore have a situation with decreased oxygen delivery to a fetus that actually has increased oxygen needs. Our results are supported by the study of Pietryga et al (34), showing a correlation between increased PI in the uterine artery and high glycosylated hemoglobin levels in women with pregestational diabetes. Interestingly, in the present study, we found that increasing fasting glucose levels correlates to decreasing blood flow also in PCOS women without preexisting diabetes. It is well documented that poor blood flow to the fetus is associated with pregnancy complications and poorer outcome for the fetus (22, 26, 35, 36). Our observation that blood flow to the placenta correlated inversely with glucose levels may in part explain why fetuses of mothers with poor glucose control are more vulnerable to stress and hypoxia during pregnancy and labor.

Doppler of the uterine artery is a widely used method to predict preeclampsia. Although its predictive value is limited, it is often considered important in the follow-up of patients at risk of preeclampsia. We found, like others that high PI correlated to preeclampsia and hypertensive disorder of pregnancy (37). In addition, we found that PI correlates at least as well to GDM as to PE. Given the present observation, one could argue that glucose control seems to be of high importance for all pregnant PCOS women, not only those with GDM. This is in accordance with the observation from the Hyperglycemia and Adverse Pregnancy Outcome study in which a continuous positive association between glucose levels and adverse pregnancy outcome was observed in a large unselected population of pregnant women (38). Doppler ultrasound might be used for the surveillance of PCOS pregnancies, although further studies are needed on this issue.

We found a weak correlation between SHBG and PI. When we adjusted for other variables with a $P \leq .10$, this correlation disappeared. Others found a significant correlation between low SHBG and preeclampsia but no correlation between SHBG and PI in the uterine artery (39). Given that second-trimester elevated androgen levels associate with subsequent preeclampsia and elevated PI is a first step toward preeclampsia (7), it is noteworthy that only androstendione at inclusion had a weak correlation to PI and that this correlation disappeared in the multivariate analyses. This was surprising to us because when planning the study, we hypothesized a correlation between androgens and PI.

The limitation of this substudy is the fact, that it is a substudy and the data presented are not primary end points of a randomized, controlled trial. However, the Doppler examination was planned based on our positive findings in the pilot study (19), and the present substudy comprises 85% of the participants in the original randomized, controlled trial (PregMet study).

In conclusion, metformin treatment from the first trimester did not affect uterine artery blood flow evaluated by PI at gestational week 19 in PCOS women. Elevated glucose levels correlate inversely to blood flow in the uterine artery and thereby also the placenta, which may partly explain why fetuses born to mothers with poor glucose control are more vulnerable to prenatal stress and hypoxia.

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