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Glucose intolerance, insulin resistance and cardiovascular risk factors in first degree relatives of women with polycystic ovary syndrome

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BACKGROUND: The aim of the present study was to evaluate insulin resistance (IR), glucose tolerance status and cardiovascular risk factors in first degree relatives of patients with polycystic ovary syndrome (PCOS). METHODS: A total of 120 family members [$Mothers_{PCOS}$ ($n = 40$), $Fathers_{PCOS}$ ($n = 38$), $Sisters_{PCOS}$ ($n = 25$) and $Brothers_{PCOS}$ ($n = 17$)] of 55 patients with PCOS and 75 unrelated healthy control subjects without a family history of diabetes or PCOS (four age- and weight-matched subgroups, i.e. $Control_{Mothers}$, $Control_{Fathers}$, $Control_{Sisters}$ and $Control_{Brothers}$) were studied. IR was assessed by homeostatic model assessment (HOMA IR), log HOMA, insulin sensivity index (ISI), the quantitative insulin sensitivity check index (QUICKI) and area under the curve for insulin during the oral glucose tolerance test (AUCI, AUCG) in with normal glucose tolerance (NGT) subjects and controls. Serum adiponectin, resistin, homocysteine and lipid levels were measured. RESULTS: The prevalence of any degree of glucose intolerance was 40% in $Mothers_{PCOS}$ and 52% in $Fathers_{PCOS}$. In total, six (15%) glucose tolerance disorders were identified in the $Control_{Mothers}$ and $Control_{Fathers}$ in first degree relatives of control subjects. The first degree relatives of PCOS patients had significantly higher serum fasting insulin, HOMA-IR, Log HOMA and AUCI levels in all subgroups than the control subjects. The control subjects had significantly elevated QUCKI, ISI levels and serum adiponectin levels compared to the first degree relatives of PCOS subjects in all subgroups. The serum Hcy and resistin levels increased significantly in both $Fathers_{PCOS}$ and $Mothers_{PCOS}$ groups but not $Brothers_{PCOS}$ and $Sister_{PCOS}$. CONCLUSION: The results of the present study support the finding that the first degree relatives of PCOS patients carry an increased risk of cardiovascular disease, as do PCOS patients.

Key words: adiponectin/family/homocysteine/insulin resistance/PCOS

Introduction

Polycystic ovary syndrome (PCOS) is the most frequent endocrine disorder seen in pre-menopausal women, affecting 5–10% of this population (Franks, 1995). It is characterized by menstrual irregularities and biochemical and/or clinical hyperandrogenism such as hirsutism, seborrhoea and acne (Homburg, 2003). Regardless of the presence of obesity, many are also insulin resistant (Dunaif *et al.*, 1989). A number of findings suggest that hyperinsulinaemia may play a central role in the development of hyperandrogenism (Dunaif *et al.*, 1989; Dunaif, 1997), Type II diabetes mellitus (DM) (Ehrman *et al.*, 1999; Legro *et al.*, 1999) and an increase in the risk of cardiovascular disease (CVD) (Wild *et al.*, 1985, 2000; Legro, 2003).

Familial aggregation of PCOS consistent with a genetic aetiology has been well documented in the literature (Hague *et al.*, 1988; Lunde *et al.*, 1989; Legro *et al.*, 1998; Govind *et al.*, 1999). Family studies of PCOS have focused mainly on ovarian morphology, menstrual disturbances, symptoms of hyperandrogenism, hyperandrogenaemia, glucose intolerance disorders and insulin resistance (IR) (Ferriman and Purdie, 1979; Carey *et al.*, 1993; Norman *et al.*, 1996; Colilla *et al.*, 2001; Kahsar-Miller *et al.*, 2001; Sir-Petermann *et al.*, 2002; Legro *et al.*, 2002; Yıldız *et al.*, 2003; Ehrman *et al.*, 2005). These studies have found higher insulin resistance, serum androgen levels, and Type II DM as well as glucose tolerance disorder in the first degree relatives of PCOS patients compared to the control groups.

Relatively few studies have investigated cardiovascular risk in the first degree relatives of PCOS patients. It was shown in one of these studies (Kaushal *et al.*, 2004) that brothers of PCOS patients had insulin resistance and endothelial dysfunction in the early stages of their life. Similarly, another study found a positive relationship between PCOS

and CVD cases in the family (Atiomo *et al.*, 2003). Elevated plasma Hcy levels are also considered to be an independent risk factor for CVD (Clarke *et al.*, 1991). Adiponectin has been postulated to play an important role in the modulation of glucose and lipid metabolism in insulin-sensitive tissues. Levels of adiponectin are also lower in patients with essential hypertension, DM, obesity and CVD (Ouchi *et al.*, 2003; Hotta *et al.*, 2000; Matsuzawa *et al.*, 2004).

The aim of the present study was to evaluate insulin resistance, glucose tolerance status and cardiovascular risk factors in first degree relatives of PCOS patients.

Material and methods

Fifty-five patients with PCOS were recruited for the study. PCOS was defined according to the Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group (2004). Patients with DM, hyperprolactinaemia, congenital adrenal hyperplasia, thyroid disorders, Cushing disease, hypertension, hepatic or renal dysfunction were excluded from the study. The ethics committee of Gazi University, Faculty of Medicine in Ankara, Turkey approved the study protocol. All patients signed informed consent forms. After obtaining permission from PCOS patients, an attempt was made to contact all first degree relatives, and a total of 120 first degree relatives [$Mothers_{PCOS}$ ($n = 40$), $Fathers_{PCOS}$ ($n = 38$), $Sisters_{PCOS}$ ($n = 25$) and $Brothers_{PCOS}$ ($n = 17$)] were studied. Three mothers and two fathers had previously been diagnosed with Type II DM. The family members of 35 women who came to the check-up centre for routine controls and who were between the ages of 18–35 years with no prior family history of PCOS or DM were contacted. A total of 75 subjects among these family members agreed to take part in the study. Four age- and weight-matched subgroups were formed to serve as the control subgroups. These subgroups were defined as $Control_{Mothers}$ ($n = 20$), $Control_{Fathers}$ ($n = 20$), $Control_{Sisters}$ ($n = 20$) and $Control_{Brothers}$ ($n = 15$). The female control subjects had no personal history of PCOS, i.e. they exhibited regular cycles and normal androgen levels. None of the women in the $Control_{Mothers}$ and $Control_{Sisters}$ groups had hirsutism score <8 (Ferriman and Gallwey, 1961) or acne. All pre-menopousal women in the control group had regular menses, i.e. every 21–35 days.

The body mass index (BMI) and waist:hip (W:H) ratio were calculated. Weight and height of the subject were measured wearing light clothing and no shoes. BMI was calculated as weight divided by height squared (kg/m^2). Waist circumference was measured at the narrowest level between the costal margin and the iliac crest, and the hip circumference was measured at the widest level over the buttocks with the subject standing and breathing normally.

A standard 75 g oral glucose tolerance test (OGTT) and insulin response to oral glucose loading were performed between 08:30–10:30, following a fasting period of 10–12 h. Glucose tolerance state was then evaluated using the criteria of the American Diabetes Association (2003). Those patients with glucose tolerance disorder were not included in the measurements of insulin resistance. The response of glucose and insulin to the OGTT was analysed by calculating the (AUCI and AUCG) using the trapezoidal method. Insulin sensivity index (ISI) was calculated the by the method of Matsuda and De Fronzo (1999). The homeostasis model (HOMA) insulin resistance index (HOMA-IR) was calculated according to the following formula: fasting glucose (mmol/l) × fasting insulin (QU/ml)/22.5 (Matthews *et al.*, 1985). Log versions of HOMA were calculated. The quantitative insulin sensitivity check index (QUICKI) was calculated according to the following formula: 1/[log fasting serum insulin (QU/ml)+log fasting plasma glucose (mg/dl)] (Katz *et al.*, 2000).

Serum levels of total cholesterol (Total-C), high density lipoprotein (HDL)-C, low density lipoprotein (LDL)-C and triglyceride (Tg) were measured using Abbott-Aeroset (Chicago, IL, USA) autoanalyser with original kits. Lipoprotein (a) [Lp (a)], Apoprotein A (Apo A) and Apoprotein B (Apo B) levels were determined by nephelometric assay using Beckman 360 protein array system. Serum levels of FSH, LH, prolactin, dehydroepiandrosterone sulphate (DHEA-S), insulin, cortisol and thyroid-stimulating hormone (TSH) were measured with specific chemiluminescence assays using the Abbott Architect system. Serum levels of 17-OH-progesterone, free testosterone, androstenedione were measured by radioimmunoassay analyser (Tosoh Bioscience, Tokyo, Japan). Serum vitamin B_{12} level was measured using Immulyte 2000 (BioDPC, CA, USA) analyser with chemiluminescence method. Folate level was determined by Tosoh analyser (Tokyo, Japan). Serum adiponectin levels were determined by enzyme-linked immunosorbent assay (Kit: B-Bridge International, Inc., CA, USA). Resistin levels were determined using human Resistin assay kit (ImmunoDiagnostik, BenSheim, Germany) which was based on a competitive enzyme immunoassay. Plasma Hcy levels were measured with high performance liquid chromatography using Chromsystems kits with fluorescence detector.

Statistical analysis

Data analysis was performed using the 10.0 PC package (SPSS, Inc., Chicago, IL, USA). All parameters are shown as the mean± SD. The unpaired *t*-test and Mann–Whitney *U*-test were used when appropriate. Bivariate correlation analysis was performed. $P<0.05$ was considered statistically significant.

Results

There were no statistically significant differences between the mean age and BMI of the controls and PCOS relatives. The clinical and hormonal characteristics of groups, of $Mothers_{PCOS}$ and $Control_{Mothers}$, $Fathers_{PCOS}$ and $Control_{Fathers}$, $Sisters_{PCOS}$, and $Control_{Sisters}$, and $Brothers_{PCOS}$ and $Control_{Brothers}$ are given in Tables I, II, III and IV respectively.

Insulin resistance–glucose tolerance state

The prevalence of any degree of glucose intolerance, including the three mothers previously diagnosed with Type II DM, was 16/40 (40%) in $Mothers_{PCOS}$. The prevalence of DM, IGT (impaired glucose tolerance), and IFG (impaired fasting glucose) in the same group was 7/40 (17.5%), 7/40 (17.5%) and 2/40 (5%) respectively. The prevalence of any degree of glucose intolerance, including the two fathers previously diagnosed with Type II DM, was 20/38 (52%) in $Fathers_{PCOS}$. The prevalence of DM, IGT and IFG in the same group was 8/38 (21%), 10/38 (26%) and 2/38 (5%) respectively. In total, six (15%) glucose tolerance disorders were identified in the $Control_{Mothers}$ and $Control_{Fathers}$ groups. While one (5%) newly diagnosed Type II DM, one (5%) newly diagnosed IGT and one (5%) newly diagnosed IFG were identified in the $Control_{Fathers}$ group, one (5%) newly diagnosed DM and two (10%) newly diagnosed IGT cases were identified in

Table I. Clinical and hormonal characteristics of the $Mothers_{PCOS}$ and $Control_{mothers}$ groups

	$Mothers_{PCOS}$ (n = 40)	Controls (n = 20)	P
Age (years)	46.38 ± 7.95	48.12 ± 9.02	NS
Body mass index (kg/m^2)	30.46 ± 6.89	29.82 ± 5.76	NS
Waist:hip ratio	0.87 ± 0.08	0.83 ± 0.07	< 0.05
Systolic blood pressure (mmHg)	144.39 ± 21.18	128.12 ± 10.83	< 0.05
Diastolic blood pressure (mmHg)	86.12 ± 9.86	76.98 ± 7.47	< 0.05
Fasting insulin (μIU/ml)	18.52 ± 6.78	10.24 ± 2.19	< 0.001
ISI_{OGTT}	3.96 ± 2.07	6.12 ± 3.92	< 0.001
AUCI (μIU/ml/min)	12.874.76 ± 3721.76	6928.29 ± 2822.91	< 0.001
AUCG (mg/ml/min)	16452.25 ± 4269.83	15.839.18 ± 4187.23	NS
HOMA-IR	3.94 ± 1.87	2.51 ± 1.19	< 0.001
QUCKI	0.321 ± 0.019	0.350 ± 0.023	< 0.001
Log HOMA	0.362 ± 0.018	0.243 ± 0.019	< 0.001
Homocysteine (μmol/l)	16.25 ± 7.86	10.98 ± 5.79	< 0.001
Adiponectin (ng/ml)	3.68 ± 0.99	5.40 ± 1.12	< 0.001
Resistin (ng/ml)	10.68 ± 4.03	7.82 ± 3.18	< 0.05
Total-C (mg/dl)	208.65 ± 46.13	157.12 ± 30.87	< 0.005
LDL-C (mg/dl)	120.81 ± 38.02	80.59 ± 17.42	< 0.005
HDL-C (mg/dl)	55.32 ± 18.01	56.74 ± 11.46	NS
Triglyceride (mg/dl)	139.79 ± 61.95	98.32 ± 40.29	< 0.005
Apoprotein A (mg/dl)	132.79 ± 26.04	142.75 ± 28.93	NS
Apoprotein B (mg/dl)	109.13 ± 29.71	85.37 ± 22.78	< 0.005
Lipoprotein (a) (mg/dl)	25.84 ± 12.85	17.86 ± 8.16	< 0.005
Vitamin B_{12} (pg/ml)	276.38 ± 41.19	285.75 ± 35.84	NS
Folic acid (ng/ml)	13.16 ± 3.18	13.08 ± 3.06	NS

Values are mean ± SD.
ISI_{OGTT} = insulin sensitivity index (oral glucose tolerance test); AUCI, AUCG = area under the curve for insulin during the OGTT; HOMA-IR = homeostatic model assessment; QUICKI = quantitative insulin sensitivity check index; C = cholesterol; LDL = low density lipoprotein; HDL = high density lipoprotein; NS = non-significant.

the $Control_{Mothers}$ group. This difference between the first degree relatives of PCOS patients and the control group was found to be statistically significant ($P<0.001$).

The first degree relatives of PCOS patients had significantly higher serum fasting insulin ($P<0.001$), HOMA-IR ($P<0.001$), log HOMA ($P<0.001$) and AUCI ($P<0.001$) levels in all subgroups than the control subjects. The control group subjects had significantly elevated ISI ($P<0.001$) and QUCKI ($P<0.001$) levels compared to the first degree relatives of PCOS patients in all subgroups.

Table II. Clinical and hormonal characteristics of the $Fathers_{PCOS}$ and $Control_{Fathers}$

	$Fathers_{PCOS}$ (n = 38)	Controls (n = 20)	P
Age (years)	51.87 ± 8.54	53.64 ± 9.02	NS
Body mass index (kg/m^2)	26.94 ± 4.22	26.86 ± 4.91	NS
Waist:hip ratio	0.84 ± 0.08	0.79 ± 0.07	< 0.05
Systolic blood pressure (mmHg)	145.92 ± 19.71	130.18 ± 12.64	< 0.05
Diastolic blood pressure (mmHg)	87.19 ± 11.57	79.23 ± 8.65	< 0.05
Fasting insulin (μIU/ml)	16.73 ± 6.51	10.18 ± 3.12	< 0.001
ISI_{OGTT}	4.32 ± 2.85	5.96 ± 3.63	< 0.001
AUCI (μIU/ml/min)	10.076.21 ± 1832.93	6583.29 ± 1152.63	< 0.001
AUCG (mg/ml/min)	13543.61 ± 2548.54	14004.61 ± 2148.84	NS
HOMA-IR	3.42 ± 1.87	2.45 ± 1.03	< 0.001
QUCKI	0.328 ± 0.018	0.351 ± 0.021	< 0.001
Log HOMA	0.350 ± 0.019	0.238 ± 0.017	< 0.001
Homocysteine (μmol/l)	13.47 ± 5.80	10.28 ± 5. 47	< 0.001
Adiponectin (ng/ml)	3.49 ± 0.92	5.83 ± 1.32	< 0.001
Resistin (ng/ml)	9.48 ± 4.92	7.01 ± 3.49	< 0.05
Total-C (mg/dl)	214.72 ± 35.72	162.78 ± 32.56	< 0.005
LDL-C (mg/dl)	142.69 ± 26.83	90.41 ± 22.38	< 0.005
HDL-C (mg/dl)	41.26 ± 9.08	52.64 ± 10.35	< 0.05
Triglyceride (mg/dl)	160.18 ± 69.52	101.04 ± 43.62	< 0.005
Apoprotein A (mg/dl)	116.27 ± 24.03	146.19 ± 26.76	< 0.05
Apoprotein B (mg/dl)	133.59 ± 32.28	96.91 ± 24.72	< 0.005
Lipoprotein (a) (mg/dl)	27.13 ± 14.72	17.54 ± 9.38	< 0.005
Vitamin B_{12} (pg/ml)	292.65 ± 59.12	290.71 ± 46.87	NS
Folic acid (ng/ml)	13.02 ± 3.51	12.85 ± 3.76	NS

Values are mean ± SD.
For abbreviations, see Table I.

Table III. Clinical and hormonal characteristics of the $Sisters_{PCOS}$ and $Control_{Sisters}$ groups

	$Sisters_{PCOS}$ (n = 25)	Controls (n = 20)	P
Age (years)	23.50 ± 7.56	24.18 ± 6.81	NS
Body mass index (kg/m^2)	26.35 ± 6.32	25.83 ± 5.59	NS
Waist:hip ratio	0.82 ± 0.08	0.81 ± 0.07	NS
Systolic blood pressure (mmHg)	124.12 ± 9.69	124.62 ± 9.72	NS
Diastolic blood pressure (mmHg)	76.23 ± 6.28	76.37 ± 6.49	NS
Fasting insulin (μIU/ml)	17.29 ± 5.85	9.92 ± 2.01	< 0.001
ISI_{OGTT}	4.02 ± 2.91	5.82 ± 3.52	< 0.001
AUCI (μIU/ml/min)	9682.52 ± 1776.62	6213.34 ± 1265.78	< 0.001
AUCG (mg/ml/min)	13678.61 ± 2527.91	13122.87 ± 21145.23	NS
HOMA-IR	3.62 ± 1.39	2.26 ± 1.09	< 0.001
QUCKI	0.325 ± 0.019	0.351 ± 0.022	< 0.001
Log HOMA	0.351 ± 0.018	0.228 ± 0.019	< 0.001
Homocysteine (μmol/l)	10.58 ± 4.87	9.64 ± 4.26	NS
Adiponectin (ng/ml)	4.63 ± 1.79	6.48 ± 2.12	< 0.001
Resistin (ng/ml)	7.26 ± 3.61	6.81 ± 3.92	NS
Total-C (mg/dl)	162.50 ± 21.04	161.38 ± 19.06	NS
LDL-C (mg/dl)	90.58 ± 18.53	90.26 ± 16.88	NS
HDL-C (mg/dl)	54.50 ± 10.67	54.63 ± 10.95	NS
Triglyceride (mg/dl)	89.56 ± 21.68	85.14 ± 20.76	NS
Apoprotein A (mg/dl)	139.12 ± 26.87	138.65 ± 28.93	NS
Apoprotein B (mg/dl)	93.51 ± 32.36	96.23 ± 24.87	NS
Lipoprotein (a) (mg/dl)	24.65 ± 5.23	16.18 ± 6.51	< 0.005
Vitamin B_{12} (pg/ml)	299.54 ± 43.87	302.57 ± 35.89	NS
Folic acid (ng/ml)	13.98 ± 3.32	13.43 ± 3.12	NS

Values are mean ± SD.
For abbreviations, see Table I.

Adiponectin–resistin

While the serum resistin levels increased significantly in both $Fathers_{PCOS}$ ($P<0.05$) and $Mothers_{PCOS}$ ($P<0.05$), the resistin levels were similar in $Brothers_{PCOS}$ and $Sisters_{PCOS}$ ($P>0.05$) when compared to their control subjects. When subjects with GI were excluded, serum resistin levels were similar in both $Fathers_{PCOS}$ and $Mothers_{PCOS}$ compared to their control groups ($P>0.05$). However, the control group subjects had significantly elevated adiponectin levels compared to the first degree relatives of PCOS patients in all subgroups ($P<0.001$). Serum adiponectin levels showed a significantly negative correlation with BMI, W:H ratio,

Table IV. Clinical and hormonal characteristics of the $Brothers_{PCOS}$ and $Control_{Brothers}$ groups

	$Brothers_{PCOS}$ (n = 17)	Controls (n = 15)	P
Age (years)	29.00 ± 11.10	28.52 ± 8.74	NS
Body mass index (kg/m^2)	22.97 ± 4.58	23.37 ± 4.62	NS
Waist:hip ratio	0.80 ± 0.08	0.80 ± 0.07	NS
Systolic blood pressure (mmHg)	126.19 ± 9.75	124.98 ± 9.77	NS
Diastolic blood pressure (mmHg)	76.48 ± 6.53	76.73 ± 6.54	NS
Fasting insulin (μIU/ml)	16.18 ± 5.67	9.28 ± 1.97	< 0.001
ISI_{OGTT}	4.27 ± 2.86	6.39 ± 3.59	< 0.001
AUCI (μIU/ml/min)	9654.26 ± 987.34	6319.27 ± 698.36	< 0.001
AUCG (mg/ml/min)	12678.63 ± 1567.91	12651.73 ± 1476.29	NS
HOMA-IR	2.74 ± 1.12	2.06 ± 0.78	< 0.001
QUCKI	0.339 ± 0.019	0.352 ± 0.021	< 0.001
Log HOMA	0.281 ± 0.019	0.210 ± 0.016	< 0.001
Homocysteine (μmol/l)	9.87 ± 4.32	9.56 ± 4.35	NS
Adiponectin (ng/ml)	4.28 ± 1.39	6.97 ± 2.11	< 0.001
Resistin (ng/ml)	7.91 ± 2.43	7.18 ± 2.89	NS
Total-C (mg/dl)	212.76 ± 42.04	160.72 ± 23.84	< 0.005
LDL-C (mg/dl)	124.15 ± 38.73	90.74 ± 18.19	< 0.005
HDL-C (mg/dl)	51.25 ± 11.17	51.76 ± 10.38	NS
Triglyceride (mg/dl)	175.12 ± 65.27	101.39 ± 38.94	< 0.005
Apoprotein A (mg/dl)	126.50 ± 14.31	138.62 ± 20.15	NS
Apoprotein B (mg/dl)	154.03 ± 35.89	92.27 ± 18.99	< 0.005
Lipoprotein (a) (mg/dl)	24.25 ± 8.36	14.58 ± 6.14	< 0.005
Vitamin B_{12} (pg/ml)	288.41 ± 29.758	292.18 ± 35.89	NS
Folic acid (ng/ml)	12.96 ± 3.51	12.78 ± 3.76	NS

Values are mean ± SD.
For abbreviations, see Table I.

HOMA-IR, log HOMA, serum insulin and AUCI levels, and a positive correlation with ISI and QUCKI levels ($P<0.005$). However, no correlation between resistin and BMI, W:H ratio HOMA-R, AUCI, ISI, QUCKI, log HOMA and serum insulin measurements was found ($P>0.05$).

Homocysteine (Hcy), lipids and blood pressure

While the plasma Hcy levels increased significantly in both $Fathers_{PCOS}$ ($P<0.001$) and $Mothers_{PCOS}$ ($P<0.001$), the Hcy values in $Brothers_{PCOS}$ and $Sisters_{PCOS}$ ($P>0.05$) non-significantly increased when compared to their control subjects. No correlation was found between plasma Hcy and BMI, W:H ratio HOMA-R, AUCI, ISI, QUCKI, log HOMA and serum insulin measurements ($P>0.05$). Serum vitamin B_{12} and folic acid levels were similar in all groups ($P>0.05$).

Serum Total-C, LDL-C, Tg, Apo B and Lp (a) levels were significantly increased in $Fathers_{PCOS}$ subjects compared to the control group ($P<0.005$). However, their HDL-C and Apo A levels were lower than the control group ($P<0.05$). In $Mothers_{PCOS}$, Total-C, LDL-C, Tg, Apo B and Lp (a) ($P<0.005$) levels were higher when compared to the control group. However, their HDL-C and Apo A levels were similar when compared the control subjects. ($P>0.05$). In $Sisters_{PCOS}$ Total-C, Tg, LDL-C, HDL-C, Apo A and Apo B were similar when compared to control subjects ($P>0.05$). However, Lp (a) level was higher than control subjects ($P<0.005$). In $Brothers_{PCOS}$ Total-C, Tg, LDL-C, Apo B and Lp (a) levels were higher than the control subjects ($P<0.005$). However, their HDL-C and Apo A levels were similar when compared to the control subjects ($P>0.05$).

Compared to the control groups, the systolic and diastolic blood pressure measurements were higher in $PCOS_{Fathers}$ and $PCOS_{Mothers}$ groups ($P<0.05$); however, in $Brothers_{PCOS}$ and $Sisters_{PCOS}$ groups this measurements were similar in their control groups ($P>0.05$).

Ten patients (26.3%) with hyperlipidaemia and eight patients (20%) with hypertension were present in PCOS-Fathers group. Nine (22.5%) patients with hyperlipidaemia and nine (22.5%) patients with hypertension were present in the $PCOS_{Mothers}$ group. Two (10%) patients with hyperlipidaemia and two (10%) patients with hypertension were present in both $Control_{Mothers}$ and $Control_{Fathers}$ subgroups. These differences were statistically significant ($P<0.05$).

Discussion

The aim of the present study was to investigate the risk factors in the first degree relatives of PCOS patients for glucose tolerance disorders and CVD. The study not only investigated classical risk factors such as DM, IR, dyslipidaemia, hypertension, but also the more recently emerged risk factors such as serum Hcy and adiponectin. IR is central to the pathogenesis of both Type II DM and PCOS, with a strong genetic basis and important implications for the management of both disorders. Family studies of PCOS have focused mainly on ovarian morphology, menstrual disturbances, symptoms of hyperandrogenism, hyperandrogenaemia, glucose intolerance disorders and IR (Ferriman and Purdie, 1979; Carey *et al.*, 1993; Norman *et al.*, 1996; Pontiroli *et al.*, 2000; Colilla *et al.*, 2001; Kahsar-Miller *et al.*, 2001; Legro *et al.*, 2002; Sir-Petermann *et al.*, 2002; Yıldız *et al.*, 2003; Ehrman *et al.*, 2005). These studies have found higher insulin resistance, serum androgen levels and Type II DM as well as glucose tolerance disorder in the first degree relatives of PCOS patients compared to the control groups. In a previous study, defects in insulin action were suggested to persist in cultured skin fibroblasts from PCOS women (Dunaif *et al.*, 1995), suggesting that these abnormalities may have a genetic root. Likewise, there is evidence of heritability of insulin levels in monozygotic twins sharing polycystic ovary morphology (Jahanfar *et al.*, 1995). In the present study, the first degree relatives of PCOS women have been shown to have a high tendency for glucose intolerance. The combined prevalence rates for IFG, IGT and diabetes were 40% in the mothers and 52% in the fathers of PCOS women. These values were reported as 19.1% for females and 17% for males and females in similar age groups in a population-based study done in Turkey (Kelestimur *et al.*, 1999). The analysis of NGT family members in our study suggest that NGT mothers, fathers, brothers, and sisters of PCOS women have IR. These findings confirm the results of preliminary studies, which suggested that hyperinsulinaemia and IR may be an important marker in family members of PCOS patients. PCOS itself has been accepted as a major risk factor for the development of Type II DM. DM screening in patients with PCOS is recommended by current American Diabetes Association guidelines (Diabetes Care, 2002). Taken together, these findings suggest that NGT first degree relatives of women with PCOS can be predicted to be at risk for developing glucose intolerance.

Similarly, the family history of heart disease is consistent with those studies which demonstrated several risk factors for heart disease in PCOS women, such as obesity, insulin resistance, hyperlipidaemia, and raised plasminogen activator inhibitor-1 (Legro *et al.*, 2003). Whether these risk factors are genetic, programmed *in utero* or environmental is unclear. Few studies have investigated the risk of CVD in the first degree relatives of PCOS patients. One of these studies (Atiomo *et al.*, 2003) investigated CVD retrospectively and a high frequency was found. Another study (Kaushal *et al.*, 2004) focused on brothers only and suggested that endothelial dysfunction, elevated IR and dyslipidaemia pose a risk of CVD in the early stages. Adiponectin is highly and specifically expressed in differentiated adipocytes and circulates at high levels in the bloodstream. Adiponectin has been postulated to play an important role in the modulation of glucose and lipid metabolism in insulin-sensitive tissues.

Levels of adiponectin are also lower in patients with essential hypertension, DM, obesity and CVD (Ouchi *et al.*, 2003; Hotta *et al.*, 2000; Matsuzawa *et al.*, 2004). Some studies have found low levels of serum adiponectin in PCOS patients (Ducluzeau *et al.*, 2003; Panidis *et al.*, 2003; Spranger *et al.*, 2004) whereas the other studies did not support this finding (Orio *et al.*, 2003b, Orio *et al.*, 2004). The present study has

found low adiponectin levels in the first degree relatives of PCOS patients when compared to the age-, sex- and BMI-matched control groups. As low adiponectin levels constitute a risk factor for (CVD), these subjects are at high risk.

Homocysteine is an intermediate formed during the breakdown of the amino acid methionine; it may undergo remethylation to methionine, or trans-sulphuration to cystathione and cysteine. Previous studies suggest that Hcy as an independent risk factor for CVD (Clarke *et al.*, 1991). Additionally, treatment to decrease the Hcy level was also found to reduce the risk for CVD. In PCOS patients, increased Hcy levels may partly explain recent findings of early atherosclerosis and an increased risk of CVD as compared with healthy control subjects (Loverro *et al.*, 2002; Schachter *et al.*, 2003; Wijeyaratne *et al.*, 2004). However, Orio *et al.* (2003a) and Boulman *et al.* (2004) found no significant difference of Hey levels between the PCOS and control groups. While the present study found high levels of Hcy in the mother and father groups, no difference was detected in the sister and brother groups. Increased serum Hcy level is an independent risk factor for CVD, which may put these subjects at a higher risk of developing CVD.

Resistin is a novel protein, discovered in pre-adipocytes that undergo differentiation into mature adipocytes. Resistin has been reported to induce insulin resistance and IGT (Kim *et al.*, 2001; Kershaw and Flier, 2004). Resistin levels were found to be significantly elevated in $Fathers_{PCOS}$ and $Mothers_{PCOS}$ groups compared with their control groups. Resistin has been shown to promote endothelial cell activation, with increased expression of the adhesion molecule VCAM-1 and the chemotactic protein VCAM-1 (Verma *et al.*, 2003). Therefore, resistin can play a role in pathogenesis of cardiovascular diseases which develop in first degree relatives of PCOS. Dyslipidaemia may be the most common metabolic abnormality in PCOS, although the type and extent of the findings have been variable (Wild *et al.*, 2000; Legro, 2003). Although various lipid parameters were high, dyslipidaemia was seen in all groups. Dyslipidaemia is an accepted risk factor for CVD. Therefore, the first degree relatives of PCOS subjects should be examined for dyslipidaemia and treated with antilipidaemic treatment. The prevalences of hypertension and hyperlipidaemia were found to be significantly increased in first degree relatives of patients with PCOS. Along with low serum adiponectin levels and high homocystein and resistin levels, the findings may imply that first degree relatives of patients with PCOS are more prone to develop DM and have higher risk of CVD.

In the present study, control groups were selected from family members without history of DM or CVD. As is well known, Type II DM is hereditary. First degree relatives of patients with Type II DM have an increased prevalance of insulin resistance and related parameters. Furthermore, insulin resistance and hyperinsulinaemia are main constituents of metabolic syndrome, which in turn leads to increased CVD (Ezenwaka *et al.*, 2004; Nyholm *et al.*, 2004; Pankow *et al.*, 2004). It was for this reason that we selected such a control group, which may not represent the general population. This may be a limiting factor for the present study since CVD risk for relatives of patients with PCOS may be found relatively elevated.

As is known, PCOS patients have increased risk of developing DM and CVD. The present study found a high frequency of glucose intolerance disorders and CVD risk in the first degree relatives of PCOS patients when compared to the control group. This finding shows that the first degree relatives of PCOS patients need also to be closely examined for DM and CVD. We could thus take preventive measures against the development of disease in these subjects.

In conclusion, the results of the present study support the finding that, similar to PCOS patients, their first degree relatives also carry an increased risk of CVD. This points to a need for the first degree relatives of PCOS patients to be monitored for DM and CVD development, and suggests that preventive measures be taken. Further, larger-scale and longitudinal studies are needed in this field.

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