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Body mass index rather than the phenotype impacts precocious ultrasound cardiovascular risk markers in polycystic ovary syndrome

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:
Pandurevic, S., Bergamaschi, L., Pizzi, C., Patton, L., Rucci, P., Corzani, F., et al. (2021). Body mass index rather than the phenotype impacts precocious ultrasound cardiovascular risk markers in polycystic ovary syndrome. EUROPEAN JOURNAL OF ENDOCRINOLOGY, 184(1), 199-208 [10.1530/EJE-20-0725].

Availability:
This version is available at: https://hdl.handle.net/11585/790097 since: 2021-01-21

Published:

DOI: http://doi.org/10.1530/EJE-20-0725

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(Article begins on next page)

10 October 2026

This is the final peer-reviewed accepted manuscript of:

Pandurevic, S., Bergamaschi, L., Pizzi, C., Patton, L., Rucci, P., Corzani, F., Cecchetti, C., Pelusi, C., Altieri, P., Vicennati, V., Di Dalmazi, G., Fanelli, F., Macut, D., Pagotto, U., & Gambineri, A. (2021). Body mass index rather than the phenotype impacts precocious ultrasound cardiovascular risk markers in polycystic ovary syndrome, European Journal of Endocrinology, 184(1), 199-208.

The final published version is available online at: https://doi.org/10.1530/EJE-20-0725

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Body mass index rather than the phenotype impacts precocious ultrasound cardiovascular risk markers in polycystic ovary syndrome

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Short title: BMI vs PCOS phenotyping for CV risk prediction

Keywords: BMI, PCOS, cardiovascular risk, IMT, FMD, EFT, intima media, flow-mediated dilation, epicardial fat

Word count: 3253

Abstract

Objective: Research into cardiovascular disease (CV) prevention has demonstrated a variety of ultrasound (US) markers predicting risk in the general population, but which have been scarcely used for polycystic ovary syndrome (PCOS). Obesity is a major factor contributing to CV disease in the general population, and it is highly prevalent in PCOS. However, it is still unclear how much risk is attributable to hyperandrogenism. This study evaluates the most promising US CV risk markers in PCOS and compares them between different PCOS phenotypes and BMI values.

Design: Women fulfilling the Rotterdam criteria for PCOS were recruited from our outpatient clinic for this cross-sectional study.

Methods: Participants (n = 102) aged 38.9±7.4 years were stratified into the four PCOS phenotypes and the three BMI classes (normal-weight, overweight, obese). They were assessed for clinical and biochemical parameters together with the following US markers: coronary intima-media thickness (cIMT), flow-mediated vascular dilation (FMD), nitroglycerine-induced dilation (NTG), and epicardial fat thickness (EFT).

Results: There was no statistical difference among the four phenotypes in terms of cIMT, FMD, NTG or EFT, however all the US parameters except NTG showed significant differences among the three BMI classes. Adjusting for confounding factors in multiple regression analyses, EFT retained the greatest direct correlation with BMI, and cIMT remained directly correlated but to a lesser degree.

Conclusions: This study showed that obesity rather than the hyperandrogenic phenotype negatively impacts precocious US CV risk markers in PCOS. In addition, EFT showed the strongest association with BMI, highlighting its potential for estimating CV risk in PCOS.

1. Introduction

Polycystic ovary syndrome (PCOS) is a multifactorial condition with a complex pathogenesis and a very polymorphic phenotype (1). In reproductive-age women, prevalences of between 6% and 20% have been estimated (2). There appears to be a higher prevalence of obesity, particularly the abdominal phenotype, insulin resistance, overt diabetes mellitus (DM), dyslipidaemias and primary arterial hypertension (HTA) in PCOS, compared to the general population (3–5). Following these findings, further studies on the long-term cardiovascular (CV) morbidity in PCOS have been performed. However conflicting data have emerged from these studies regarding the incidence of CV events in what was perceived as a high-risk population (6–9).

Pathophysiologically, vascular failure is preceded by atherosclerosis, which starts decades before the occurrence of the first obstructions of clinical relevance (10). The search for a cost-effective, reliable and non-invasive indicator of this precocious process of vascular damage has spawned multiple ultrasound (US) CV risk markers, of which arguably the most promising are carotid intima media thickness (cIMT), flow-mediated vessel dilation (FMD), and epicardial fat thickness (EFT).

In the general population, cIMT has been shown to be a better predictor of CV events than the Framingham Risk Score. In fact, cIMT has a specifically higher precision in women, and it has also been shown to be significantly higher in PCOS compared to controls (11,12). FMD has also been proven to be a good predictor of future CV events in the general population, and to be significantly impaired in PCOS patients (13,14). However, a lack of androgen profiling, a lack of comparisons between normal weight and overweight or obese PCOS patients, and disparate PCOS classifications in published studies preclude the association of these US markers with obesity, hyperandrogenism or different PCOS phenotypes in general, which may be the missing variables in estimating CV risk in PCOS.

One study, conducted on menopausal women in the general population, demonstrated a positive correlation between cIMT and circulating androgens, specifically dehydroepiandrosterone sulphate (DHEAS) and androstenedione, thus suggesting a potential association between the hyperandrogenic phenotype and worse US CV risk markers also in PCOS (15).

EFT has been relatively recently developed as an inexpensive, fast analogue for visceral fat measurement, as well as an extensively studied CV and metabolic risk factor, and it also correlates well with the current gold standard – abdominal MRI (16). EFT is an independent predictor of insulin resistance, adipocytokine levels and clinical coronary artery disease in the general population (17,18). In the PCOS population however, to date only six small-scale studies on EFT have been published , all on young subjects, with conflicting results on the value of EFT in PCOS compared to controls, and no consideration of PCOS phenotypes (19–24).

Overall, existing studies on cIMT, FMD and EFT have either compared obese PCOS patients with obese controls, or PCOS and controls, without stratifying for BMI. Since obesity is one of the major factors contributing to CV morbidity, it merits consideration in PCOS when investigating CV risk markers.

The aim of this cross-sectional study was to investigate the possible differences in the expression of precocious US CV risk markers among different PCOS phenotypes and BMI classes in reproductive age PCOS women. With this approach, we hoped to gain insights into the risk profile of PCOS patients for simpler screening and long-term follow-up.

2. Materials and methods

2.1. Participants

This cross-sectional study originates from the analysis of data collected in 2009 in the context of a larger, ongoing longitudinal study aimed at estimating the prevalence and incidence of metabolic and CV diseases in a population of PCOS followed at the Endocrinology Unit of St. Orsola Hospital in Bologna, Italy. One hundred and two Caucasian PCOS women in reproductive age were included. The diagnosis of PCOS was made according to the Rotterdam criteria (25). Hyperandrogenism (HA) was defined as any of the following: presence of hirsutism, defined by the modified Ferriman-Gallwey score ≥8 (26), total testosterone above 0.7ng/mL, or free testosterone calculated by the Vermeulen formula higher than 9.52 pg/mL. Polycystic ovarian morphology (PCOM) was defined as presence of 12 or more follicles 2–9 mm in diameter, and/or increased ovarian volume >10 mL in at least one ovary, according to the 2004 ESHRE/ASRM recommendations (25). Oligo/anovulation was diagnosed with the occurrence of menstrual cycles lasting more than 35 days or less than 21 days, supported by the measurement of luteal phase progesterone being <2 ng/mL.

Exclusion criteria were: hyperprolactinaemia, congenital adrenal hyperplasia, Cushing's syndrome, androgen-secreting tumours, thyroid disease, premature ovarian failure or other specific causes of amenorrhoea, DM, CV, renal or liver diseases. None of the subjects included in the study had taken estroprogestins, insulin sensitisers or other drugs that could interfere with steroid levels in the blood for at least three preceding months. The study was approved by the Ethics Committee of the Emilia Center Area of Emilia-Romagna Region (CE-AVEC), and each woman gave informed consent.

2.2. PCOS phenotyping

Participants were grouped into four PCOS phenotypes based on the following criteria (27):

A: HA + oligo/anovulation + PCOM

B: HA + oligo/anovulation

C: HA + PCOM

D: oligo/anovulation + PCOM

In relation to BMI, these PCOS phenotypes were also subdivided into normal weight (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2), and obese(≥30 kg/m^2) classes, based on the WHO criteria for obesity (28).

2.3. Protocol

Participants underwent a complete medical examination, anthropometric measurements, blood sampling for metabolic parameters and hormones, and US study. For women with a regular menstrual period or mild oligomenorrhoea, blood samples were taken after an overnight fast in the first week following the start of spontaneous bleeding. Participants with moderate to severe oligo- and amenorrhoea were given oral medroxyprogesterone acetate to induce bleeding, after which the same protocol was followed. An oral glucose tolerance test (OGTT) with 75 g of glucose (Curvosio, Sclavo, Cinisello Balsamo, Italy) was performed to diagnose DM, based on glucose measurements at the start and at the 120 min mark of the test, according to American Diabetes Association criteria (29). US examination of cIMT, FMD, nitroglycerine test (NTG) and EFT were performed during the same examination by a single expert physician, who was blinded to the clinical data of the patients.

2.4. Anthropometric and clinical measurements

Participants had a physical examination which included body measurements (height, weight, waist circumference) and blood pressure. Height was measured without shoes and rounded to the nearest 0.5 cm; weight was measured in the morning, fasting, without clothes, upon emptying the bladder and bowels. BMI was calculated as weight (kg) divided by the square of height (m). Waist circumference was measured with the subjects standing, with a 1 cm wide measuring tape, according to WHO guidelines (28). Blood pressure was measured twice in the supine position, in the morning, after at least 3 min of rest before measurement, taking the average of two. In the case they differed >10mmHg, a third measurement was done, and the outlier rejected. Women were instructed not to drink coffee or water 3h before the examination. Patients were defined as having HTA if any of the following applied: use of antihypertensive medication; systolic arterial blood pressure ≥ 140 mmHg; diastolic pressure ≥ 90mmHg. Similarly, dyslipidaemia was defined as the presence of any of the following: use of hypolipidemic drugs; blood triglycerides ≥ 150 mg/dL; blood LDL ≥ 100 mg/dL.

2.5. Biochemical assays

Glucose, insulin, triglycerides, total and high-density lipoprotein (HDL) cholesterol, TSH, prolactin, LH, FSH, oestradiol and testosterone were measured by Modular Analytics E170 (Roche Diagnostics, Mannheim, Germany) and SHBG was measured by Immulite 2000 (Siemens Healthcare Diagnostics, Deerfield, Illinois), as reported elsewhere (30)

LDL was calculated using the Friedewald formula (31). Insulin resistance was calculated using the homeostatic model assessment of insulin resistance (HOMA-IR) index (32). Free testosterone was calculated using the formula by Vermeulen (33).

2.6. Ultrasound measurements

2.6.1. cIMT

To measure early atherosclerosis, we sonographically measured IMT of the common carotid artery wall (34). Measurement of cIMT was carried out using B-mode US image acquisition (Siemens 2004 US machine with the linear probe at 10 MHz) between 08:00h and 09:30h.

Image acquisition included the evaluation of right and left common carotid arteries, 1 cm proximal to the carotid bulb. The left and right far walls of the carotid artery segments were visualised with standardized magnification (2 cm × 2 cm). The sonographer used different scanning angles to identify the maximum thickness of the IMT 1 cm proximal to the carotid bulb to be measured. IMT was defined for the common carotid artery as the mean of the maximum wall thicknesses for the near and far walls on the right and left common carotid segments: (maximum left near wall + maximum left far wall + maximum right near wall + maximum right far wall) / 4. For each segment, IMT was defined as the average of three measurements. Intra-observer coefficient of variation was 4.5±1.9%.

2.6.2. FMD

The procedure was performed using the linear 10 MHz probe of a vascular US system (Siemens 2004 US machine). With the probe above the cubital fossa, a measurement of the average diameter of the brachial artery at rest was obtained. Then, 3-5 cm above the cubital fossa, the sphygmomanometer cuff was placed and inflated rapidly to above the systolic pressure (> 300 mmHg) and held for 5 minutes. The cuff was then rapidly deflated, with simultaneous repeated measurements of the arterial diameter taken every 20 seconds for 3 minutes. The FMD was calculated as the percentage difference between the maximal diameter after deflation of the cuff and the average diameter in basal conditions. After a 15-minute rest period following FMD, we repeated the measurements before and 3-4 minutes after administering 0.4 mg of sublingual nitroglycerine. Vascular reactivity (NTG) was calculated as the percentage difference between the maximal diameter post-nitroglycerine and the average diameter at rest. Intra-observer coefficient of variation was 1.6±1.0% for NTG and 1.2±0.6% for FMD.

2.6.3. EFT

Measurements were taken using with the convex 3.5 MHz probe positioned between the 3rd and 4th intercostal space along the left parasternal line, with the patient lying in the left lateral decubitus position. Measurements

were taken at the end-diastolic part of the cardiac cycle, following the peak of the QRS complex on ECG. 2D US was performed during normal respiration. Epicardial fat thickness was measured above the free part of the right ventricle, in the long and the short axis. Epicardial fat was identified as the anechogenic space inside the pericardial line; the thickness was measured perpendicular to the ventricle muscle wall in the end-diastole part of the cardiac cycle, as an average of three cycles. Measurements were standardised using the aortic annulus as the anatomic reference.

2.7. Statistical analysis

Continuous variables were summarized using mean ± standard deviation (SD) or median and interquartile range, as appropriate according to the distribution of the variable. Categorical variables were summarized using absolute and percentage frequencies.

The anthropometric, biochemical, and CV US measurements were compared across the four PCOS phenotypes and the three BMI classes (normal weight, overweight, obese) using one-way analysis of variance (ANOVA) when the assumptions for this analysis were met. Otherwise, Welch's test was used. Following a significant ANOVA F or Welch's test, post-hoc pairwise tests were conducted using the Bonferroni or Games-Howell test with a correction to the probability level for multiple comparisons. Free T was compared among the four PCOS phenotypes using the Kruskal-Wallis test. The relationship of CV US measures with BMI, used as a continuous variable, was investigated using Pearson's correlation coefficient and multiple linear regression models, in order to adjust for potential confounders. These included age, waist circumference, type 2 DM, smoking, HTA, fasting glucose, fasting insulin, free testosterone, HOMA2-IR, SHBG and FSH. A p value of <0.05 was considered statistically significant. All statistical analyses were carried out using IBM SPSS, version 25.0.

3. Results

The study sample was comprised of 102 PCOS women with a mean age of 38.9±7.4 years. A total of 29 women (28.4%) were overweight and 38 (37.2%) were obese. A total of 26 patients (25.5%) were affected by HTA, 58 (56.9%) by dyslipidaemia, and 13 (12.7%) by type 2 DM. A total of 27 patients (26.5%) used metformin as an insulin-sensitizer and 78 (76.5%) had taken estroprogestins in the past. The four PCOS phenotypes produced subgroups of 38, 7, 46 and 11 women, phenotypes A to D respectively, who had a similar age, BMI and waist circumference, as well as a similar prevalence of smoking, HTA, dyslipidaemia, type 2 DM, and previous treatments with metformin or estroprogestins (Tables 1 and 2).

No significant difference was found when biochemical parameters were compared among phenotypes (Tables 1 and 2), except for total and free testosterone, which were significantly higher in phenotypes A and C than in

phenotype D (Table 2). The four PCOS phenotypes had similar values of cIMT, FMD, NTG, and EFT (Table 3).

Conversely, when the study sample was subdivided into the three BMI classes (normal weight n=35, overweight n=29, obese n=38) significant differences appeared in all the CV US measurements except for NTG which had a borderline significance (Table 4). In particular, cIMT was significantly higher in obese than overweight and normal weight participants; FMD was significantly lower in obese than in normal weight and overweight participants; and EFT showed a significant increasing gradient with increasing BMI class (Table 4). When the BMI was used as a continuous variable, it significantly, directly and strongly correlated with cIMT (Pearson's $r = 0.604$, $p < 0.001$) and EFT ($r = 0.695$, $p < 0.001$), and inversely and more weakly with FMD (rho = -0.290, $p = 0.004$) and NGT (rho = -0.243, $p = 0.02$).

All the other variables investigated except for age, LH, total testosterone, oestradiol, and prevalence of smoking and previous estroprogestin users differed among BMI classes (Tables 5 and 6). In particular, free testosterone was significantly higher and SHBG was significantly lower in obese and overweight than in normal weight participants; FSH was significantly higher in overweight than in normal weight participants; fasting glucose and the prevalence of type 2 DM and of dyslipidaemia were significantly higher in obese than in normal weight participants; and waist circumference, insulin, HOMA-IR and systolic blood pressure showed significant increases with increasing BMI classes (Tables 5 and 6). Moreover, diastolic blood pressure and prevalence of HTA were significantly higher in obese than in normal weight and overweight participants (Table 5).

~~The relationships BMI-cIMT and BMI-EFT remained significant after adjusting for age, DM, smoking, HTA, SHBG, FSH and free testosterone by multiple regression analyses, whereas the relationships BMI-FMD and BMI-NTG were no longer significant (Supplemental Table).~~ Significant correlation was found between BMI and all US CV markers using the parametric Pearson's test; when adjusting for age, DM, smoking, HTA, SHBG, FSH and free testosterone in the multiple regression analysis, BMI-cIMT and BMI-EFT pairs remained significant (Supplemental Table).

4. Discussion

To the best of our knowledge, this is the largest cross-sectional study evaluating US CV risk parameters in PCOS across different PCOS phenotypes and different BMI classes. In addition, this is the first study to simultaneously measure the most promising precocious US CV risk markers in PCOS, that is: cIMT, FMD, EFT and NTG. Interestingly, we found no significant differences in these US measurements between the four PCOS phenotypes, however we found significant differences between the three BMI classes. In particular, cIMT and FMD showed a significant difference between obese and both overweight and normal weight

patients, while EFT showed significant differences among the three classes of BMI. In addition, after adjusting for the effect of age, DM, smoking, hypertension and for levels of SHBG, FSH and of free testosterone, EFT showed the greatest direct correlation with BMI, cIMT remained directly correlated, though to a lesser degree, while FMD showed no relationship with BMI.

The fact that there the values of cIMT, FMD, EFT and NTG were similar among the four PCOS phenotypes was unexpected because hyperandrogenism has been suggested to be a risk factor for CV events both in the general population and in PCOS (35,36), although some studies do not support this hypothesis (15,37,38).

One plausible explanation for this result could be the relatively young age of our population, which consisted of women in reproductive age. With advancing age and prolonged exposure to hyperandrogenaemia, it is possible that these parameters could change in a significantly different way, depending on the phenotype. At the time of the study, however, only three of our patients had a cIMT value above 1mm, considered by many as the cut-off for clinical significance (39). There are no similarly agreed-upon reference ranges for FMD, EFT or NTG.

Another unexpected finding was that our four PCOS phenotypes did not differ in terms of anthropometry or metabolic parameters, or in the prevalence of HTA, dyslipidaemia or type 2 DM. These results are apparently in contrast with some of the few studies that have compared the PCOS phenotypes for metabolic alterations and which investigated a comparably aged PCOS population (40,41). However, in these studies, the BMI differed significantly among the PCOS phenotypes, and when the comparison among phenotypes was adjusted for BMI (35) or body fat (34) the differences disappeared.

Obesity is known to have an important role in the metabolic disturbances of PCOS (42,43), and our study confirms these findings. In fact, our obese PCOS subgroup had the highest prevalence of type 2 DM, dyslipidaemia, hypertension, as well as higher fasting insulin levels and HOMA-IR score than overweight and normal weight subgroups.

In addition, our obese PCOS subgroup had higher free testosterone than the normal weight subgroup. Since total testosterone was comparable among BMI classes, the difference in free testosterone stems from a significantly lower SHBG in obese patients. Low SHBG is a recognised feature of PCOS (38), particularly in the presence of insulin resistance, which accompanies almost all obese PCOS women when measured by the euglycaemic-hyperinsulinemic clamp (44,45). It is also considered a risk factor for type 2 DM (46), as well as a predictor of the therapeutic response to metformin (47). Whether low SHBG is also a CV risk factor is unknown, but this important question is not answered in this study.

However, our study does suggest that overweight, and especially obese PCOS subjects could be exposed to a higher risk of CV events later in life, because we found a quantifiable association between BMI and early cardiovascular alterations detected by US.

When the BMI was used as a continuous variable, it significantly, directly and strongly correlated with cIMT and EFT, and inversely, albeit more weakly, with FMD and NTG. In addition, the relationship BMI-cIMT and BMI-EFT remained significant, after adjusting for the other clinical and biochemical variables that deteriorated with increasing body weight.

This result supports the hypothesis that excess adipose tissue may directly contribute to early endothelial dysfunction, probably through the maintained production of adipocytokines and a chronic low-grade inflammation (41,42,48). Many studies have demonstrated that obesity in PCOS is almost always characterized by an abdominal phenotype (49). Accordingly, in the present study waist circumference, which is a known CV risk factor, was found to have a high collinearity with BMI in the multiple regression analysis, thus making it superfluous in this specific analysis.

In conclusion, this study demonstrates that obesity, rather than the hyperandrogenic phenotype, negatively impacts precocious ultrasound CV risk markers in PCOS, thus suggesting that the main driver of CV events in PCOS could be the concomitant metabolic disorders, and primarily obesity. In addition, compared with other US markers, we found that EFT has the strongest adjusted association with BMI, highlighting its potential for estimating CV risk in PCOS.

Conflict of interest: The authors have no conflict of interest to declare

Funding: This study was supported by PRIN 2017 Prot. 2017ATZ2YK.

Author contribution statement: SP and AG interpreted data and wrote the article; CP and LP acquired data and revised the article; PR performed statistical data analysis and revised the article, LP and AG designed the study; FC, CC, CP, PA, VV, GDD, FF, DM, UP revised the article. All the co-authors approved the final version of the manuscript.

Acknowledgements: Women Work for Women healthcare group members collaborated in the study and approved the final version of the manuscript: Cristina Antinozzi, Stefania Basili, Angela Di Baldassarre, Susanna Dolci Jannini.

This paper is dedicated to Prof. Renato Pasquali, for his constant commitment to endocrinology and the study of PCOS.

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