Maternal serum androgens in pregnant women with polycystic ovarian syndrome: possible implications in prenatal androgenization

T.Sir-Petermann[1,5], M.Maliqueo[1], B.Angel[1], H.E.Lara[2], F.Pérez-Bravo[3] and S.E.Recabarren[4]

[1]Division of Endocrinology, Department of Internal Medicine, San Juan de Dios Hospital, School of Medicine, University of Chile, [2]Laboratory of Neurobiochemistry, Faculty of Chemistry and Pharmaceutical Sciences, University of Chile, [3]Laboratory of Energy Metabolism and Stable Isotopes, INTA, University of Chile, Santiago and [4]Laboratory of Animal Physiology and Endocrinology, School of Veterinary Medicine, University of Concepción, Chillán, Chile

[5]To whom correspondence should be addressed at: Las Palmeras 299, Interior Quinta Normal, Casilla 33052, Correo 33, Santiago, Chile. E-mail: tsir@machi.med.uchile.cl

BACKGROUND: The aim of this study was to evaluate the peripheral serum androgen concentrations in normal and polycystic ovarian syndrome (PCOS) women during pregnancy, in order to establish if PCOS may induce gestational hyperandrogenism and therefore constitute a potential source of androgen excess for the fetus. METHODS: Twenty pregnant PCOS (PPCOS) women and 26 normal pregnant (NP) women of similar age with singleton pregnancies were selected for the study. During gestational weeks 10–16 and 22–28, a 2 h, 75 g oral glucose tolerance test (OGTT) was performed. For the OGTT, glucose and insulin were measured in each sample and testosterone, androstenedione, dehydroepiandrosterone sulphate (DHEAS), estradiol, progesterone and sex hormone-binding globulin were determined in the fasting sample. RESULTS: In the first study period (gestational weeks 10–16), the levels of androstenedione, testosterone and DHEAS and the free androgen index tended to be higher in the PCOS group. These differences became significant in the second study period (gestational weeks 22–28). In this second period, 2 h insulin concentrations were also significantly higher in PPCOS than in NP women. CONCLUSIONS: The present study demonstrates a significant increase in androgen concentrations during pregnancy in PCOS women. We propose that these androgen concentrations could provide a potential source of androgen excess for the fetus, without leading to fetal virilization.

Key words: androgens/fetal androgen excess/insulin/PCOS/pregnancy

Introduction

Polycystic ovarian syndrome (PCOS) is one of the most common endocrine disorders affecting women of reproductive age (Adams *et al*., 1986; Hull *et al*., 1987; Franks 1995; Knochenhauer *et al*., 1998). The most widely accepted definition of PCOS is the association of chronic anovulation and hyperandrogenism in the absence of specific diseases of the ovaries, adrenals, and pituitary (Zawadzky and Dunaif, 1992).

PCOS is probably the result of several pathophysiological pathways: increased LH levels (Yen *et al*., 1970; Rebar *et al*., 1976; Kazer *et al*., 1987; Berga *et al*., 1993; Sir-Petermann *et al*., 1993), ovarian abnormalities (Ehrmann *et al*., 1995; Gilling-Smith, 1997), peripheral insulin resistance and compensatory hyperinsulinaemia independent of obesity (Dunaif *et al*., 1989; Franks, 1995; Holte, 1996; Dunaif, 1997; Sir-Petermann *et al*., 1998), all leading to increased ovarian androgen secretion. The complexity of PCOS pathophysiology is of such magnitude that the aetiology remains unknown.

Studies have shown that an experimentally induced prenatal androgen excess in fetal female monkeys results in changes that, in adulthood, resemble those observed in PCOS women (Abbott *et al*., 1997, 1998; Eisner *et al*., 2000). In humans, clinical observations have established that women with a classical 21-hydroxylase deficiency exhibit, in adulthood, features that closely mimic those found in PCOS, such as anovulation, ovarian hyperandrogenism, LH hypersecretion, polycystic-appearing ovaries and insulin resistance, despite the normalization of the adrenal androgen excess after birth (Hague *et al*., 1990; Barnes *et al*., 1994). These observations suggest that androgen excess during early life, whether derived from fetal or maternal sources, may provide one possible mechanism to explain the occurrence of PCOS in adulthood.

On the other side, we have recently demonstrated that during lactational amenorrhoea in breastfeeding women with PCOS, the ovarian size and the androstenedione levels were greater than those observed in normal lactating women, suggesting that in these patients, the ovaries were persistently stimulated during pregnancy and therefore could constitute a maternal

source of androgen excess for the fetus (Sir-Petermann *et al*., 2001).

The aim of this study was to evaluate the serum androgen concentrations in normal and PCOS women during pregnancy, in order to establish if PCOS may induce gestational hyperandrogenism and therefore constitute a potential source of androgen excess for the fetus.

Materials and methods

Subjects

Twenty pregnant women with PCOS (PPCOS) were selected for the study from PCOS patients attending the Unit of Reproductive Medicine, University of Chile, who desired fertility, and were placed on a treatment programme as described previously (Sir-Petermann *et al*., 2001). Preconceptional inclusion criteria were: chronic oligo- or amenorrhoea, clinical signs of hyperandrogenism with no virilization, clinical signs of hyperinsulinaemia (waist:hip ratio >0.85), serum testosterone concentration >0.6 ng/ml and/or free androgen index (FAI) >5.0, different grades of hyperinsulinaemia evaluated by an oral glucose tolerance test (OGTT), and a characteristic ovarian morphology on ultrasound based on previously described criteria (Adams *et al*., 1986). A normal LH:FSH ratio was not considered an exclusion criterion. All women were amenorrhoeic and anovulatory according to progesterone measurements and ultrasound examinations. Hyperprolactinaemia, androgen-secreting neoplasm, Cushing's syndrome and attenuated 21-hydroxylase deficiency, as well as thyroid disease, were excluded by appropriate tests.

By design, 26 normal pregnant (NP) women of similar age acted as a control group. Each one had a history of regular 28–32 day menstrual cycles, absence of hirsutism and other manifestations of hyperandrogenism, absence of galactorrhoea, thyroid dysfunction and family history of diabetes. All were healthy and were not receiving any drug therapy. These women were recruited from the antenatal care unit of the same hospital since the 12th week of gestation and they belonged to the same geographical city area as the patients.

Prior to the study, informed consent was obtained from all subjects. This study was approved by the local ethics committee.

Study protocol

The women were admitted to the Clinical Research Center in the morning (08:30–09:00) after an overnight fast of between 8 and 12 h. During gestational weeks 10–16 and 22–28, a 2 h, 75 g OGTT was performed in accordance with published criteria (World Health Organization, 1999). Glucose was measured in the fasting sample and 2 h postload. Pregnant women who met the World Health Organization criteria for diabetes mellitus (fasting glucose values >126 mg/dl; 2 h glucose postload >140 mg/dl) were classified as having gestational diabetes mellitus.

Serum insulin was measured in each sample. Serum total testosterone, androstenedione, dehydroepiandrosterone sulphate (DHEAS), estradiol (E_2), progesterone and sex hormone-binding globulin (SHBG) were determined in the fasting sample.

Assays

Serum glucose was determined by the glucose oxidase method (Photometric Instrument 4010; Roche, Basel, Switzerland). The intra-assay coefficient of variation (CV) of this method was <2.0%. Serum insulin, testosterone, DHEAS, androstenedione and progesterone were assayed by radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA, USA), SHBG concentration was measured by immunoradiometric assay using commercial kits (Diagnostic Products Corp.). E_2 was measured by electrochemiluminiscence (Roche). The intra- and inter-assay CV respectively were 5 and 8% for insulin, 7.0 and 11% for testosterone, 6.6 and 4.9% for DHEAS, 3.7 and 4.9% for androstenedione, 4.8 and 9.2% for progesterone, 3.8 and 7.9% for SHBG and 2.7 and 5% for E_2.

Statistical evaluation

Comparisons within groups were performed by two-way analysis of variance, with previous logarithmic transformation of the data. Differences between groups were sought by Mann–Whitney test. The significance level was set at 5%. Results are expressed as medians and ranges.

Results

Table I shows the clinical characteristics of the two groups of pregnant women. By design, age was not different between both groups. No difference was found in the duration of gestation, weight gain during pregnancy, birthweight, or systolic and diastolic blood pressure between the groups. In two pregnant women with PCOS (10.5%), pre-eclampsia was diagnosed. There were significant differences in the body mass index (BMI) at the initiation and in the third trimester of pregnancy between PPCOS and NP women.

Regarding the sex distribution of the newborn babies, no significant difference was found between the groups (NP: 52% female and 48% male; PPCOS: 50% female and 50% male). No pregnant patients developed the classic signs and symptoms of virilization and the female infants born to these patients were non-virilized.

Table II shows the endocrine characteristics during the two study periods in NP women. In NP women, E_2 and progesterone concentrations increased significantly between the two study periods. No significant changes in the serum concentrations of testosterone, androstenedione or DHEAS were observed. SHBG decreased significantly and the FAI increased significantly during the study.

Table III shows the endocrine characteristics during the two study periods in PPCOS women. In PPCOS women, an increase in the serum concentrations of E_2 and progesterone was observed. No significant changes in the serum concentrations of testosterone, androstenedione or DHEAS were observed. SHBG decreased significantly while the FAI increased significantly during the study.

Comparing both groups of pregnant women (Figure 1), in the first study period (gestational weeks 10–16), the levels of androstenedione, testosterone and DHEAS and the FAI were not significantly different between the groups, although they tended to be higher in the PCOS group. These differences become significant in the second study period (gestational weeks 22–28). SHBG, E_2 and progesterone concentrations were not significantly different between both groups in either of the study periods.

In both groups of pregnant women, no differences were noted in DHEAS, androstenedione or total testosterone concentrations between mothers with female fetus and mothers with male fetus during either of the study periods (Table IV).

The fasting and 2 h glucose and insulin levels of both groups of pregnant women during the two study periods are

Table I. Clinical characteristics of normal pregnant (NP) and pregnant polycystic ovarian syndrome (PPCOS) women

	NP (n = 26)	PPCOS (n = 20)
Age (years)	26.70 (16.0–38.0)	26.80 (16.0–35.0)
Duration of gestation (weeks)	38.6 (37.0–41.0)	38.6 (37.0–40.0)
Initial body mass index (kg/m^2)	24.4 (20.1–28.9)	29.4 (25.1–34.2)*
Body mass index in the third trimester (kg/m^2)	25.9 (22.1–31.1)	29.7 (24.2–35.8)*
Weight gain during pregnancy (kg)	12.6 (6.9–18.3)	14.4 (11.2–21.0)
Birthweight (g)	3435 (2880–4410)	3464 (2890–4420)
Systolic blood pressure (mmHg)	113.1 (90.0–135.0)	116.7 (100.0–140.0)
Diastolic blood pressure (mmHg)	69.0 (60.0–84.0)	75.3 (60.0–90.0)

Values are medians and ranges.
*$P < 0.05$ between NP and PPCOS.

Table II. Endocrine characteristics of normal pregnant women during gestational weeks 10–16 and 22–28

	Weeks 10–16	Weeks 22–28
DHEAS (μg/dl)	117.48 (39.4–217.56)	94.97 (73.49–177.06)
Androstenedione (ng/ml)	2.67 (0.81–4.83)	3.60 (0.81–4.83)
Testosterone (ng/ml)	0.92 (0.52–2.19)	1.14 (0.62–1.51)
Sex hormone-binding globulin (nmol/l)	104.06 (73.49–174.80)	79.13 (60.22–177.01)*
Free androgen index	2.66 (1.37–5.74)	4.97 (1.38–8.07)*
Estradiol (pg/ml)	7242.0 (2097.6–22 878.0)	20 405.0 (12 504.0–30 070.0)*
Progesterone (ng/ml)	48.28 (31.85–90.43)	86.97 (53.72–134.78)*

Values are medians and ranges.
*$P < 0.05$ between weeks 10–16 and weeks 22–28.
DHEAS = dehydroepiandrosterone sulphate.

Table III. Endocrine characteristics of pregnant women with PCOS during gestational weeks 10–16 and 22–28

	10–16 weeks	22–28 weeks
DHEAS (μg/dl)	168.25 (35.47–400.84)	132.54 (39.76–265.32)
Androstenedione (ng/ml)	4.33 (1.71–6.00)	4.94 (1.65–13.91)
Testosterone (ng/ml)	1.38 (0.75–2.14)	1.66 (0.58–3.82)
Sex hormone-binding globulin (nmol/l)	168.0 (39.76–197.40)	74.82 (39.99–166.70)*
Free androgen index	3.60 (1.55–18.05)	7.00 (2.81–15.83)*
Estradiol (pg/ml)	4860.0 (368.2–19 284.0)	20 592.0 (8688.0–31 740.0)*
Progesterone (ng/ml)	42.83 (26.40–74.71)	88.10 (47.28–144.76)*

Values are medians and ranges.
*$P < 0.05$ between weeks 10–16 and weeks 22–28.
DHEAS = dehydroepiandrosterone sulphate.

shown in Figure 2. Fasting and 2 h glucose concentrations were not significantly different between groups (Figure 2A). In both periods, fasting insulin was not significantly different between PPCOS and NP women. However, in both periods, 2 h insulin levels were significantly higher in PPCOS compared with NP women (Figure 2B).

The incidence of gestational diabetes according to World Health Organization criteria (1999) was significantly higher ($P = 0.02$) in the PCOS group (15%) than in the control group (0%).

Discussion

In this study, we evaluated the concentration of serum androgens during pregnancy in a group of PPCOS women, compared with that observed in NP women. PPCOS women showed significantly higher concentrations of androgens than NP women. However, the profile of androgen concentrations and other sexual steroids such as E_2 and progesterone during pregnancy was similar in both groups, suggesting that in pregnant women with PCOS, the function of the fetal–placental unit may be normal and that probably the high androgen levels detected in these women could be of maternal origin, although a placental source of androgens cannot be totally discarded.

During normal pregnancy, an increase in the circulating levels of some androgens has been described (Mizuno *et al*., 1968; Rivarola *et al*., 1968; Berger *et al*., 1984; McClamrock and Adashi, 1992). Testosterone concentration increases in the first trimester of pregnancy with further increments being

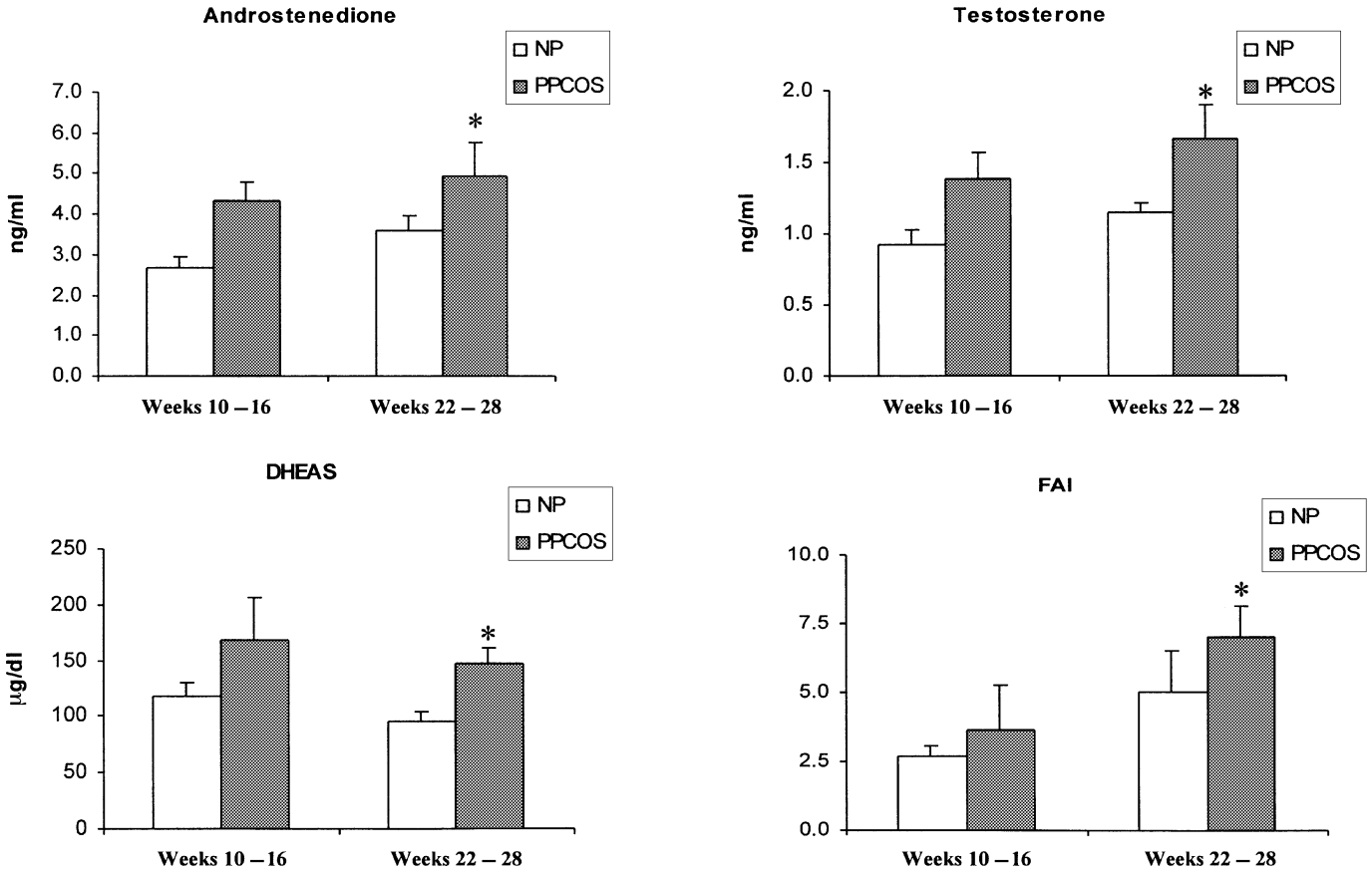

Figure 1. Median serum concentrations of androstenedione, testosterone and dehydroepiandrosterone sulphate (DHEAS) and free androgen index (FAI) in normal pregnant (NP) and pregnant polycystic ovarian syndrome (PPCOS) women during gestational weeks 10–16 and 22–28. Values are medians ± SEM. *$P < 0.05$ adjusted by body mass index.

Table IV. Androgen serum concentrations in normal pregnant (NP) and pregnant polycystic ovarian syndrome (PPCOS) women according to sex distribution of the newborn babies during gestational weeks 10–16 and 22–28

	Female		Male	
	10–16 weeks	22–28 weeks	10–16 weeks	22–28 weeks
NP				
DHEAS (µg/dl)	131.8 (39.4–217.6)	75.4 (34.1–134.6)	105.9 (97.7–153.7)	94.9 (35.9–143.4)
Androstenedione (ng/ml)	2.77 (1.78–4.61)	2.00 (1.65–4.67)	2.86 (2.45–3.99)	3.78 (0.81–4.28)
Testosterone (ng/ml)	0.90 (0.58–1.34)	1.02 (0.62–1.51)	0.92 (0.78–1.17)	1.05 (0.62–1.29)
PPCOS				
DHEAS (µg/dl)	192.9 (140.8–398.6)	132.5 (59.4–277.6)	188.8 (35.5–400.8)	147.2 (91.7–217.7)
Androstenedione (ng/ml)	4.66 (2.22–6.00)	4.42 (1.65 –13.91)	2.51 (1.71–3.97)	5.29 (2.65–10.52)
Testosterone (ng/ml)	1.47 (0.88–2.13)	2.27 (0.58–3.19)	1.32 (0.75–2.14)	1.50 (0.95–3.82)

Values are medians and ranges.
DHEAS = dehydroepiandrosterone sulphate.

noted through term (Bammann *et al.*, 1980, Saez *et al.*, 1972) and androstenodione levels increase in the latter part of pregnancy (Mizuno *et al.*, 1968; Rivarola *et al.*, 1968). On the contrary, DHEAS decreases in the maternal circulation with advancing gestation (Rivarola *et al.*, 1968; Gant *et al.*, 1971; Buster *et al.*, 1979). Interestingly, during normal pregnancy, the increase in the circulating levels of androgens does not lead to fetal virilization. This phenomenon has been attributed to the high levels of E_2, progesterone and SHBG during pregnancy that interfere with the biological activity of androgens (Forest *et al.*, 1971; Hensleigh *et al.*, 1975; Berger *et al.*, 1984), and to the placental aromatase enzyme that rapidly converts androstenedione to estrone and 16-hydroxytestosterone to estriol (Smith and Axelrod, 1969).

The possible role of PCOS as a cause of prenatal androgen excess has not been routinely evaluated and, according to the antecedents obtained from the literature, PCOS is described as a sporadic cause of virilization during pregnancy

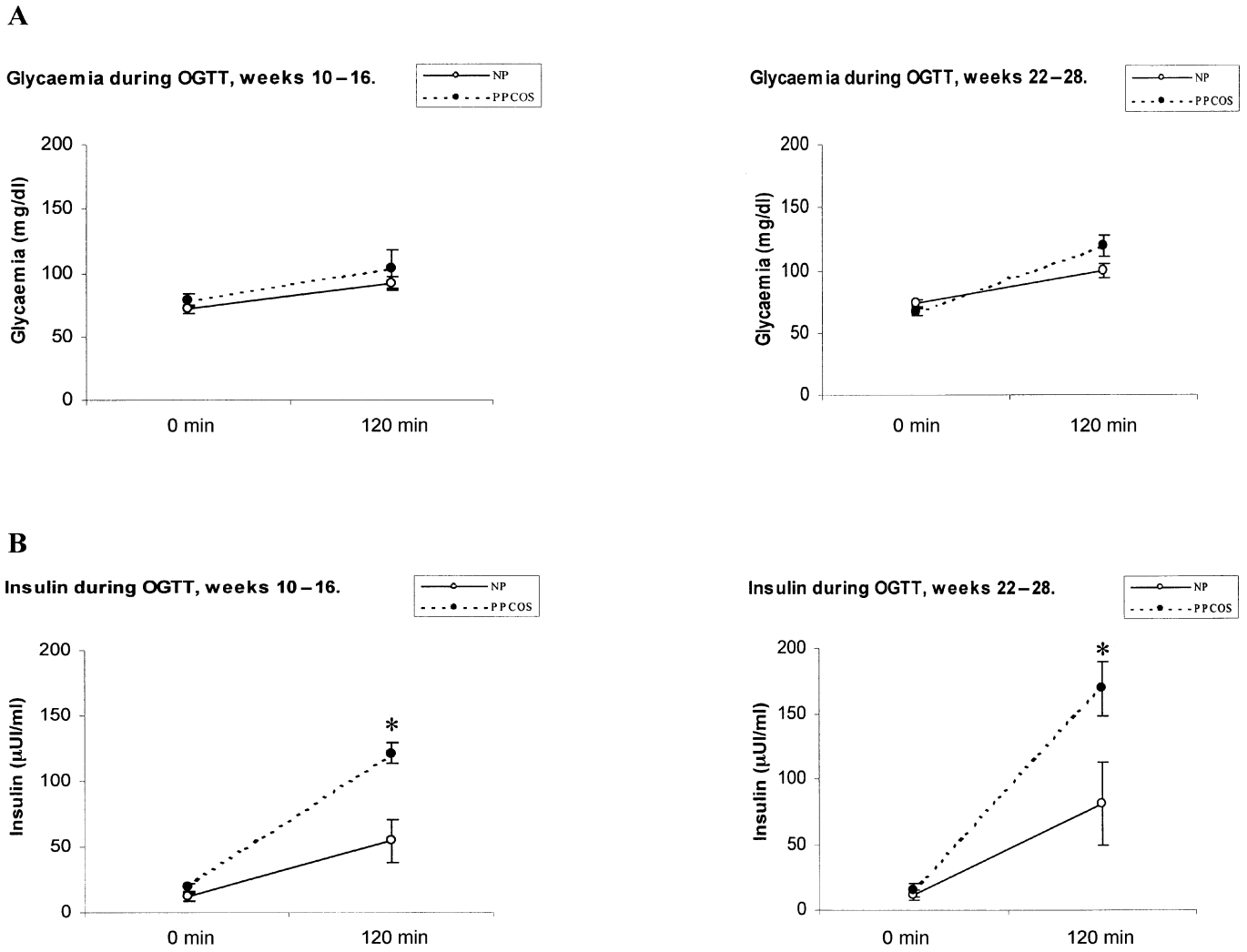

Figure 2. Median serum fasting and 2 h glucose (**A**) and insulin (**B**) concentrations in normal pregnant (NP) and pregnant polycystic ovarian syndrome (PPCOS) women during gestational weeks 10–16 and 22–28. Values are medians ± SEM. *$P < 0.05$ adjusted by body mass index. OGTT = oral glucose tolerance test.

(Magendantz *et al*., 1972; Fayez *et al*., 1974; Bilowus *et al*., 1986; McClamrock and Adashi, 1992; Ben-Chetrit and Greenblatt., 1995; Sarlis *et al*., 1999).

In the present study, normal and PPCOS women showed similar dynamics in the androgen concentrations during pregnancy; however, PPCOS women exhibited higher concentrations of testosterone, androstenedione and DHEAS than NP women. Similar to what was described previously for normal pregnancy (Berger *et al*., 1984), the increase in androgen levels during pregnancy in these PCOS women was without fetal virilization, probably because the androgen levels observed in the present study were lower than those reported in PCOS patients who exhibited a clinical virilization during pregnancy (Magendantz *et al*., 1972; Fayez *et al*, 1974; Ben-Chetrit and Greenblatt, 1995; Sarlis *et al*., 1999). Nevertheless, the absence of fetal virilization does not rule out that these fetuses were exposed to high androgen levels during their intrauterine life. A considerable variability in fetal response to endogenous fetal or maternal androgens has been described, the time of exposure in relation to fetal age being the factor which determines the degree of virilization. According to one study (Grumbach and Ducharme, 1960), fetal virilization occurs during a critical period between weeks 8 and 13 of gestation and results in labioscrotal fusion and urogenital sinus formation. In the present study, the highest androgen levels observed in PPCOS women were later than week 16 of gestation which could explain in part the absence of fetal virilization. On the other hand, studies suggest that experimentally induced prenatal androgen excess in fetal female monkeys, without external virilization, during primate neural differentiation may permanently alter the pattern of LH secretion in the presence of cyclic gonadotrophin release (Dumesic, 1997). This induced prenatal androgen excess regardless of gestational timing also perturbs the insulin–glucose homeostasis, with androgen excess in early and late gestation impairing pancreatic β-cell function and altering insulin sensitivity respectively (Eisner *et al*., 2000). Therefore, it does not seem unreasonable to speculate that the high androgen levels, observed in PPCOS women, could have an impact on the fetal physiology, even though they do not masculinize the external genitalia.

Although the origin of the androgen increase during normal pregnancy remains uncertain, the androgens are probably

produced mainly by the maternal ovaries and less probably by the placenta. While human placenta lacks 17β-hydroxylase and 17,20-desmolase (Christensen, 1974), it does express 17β-hydroxysteroid dehydrogenase (17β-HDS) (Takeyama *et al*., 1998) and aromatase as well as 3β-hydroxysteroid dehydrogenase (3β-HDS) (Mason *et al*., 1993). It can therefore synthesize androstenedione from adrenal or ovarian DHEAS and can undertake the onward synthesis of both testosterone and E_2. Normally, androgens synthesized by the placenta are rapidly converted to estrogens due to the activity of the placental aromatase and, therefore they probably contribute only slightly to the androgen increase observed in normal pregnancy. However, when the enzyme capacity of the placental aromatase is overpassed, androgens of placental origin could increase. In human cytotrophoblasts, insulin has been shown to inhibit aromatase (Nestler, 1987, 1990) and stimulate 3β-HDS activities (Nestler, 1989, 1990). Therefore, in PPCOS patients in which insulin levels are significantly increased, this could be a mechanism to explain in part the high androgen concentrations observed in these patients during gestation. In PPCOS women, it is likely that the elevated androgen levels also reflect an increase in androgen production by the maternal theca-interstitial cells. In a recent study (Sir-Petermann *et al*., 2001), we demonstrated that after delivery the androstenedione levels and the ovarian volume of PCOS patients were increased, suggesting that these ovaries were persistently stimulated during pregnancy. It is possible that in PPCOS patients, not only placental human chorionic gonadotrophin may stimulate androgen production, as has been previously suggested (Di Zerega and Hodgen 1979; Penny *et al*., 1979), but also that androgen production could be stimulated by the insulin levels which are significantly increased during pregnancy in these patients, compared with NP women. This assumption is in accordance with a recent study in which the role of insulin on hyperandrogenaemia during pregnancy is discussed (Sarlis *et al*., 1999). However, the possible contribution of the maternal adrenal gland to the elevated androgen levels observed in PPCOS women cannot be ruled out, due to the high levels of DHEAS observed during pregnancy in these patients. Abnormalities in adrenocortical steroidogenesis have been described in a high percentage of PCOS patients (Givens *et al*., 1975; Carmina *et al*., 1995; Azziz 1997), and it may be possible that during pregnancy these abnormalities persist. It is unlikely that the source of the increase in androgen levels is of fetal origin, because no differences were noted in total testosterone levels between mothers with female fetus and mothers with male fetus during any of the two periods in which measurements were made

Finally, regarding the clinical characteristics of PPCOS women, it is interesting to emphasize that only two patients presented elevated blood pressure during pregnancy. This observation is in disagreement with previous studies that proposed that high androgen levels during pregnancy may be implicated in the pathogenesis of pre-eclampsia (Urman *et al*., 1997; Serdar Serin *et al*., 2001). On the other hand, the prevalence of gestational diabetes in this group of PPCOS women was similar to that reported in the literature (Gjonnaess, 1989; Wortsman *et al*., 1991; Lanzone *et al*., 1995, 1996; Lesser and Garcia, 1997).

In summary, the present study demonstrates a significant increase of androgen levels during pregnancy in PCOS women. We propose that these androgen concentrations could provide a prenatal androgen excess to the fetus. Further studies are needed to evaluate the long effect of this prenatal androgen excess on baby girls born to PCOS mothers, especially if they are insulin resistant, to help explain the aetiology of the PCOS during adult life.

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