Evaluation of metabolic syndrome frequency and premature carotid atherosclerosis in young women with polycystic ovary syndrome

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BACKGROUND: The aim of this study was to evaluate metabolic syndrome frequency, cardiovascular risk profile and premature carotid artery atherosclerosis in patients with polycystic ovary syndrome (PCOS) especially during early adulthood. METHODS: A case–control study was conducted on 43 young women (18–22 years of age) with PCOS and 43 age-matched volunteer controls. Anthropometrical measurements, hormone levels, lipid and glucose profile were obtained from all subjects. Two different criteria were used to assess metabolic syndrome (MS) frequency. Common carotid artery intima-media thickness (IMT) was measured and stepwise multiple linear regression analysis was used to identify the independent cardiovascular risk factors that predict IMT. RESULTS: MS was diagnosed in 11.6% (n = 5) of women with PCOS compared to no cases in the control group (P = 0.02). The mean IMT was significantly higher in PCOS subjects (0.746 ± 0.106 mm) compared to controls (0.608 ± 0.105 mm, P < 0.001). Among many cardiovascular risk factors evaluated, the diagnosis of PCOS, increased body mass index and decreased sex hormone-binding globulin were significant independent predictors of increased IMT. CONCLUSIONS: These findings indicate that adolescence may be a more appropriate time to intervene for PCOS patients, as many cardiovascular risks are already present during early adulthood. As far as IMT is concerned, mechanisms other than hyperandrogenaemia and obesity might be operating as causative factors.

Key words: cardiovascular/metabolic syndrome/PCOS/risk

Introduction
Polycystic ovarian syndrome (PCOS) is one of the most common metabolic disturbances affecting ~5% of women (Guzick, 2004). Although not a diagnostic criteria for PCOS, these women frequently carry several risk factors for future coronary heart disease, stroke or type 2 diabetes, such as obesity, insulin resistance and dyslipidaemia (Rotterdam ESHRE–ASRM-sponsored PCOS consensus workshop group, 2004).

Metabolic syndrome is another cluster of endocrine disturbances including obesity, insulin resistance, dyslipidaemia and hypertension, predisposing the individual to greater risk for developing cardiovascular disease and type-2 diabetes (Kohen-Avramoglu et al., 2003). Metabolic syndrome affects 4–8% of adolescents according to the definition criteria used (Goodman et al., 2004). In a recent study, the prevalence of metabolic syndrome among 20–29 year old PCOS patients was reported to be 8-fold greater than age-stratified population controls (Apridonidze et al., 2004).

Carotid intima-media thickness has been positively associated with the prevalence and incidence of stroke and myocardial infarction (Bots et al., 1997). Middle-aged women >45 years of age and women ~30 years of age with PCOS were shown to have increased carotid intima-media thickness (IMT) when compared to controls (Talbott et al., 2000; Lakhani et al., 2004).

Despite the above studies, little is known about the pathophysiological basis and the time of onset of these clinically detectable changes. In this study, we aimed to evaluate metabolic syndrome prevalence and carotid IMT in the first 5 years of adulthood among women with PCOS.

Materials and methods
The study was designed as a prospective case–control study in Kocaeli University Department of Obstetrics and Gynecology between January 1, 2002 and January 1, 2004. The University Ethics Committee approved the study protocol and informed written consent was obtained from all the subjects. A total of 43 PCOS cases between 18 and 22 years of age and 43 age-matched control subjects were included.

The diagnosis of PCOS was made according to the criteria of the Rotterdam ESHRE–ASRM-sponsored PCOS consensus workshop group (2004) (Table I) when two out of three criteria was present:
The results are presented as numbers and percentages.

oligo/menorrhoea (fewer than six menstrual periods in the preceding year) and/or anovulation; clinical and/or biochemical signs of hyperandrogenism; presence of $\geq 12$ follicles in each ovary measuring 2–9 mm in diameter and/or increased ovarian volume (>10 ml). Type 2 diabetes, hyperprolactinaemia, hypogonadotropic hypogonadism, thyroid disease, congenital adrenal hyperplasia, androgen-secreting tumours and Cushing’s syndrome were ruled out by appropriate initial laboratory work-up. As all the patients were initially diagnosed to have PCOS, none had taken oral contraceptives, antiandrogens or any medication that could influence carbohydrate metabolism. Clinical evidence of hyperandrogenism was a Ferriman–Gallwey score $\geq 8$, indicating hirsutism and/or presence of acne (Gallwey, 1962). Biochemical hyperandrogenism was defined as total testosterone and free androgen index $>95$th percentile for the control group studied, which were 3.8 nmol/l and 7% respectively.

The control group consisted of regularly menstruating (26–32 day cycles) healthy, age-matched female medical students or nurses without evidence of above-mentioned endocrine disturbances based on the similar initial laboratory work-up as the PCOS patients. No restrictive diet was recommended and none of the subjects engaged in intensive aerobic activity. Women who had respiratory or cardiovascular disease, or who were taking medication such as aspirin that restricted diet was recommended and none of the subjects engaged in intensive aerobic activity. Women who had respiratory or cardiovascular disease, or who were taking medication such as aspirin that could influence vascular resistance, were excluded from PCOS cases and controls.

Initial physical examination included weight, height, waist and hip circumferences to calculate waist:hip ratio (WHR) and body mass index (BMI). Resting systolic (SBP) and diastolic (DBP) were measured by the same nurse with a sphygmomanometer in the supine position to allow pulse and blood pressure to stabilize. Ambient light and temperature were controlled throughout the procedure. The right and left common carotid artery, the carotid bifurcation–bulb areas were scanned from multiple planes. Images were obtained from the distal portions of both common carotid arteries, 1–2 cm proximal to the carotid bulb and immediately proximal to the origin of the bifurcation. IMT across 1 cm segments of the near and far walls of both common carotid arteries was measured as the distance between the junction of the lumen and intima and the junction of the media and adventitia and averaged. The intra-observer error was $<0.03$ mm.

Table I. The distribution of the clinical and biochemical diagnostic criteria of the polycystic ovary syndrome (PCOS) women compared to the controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>PCOS ($n = 43$)</th>
<th>Control ($n = 43$)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligo/amenorrhoea</td>
<td>43 (100)</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Clinical hyperandrogenism</td>
<td>38 (88)</td>
<td>8 (18)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hirsutism</td>
<td>38 (88)</td>
<td>5 (11)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Acne</td>
<td>8 (18)</td>
<td>6 (14)</td>
<td>0.6</td>
</tr>
<tr>
<td>Biochemical hyperandrogenism</td>
<td>31 (72)</td>
<td>2 (4.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total testosterone $&gt;3.8$ mmol/l</td>
<td>18 (42)</td>
<td>1 (2.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Free androgen index $&gt;7$%</td>
<td>29 (68)</td>
<td>1 (2.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Polycystic ovaries on ultrasound</td>
<td>41 (95)</td>
<td>4 (9)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The results are presented as numbers and percentages.

An oral glucose tolerance test (OGTT) was performed between 08:00 and 10:00 after a 3 day, 300 g carbohydrate diet and an overnight fast of 10–14 h. A 75 g oral glucose load was administered, and blood samples for glucose and insulin determinations were collected through an i.v. cannula at 0, 30, 60, 90 and 120 min. Serum glucose was measured by using glucokinase technique. Plasma insulin levels were measured by chemiluminescent enzyme immunoassay (Immulite 1000 Analyzer; Diagnostic Products Corp., Los Angeles, CA, USA) with intra- and inter-assay coefficients of variation $<6.4\%$. Lipid analysis in fasting serum was performed for all patients and included total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) and triglycerides. These parameters were measured by commercial enzymatic methods (Aeroset automated analyzer; Abbott laboratories, Abbott, IL, USA). OGTT results were evaluated according to the criteria of Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (Lepor, 2004); impaired fasting glucose if fasting plasma glucose level is 100–125 mg/dl, impaired glucose tolerance if 120 min plasma glucose is 140–199 mg/dl. A ratio of fasting glucose to fasting insulin $<4.5$ was diagnosed as insulin resistance (Legro et al., 1998). Homeostatic model assessment (HOMA) (Matthews et al., 1985) is applied by using the formula: insulin resistance (HOMA-IR) = [fasting insulin (IU/l) $\times$ fasting glucose (mmol/l)]/22.5.

All regularly menstruating women were scanned on cycle days 3–5 whereas oligo/amenorrhoeic women were scanned between days 3 and 5 after progesterin-induced withdrawal bleeding using Siemens Versa plus ultrasonography machine. The serum concentrations of estradiol ($E_2$), FSH, LH, testosterone, sex hormone-binding globulin (SHBG), prolactin, thyroid-stimulating hormone (TSH), cortisol, 17-OH-progesterone and dehydroepiandrosterone sulphate (DHEA-S) were measured by chemiluminescent enzyme immunoassay (Immulite 2000, Diagnostic Products Corp.). The intra- and inter-assay coefficients of variation were $<7\%$ for all assays performed. Free androgen index (FAI) was calculated using the following formula: testosterone (nmol/l)/SHBG (nmol/l) $\times 100$ (Morley et al., 2002).

An experienced sonographer (A.D.), who was unaware of the nature of the study and the diagnosis of the patients, measured the IMT of the common carotid artery. High resolution B-mode ultrasound images were obtained using a 7.5 MHz transducer (Power Vision 8000; Toshiba, Japan). The subjects rested for 15 min in supine position to allow pulse and blood pressure to stabilize. Blood pressure was measured as the distance between the junction of the lumen and intima and the junction of the media and adventitia and averaged. The intra-observer error was $<0.03$ mm.

Metabolic syndrome (MS) was assessed both by National Cholesterol Education Program Adult Treatment Panel III (NCEP) (Goodman et al., 2004) and by revised World Health Organization (WHO) definition (Balkau and Charles, 1999). Metabolic syndrome was diagnosed if three of the five factors were present: hypertension (diastolic $\geq 85$ mmHg, systolic $\geq 130$ mmHg), central obesity (waist $\geq 88$ cm), HDL $\leq 50$ mg/dl, triglycerides $\geq 150$ mg/dl, fasting glucose $\geq 110$ mg/dl). For WHO-defined MS, fasting glucose $\geq 110$ mg/dl or hyperinsulinaemia $\geq 20$ mlU/l was required plus two of the additional parameters: hypertension parameter (diastolic $\geq 85$ mmHg, systolic $\geq 130$ mmHg), central obesity parameter (WHR $>0.85$ and/or BMI $>30$ kg/m$^2$), dyslipidaemia parameter (HDL $\leq 39$ mg/dl and/or triglycerides $\geq 150$ mg/dl) and presence of microalbuminuria.

Statistical analysis

We analysed the distribution of continuous variables with the Shapiro–Wilks normality tests. Continuous variables were expressed as mean $\pm$ SD and compared using Student’s $t$-test between the groups. Categorical data were expressed as numbers (percentages) and compared by using the $\chi^2$-test or Fisher’s exact test where suitable. $P < 0.05$ was accepted as statistically significant. Stepwise multiple linear regression analysis was used to identify
The fasting insulin, fasting glucose and lipid levels and response to 75 g OGTT in women with and without PCOS are presented in Table III. Mean fasting insulin level and HOMA-IR level were significantly higher in PCOS patients while the fasting glucose:fasting insulin ratio was significantly lower than the control group. The criteria of insulin resistance or decreased insulin sensitivity such as fasting glucose:fasting insulin < 4.5 was significantly more frequent in the PCOS group when compared to the controls.

We found significantly lower mean HDL level and higher mean VLDL level in PCOS patients (Table III). Although the mean total cholesterol level was similar in the two groups, the frequency of women with total cholesterol:HDLC ratio > 5, which is a risk factor for coronary heart disease, was significantly higher in the PCOS group when compared to the control group.

Metabolic syndrome frequency according to NCEP criteria was very low among PCOS patients (2.3%), which was nil among the controls (Table IV). WHO criteria more frequently labelled women as the metabolic syndrome among PCOS patients (11.6%) compared to no cases among the control group. Carotid artery ultrasound measurements in women with and without PCOS revealed that the mean carotid artery IMT was significantly higher while the pulsatility index, resistance index and systole:diastole ratios were significantly lower in the PCOS group when compared to the control group (Table IV). The 10th, 50th and 90th percentiles of carotid artery IMT were 0.50, 0.60, 0.71 mm for the control group respectively and 0.65, 0.70, 0.88 mm for the PCOS group.

In the multivariate linear regression model, increased BMI ($\beta = 0.47$, $P < 0.001$), the diagnosis of PCOS ($\beta = 0.29$, $P = 0.01$) and decreased SHBG ($\beta = -0.28$, $P = 0.01$) were independent predictors of increased IMT (adjusted $R^2 = 0.57$, standard error of the estimate = 0.08).

### Discussion

Our findings suggest that young women with PCOS have more frequent cardiovascular risk factors such as increased...
than in the controls (Solomon relative risk for coronary heart disease was 1.5-fold higher in a study, using menstrual irregularity as a marker for PCOS, family (Kohen-Avramoglu lar level, suggesting different degrees of genetic and func-
tid artery intima-media thickness are already present in early

<table>
<thead>
<tr>
<th>Variable</th>
<th>PCOS (n = 43)</th>
<th>Control (n = 43)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic syndrome (NCEP)</td>
<td>1 (2.3)</td>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td>Metabolic syndrome (WHO)</td>
<td>5 (11.6)</td>
<td>0</td>
<td>0.02²a</td>
</tr>
<tr>
<td>Intima-media wall thickness</td>
<td>0.746 ± 0.106</td>
<td>0.608 ± 0.105</td>
<td>&lt;0.001b</td>
</tr>
<tr>
<td>(range in mm)</td>
<td>(0.50–1)</td>
<td>(0.45–0.80)</td>
<td></td>
</tr>
<tr>
<td>Pulsatility index</td>
<td>1.06 ± 0.44</td>
<td>1.73 ± 0.44</td>
<td>&lt;0.001b</td>
</tr>
<tr>
<td>Resistance index</td>
<td>0.54 ± 0.1</td>
<td>0.71 ± 0.11</td>
<td>&lt;0.001b</td>
</tr>
<tr>
<td>Systole/diastole</td>
<td>2.3 ± 0.3</td>
<td>3.7 ± 1.1</td>
<td>&lt;0.001b</td>
</tr>
</tbody>
</table>

Values are mean ± SD and numbers and percentages.

²Compared to control group, Fisher’s test.
³Compared to control group, Student’s t-test.

NCEP = National Cholesterol Education Program Adult Treatment Panel III; WHO = World Health Organization; PCOS = polycystic ovarian syndrome.

IMT and MS frequency compared to the controls. Although there is a clear association between MS and PCOS, which can be defined as intertwined insulin resistance syndromes, an underlying common and predictive vascular risk parameter both in PCOS and MS is unknown.

Studies on the association of PCOS and vascular diseases yielded conflicting results. In one study, deaths due to circu-
latory disease were found not to increase among women with PCOS (Pierpoint et al., 1998). In a follow-up study on PCOS patients, the same investigators found an insignificant increase in coronary heart disease but a significant increase in cerebrovascular disease (Wild et al., 2000). In a larger study, using menstrual irregularity as a marker for PCOS, relative risk for coronary heart disease was 1.5-fold higher than in the controls (Solomon et al., 2002).

We found that higher frequency of MS and increased caro-
tid artery intima-media thickness are already present in early adulthood in PCOS patients. This is not surprising as both MS and PCOS were linked to insulin resistance at the cellular level, suggesting different degrees of genetic and func-
tional defects, also affecting other members of the same family (Kohen-Avramoglu et al., 2003; Yildiz et al., 2003). Both PCOS and MS can be detected during adolescence (Kent and Legro, 2002; Goodman et al., 2004). Screening programmes including a family history may help to identify adolescents at risk for future cardiovascular and endocrine disturbances.

Inconsistent findings on the association between PCOS and cardiovascular disease suggest that therapeutic interventions such as oral contraceptive use and weight loss might modify the natural course of the disease. In addition, presence and degree of individual confounding variables such as BMI, WHR, smoking, hypertension, insulin resistance and dyslipi-
daemia, which are not diagnostic criteria for PCOS, although some are for MS, might contribute to the variable translation of risk factors into disease. Our finding that increased BMI, decreased SHBG and presence of PCOS are all independent predictors of increased IMT support these hypotheses. Viscoelastic disturbances in the arteries and endothelial dys-
fuction in PCOS patients may begin earlier than expected (Lakhani et al., 2002) and may be aggravated by long-
standing exposure to adverse hormonal, cytokine and lipid milieu or addition of absolute cardiovascular risk factors around menopause, after which coronary artery disease and stroke risk increases (Sutton-Tyrell et al., 1998; Matthews et al., 2001).

Screening for both syndromes has several benefits: (i) women with PCOS and the MS have increased hyperan-
drogenaemia, lower serum SHBG and more severe insulin resistance (Apridonidze et al., 2004); (ii) we can modify the progress of both syndromes by lifestyle modifications such as low fat diet and exercise programmes. Obese adolescents with PCOS have lower SHBG, HDL-cholesterol and higher fasting insulin, androgens, LDL-cholesterol compared to non-obese adolescents with PCOS (Silfen et al., 2003). Weight loss decreases LDL-cholesterol, fasting insulin, leptin and C-reactive protein (Brook et al., 2004). Also, oral contracep-
tive use with or without addition of metformin decreases BMI and WHR, improves insulin sensitivity, increases SHBG levels and decreases free androgen index significantly (Elter et al., 2002; Cibula et al., 2004). If these measures are taken during adolescences they can modify increased IMT during early adulthood, as we found that BMI, SHBG and PCOS are all significant predictors.

PCOS itself was an independent predictor of IMT in our study. Previous studies showed that, even in young women with PCOS, high basal insulin levels are correlated with non-
restrictive type of diastolic dysfunction and HOMA values are related to left ventricular mass index (Tiras et al., 1999; Orio et al., 2004a). Young normal weight PCOS patients also have higher C-reactive protein levels, which is an indicator of chronic inflammation and inversely related to endo-
thelium-dependent and -independent vasodilation (Tarkun et al., 2004). In a recent study, young, normal weight, non-
dyslipidaemic, non-hypertensive women with PCOS were shown to have higher endothelin-1 levels and common caro-
tid artery intima-media thickness (Orio, 2004b). Furthermore, a study conducted on women with PCOS aged <35 years stated that after adjusting for blood pressure, BMI, choles-
terol and insulin, PCOS remains an independent risk for arterial disease (Lakhani et al., 2004). Our findings further support this hypothesis and suggest that PCOS may cause increased IMT due to high C-reactive protein and endothelin levels. Similar inflammatory mechanisms have been suggested to explain the relationship between MS and prema-
ture atherosclerosis respectively (Tracy, 2003; Ridker et al., 2004).

We have found a higher mean IMT in both controls and PCOS group than reported by other small sample-sized studies on 12, 20 and 30 young women (Lakhani et al., 2002, 2004; Orio et al., 2004b). As our sample size (n = 43 in both groups) is also relatively small, it is always prone to some selection errors. Also, our centre is a tertiary referral centre where mostly severe cases of PCOS with clinical hyperandro-
genaemia are referred which might explain the differences in populations. As all cases were initial diagnosis of PCOS, none had any treatment up to study date. In addition to these, our diagnosis of PCOS was made according to recently formulated Rotterdam ESHRE–ASRM criteria, which was not the case in other studies.
On the other hand, our measurements are not out of line. In a cross-sectional study, Stein et al. (2004) found that carotid IMT increased by 0.005 mm/year (CI: 0.002–0.008) in white females; 50th percentile was 0.61 mm in a 25 year old white female. Our control group is an average of 5 years younger and the 50th percentile can be expected to be ~0.60 mm, which was 0.61 mm in our study. The 90th percentile of carotid IMT in 25 year old white females was 0.759 (Stein et al., 2004), which can be estimated to be ~0.73 in a group composed of women 5 years younger. Our finding of 0.746 mm average carotid IMT indicates that almost half of the young women with PCOS have IMT above the 90th percentile.

We conclude that cardiovascular risk factors such as metabolic syndrome and increased common carotid artery intima-media thickness are already present as early as the first few years after adolescence. Screening during adolescence and controlling the BMI and PCOS with exercise or medical therapies may alter and modify the risk factors for future cardiovascular and cerebrovascular disease.

References
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