

## Endocrino-metabolic features in women with polycystic ovary syndrome during pregnancy

Giancarlo Paradisi<sup>1</sup>, Anna Maria Fulghesu<sup>1</sup>, Sergio Ferrazzani<sup>1</sup>, Simonetta Moretti<sup>1</sup>, Caterina Proto<sup>2</sup>, Liberato Soranna<sup>1</sup>, Alessandro Caruso<sup>1</sup> and Antonio Lanzone<sup>2,3</sup>

<sup>1</sup>Department of Obstetrics and Gynecology, Università Cattolica del Sacro Cuore, Largo Agostino Gemelli, 1, 00168 Rome, Italy and <sup>2</sup>Oasi, Institute of Research, Via Conte Ruggero 73, 94018 Troina (Enna), Italy

<sup>3</sup>To whom correspondence should be addressed

To elucidate the mechanism of metabolic adaptation of women with polycystic ovary syndrome (PCOS) during pregnancy, the endocrino-metabolic features of a group of PCOS patients with or without gestational diabetes were studied longitudinally during the three trimesters of gestation. Oral glucose tolerance test (OGTT, 100 g) and hyperinsulinaemic–euglycaemic clamp were performed throughout the study. Plasma concentrations of insulin and glucose were determined by radioimmunoassay and glucose oxidase technique, respectively. Five of 13 PCOS patients developed gestational diabetes (GD) at the third trimester (PCOS-GD), while the other eight patients did not develop any alteration of glucose metabolism (PCOS-nGD). Both fasting glucose and insulin plasma concentrations did not change significantly during pregnancy and no difference was seen between the two groups. On the contrary PCOS-GD group early exhibited higher values of area under the curve (AUC) for glucose and insulin response to OGTT with respect to those found in PCOS-nGD group. This difference was already significant in the first gestational trimester. Moreover insulin sensitivity value (M) was significantly lower in the first trimester of gestation in PCOS-GD as compared with that found in PCOS-nGD group. However, as gestation proceeded, M value decreased in PCOS-nGD group and the difference from PCOS patients developing gestational diabetes was not sustained into the second and third trimesters. Both groups had similar body mass index values and AUC insulin increase from first to third trimester of gestation. It is concluded that early alteration of insulin sensitivity and secretion constitute specific risk factors in PCOS patients for the development of abnormalities of glucose tolerance.

**Key words:** insulin metabolism/insulin resistance/PCOS/pregnancy

### Introduction

It is well known that hyperinsulinaemia, obesity and hyperandrogenaemia are common features of patients affected by

polycystic ovary syndrome (PCOS) (Dunaif *et al.*, 1989; Lanzone *et al.*, 1990; Dale *et al.*, 1992; Holte *et al.*, 1994). Clinically, such abnormalities may lead to impairment of glucose tolerance for the development of non-insulin dependent diabetes mellitus (Joslin's, 1985; Barbieri *et al.*, 1988; Dunaif *et al.*, 1989; Conway *et al.*, 1992; Falcone *et al.*, 1992). Moreover, recent data indicate that PCOS subjects have a heterogeneous pattern of insulin secretion. A large number of obese patients are affected by hyperinsulinism and a significant proportion of lean subjects also shows this feature (Joslin's, 1985; Lanzone *et al.*, 1990; Falcone *et al.*, 1992; Holte *et al.*, 1994).

It is commonly held that pregnancy induces insulin resistance (Knopp *et al.*, 1981). Therefore, this condition may constitute in PCOS an additional risk for impaired carbohydrate metabolism. In fact, we previously demonstrated that the PCOS population was at higher risk for developing carbohydrate abnormalities during pregnancy than a normal population of similar reproductive age (Lanzone *et al.*, 1995); moreover, only those patients with abnormal pregestational insulin secretion developed impaired gestational glucose tolerance (IGGT) or gestational diabetes (Lanzone *et al.*, 1996). Moreover, IGGT or gestational diabetes (GD) occurred in 2–3% of pregnant women and affected fetal well-being in terms of increased incidence of malformations, abortions as well as fetal morbidity and perinatal mortality (Caruso, 1994).

In the present study we investigated the endocrino-metabolic features of a group of PCOS patients followed longitudinally during pregnancy with or without gestational diabetes. Their data are compared with those obtained from a control group and patients with gestational diabetes.

The aim of the present study was to elucidate further the mechanism of metabolic adaptation of PCOS women during pregnancy and the early derangement of glycaemic homeostasis in relation to endocrino-metabolic features.

### Materials and methods

We evaluated 13 consecutive pregnant patients affected by PCOS. All of these women were in good health and euthyroid, and none had taken any medication known to affect carbohydrate metabolism. Informed consent was obtained from each woman. This study protocol was approved by our Ethical Institutional Board.

All patients had had spontaneous puberty onset and all had been affected by oligoamenorrhoea with chronic anovulation since puberty. Obesity was defined as a body mass index (BMI) >25, calculated as the ratio between habitual weight (kg) and height<sup>2</sup> (m). PCOS was diagnosed by the finding of bilaterally normal or enlarged ovaries with the presence of at least 7–10 microcysts per ovary (<6 mm in diameter), associated with a moderate–marked increase of stroma at

the time of ultrasonography, and if at least two of the following clinical or endocrine factors were present: amenorrhoea or oligomenorrhoea, hirsutism, and plasma androgen concentrations at the upper limits of, or above, the normal range (Fulghesu *et al.*, 1993). The normal ranges were 2.0–6.5 nmol/l for androstenedione and 0.6–2.0 nmol/l for testosterone. A normal luteinizing hormone (LH)/follicle stimulating hormone (FSH) ratio was not considered an exclusion criterion. The presence of enzymatic defects of the adrenal gland was excluded by an adrenocorticotrophic hormone stimulation test (New *et al.*, 1983).

All patients became pregnant after pharmacological induction of ovulation. All received treatment with pure FSH (Metrodin; Serono, Milano, Italy) 3–5 days after the induced menses. Ultrasonographic monitoring of ovulation induction was done as well as and daily determinations of plasma oestradiol. Adequate follicular maturation was considered to be at least two follicles of 17 mm diameter. When this stage was reached, human chorionic gonadotrophin (HCG, Profasi 5000 IU; Serono) was administered to obtain the ovulation. Only moderate hyperstimulation was observed in all cases. All pregnancies were singleton.

Gonadotrophins were assayed by a double-antibody technique. All steroids were determined by a dextran–charcoal technique.

The patients were all Caucasian. On interview, pre-pregnancy weight, family history of diabetes, smoking habits and parity were recorded, and height, current weight, maternal age and arterial blood pressure were measured. No patient had a personal history of diabetes mellitus. None of them was a smoker during pregnancy. Before admission to the hospital they were following a diet of standard composition (the Italian diet typically contains 45% carbohydrate, 40% fat and 15% protein). Hypertension during pregnancy was defined as diastolic blood pressure >90 mmHg in two consecutive measurements 6 h apart with the patient resting in the semirecumbent position. The first and fourth Korotoff sounds were used to determine systolic and diastolic blood pressure, respectively. Neonatal data included birthweight, birth percentile, 1 and 5 min Apgar scores and gender of newborns.

The following studies were sequentially performed during a 2 day protocol: day 1, oral glucose tolerance test (OGTT); day 2, hyperinsulinaemic–euglycaemic clamp. The study was performed longitudinally at first (10–12 weeks), second (20–24 weeks) and third trimester (29–32 weeks) of gestation.

The OGTT with 100 g of glucose was performed after 48 h of standard diet containing at least 250 g of carbohydrate per day and following overnight fast. After the collection of a basal sample just before the load, the other samples were collected at 30, 60, 90, 120 and 180 min following the oral glucose consumption. Insulin and glucose plasma concentrations were assayed in each blood sample. Plasma samples for glucose concentrations were collected in tubes containing an inhibitor of glycolysis (sodium fluoride) to be analysed within 5 h. Plasma samples for insulin concentrations were placed in tubes standing in ice, centrifuged for 10 min at 1000 g using a 4226 ALC Centrifuge (ALC, Milan, Italy) and remained frozen at –30°C until assayed. Insulin and glucose plasma values were also expressed as the area under the curve (AUC) after the glucose ingestion, calculated by the trapezoidal rule and expressed as pmol/l×180 min and mmol/l×180 min respectively. Normal glucose tolerance was evaluated by the criteria of O'Sullivan and Mahan (1964). Gestational diabetes was diagnosed when two or more blood glucose values were >105 mg/dl at time 0, 189 mg/dl at 60 min, 165 mg/dl at 120 min and 144 mg/dl at 180 min (conversion factor for SI units = 0.0555).

The hyperinsulinaemic–euglycaemic clamp was performed after a 10 h overnight fast as described by De Fronzo *et al.* (1979) to estimate peripheral insulin sensitivity. At 0800 h, an i.v. catheter was placed in the antecubital vein for the infusion of glucose and insulin. Another

catheter was placed in the dorsal vein of the contralateral hand for blood withdrawal and warmed to 65°C with a warming box. A primed constant infusion of insulin (Actrapid HM, Novo Nordisk, Copenhagen, Denmark) 40 mU/min to achieve a high concentration of insulin (~100 µU/ml; range 80–120) was performed. A variable infusion of 20% glucose was adjusted, on the basis of plasma glucose samples drawn every 5 min, to maintain plasma glucose at 100 mg/dl. The glucose infusion rate during the last 60 min of a 2 h infusion was then taken as the estimate of peripheral insulin sensitivity and measured as M (mg/kg/min).

Since GD was generally diagnosed at the third trimester of gestation (Caruso, 1994), a group of 10 consecutive pregnant controls (CTR) and nine consecutive pregnant patients with GD were also investigated at third trimester of gestation with the same clinical protocol. Results of these two groups have been compared with those obtained from PCOS groups at the same age of gestation.

Insulin plasma concentrations were determined by a commercial radioimmunoassay kit (Radim, Pomezia, Italy). The intra- and inter-assay coefficients of variations were <8 and 15% respectively. Glucose plasma concentrations were determined by the glucose oxidase technique with a glucose analyser (Beckman, Fullerton, CA, USA).

The analysis of variance (ANOVA) with Fisher's protected least significant difference test, the Kruskal–Wallis test, and  $\chi^2$  analysis were used when appropriate. Two-tailed *P* values of < 0.05 were considered significant.

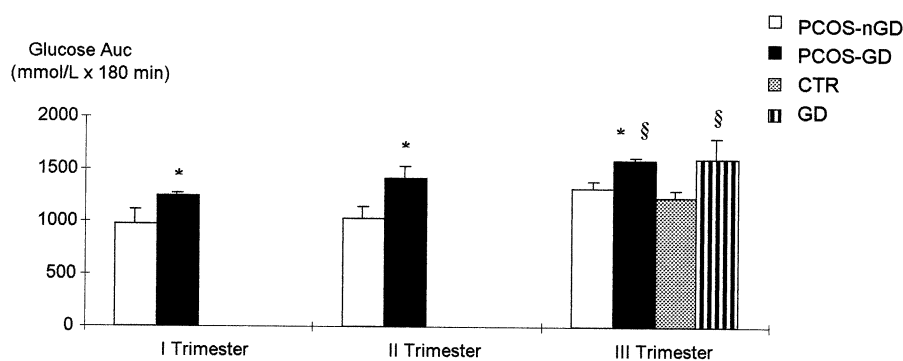
## Results

Five of 13 PCOS patients developed gestational diabetes at third trimester of gestation (PCOS-GD group); data from these patients were compared with those obtained from the other PCOS patients who did not show any alteration of glucose tolerance (PCOS-nGD group).

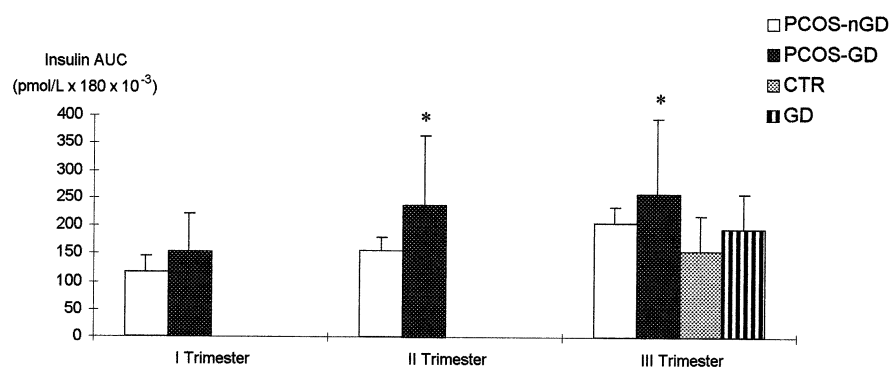
Fasting plasma glucose concentrations were similar between the two groups during the course of gestation (PCOS-nGD versus PCOS-GD: first trimester:  $4.1 \pm 0.2$  versus  $4.3 \pm 0.2$  mmol/l; second:  $4.1 \pm 0.2$  versus  $4.2 \pm 0.2$ ; third:  $4.1 \pm 0.3$  versus  $4.2 \pm 0.3$ ). However, at third trimester of gestation glycaemic values were higher in GD patients ( $4.8 \pm 0.6$  mmol/l; *P* < 0.05) in respect to those found in other groups (controls:  $4.0 \pm 0.2$  mmol/l). On the other hand, glucose AUC values after OGTT were significantly greater in all trimesters of pregnancy in those PCOS patients who developed GD (Figure 1). When data were compared at third trimester of gestation with those found in other groups, glucose AUC values were higher in GD and PCOS-GD in respect to control and PCOS-nGD groups. No difference in AUC glucose value was seen among GD and PCOS-GD subjects.

Also fasting insulin plasma concentrations were superimposable during all trimesters of gestation in the two PCOS groups (PCOS-nGD versus PCOS-GD: first trimester:  $71.7 \pm 28.7$  versus  $71.2 \pm 14.3$  pmol/l; second:  $71.2 \pm 14.3$  versus  $100.4 \pm 57.4$ ; third:  $136.3 \pm 57.4$  versus  $129.1 \pm 57.4$ ). At third trimester all PCOS patients ( $136.3 \pm 57.4$  pmol/l) had significantly higher plasma fasting insulin concentrations in respect to controls ( $86.1 \pm 22.2$  pmol/l; *P* < 0.05), whereas no difference among GD ( $93.2 \pm 35.8$  pmol/l) and PCOS or control groups was found.

Figure 2 shows the insulin AUC values after OGTT in the



**Figure 1.** Glucose area under the curve (AUC) after the oral glucose tolerance test (OGTT) in polycystic ovary syndrome (PCOS) subjects during pregnancy in relation to their metabolic status. \*The AUC values in PCOS patients who developed GD (PCOS-GD) were significantly greater ( $P < 0.001$ ) in all trimesters than the AUC values in PCOS patients who did not show any alteration of glucose tolerance (PCOS-nGD). §At the third trimester glucose AUC values were significantly higher in PCOS-GD and in patients with gestational diabetes (GD) than the control (CTR) and PCOS-nGD groups (PCOS-GD versus CTR:  $P < 0.0001$ ; GD versus CTR:  $P < 0.001$ ; GD versus PCOS-nGD:  $P < 0.001$ ). No difference was found between GD and PCOS-GD groups.



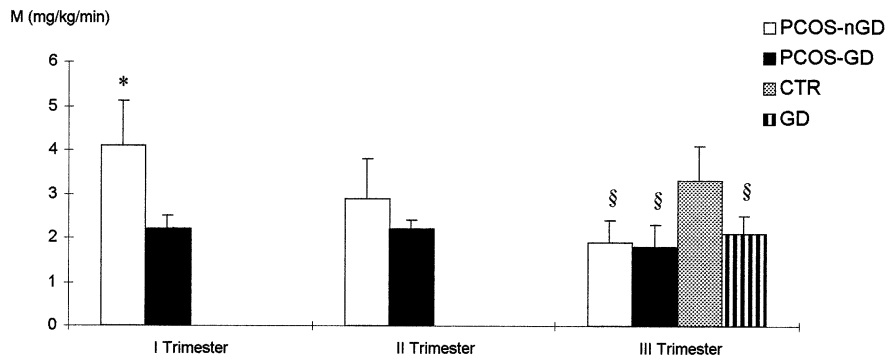
**Figure 2.** Insulin area under the curve (AUC) after glucose load in the studied groups. \*The insulin AUC values were higher in PCOS patients who developed GD (PCOS-GD) in respect to PCOS patients who did not show any alteration of glucose tolerance (PCOS-nGD) and control (CTR) subjects; this difference, starting from the second trimester of pregnancy, was statistically significant (second trimester: PCOS-GD versus PCOS-nGD:  $P < 0.05$ ; third trimester: PCOS-GD versus PCOS-nGD:  $P < 0.04$ ; PCOS-GD versus CTR:  $P < 0.04$ ).

studied groups. First trimester insulin AUC values had a non-significant trend towards higher values in PCOS-GD than in the PCOS-nGD group; however, a statistical difference was attained starting from the second trimester. Moreover, at third trimester, the highest statistically significant insulin AUC values were seen in PCOS-GD patients as compared with those found in PCOS-nGD and control subjects, whereas the difference was at the limit of significance in comparison with the values found in GD women.

The increment of insulin AUC after OGTT in PCOS groups was also analysed. In all patients a remarkable increase of insulin AUC was seen at second trimester of gestation (PCOS-nGD  $37\,779 \pm 37\,102$  pmol/l $\times$ 180 min, increase  $22 \pm 22\%$ ; PCOS-GD  $82\,994 \pm 103\,714$  pmol/l $\times$ 180 min,  $23 \pm 32\%$ ); at third trimester this increment was less pronounced (PCOS-nGD:  $16\,689 \pm 3056$  pmol/l $\times$ 180 min,  $8 \pm 15\%$ ; PCOS-GD:  $64\,144 \pm 19\,745$  pmol/l $\times$ 180 min,  $7 \pm 7\%$ ). No significant difference was found among PCOS groups either as absolute or percentage increase of insulin AUC (PCOS-nGD versus PCOS-GD:  $54\,687 \pm 38\,881$  pmol/l $\times$ 180 min,  $30 \pm 18\%$  versus  $104\,683 \pm 139\,998$  pmol/l $\times$ 180 min,  $28 \pm 33\%$ ).

Figure 3 indicates the values of insulin sensitivity (M, see Materials and methods) in all studied groups. At first trimester of gestation PCOS-nGD subjects exhibited a greater M value than those found in the PCOS-GD group. However, as gestation proceeded, M values progressively decreased in the PCOS-nGD group and the difference with patients developing GD was not maintained at second trimester. In the PCOS-GD group, M values were always very low and they did not change during pregnancy. At third trimester, GD and all PCOS subjects had significantly lower M values with respect to controls ( $P < 0.001$ ). No difference was detected between GD and PCOS subjects.

Table I shows the clinical characteristics of the studied groups. All groups were superimposable for the age of patients, except for GD versus PCOS-nGD patients. Moreover, no significant difference was seen between the PCOS groups. As indicated, the only differences between GD and PCOS groups were the greater values of maternal weight gain and parity found in GD subjects; the dosage and the duration of insulin therapy were similar in PCOS-GD and GD groups. On the other hand, controls exhibited significantly lower values of



**Figure 3.** Values of insulin sensitivity in all studied groups. \*At the first trimester of pregnancy PCOS-nGD subjects showed significantly greater M values than PCOS-GD ( $P < 0.03$ ). At the second trimester of pregnancy M values progressively decreased in PCOS-nGD patients and the difference with subjects developing GD was not maintained. §At the third trimester of pregnancy GD and all PCOS subjects had significantly lower M values with respect to CTR patients (PCOS-nGD versus CTR:  $P < 0.001$ ; PCOS-GD versus CTR:  $P < 0.001$ ; GD versus CTR:  $P < 0.001$ ). No difference was detected between GD and PCOS subjects.

**Table I.** Clinical characteristics of studied groups

	CTR (n = 10)	GD (n = 9)	PCOS-nGD (n = 8)	PCOS-GD (n = 5)
Age (years)	30.9 ± 4.6	34.1 ± 3*	28 ± 2.9	32.2 ± 6.3
BMI (kg/m <sup>2</sup> )	21.3 ± 2.5†	25.6 ± 3.9	28.3 ± 3.2	28.3 ± 0.7
Weight gain (kg)	10.50 ± 2.0	14.9 ± 4.0**	8.4 ± 2.4	9.2 ± 0.4
Max SBP (mmHg)	123 ± 16	133 ± 5	136 ± 7	128 ± 8
Max DBP (mmHg)	80 ± 10	85 ± 7	87 ± 4	82 ± 3
Mean DBP (mmHg)	69 ± 7†	74 ± 5	75 ± 1	72 ± 4
Week of delivery	38.4 ± 1.3†††	39.4 ± 1.5	39.9 ± 1.9	40.2 ± 0.5
Birthweight (g)	2890 ± 339†	3505 ± 464	3345 ± 585	3510 ± 241
Birthweight percentile	34.3 ± 20.2†	67 ± 23.7	56.7 ± 29.4	67.6 ± 17.1
Macrosomia	0/10	1/9	0/8	0/5
Parity	1.7 ± 0.7	2.0 ± 1.0***	1.1 ± 0.4	1.2 ± 0.5
Pregravid weight (kg)	53.6 ± 6.1†	65.5 ± 12.7	73.7 ± 13.6	76.6 ± 3.6
Insulin therapy (IU/day)		24.6 ± 4.1		21.2 ± 1.6
Days of insulin therapy		59.9 ± 14.5		61.6 ± 11.5

BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure. For other abbreviations, see Figure 1.

Values are expressed as mean ± SD.

† $P < 0.05$  CTR versus other groups; †† $P < 0.05$  CTR versus GD and PCOS-nGD; ††† $P < 0.05$  CTR versus PCOS-nGD and PCOS-GD; \* $P < 0.01$  GD versus PCOS-nGD; \*\* $P < 0.002$  GD versus other groups; \*\*\* $P < 0.05$  GD versus PCOS-nGD and PCOS-GD.

BMI, diastolic blood pressure, week of delivery, birthweight, birthweight percentile and pregravid weight as compared to those found in other groups.

**Discussion**

This paper is the first in the literature showing a perspective longitudinal study of PCOS patients during pregnancy. It extends our previous observations, where it was found that PCOS pregnant patients constitute a group of subjects at high risk of developing abnormalities of carbohydrate metabolism.

The objective of the present work is to analyse, beyond the simple insulin secretion, the metabolic features of these subjects during pregnancy by a perspective longitudinal study in order to verify the possible early metabolic derangement, leading to gestational diabetes. Our results show that already at first trimester of pregnancy PCOS patients who further developed gestational diabetes had significant differences with respect to unaffected subjects. In fact PCOS-GD group showed, although in the normal range, higher glucose plasma concentrations

after OGTT as compared to PCOS-nGD group. Also insulin OGTT-induced secretion was higher in this group. Moreover, at first trimester PCOS-GD subjects had a reduced insulin sensitivity compared with PCOS-nGD subjects. Indirectly, these results agree with our previous observations where we documented that only pregestational hyperinsulinaemic PCOS patients may develop gestational diabetes (Lanzone *et al.*, 1966). In the present series the pregestational study was not performed; however, it is presumed that the profound metabolic changes of pregnancy, mostly due to the increasing concentrations of human placental lactogen, do not occur significantly at this stage of gestation and thus our data may in part reflect the pregestational condition of these patients.

Our data suggest that the primary event, enhancing a metabolic condition of risk for gestational glucose intolerance in PCOS, is the reduced peripheral insulin sensitivity, which in turn determines the compensatory hyperinsulinaemia. Several observations support this hypothesis, as set out below.

Some reports demonstrated that in PCOS glucose intolerance is present mainly in obese non-pregnant PCOS subjects, sug-

gesting that insulin and obesity may have an additive deleterious effect (Harris *et al.*, 1987; Dunaif *et al.*, 1989); however, our previous findings suggested that in pregnancy the impact of obesity on the incidence of gestational diabetes seems to be superimposable in PCOS on that observed in the general pregnant population (Freinkel *et al.*, 1985; Lanzone *et al.*, 1995). Moreover, this hypothesis is further supported by the fact that, in spite of different insulin sensitivity, both PCOS-GD and PCOS-nGD showed similar BMI. Therefore, although obesity is known to impair insulin sensitivity, this study confirms that in pregnancy the risk of developing glucose intolerance in PCOS is represented by impaired insulin sensitivity and hyperinsulinism.

Second, both PCOS-GD and PCOS-nGD groups exhibited a similar absolute insulin increase to OGTT during the course of gestation. This suggests that in PCOS-GD patients, insulin secretion by the pancreas is not reduced: however, since impairment of insulin sensitivity is present at an early stage of gestation in such subjects, we suggest that the insulin secretion might be impaired in relation to the growing metabolic requirements during the course of gestation. This hypothesis is also supported by the fact that PCOS-GD patients exhibited the highest values of insulin in response to OGTT as compared to a group of patients with gestational diabetes, suggesting that from a pathophysiological point of view they may represent a specific subgroup.

Finally, it is interesting to note that, beyond the glucose tolerance, the two PCOS groups did not show any clinical difference for other maternal and fetal parameters: this observation supports the idea of an early alteration of metabolic status as the principal variable inducing changes of glucose tolerance.

Compared to PCOS-GD subjects, GD patients showed a significant increase of maternal weight gain; this finding coupled with the observation of an insulin secretion in gestational diabetes patients similar to that found in controls and PCOS-nGD groups suggests that in GD, non-PCOS subjects, overweight during pregnancy could be more important than insulin metabolism in determining glucose intolerance (Freinkel *et al.*, 1985; Caruso *et al.*, 1994). Dosage and duration of insulin therapy were similar in all diabetic subjects, thus indicating an homogeneous clinical approach in the different groups.

Control patients exhibited significantly lower values for diastolic blood pressure and birthweight percentile compared with the other studied groups; however, these patients also had significantly lower values of BMI compared with PCOS and GD subjects. Thus, part of the difference found in this study between controls and other patients could be ascribed to this feature. It should be noted, however, that PCOS-nGD patients exhibited at third trimester of gestation a 40% reduction of M value compared with non-diabetic controls. However, it should be noted that overweight and obesity are either intrinsic features of patients with PCOS, varying in the percentage increase compared with all patients from 40 to 80%, or significant risk factors for the development of gestational diabetes (Caruso *et al.*, 1994). Therefore, it is difficult to plan such a study matching controls, PCOS and GD patients for BMI.

In conclusion, our study analysed longitudinally the endocrine-metabolic features of pregnant PCOS women in relation to their risk of developing abnormalities of glucose tolerance. Early alteration of insulin sensitivity and secretion constitute specific factors of risk for this population. Early identification of these features may lead to a better characterization and early diagnosis of glucose intolerance.

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