C-reactive protein and homocysteine levels are associated with abnormal heart rate recovery in women with polycystic ovary syndrome

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Objective: To determine heart rate recovery (HRR) in patients with polycystic ovary syndrome (PCOS) and its relation to C-reactive protein (CRP) and homocysteine (Hcy) levels.
Design: Prospective clinical study.
Setting: University hospital.
Patient(s): Sixty-eight women with PCOS and 68 healthy women were included this study.
Intervention(s): Heart rate recovery was evaluated. We measured serum levels of CRP and Hcy. The presence of insulin resistance was investigated using homeostasis model assesment (HOMA-IR).
Main Outcome Measure(s): Heart rate recovery, CRP, Hcy.
Result(s): Heart rate recovery was significantly decreased in women with PCOS compared with control group women. Subjects with abnormal HRR had significantly greater levels of CRP and Hcy. The PCOS patients with HRR in the top tertile compared with the bottom quartile tended to have lower mean CRP and Hcy levels. The HRR was significantly and negatively correlated with age, CRP, Hcy, HOMA-IR, and body mass index. C-reactive protein and Hcy are independent determinants of HRR.
Conclusion(s): The CRP and Hcy levels may affect the development and progression of abnormal HRR in PCOS. (Fertil Steril® 2010;94:230–5. ©2010 by American Society for Reproductive Medicine.)

Key Words: Heart rate recovery, CRP, homocysteine, polycystic ovary syndrome

The polycystic ovary syndrome (PCOS) is a common endocrine-metabolic disorder that occurs in about 7% of reproductive-age women (1). A significant majority of women have multiple cardiovascular risk factors, such as insulin resistance (IR), dyslipidemia, and hypertension (2). Other markers of cardiovascular disease, such as C-reactive protein (CRP) and homocysteine (Hcy), have been found to be elevated in women with PCOS (3–7).

Heart rate recovery (HRR) is a marker of cardiac autonomic function and is directly correlated with parasympathetic activity (8). Abnormal HRR might play a role, because cardiovascular autonomic dysfunction is associated with sharply increased cardiovascular mortality (9–11). The effect of PCOS on cardiovascular mortality is currently unclear. Mounting evidence indicates that several cardiovascular risk factors are clearly present and higher in PCOS compared with healthy women (2–7). The HRR was impaired in young overweight PCOS women compared with healthy subjects (12). In PCOS women, abnormal HRR was inversely correlated to body mass index (BMI) and in the area under the curve for insulin (12). However, the mechanism underlying abnormal HRR in PCOS women have not been elucidated. Raised CRP and Hcy levels are considered to be risk factors for cardiovascular disease in PCOS women (3–7).

To date, there are no data regarding CRP and Hcy in relation to HRR assessment in PCOS patients in the literature. In view of these observations, we have evaluated HRR and its relation to CRP and Hcy levels in women with PCOS.

MATERIALS AND METHODS
Patients

The study group consisted of 68 PCOS and 68 control subjects. Each control was defined as age- and BMI-matched with a PCOS case when the age and BMI differences between case and control were <2 years and <1 kg/m², respectively. The majority of the control group consisted of students or hospital staff. Control subjects had normal menstrual cycles and no clinical or biochemical features of hyperandrogenism. The healthy state of the control subjects were determined by medical history, physical and pelvic examination, and complete blood chemistry. They were recruited from hospital staff and students. The diagnosis of PCOS was made according to the Rotterdam European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine–sponsored PCOS Consensus Workshop Group (13). Specifically, all eligible patients presented with at least two of three following criteria: 1) chronic anovulation; 2) hyperandrogenism (hirsutism,

acne) and/or hyperandrogenemia; and 3) polycystic ovaries. The presence of polycystic ovarian appearance was determined ultrasonographically (14). All subjects had irreguler menses, and 69% of participants had eight or fewer spontaneous cycles per year. Normal ovulatory state was confirmed by transvaginal ultrasonography and plasma progesterone (P) assay detected during the luteal phase of the cycle.

All subjects underwent baseline testing of TSH, PRL, 17OH-P, and glucose during a 2-hour oral glucose tolerance test (OGTT). None of the subjects had a thyroid disorder, diabetes mellitus, congenital adrenal hyperplasia, androgen-secreting tumors, or signs or symptoms of other androgen-secreting tumors, or signs or symptoms of other endocrinopathies. Patients and control subjects were excluded if they had used any oral contraceptive agents, antiandrogen agents, oral hypoglycemic agents, antilipidemic drugs, hypertensive medications, insulin sensitizers, or drugs that might interfere with cardiac autonomic activity in the preceding 6 months. Treatment in the last 3 months via exercise and diet were also accepted as criteria for exclusion. All subjects gave written informed consent according to the Helsinki Committe requirements, and the Institutional Review Boards of hospitals approved the study.

Venous blood collections were carried out in the follicular phase of a spontaneous cycle or after medroxyprogesterone-induced menstruation. After a 3-day 300-g carbohydrate diet and 12-hour overnight fasting, samples were obtained for the measurement of total T, 17OH-P, DHEAS, PRL, and TSH. Complete serum biochemistry and lipid profiles were also obtained. Then all patients underwent a 2-hour OGTT with a 75-g glucose load, with determinations of both glucose and insulin at baseline (before glucose load) and 120 minutes after load. Baseline and post-treatment serum levels of insulin were measured using an electrochemiluminescence immunoassay (Hitachi Elecsys 2010; Roche Diagnostics, Mannheim, Germany). Glucose tolerance test was evaluated by using the criteria of the American Diabetes Association, and impaired glucose tolerance (IGT) was defined as a 2-hour post-load glucose of 140 mg/dL to <200 mg/dl (15). Insulin resistance (IR) was evaluated using the homeostasis model assessment of insulin resistance index (HOMA-IR), defined as fasting glucose (mg/dL) × fasting insulin (μU/mL) × 0.055 ÷ 22.5 (16, 17), which has been shown to correlate well with IR evaluated using the clamp technique. Plasma glucose was determined with enzyme electrode in an EBIO analyzer (enzymatic amperometic principle, enzyme glucose hexokinase; Cobas Integra 400 Plus; Roche Diagnostics). Levels of total cholesterol and triglycerides were determined with enzymatic colorimetric assays (Roche Diagnostics). High-density lipoprotein (HDL) and low-density lipoprotein (LDL) were determined by colorimetric method using the Cobas Integra 400 Plus autoanalyzer. The intra- and interassay coefficients of variation were <5% for all of the assays. Samples were immediately centrifuged, and serum was separated and frozen at -20°C until assayed.

Homocysteine was measured as total Hcy by high performance liquid chromatography (Cromosystem, Mannheim, Germany). In this technique, the reagent kit allows the specific determination of total Hcy in plasma. Sample preparation is simply a reduction step for releasing Hcy from its protein binding. The Hcy level of the plasma was measured by fluorescense detection. Specifications were: linearite, up to 200 μmol/L, intraassay reproducibility <2%, recovery >98%. Serum CRP was measured by latex immunoturbidometric methodology on an automated clinical analyzer system (Cobas Integra; Roche Diagnostic).

Exercise Stress Test Protocol

Subjects underwent a maximal graded exercise test on an electronic treadmill (Kardiosis, Ankara, Turkey). Subjects were instructed to fast for 4–6 hours before exercise. Subjects underwent a symptoms-limited cardiopulmonary exercise test with Bruce treadmill protocol. Continuos, 12-lead electrocardiographic monitoring was performed throughout testing. ST-Segment changes were evaluated during the test using Mason et al.'s adaptation (18). Blood pressure (BP) was measured by arm-cuff sphygmomanometry during the last 30 seconds of each work stage. Participants exercised until limiting symptoms (dyspnea, dizziness, fatigue, leg cramps) or a medical contraindication such as ST-segment depression of >0.3 mV, or systolic BP >230 mm Hg developed, or a drop of >20 mm Hg in systolic BP occurred. Patients were ambulated briefly during a cool-down period of 2 minutes. To avoid the effect of manifest ischemia on HRR, only patients whose exercise tests terminated due to reached target heart rate, fatigue, or dyspnea were taken into analysis. All other reasons for termination of exercise resulted in exclusion of patients from the study. Blood pressure was recorded at baseline, every 2 minutes during exercise, and at the end of the recovery period. Heart rate was measured at rest, during each minute of exercise, and at the start of the recovery period. The HRR was calculated as the difference between heart rate at peak exercise and heart rate after the first minute of the cool-down period. Abnormal HRR was defined as ≤ 18 beat/min for standard exercise testing (8).

Statistical Analysis

Data analysis was performed by using SPSS for Windows, version 11.5. Shapiro-Wilk test was used to detect whether the continuous variables were normally distributed or not. Descriptive statistics were shown as mean ± SD for continuous data. Groups were compared using Student t or Mann-Whitney U test as appropriate. The differences among HRR tertile groups regarding for CRP and Hcy were evaluated by using one-way analysis of variance (ANOVA) or Kruskal-Wallis test. When the P value from the one-way ANOVA or Kruskal-Wallis test statistics were statistically significant, post hoc Tukey or Kruskal-Wallis multiple comparison test, respectively, were used to know which group diffeed from which others. Degrees of association between continuous variables were calculated by Pearson correlation coefficient. Stepwise multiple linear regression (MLR) was used to find the major determinants of HRR among those variables

showing significant correlations. A *P* value of <.05 was considered to be statistically significant.

RESULTS

The HRR was significantly decreased in women with PCOS compared with healthy control subjects ($P<.001$). Baseline heart rate was significantly increased in subjects with abnormal HRR compared with subjects with normal HRR (103 ± 12 vs. 84 ± 14; $P<.001$) (data not shown). Total T, fasting insulin, and HOMA-IR were significantly higher in patients with PCOS compared with control subjects ($P<.05$). The HDL cholesterol level was significantly lower in patients with PCOS compared with control subjects ($P<.01$). None of the patients had diabetes or impaired glucose tolerance. Serum LH, FSH, total cholesterol, LDL cholesterol, triglycerides, fasting glucose levels, and BMI were similar in both groups (Tables 1 and 2).

To evaluate the association between abnormal HRR, CRP, and Hcy, subjects were divided into tertiles according to HRR (tertile 1 <18 beats/min, tertile 2 18–30 beats/min, and tertile 3 >30 beats/min). There was statistical difference for CRP and Hcy levels in tertile subgroups ($P<.01$). The PCOS patients with HRR in the top tertile compared with the bottom tertile tended to have lower mean CRP and Hcy levels (Table 3).

In the Pearson correlation test, HRR was significantly and negatively correlated with age ($r = -0.37$; $P<.01$), CRP ($r = -0.54$, $P<0.01$), Hcy ($r = -0.39$; $P<.01$), HOMA-IR ($r = -0.28$; $P<.05$), and BMI ($r = -0.44$; $P<.01$). The CRP levels were positively and significantly correlated with baseline heart rate in subjects with abnormal HRR ($r = 0.48$; $P<.01$). No correlation was found for the other parameters.

Stepwise MLR analysis was carried out to introduce HRR as a dependent variable and age, BMI, CRP, Hcy, and HOMA-IR as independent variables. Stepwise MLR analysis revealed that CRP, Hcy, HOMA-IR, and BMI are independent determinants of HRR in PCOS patients (Table 4). This model explains 68.4% of variation of HRR.

DISCUSSION

In the present study, we determined that HRR was significantly decreased in women with PCOS compared with healthy controls. Plasma CRP and Hcy levels were found to be associated with abnormal HRR in PCOS. Pearson correlation analysis showed that abnormal HRR was significantly and inversely correlated with CRP, Hcy, fasting insulin, HOMA-IR, and BMI. Stepwise MLR analysis revealed that CRP, Hcy, HOMA-IR, and BMI are independent determinants of abnormal HRR in PCOS patients. To the best of our knowledge, this is the first study reporting the relation between HRR after exercise, an indicator of cardiac autonomic activity, CRP, and Hcy, in addition to BMI and IR, in PCOS.

Heart rate recovery is a marker of autonomic function and is directly correlated with parasympathetic activity (19–21). HRR has been identified as a powerful independent predictor

TABLE 1

Clinical, biochemical, and metabolic characteristics between polycystic ovary syndrome (PCOS) patients and control subjects.

	PCOS (n = 68)	Control (n = 68)	*P* value
Age (y)	24.2 ± 4.8	24.4 ± 3.9	NS
FSH (IU/L)	6.9 ± 3.5	5.8 ± 3.6	NS
LH (IU/L)	5.4 ± 2.8	4.1 ± 1.7	NS
BMI (kg/m^2)	23.4 ± 2.6	24.1 ± 2.7	NS
Total T (ng/mL)	0.68 ± 0.34	0.29 ± 0.11	<.05
Total C (mg/dL)	164 ± 31	168 ± 29	NS
LDL-C (mg/dL)	101 ± 26	98 ± 23	NS
HDL-C (mg/dL)	52 ± 19	61 ± 19	<.01
TG (mg/dL)	101 ± 42	97 ± 79	NS
Fasting insulin (μIU, Min/Ml)	17.8 ± 4.8	8.9 ± 3.2	<.05
Fasting glucose (mg/Ml)	91.2 ± 7.9	88.6 ± 9.4	NS
HOMA-IR	3.7 ± 1.2	1.3 ± 0.7	<.01

Note: Data are expressed as mean $\pm$ SD. Statistical significance was defined as $P<.05$. BMI = body mass index; C = cholesterol; HDL-C = high-density lipoprotein cholesterol; HOMA-IR = homeostasis model assessment of insulin resistance; LDL-C = low-density lipoprotein cholesterol; NS = nonsignificant; TG = triglycerides.

Kaya. PCOS, CRP, homocysteine, and heart rate recovery. Fertil Steril 2010.

of cardiovascular and all-cause mortality in healthy adults (9, 10, 21). A delayed decrease in heart rate during the first minute after graded exercise has been found, independent of workload, to be a powerful predictor of overall mortality, of the presence or absence of myocardial perfusion defects, and of changes in heart rate during exercise (20, 21). PCOS seems to be characterized by several alterations that could increase the risk for cardiovascular diseease (1–6). It is possible that these findings are due in part to abnormal HRR in PCOS patients. In the present study, when PCOS women were compared with healthy control women, HRR was found to be lower in the PCOS group. These results suggest that abnormal HRR is increased by PCOS.

More recently, Giallauria et al. (12) demonstrated that PCOS patients, after exercise, had abnormal HRR compared with healthy control subjects. Those authors suggested that abnormal HRR may be considered to be a further marker of cardiovascular risk in PCOS. To date, the full mechanism underlying abnormal HRR in PCOS women has not been elucidated. Abnormal HRR after exercise testing is associated with inflammatory markers, which could contribute to the high incidence of cardiovascular disease (22). Kelly et al. (3) has shown that CRP concentration is significantly

increased in PCOS women. CRP may be directly involved in the atherogenic process by promoting endothelial dysfunction and complement activation (3). Homocysteine levels are also considered to be a risk factor for cardiovascular disease (4). Several studies have examined the relationship between PCOS and serum Hcy levels, with most finding that the serum Hcy levels were significantly higher in PCOS women (4, 6, 7). Hyperhomocysteinemia is associated with atherosclerotic coronary, cerebral, and peripheral vascular disease (23–25). It is possible that abnormal HRR is due in part to an increase in systemic inflammation and Hcy levels in PCOS patients.

The studies conducted so far have not specifically addressed the association between hyperhomocysteinemia and cardiac autonomic dysfunction in PCOS patients. In the present study, subjects with abnormal HRR had significantly greater levels of Hcy. In Pearson correlation analysis, abnormal HRR was significantly and negatively correlated with serum Hcy levels. In addition, in the MLR analysis, serum Hcy is an independent predictor for HRR (β coefficient 0.041;

$P<.001$; 95% confidence interval [CI] 0.026–0.081). As levels of serum Hcy levels increased, linear decreases at the start of the recovery period were observed. We found that subjects in the lowest tertile of HRR were more likely to have higher Hcy levels. These findings reveal that there is a direct relation between increased Hcy levels and abnormal HRR in PCOS women. These results may suggest that abnormal HRR may be related to high levels of Hcy. Therefore, hyperhomocysteinemia may contribute to the pathogenesis of abnormal HRR in PCOS women. In other words, abnormal HRR may be the mechanism underlying the greater susceptibility of PCOS individuals to the adverse effects of hyperhomocysteinemia.

Recent cross-sectional studies have suggested that cardiac autonomic nervous activity, as assessed by heart rate variability, is related to inflammatory markers such as CRP (22). In the present report, we found that HRR was significantly and negatively correlated with CRP ($P<0.01$). Levels of CRP were found to be higher in subjects with abnormal HRR than in those with normal HRR. Patients in the highest tertile of HRR were more likely to have lower CRP levels. In stepwise MLR analysis, CRP is an independent risk factor for HRR in PCOS women (β coefficient 0.09; $P=.003$; 95% CI 0.03–0.16. Thus, abnormal HRR after exercise testing is associated with CRP levels, which could contribute to high incidence of cardiovascular disease.

Baseline heart rate was significantly increased in subjects with abnormal HRR compared with normal HRR subjects. The CRP levels were positively and significantly correlated with baseline heart rate in subjects with abnormal HRR. We assume that increased CRP levels, being inversely related to HRR, may have an effect on the heart rate through a decrease in parasympathetic activity. Considering these findings, decreased parasympathetic nerve system activity may be related to systemic inflammation in PCOS. The CRP level is an independent risk factor for the development of hypertension (27). CRP may be directly involved in the atherogenic

Stepwise multiple linear regression analysis of relationship between HRR and selected variables.

Independent variable	Coefficient of regression (β)	P value	95% CI for bound		Adjusted R^2
			Lower bound	Upper bound	
CRP	0.09	.003	0.03	0.16	68.4%
Hcy	0.041	<.001	0.026	0.081	
HOMA-IR	0.15	<.001	0.09	0.22	
BMI	0.02	<.001	0.01	0.09	

Note: CI = confidence interval; other abbreviations as in Tables 1 and 2.

Kaya. PCOS, CRP, homocysteine, and heart rate recovery. Fertil Steril 2010.

process by promoting endothelial dysfunction and complement activation (26). The presence of a low-grade chronic inflammatory state has been documented in women with PCOS (3, 26, 28). Holte et al. (29) demonstrated that women with PCOS have an increased prevalence of labile blood pressure, which may indicate a prehypertensive state. Therefore, decreased parasympathetic nerve system activity through systemic inflammation may be responsible for both the increase in heart rate and blood pressure, as well as for the atherogenic process in PCOS women.

Body mass index is an independent predictor of abnormal HRR (30). Investigators have determined an association between autonomic nervous system dysfunction as estimated by abnormal HRR and BMI (12). However, we could also determine a correlation between BMI and HRR by univariate and multivariate analyses in PCOS. This situation suggests that BMI might play an important role in the development of abnormal HRR in patients with PCOS.

Insulin resistance and compensatory hyperinsulinemia are associated with autonomic imbalance with increased sympathetic activity and reduced parasympathetic activity (31–33). Earlier studies have shown that the enhancement of parasympathetic tone may decrease the incidence of malignant ventricular arrhythmias and sudden cardiac death (34). In the present study, we also found an association in abnormal HRR and fasting insulin and IR (based on HOMA-IR). In the present study, baseline heart rate was significantly increased in subjects with abnormal HRR compared with normal HRR subjects. This relationship may be explained in part by insulin resistance, as shown in recent studies (32, 33).

Reduced HRR may be a predictor of increased cardiovascular disease in PCOS patients (12). In the present study, we clearly demonstrated that an association exists between CRP and Hcy levels and cardiac autonomic function in PCOS patients. Abnormal HRR might a play role, because cardiovascular autonomic dysfunction, even if subclinical, is associated with sharply increased cardiovascular mortality. The mechanism underlying the greater susceptibility of PCOS individuals to the adverse effects of inflammation and hyperhomocysteinemia may be related to abnormal HRR. These findings may have important implications in the long term regarding cardiovascular complications associated with inflammation and hyperhomocysteinemia in PCOS. Raised CRP and hyperhomocysteinemia might play an important role in the development and progression of abnormal HRR.

Women with PCOS are characterized by clinical and/or biochemical hyperandrogenism (1, 35–37). In the present study also, total T levels were higher in PCOS women than in control subjects. There is little evidence to substantiate the association between hyperandrogenism and cardiovascular events (36, 37). Univariate and multivariate analyses were conducted to reveal no correlation between total T and HRR in PCOS. Therefore, it can be speculated that total T might not affect HRR in PCOS.

The results of the present study support the associations between CRP, Hcy, and HRR in PCOS patients. Because this study was cross-sectionally designed, whether impaired autonomic function is the cause or effect of systemic inflammation and hyperhomocysteinemia could not be determined. A large prospective longitudinal study will be necessary to clarify the relationship between CRP, Hcy, and HRR in PCOS patients.

In conclusion, increased CRP and hyperhomocysteinemia may be the determining factor for abnormal cardiac autonomic function in PCOS, along with other known risk factors, such as BMI, insulin, and insulin resistance. Raised CRP and hyperhomocysteinemia is associated with abnormal HRR, which could contribute to high incidence of cardiovascular disease in PCOS patients.

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