

Different phenotypes of polycystic ovary syndrome by Rotterdam criteria are differently steroidogenic but similarly insulin resistant

In traditional Chinese medicine, polycystic ovary syndrome (PCOS) is considered an anovulation disorder related to ovarian insulin resistance. The three phenotypes of PCOS, according to the Rotterdam criteria, are differently steroidogenic but similarly insulin resistant, suggesting a similar involvement of insulin resistance/hyperinsulinemia in different compartments of the PCOS ovary, namely, overactive theca and/or granulosa cells. (*Fertil Steril*® 2010;93:1362–5. ©2010 by American Society for Reproductive Medicine.)

Polycystic ovary syndrome (PCOS) is a disorder that affects approximately 5% to 10% of reproductive-aged women and is characterized by irregular cycle, infertility, and excessive androgens (1–3). Most clinicians recognize the wide spectrum of phenotypes that PCOS encompasses. However, what remain greatly controversial are the appropriate diagnostic criteria. Both the 2003 Rot-

terdam consensus workshop (4) and the Androgen Excess Society (5) concluded that PCOS is a syndrome of ovarian dysfunction with three cardinal features: [1] hyperandrogenism, [2] anovulation, and [3] polycystic ovary (PCO) morphology. But they differ on the diagnostic criteria of PCOS. The new Rotterdam criteria require two of the three cardinal features, while the criteria of the Androgen Excess Society regard hyperandrogenism as necessary for a diagnosis, along with either anovulation or PCO morphology.

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Cycle irregularity and infertility due to anovulation, called a TianGui disorder (TGD) in traditional Chinese medicine, constitutes the main, and often the only, reason for PCOS patients to seek medical care within the Chinese mainland (6). Traditional medicine has long emphasized that anovulation is central to PCOS, a concept that has been at variance with the American and European opinion that hyperandrogenism or sonographic PCO appearance is more important (4–6). Insulin resistance is not included in the current diagnostic criteria of PCOS, but it is an important characteristic of PCOS. Insulin resistance appears in both obese and nonobese women with PCOS with a prevalence exceeding 50% to 70%, and some clinicians even consider all women with PCOS to exhibit insulin resistance and compensatory hyperinsulinemia (7). Thus, we designed this study to explore glucose metabolism and steroidogenesis among anovulatory women with the different phenotypes of PCOS according to the Rotterdam criteria. These differences can help examine the biologic implications of the Rotterdam criteria, with a focus on traditional Chinese medicine's emphasis on anovulation as the primary characteristic of PCOS.

A total of 60 women were recruited and divided into four groups according to the Rotterdam criteria's three typical characteristics: [1] chronic anovulation, [2] biochemical characteristics of hyperandrogenism, and [3] polycystic ovary morphology. Group 1 contained 21 women with all features of 1, 2, and 3; group 2 was 14 women with 1 and 2 only; group 3 was 17 women with 1 and 3 only; group 4 served as a control, containing eight volunteers who exhibited none of the criteria. Women in four groups were matched for age (<35 years old), body mass index,

and waist/hip ratio. All women underwent the following two tests: a 3-hour 75-g oral glucose tolerance test (OGTT), as in our previous work (8), and an ovarian stimulation test (OST) during the early follicular phase of spontaneous or progesterone-induced cycle. The OST was performed by giving a single IM injection of human chorionic gonadotropin (hCG, 5000 IU; First Biochemical Pharmaceuticals, Shanghai, China) plus human menopausal gonadotropin (hMG, 150 IU; Libao, Zhuhai, China) on a separate day of OGTT. Blood samples from all patients were collected just before the injection (basal, 0 hours), and at 3, 8, 12, 18, and 24 hours after OST. Glucose concentrations and all hormones were analyzed as previously reported elsewhere (8, 9). To evaluate the functional state of ovarian enzymes acting in steroidogenesis, product/precursor ratios of absolute levels at time points before and after OST were employed as appropriate (9, 10). We also use the ratios of the net product increment from baseline values versus basal precursors to evaluate the functional activity of these enzymes (9, 10). The methods for the multiple comparisons were analysis of variance (ANOVA) and analysis of covariance (ANCOVA) to identify group differences, with the Bonferroni correction when appropriate. Logarithmic transformation of data was performed for measures that were not normally distributed.

In general, there were comparable luteinizing hormone, follicle-stimulating hormone (FSH), and luteinizing hormone/FSH levels in patients between groups 1 and 2 or between groups 3 and 4. By design, groups 1 and 2 differed from groups 3 and 4 in levels of basal testosterone (2.09 ± 0.14 and 2.09 ± 0.12 vs. 1.22 ± 0.13 , 0.73 ± 0.14 nmol/L) and sex hormone-binding globulin (31.90 ± 4.65 and 45.20 ± 5.68 vs. 67.10 ± 7.20 and 61.90 ± 13.50 nmol/L), resulting in statistically significantly higher free androgen index (10.76 ± 1.57 and 6.44 ± 0.70 vs. 3.52 ± 0.62 and 1.58 ± 0.48). The glucose, C-protein, and glucagon levels at all time points during OGTT were not different among the four groups. As for the insulin responses after glucose load, groups 1, 2, and 3 were comparable and were higher than group 4, with statistical significance at the 3-hour point between groups 3 and 4, resulting in lower insulin/glucose ratios at the same point in group 4 than the other three PCOS groups.

During OST, as shown in Figure 1, the steroidogenesis of progesterone, 17-hydroxyprogesterone, androstenedione, testosterone, and estradiol (E_2) in groups 3 and 2 was generally intermediate between groups 1 and 4; women in group 2 at some points had similarly androgenic patterns compared with group 1 in 17-hydroxyprogesterone, androstenedione, and testosterone responses, but a similarly estrogenic response compared with group 3; the three anovulatory PCOS groups showed a one to two times greater E_2 response than group 4 (control). The 17-hydroxylase and 17,20 lyase functional states/activities ranked as follows: group 1 > group 2 > group 3 > group 4, with statistically significant differences at some time points among the four groups.

Aromatase states during OST, as suggested by E_2 /testosterone levels at respective time points, were unexpectedly found to be approximately one times higher in group 3 than in the other three groups at most time points. In contrast, estrogenic enzyme activities in response to OST were higher in all three

PCOS groups than in group 4. It is interesting that when we examined correlations of carbohydrate and steroid metabolism by linear regression analysis, we found a statistically significant positive correlation between the area under curve of insulin response to OGTT and the total E_2 increment during OST in the women from the three PCOS groups as a whole ($r = 0.50$, $P < .05$).

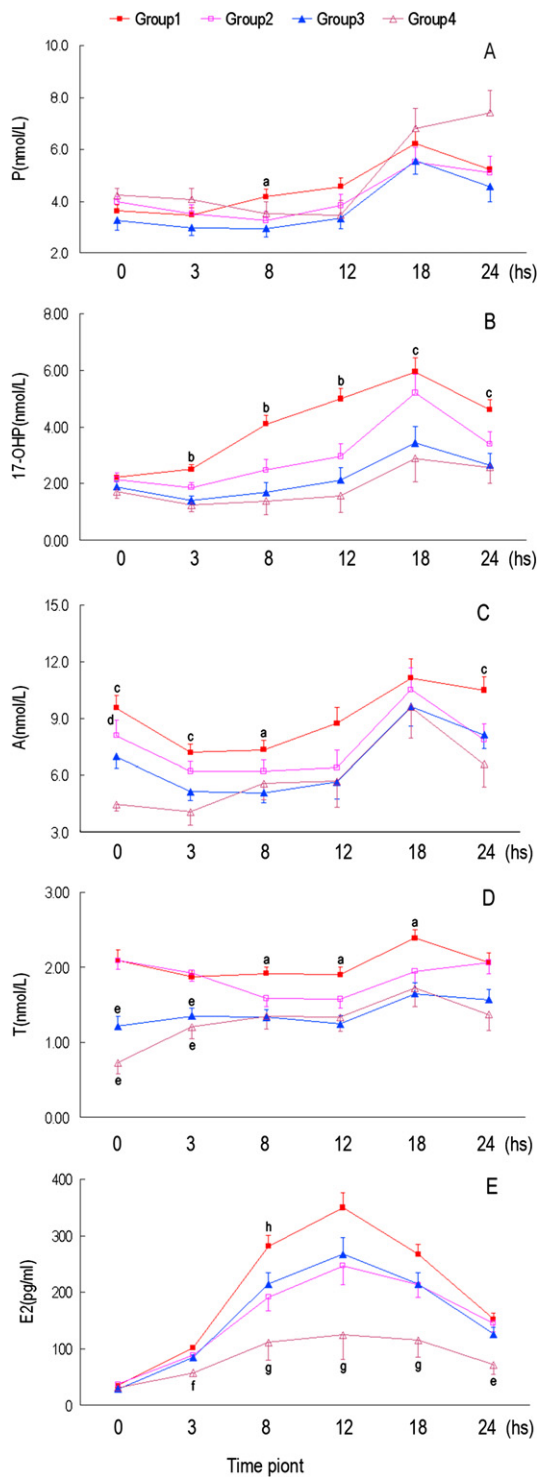
Polycystic ovary syndrome is the major cause of anovulatory infertility, menstrual disturbances, and hirsutism. Currently, two definitions of PCOS are in international use, the U.S. National Institutes of Health 1990 criteria (11) and Rotterdam 2003 (4) criteria. As defined in traditional Chinese medicine, PCOS has been considered an anovulation disorder since the Yuan Dynasty (AD 1281) (6). During the past two decades, even with availability of sonography and hormone measurement in regional centers, the practical criteria for diagnosis within mainland China includes clinical anovulation, together with PCO appearance and/or hyperandrogenism. This ovulation-oriented criterion obviously is consistent with understanding of ovarian physiology controlling the menstrual cycle and reproduction as well as current recognition of the ethnic difference in clinical PCOS features between China and Western societies (12).

Whereas there is little difference in the characteristics of theca cells between follicles obtained from ovulatory women compared with anovulatory women with polycystic ovaries, there are major differences according to ovulatory status in granulosa cell steroidogenesis (13). Granulosa cells cultured from follicles derived from anovulatory women with PCOS are 6 to 10 times more responsive to FSH in terms of E_2 production compared with those derived from a similar spectrum of follicles from women with polycystic ovaries and regular cycles (14). Thus, anovulation in PCOS is supposed to result from intrinsically higher E_2 responsiveness to FSH in the ovary but arrested growth of antral follicles due to down-regulated FSH levels by higher E_2 levels (6, 13).

Although we did not reveal frankly different FSH levels in the four groups, the three anovulatory PCOS groups showed one to two times more estrogenic response to OST than the control group, similar to a previous report (15). Furthermore, we found different mechanisms underlying the overactive E_2 response profiles in the three anovulatory PCOS groups: group 2 produced twofold E_2 because of the greater availability of precursor androgens from the theca layer and group 3 because of the more intensive driving by aromatase enzyme function from granulosa layer; group 1 shared both these two features from two compartments, resulting in their almost three times higher E_2 level compared with ovulatory control group 4. These differences clearly elucidate the biologic implications of theca and/or granulosa cells in the Rotterdam criteria, with a focus on anovulation as in traditional Chinese medicine's concept of PCOS.

In our experiment, the obviously higher activity in the theca cells of groups 1 and 2 demonstrates their common overactive profile of androgen production and concomitant aromatized E_2 potential, but the degree of theca overactivity in group 2 was lower than that of group 1 with the diagnosis of anovulatory PCOS. On the other hand, the comparable androgenic

FIGURE 1



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FIGURE 1 Continued

Responses during the ovarian stimulation test: (A) progesterone, (B) 17-hydroxyprogesterone, (C) androstenedione, (D) testosterone, and (E) estradiol. Sixty participants were divided into four groups with or without the three typical characteristics of the Rotterdam criteria: [1] chronic anovulation, [2] biochemical hyperandrogenism, and [3] polycystic ovaries. Group 1 (n = 21) met criteria 1, 2, and 3. Group 2 (n = 14) met criteria 1 and 2. Group 3 (n = 17) met criteria 1 and 3. Group 4 (n = 8), control, met no PCOS criteria. ^aP < .05 versus group 3. ^bP < .05 versus groups 2, 3, and 4. ^cP < .05 versus groups 3 and 4. ^dP < .05 versus group 4. ^eP < .05 versus groups 1 and 2. ^fP < .05 versus groups 1, 2, and 3. ^gP < .05 versus groups 1 and 3. ^hP < .05 versus group 2.

response of patients in groups 3 and 4 suggests normal theca functionality with only mild dysregulation in 17,20 lyase activity but different granulosa functionality. Due to the increased androgen production in both ovulatory and anovulatory conditions for women, it is unlikely that hyperandrogenism is the major cause of anovulation unless the resultant estrogen is elevated enough to down-regulate the pituitary FSH level below the threshold for subsequent follicular dominance and ovulation (13, 16).

Polycystic ovary syndrome affects both reproduction and energy homeostasis. As shown in our study, the three groups of anovulatory PCOS patients had generally normal glucose tolerance during OGTT, but comparable hyperinsulinemia and thus insulin resistance in spite of their different steroidogenesis. Such a contribution of insulin resistance to ovary overactivity and anovulation in our study is in agreement with the traditional Chinese medicine concept that PCOS is a disorder of an “insulin resistant” ovary, namely, “phlegm affected BaoGong (PABG)” (6, 17–19). The application of insulin-sensitizing agents in PCOS patients results in improvement in both insulin resistance and ovulation (20, 21). Furthermore, the beneficial effect of metformin on steroidogenesis in PCOS has been confirmed as occurring directly within the ovary, as shown by in vitro experiments with cultured granulosa/theca cells (22–25). Insulin resistance thus plays a causal role in the overactivity of theca and the granulosa compartments within the PCOS ovary.

The difference in ovarian phenotypes in our study in contrast to the Rotterdam criteria can be explained reasonably by an involvement (or existence) of similar insulin resistance/hyperinsulinemia in different compartments of PCOS ovary that present overactive theca and/or granulosa, both of which are found to be the main target of hyperinsulinemia. Of note, the small number of patients in our pilot study is a limitation, and caution should be exercised in interpretation due to use of steroid ratios to estimate true enzyme activities in vivo.

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