How Metformin Acts in PCOS Pregnant Women

Insights into insulin secretion and peripheral action at each trimester of gestation

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OBJECTIVE—Metformin has been reported to reduce the risk of gestational diabetes (GD) in women with polycystic ovarian syndrome (PCOS). However, little is known about the mechanisms of action of this drug during pregnancy. In the attempt to fill this gap, we performed a prospective longitudinal study providing a detailed examination of glucose and insulin metabolism in pregnant women with PCOS undergoing metformin therapy.

RESEARCH DESIGN AND METHODS—We enrolled 60 women with PCOS who conceived while undergoing metformin treatment. An oral glucose tolerance test and a euglycemic-hyperinsulinemic clamp were performed at each trimester of gestation in 47 ongoing pregnancies.

RESULTS—Twenty-two of the study subjects had development of GD despite the treatment. At baseline, insulin sensitivity was comparable between women who had development of GD and women who did not. A progressive decline in this parameter occurred in all subjects, independently of the trimester of GD diagnosis. Insulin secretion was significantly higher during the first trimester in patients with an early failure of metformin treatment. Women with third trimester GD and women with no GD exhibited a significant increase in insulin output as gestation proceeded. All newborns were healthy and only one case of macrosomia was observed.

CONCLUSIONS—Women with PCOS who enter pregnancy in a condition of severe hyperinsulinemia have development of GD earlier, independently of metformin treatment. The physiologic deterioration of insulin sensitivity is not affected by the drug and does not predict the timing and severity of the glycemic imbalance. Despite the high incidence of GD observed, the drug itself or the intensive monitoring probably accounted for the good neonatal outcome.

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Women affected by polycystic ovarian syndrome (PCOS) show an increased prevalence of obesity, mostly of the central type, and abnormalities of glucose-insulin homeostasis, which are frequently found also in the absence of excessive body weight (1). The coexistence of these metabolic features is known to worsen the endocrine profile and the ovulatory function during reproductive age; later in life, it predisposes affected women to the development of diabetes mellitus and cardiovascular diseases. If a pregnancy occurs, then the presence of insulin resistance and hyperinsulinemia are responsible for a higher rate of obstetric complications [gestational diabetes (GD), early pregnancy loss, hypertensive disorders, preterm birth, perinatal mortality] (2,3). It is commonly believed that pregnancy itself represents a stressful clinical condition for the glycemic homeostasis by inducing a physiologic insulin resistance aimed at ensuring a preferential continuous supply of nutrients for feto-placental oxidative requirements. This condition may represent in women with PCOS an additional risk for impaired carbohydrate metabolism. Pancreatic function, which fulfills a compensatory adaptation before pregnancy, may fail to further overcome the insulin resistance induced by the gestational hormonal changes. Consistently, the incidence of GD in women with PCOS is increased compared with the general population, with an odds ratio ranging from 1.15 to 26.20 in different case series (4).

PCOS affects up to 10–12% of females, and more and more often these females become pregnant thanks to the advances in reproductive medicine. Hence, in the last years, the increased maternal and fetal morbidity rates have become a public health concern. In an aim to cope with this problem, the primary effort undoubtedly should be made in ameliorating the metabolic status of women with PCOS before they begin a pregnancy (5). When a preventive strategy is not feasible or has not been correctly pursued, the use of the insulin-sensitizing agent metformin has been proposed to modulate insulin sensitivity during pregnancy in women with PCOS.

During the past decade, several studies have reported beneficial effects of metformin treatment during pregnancy in PCOS patients in terms of prevention of early pregnancy loss and reduction of adverse pregnancy outcomes (6–10). In particular, most of them have claimed that metformin may reduce the risk of GD in women with PCOS to that expected in the general population, and some investigators even reported that this treatment may eliminate the occurrence of GD in women with PCOS (11). However, the majority of literature reports consist of retrospective studies burdened with selection bias and lacking universally recognized criteria for the diagnosis of GD.

In this scenario, little is known regarding the mechanisms by which metformin could exert its effects. In an aim to

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fill this gap, we present the first prospective longitudinal study providing a detailed examination of glucose and insulin metabolism in pregnant women with PCOS undergoing metformin administration. An analysis of the obstetrical outcomes is provided.

RESEARCH DESIGN AND METHODS—In this pilot prospective study, we enrolled 60 women affected by PCOS who conceived while undergoing metformin treatment.

The inclusion criteria were as follows: PCOS diagnosed before pregnancy; age 18–40 years; metformin treatment for at least 3 months before beginning the pregnancy; and gestational age between 5 and 12 weeks. The exclusion criteria were the following: diabetes mellitus [excluded with an oral glucose tolerance test (OGTT) before starting metformin treatment]; significant liver or renal impairment; neoplasms and other endocrine diseases; and the use of drugs other than metformin able to interfere with glucose and insulin metabolism.

All subjects were volunteers and informed consent was obtained from each patient. The study protocol was approved by the Ethical Board of our department.

In accordance with Rotterdam Consensus Conference (12), PCOS was diagnosed in the presence of at least two of the following criteria: irregular menstrual cycles (or amenorrhea); clinical or biochemical evidence of hyperandrogenism; and ultrasound assessment of polycystic ovary (presence of ≥12 follicles in each ovary measuring 2 ± 9 mm in diameter or increased ovarian volume) after exclusion of other etiologies.

All the enrolled subjects were using metformin from a minimum of 3 months to a maximum of 6 months before becoming pregnant (treatment dosage range, 1,500–2,550 mg/day). Out of the 60 included pregnant women, 52 conceived with metformin alone, 7 patients required the addition of a pharmacological induction of ovulation, and 1 woman conceived after an intracytoplasmic sperm injection cycle for concomitant male factor. All pregnancies were singleton, with the exception of one patient who presented with a twin gestation after ovarian stimulation with gonadotrophins. Enrolled women were advised to continue metformin throughout pregnancy.

Clinical laboratory tests were sequentially and longitudinally performed during the first (10–12 weeks of gestation), second (20–22 weeks of gestation), and third trimesters (30–32 weeks of gestation). An OGTT with 100 g glucose was performed after 48 h of standard diet and after overnight fast. Blood samples were collected basally and, after the ingestion of 100 g glucose, at 30, 60, 90, 120, 180, and 240 min. Insulin, glucose, and C-peptide were assayed in all samples. Insulin, C-peptide, and glucose responses to the stimuli are expressed as the area under the curve (AUC). A normal insulinemic response to OGTT was defined by a threshold AUC value of 15,000 μIU/mL/240 min, as previously described (13). On the next day, a euglycemic-hyperinsulinemic clamp was performed to estimate peripheral insulin sensitivity. Peripheral glucose utilization and expression of insulin sensitivity were measured as M (mg/kg/min), with a threshold value for insulin resistance determined to be 4.5 mg/kg/min (14).

Pregnancies were monitored as requested by the obstetric observation protocol of our department. Laboratory studies were performed to check blood cells and hepatic and kidney functions. The patients were subjected to obstetric ultrasonographic evaluations, and systolic blood pressure and diastolic blood pressure were determined using the first and the fourth Korotoff sounds.

In accordance with Carpenter and Coustan criteria (15), GD was diagnosed when two or more blood glucose values were >95 mg/dL at time 0 min, >180 mg/dL at 60 min, >155 mg/dL at 120 min, and >140 mg/dL at 180 min. Impaired glucose tolerance (IGT) was diagnosed when one of the blood glucose values was higher than the threshold value. If GD was diagnosed, then patients stopped metformin treatment, started therapy with insulin, and exited the study protocol. If IGT was diagnosed, then women continued the drug intake and repeated the laboratory work-up during the next trimester.

Data were collected regarding the rate of spontaneous abortions, live births, intrauterine and congenital malformations, and other adverse pregnancy outcomes (hypertensive disorders, polyamnios, macrosomia, cesarean delivery). Neonatal data included birth weight, 1-min and 5-min Apgar scores, and motor and social development during the first year of life.

Statistical methods

All results are presented as mean ± SD. Distribution of the data was tested by the Kolmorov-Smirnov test. We found that the variables were not normally distributed. The data from the study subgroups were compared by using Mann-Whitney *U* test. In the same group, the data were compared longitudinally over time by one-way repeated measures ANOVA (Friedman test). $P < 0.05$ was considered statistically significant.

RESULTS—At the beginning of the study, the mean age of our PCOS patients was 32.06 ± 4.2 years (range, 24–40) and the mean BMI was 28.67 ± 5.80 kg/m^2. Five women dropped-out immediately after inclusion. The cited reasons for discontinuation were long distance to the investigation center and time-consuming procedures. None dropped-out because of side effects. Among the 55 patients who continued metformin during pregnancy, 6 first-trimester spontaneous abortions (10.90%) and 2 spontaneous abortions during the second trimester (both for cervical incontinence) occurred (3.65%). There were 47 live birth pregnancies with 48 normal live births (46 singleton pregnancies and 1 twin gestation; 85.45%).

Figure 1 shows the incidence of GD and IGT at each trimester of pregnancy in the 47 studied PCOS women undergoing metformin treatment. During the first trimester, six women had development of GD and nine showed IGT. Thirty-two patients did not show any alteration of glucose metabolism. Whereas the six diabetic women stopped metformin therapy and started insulin treatment, the remaining 41 patients continued to use the drug and repeated the laboratory work-up during the second hospitalization. The results obtained from the OGTT at 20–22 weeks of gestation showed that 11 PCOS patients had development of GD and then switched to the insulin therapy. Six women with IGT and 24 patients exhibited a normal glycemic response. Of the 30 patients who did not exhibit alteration of glycemic homeostasis during the first and second trimesters, during the third trimester GD occurred in 5 patients, 3 had development of IGT, and 22 patients did not show any alteration of glucose metabolism. In summary, 22 PCOS women (46.8%) had diabetes diagnosed during pregnancy, whereas the remaining 25 patients (53.2%) did not have development of evident imbalances of carbohydrate homeostasis.

Table 1 shows the clinical and metabolic features at the time of enrollment of

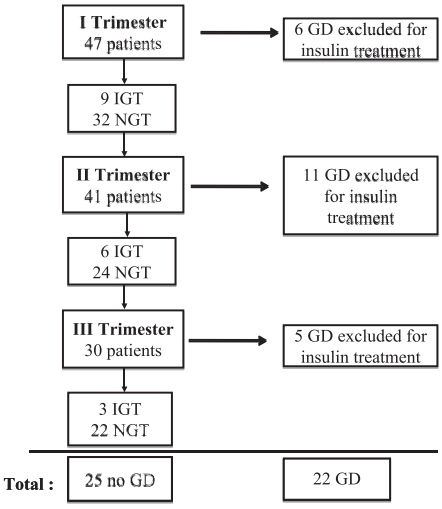

Figure 1—*Flow chart of incidence of GD, IGT, and normal glucose tolerance (NGT) during the three trimesters in studied PCOS patients.*

the patients who later had development of GD (GD group) compared with those of PCOS women who did not have development of GD and continued metformin until delivery (no GD group). The two groups did not differ in terms of age and BMI. The mean increment in BMI was 4.58 ± 0.67 kg/m^2 in patients in GD group and 3.82 ± 0.65 kg/m^2 in women in the no GD group (data not shown). Glucose, insulin, and C-peptide AUC values after OGTT during the first trimester were significantly higher in GD patients compared with the nondiabetic group ($P < 0.01$ for all the parameters). Despite this finding, the peripheral glucose utilization as determined by the clamp was comparable between the two groups.

Table 2 depicts age, BMI, AUC insulin, and M values during each trimester in patients grouped in accordance with the metabolic pregnancy outcomes. No differences were found in age and BMI in the subgroups.

Only women who had development of GD during the first trimester showed insulin AUC values markedly above the normal range and significantly higher compared with those found in patients who had development of GD during the second trimester ($P < 0.05$) and with those found in women in the no GD group ($P < 0.01$). The insulin secretion after glucose load significantly increased as pregnancy proceeded in patients without GD and those with GD diagnosed during the third trimester ($P < 0.01$ and $P < 0.05$, respectively).

The evaluation of the peripheral glucose utilization obtained by the euglycemic-hyperinsulinemic clamp documented mild insulin resistance in all studied patients at the time of the first determination. Although there was a trend toward decrease in the peripheral insulin sensitivity in all the studied groups, this modification did not attain the statistical significance and no differences were found in this parameter among subgroups.

No major maternal adverse effects possibly or probably related to metformin were observed during the study. In particular, there was no maternal lactic acidosis or hypoglycemia.

Three women had development of gestational hypertension that was treated successfully with pharmacological interventions. None had development of preeclampsia. Three cases of hepatosis occurred, one in a woman with GD since the first trimester and two in patients who did not show any alteration of glucose tolerance and who continued metformin until delivery.

Neither alterations of uterine and umbilical artery fluximetric profiles nor evidence of intrauterine growth retardation was detected by ultrasonography. There have been 47 at-term gestations (>37 weeks); only one case of preterm vaginal delivery occurred at week 31 of gestation because of premature rupture of membranes. Regarding modes of delivery, 16 women had cesarean deliveries (29%).

All 48 newborns were healthy. There have been no congenital defects and episodes of neonatal hypoglycemia did not occur. The mean birth weight was 3,224.85 ± 423.72 g. In the enrolled cohort, we observed just one case of macrosomia (4,500 g) in a newborn delivered vaginally from a patient with GD diagnosed during the first trimester. Median Apgar scores were 8 or 9. Neonatal motor development and social development were normal for all newborns.

Table 1—*Age, BMI, and metabolic features at the time of enrollment of women with or without GD*

	GD group (n = 22)	No GD group (n = 25)
Age (years)	32.64 ± 4.33	31.56 ± 3.74
BMI (kg/m^2)	28.71 ± 5.11	28.64 ± 6.45
AUC glucose (μIU/mL/240 min)	30,820.91 ± 4,463.00	25,434.00 ± 3,512.59*
AUC insulin (μIU/mL/240 min)	18,266.93 ± 8,036.62	12,948.48 ± 5,441.95*
AUC C-peptide (μIU/mL/240 min)	2,079.34 ± 850.66	1,609.08 ± 426.93*
M (mg/kg/min)	3.38 ± 0.90	3.71 ± 1.45

*$P < 0.01$ considered significant.

CONCLUSIONS—At variance with previous reports of metformin in pregnant women with PCOS, in the current study the gluco-insulinemic metabolism was thoroughly and longitudinally examined by the evaluation of the glucose, insulin, and C-peptide responses to OGTT and by the euglycemic-hyperinsulinemic clamp technique, which is to date considered the gold standard for the determination of peripheral insulin sensitivity. This approach allowed us to detect a state of more marked hyperinsulinemia during the first trimester in the patients destined for the development of GD despite metformin treatment compared with euglycemic subjects. Furthermore, we analyzed the characteristics of study subjects in relation to the gestational trimester in which GD was diagnosed. Interestingly, those women who had development of GD already during the first trimester presented an early exaggerated insulin secretion, with AUC insulin values markedly higher compared with those of patients with development

Table 2—*Age, BMI, insulin AUC, and M values during the three trimesters in four groups of patients*

	GD first trimester (n = 6)	GD second trimester (n = 11)	GD third trimester (n = 5)	No GD (n = 25)
Age (years)	32.83 ± 3.76	31.73 ± 4.92	34.40 ± 3.71	31.56 ± 3.74
BMI (kg/m^2)	29.39 ± 8.09	28.85 ± 4.83	27.59 ± 2.24	28.64 ± 6.45
AUC insulin, first trimester (μIU/mL/240 min)	24,450.75 ± 6,837.44	15,947.05 ± 7,850.73*	15,950.10 ± 6,905.83	12,948.48 ± 5,441.95†
AUC insulin, second trimester (μIU/mL/240 min)		20,023.64 ± 7,904.15	16,434.60 ± 5,286.77	15,084.84 ± 6,117.28
AUC insulin, third trimester (μIU/mL/240 min)			22,192.80 ± 4,669.57‡	19,781.58 ± 10,356.21§
M clamp, first trimester (mg/kg/min)	3.09 ± 0.45	3.46 ± 1.09	3.53 ± 0.92	3.71 ± 1.45
M clamp, second trimester (mg/kg/min)		2.77 ± 0.46	2.96 ± 0.83	3.25 ± 1.08
M clamp, third trimester (mg/kg/min)			2.70 ± 0.81	2.96 ± 0.72

Intergroup significance: *$P < 0.05$ vs. GD during first trimester; †$P < 0.01$ vs. GD during first trimester. Intragroup significance: ‡$P < 0.05$ during the three trimesters; §$P < 0.01$ during the three trimesters.

of diabetes later and, mostly, compared with nondiabetic women. However, the comparison between patients with development of GD during the first and the third trimesters did not achieve statistical significance, probably because of bias attributable to the small number of subjects. Therefore, it could be speculated that an early alteration of insulin secretion may represent the stronger predictive factor for the development of diabetes in PCOS patients as pregnancy proceeds. In accordance with this hypothesis, a previous study by our group documented a 100% diagnosis of an impairment of glucose metabolism during pregnancy in a small group of hyperinsulinemic PCOS patients (16). Metformin does not seem to affect this trend.

In this regard, metformin was supposed to prevent GD by protecting the reserve of pancreatic β-cells by reducing insulin resistance and through an effect on weight gain. At variance, in the current study, we did not observe significant differences in the percentage increase in insulin secretion in relation to the metabolic outcome and variations in this adaptive mechanism seem to play a less relevant role in the development of GD compared with basal hyperinsulinemia. Results from clamp examination indicate that metformin does not prevent the appearance of physiologic insulin resistance during pregnancy. Our patients who showed a defective peripheral glucose utilization already during the first determination experienced a further decline in insulin sensitivity. Participants who had development of GD did not differ from nondiabetic women in this parameter, irrespective of the trimester of gestation considered. Similarly, the increment in BMI, which was comparable with that reported in the literature for pregnant women with PCOS using metformin, did not seem to predict the deterioration of glycemic control.

The evaluation of metformin efficacy in preventing GD was not an end point of our study that was primarily planned to gain insight into the mechanisms of metformin-protective effects. Nevertheless, the observed high prevalence of treatment failure is somehow worth considering, notwithstanding the relatively small sample. In 2002, Glueck et al. (17) first observed a 10-fold reduction of the prevalence of GD in PCOS patients using metformin compared with previous untreated pregnancies. The same authors and other investigators published analogous reports and almost unanimously confirmed a breakthrough in the prevention of PCOS-related pregnancy complications (8,18–21). Recently, De Leo et al. (11) even claimed no case of GD in 98 hyperinsulinemic pregnant women with PCOS treated with metformin compared with a 13% incidence in healthy women. By contrast, and in keeping with our data, the only previous landmark, randomized, placebo-controlled study published to date failed to document a positive effect of metformin on the risk of GD in women with PCOS (22).

The disagreement with the reports documenting a positive profile of action for metformin could be explained on the basis of some methodological aspects that distinguish our study from the previous ones. The most evident discrepancy relies on the extremely variable diagnostic criteria for GD. Some of the studies that reported a reduced GD incidence in PCOS subjects using metformin are exclusively based on unconventional approach, such as the measurement of postprandial glycemia or fasting glycemia, which possess a very low sensitivity for the diagnosis of diabetes, particularly in the PCOS population (23).

Other studies are limited to the screening between weeks 26 and 28 of gestation with a 50-g oral glucose challenge test and, only in case of an abnormal response, an OGTT performed following, alternatively, World Health Organization or National Diabetes Data Group criteria. In this regard, even if the preferable diagnostic criteria for GD are still debated, we adopted Carpenter and Coustan criteria, which consider lower threshold values for the diagnosis and are able to identify ~40% diabetic pregnant women who would be missed by National Diabetes Data Group criteria and thus would have development of a higher rate of gestational complications (24,25). Hence, the hypothesis of an underdiagnosis or misdiagnosis of GD in a large number of previously published studies seems to be not completely ruled out.

It is also noteworthy that previous studies in this field are often retrospective and multicentric, and, in most of them, diagnostic tests were managed and assessed in collaboration with personal obstetricians of the patients. These aspects may impair the diagnostic

accuracy not only of GD but also of PCOS itself. This could imply that the putative efficacy of metformin in preventing GD could be amplified by an ultimate overestimation of the PCOS diagnosis and, therefore, of the metabolic risk among participants. In the current investigation, pregnancies were prospectively monitored in a single academic center, in hospitalization regimen, and the presence of PCOS was rigorously ascertained in the pregestational state, thus assuring the selection of a group of patients really at high risk for development of GD. Moreover, the reevaluation of subjects during each trimester is expected to offer solid accuracy of data and may be recommended in populations at high risk. Previous investigators reported that repeating the OGTT 1 month after a previous test allows the detection of ~30% conversion to GD in predisposed women (26).

The close longitudinal monitoring of patients was also the background for a novel result from our study, the high rate of GD diagnosis in an early phase of pregnancy. In fact, >77% of cases of metformin failure in preventing GD occurred before week 22 of gestation. Although the small sample did not allow us to draw conclusions on the effective prevalence of early gestational glucose impairment in the PCOS population, the clinical importance of this finding should not be undervalued. In this group, the missed diagnosis and treatment of diabetes would have exposed the fetuses to unquantifiable episodes of maternal hyperglycemia until the time of the standard screening for GD in the third trimester. In this regard, the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study recently has suggested that obstetric and neonatal outcomes may change in a "continuum" of risk without clear thresholds in direct proportion to maternal glucose levels, irrespective of the period of pregnancy considered (27).

Regarding further pregnancy outcomes, our data documented a cumulative low rate of early spontaneous abortion, hypertensive disorders, and preterm birth in comparison with those reported in the literature describing PCOS patients not treated with metformin. In line with previous larger studies in the general population as well as in PCOS subjects, neonatal outcomes from our patients are reassuring regarding possible adverse effects of the drug on infant health.

In conclusions, our study does not confirm the reduction of GD occurrence in PCOS subjects undergoing metformin treatment throughout pregnancy. On the contrary, we observed a high rate of GD, in most cases, in an early phase of gestation. Even if the small sample and underevaluation may limit the generalizability of this finding, our data strongly support the importance of early and close metabolic monitoring of women with PCOS during gestation.

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References

1. Ehrmann DA. Polycystic ovary syndrome. N Engl J Med 2005;352:1223–1236
2. Boomsma CM, Fauser BC, Macklon NS. Pregnancy complications in women with polycystic ovary syndrome. Semin Reprod Med 2008;26:72–84
3. Boomsma CM, Eijkemans MJ, Hughes EG, Visser GH, Fauser BC, Macklon NS. A meta-analysis of pregnancy outcomes in women with polycystic ovary syndrome. Hum Reprod Update 2006;12:673–683
4. Kjerulff LE, Sanchez-Ramos L, Duffy D. Pregnancy outcomes in women with polycystic ovary syndrome: a meta-analysis. Am J Obstet Gynecol 2011; 204: 558.e1-6.
5. Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. Consensus on women's health aspects of polycystic ovary syndrome (PCOS). Hum Reprod 2012;27:14–24
6. Jakubowicz DJ, Iuorno MJ, Jakubowicz S, Roberts KA, Nestler JE. Effects of metformin on early pregnancy loss in the polycystic ovary syndrome. J Clin Endocrinol Metab 2002;87:524–529
7. Glueck CJ, Phillips H, Cameron D, Sieve-Smith L, Wang P. Continuing metformin throughout pregnancy in women with polycystic ovary syndrome appears to safely reduce first-trimester spontaneous abortion: a pilot study. Fertil Steril 2001; 75:46–52
8. Glueck CJ, Bornovali S, Pranikoff J, Goldenberg N, Dharashivkar S, Wang P. Metformin, pre-eclampsia, and pregnancy outcomes in women with polycystic ovary syndrome. Diabet Med 2004;21:829–836
9. Glueck CJ, Wang P, Goldenberg N, Sieve-Smith L. Pregnancy outcomes among women with polycystic ovary syndrome treated with metformin. Hum Reprod 2002;17:2858–2864
10. Lord JM, Flight IH, Norman RJ. Metformin in polycystic ovary syndrome: systematic review and meta-analysis. BMJ 2003;327:951–953
11. De Leo V, Musacchio MC, Piomboni P, Di Sabatino A, Morgante G. The administration of metformin during pregnancy reduces polycystic ovary syndrome related gestational complications. Eur J Obstet Gynecol Reprod Biol 2011;157:63–66
12. Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised consensus on diagnostic criteria and long term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004;19: 41–47
13. Ciampelli M, Leoni F, Cucinelli F, et al. Assessment of insulin sensitivity from measurements in the fasting state and during an oral glucose tolerance test in polycystic ovary syndrome and menopausal patients. J Clin Endocrinol Metab 2005;90:1398–1406
14. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979;16:801–810
15. Carpenter MW, Coustan DR. Criteria for screening tests for gestational diabetes. Am J Obstet Gynecol 1982;144:768–773
16. Paradisi G, Fulghesu AM, Ferrazzani S, et al. Endocrino-metabolic features in women with polycystic ovary syndrome during pregnancy. Hum Reprod 1998;13: 542–546
17. Glueck CJ, Wang P, Kobayashi S, Phillips H, Sieve-Smith L. Metformin therapy throughout pregnancy reduces the development of gestational diabetes in women with polycystic ovary syndrome. Fertil Steril 2002;77:520–525
18. Glueck CJ, Pranikoff J, Aregawi D, Wang P. Prevention of gestational diabetes by metformin plus diet in patients with polycystic ovary syndrome. Fertil Steril 2008;89:625–634
19. Khattab S, Mohsen IA, Aboul Foutouh I, et al. Can metformin reduce the incidence of gestational diabetes mellitus in pregnant women with polycystic ovary syndrome? Prospective cohort study. Gynecol Endocrinol 2011;27:789–793
20. Vanky E, Salvesen KA, Heimstad R, Fougner KJ, Romundstad P, Carlsen SM. Metformin reduces pregnancy complications without affecting androgen levels in pregnant polycystic ovary syndrome

women: results of a randomized study. Hum Reprod 2004;19:1734–1740
21. Begum MR, Khanam NN, Quadir E, et al. Prevention of gestational diabetes mellitus by continuing metformin therapy throughout pregnancy in women with polycystic ovary syndrome. J Obstet Gynaecol Res 2009;35:282–286
22. Vanky E, Stridsklev S, Heimstad R, et al. Metformin versus placebo from first trimester to delivery in polycystic ovary syndrome: a randomized, controlled multicenter study. J Clin Endocrinol Metab 2010;95:E448–E455
23. Legro RS, Kunselman AR, Dodson WC, Dunaif A. Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. J Clin Endocrinol Metab 1999;84:165–169
24. Berggren EK, Boggess KA, Stuebe AM, Jonsson Funk M. National Diabetes Data Group vs Carpenter- Coustan criteria to diagnose gestational diabetes. Am J Obstet Gynecol 2011;205:253.e1–e7
25. Sermer M, Naylor CD, Gare DJ, et al. Impact of increasing carbohydrate intolerance on maternal-fetal outcomes in 3637 women without gestational diabetes. The Toronto Tri-Hospital Gestational Diabetes Project. Am J Obstet Gynecol 1995;173:146–156
26. Neiger R, Coustan DR. The role of repeat glucose tolerance tests in the diagnosis of gestational diabetes. Am J Obstet Gynecol 1991;165:787–790
27. Metzger BE, Lowe LP, Dyer AR, et al.; HAPO Study Cooperative Research Group. Hyperglycemia and adverse pregnancy outcomes. N Engl J Med 2008;358:1991–2002