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The polycystic ovary syndrome (pcos) status and cardiovascular risk in young women

Research Article

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Abstract: The study investigated the presence of early vascular damage and chronic inflammation, and their relationships with hormonal and metabolic parameters in 45 young women with PCOS in comparison with thirty-two healthy age-matched controls. Hormonal and metabolic profiles, high sensitivity C-reactive protein (hsCRP), tumoral necrosis factor-alpha (TNF-α), endothelin-1 (ET-1), brachial flow-mediated vasodilation (FMD) and carotid intima-media thickness (CIMT) were determined in both groups. Compared with the controls, women with PCOS had significantly lower FMD and respectively higher ET-1 levels ($p=0.001$). No differences were observed between the groups in terms of CIMT or inflammatory markers. In the PCOS group, ET-1 levels were significantly correlated with only testosterone concentrations ($r = 0.31$, $p = 0.037$), whereas the hsCRP levels were independently predicted only by body mass index (BMI). Within the total group, the PCOS status was the sole significant predictor of ET-1 levels and the only independent predictor of FMD. In conclusion, there is evidence of endothelial dysfunction associated with increased levels of androgen hormones in young women with PCOS. The combination of endothelial dysfunction and coexistent obesity promoting inflammation contributes to the progression of atherogenesis in PCOS. The PCOS status should be regarded as a predictor marker of cardiovascular risk, along with well-known cardiovascular risk factors.

Keywords: *Polycystic ovary syndrome • Endothelin-1 • Flow-mediated vasodilation • High-sensitive C-reactive protein • Obesity*

1. Introduction

Polycystic ovary syndrome (PCOS) is an extremely heterogeneous and complex disease, characterized not only by clinical and/or biochemical hyperandrogenism, ovulatory dysfunction, or polycystic ovaries but also by several metabolic and endothelial alterations. Obesity is present in 40-60% of the women with this pathology and 50-70% of the subjects with PCOS, either obese or of normal weight, have evidence of resistance to insulin [1]. Moreover, it has been suggested that PCOS represents a female subtype of metabolic syndrome, carrying a high risk for developing type 2 diabetes mellitus and atherosclerosis with a higher probability for coronary

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and cerebrovascular events [2]. Endothelial dysfunction and carotid intima-media thickness (CIMT) have been regarded as early features of atherosclerosis, and their assessment, as well as the evaluation of inflammatory markers, can be regarded as useful prognostic tools for the detection of pre-clinical cardiovascular disease. Up to the present, the results on this topic have been controversial as far as the presence of arterial structural and functional alterations or of chronic inflammation in PCOS are concerned. For example, there are reports which have demonstrated that PCOS is associated with endothelial dysfunction or increased CIMT even from a young age, while other reports have stated the contrary [3-16]. Additionally, there are authors who consider PCOS as a pro-inflammatory state due to elevated plasma concentrations of inflammatory mediators of atherogenesis, such as interleukin (IL)-6, IL-18, soluble intercellular adhesion molecule-1 (sICAM-1), sE-selectin, high sensitivity C-reactive protein (hsCRP), tumoral necrosis factor-alpha (TNF-α), monocyte chemotactic protein-1 (MCP-1), and plasminogen activator inhibitor-1 (PAI-1)[17-19]. However, there are also studies which have not confirmed the elevated levels of inflammatory markers in PCOS [20,21]. Moreover, a problem that has not yet been settled is the determining factors for vascular abnormalities and low-grade chronic inflammation in PCOS. Are they related to the PCOS status, to hyperandrogenemia, or to insulin resistance and/or obesity/adiposity, which usually accompanies this disorder, and to all the factors that derive from obesity? The purpose of this study was to investigate the presence of chronic inflammation, endothelial dysfunction, and of anatomic markers for sub-clinical cardiovascular disease, as well as their relationships with the characteristic hormonal and metabolic alterations of the syndrome in a population of young women with PCOS. Eventually, we sought to establish which endocrine and/or metabolic parameter may independently predict increased cardiovascular risk in PCOS.

2. Material and Methods

2.1. Study protocol

We prospectively studied 45 women with PCOS (age: 23.11± 4.14 (15-23), body mass index (BMI): 28.41±5.97 (15.62-40.74) kg/m^2), who were presented to our clinic. The study protocol was approved by the local ethics board and informed written consent was obtained from all participants. The diagnosis of PCOS was based on the AES 2006 guideline [22]. Hyperandrogenism was defined as hirsutism and/or as an elevated total testosterone concentration. Menstrual irregularities were defined as oligomenorrhea (eight or fewer menses per year) or amenorrhea (absence of menstruation for 3 consecutive months). Polycystic ovaries were defined by the ultrasound appearance of 12 or more follicles in each ovary measuring 2-9 mm in diameter and/or ovarian volume greater than 10 ml. Secondary causes of hyperandrogenism, such as hyperprolactinemia, thyroid disease, androgen-secreting tumors, and congenital adrenal hyperplasia were excluded in all patients. Specific adrenal disorders (such as Cushing's syndrome) were ruled out clinically and, where indicated, biochemically. The presence and extent of hirsutism were quantified using the Ferriman-Gallwey (F-G) score. Exclusion criteria for PCOS women included current or previous use (within 6 months) of oral contraceptives, anti-androgens, infertility medications, and drugs known to affect carbohydrate-lipid metabolism, as well as personal history of diabetes. There were no concurrent minor infections reported during the study or during the month preceding the study, and none of the subjects had taken anti-inflammatory drugs during the previous month.

A control group composed of 32 women (age: 23.06 ±5.37 (15-34), BMI: 25.87± 6.89 (16.88-42.86) kg/m^2) was selected as to be similar to the group of patients in terms of age and BMI. In the end though, our intention was not fully accomplished because there was still a significant difference in BMI between the groups. They all had regular menstrual cycles (defined as 26-32 days in length) and no signs of hyperandrogenism. No formal confirmation of the ovulatory status was done. The same exclusion criteria for the test group was used for the control group.

A complete history and physical examination, including BMI and waist hip ratio (WHR) were determined by the same doctor. An F-G score of 8 or greater was considered to be hirsutism. The BMI was calculated as weight (kilograms) divided by height (meters) squared (kg/m^2). The BMI values that were >25 kg/m^2 and < 30 kg/m^2 were considered as overweight, and the BMI values ≥30 kg/m^2 were considered as obese. The 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 while the subjects were standing and breathing normally. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in the right arm, with the subjects in a seated position. The average of two measurements taken with a mercury sphygmomanometer was used. All of the patients presented normal blood pressure (systolic, ≤130

mmHg; diastolic, ≤ 80 mmHg). Thirteen (28.88%) of the PCOS subjects and four (12.5%) of the controls were smokers.

2.2. Assay methods

All the blood samples were obtained in the morning between 08:00 and 10:00, after an overnight fasting and during early follicular phase (days 2-5) of a spontaneous or progestin-induced menstrual cycle (in thirty-one PCOS subjects (68.88%) menstruation was induced using dydrogesterone, 20 mg/day for 5 days). Blood samples were immediately centrifuged and the serum obtained was stored at -80°C until the time of assay. Serum fasting glucose (GLU, mg/dl) was measured using the glucose oxidase color method (Glucose GOD/PAP; Diagnosticum Zrt, Budapest, Hungary). Total cholesterol (TC, mg/dl) and triglycerides (TG, mg/dl) were measured by an enzymatic, colorimetric method (Diagnosticum Zrt, Budapest, Hungary).

All other measurements were performed with the ELISA TECAN auto-analyzer, unless otherwise stated. Insulin (INS, μU/ml), total testosterone (TT, ng/ml), estradiol (E_2, pg/ml), sex hormone-binding globulin (SHBG, nmol/l), endothelin-1 (ET-1, pg/ml), hsCRP (mg/l), and TNF-α (pg/ml) were all measured using commercial enzyme –linked immunosorbent assay kits from DRG Instruments in Marburg, Germany. The intra-assay coefficients of variation (CV) for low and high values for INS, TT, E2, SHBG, ET-1 and TNF-α, respectively were as follows: 2.6% and 1.8%, 4.1% and 3.3%, 6.8% and 4.4%, 8.6% and 5.3%, 8.8% and 6.7%, and 6.6% and 6.3%, respectively. The intra-assay coefficients of variance for hsCRP were 6.9% for CRP values <1.0 mg/l and 4.1% for CRP values >3.0 mg/l, respectively. The minimal detectable concentrations for adiponectin, ET-1, hsCRP and TNF-α, respectively were 0.41 pg/ml, 0.02 μg/ml, and 0.7 pg/ml, respectively.

The free androgen index (FAI) was calculated according to the equation: FAI (%) = TT (ng/ml) x 3.47 X 100/SHBG (nmol/l). Insulin resistance was estimated by the quantitative insulin resistance index: the homeostasis model assessment of insulin resistance (HOMA-IR) defined fasting glucose (mg/dl) x insulin (μU/ml)/405 and by the quantitative insulin sensitivity check index (QUICKI) defined as 1/ [log (fasting insulin) + log (fasting glucose)].

Body composition was assessed by whole-body dual-energy X-ray-absorptiometry (DXA) with a DPX-NT (GE, Madison, USA) device. Fat-free mass was automatically determined as the difference between total body weight and bone mineral content and fat mass. Both the total and segmental fat mass and fat-free mass were expressed as percentage of weight. The coefficient of variance, evaluated at 3% for total fat mass, was determined by measurements on 10 patients, each one evaluated 3 times.

2.3. Carotid measurement technique

Carotid artery ultrasound imaging was performed by the same experienced cardiologist, who was blinded to PCOS status, using a color Doppler (AGILENT SONOS 4500) with a high-resolution 10-Mhz linear probe. Overall single maximum IMT was used as a measure of CIMT, which represented one of the three most frequently used IMT measurements in clinical trials, as appropriate [23]. Longitudinal images were obtained from the distal portion of both common carotid arteries, 2 cm proximal to the carotid bulb, immediately proximal to the origin of the bifurcation, the carotid bulb, and the internal carotid artery on both right and left sides. For each location, the sonographer imaged the vessel in multiple planes and then focused on the interfaces required to measure IMT and also any area of focal plaque, and the maximum thickness (on both sides) was noted. CIMT was defined as the distance between the junction of the lumen and intima, and that of the media and adventitia. The IMT was measured during end-diastole from the B-mode screen and only the posterior (far) carotid wall measurements were taken. The coefficient of variation between visits was 5%.

2.4. Hemodynamic studies

Flow-mediated dilation (FMD) was measured in all subjects by the same cardiologist, during the same visit as the carotid IMT, and using the same echographic vascular linear probe as previously described. Each patient was taken into a quiet, temperature-controlled room at 20-25°C. After resting in a supine position for 15 minutes, the right brachial artery was identified and its position was marked at about 5 cm above the elbow joint. The diameter (measured in mm) of the artery was measured at end-diastole. After the resting measurement, limb flow occlusion was produced by inflating a standard sphygmomanometer cuff on the upper arm to 50 mmHg above systolic pressure for 5 minutes. This caused ischemia and consequently, dilatation of downstream resistance vessels. Subsequent cuff deflation induced a brief high-flow state through the brachial artery (reactive hyperemia) for the release of the endothelial nitric oxide to accommodate the dilated resistance vessels. The brachial artery was scanned continuously for 90 seconds after cuff deflation and the measurements were performed during the 30-90 seconds interval. The vessel's diameter was measured at least twice at the same point as that of the resting measurement, and the

Table 1. Anthropometric, hormonal and metabolic profile in PCOS and control women. Data are given as mean ± SE; p<0.05 was considered statistically significant.

Variables	PCOS (n=45)	Controls (n=32)	p-value
Age	23.11 ± 4.14	23.06 ± 5.37	0.876
BMI (kg/m^2)	28.41 ± 5.97	25.87 ± 6.89	0.043
WHRTG (mg/dl)	0.84 ± 0.06	0.80 ± 0.07	0.006
TC (mg/dl)	97.82 ± 38.99	88.22 ± 40.41	0.207
Glucose (mg/dl)	199.91 ± 22.13	189.53 ± 23.25	0.030
HOMA-IR	84.60 ± 8,13	84.0 ± 7.11	0.760
QUICKI	3.39 ± 1.83	2.73 ± 1.27	0.072
Insulin (μU/ml)	0.32 ± 0.02	0.33 ± 0.03	0.165
Total fat mass (%)	15.95 ± 7.69	13.09 ± 5.69	0.085
Trunk fat mass (%)	43.48 ± 8.06	39.88 ± 10.94	0.196
Fat-free mass (kg)	45.24 ± 9.19	40.79 ± 11.88	0.133
TT (ng/ml)	39.06 ± 6.12	37.86 ± 5.22	0.342
FAI (%)	1.033 ± 1.052	0.631 ± 0.439	<0.001
Estradiol (pg/ml)	11.33 ± 1.61	4.50 ± 0.84	<0.001
SHBG (nmol/l)	50.51±59.03	38.92±31.59	0.315
	51.10±37.26	73.84±40.23	0.013

PCOS - polycystic ovary syndrome; BMI - body-mass index; WHR – waist-to-hip ratio; TG – triglycerides; TC – total cholesterol; HOMA-IR – homeostasis model assessment index of resistance; QUICKI - quantitative insulin sensitivity check index; TT – total testosterone; FAI – free androgen index; SHBG – sex hormone binding globulin.

maximal diameter (diameter during reactive hyperemia) was defined again. FMD was calculated as the maximum percentage change in vessel size from the baseline. The coefficient of variation for repeated measurements of resting arterial diameter was 2.3%.

2.5. Statistical methods

Results are reported as mean values ± SE. After previously testing the distribution of continuous variables with the Kolmogorov-Smirnof test and including them in the group of not normally distributed variables, non-parametric tests such as Mann-Whitney and Kruskall-Wallis were used, as appropriate, in order to test the differences between continuous variables. Bivariate correlations were performed calculating the Spearman rho coefficient. In addition, simple and multiple regression analysis were performed to determine which variables predicted ET-1, FMD%, or hsCRP levels. Therefore, in the stepwise multiple regression analysis, variables whose correlation with vascular parameters achieved near statistical significance (p<0.1) were entered as independent variables to assess the magnitude of their individual effects on vascular parameters, as dependent variables. Both the adjusted R^2 values, corresponding to the entire model, and the values corresponding to each component part of the model were determined. Additionally, the level of the statistic significance corresponding to each component variables of the model was calculated and the independent predictors were identified according to it. Furthermore, adjustments for the possible confounding factors were also performed. P values of <0.05 were considered statistically significant.

3. Results

3.1. Descriptive variables in PCOS cases and controls

The anthropometric characteristics, the main metabolic and hormonal pattern, and the hemodynamic and inflammatory profile of PCOS patients and normal women are illustrated in Table 1 and Table 2, respectively.

As anticipated in PCOS, TT and FAI were significantly increased. Furthermore, women suffering from PCOS had a slightly higher BMI, WHR and mean serum levels of TC, but a lower SHBG levels than their corresponding age-matched healthy controls. However, there were no differences between the PCOS patients and controls in terms of body composition, e.g. total and trunk fat mass and fat-free mass. Metabolic assessment of study participants showed that young PCOS women had a tendency towards higher fasting insulin levels with borderline differences in HOMA-IR index when compared to healthy controls, but there were no differences in regard to fasting glucose levels between the two populations. ET-1 levels were significantly higher, while the FMD levels were significantly lower in the PCOS women compared to the control women. CIMT levels were similar between the two groups.

Table 2. Haemodynamic and inflammatory profile of PCOS and control women. Data are given as mean ± SE. P<0.05 was considered statistically significant.

Variables	PCOS (n=45)	Controls (n=32)	p-value
Baseline arterial diameter (mm)	3.09 ± 0.38	2.89 ± 0.46	0.081
FMD (%)	4.13 ± 6.54	12.47 ± 12.01	0.001
ET-1 (pg/ml)	3.46 ± 6.34	1.18 ± 1.34	0.001
Max CIMT (mm)	0.55 ± 0.11	0.55 ± 0.1	0.89
SBP (mmHg)	106.63 ± 13.61	106.56 ± 12.01	0.88
DBP (mmHg)	71.51 ± 10.03	69.84 ± 9.79	0.45
HsCRP (mg/L)	4.75 ± 3.56	3.51 ± 3.13	0.099
TNF-α (pg/ml)	8.20 ± 5.49	7.26 ± 2.54	0.90

The patients with PCOS had higher levels of TNF-α and hsCRP than the control group, but these were not statistically significant.

Since several parameters are known to be correlated with BMI, and because BMI demonstrated a difference between PCOS and controls, we separated our groups according to BMI, creating two groups: overweight and obese (BMI>25kg/m^2) subjects, and non-obese (BMI≤25kg/m^2) subjects. Twenty-eight women with PCOS were overweight and obese, whereas seventeen were non-obese. Thirteen controls were overweight and obese, and nineteen were non-obese. There was a borderline difference between the two study populations in terms of smoking habit (p=0.067), but this did not significantly influence our results.

3.2. Hemodynamic profile and relationships to hyperandrogenemia, body composition and indices of insulin resistance

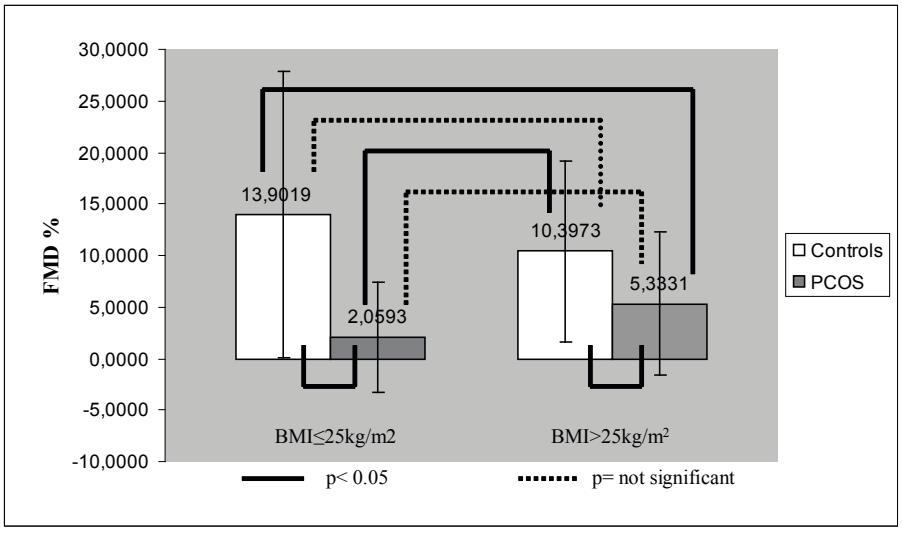
As shown in Table 2, ET-1 levels were about three fold higher in the PCOS women when compared with the age-matched control women. The differences in serum ET-1 were maintained between PCOS and weight-matched controls, no matter if analysis was applied to lean (BMI ≤25kg/m^2) or to overweight and obese women (BMI >25 kg/m^2) (p<0.05). These suggest that the process of endothelial dysfunction is largely independent of obesity.

In the whole studied population, ET-1 values were positively related to TT concentrations (r = 0.44, p <0.001), FAI (r = 0.45, p <0.001), BMI (r = 0.24, p = 0.035), and WHR (r = 0.35, p = 0.002), and negatively related to SHBG levels (r = -0.26, p = 0.019). The same relationship between ET-1 and androgen levels was described when evaluating women with PCOS and controls separately: in women with PCOS, ET-1 levels correlated significantly only with TT concentrations (r = 0.31, p = 0.037), whereas in controls, significant correlations were found with both TT concentrations (r = 0.35, p = 0.05) and FAI (r = 0.45, p = 0.009), but not with any other variable. Interestingly enough, univariate linear regression results of ET-1 and PCOS status, TT and FAI, respectively, in the total group showed that only PCOS status (b = 0.227, p = 0.049, adjusted R^2 = 3.9%) was a significant predictor of ET-1 levels. The multiple linear regression stepwise method was used in the total populations (test group + control group) to determine the independent predictors of ET-1 among the above parameters, which were found to correlate with ET-1. None of these variables were an independent determinant of serum ET-1.

FMD% was significantly lower in PCOS subjects compared with controls, irrespective of the presence of obesity (p<0.05) (Figure 1). In the total group, FMD values were negatively and significantly related to WHR (r = -0.25, p = 0.041), insulin (r = -0.29, p = 0.01), HOMA-IR (r=-0.25, p=0.03) and FAI (r = -0.24, p = 0.046). Negative, but of borderline significance correlations were also found between FMD and TC levels (r = -0.23, p = 0.059), TG levels (r = -0.23, p = 0.057), and ET-1 (r = -0.21, p = 0,084). After analyzing separate PCOS individuals and controls, there was, however, no significant association between FMD and studied parameters among the groups. In multiple regression analyses for the total population, only the presence of PCOS was an independent predictor of FMD levels (p<0.003), with this variable alone explaining 15.9% of FMD variability (p<0.001).

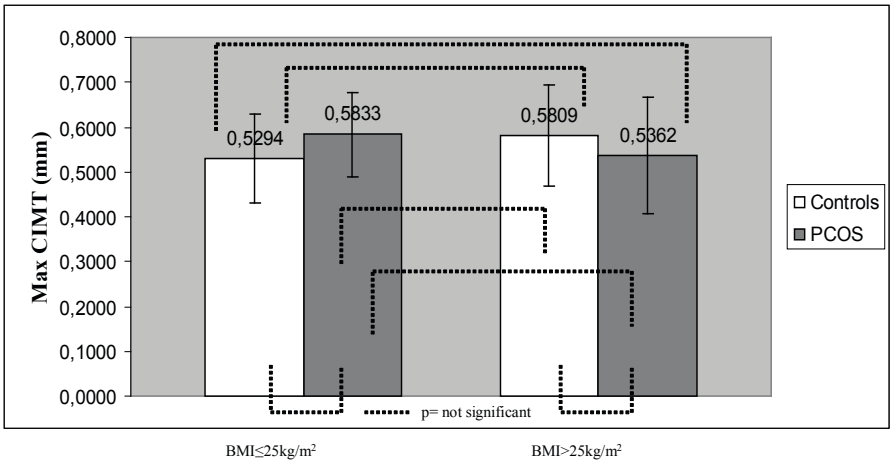
No differences were observed between the two groups in CIMT, even after being stratified according to BMI (Figure 2). Considering the whole studied population, controls, and the PCOS group, significant positive correlations were noted only between CIMT and age and CT levels (p<0.05) in each mentioned group.

Figure 1. FMD % in PCOS and control subgroups stratified according to BMI in non-obese (BMI≤25kg/m²) and respectively, overweight and obese (BMI>25kg/m²) subjects.



Data are given means ± SE, p<0.05 statistically significant;

Figure 2. Max CIMT in PCOS and control subgroups stratified according to BMI in non-obese (BMI≤25kg/m²) and respectively, overweight and obese (BMI>25kg/m²) subjects.



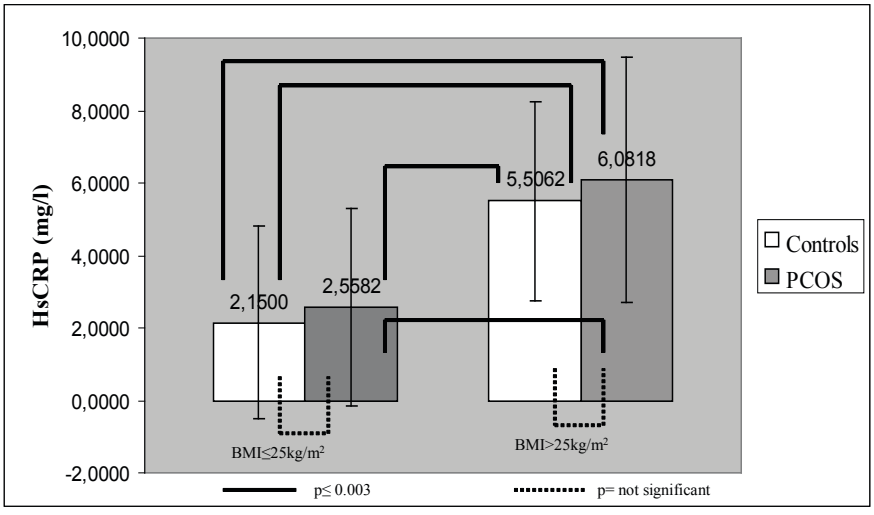
Data are given means ± SE, p<0. 05 statistically significant;

3.3. Inflammatory markers and relationships to hyperandrogenemia, body composition and indices of insulin sensitivity

HsCRP, but not TNF-α, was significantly higher in the groups with BMI >25kg/m², compared with the groups with BMI <25 kg/m², whether or not they had PCOS (Figure 3. p ≤0.003), but neither was significantly different when women with PCOS were compared with their respective weight-matched controls.

HsCRP significantly correlated with BMI, total fat mass, trunk fat mass (p<0.001), WHR, fasting insulin, HOMA-IR, QUICKI, and CT (p<0.05) in both PCOS and the total group. Regarding the controls, significant associations were found only with BMI, total fat mass, trunk fat mass (p<0.001), WHR, and TG (p<0.05). However, we found, no correlations between hsCRP levels and ET-1, FMD, max CIMT, or fasting glucose in any of the studied groups: PCOS, controls, the PCOS subjects and controls taken together. As far as markers of hyperandrogenemia were concerned, a significant and positive association to FAI was observed in the total group (r= 0.26, p=0.022), whereas in PCOS, there was no correlation among them and moreover, in the test group, hsCRP negatively correlated with total testosterone (r= -0.30, p=0.044). Multivariate linear regression models were carried out in PCOS in order to find the parameters independently associated with the levels of hsCRP, and to quantify the magnitude of their individual effects on

Figure 3. High-sensitivity CRP in PCOS and control subgroups stratified according to BMI in non-obese (BMI≤25kg/m²) and respectively, overweight and obese (BMI>25kg/m²) subjects.



Data are given means ± SE, p<0. 05 statistically significant;

this inflammation marker. We observed that the BMI remained the only significant predictor of hsCRP in PCOS, independent of any parameters included in the models: total fat mass, trunk fat mass, TC, QUICKI, and TT. When BMI was entered as the univariate predictor of the hsCRP variable, the regression coefficient was 0.65 (p <0.001). This factor alone explained 41% of hsCRP variability. Notably, the inclusion of QUICKI, TC, and TT in separate models basically did not change the BMI-hsCRP relationship. On the contrary, the addition of trunk fat mass and total fat mass in particular, appeared to attenuate the relationship of BMI on hsCRP. In the model including BMI and total fat mass, BMI explained only 6.33% of hsCRP variability.

No group of study reported a significant correlation between levels of TNF-α and cardiovascular risk factors evaluated in this study (results not shown).

4. Discussion

We clearly demonstrated impaired endothelial function in the present study, assessed by hemodynamic (FMD) and biochemical methods (ET-1) in PCOS women. Consistent with our results, most of the studies, but not all [8,12], demonstrated impaired endothelial function in PCOS using either noninvasive endothelium-dependent (FMD) and–independent (glyceroltrinitrate, GTN) changes in the brachial artery or invasive methodology [4,6,10,11,24] compared with the controls. Among several circulating endothelium-derived vasoactive molecules, ET-1 is considered one of the best indices of abnormal vascular reactivity, playing an important role in the early events of endothelial dysfunction [4]. With regard to ET-1 levels, we confirm the other authors' results on increased levels of ET-1 in PCOS women [3,4,9,10]. Furthermore, impaired vascular reactivity and increased level of ET-1 were linked to markers of hyperandrogenism (TT, FAI). Conflicting results have been provided regarding the factors associated with impaired vascular reactivity in PCOS. The relationship with hyperandrogenemia is especially controversial, since the effect of the androgens was described as either adverse [10,25], neutral [24,26], or even protective in patients with PCOS [27]. However, the reports are more constant with regard to ET-1 and the deleterious effect that markers of hyperandrogenemia have on it [3,9,10]. The mechanisms by which hyperandrogenemia might affect endothelial function are complex and controversial. It is possible that testosterone has a direct effect on the vessel wall, as indicated by the localization of androgen receptor (AR) in endothelial and vascular smooth muscle cells [28], influencing vasodilatation by endothelium-dependent and endothelium-independent mechanisms, by genomic and non-genomic effects [29,30]. Zitzmann et al. observed that the number of CAG repeats in the AR gene showed a significant positive basic correlation with FMD, GTN-induced vasodilatation. Therefore, a low number of CAG repeats in the AR gene appear to put men and women at an increased risk for developing cardiovascular disease [30], insulin resistance, and dyslipidemia respectively, [31] and it may also enhance hyperandrogenity in PCOS [32]. By design, hirsutism and/or the presence of elevated TT levels were part of the selection criteria for PCOS. In contrast to most of the studies which used

Rotterdam criteria though, we used AES 2006 guideline in recruiting our test group [22], which in turn might account for our results. Perhaps the most intriguing find in our study was the negative correlation between the biochemical markers of hyperandrogenemia and both indices of endothelial dysfunction (ET-1, FMD %) across all subjects, suggesting that increased androgen levels may impair endothelial functions. The predictive value of PCOS presence revealed by regression analysis on both FMD% and ET-1 values may prove to be of clinical relevance. Therefore, we might speculate that some genetic abnormalities, such as polymorphisms in AR or other yet unidentified genetic abnormalities aggravated by the higher levels of androgens found in PCOS subjects, may be responsible for impaired endothelial function observed in our test group in comparison with our control group. Several lines of evidence, but not all [8,10,12,27,33], revealed an association between insulin resistance and endothelial dysfunction markers in PCOS women [4,7,9,11,34]. We observed however, significant correlations of FMD with insulin concentrations and HOMA-IR, but only in the entire population studied. In the view of all these findings, androgen excess exposure seems to be sufficient enough to cause endothelial dysfunction in our PCOS women.

With regard to CIMT, in line with other studies [8,13,15,34], we could not find evidence of an altered arterial structure in our group of young subjects with PCOS when compared to the controls. Likewise, in particular, Talbott et al. demonstrated a difference in carotid IMT between middle-aged women with PCOS (≥45 years) and age-matched controls, but not in younger women (group aged 30 to 45 years) [13]. It appears that our PCOS women have evidence of endothelial dysfunction, but not morphological evidence of carotid atherosclerosis. This is not surprising when the young age of our PCOS women is taken into account. Endothelial dysfunction has been shown to occur earlier in the atherosclerosis development and progression, preceding the onset of increased CIMT, which seems to be more evident in middle-aged and older women [27,34]. In this study, hyperandrogenemia seems to exert its effect on the vascular system through the endothelium, but not the intima, as evidenced by its association with only FMD and ET-1.

Similar to the results of others [20,21,35], we found no evidence of increased levels of either hsCRP or TNF-α in a young, euglycaemic, eulipidemic, and normotensive population of PCOS women compared with controls. The most published data, but not all [20,34-36], demonstrated increased levels of hsCRP in women with PCOS [10,11,37-39], which are primarily dependent upon co-existent obesity [40]. Obesity, and specifically increased central adiposity, which is known to secrete many pro-inflammatory markers (IL-1, IL-6, IL-18, TNF-α), is a common PCOS feature [41]. We found that, despite the significantly increased BMI in the PCOS group compared to the control group, both total fat mass (%) and trunk fat mass (%) were similar and elevated in the two populations. Additionally, there were only borderline differences in terms of insulin concentrations and HOMA-IR between the PCOS subjects and controls. Therefore, these metabolic similarities probably could explain the lack of differences in terms of inflammatory markers among the two populations from our study. The increased body adiposity found in both the PCOS group and control group is most likely the explanation for the elevated levels of hsCRP that were found in our control group, as well. It should be also mentioned that in all the studies which have noted higher levels of hsCRP in PCOS women compared with controls, the subjects either were not matched for trunk fat mass as evaluated by DXA or abdominal CT[37,42-44], or if they were matched for BMI, the PCOS women might have had a higher proportion of visceral fat, since they did not carry out direct measurements of fat and lean body mass by scanning [15,45]. Furthermore, hsCRP was significantly and positively correlated with variables of obesity and insulin resistance, and CT, and negatively correlated with TT in PCOS, suggesting that obesity and metabolic alterations, rather than hyperandrogenemia, have a negative impact on markers of chronic inflammation in PCOS subjects. Additionally, as shown by the multiple regression analyses, BMI was a strong and the only independent predictor of hsCRP levels in PCOS. Therefore, we concluded that PCOS is not associated with low-grade chronic inflammation per se, and the levels of hsCRP in this disease are solely related to obesity. Additionally, similar to Puder et al.' and Shroff et al.' findings [35,42], we noted that total fat mass in particular reduced the BMI-related contribution to hsCRP variability the most, suggesting that at least part of the observed association of BMI and hsCRP in PCOS may have been driven by total fat mass. With similar results to our own, a very recent study by Tosi et al. noted a negative correlation between TT and hsCRP levels in the studied population (controls, PCOS women, and women with idiopathic hyperandrogenism) [44]. In PCOS subjects, either no correlation [11,42,45] or a positive association between increased androgen levels and indices of chronic inflammation [10,39] were described. Therefore, our results suggest an interesting dissociative effect of androgens on inflammatory markers and endothelial function in PCOS subjects that is not easily explainable. High testosterone and dihydrotestosterone (DHT) levels have been found to increase TNF-α-induced VCAM-1

and E-selectin expression in endothelial cells [46]. On the contrary, Norata et al., using a lower dose of DHT than in the previous studies, reported that DHT can decrease TNF-α-induced luciferase activity of the Cox-2 promoter, suggesting that DHT can modulate pro-inflammatory gene expression [47]. Likewise, a study carried out in an animal model reported a protective effect of androgens on chronic inflammation [48]. We could hypothesize that androgens might have a direct anti-inflammatory effect in PCOS, but this action is weak and is probably lost at a significantly higher concentration and masked in PCOS women by the prevalent effect of BMI and especially fat mass.

The limitations of our study included the small number of subjects in each group, which might have also limited our ability to detect associations between the markers of endothelial dysfunction such as FMD and other parameters such as androgens and insulin resistance indices among the PCOS women and controls, taken separately. The research should be further carried out using an enlarged sample size for validation of the present results. One further limitation would be that GTN was not included as a test of endothelium-independent dilatation and thus, vascular smooth muscle cell injury could not be excluded and the demonstrated effect could not necessarily be localized to the endothelial cell as opposed to the smooth muscle. Moreover, part of our PCOS subjects were evaluated during a spontaneous menstrual cycle, while the rest of them were assessed during a progestin induced menstrual cycle. However, the period which dydrogesterone was administered was short and we support the idea that this limitation does not alter our results.

5. Conclusion

Our data show that young, non-dyslipidemic, non-hypertensive, and euglycaemic women with PCOS have altered endothelial function as documented by both impaired vascular reactivity and increased ET-1 levels, but not increased CIMT levels or evidence of low grade chronic inflammation, suggesting early functional pre-atherosclerotic vascular impairment. Furthermore, the combination of the endothelial dysfunction, which is related to both the PCOS status and hyperandrogenemia, and the co-existent obesity promoting inflammation, can contribute significantly to the progression of atherogenesis. We also propose that the PCOS status should be regarded as a predictor marker of cardiovascular risk, alongside other well-known cardiovascular risk factors. More long-term studies will be needed to determine the significance of the finding of early endothelial disease in women with PCOS. We found significant correlations between hyperandrogenemia and indices of endothelial dysfunction across all subjects, suggesting that increased androgen levels, besides insulin resistance may impair endothelial function. Further studies are needed to assess the impact of androgen hormones on arterial function and structure, and to define the mechanisms by which male hormone pattern determines vascular damage in women.

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