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Androgen excess is associated with the increased carotid intima-media thickness observed in young women with polycystic ovary syndrome

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BACKGROUND: We evaluated carotid intima-media thickness (CIMT) as an early marker of atherosclerosis, as well as its main determinants among androgen excess, obesity and insulin resistance, in patients with polycystic ovary syndrome (PCOS). METHODS: We selected 40 PCOS patients and 20 non-hyperandrogenic women who were similar in terms of age and grade of obesity. Complete clinical, metabolic and hormonal profiles and left common CIMT measurements were obtained. RESULTS: Patients with PCOS presented with increased mean CIMT values when compared with controls ($F = 8.575$; $P = 0.005$), and this was independent of obesity. Five PCOS patients but no controls had increased CIMT values. CIMT correlated directly with serum total and free testosterone, androstenedione and dehydroepiandrosterone-sulfate levels and mean 24-h heart rate (HR), and inversely with the insulin sensitivity index (ISI), but no correlation was observed with the body mass index (BMI). Multiple stepwise linear regression models showed that in PCOS patients, the main determinants of CIMT were serum total testosterone or androstenedione concentrations, with no influence of ISI or the mean 24-h HR. CONCLUSIONS: Compared with control women, PCOS patients present with an increased CIMT, independent of obesity and related directly to androgen excess; this suggests that hyperandrogenism is associated with atherosclerosis and cardiovascular risk in these women.

Keywords: hyperandrogenism; androgen excess; obesity; insulin resistance; atherosclerosis

Introduction

Classic and non-classic risk factors for atherosclerotic cardiovascular disease, including obesity and insulin resistance (Gambineri and Pasquali, 2006), disorders of glucose tolerance (Legro *et al*., 1999), dyslipidemia (Legro *et al*., 2001), disordered blood pressure regulation (Luque-Ramírez *et al*., 2007), low-grade chronic inflammation (Kelly *et al*., 2001) and a prothrombotic state (Yildiz *et al*., 2002), cluster in patients with the polycystic ovary syndrome (PCOS).

Early atherosclerotic lesions are characterized by accumulation of lipid-rich macrophages and proliferation of smooth muscle cells within the intima layer of the arterial walls, resulting in reduced arterial elasticity and progressive narrowing of the lumen (Rackley, 2007). Ultrasound measurement of the common carotid intima-media thickness (CIMT) is a validated, sensitive and specific non-invasive marker of the early preclinical phases of systemic atherosclerosis and an increased CIMT is an independent predictor of the occurrence of major cardiovascular events later in life (Lorenz *et al*., 2007).

Age, body mass index (BMI), blood pressure, male sex and low-density lipoprotein (LDL) cholesterol are among the many determinants of increased CIMT in young adults in the general population (Rajala *et al*., 2002; Oren *et al*., 2003). Not surprisingly, CIMT has been reported to be increased in PCOS patients (Guzick *et al*., 1996; Talbott *et al*., 2000, 2004a), who might also develop coronary artery calcification more frequently than non-hyperandrogenic women (Talbott *et al*., 2004b). Although obesity, insulin resistance and chronic inflammation appear to contribute to these carotid artery abnormalities, it is noteworthy that PCOS is also independently associated with increased CIMT and coronary artery calcification (Talbott *et al*., 2004a,b).

In the present study, we aimed to delineate the relative importance that obesity, insulin resistance and androgen excess play on the increase in CIMT observed in PCOS by comparing a group of young PCOS patients with a control group of healthy women selected in order to be similar in terms of age, obesity and frequency of smokers.

Materials and Methods

Patients

We recruited 40 consecutive women presenting with PCOS. The diagnosis of PCOS was based on the presence of clinical and/or biochemical hyperandrogenism, oligo-ovulation and exclusion of secondary etiologies (Zawadzki and Dunaif, 1992). Hirsutism was defined by a modified Ferriman–Gallwey score of >7 (Hatch *et al*., 1981), and oligomenorrhea [more than six cycles longer than 36 days in the previous year (Goodman, 1999)], amenorrhea [absence of menstruation for three consecutive months (Goodman, 1999)] and luteal phase progesterone measurements <12.7 nmol/l in women with regular menstrual cycles were considered indicative of oligo-ovulation. We also excluded hyperprolactinemia (serum prolactin levels below 24 μg/l), thyroid dysfunction (serum thyrotropin levels within the normal range), congenital adrenal hyperplasia (1–24 adrenocorticotropin-stimulated serum 17-hydroxyprogesterone levels below 30 nmol/l) and virilizing tumors in all the patients.

The control group of 20 women, selected in order to be similar in terms of age and BMI with the PCOS group, was composed by 8 healthy female volunteers and 12 consecutive patients attending the clinical practices of the authors solely for treatment of obesity. None of the controls had signs or symptoms of hyperandrogenism, menstrual dysfunction, a history of infertility or any comorbidity associated with obesity. None of the patients or controls had either a personal history of hypertension or cardiovascular events, sleep apnea, nor had any received treatment with oral contraceptives, antiandrogens, insulin sensitizers, statins or antihypertensive drugs for the previous six months.

Data from most of the patients and controls regarding the impact of obesity on blood pressure regulation in PCOS have been reported previously (Luque-Ramírez *et al*., 2007). Written informed consents were obtained from all the participants, and the study was approved by the local Ethics Committee.

Study protocol

Clinical and anthropometrical variables, including the hirsutism score, BMI and waist-to-hip ratio (WHR) were determined by a single investigator in all the subjects. The WHR was calculated by dividing the minimal waist circumference by the hip circumference at the level of greater trochanters, using a non-stretchable measuring tape. The percentage of body fat respect to total body weight was estimated using a validated (Loy *et al*., 1998) body fat monitor (Omron BF 300, Omron Corporation, Kyoto, Japan).

Serum and plasma samples were obtained between Days 5 and 10 of the menstrual cycle, or during amenorrhea after excluding pregnancy. After a 3-day, 300-g carbohydrate diet and 12-h overnight fasting, samples were obtained for the measurement of total testosterone, 17-hydroxyprogesterone, androstenedione, dehydroepiandrosterone-sulfate (DHEAS), prolactin, thyrotropin, sex hormone-binding globulin (SHBG) and calculated free testosterone (Vermeulen *et al*., 1999). A complete hemogram, serum biochemistry and lipid profiles were also obtained. Then, a 75-g oral glucose tolerance test was performed, and samples were obtained for measurement of serum insulin and plasma glucose at 0, 30, 60, 90 and 120 min. Samples were immediately centrifuged, and serum was separated and frozen at −20°C until assayed as described elsewhere (Escobar-Morreale *et al*., 1997, 2000). The composite insulin sensitivity index (ISI) and the areas under curve (AUC) for glucose and insulin were calculated from the circulating glucose and insulin concentrations during the oral glucose tolerance test as previously described (Tai, 1994; Matsuda and DeFronzo, 1999).

Blood pressure measurement

Ambulatory blood pressure monitoring was performed for 24 h with an A&D TM2430EX oscillometric device (A&D Company Ltd., Tokyo, Japan). Systolic (SBP), diastolic (DBP) and mean blood pressure (MBP), as well as HR, were measured every 20 min during daytime and every 30 min during nighttime. The data regarding ambulatory blood pressure monitoring have been previously reported in detail (Luque-Ramírez *et al*., 2007).

Common CIMT measurement

Imaging was conducted using a high-resolution 7.5-MHz phased-array transducer (Imagepoint-Hx, Hewlett-Packard, Andover, MA, USA) by the same trained operator (C.M.-A.) in all the PCOS patients and controls. Under controlled light and temperature conditions, studies were performed by positioning the women in a supine position with a 35 degrees incline of the head and torso, and a 45 degrees right-turn of the head. The left common carotid artery was explored in B-mode in longitudinal and transversal planes, to rule out the presence of plaque that might interfere with CIMT measurements. The posterior carotid wall at 1 cm of the common carotid bulb was imaged and CIMT was estimated by visual assessment of the distance between the lumen/intima and intima/adventicia interphases in longitudinal frames acquired during arterial diastole as described (Pignoli *et al*., 1986). The intraobserver coefficient of variation for CIMT was 10.8%. Increased CIMT was defined by values at or above the 75th percentile of the CIMT measurements of women in the same age range derived from a separate Spanish community cohort composed of 34 healthy ≤35-year-old women (mean CIMT: 0.41 mm, SD: 0.10 mm, 75th percentile: 0.53 mm) and 24 36–45-year-old women (mean CIMT: 0.47 mm, SD: 0.13 mm, 75th percentile: 0.58 mm) (Junyent *et al*., 2005) who were similar to our population of PCOS patients and controls in terms of race, ethnic and geographic background.

Statistical analysis

Data are shown as means ± SD, or raw numbers and percentages, as appropriate. For continuous variables, normality was assessed by the Kolmogorov–Smirnov test, and logarithmic or square root transformations were applied as needed to ensure a normal distribution. The comparisons between PCOS patients and controls were analysed by unpaired *t*-test.

Obesity was graded into three groups according to BMI: lean (BMI <25.0 kg/m^2), overweight (BMI 25.0–29.9 kg/m^2) and obese (BMI ≥30.0 kg/m^2). We used a general linear model to evaluate the differences in continuous variables depending on the grade of obesity and patient or control status, and the possible interaction between these independent variables. The differences between the grades of obesity were then identified by the Bonferroni's test for multiple comparisons. For discontinuous variables, the χ^2 test or Fisher's exact test were applied, as appropriate. The relationship between continuous variables and the CIMT were assessed by Pearson's correlation analysis. Multiple linear regression analysis using a stepwise method (probability for entry ≤0.05, probability for removal ≥0.10) for the introduction of independent variables was used to identify the main determinants of CIMT values among the variables showing a statistically significant correlation with this atherosclerosis marker. This method involves the sequential introduction of independent variables as follows: in stage one, the independent variable best correlated with the dependent variable is included in the equation; in the second stage, the next independent variable with the highest partial correlation with the dependent variable, controlling for the first independent variable, is entered. This process is repeated, at each stage partialling for

previously entered independent variables, until the addition of a remaining independent does not increase the coefficient of determination (R^2, which indicates the proportion of the variability in the dependent variable explained by the model) by a significant amount or until all independent variables are entered. Analyses were performed using SPSS 10 for Macintosh (SPSS Inc, Chicago, Illinois). $P < 0.05$ was considered statistically significant.

Results

The groups of patients with PCOS and non-hyperandrogenic controls had similar frequencies of smokers [lean: four PCOS patients (36.4%) versus three controls (37.5%), $\chi^2 = 0.003$, $P > 0.995$); overweight: six PCOS patients (46.2%) versus three controls (75%), $\chi^2 = 1.022$, $P = 0.576$; obese: seven PCOS patients (43.8%) versus three controls (37.5%), $\chi^2 = 0.086$, $P > 0.995$)] and of subjects with a family history of cardiovascular disease [lean: one PCOS patient (9.1%) versus 0 controls (0.0%), $\chi^2 = 0.768$, $P > 0.995$; overweight: four PCOS patients (30.8%) versus 0 controls (0.0%), $\chi^2 = 1609$, $P = 0.519$; obese: three PCOS patients (20.0%) versus three controls (37.5%, $\chi^2 = 0.829$, $P = 0.621$)].

PCOS patients presented with increased serum total and free testosterone, androstenedione and DHEAS levels and insulin AUC of the oral glucose tolerance test and reduced ISI, compared with non-hyperandrogenic controls (Table I).

There was no interaction between the PCOS or control status and the grade of obesity of the women studied here for any of the continuous variables studied here (Table II), meaning that the differences between patients and controls were similar in lean, overweight and obese women and, conversely, the differences between lean, overweight and obese women were equally present in PCOS patients and controls.

There was a graded increase in BMI, waist circumference, WHR and fat mass, and a graded decrease in the ISI with increasing grades of obesity, reaching statistically significant differences between lean, overweight and obese women (Table II). Both overweight and obese women had similarly reduced serum SHBG and high-density lipoprotein (HDL) cholesterol levels compared with lean women (Table II), whereas serum free testosterone, triglycerides and the AUC of insulin during the oral glucose tolerance test were higher only in the obese women compared with lean and overweight groups (Table II). Finally, compared with lean individuals, overweight women presented with increased serum LDL cholesterol levels, and obese women had increased mean 24-h SBP and HR readings in ambulatory blood pressure monitoring and an increased AUC of glucose during the oral glucose tolerance test (Table II).

Five PCOS patients (four were overweight and one was obese), but no control, presented with CIMT values above the reference range. Furthermore, as shown in Table I and Fig. 1, the mean CIMT values were increased in PCOS patients compared with controls ($t = 2.939$; $F = 8.575$; $P = 0.005$); this was independent of obesity ($F = 0.757$; $P = 0.474$ for the interaction) and CIMT did not change as a function of the grade of obesity ($F = 0.111$; $P = 0.895$).

When considering PCOS patients and non-hyperandrogenic control women as a whole, CIMT values showed direct correlations with serum androgens and the mean 24-h HR and an inverse correlation with the ISI, but no correlation with the BMI (Table III).

In order to explore which of these variables was the major determinant of CIMT in our population, we used multivariate linear regression models in which CIMT values were

Table I. Anthropometric variables, hormone and lipid profiles, blood pressure measurements and CIMT of PCOS patients and control women.

	Patients ($n = 40$)	Controls ($n = 20$)	P-value
Age (years)	24.5 ± 5.8	27.2 ± 6.8	0.121
BMI (kg/m^2)	29.4 ± 6.3	28.2 ± 6.9	0.526
Waist circumference (cm)	86 ± 16	84 ± 14	0.667
WHR	0.81 ± 0.10	0.78 ± 0.08	0.273
Fat mass (% of body weight)	32.5 ± 7.5	32.0 ± 9.0	0.797
Hirsutism score	11 ± 5	2 ± 2	<0.001
Total testosterone (nmol/l)	2.0 ± 0.7	1.2 ± 0.4	<0.001
SHBG (nmol/l)	32 ± 17	40 ± 16	0.073
Free testosterone (pmol/l)	42 ± 21	20 ± 6	<0.001
Androstenedione (nmol/l)	12.7 ± 3.6	7.2 ± 2.2	<0.001
DHEAS (μmol/l)	6.6 ± 2.7	4.0 ± 1.7	<0.001
AUC—insulin (pmol/l/120 min)	67 554 ± 38 983	34 835 ± 14 602	<0.001
AUC—glucose (mmol/l/120 min)	847 ± 180	843 ± 135	0.918
ISI	4.7 ± 3.7	8.7 ± 4.7	<0.001
Total cholesterol (mmol/l)	4.1 ± 0.6	4.1 ± 0.8	0.805
HDL cholesterol (mmol/l)	1.1 ± 0.3	1.1 ± 0.3	0.494
LDL cholesterol (mmol/l)	2.6 ± 0.6	2.7 ± 0.8	0.618
Triglycerides (mmol/l)	1.1 ± 0.8	0.8 ± 0.4	0.282
Mean 24-h SBP (mmHg)	114 ± 11	113 ± 10	0.876
Mean 24-h DBP (mmHg)	68 ± 7	70 ± 7	0.260
Mean 24-h MBP (mmHg)	82 ± 9	84 ± 8	0.489
Mean 24-h HR (bpm)	75 ± 10	76 ± 9	0.921
CIMT (mm)	0.41 ± 0.11	0.33 ± 0.08	0.005

Data are means ± SD. Data were submitted to unpaired t-test. AUC, area under of the curve of the oral glucose tolerance test; DHEAS, dehydroepiandrosterone-sulfate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure.

Table II. Anthropometric variables, hormone and lipid profiles, and blood pressure measurements of PCOS patients and control women, classified according to their grade of obesity.

	Lean		Overweight		Obese		PCOS versus controls		Grade of obesity		Interaction	
	Patients ($n = 11$)	Controls ($n = 8$)	Patients ($n = 13$)	Controls ($n = 4$)	Patients ($n = 16$)	Controls ($n = 8$)	F	P-value	F	P-value	F	P-value
Age (years)	23.0 ± 5.4	24.8 ± 6.0	23.6 ± 4.6	29.3 ± 10.3	26.3 ± 6.7	28.5 ± 5.8	3.38	0.071	1.67	0.197	0.42	0.662
BMI (kg/m^2)	22.2 ± 2.0	21.3 ± 1.3	27.5 ± 1.8	27.4 ± 1.5	35.8 ± 3.9	35.5 ± 3.2	0.31	0.583	132.65	<0.001*,**,***	0.09	0.912
Waist circumference (cm)	70 ± 7	71 ± 7	81 ± 9	80 ± 4	100 ± 13	98 ± 8	0.05	0.825	49.78	<0.001*,**,***	0.15	0.862
WHR	0.73 ± 0.06	0.73 ± 0.06	0.79 ± 0.07	0.79 ± 0.04	0.88 ± 0.09	0.83 ± 0.08	0.60	0.443	15.21	<0.001*,**,***	1.01	0.344
Fat mass (% of body weight)	23.4 ± 5.0	22.7 ± 3.9	31.9 ± 2.5	32.0 ± 1.0	39.3 ± 3.0	41.2 ± 3.4	0.20	0.658	121.91	<0.001*,**,***	0.72	0.493
Total testosterone (nmol/l)	1.9 ± 0.7	1.4 ± 0.4	2.0 ± 0.7	0.9 ± 0.2	2.2 ± 0.7	1.1 ± 0.2	26.23	<0.001	0.68	0.509	1.31	0.279
SHBG (nmol/l)	49 ± 18	52 ± 11	29 ± 6	43 ± 15	21 ± 13	27 ± 11	3.94	0.052	21.64	<0.001**,***	0.71	0.486
Free testosterone (pmol/l)	28 ± 12	19 ± 5	38 ± 13	14 ± 1	55 ± 24	23 ± 7	25.31	<0.001	6.08	0.004*,**	2.84	0.067
Androstenedione (nmol/l)	12.0 ± 3.6	8.5 ± 2.0	12.5 ± 3.9	5.4 ± 1.1	13.5 ± 3.4	6.8 ± 2.2	40.38	<0.001	0.74	0.483	1.64	0.203
DHEAS (μmol/l)	5.5 ± 2.9	4.2 ± 2.2	7.6 ± 2.4	4.2 ± 1.9	6.5 ± 2.6	3.7 ± 0.9	13.58	0.001	0.81	0.452	0.85	0.435
AUC—insulin (pmol/l/120 min)	47 953 ± 26 631	24 520 ± 7312	53 252 ± 21 883	33 466 ± 6620	98 243 ± 43 715	48 719 ± 14 363	14.64	<0.001	9.50	<0.001*,**	1.50	0.232
AUC—glucose (mmol/l/120 min)	770 ± 169	759 ± 89	793 ± 92	939 ± 150	943 ± 204	877 ± 131	0.27	0.603	4.66	0.014**	1.90	0.159
ISI	7.8 ± 4.8	12.7 ± 4.1	5.0 ± 2.7	9.0 ± 1.1	2.3 ± 1.0	4.5 ± 1.6	19.50	<0.001	26.60	<0.001*,**,***	1.15	0.324
Total cholesterol (mmol/l)	3.9 ± 0.6	3.8 ± 0.4	4.0 ± 0.7	4.8 ± 1.1	4.3 ± 0.6	4.2 ± 0.9	0.90	0.348	3.04	0.056	1.95	0.152
HDL cholesterol (mmol/l)	1.4 ± 0.4	1.2 ± 0.3	1.0 ± 0.2	1.2 ± 0.2	0.9 ± 0.2	1.0 ± 0.2	0.34	0.562	7.75	0.001**,***	2.46	0.095
LDL cholesterol (mmol/l)	2.2 ± 0.5	2.3 ± 0.5	2.6 ± 0.7	3.3 ± 1.0	2.8 ± 0.6	2.7 ± 0.7	1.53	0.222	4.78	0.012***	1.28	0.288
Triglycerides (mmol/l)	0.7 ± 0.3	0.6 ± 0.2	0.8 ± 0.3	0.7 ± 0.1	1.5 ± 1.0	1.0 ± 0.4	1.69	0.200	7.34	0.002*,**	0.62	0.542
Mean 24-h SBP (mmHg)	106 ± 8	111 ± 7	115 ± 8	114 ± 13	118 ± 12	116 ± 11	0.03	0.853	3.77	0.029**	0.75	0.475
Mean 24-h DBP (mmHg)	64 ± 6	67 ± 4	69 ± 7	69 ± 9	69 ± 7	73 ± 8	1.66	0.203	3.13	0.052	0.17	0.847
Mean 24-h MBP (mmHg)	77 ± 7	81 ± 5	84 ± 7	84 ± 10	84 ± 11	87 ± 9	0.75	0.391	2.40	0.100	0.22	0.802
Mean 24-h HR (bpm)	70 ± 6	70 ± 8	75 ± 9	77 ± 10	79 ± 11	81 ± 8	0.21	0.649	5.93	0.005**	0.05	0.949

Data are means ± SD. Data were submitted to two-way analysis of variance followed by the Bonferroni's test. *$P < 0.05$ comparing obese with overweight women; **$P < 0.05$ comparing obese with lean women; ***$P < 0.05$ comparing overweight with lean women. SHBG, sex hormone-binding globulin.

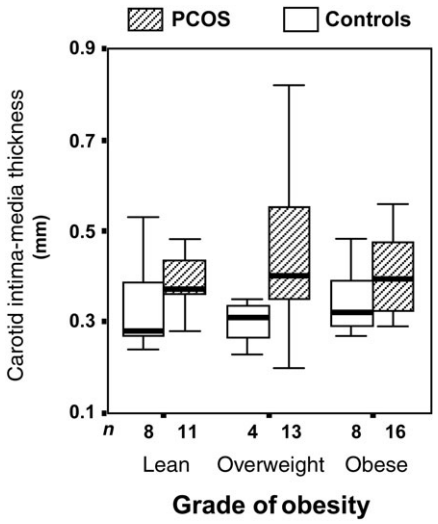

Figure 1: CIMT depending on PCOS status and grade of obesity Data were analysed by a general linear model including PCOS status and grade of obesity as independent variables. The box-plot includes the median (horizontal line) and the interquartile range (box), and the whiskers indicate the fifth and 95th percentiles. CIMT was increased in PCOS patients compared with controls ($F = 8.575$; $P = 0.005$); this was independent of obesity ($F = 0.757$; $P = 0.474$ for the interaction) and CIMT did not change as a function of the grade of obesity ($F = 0.111$; $P = 0.895$)

Table III. Determinants of left common CIMT by Pearson's correlation analysis considering patients and controls as a whole.

Variables	Coefficient of correlation (r)	P-value
Age	0.020	0.880
BMI	0.114	0.385
Waist circumference	0.094	0.476
WHR	0.208	0.110
Fat mass	0.093	0.483
Total testosterone	0.461	<0.001
SHBG	−0.198	0.129
Free testosterone	0.404	0.001
Androstenedione	0.576	<0.001
DHEAS	0.271	0.036
AUC—insulin	0.226	0.082
AUC—glucose	0.044	0.740
ISI[a]	−0.260	0.045
Total cholesterol	0.011	0.934
HDL cholesterol	−0.185	0.157
LDL cholesterol	0.119	0.368
Triglycerides[a]	−0.041	0.758
Mean 24-h SBP	0.156	0.238
Mean 24-h DBP	0.138	0.299
Mean 24-h MBP	0.133	0.316
Mean 24-h HR	0.281	0.031

[a]Logarithmic transformation was applied to these variables in order to ensure normality before correlation analysis.

introduced as the dependent variable, and serum androgen concentrations (either total or free testosterone or androstenedione levels were evaluated in separate models, because serum androgen levels were not independent of one another), the ISI and the mean 24-h HR were introduced as independent variables using a stepwise method. After evaluating the relative contributions of the independent variables to the variability in CIMT, while controlling for all of them at each step of the regression model, only serum androgen levels and 24-h HR were retained as statistically significant contributors to the variability in CIMT, whereas the ISI was excluded from all models (Table IV).

We then repeated the linear regression analysis considering PCOS and control groups separately. In PCOS patients, the models retained only serum androgen levels as significant predictor of CIMT (with the exception of the model containing free testosterone levels as dependent variable, which did not reach statistical significance, Table IV), whereas in non-hyperandrogenic controls the models retained only the mean 24-h HR as significant predictor of this atherosclerosis marker (Table IV). Of note, the ISI was not retained by any stepwise regression model.

Discussion

Our present results show that CIMT is increased in young PCOS patients when compared with non-hyperandrogenic women, and that this increase is not related to the frequent association of this syndrome with increased weight and obesity (Alvarez-Blasco *et al*., 2006; Gambineri and Pasquali, 2006). The fact that CIMT values reached values above the reference range only in a minority of PCOS patients may be related to the use of a population-based cohort to establish the reference range. Because the population-based cohort was not as carefully selected as the study groups in order to be similar to the PCOS patients in terms of age, weight and prevalence of smokers (additionally hyperandrogenism was not ruled out in these control women), the impact of androgen excess on CIMT may have been even underestimated by the direct comparison with the reference range. Nevertheless, it should be noted that the relationship between CIMT and cardiovascular risk is continuous (Lorenz *et al*., 2007) and therefore, the increased mean CIMT values in PCOS patients provide further evidence of subclinical cardiovascular disease in these women. In conceptual agreement, an increased prevalence of coronary artery calcification has been reported in PCOS patients (Christian *et al*., 2003; Talbott *et al*., 2004b).

Our results strongly suggest that androgen excess, which is central to the pathogenesis of PCOS (Azziz *et al*., 2006), is the major determinant of increased CIMT in these women. Further analysis of the data confirmed that CIMT correlates with serum androgen levels, HR and with insulin resistance (given the inverse correlation with the ISI). Importantly, other classic cardiovascular risk factors such as obesity, abdominal adiposity, circulating lipid levels and blood pressure were not related to CIMT in the young women studied here.

Furthermore, when studying patients and controls as a whole, only serum androgen levels and HR determined CIMT in a stepwise multiple regression model and, on the contrary, the influence of insulin resistance was no longer maintained when considering simultaneously the influence of androgens and HR. Of note, serum androgen levels and average 24-h HR explained 22–39% of the variability in CIMT in the young women studied here. Furthermore, serum androgen levels appear to be a main determinant of CIMT

Table IV. Multivariate regression models predicting CIMT from serum androgen levels, ISI and mean 24-h HR.

	Dependent variables		
Independent variable: CIMT	TT, ISI, 24-h HR	FT, ISI, 24-h HR	A, ISI, 24-h HR
PCOS patients & Controls ($n = 60$)			
Model statistics	$R^2 = 0.295$, $P < 0.001$	$R^2 = 0.219$, $P = 0.001$	$R^2 = 0.388$, $P < 0.001$
Predictive variable	TT, $\beta = 0.465$, $P < 0.001$ 24-h HR, $\beta = 0.282$, $P = 0.015$	FT, $\beta = 0.376$, $P = 0.003$ 24-h HR, $\beta = 0.240$, $P = 0.048$	A, $\beta = 0.557$, $P < 0.001$ 24-h HR, $\beta = 0.239$, $P = 0.026$
PCOS patients separately ($n = 40$)			
Model statistics	$R^2 = 0.163$, $P = 0.011$	$R^2 = 0.135$, $P = 0.163$	$R^2 = 0.316$, $P < 0.001$
Predictive variable	TT, $\beta = 0.404$, $P = 0.011$	None	A, $\beta = 0.562$, $P < 0.001$
Controls separately ($n = 20$)			
Model statistics	$R^2 = 0.211$, $P = 0.041$	$R^2 = 0.211$, $P = 0.041$	$R^2 = 0.211$, $P = 0.041$
Predictive variable	24-h HR, $\beta = 0.460$, $P = 0.041$	24-h HR, $\beta = 0.460$, $P = 0.041$	24-h HR, $\beta = 0.460$, $P = 0.041$

Dependent variables were introduced using a stepwise (probability for entry ≤ 0.05, probability for removal ≥ 0.10) model. After evaluating the relative contributions of the independent variables to the variability in CIMT, while controlling for all of them at each step, the ISI was not retained by any stepwise model. A, serum androstenedione levels; β, standardized regression coefficient; CIMT, carotid intima-media thickness; FT, serum free testosterone levels; HR, heart rate; ISI, insulin sensitivity index; R^2, coefficient of determination, TT, serum total testosterone levels.

when studying PCOS patients separately (explaining 16–32% of its variability), whereas in non-hyperandrogenic women, it is the HR which is the main determinant of this early marker of atherosclerosis (explaining 21% of its variation). Increased HR is a marker of sympathetic hyperactivity (Grassi *et al*., 1998), which is a recognized mechanism in the pathogenesis of atherosclerosis and vascular damage (Pauletto *et al*., 1991).

In conceptual agreement with the role of androgen excess in the increase in CIMT in PCOS, *in vitro* studies indicate that testosterone exerts proatherogenic effects on macrophage function by facilitating the uptake of modified lipoproteins, yet these effects are partially counterbalanced by an antiatherogenic effect by stimulating efflux of cellular cholesterol to HDL (Wu and von Eckardstein, 2003). Moreover, administration of testosterone to female cynomolgus monkeys clearly results in exacerbation of diet-induced atherosclerosis (Adams *et al*., 1995) although in humans, the precise role of endogenous androgens in the development of atherosclerosis is unclear at present, despite the obvious sex-specific differences in atherosclerotic cardiovascular disease (Wu and von Eckardstein, 2003).

On the contrary, considering that the mean CIMT values were increased in PCOS patients irrespective of the grade of obesity, being present in lean, overweight and obese patients, obesity did not exert a statistically significant influence on CIMT in our series of young women, in sharp contrast with the major influence of obesity on the abnormalities in blood pressure regulation that we have recently reported in these women (Luque-Ramírez *et al*., 2007). Furthermore, insulin resistance only had a minor influence on CIMT, which did not retain statistical significance when also considering the influence of androgen excess and HR.

Our results illustrate the concept that the increased cardiovascular risk profile of PCOS patients has a multifactorial origin that does not only result from the metabolic abnormalities frequently associated with this common disorder. While insulin resistance, chronic inflammation and obesity are undoubtly involved in the association with some of these risk factors (Legro, 2003), our present results suggest that androgen excess plays a substantial independent role on the pathogenesis of atherosclerosis in PCOS. Therefore, amelioration of androgen excess should be also considered as a target for cardiovascular prevention, and not only as a way of improving hyperandrogenic symptoms, when deciding the best therapeutic strategy for PCOS patients. However, because we did not evaluate ovarian morphology in our series, our present results should not be extrapolated to non-hyperandrogenic PCOS patients in whom this diagnosis is solely based on the presence of polycystic ovaries on ultrasound and oligomenorrhea (The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group, 2004).

In summary, we here report that young PCOS patients present with increased CIMT values when compared with non-hyperandrogenic women selected in order to be similar in terms of age and grade of obesity. This suggests that hyperandrogenism is associated with atherosclerosis and cardiovascular risk in these women.

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