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Normal Ovulatory Women with Polycystic Ovaries Have Hyperandrogenic Pituitary-Ovarian Responses To Gonadotropin-Releasing Hormone-Agonist Testing*

PETER L. CHANG, STEVEN R. LINDHEIM, CHERI LOWRE, MICHEL FERIN, FRANK GONZALEZ, LARS BERGLUND, ENRICO CARMINA, MARK V. SAUER, AND ROGERIO A. LOBO

Department of Obstetrics and Gynecology (P.L.C., S.R.L., C.L., M.F., E.C., M.V.S., R.A.L.), Division of Reproductive Endocrinology, Columbia University, College of Physicians and Surgeons, New York, New York 10032; Department of Gynecology and Obstetrics (F.G.), Division of Reproductive Endocrinology, State University of New York at Buffalo, School of Medicine and Biomedical Sciences, Buffalo, New York 14214; Department of Medicine (L.B.), Columbia University, College of Physicians and Surgeons, New York, New York 10032

ABSTRACT

Women with polycystic ovary syndrome (PCOS) have chronic anovulation and hyperandrogenism and frequently have abnormalities in their lipid profiles and insulin/insulin-like growth factor axis that increase their lifetime risk for cardiovascular disease. Normal ovulatory women may have polycystic ovaries on ultrasonography and yet lack the clinical features of PCOS. To further explore whether ovulatory women without clinical/biochemical hyperandrogenism but with polycystic appearing ovaries (ov-PAO) have subclinical features of PCOS, we prospectively characterized 26 ov-PAO women and matched them by age and body mass index to 25 ovulatory women with normal appearing ovaries (ov-NAO) and to 22 women with PCOS. After an overnight fast, all women had baseline endocrine and metabolic assessments. In addition, a subset of each group of women underwent GnRH-agonist (leuprolide acetate 1 mg sc) testing, ACTH stimulation, and an insulin tolerance test (ITT). At baseline, ov-PAO and ov-NAO women had similar endocrine profiles (LH, LH:FSH, androstenedione, and DHEAS). Compared with ov-NAO, 31% of ov-PAO women had reduced glucose responses after insulin (K_{itt}), suggesting mild insulin resistance, and 35% had high density lipoprotein levels below 35 mg/dL, a level considered to represent significant cardiovascular risk. After GnRH-agonist, ov-PAO women had response patterns in LH, total testosterone, and 17-hydroxyprogesterone (17-OHP) that were intermediate between ov-NAO and women with PCOS. Ovarian responses were above the normal range in 30–40% of women with ov-PAO. In ov-PAO, peak responses of LH after leuprolide correlated with triglyceride levels ($P < 0.05$) and peak responses of 17-OHP correlated inversely with Kitt values ($P < 0.05$). No significant differences were noted with ACTH testing. In conclusion, occult biochemical ovarian hyperandrogenism may be uncovered using GnRH-agonist in ovulatory women with ov-PAO, while adrenal responses remain normal. Subtle metabolic abnormalities may also be prevalent. (*J Clin Endocrinol Metab* **85:** 995–1000, 2000)

POLYCYSTIC ovary syndrome (PCOS) is a heterogeneous clinical disorder characterized primarily by chronic anovulation and hyperandrogenism (1, 2). It is a commonly encountered endocrinopathy with a prevalence of approximately 5–7% of women of reproductive age (3). The disorder is associated with significantly increased risks of hypertension, impaired glucose tolerance, and cardiovascular disease (4–6). Characteristics of PCOS that are thought to contribute to these disorders are hyperinsulinemia or insulin resistance, hyperandrogenism, and abnormal lipid/lipoprotein profiles (4, 6–8).

Strict criteria for the ultrasonographic diagnosis of the polycystic ovary have been established and include the presence of ten or more peripherally oriented cysts in one sonographic plane, each 2–8 mm in diameter, arranged around a dense stroma. The central stromal mass should occupy at least 25% of the total volume (9–10). While this sonographic finding has been used as the sole criterion for the diagnosis of PCOS, polycystic appearing ovaries (PAO) can be encountered in several endocrinopathies such as hypothyroidism, Cushing's disease, congenital adrenal hyperplasia, and hypothalamic amenorrhea (11), and may occur in 16–25% of normal women (11–13). While women diagnosed with PCOS typically have polycystic ovaries, the disorder requires the presence of chronic anovulation and clinical/biochemical hyperandrogenism.

Normal ovulatory women with the isolated finding of polycystic ovaries on ultrasound are not considered to have PCOS if they are asymptomatic and have normal serum androgens (ovarian and adrenal), LH, and LH:FSH ratios. Nevertheless, we have noted that some women have subtle metabolic abnormalities that occur in PCOS such as elevated fasting insulin, decreased insulin-like growth factor binding protein-1 (14), as well as exaggerated ovarian responses to injected gonadotropins (15) similar to women with PCOS. In these previous studies, the

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Address correspondence and requests for reprints to: Peter L. Chang, M.D., Assistant Professor, Department of Obstetrics & Gynecology, Division of Reproductive Endocrinology, College of Physicians & Surgeons, Columbia University, 630 West 168th Street, PH 16–28, New York, New York 10032. E-mail: pc174@columbia.edu.

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sample size was relatively small, and confirmation was needed to ascertain whether asymptomatic women with the isolated finding of PAO do in fact exhibit subtle endocrinologic and metabolic abnormalities such as insulin resistance and how frequently this may occur. Because women with PAO constitute a large segment of the general population and because cardiovascular disease is the leading cause of death in women, we considered it important, from a public health perspective, to determine whether normal women with PAO may share some of the risks associated with PCOS.

We performed a prospective study to further explore whether women with PAO have similar endocrine and metabolic profiles as found in PCOS. For the first time, we assessed dynamic pituitary-ovarian responses to GnRH-agonist as well as adrenal responses to ACTH stimulation. GnRH-agonist testing was chosen along with ACTH testing because of the well described endocrine changes known to occur in women with PCOS. Additionally, we assessed insulin resistance using the insulin tolerance test, which has been shown to correlate well with the euglycemic/hyperglycemic clamp studies and the frequently sampled iv glucose tolerance test (16–17). Finally, we assessed whether the provoked endocrine responses correlated with certain metabolic profiles known to be abnormal in PCOS.

Materials and Methods

Subjects

A total of 73 women were recruited and divided into 3 groups: group I) 22 women with characteristic hyperandrogenism, chronic anovulation, and polycystic ovaries on ultrasound (PCOS); group II) 26 ovulatory women with no characteristics of PCOS but with the isolated ultrasound finding of polycystic-appearing ovaries (ov-PAO) defined by the presence of ten or more peripherally oriented cysts in one sonographic plane, each 2–8 mm in diameter, arranged around a dense stroma; and group III) 25 ovulatory women with normal-appearing ovaries (ov-NAO) on ultrasound and no characteristics of PCOS. All women were matched for age (<35 yr) and had normal body mass index (BMI < 26 kg/m^2). Specifically, this was a group of nonobese women with PCOS recruited from our infertility/endocrine clinic. Ov-NAO and ov-PAO women were recruited from our pool of egg donors, and all had regular menses every 26–34 days, with no signs of hyperandrogenism. All subjects were screened,and no other endocrine disturbances (thyroid, adrenal) or medical illnesses were found. Informed consent was obtained from all subjects. The study was approved by the Institutional Review Board of Columbia University, College of Physicians & Surgeons.

Protocol

All patients were examined by a transvaginal ultrasound during the early follicular phase. Their weight, height, and waist-to-hip ratio were recorded. After 3 days of a high carbohydrate diet and followed by an overnight fast, baseline blood samples were obtained in the morning, at 0800–1000 h, in the midfollicular phase (cycle days 4–9) for FSH, LH, total testosterone (T), androstenedione (A4), dehydroepiandrosterone sulfate (DHEAS), insulin-like growth factor-1 (IGF-1), insulin-like growth factor binding protein-1 (IGFBP-1), insulin, glucose, cholesterol, triglyceride, high density lipoprotein (HDL) cholesterol, and low density lipoprotein (LDL) cholesterol measurements. A subset of women from the 3 groups (10 ov-NAO, 14 ov-PAO, and 6 PCOS) underwent insulin tolerance testing, GnRH-agonist testing with leuprolide acetate, and ACTH stimulation. For the insulin tolerance test, 0.1 μ/kg of regular insulin was administered iv, and glucose levels were obtained at 0, 3, 6, 9, 12, and 15 min. At this time, a 50 μL bolus of 50% dextrose was administered iv to prevent hypoglycemia. The rate constant for plasma glucose disappearance (K_{itt}), an accurate marker of insulin resistance, was then calculated according to the method of Bonora (16). For GnRH-agonist testing, leuprolide acetate (1 mg) was administered sc, and serum LH, 17-OH progesterone (17-OHP), and T measurements were obtained at 0, 1, 2, 4, and 24 h. For the ACTH stimulation test, serum levels of A4, T, cortisol, and dehydroepiandrosterone (DHEA) were measured at 0, 30, 60, and 120 min after cosyntropin (0.25 mg) iv administration.

Assays

Serum A4, 17-OHP, and DHEA were measured by commercial RIA methods (Diagnostics Systems Laboratories, Inc. (DSL), Webster, TX). Serum levels of T, sex hormone binding globulin (SHBG), DHEAS, cortisol, FSH, LH, and insulin were measured by chemiluminescent enzyme immunoassays (Immulite, Diagnostic Products Corporation, Los Angeles, CA). IGF-1 and IGFBP-1 were measured by enzyme-linked immunosorbent assays (DSL). Glucose was quantified by glucose oxidase method. Plasma levels of cholesterol and triglycerides were determined by standardized enzymatic procedures (Roche Molecular Biochemicals, Mannheim, Germany) on a Hitachi 705 automated spectrophotometer. HDL cholesterol levels were measured after precipitation of plasma apoB-containing lipoproteins with phosphotungstic acid (18), and LDL cholesterol levels were calculated by the Friedewald formula (19). In all assays, intra-assay and interassay coefficients of variation did not exceed 6% and 13%, respectively.

Statistics

All data are expressed as the mean ± SEM. Statistical analyses were performed using SPSS, Inc.-PC. Statistical differences between groups for baseline variables were determined by ANOVA with Tukey *post hoc*. Correlations were analyzed using the Pearson product moment correlation. Differences between groups in the temporal course of provoked responses were analyzed by repeated measures ANOVA, with baseline hormonal levels entered as a continuous covariate. A P value of less than 0.05 was considered significant. Additionally, 95% confidence intervals in normal women (ov-NAO) were calculated to assess abnormal responses in the ov-PAO group.

Results

The three groups did not differ in age, BMI, or waist-to-hip ratios (Table 1). As expected, significant differences in the baseline endocrine profile of PCOS were observed when compared to ov-NAO and ov-PAO groups (Fig. 1). Specifically, serum LH, LH:FSH, and androgens (T, free testosterone index, A4, and DHEAS) were all significantly elevated in PCOS. The endocrine profiles of ov-PAO women did not differ as a group compared with those of ov-NAO. Serum total testosterone in the ov-PAO group (0.50 ± 0.04 ng/mL) was significantly higher than in ov-NAO (0.29 ± 0.02 ng/mL), but all individual values remained in the normal range.

Fasting metabolic profiles (Fig. 2) revealed significantly elevated fasting insulin (16.2 ± 5.3 mIU/mL) and decreased IGFBP-1 (38.3 ± 5.6 ng/mL) only in the PCOS group. In this nonobese group of subjects, fasting serum cholesterol, triglyceride, and LDL were not different between groups. Although HDL was lower in PCOS, it was not statistically

TABLE 1. Age, BMI, and waist-to-hip ratios for ov-NAO, ov-PAO, and PCOS groups

Groups	n	Age (yr)	BMI (Kg/m^2)	Waist-to-hip ratio
ov-NAO	25	30.2 ± 0.8	22.1 ± 0.4	0.78 ± 0.01
ov-PAO	26	28.0 ± 0.7	21.5 ± 0.3	0.77 ± 0.01
PCOS	22	26.6 ± 1.7	22.8 ± 0.6	0.81 ± 0.01

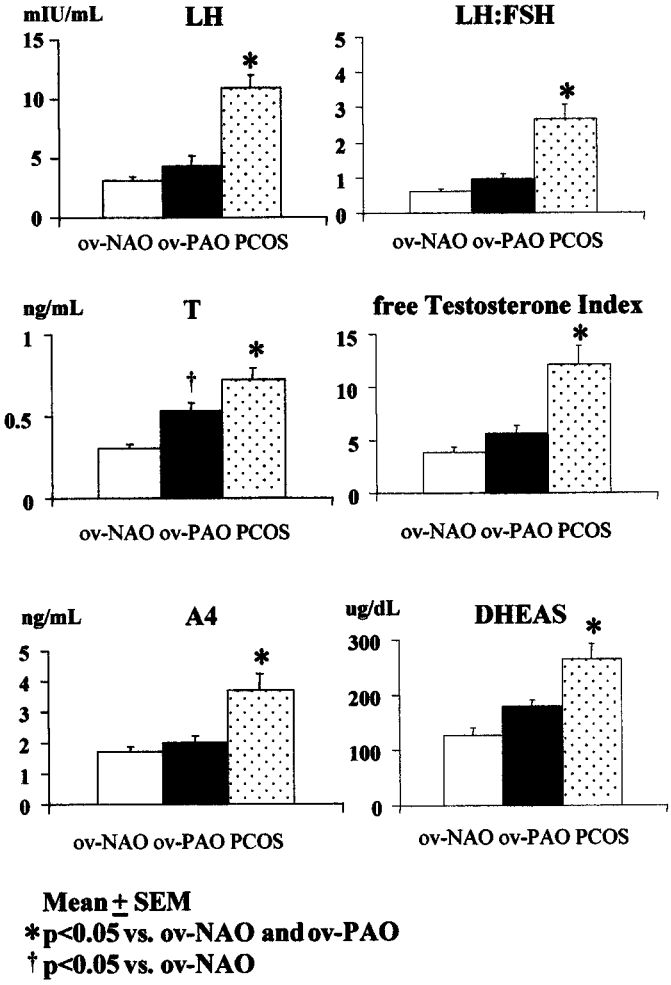

FIG. 1. Baseline endocrine profiles for ov-NAO, ov-PAO, and PCOS groups. While significant differences were noted in PCOS, ov-PAO did not differ from ov-NAO in serum LH, LH:FSH, free testosterone index, androstenedione, and DHEAS. Although serum total testosterone in ov-PAO was statistically higher than ov-NAO, all individual values remained in the normal range.

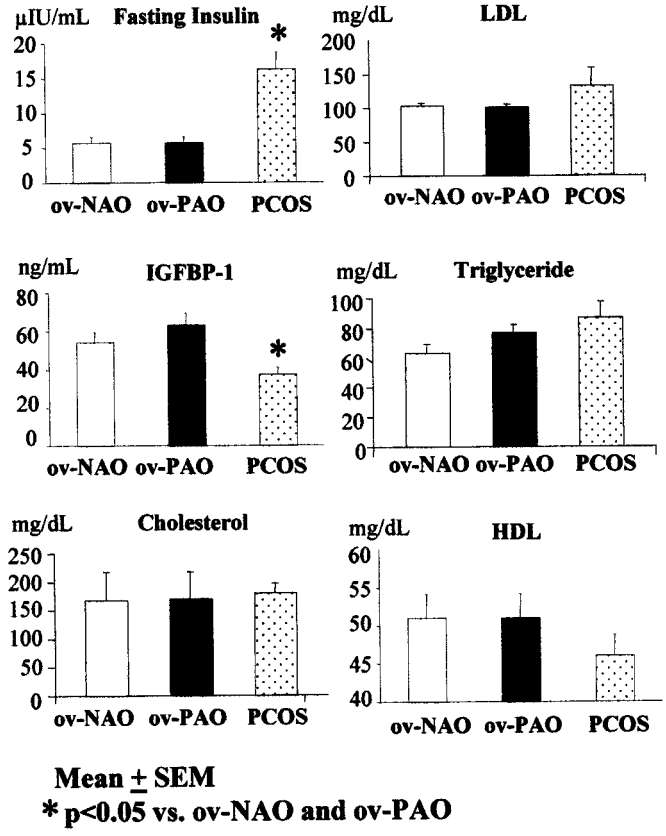

FIG. 2. Baseline fasting metabolic profiles in ov-NAO, ov-PAO, and PCOS.

different. However, compared to none in ov-NAO, 15% of women with PAO had HDL levels below 35 mg/dL (Fig. 3). K_{itt} values in ov-PAO and ov-NAO did not differ significantly, but 31% of ov-PAO women had values that were below the normal range (3.1%/min), suggesting mild insulin resistance in a subset of ov-PAO women (Fig. 3).

By ANOVA with repeated measures, GnRH-agonist provoked LH responses were similar in the three groups (Fig. 4, *left panel*). However, 15% of ov-PAO women had increased LH values at 4 and 24 h that were above the upper 95% confidence interval of ov-NAO (Fig. 4, *right panel*). This peak response of LH in ov-PAO correlated with triglyceride levels ($r = 0.577$, $P < 0.05$). Serum 17-OHP responses to GnRH-agonist were significantly increased in PCOS ($P < 0.05$), and although an apparent intermediate response was noted in the ov-PAO group (Fig. 4, *left panel*), it failed to reach statistical significance. At 2 and 4 h, 38% of ov-PAO women had 17-OHP responses that were above the upper 95% confidence interval of ov-NAO (Fig. 4, *right panel*). This increased response was inversely correlated with K_{itt} ($r = -0.594$, $P < 0.05$) (Fig. 5). Similarly, the total testosterone response was increased in PCOS ($P < 0.05$) and intermediate in ov-PAO (Fig. 4, *left panel*). At 2 and 4 h, 62% and 46% of ov-PAO subjects had T values above the upper 95% confidence interval of ov-NAO (Fig. 4, *right panel*).

Cortisol, A4, DHEA and T responses to ACTH stimulation testing were not significantly different between the three groups (Fig. 6).

Discussion

One of the most characteristic biochemical abnormalities in PCOS is inappropriate gonadotropin secretion (20–21) and exaggerated ovarian responses to gonadotropin (22). Previously, we have found that ovulatory women with the isolated finding of PAO (ov-PAO) displayed a similarly exaggerated ovarian sensitivity to human menopausal gonadotropin (hMG) stimulation in terms of peak estradiol, number of follicles, and number of oocytes per hMG ampules (15). In the present study, both ovulatory groups (ov-NAO and ov-PAO) had similar baseline values of serum gonadotropins (LH, LH:FSH ratio) and androgens that were significantly lower than those of PCOS.

The GnRH-agonist, leuprolide acetate, stimulated both pituitary and ovarian secretion in all three groups of women. Maximal gonadotropin responses occurred within 4 h and were similar to those reported elsewhere (22). While basal levels of LH in the ov-PAO group were normal, LH responses

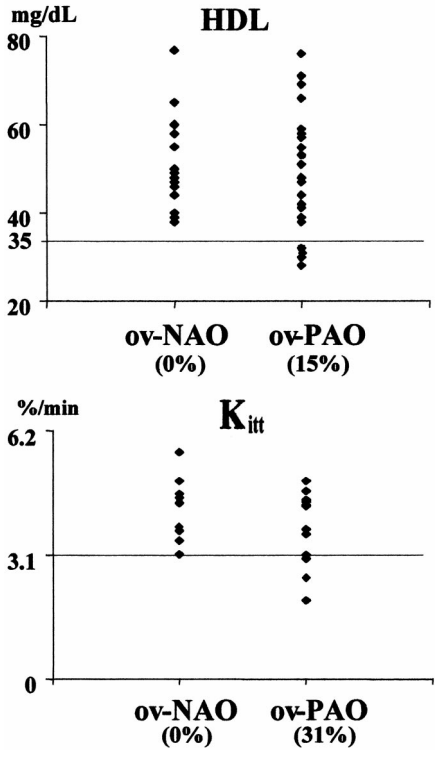

FIG. 3. Scattergrams of individual ov-PAO's HDL levels (normal > 35 mg/dL) and K_{itt} values (normal > 3.1%/min.) compared with those of ov-NAO.

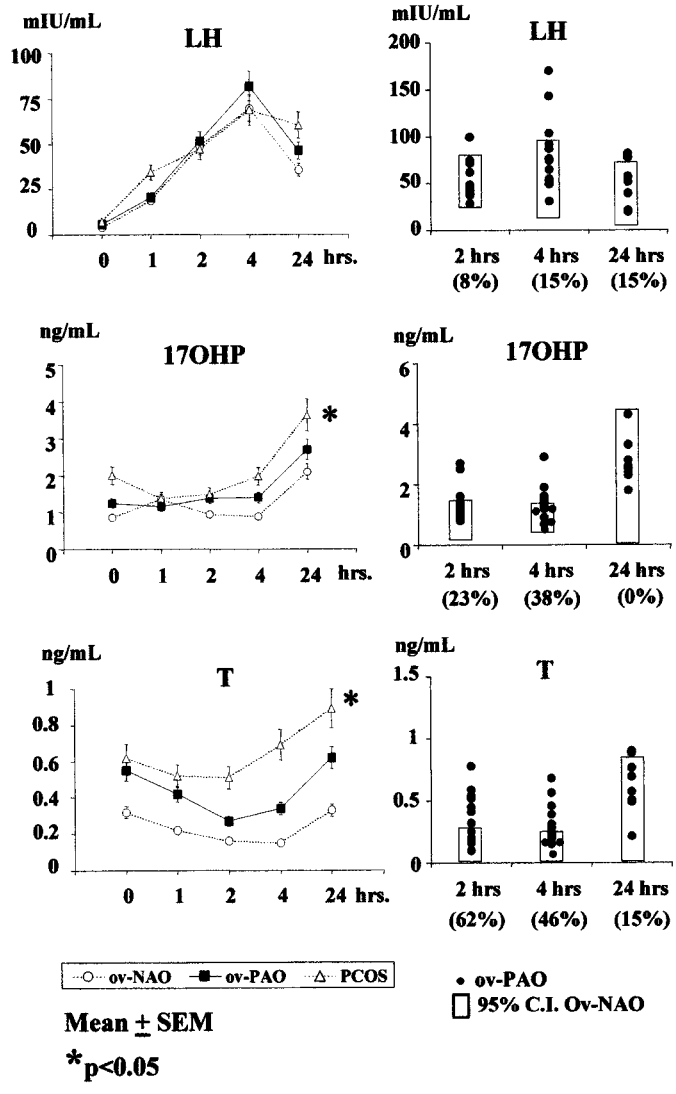

FIG. 4. GnRH-agonist provoked responses of LH, 17-OHP, and T (*left panel*) analyzed by repeated measures ANOVA, with baseline hormonal levels entered as continuous covariate. Scattergrams of provoked LH, 17-OHP, and T responses in ov-PAO and the proportions (*shown in parentheses*) that were over the 95% confidence interval in ov-NAO (*right panel*).

to GnRH-agonist were exaggerated only in a subset of ov-PAO women. Because LH pulse characteristics have not been studied in ov-PAO women, it remains unclear if this exaggerated response to GnRH-agonist reflects an underlying abnormality of 24-h secretion of LH, which is similar to that of PCOS. Increased ovarian androgen secretion occurred in response to GnRH-agonist stimulated release of LH and FSH in all three groups. These provoked responses were significantly increased in PCOS and, although intermediate in ov-PAO, these responses failed to reach statistical significance when compared with ov-NAO. However, provoked ovarian androgenic responses were exaggerated in 30–40% of women with ov-PAO. These increased androgenic responses are consistent with previous findings of exaggerated testosterone and androstenedione responses after hMG and hCG stimulation in two ov-PAO women (14). These findings may represent an increased ovarian sensitivity to GnRH-agonist and gonadotropin stimulation in ov-PAO women, and they suggest an abnormal regulation of their 17-hydroxylase and C-17,20-lyase in the ovarian Δ^4 pathway, which is similar to that of PCOS (22). Although ov-PAO women are not hyperandrogenic at baseline testing, GnRH-agonist testing can uncover exaggerated androgenic responses (occult hyperandrogenism) in a large subgroup of women with PAO.

Women with PCOS are known to have increased risks for cardiovascular disease (4–6, 23–25). It has been estimated that women with PCOS have a 7-fold relative risk for myocardial infarction (6). By the time women with PCOS reach perimenopause, up to 40% will have developed hypertension, and 16% will have become diabetic (5). In young women with PCOS, these increased risks are associated with insulin resistance and abnormal lipid profiles (4, 6, 8). We and others have found reduced levels of IGFBP-I to be another characteristic feature of women with PCOS (14, 26–29). This decreased level of IGFBP-1 in turn may increase the ratio of IGF-I:IGFBP-1 levels and may possibly lead to increased bioavailable levels of free IGF-I. High levels of insulin and bioavailable IGF-I may lead to LH augmentation of androgen biosynthesis and secretion (30–31), as well as to pituitary release (31–32). Furthermore, elevated fasting insulin levels have been implicated in the alterations of ovarian morphology such as polycystic changes (33). Previously, we have found that ov-PAO women have elevated fasting insulin levels and reduced levels of IGFBP-1 (14). In the present larger series of lean

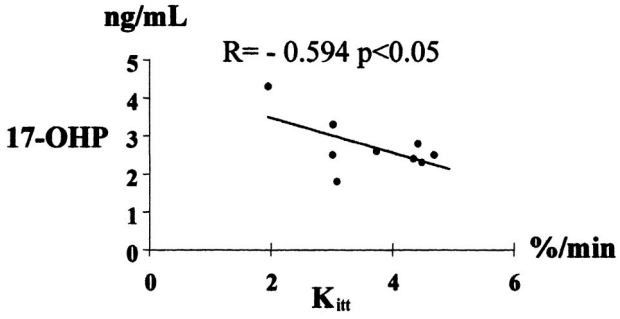

FIG. 5. Ov-PAO peak 17-OHP responses to leuprolide correlate inversely with K_{itt}, suggesting greater hyperandrogenic responses with mild insulin resistance.

women with PAO, we failed to confirm our previous findings regarding alterations of the insulin-IGF axis, but we found that up to one third have subtle metabolic abnormalities. While the average levels in ov-PAO did not differ as a group when compared with ov-NAO, 15% of women with ov-PAO had HDL levels below 35 mg/dL, a level considered to constitute significant cardiovascular risk (34). In fact, the average HDL level for white females is 55 mg/dL (35), and a level below 35 mg/dL corresponds to the lowest 5th percentile for the general female population (36). In addition, 31% of ov-PAO had Kitt levels below normal. Moreover, Kitt levels were inversely correlated with increased 17-OHP response to GnRH-agonist in the ov-PAO group, suggesting a greater androgenic response with insulin resistance. The women with PCOS in this study were not obese and were matched by BMI and age to the ovulatory groups. It may be for this reason that the overall prevalence of metabolic abnormalities was not high.

When the adrenals were stimulated with a pharmacologic dose of ACTH, no significant differences were noted in the response pattern between the three groups of women. Previous investigators have used pharmacologic, physiologic, or endogenous ACTH for disclosing subclinical abnormalities in adrenal steroidogenesis in some women with PCOS (37–41), while others have failed to note such significant changes (42–43). Although women with PCOS in this study had elevated adrenal androgens (DHEAS) at baseline, they did not exhibit significant adrenal hyperandrogenic responses to ACTH when compared with ov-PAO and control groups. Because ov-PAO subjects in this study had normal adrenal responses to ACTH, but some abnormal ovarian responses to GnRH-agonist, it suggests that this resemblance to PCOS may be linked to this altered ovarian morphology, which may present with subtle ovarian but not adrenal hyperandrogenism.

While it is not entirely clear if asymptomatic women with this isolated ultrasound finding exhibit all of the endocrine abnormalities and cardiovascular risk factors of PCOS, the present data confirm and extend our previous findings in that up to one third of women with PAO may have subtle findings consistent with PCOS. Because these responses were provoked, some women with PAO may merely carry a risk for the full development of PCOS. Nevertheless, because many of these subjects had metabolic abnormalities linked to cardiovascular disease, and

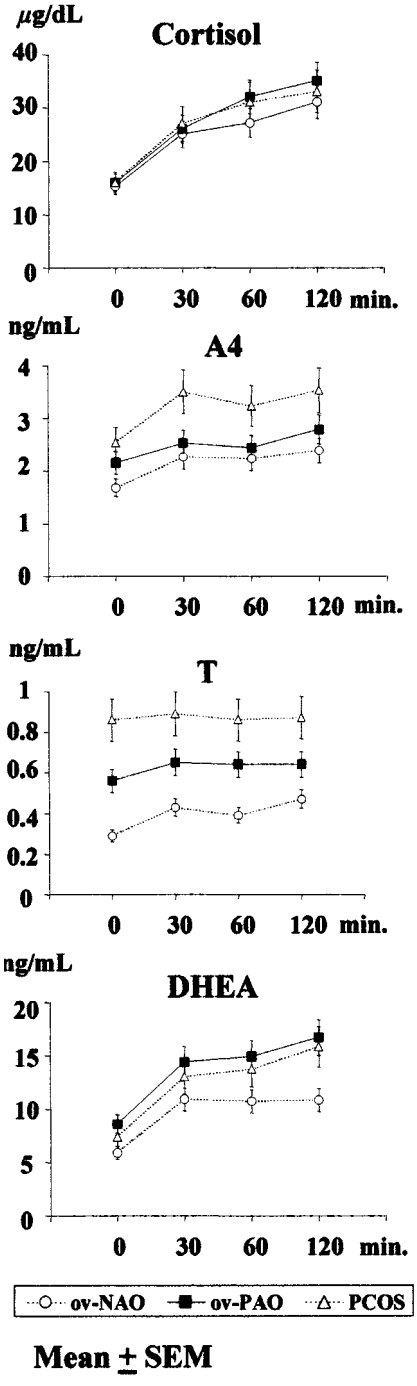

FIG. 6. Adrenal responses of cortisol, A4, T, and DHEA to ACTH stimulation were not significantly different among the three groups by repeated measures ANOVA with baseline hormonal levels entered as continuous covariate.

because cardiovascular disease is the leading cause of death in women, our findings suggest that monitoring all women with polycystic ovaries for these risks may be important in the health care of women.

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