

Alma Mater Studiorum Università di Bologna
Archivio istituzionale della ricerca

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>

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>).
When citing, please refer to the published version.

(Article begins on next page)

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>

Rights / License:

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)

When citing, please refer to the published version.

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

3

4 ¹Srdjan Pandurevic, ² Luca Bergamaschi, ²Carmine Pizzi, ¹Laura Patton, ³Paola Rucci, ¹Francesca Corzani,
5 ¹Carolina Cecchetti, ¹Carla Pelusi, ¹Paola Altieri, ¹Valentina Vicennati, ¹Guido Di Dalmazi, ¹Flaminia Fanelli,
6 ⁴Djuro Macut, ¹Uberto Pagotto, ¹Alessandra Gambineri, and on behalf of the Women Work for Women
7 healthcare group.

8

9 ¹Unit of Endocrinology and Prevention and Care of Diabetes, Dept. of Medical and Surgical Sciences, S. Orsola
10 Hospital, University of Bologna, Italy

11 ²Unit of Cardiology, Dept. of Specialistic, Diagnostic and Experimental Medicine, S. Orsola Hospital, University
12 of Bologna, Italy

13 ³Department of Biomedical and Neuromotor Sciences, University of Bologna, Italy

14 ⁴Clinic of Endocrinology, Diabetes and Metabolic Diseases, Faculty of Medicine, University of Belgrade, Serbia

15

16 Corresponding author: Alessandra Gambineri MD, PhD

17 email: alessandra.gambiner3@unibo.it

18 Postal address: Unit of Endocrinology and Prevention and Care of Diabetes, Dept. of Medical and Surgical
19 Sciences, S. Orsola Hospital, Via Massarenti 9, 40138 Bologna, Italy

20 Other contact info Phone: +39 051 2144 628.

21

22 Short title: BMI vs PCOS phenotyping for CV risk prediction

23

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

26

27 Word count: 3253

28 **Abstract**

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

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

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

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

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

48

49 **1. Introduction**

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

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

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

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

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

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

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

87 **2. Materials and methods**

88 **2.1. Participants**

89 This cross-sectional study originates from the analysis of data collected in 2009 in the context of a larger,
90 ongoing longitudinal study aimed at estimating the prevalence and incidence of metabolic and CV diseases in
91 a population of PCOS followed at the Endocrinology Unit of St. Orsola Hospital in Bologna, Italy. One hundred
92 and two Caucasian PCOS women in reproductive age were included. The diagnosis of PCOS was made
93 according to the Rotterdam criteria (25). Hyperandrogenism (HA) was defined as any of the following: presence
94 of hirsutism, defined by the modified Ferriman-Gallwey score ≥ 8 (26), total testosterone above 0.7ng/mL, or
95 free testosterone calculated by the Vermeulen formula higher than 9.52 pg/mL. Polycystic ovarian morphology
96 (PCOM) was defined as presence of 12 or more follicles 2–9 mm in diameter, and/or increased ovarian volume
97 >10 mL in at least one ovary, according to the 2004 ESHRE/ASRM recommendations (25). Oligo/anovulation
98 was diagnosed with the occurrence of menstrual cycles lasting more than 35 days or less than 21 days,
99 supported by the measurement of luteal phase progesterone being <2 ng/mL.

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

106 **2.2. PCOS phenotyping**

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

108 A: HA + oligo/anovulation + PCOM

109 B: HA + oligo/anovulation

110 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/m²), overweight (25-29.9 kg/m²), and obese (≥30 kg/m²) 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 (Curvoso, 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 $4.5 \pm 1.9\%$.

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 $1.6 \pm 1.0\%$ for NTG and $1.2 \pm 0.6\%$ 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

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

179 2.7. Statistical analysis

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

183 The anthropometric, biochemical, and CV US measurements were compared across the four PCOS
184 phenotypes and the three BMI classes (normal weight, overweight, obese) using one-way analysis of variance
185 (ANOVA) when the assumptions for this analysis were met. Otherwise, Welch's test was used. Following a
186 significant ANOVA F or Welch's test, post-hoc pairwise tests were conducted using the Bonferroni or Games-
187 Howell test with a correction to the probability level for multiple comparisons. Free T was compared among
188 the four PCOS phenotypes using the Kruskal-Wallis test. The relationship of CV US measures with BMI, used
189 as a continuous variable, was investigated using Pearson's correlation coefficient and multiple linear
190 regression models, in order to adjust for potential confounders. These included age, waist circumference, type
191 2 DM, smoking, HTA, fasting glucose, fasting insulin, free testosterone, HOMA2-IR, SHBG and FSH. A p value
192 of <0.05 was considered statistically significant. All statistical analyses were carried out using IBM SPSS,
193 version 25.0.

194 3. Results

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

202 No significant difference was found when biochemical parameters were compared among phenotypes (Tables
203 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

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

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

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

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

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

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

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

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

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

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

283

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

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

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

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

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

295

296 **References**

- 297 1. Urbanek M. The genetics of the polycystic ovary syndrome. *Nat Clin Pract Endocrinol Metab*
298 [Internet]. 2007;3(2):103–11. Available from:
299 <http://www.nature.com/doi/10.1038/ncpendmet0400>
- 300 2. Lizneva D, Suturina L, Walker W, Brakta S, Gavrilova-Jordan L, Azziz R. Criteria, prevalence, and
301 phenotypes of polycystic ovary syndrome. *Fertil Steril* [Internet]. 2016;106(1):6–15. Available from:
302 <http://dx.doi.org/10.1016/j.fertnstert.2016.05.003>
- 303 3. Glueck CJ, Morrison JA, Goldenberg N, Wang P. Coronary heart disease risk factors in adult
304 premenopausal white women with polycystic ovary syndrome compared with a healthy female
305 population. *Metabolism* [Internet]. 2009;58(5):714–21. Available from:
306 <http://dx.doi.org/10.1016/j.metabol.2009.02.005>
- 307 4. Daan NMP, Louwers Y V., Koster MPH, Eijkemans MJC, De Rijke YB, Lentjes EWG, Fauser BCJM,
308 Laven JSE. Cardiovascular and metabolic profiles amongst different polycystic ovary syndrome
309 phenotypes: Who is really at risk? *Fertil Steril* [Internet]. 2014;102(5):1444-1451.e3. Available from:
310 <http://dx.doi.org/10.1016/j.fertnstert.2014.08.001>
- 311 5. Glinborg D, Rubin KH, Nybo M, Abrahamsen B, Andersen M. Cardiovascular disease in a nationwide
312 population of Danish women with polycystic ovary syndrome. *Cardiovasc Diabetol* [Internet].
313 2018;17(1):1–12. Available from: <https://doi.org/10.1186/s12933-018-0680-5>
- 314 6. Wild S, Pierpoint T, McKeigue P, Jacobs H. Cardiovascular disease in women with polycystic ovary
315 syndrome at long- term follow-up: A retrospective cohort study. *Clin Endocrinol (Oxf)*.
316 2000;52(5):595–600.
- 317 7. Iftikhar S, Collazo-Clavell ML, Roger VL, Sauver JS, Brown JD, Cha S, Rhodes DJ. Risk of
318 cardiovascular events in patients with polycystic ovary syndrome. *Neth J Med*. 2012;70(2):74–80.
- 319 8. Schmidt J, Landin-Wilhelmsen K, Brännström M, Dahlgren E. Cardiovascular disease and risk factors
320 in PCOS women of postmenopausal age: A 21-year controlled follow-up study. *J Clin Endocrinol*
321 *Metab*. 2011;96(12):3794–803.
- 322 9. Gunning MN, Fauser BCJM. Are women with polycystic ovary syndrome at increased cardiovascular
323 disease risk later in life? *Climacteric*. 2017;20(3):222–7.
- 324 10. Hong YM. Atherosclerotic cardiovascular disease beginning in childhood. *Korean Circ J*.
325 2010;40(1):1–9.
- 326 11. Polak J, Pencina M, Pencina K, O'Donnell C, Wolf P, D'Agostino R. Carotid-wall intima-media

327 thickness and cardiovascular events. *N Engl J Med*. 2011;365(3):213–21.

328 12. Meyer ML, Malek AM, Wild RA, Korytkowski MT, Talbott EO. Carotid artery intima-media thickness in
329 polycystic ovary syndrome: A systematic review and meta-analysis. *Hum Reprod Update*.
330 2012;18(2):112–26.

331 13. Yeboah J, Folsom AR, Burke GL, Johnson C, Polak JF, Post W, Lima JA, Crouse JR, Herrington DM.
332 Predictive value of brachial flow-mediated dilation for incident cardiovascular events in a population-
333 based study: The multi-ethnic study of atherosclerosis. *Circulation*. 2009;120(6):502–9.

334 14. Guleria AK, Syal SK, Kapoor A, Kumar S, Tiwari P, Dabadghao P. Cardiovascular disease risk in
335 young Indian women with polycystic ovary syndrome. *Gynecol Endocrinol*. 2014;30(1):26–9.

336 15. Meun C, Franco OH, Dhana K, Jaspers L, Muka T, Louwers Y, Ikram MA, Fauser BCJM, Kavousi M,
337 Laven JSE. High Androgens in Postmenopausal Women and the Risk for Atherosclerosis and
338 Cardiovascular Disease: The Rotterdam Study. *J Clin Endocrinol Metab*. 2018;103(4):1622–30.

339 16. Iacobellis G, Assael F, Ribaudo MC, Zappaterreno A, Alessi G, Di Mario U, Leonetti F. Epicardial fat
340 from echocardiography: A new method for visceral adipose tissue prediction. *Obes Res*.
341 2003;11(2):304–10.

342 17. Iacobellis G, Lonn E, Lamy A, Singh N, Sharma AM. Epicardial fat thickness and coronary artery
343 disease correlate independently of obesity. *Int J Cardiol [Internet]*. 2011;146(3):452–4. Available from:
344 <http://dx.doi.org/10.1016/j.ijcard.2010.10.117>

345 18. Malavazos AE, Ermetici F, Coman C, Corsi MM, Morricone L, Ambrosi B. Influence of epicardial
346 adipose tissue and adipocytokine levels on cardiac abnormalities in visceral obesity. *Int J Cardiol*.
347 2007;121(1):132–4.

348 19. Soydinc E, Soydinc S, Ariturk Z, Tekbas E, Cakici M, Islamoglu Y, Ercan S, Sari I, Davutoglu V.
349 Increased epicardial fat thickness is related with body mass index in women with polycystic ovary
350 syndrome. *Eur Rev Med Pharmacol Sci*. 2013;17(15):2111–3.

351 20. Arpaci D, Gurkan Tocoglu A, Yilmaz S, Ergenc H, Tamer A, Keser N, Gunduz H. The relationship
352 between epicardial fat tissue thickness and visceral adipose tissue in lean patients with polycystic
353 ovary syndrome. *J Ovarian Res [Internet]*. 2015;8(1):4–9. Available from:
354 <http://dx.doi.org/10.1186/s13048-015-0197-4>

355 21. Aydogdu A, Uckaya G, Tasci I, Baysan O, Tapan S, Bugan B, Serdar M, Akbulut H, Aydogan U,
356 Sonmez A, et al. The relationship of epicardial adipose tissue thickness to clinical and biochemical
357 features in women with polycystic ovary syndrome. *Endocr J*. 2012;59(6):509–16.

- 358 22. Cakir E, Doğan M, Topaloglu O, Ozbek M, Cakal E, Vural MG, Yeter E, Delibasi T. Subclinical
359 atherosclerosis and hyperandrogenemia are independent risk factors for increased epicardial fat
360 thickness in patients with PCOS and idiopathic hirsutism. *Atherosclerosis*. 2013;226(1):291–5.
- 361 23. Sahin SB, Cure MC, Ugurlu Y, Ergul E, Gur EU, Alyildiz N, Bostan M. Epicardial adipose tissue
362 thickness and NGAL levels in women with polycystic ovary syndrome. *J Ovarian Res*. 2014;7(1):2–7.
- 363 24. Demir B, Cengiz H, Ungan I, Gedikbasi A, Karakoç G, Demir E, Demir N. The relationship between
364 epicardial adipose tissue thickness and oxidative stress parameters in patients with isolated
365 polycystic ovary syndrome. *Gynecol Endocrinol*. 2015;31(7):531–5.
- 366 25. The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003
367 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Vol.
368 81, *Fertility and Sterility*. 2004.
- 369 26. Hatch R, Rosenfield RL, Kim MH, Tredway D. Hirsutism: Implications, etiology, and management. *Am*
370 *J Obstet Gynecol* [Internet]. 1981;140(7):815–30. Available from: [http://dx.doi.org/10.1016/0002-](http://dx.doi.org/10.1016/0002-9378(81)90746-8)
371 [9378\(81\)90746-8](http://dx.doi.org/10.1016/0002-9378(81)90746-8)
- 372 27. Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, Piltonen T, Norman RJ, Andersen M,
373 Azziz R, et al. Recommendations from the international evidence-based guideline for the assessment
374 and management of polycystic ovary syndrome. *Clin Endocrinol (Oxf)*. 2018;89(3):251–68.
- 375 28. Akram D, Astrup A, Atinmo T, Boissin J, Bray G, Carroll K, Chitson P, Chunming C, Dietz W, Hill J, et
376 al. Obesity: Preventing and Managing the Global Epidemic [Internet]. WHO Technical Report Series.
377 2000. Available from: https://www.who.int/nutrition/publications/obesity/WHO_TRS_894/en/
- 378 29. Riddle MC, Bakris G, Blonde L, Boulton AJM, D'Alessio D, DiMeglio LA, Gonder-Frederick L, Hood
379 KK, Hu FB, Kahn SE, et al. Standards of medical care in diabetes. *Diabetes Care* [Internet].
380 2020;1(1). Available from: [https://professional.diabetes.org/content-page/practice-guidelines-](https://professional.diabetes.org/content-page/practice-guidelines-resources)
381 [resources](https://professional.diabetes.org/content-page/practice-guidelines-resources)
- 382 30. Fanelli F, Gambineri A, Belluomo I, Repaci A, Di Lallo VD, Di Dalmazi G, Mezzullo M, Prontera O,
383 Cuomo G, Zanolli L, et al. Androgen profiling by liquid chromatography-tandem mass spectrometry
384 (LC-MS/MS) in healthy normal-weight ovulatory and anovulatory late adolescent and young women. *J*
385 *Clin Endocrinol Metab*. 2013;98(7):3058–67.
- 386 31. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the Concentration of Low-Density Lipoprotein
387 Cholesterol in Plasma, Without Use of the Preparative Ultracentrifuge. *Clin Chem*. 1972;18(6):499–
388 502.

- 389 32. Levy JC, Matthews DR, Hermans MP. Correct homeostasis model assessment (HOMA) evaluation
390 uses the computer program [3]. *Diabetes Care*. 1998;21(12):2191–2.
- 391 33. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of
392 free testosterone in serum. *J Clin Endocrinol Metab*. 1999;84(10):3666–72.
- 393 34. Pizzi C, Costa GM, Santarella L, Flacco ME, Capasso L, Bert F, Manzoli L. Depression symptoms
394 and the progression of carotid intima-media thickness: A 5-year follow-up study. *Atherosclerosis*
395 [Internet]. 2014;233(2):530–6. Available from: <http://dx.doi.org/10.1016/j.atherosclerosis.2014.01.012>
- 396 35. Sutton-Tyrrell K, Wildman RP, Matthews KA, Chae C, Lasley BL, Brockwell S, Pasternak RC, Lloyd-
397 Jones D, Sowers MF, Torr  ns JI, et al. Sex hormone-binding globulin and the free androgen index
398 are related to cardiovascular risk factors in multiethnic premenopausal and perimenopausal women
399 enrolled in the study of women across the nation (SWAN). *Circulation*. 2005;111(10):1242–9.
- 400 36. Vryonidou A, Papatheodorou A, Tavridou A, Terzi T, Loi V, Vatalas IA, Batakis N, Phenekos C,
401 Dionyssiou-Asteriou A. Association of hyperandrogenemic and metabolic phenotype with carotid
402 intima-media thickness in young women with polycystic ovary syndrome. *J Clin Endocrinol Metab*.
403 2005;90(5):2740–6.
- 404 37. Merz CNB, Shaw LJ, Azziz R, Stanczyk FZ, Sopko G, Braunstein GD, Kelsey SF, Kip KE, Cooper-
405 Dehoff RM, Johnson BD, et al. Cardiovascular Disease and 10-Year Mortality in Postmenopausal
406 Women with Clinical Features of Polycystic Ovary Syndrome. *J Women's Heal*. 2016;25(9):875–81.
- 407 38. Dokras A, Jagasia DH, Maifeld M, Sinkey CA, VanVoorhis BJ, Haynes WG. Obesity and insulin
408 resistance but not hyperandrogenism mediates vascular dysfunction in women with polycystic ovary
409 syndrome. *Fertil Steril*. 2006;86(6):1702–9.
- 410 39. O'Leary DH, Bots ML. Imaging of atherosclerosis: Carotid intima-media thickness. *Eur Heart J*.
411 2010;31(14):1682–9.
- 412 40. Moghetti P, Tosi F, Bonin C, Di Sarra D, Fiers T, Kaufman JM, Giagulli VA, Signori C, Zambotti F,
413 Dall'Alda M, et al. Divergences in insulin resistance between the different phenotypes of the
414 polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2013;98(4):628–37.
- 415 41. Bo  i  -Anti   I, Ili   D, Bjeki  -Macut J, Bogavac T, Vojnovi  -Milutinovi   D, Kastratovic-Kotlica B, Mili   N,
416 Stanojlovi   O, Andri   Z, Macut D. Lipid accumulation product as a marker of cardiometabolic
417 susceptibility in women with different phenotypes of polycystic ovary syndrome. *Eur J Endocrinol*.
418 2016;175(6):551–60.
- 419 42. Gambineri A, Pelusi C, Vicennati V, Pagotto U, Pasquali R. Obesity and the polycystic ovary

syndrome. *Int J Obes*. 2002;26:883–96.

43. Borrueal S, Fernández-Durán E, Alpañés M, Martí D, Álvarez-Blasco F, Luque-Ramírez M, Escobar-Morreale HF. Global adiposity and thickness of intraperitoneal and mesenteric adipose tissue depots are increased in women with polycystic ovary syndrome (PCOS). *J Clin Endocrinol Metab*. 2013;98(3):1254–63.

44. Deswal R, Yadav A, Dang AS. Sex hormone binding globulin - an important biomarker for predicting PCOS risk: A systematic review and meta-analysis. *Syst Biol Reprod Med* [Internet]. 2018;64(1):12–24. Available from: <https://doi.org/10.1080/19396368.2017.1410591>

45. Tosi F, Bonora E, Moghetti P. Insulin resistance in a large cohort of women with polycystic ovary syndrome: A comparison between euglycaemic-hyperinsulinaemic clamp and surrogate indexes. *Hum Reprod*. 2017;32(12):2515–21.

46. Gambineri A, Patton L, Altieri P, Pagotto U, Pizzi C, Manzoli L, Pasquali R. Polycystic ovary syndrome is a risk factor for type 2 diabetes: Results from a long-term prospective study. *Diabetes*. 2012;61(9):2369–74.

47. Wassell J, Michail M, Soliman N, Wardle PG. The Value of Sex Hormone Binding Globulin (SHBG) in Predicting Treatment Response in Polycystic Ovary Syndrome (PCOS). *Clin Lab* [Internet]. 2011;1–2:95–8. Available from: <https://www.clin-lab-publications.com/article/700>

48. Macut D, Antić IB, Bjekić-Macut J. Cardiovascular risk factors and events in women with androgen excess. *J Endocrinol Invest*. 2015;38(3):295–301.

49. Conway G, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Franks S, Gambineri A, Kelestimur F, Macut D, Micic D, Pasquali R, et al. The polycystic ovary syndrome: A position statement from the European Society of Endocrinology. *Eur J Endocrinol*. 2014;171(4):P1–29.