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Influence of insulin resistance on total renin level in normotensive women with polycystic ovary syndrome

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Objective: To evaluate the influence of insulin resistance on the plasma total renin level in normotensive women with polycystic ovary syndrome (PCOS).

Design: Prospective, controlled study.

Setting: University hospital.

Patient(s): Twenty-five normotensive women with PCOS were compared with 11 normotensive control women with regular cycles and no features of PCOS.

Intervention(s): Clinical, ultrasonographic, and hormonal findings were used to define PCOS. Insulin resistance was estimated by continuous infusion of glucose with model assessment in the early follicular phase.

Main Outcome Measure(s): Plasma levels of total renin and angiotensin II and serum levels of gonadotropins, DHEAS, total T, free T, 17 α-hydroxyprogesterone, and PRL were determined.

Result(s): Plasma concentrations of angiotensin II were similar in the PCOS group and the control group. The concentration of total renin in plasma was higher in women with PCOS than in healthy women independent of insulin resistance. The sensitivity and specificity of the plasma total renin level to diagnose women with PCOS were calculated as 80% and 71.4%, respectively.

Conclusion(s): The plasma total renin level is higher in normotensive women with PCOS than in healthy women independent of insulin resistance. (Fertil Steril® 2000;73:261–5. ©2000 by American Society for Reproductive Medicine.)

Key Words: Total renin, insulin resistance, PCOS

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Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age. Recent prevalence studies have suggested that 5%–10% of premenopausal women have the full syndrome of hyperandrogenism, chronic anovulation, and polycystic ovaries (1). Insulin resistance, defined as a diminished effect of endogenous or exogenous insulin on glucose metabolism, is highly prevalent in women with PCOS (2, 3).

It has been assumed that angiotensin II has diabetogenic effects on glucose disposal in the same way as other counterregulatory hormones (4). It has been shown, however, that activation of the renin-angiotensin system in renovascular hypertension is not associated with insulin resistance. Conversely, insulin-resistant subjects have higher plasma renin activity, perhaps as part of a compensatory response to the reduction in glucose uptake (5). It has been concluded that the renin-angiotensin system is a potential source of insulin resistance because angiotensin infusion increases peripheral resistance and could diminish blood flow to skeletal muscle, which is the primary target of insulin action (6).

It has been reported that the plasma total renin level is higher in women with PCOS than in healthy women independent of body mass index (BMI), age, and serum insulin level (7). We measured the insulin resistance by continuous infusion of glucose with model assess-

ment and investigated whether the plasma total renin level is independent of insulin resistance in women with PCOS.

MATERIALS AND METHODS

Subjects

The study group included 25 women with PCOS, aged 25.81 ± 2.04 years (mean ± SD). The control group included 11 healthy women, aged 29.44 ± 1.55 years, with regular menstrual cycles (intervals between 21 and 35 days). Each woman underwent a screening history and physical examination. All subjects were free of medical illnesses and had not received any medications for ≥3 months before enrollment.

A 2-hour, 75-g oral glucose tolerance test was performed, and subjects were excluded if their fasting plasma glucose level was >115 mg/dL and/or their 120-minute plasma glucose level was >140 mg/dL. Blood pressure was measured, and subjects were excluded from the study if it was ≥140/90 mm Hg. Weight and height were obtained to calculate BMI (weight [kg]/height $[m]^2$). Obesity was defined as a BMI >25 kg/m^2. Oligomenorrhea was defined as an intermenstrual interval of >35 days and amenorrhea as the absence of menstruation for ≥6 months. Hirsutism was assessed by the protocol of Ferriman-Gallwey, and a woman with a score of >7 was considered clinically hirsute (8, 9).

Healthy women were not hirsute or infertile, had normal-appearing ovaries on ultrasound scan, and had normal serum androgen levels (T <81 ng/dL; free T <3.17 pg/mL; and DHEAS <430 μg/dL for age <20 years, <380 μg/dL for ages 20–29 years, and <270 μg/dL for ages 30–39 years). Baseline serum levels of FSH, LH, PRL, and 17α-hydroxyprogesterone (17-OHP) also were determined.

Polycystic ovary syndrome was defined as the presence of bilateral polycystic ovaries on ultrasound examination (10), oligomenorrhea or amenorrhea and/or chronic anovulation (11), and hyperandrogenemia (high serum T and/or free T and/or 17-OHP) (12).

All of the women were euthyroid (serum TSH level 0.35–5.5 μIU/mL). Their serum PRL levels were <40 ng/mL. Because mild hyperprolactinemia (PRL level <40 ng/mL) has been described in women with PCOS, women with mild hyperprolactinemia were recruited into the study unless their history, physical examination, or computed tomographic examination showed any other reason for the hyperprolactinemia (13).

In the PCOS group, if the basal serum 17-OHP level was ≥2 ng/mL, an ACTH stimulation test was performed to exclude patients with late-onset congenital adrenal hyperplasia (14). Serum 17-OHP levels were determined before and 30 minutes after an IV injection of 0.25 mg of synthetic ACTH (Synacthen; Ciba, Basel, Switzerland).

Women who had an increase of ≥3.3 ng/mL in the serum 17-OHP level after ACTH stimulation were excluded from the study. A serum cortisol level of >5 μg/dL after an overnight dexamethasone suppression test also was an exclusion criterion (15). Women were excluded from the study if an adnexal mass was noted on pelvic ultrasonography. All hormonal investigations and ultrasound examinations were performed in the early follicular phase (days 3–5) of spontaneous bleeding or withdrawal bleeding induced with medroxyprogesterone acetate.

All ultrasound examinations were performed by one operator, either transabdominally or transvaginally (3.5-MHz and 5-MHz sector probes, respectively; Kretz, Zipf, Austria). Polycystic-appearing ovaries were defined sonographically as the presence of multiple (≥10), small (2–8 mm in diameter) follicles in the periphery and increased stromal echogenicity as described by Adams et al. (16). The study was approved by the institutional review board at Istanbul University, and written informed consent was obtained from each subject.

Hormonal Assays

Blood samples were obtained through venipuncture and centrifuged within 2 hours after withdrawal. Serum was stored at −20°C and was assayed for FSH, LH, and PRL with chemiluminescent enzyme immunoassay kits (Chiron Diagnostics Corporation, East Walpole, MA). Assays for T, free T, DHEAS, and 17-OHP were performed with commercially available RIA kits (Coat-A-Count; Diagnostic Products Corporation, Los Angeles, CA).

Blood samples for angiotensin II assessment were collected into cold tubes containing ethylenediaminetetraacetic acid (EDTA) and centrifuged at 4°C to separate plasma. The samples were frozen immediately at −20°C until they were assayed with an RIA kit (Euro-Diagnostica, Arnhem, the Netherlands). Blood samples for total-renin assessment were drawn at room temperature, and EDTA-containing plasma samples were stored in a nondefrosting freezer at −20°C until assayed with an immunoradiometric assay kit (Nichols Institute Diagnostics, Wijchen, the Netherlands).

The glucose oxidase method was used for glucose determination in serum (Pointe Scientific Inc., Lincoln Park, MI), and the RIA kit provided by Diagnostic Products Corporation (Coat-A-Count) was used for quantitative insulin measurement in serum.

The intraassay and total coefficients of variation for the low, middle, and high serum values were as follows: 2.8%, 1.7%, and 1.2% and 4.6%, 4.5%, and 4.7% for FSH; 4.7%, 4.5%, and 4.4% and 6.3%, 5.2%, and 5% for LH; and 3.6%, 4%, and 4.5% for PRL. The intraassay coefficients of variation for different serum values were between 5% and 12% for T, 3.2% and 4.3% for free T, 6% and 9.8% for DHEAS, 3.5% and 6.7% for 17-OHP, 3.9% and 8.6% for angiotensin II, and 1.6% and 2.5% for total renin. Interassay coefficients of variation were between 7.3% and 11% for T, 3.4% and

TABLE 1

Demographic and hormonal data for women with PCOS and healthy women.

Variable	PCOS	Control	*P* value
Age (y)	25.8 ± 2.0	29.4 ± 1.5	NS
BMI (kg/m^2)	31.6 ± 7.3	26.7 ± 3.9	.01
FSH level (mIU/mL)	5.4 ± 1.7	7.5 ± 2.5	.03
LH level (mIU/mL)	11.7 ± 6.5	8.7 ± 4.4	NS
LH-FSH ratio	2.1 ± 0.9	1.2 ± 0.5	.001
Free T level (pg/mL)	3.2 ± 1.9	1.3 ± 0.5	.05
DHEAS level (μg/dL)	238 ± 76	130 ± 60	.001
PRL level (ng/mL)	15.0 ± 9.8	12.7 ± 6.5	NS
T level (ng/mL)	74.2 ± 33.4	28.8 ± 17.2	.001
17-OHP level (μg/L)	1.4 ± 1.0	0.8 ± 0.5	.02
Total renin level (ng/L)	242.3 ± 79.8	166.9 ± 54.9	.03
Angiotensin II level (μg/L)	62.4 ± 35.0	45.8 ± 31.2	NS

Note: All values are means ± SD. NS = not significant.

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5.5% for free T, 7.3% and 9.5% for DHEAS, 8.5% and 11% for 17-OHP, 2.5% and 8.3% for angiotensin II, 4.4% and 9.9% for total renin, 1.6% and 5.5% for insulin, and 1.4% and 1.9% for glucose.

Continuous Infusion of Glucose With Model Assessment

The subjects were put on a diet that consisted of 50% carbohydrate, 30% fat, and 20% protein and were told to avoid strenuous exercise for 3 days until the test was performed. After a 10- to 12-hour overnight fast, 18-gauge IV cannulas were placed in both antecubital veins. One arm was kept warm with an electric heating pad at 60°C so that the blood samples, taken from the indwelling venous cannula in the antecubital region, were similar to arterial blood. A continuous IV infusion of 5 mg glucose per kilogram ideal body weight per minute was then started and continued for 60 minutes.

Blood samples were taken at 50, 55, and 60 minutes for determination of plasma glucose and insulin concentrations. The concentrations were interpreted with use of a mathematical model of glucose and insulin homeostasis to estimate insulin resistance (17). Insulin resistance was calculated by the formula: R = A/[22.5 (2.71828 − LN{B})], where R = insulin resistance; A = mean plasma insulin concentration; and B = mean glucose concentration (18). Insulin resistance of ≥2 was the criterion for positive insulin resistance according to the continuous infusion of glucose with model assessment procedure (17).

Statistical Analysis

Statistical analysis was performed with the SPSS/PC package (SPSS Inc; Chicago, IL). All data are presented as the mean ± SD. Normal distribution of the age, BMI, and hormonal data of the two groups was tested with the Kolmogorov-Smirnov test. Because these variables were not normally distributed, Mann-Whitney *U* test was used to compare the PCOS and control groups. Results of angiotensin II and total renin measurements were compared by the Mann-Whitney *U* test in two groups: PCOS with insulin resistance and PCOS without insulin resistance.

The sensitivity and specificity of the plasma total renin level to diagnose women with PCOS were calculated as follows. For sensitivity, the number of women with PCOS with a positive test (plasma total renin level ≥195 ng/dL) was divided by the total number of women with PCOS; and for specificity, the number of control women with a negative test (plasma total renin level <195 ng/dL) was divided by the total number of control women. Pearson's correlation coefficients were used for correlation analysis. $P<.05$ was considered statistically significant.

RESULTS

Women with PCOS and controls were of similar age but had different BMIs. The mean age of the women with PCOS was 25.81 ± 2.04 years (range, 18–34 years) and of the healthy subjects was 29.44 ± 1.55 years (range, 22–35 years). The mean for BMI was 30.67 ± 7.31 kg/m^2 in the study group and 26.69 ± 3.89 kg/m^2 in the controls. Of the women with PCOS, 20 (80%) were hirsute, 18 (72%) were obese, and 12 (48%) were infertile. One patient had acanthosis nigricans, and 2 had mild hyperprolactinemia.

The hormonal profiles of the women in both groups are shown in Table 1. The women with PCOS had a higher LH-FSH ratio; higher serum levels of T, free T, 17-OHP, DHEAS, total renin, and insulin; and lower levels of FSH. Angiotensin II, LH, and PRL concentrations of the two groups were similar ($P>.05$).

Insulin resistance was determined in 12 women with

TABLE 2

Continuous infusion of glucose with model assessment for 25 women with PCOS and 11 controls.

Variable	PCOS (n = 25)	Control (n = 11)	*P* value
CIGMA (R)	1.96 ± 1.53 (1.33–2.60)	0.55 ± 0.24 (0.40–0.71)	.0001

Note: All values are means ± SD (range). CIGMA = continuous infusion of glucose with model assessment; R = insulin resistance.

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PCOS (48%). None of the women in the control group had insulin resistance. Results of continuous infusion of glucose with model assessment for the women in both groups are shown in Table 2. The total renin and angiotensin II levels of the insulin-resistant women with PCOS were similar to those of the women with PCOS who were not insulin resistant (Table 3). The sensitivity and specificity of the plasma total renin level to diagnose women with PCOS were calculated as 80% and 71.4%, respectively.

In the whole group (n = 36), positive correlations were found between insulin resistance and both LH-FSH ratio ($r = .5$, $P = .03$) and LH ($r = .39$, $P = .03$). We also found a weak positive correlation between plasma total renin level and LH in women with PCOS ($r = .27$, $P = .913$). No correlation was found between total renin and insulin resistance or between angiotensin II and insulin resistance.

DISCUSSION

The study of Balen et al. (18) included the largest population with PCOS in the literature. In addition, the study reported by Conway et al. (19) included another large group with PCOS. The proportions of overweight patients in these studies were 35% and 39%, respectively, but the rate was 72% in our study. There may be other factors besides PCOS contributing to the difference in the percentage of overweight patients, such as nutritional status and race. Furthermore, there were only 25 patients with PCOS in our study.

TABLE 3

Comparison of angiotensin II and total renin levels between women with and without insulin resistance among those with PCOS.

PCOS group (n = 25)	Angiotensin II level (μg/L)	Total renin level (ng/L)
IR (+) (n = 12)	52.27 ± 20.1	223 ± 49
IR (−) (n = 13)	70.2 ± 47.2	277 ± 102
P value	NS	NS

Note: All values are means ± SD. IR = insulin resistance; NS = not significant.

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This is a small series compared with the studies by Balen et al. (18) and Conway et al. (19). Therefore, the group in our study may represent severe cases of PCOS. This may have led to the higher percentage of overweight patients.

We also compared the incidence of infertility in our study with the studies of Balen et al. (18) and Conway et al. (19). The rates of infertility among women with tested fertility in these studies were 75% and 29%, respectively. The difference between these large series seems to be so much for the incidence of infertility. It is not unexpected that the percentage of patients with infertility was 48% in our study.

The current study shows that the plasma total renin concentration is elevated in women with PCOS compared with healthy women. It has been demonstrated that the concentration of total renin is higher in women with PCOS than in women having tubal infertility during gonadotropin stimulation (20). Consequently, these results suggest increased activity of the ovarian renin-angiotensin system in women with PCOS. We found a weak positive correlation between plasma total renin level and LH in women with PCOS, which parallels results from the study of Jaatinen et al. (7). It is apparent that renin production by the ovary is subject to complex regulation that may involve both autocrine and paracrine regulatory factors.

Sealey et al. (21) demonstrated that the initial rise in LH preceded the initial rise in plasma prorenin and total renin during the menstrual cycle (21). Thus, we collected all the blood samples for total renin in the early follicular phase to avoid the fluctuations during the menstrual cycle.

It has been demonstrated that the total renin concentration is independent of BMI, age, and insulin in women with PCOS (7). In this study, we measured the insulin resistance and determined that the plasma total renin concentration is also independent of insulin resistance in PCOS.

There was a statistically significant positive correlation between insulin resistance and LH-FSH ratio. This finding supports the fact that hyperinsulinemia stimulates gonadotropins in the pathogenesis of PCOS (22). The insulin resistance in primary hypertension is generally thought to be peripheral, and angiotensin II can diminish skeletal muscle blood flow, depriving the subject of an important site of insulin action and resulting in less glucose uptake (23, 24). Normotensive women with PCOS were included in this study because of the findings that physiologic administration of angiotensin II did not alter insulin sensitivity in normotensive subjects (6, 23, 25).

In our study, angiotensin II concentrations were similar in women with PCOS who were insulin resistant and women who were not insulin resistant. This finding suggests that the renin-angiotensin system has no role in insulin resistance in normotensive women with PCOS. New studies comparing plasma total renin and angiotensin II concentrations in hypertensive and normotensive subjects who are insulin resis-

tant might reveal the effect of the renin-angiotensin system on insulin resistance in women with PCOS.

In summary, it seems that insulin resistance has no effect on the plasma total renin level in PCOS. The plasma total renin level is elevated in women with PCOS independent of age, BMI, and insulin resistance. Currently, use of the plasma total renin level alone as a diagnostic marker in PCOS is not valuable because the clinical findings of PCOS are heterogeneous and the pathogenesis is unclear. However, it can be used together with other variables to diagnose women with PCOS.

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