

Relationship of Biological Markers of Body Fat Distribution and Corticosteroidogenic Enzyme Activities in Women with Polycystic Ovary Syndrome

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ABSTRACT

The aim of the study is to determine the impact of different anthropometric measurements of fat distribution on baseline sex-steroid concentrations and corticosteroidogenic enzyme activity in women with polycystic ovary syndrome compared to those with regular menstrual cycles. The current cross-sectional study included 106 normal cycling controls and 268 polycystic ovary syndrome patients. Patients with polycystic ovary syndrome, diagnosed by Rotterdam criteria, were divided in normoandrogenemic ($n = 91$) and hyperandrogenemic ($n = 177$). Anthropometric, biochemical, and hormone parameters were assessed and correlated with corticosteroidogenic enzyme activities in all three groups. Corticosteroidogenic enzyme activities were calculated using product-to-precursor ratios. Regarding sex-steroids individually, anthropometric parameters correlated with the concentrations of several androgens in polycystic ovary syndrome patients, most of them in patients with biochemical hyperandrogenism. The androgen precursors androstenedione, 17-hydroxyprogesterone, and dehydroepiandrosterone were less correlated with anthropometric parameters. The 17,20 lyase activity, in both $\Delta 4$ and $\Delta 5$ pathways, correlated with several anthropometric measurements in normo- and hyperandrogenemic polycystic ovary syndrome patients. The 17,20 lyase enzyme activity ($\Delta 4$ pathway) also correlated with conicity index, visceral adiposity index, and lipid accumulation product in the control group. 17-Hydroxylase activity positively correlated with waist-height ratio in both polycystic ovary syndrome groups. In contrast, 17-hydroxylase negatively correlated with the conicity index. Anthropometric markers of adiposity are associated with androgen levels and their precursors in blood. Body fat distribution correlates with the activities of some steroidogenic enzyme in both normo- and hyperandrogenemic polycystic ovary syndrome phenotypes. The molecular mechanisms involved in these associations are largely unclear and more investigations are required.

Introduction

Polycystic ovary syndrome (PCOS) is characterized by infrequent menses, amenorrhea, infertility, weight gain, dyslipidemia, dysglycemia, and hyperandrogenemia. PCOS patients with hyperandro-

genemia may have a higher risk for cardiovascular disease (CVD) than those with normoandrogenemia phenotype [1]. Moreover, there is a strong association between androgens and their precursors and obesity [2]. Hyperandrogenism is also associated with the

amount of fat localized in the trunk [3] and the administration of testosterone has been shown to increase visceral fat accumulation [4]. Therefore, hyperandrogenism may increase abdominal visceral adiposity in women and hyperandrogenic PCOS patients are more likely to be obese [5].

Several anthropometric parameters have been used as markers for a higher risk of type II diabetes mellitus, and CVD, mainly when hyperandrogenism is associated with abnormal fat deposition [6]. Additionally, it was demonstrated that body mass index (BMI), waist circumferences (WC)/hip ratio (WHR), and conicity index (CI) have a strong correlation with total fat mass and central abdominal fat, as diagnosed with dual X-ray absorptiometry (DEXA) [7]. Visceral adiposity index (VAI) has recently replaced computerized tomography (CT) as a surrogate marker for visceral adiposity in clinical studies [8]. In prepubertal and pubertal girls, progesterone (P4), 17-hydroxypregnenolone (17-OHPE), total testosterone (T), and androstenedione (A4) increase with obesity. VAI and fat mass (FM) seem to be positively correlated with T [9], while WHR positively correlated with A4 [10]. In contrast, dehydroepiandrosterone (DHEA) seems to be negatively related to total and visceral adiposity in non-PCOS subjects [11].

The net effects of the degree or the distribution of adiposity on steroidogenic enzyme activities remain unclear. Adipokines were shown to modulate steroidogenic enzymes, such as the steroidogenic acute regulatory protein (StAR), P450 side chain cleavage enzyme (CYP11A1), and 3 β -hydroxysteroid dehydrogenase (3 β -HSD) [12, 13]. Further, adiponectin has been shown to reduce the production of A4 in theca cells [14]. Androgens increase visceral fat mass through the inhibition of lipolysis and the stimulation of lipogenesis [15]. Though the existence of individual variations in steroid levels in adipose tissue [16], it is less clear whether and to what extent the levels of these steroid hormones vary within the same individual. In vitro and in vivo studies suggest different steroidogenic capacity between abdominal adipose tissue and adipose tissue from other locations [17–20]. Further, 17 β -hydroxysteroid dehydrogenase (17-HSD) activity in abdominal adipose tissue may favor visceral adiposity and metabolic syndrome [21]. The present study was designed to examine the association of different anthropometric parameters as surrogate markers of adipose tissue distribution with androgen concentrations and steroidogenic enzyme activities in PCOS. These possible associations were assessed between PCOS women with high androgen levels in blood, PCOS women with normal levels of androgen and, also in non-PCOS women with normal menstrual cycles.

Subjects and Methods

Study design and subjects

This cross-sectional study, using accessibility sampling, enrolled 268 patients diagnosed with PCOS using the Rotterdam criteria, and 106 normal cycling controls who presented to the Júlio Muller University Hospital and Tropical Institute of Reproductive Medicine in Cuiabá, MT, Brazil from January 2012 to June 2017. A specific signed informed consent approved by the local Committee for Ethics in Research was signed by each participant before data collection in the course of routine clinical assistance in both Institutions.

PCOS patients were divided in normoandrogenic PCOS (NA-PCOS, n = 91) and hyperandrogenic PCOS (HA-PCOS, n = 177). NA-PCOS, HA-PCOS and normal cycling women were compared to assess whether they present differences in the corticosteroidogenic enzyme activities among them. According to several robust publications, biochemical hyperandrogenic PCOS may be defined by the presence of one or more of the following biochemical parameters: T \geq 2.1 nmol/l, free T \geq 0.027 pmol/l, DHEA \geq 6.7 μ mol/l, A4 \geq 8.6 nmol/l, and free androgen index (FAI) \geq 6 [22–25]. Patients who had used sex steroids or insulin sensitizing drugs over the last six months or who did not fulfill the Rotterdam criteria were excluded. In addition, patients with TSH levels \geq 4.2 μ U/l, prolactin \geq 1.086 pmol/l, and 17-OHP4 \geq 6 nmol/l were also excluded.

Measurement of anthropometric characteristics

The subjects were weighed on an electronic scale, and height was measured using a Harpenden stadiometer (Holtain Limited, Crymch, Dyfed, UK). The waist circumference (WC) was measured at the midway point between the lower rib margin and the iliac crest, and the hip was measured at the widest circumference at the iliac crest. BMI was calculated as body weight (kg)/height (m²). Lean body mass (LBM) was calculated using the James equation: $[1.07 \times \text{weight (kg)}] - 148 \times \text{weight (kg)}^2 / [100 \times \text{height (m)}^2]$ [26]. Fat mass (FM) was calculated as body weight minus LBM. Abdominal adiposity was estimated using the conicity index (C index): $\text{WC (m)} / (0.109 \times \text{square root of body weight (kg)} / \text{height (m)})$ [27]. The visceral adiposity index (VAI) was estimated using the equation: $\text{WC} / [36.58 + (1.89 \times \text{BMI})] \times (\text{TG} / 0.81) \times (1.52 / \text{HDL-C})$ [28]. Lipid accumulation product (LAP) was calculated as established for women $[\text{WC (cm)} - 58] \times \text{TG (mmol/l)}$ [29].

Biochemical and hormone analysis

All patients with regular cycles were tested in the early follicular phase of the menstrual cycle (days 3–5 of the cycle). Patients with infrequent menses or amenorrhea had their blood collected at any time provided the progesterone was less than 6.4 nmol/l. Triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and total cholesterol (TC) levels were measured using an enzymatic assay (Wiener Laboratories, Rosário, Argentina). Low-density lipoprotein cholesterol (LDL-C) was calculated as $\text{TC} - (\text{HDL-C} + \text{TG} / 5)$ [30]. Glucose concentration was analyzed using the glucose oxidase technique (Beckman Glucose Analyses, Fullerton, CA, USA). Impaired glucose tolerance (IGT), or prediabetes, was defined by a single abnormal parameter as follows: fasting plasma glucose (FPG) between 100 mg/dl (5.5 mmol/l) and 126 mg/dl (6.99 mmol/l); 2-hour oGTT glucose value between 140 mg/dl (7.8 nmol/l) and 199 mg/dl (11.0 nmol/l) [7]. Type II diabetes mellitus (T2DM) was defined as fasting glucose \geq 6.99 nmol/l or over 7.8 nmol/l at 120 min after 75 g dextrose ingestion. Insulin resistance was defined using fasting insulin levels $>$ 12.2 μ U/ml (84.7 pmol/l) [26]; and/or homeostasis model assessment of insulin resistance (HOMA-IR) \geq 2.7 [31]. The homeostatic model for insulin resistance and tissue sensitivity to insulin (HOMA-IR) was calculated using a free online program [32]: $[\text{glucose (nmol/l)} \times \text{insulin (}\mu\text{U/ml)}] / 22.5$. Oral glucose tolerance test (OGTT) was performed by measuring glucose before and 120 min after 75 g oral dextrose. All hormone measurements were detailed in a recent publication [33]. The level of 17-OHPE was

measured with an HPLC/MS/MS (Labco Nous Advanced Special Diagnostics, SP, Brazil). The sensitivity was 0.033 nmol/l, and intra- and inter-assay coefficients of variation were between 10.4% and 12.9%, respectively. FAI was calculated as the T (nmol/l)/SHBG (nmol/l) × 100. Free estrogen index (FEI) was estimated as 100 × E2pmol/l/272.14 × SHBG [28].

Statistical analysis

Results are presented as percentages or mean \bar{x} and standard deviation (SD) or number and percentage (n%). Comparison of ethnicities was performed using the chi-square test. Proportions of IGT, IR, and HOMA-IR among groups were also compared using the chi-square test followed by the Dunn test with Bonferroni adjustments. Baseline differences between more than two independent continuous variables were assessed using one-way analysis of variance (ANOVA), followed by the Tukey post hoc and F tests. The re-

lationship between two Gaussian distributed variables was examined using the Pearson's correlation coefficient (r). Further, a stepwise-multiple linear regression analysis was performed to estimate the relationship between enzyme activity and anthropometric parameters. The product/precursor ratio was used as the criterion variable and anthropometric, endocrine, and metabolic variables that presented a simple significant correlation with the criterion variable were used as predictor variables. The Durbin-Watson test was used to verify correlation between residuals. All statistical procedures were performed with SPSS version 17 (SPSS Inc., Chicago, IL, USA). All tests were two-sided, and p-values < 0.05 were considered statistically significant.

Results

Baseline characteristics of normo- and hyperandrogenic PCOS patients and normal cycling controls

Ethnicity, clinical, and anthropometric features of each group are summarized in ► **Table 1**. Regarding ethnicity, no differences were found among groups ($\chi^2 = 2.538$, $p = 0.281$). Blood pressures, both SBP and DBP, were higher in HA-PCOS group than in controls and NA-PCOS ($p < 0.001$, and $p = 0.004$, respectively). BMI, WC, WHR, and CI, LBM, FM, and LAP, variables commonly associated with higher risk for long-term disease, were significantly increased in HA-PCOS patients when compared with controls and NA-PCOS groups (► **Table 1**). All data regarding carbohydrate metabolism are shown in ► **Table 2**. IFG had similar prevalence in controls and NA-PCOS (4.1 vs. 4.5%, respectively; $p = 0.976$). In HA-PCOS patients IFG was more frequent than in controls and in NA-PCOS. In the whole group of PCOS, IFG was higher than in NA-PCOS and similar to HA-PCOS (12.3 vs. 15.9%, $p = 0.352$; data not shown).

Glucose intolerance (GI, glucose ≥ 7.8 nmol/l at 120 in TTOG) in the whole group of PCOS had a prevalence of 22.1%. Other results regarding GI are shown in ► **Table 2**. Using the cut-off values of 84.7 pmol/l, the insulin resistance was more prevalent in NA-PCOS (43.9%), HA-PCOS (57.8%), and in PCOS as a group (52.4%) than in controls (12.8%) ($p < 0.001$ for all comparisons). Otherwise, NA-PCOS presented lower prevalence of IR than HA-PCOS ($p < 0.001$), and than PCOS women as a group ($p < 0.001$). The baseline levels of fasting insulin in all PCOS groups were significantly higher than in controls (► **Table 2**). In the control group, no women presented HOMA-IR over 2.7. HOMA-IR was ≥ 2.7 in 10% NA-PCOS, 30.6% in HA-PCOS, and in 23.8% in the whole group of PCOS women. There were no difference in HOMA-IR between controls and NA-PCOS women ($p = 0.144$). However, HOMA-IR was higher in HA-PCOS than in controls (30.6% vs. 0%, $p < 0.001$), NA-PCOS (30.6% vs. 10%, $p < 0.001$) and in the whole PCOS group (30.6% vs. 23.8%, $p = 0.003$). FPG, insulin, and HOMA-IR were also higher in HA-PCOS than in NA-PCOS. On the other hand, HOMA-5 was lower in the NA-PCOS and HA-PCOS groups. C-peptide was higher in NA-PCOS and HA-PCOS when compared with controls (► **Table 2**).

► **Table 3** compares sex-steroids concentrations and steroidogenic enzymatic activities between normal cycling women and women with PCOS, either normoandrogenic or hyperandrogenic. Though E2 had been similar in all groups, FEI shows statisti-

► **Table 1** Comparison of clinical and anthropometric baseline characteristics of women with polycystic ovary syndrome and normal cycling non-PCOS women.

Variable	Normal cycling women	NA-PCOS group	HA-PCOS group
Age (years)	30.6 (4.73)	29.1 (5.40)	26.9 (5.00) ^{a,b}
Ethnicity n (%)			
White	85 (80.1)	119 (71.7)	70 (76.9) ^{NS}
Afro descendent	14 (13.2)	26 (15.7)	10 (11.0) ^{NS}
Others	6 (5.7)	21 (12.6)	11 (12.1) ^{NS}
Blood pressure (mmHg)			
SBP	112.9 (9.3)	114.4 (13.1)	118.3 (10.9) ^{a,c}
DBP	73.0 (7.3)	73.4 (9.7)	76.2 (10.6) ^{a,d}
Anthropometric parameters			
Body weight (kg)	61.6 (9.4)	75.0 (199.4)	75.7 (16.5) ^{a,d}
BMI (kg/m ²)	23.5 (3.4)	28.9 (7.0)	30.3 (6.7) ^{a,d}
WC (cm)	73.5 (8.3)	86.3 (15.0)	88.2 (14.6) ^{a,d}
WHR (cm)	0.75 (0.06)	0.80 (0.07)	0.82 (14.6) ^{a,d}
CI (% pg/ml.nmol)	1.09 (0.07)	1.16 (0.09)	1.18 (0.1) ^{a,d}
LBM (kg)	43.42 (4.44)	46.92 (6.69)	45.77 (5.62) ^{a,e}
FM/LBM ratio	0.41 (0.11)	0.59 (0.22)	1.80 (0.73) ^{b,c,d}
FM (kg)	18.18 (5.88)	28.57 (13.92)	29.71 (11.96) ^{a,d}
VAI	1.16 (0.77)	1.77 (1.61)	2.66 (2.43) ^{a,b}
LAP	14.65 (12.90)	32.82 (28.62)	52.25 (48.62) ^{c,d,f}

Data are expressed as mean and standard deviation \bar{x} (SD) or n (%). ANOVA followed by Tukey post hoc test was used for continuous variables and χ^2 test followed by Dunn test with Bonferroni adjustments for categorical variables. SBP: Systolic blood pressure; DBP: Diastolic blood pressure; BMI: Body mass index; WC: Waist circumferences; WHR: Hip ratio; CI: Conicity index; LBM: Lean body mass; FM/LBM: Fat mass/ lean body mass ratio; FM: Fat mass; VAI: Visceral adiposity index; LAP: Lipid accumulation product. NS: $p > 0.05$; ^a Control vs. NA-PCOS, $p < 0.001$; ^b NA-PCOS vs. HA-PCOS, $p = 0.004$; ^c NA-PCOS vs. HA-PCOS, $p < 0.001$; ^d control vs. HA-PCOS, $p < 0.001$; ^e Control vs. HA-PCOS, $p = 0.004$; ^f Control vs. NA-PCOS, $p = 0.003$.

► Table 2 Comparison of baseline carbohydrate metabolic markers in women with polycystic ovary syndrome and non-PCOS women with regular menstrual cycles.

Variable*	Normal cycling women	NA-PCOS group	HA-PCOS group
FPG (nmol/l)	4.7 (0.5)	4.8 (0.4)	4.9 (0.5) ^a
IFG (%)	4.1	4.5	15.9 ^{b,c}
GI (%)	3.7	13.8	25.3 ^{a,b,c}
Fasting insulin (μmol/l)	48.1 (3.7)	83.5 (5.8)	101.8 (10.8) ^{a,b,e}
HOMA-IR ≥ 2.7 (%)	0	10.0	30.6 ^{a,b}
HOMA-IR	0.95 (0.2)	1.56 (0.1)	1.95 (0.2) ^{a,c,f}
HOMA%B	106.9 (44.8)	142.4 (79.1)	154.9 (66.9) ^{a,g}
HOMA-S	132.5 (70.3)	79.3 (53.1)	69.9 (49.5) ^{a,f}
Pep-C (nmol/l)	0.53 (0.2)	0.68 (0.3)	0.90 (0.4) ^{a,d,h}

* Categorical variables age given in proportions (%) and continuous variables are given in \bar{x} (SD). ANOVA followed by Tukey post hoc test was used for continuous variables and χ^2 test followed by Dunn test with Bonferroni adjustments for categorical variables. FPG: Fasting plasma glucose; IFP: Impaired fasting glucose; GI: Glucose intolerance; HOMA: Homeostatic assessment model; Pep-C: C-peptide. ^a Controls vs. HA-PCOS, $p < 0.001$; ^b Controls vs. HA-PCOS, $p = 0.008$; ^c NA-PCOS vs. HA-PCOS, $p = 0.007$; ^d NA-PCOS vs. HA-PCOS, $p < 0.001$; ^e NA-PCOS vs. HA-PCOS, $p = 0.030$; ^f Controls vs. NA-PCOS, $p < 0.001$; ^g Controls vs. NA-PCOS, $p = 0.002$; ^h Controls vs. NA-PCOS, $p = 0.003$.

cally significant higher levels in NA-PCOS and HA-PCOS women than in controls ($p < 0.001$ and $p < 0.001$, respectively). As expected, all androgen hormones were also higher in HA-PCOS women than in controls and NA-PCOS subjects. Regarding the enzyme activities, 17,20 lyase activity ($\Delta 4$ pathway) was higher in HA-PCOS than in NA-PCOS and controls ($p < 0.001$ and $p < 0.001$, respectively). 17-hydroxylase activity ($\Delta 4$) was higher in NA-PCOS and HA-PCOS than in controls ($p < 0.0001$ and $p = 0.025$, respectively). The 3- β HSD had higher activity in the conversion of DHEA into A4 in HA-PCOS and NA-PCOS groups ($p < 0.001$ for both comparisons). Further, 21-hydroxylase activity was lower in HA-PCOS than in normal cycling women ($p < 0.001$) and in NA-PCOS ($p = 0.025$). The combined activity of 21-hydroxylase and 11-hydroxylase was also lower in HA-PCOS than in NA-PCOS and controls ($p < 0.001$ and $p = 0.015$, respectively).

Simple correlation between anthropometric parameters and androgens concentrations

Possible simple correlations between anthropometric measurements and a particular androgen concentration are summarized in this section. BMI presented a negatively and significant correlation with 17-OHPE in controls ($r = -0.201$, $p = 0.049$). In NA-PCOS, BMI was negatively correlated with DHEA ($r = -0.245$, $p = 0.033$) but in HA-PCOS BMI was positively correlated with total testosterone ($r = 0.199$, $p = 0.010$). WHR was positively correlated with total tes-

tosterone in controls ($r = 0.215$, $p = 0.037$), and negatively correlated with A4 ($r = -0.384$, $p < 0.001$), and DHEA ($r = -0.276$, $p = 0.016$) in the NA-PCOS group. On the other hand, in HA-PCOS WHR was negatively correlated with 17-OHPE ($r = -0.237$, $p = 0.002$). The WHtR was negatively correlated with 17-OHPE ($r = -0.201$, $p = 0.048$) in controls, negatively correlated with A4 ($r = -0.249$, $p = 0.019$) in NA-PCOS, and negatively ($r = -0.167$) and positively ($r = 0.116$) correlated with 17-OHPE ($p = 0.033$), and total testosterone ($p = 0.006$), respectively.

The conicity index (CI) negatively correlated with A4 ($r = -0.249$, $p = 0.027$) in NA-PCOS and with 17-OHPE ($r = -0.175$, $p = 0.027$) in HA-PCOS. LBM was correlated only with A4 ($r = 0.200$, $p = 0.014$) in the HA-PCOS group. FM was negatively correlated with 17-OHP4 ($r = -0.205$, $p = 0.050$) in controls and with total testosterone ($r = 0.192$, $p = 0.014$) in HA-PCOS. LBM/FM ratio was negatively correlated with total testosterone in HA-PCOS ($r = -0.176$, $p = 0.024$). VAI was negatively correlated with 17-OHP4 ($r = -0.211$, $p = 0.049$) and positively correlated with A4 ($r = 0.251$, $p = 0.020$) in controls. In NA-PCOS VAI was positively correlated with 17-OHPE ($r = 0.425$, $p < 0.001$), and negatively correlated with 17-OHPE ($r = -0.175$, $p = 0.036$) in the HA-PCOS group. Finally, LAP was positively correlated with 17-OHPE in NA-PCOS ($r = 0.417$, $p < 0.001$), and negatively correlated with 17-OHPE ($r = -0.199$, $p = 0.015$) in the HA-PCOS group.

Simple correlation between anthropometric measures and corticosteroidogenic enzyme activity

Associations between anthropometric parameters and enzyme activities are shown in ► **Table 4**. In both NA-PCOS and HA-PCOS patients, the $\Delta 5$ pathway 17,20 lyase negatively correlated with CI, and LAP, but in this $\Delta 5$ pathway, 17,20 lyase correlated positively with WHR in the HA-PCOS group. In controls 17,20 lyase ($\Delta 5$) did not present correlation with any anthropometric measurement (► **Table 4**). In the $\Delta 4$ pathway in normal cycling women, 17,20 lyase activity was positively correlated with CI, VAI, and LAP. In HA-PCOS, this enzyme in the $\Delta 4$ pathway positively correlated with FM and the LBM/FM ratio.

In normal cycling women, 17-hydroxylase activity, in the conversion of P4 into 17-OHP4, presented a negative simple correlation with CI, but in HA-PCOS patients this enzyme was positively correlated with WHR. A non-significant trend was observed between 17-hydroxylase activity, body weight ($p = 0.052$) and BMI ($p = 0.071$) in the HA-PCOS group. In this HA-PCOS group, in the conversion of 17-OHPE into 17-OHP4, the 3 β -HSD activity was positively correlated with WC ($p = 0.049$) and WHR ($p = 0.023$) and negatively correlated with VAI ($p = 0.012$). In NA-PCOS, 3 β -HSD activity also negatively correlated with VAI. In the conversion of DHEA into A4, 3 β -HSD activity positively correlated with WHR in HA-PCOS patients and negatively correlated with WHtR in normal controls. In both NA-PCOS and HA-PCOS patients, the activity of 21-hydroxylase did not correlate with anthropometric parameters. On the contrary, in controls, 3 β -HSD was positively correlated with BMI, WHtR, CI, and LAP. 11 β -Hydroxylase also did not correlate with any anthropometric parameter in PCOS but was negatively correlated with WHR and CI in normal controls.

► **Table 3** Comparison of endocrine characteristics between women with polycystic ovary syndrome and women with normal regular cycles.

Variable *	Control \bar{x} (SD)	Normoandrogenemic PCOS \bar{x} (SD)	Hyperandrogenemic PCOS \bar{x} (SD)
P4 (nmol/l)	1.54 (1.02)	1.21 (0.78)	2.10 (1.10) ^{NS}
E2 (pmol/l)	181.85 (99.32)	179.61 (82.84)	191.81 (79.18) ^{NS}
FEI (%) (ng/nmol)	0.35 (0.22)	0.43 (0.27)	0.72 (0.47) ^{a,b}
T (nmol/l)	1.00 (0.54)	1.11 (0.39)	2.34 (1.08) ^{a,b}
fT (nmol/l)	0.013 (0.01)	0.021 (0.02)	0.057 (0.04) ^{a,c}
FAI (%)	2.14 (1.90)	2.59 (1.36)	8.87 (5.81) ^{a,b}
F (μ mol/l)	353.17 (143.74)	321.87 (131.67)	342.33 (141.26) ^{NS}
A4 (nmol/l)	5.20 (2.39)	4.96 (1.91)	10.39 (5.104) ^{a,b}
DHEA (nmol/l)	15.23 (8.41)	14.98 (8.68)	18.01 (10.31) ^{a,b}
DHEAS (μ mol/l)	4.15 (1.93)	3.69 (1.53)	5.41 (2.36) ^{a,b}
17OHP4 (nmol/l)	2.56 (1.23)	2.78 (1.51)	3.54 (1.78) ^{a,b}
17OHPE (nmol/l)	4.98 (4.15)	5.19 (4.33)	6.67 (5.50) ^d
Comp S (nmol/l)	6.88 (2.77)	6.26 (2.86)	7.18 (4.44) ^{NS}
P450, 17 α			
17,20 lyase (Δ 5)	4.92 (1.23)	4.59 (0.92)	4.49 (1.14) ^{NS}
17,20 lyase (Δ 4)	2.17 (0.15)	2.10 (0.12)	3.11 (0.29) ^{a,c}
17-hydroxylase (Δ 4)	2.24 (1.54)	3.14 (2.73)	2.90 (2.28) ^{b,e}
3 β HSD (A4/DHEA)	0.36 (0.22)	0.41 (0.25)	0.68 (0.44) ^{a,b}
3 β HSD (17-OHP4/17-OHPE)	0.98 (0.17)	1.02 (0.46)	0.98 (0.38) ^{NS}
21-Hydroxylase	2.86 (0.16)	2.41 (0.13)	2.05 (0.18) ^a
11-Hydroxylase	53.14 (2.26)	53.37 (2.28)	56.43 (7.86) ^{NS}
F/17-OHP4	156.68 (79.11)	137.46 (66.06)	120.26 (8.937) ^{a,d}

* ANOVA test followed by the Tukey post hoc test. P4: Progesterone; E2: Estradiol; FEI: Free estrogen index; T: Total testosterone; fT: Free testosterone; FAI: Free androgen index; F: Cortisol; A4: Androstenedione; DHEA: Dehydroepiandrosterone; DHEAS: Dehydroepiandrosterone sulfate; 17OHP4: Hydroxyprogesterone; 17OHPE: Hydroxypregnenolone; comp S: Compound S. NS: $p > 0.05$; ^a Control vs. HA-PCOS, $p < 0.001$; ^b Control vs. NA-PCOS, $p < 0.001$; ^c NA-PCOS vs. HA-PCOS, $p < 0.001$; ^d Control vs. HA-PCOS, $p = 0.015$; ^e Control vs. HA-PCOS, $p = 0.025$.

Stepwise multiple regression

The stepwise multiple regression models, including product-to-precursor ratios as criteria (dependent) variables and anthropometric parameters that showed significant simple correlation with these ratios as predictors (independency variables) are shown in ► **Table 5**. In the NA-PCOS group, 17,20 lyase activity in the conversion of 17-OHPE into DHEA (Δ 5 pathway) was facilitated by BMI and LAP (Adj $R^2 = 0.106$, $t = 2.179$, $p = 0.033$). In HA-PCOS patients, in the conversion of 17-OHP4 into A4 (Δ 4 pathway), 17,20 lyase activity was positively favored by FM (Adj $R^2 = 0.029$, $t = 2.175$, $p = 0.032$). In controls, LAP was correlated with Δ 4 pathway 17,20 lyase action (Adj $R^2 = 0.016$, $t = 2.213$, $p = 0.030$).

In controls, WC may facilitate the 17-hydroxylase activity in the conversion of P4 into 17-OHP4 (Adj $R^2 = 0.073$, $t = -2.608$, $p = 0.011$). In NA-PCOS, 17-hydroxylase, Δ 4 pathway, was positively favored by WHtR (Adj $R^2 = 0.047$, $t = 2.129$, $p = 0.037$). In HA-PCOS, the activity of 3 β -HSD was influenced only by WHR (Adj $R^2 = 0.050$, $t = 2.878$, $p = 0.005$) in the conversion of 17-OHPE into 17-OHP4. The activity of 21-hydroxylase was shown to be influenced only by LAP (Adj $R^2 = 0.067$, $t = 2.522$, $p = 0.014$) in normal controls. In PCOS patients, either normo- or hyperandrogenemic groups, anthropo-

metric parameters did not influence either 21-hydroxylase or 11 β -hydroxylase activities when a stepwise multiple regression model was applied.

Discussion

The association between hyperandrogenism and adipose tissue dysfunction, either in its amount or in its centralized distribution, is still debatable. Considering the high prevalence of obesity and hyperandrogenism in PCOS, the current study examined the impact of various anthropometric, proved markers of fat mass distribution, on corticosteroidogenic enzyme activities; secondarily, compared the levels of baseline sex-steroid and enzyme activities in NA-PCOS, HA-PCOS, and normal cycling women. Most anthropometric, metabolic and hormonal characteristics found in these groups confirmed previous reports [34, 35]. Insulin levels showed different results between NA-PCOS and HA-PCOS and this seems to have clinical significance since insulin resistance is more frequent and intense in the HA-PCOS groups [36]. A positive correlation between some anthropometric parameters and T concentration was demonstrated in HA-PCOS, indicating that high T levels leads to

▶ Table 4 Simple correlations between anthropometric parameters and steroidogenic enzyme activities in normo- and biochemical hyperandrogenemic polycystic ovary syndrome and normal cycling women.

Adiposity indicators	17,20 Lyase (A5) (DHEA/17-OHPE) r (p)	12,20 Lyase (A4) (A4/17-OHP4) r (p)	17-Hydroxylase (17-OHP4/P4) r (p)	3β-HSD II (17-OHP4/17-OHPE) r (p)	3β-HSD II (A4/DHEA) r (p)	21-Hydroxylase (S/17-OHP4) r (p)	11β-Hydroxylase (F/S) r (p)
BMI (kg/m ²)							
Controls	-0.195 (0.889)	0.107 (0.320)	-0.197 (0.076)	0.015 (0.886)	-0.089 (0.428)	0.222 (0.043) *	0.034 (0.759)
WHR							
Controls	-0.187(0.081)	0.147 (0.168)	-0.095 (0.393)	-0.102 (0.333)	0.094 (0.399)	0.115 (0.299)	-0.291 (0.006) *
HA-PCOS	0.217 (0.030) *	0.105 (0.204)	0.184 (0.028) *	0.180 (0.023) *	0.197 (0.031) *	0.085 (0.470)	-0.083 (0.346)
WHR							
Controls	-0.040 (0.713)	0.164 (0.127)	-0.207 (0.062)	-0.066 (0.533)	-0.022 (0.045) *	0.252 (0.021) *	-0.119 (0.277)
C Index							
Controls	-0.007 (0.949)	0.218 (0.042) *	-0.242 (0.030) *	-0.060 (0.573)	0.065 (0.566)	0.248 (0.025) *	-0.249 (0.023) *
NA-PCOS	-0.294 (0.012) *	-0.102 (0.376)	-0.062 (0.599)	-0.196 (0.078)	-0.018 (0.832)	-0.009 (0.940)	-0.130 (0.291)
HA-PCOS	-0.294 (0.012) *	-0.102 (0.376)	-0.062 (0.599)	-0.196 (0.078)	-0.018 (0.882)	-0.009 (0.940)	-0.130 (0.291)
FM							
HA-PCOS	0.055 (0.589)	0.212 (0.010) *	-0.037 (0.662)	0.048 (0.549)	0.117 (0.207)	0.028 (0.749)	-0.009 (0.916)
LBM/FM							
HA-PCOS	-0.041 (0.683)	0.231 (0.005) *	-0.067 (0.429)	0.098 (0.217)	-0.106 (0.251)	-0.014 (0.868)	-0.071 (0.423)
VAI							
Controls	-0.061 (0.584)	0.247 (0.026) *	-0.105 (0.765)	-0.162 (0.136)	-0.092 (0.421)	0.153 (0.185)	-0.142 (0.212)
NA-PCOS	-0.319 (0.006) *	0.058 (0.616)	0.104 (0.378)	-0.277 (0.012) *	-0.024 (0.843)	0.033 (0.782)	0.154 (0.214)
HA-PCOS	-0.319 (0.016) *	0.058 (0.616)	0.104 (0.378)	-0.277 (0.012) *	-0.024 (0.843)	0.033 (0.782)	0.154 (0.214)
LAP							
Controls	-0.076 (0.496)	0.237 (0.031) *	-0.143 (0.216)	-0.129 (0.233)	-0.027 (0.813)	0.264 (0.020) *	-0.137 (0.227)
NA-PCOS	-0.312 (0.008) *	0.121 (0.293)	0.021 (0.856)	-0.198 (0.074)	0.182 (0.134)	0.055 (0.640)	0.013 (0.919)
HA-PCOS	-0.267 (0.023) *	0.118 (0.304)	-0.024 (0.838)	-0.114 (0.310)	0.067 (0.583)	0.216 (0.065)	0.010 (0.934)

* p<0.005; ** p<0.001.

visceral adiposity [37, 38]. Otherwise, a negative correlation between A4 and DHEA with a few anthropometric markers was found in this hyperandrogenemic group. In NA-PCOS group, and in controls, 17-OHPE was also negatively correlated with VAI and LAP. In normal cycling controls, 17-OHP4 showed positive correlation with FM and VAI.

As to the enzyme activities, the 17,20 lyase ($\Delta 5$ pathway) was negatively correlated with lipids and central obesity in both groups of PCOS patients. Similarly, the activity of 3β-HSD enzyme was negatively correlated with VAI in both PCOS groups. Applying a multivariate stepwise regression, the activity of 17,20 lyase ($\Delta 5$ pathway) was influenced by BMI and LAP in NA-PCOS women. In HA-PCOS group, 17,20 lyase ($\Delta 4$ pathway) was enabled by FM. The 3β-HSD activity, in the conversion of 17-OHPE into 17-OHP4, was correlated with WHR. However, the activity of 3β-HSD in the conversion of DHEA into A4 was not influenced by any anthropometric surrogates, in any group of patients.

A few points should be considered as possible weaknesses of the current results. The cross-sectional design of this study does not prove a direct causality for the examined correlations. Anthropometric measures might be less sensitive and accurate when com-

pared to DEXA, CT or MRI in quantifying fat deposition, but their general usability must be considered. Furthermore, in clinical settings anthropometric measurement is a more cost-effective and feasible method for measuring body composition, and provides comparable estimates of fat tissue [39]. Therefore, the use of anthropometric parameters as surrogates for adipose tissue distribution in PCOS is largely accepted in most clinical studies [40–43]. The use of product-to-precursor ratio instead of a molecular biology technique to examine enzymatic activity must also be considered in clinical studies. The hormone levels measured are the net of production and degradation at a given moment, but both precursor and product may be changed after cell secretion. Markers of adipocyte function or dysfunction were not measured in the blood for direct comparison, however some anthropometric parameters have already been associated with the concentrations of a number of adipocyte products [44–47]. Despite these weaknesses, the current study has multiple scientific strengths. It included a number of patients who were carefully and prospectively evaluated. Furthermore, the present study added many novel insights to the complex relationship between total body fat, body shape, fat distribution, and steroidogenic enzyme activity. Secondarily, verified

► **Table 5** Multivariate stepwise regression correlating anthropometric adiposity indicators with steroidogenic enzyme activities in normo- and biochemical hyperandrogenemic PCOS and normal cycling women.

Dependent variables	Adiposity predictors	R	R ²	Adjusted r ²	Standard error estimate	Durbin-Watson	Standardized Beta coefficient	t	p
DHEA/17-OHPE (constant)									
NA-PCOS	BMI, LAP	0.362	0.131	0.106	4.6501	2.074	0.283	2.179	0.033
A4/17-OHP4 (constant)									
Controls	LAP	0.242	0.058	0.046	1.1695	1.984	0.242	2.213	0.030
HA-PCOS	FM	0.190	0.036	0.029	2.21757	1.885	0.190	2.175	0.032
17-OHP4/P4 (Constant)									
Controls	WC	0.292	0.085	0.073	1.3361	2.108	-0.292	-2.608	0.011
NA-PCOS	WHR	0.245	0.060	0.047	2.6851	1.743	0.245	2.129	0.037
17-OHP4/17-OHPE (Constant)									
HA-PCOS	WHR	0.240	0.057	0.050	1.8732	1.427	0.240	2.878	0.005
S/17-OHP4 (constant)									
Controls	LAP	0.281	0.079	0.067	1.2464	1.755	0.281	2.522	0.014

baseline sex-steroid concentrations and adiposity relationship in PCOS patients, with either normal or higher levels of androgen.

Visceral adipose tissue (WAT) differentiation, proliferation, abdominal centralization, and hypertrophy are driven by testosterone levels [48–51]. Adrenal androgens were already found to correlate with adipose tissue in hyperandrogenemic states [52]. The finding of positive correlation between T, fat mass, and waist circumference/height ratio in HA-PCOS are consistent with the results of other reports that have previously demonstrated to exist association between T, FM [7, 18] and WHR [53]. DHEA was shown to be positively correlated with general body fat mass [40] but the negative correlation between DHEA and WHR in NA-PCOS indicates that the synthesis of this precursor may be diminished in the presence of central adiposity. On the other hand DHEA was already shown to be negatively correlated with visceral adiposity [11]. Interleukin-6 (IL-6), a product of adipocytes with a higher level of expression in PCOS, was shown to increase DHEA syntheses and secretion [54]. It is not clear whether this adipokine increased the activity of the enzyme P450 17 α in the Δ 5 pathway. The negative correlation of A4 with some adiposity markers in NA-PCOS, and with LBM in HA-PCOS suggest that adiposity may worsen A4 syntheses, probably because leptin and adiponectin may downregulate the expression of steroidogenic enzymes [12, 55]. In a few studies, A4 was positively correlated with WHR [10] and FM [40]. The mechanisms behind these associations are not totally clear, but may involve lower activity of 3 β -HSD in the conversion of DHEA into A4 or higher 17,20 lyase activity in the Δ 4 pathway.

Adiponectin was shown to decrease A4 syntheses in bovine theca cells in vitro [14]. Furthermore, the acute administration of adiponectin was demonstrated to decrease A4 secretion [56]. Their findings explain the lower levels of adiponectin and the higher levels of A4 in PCOS subjects. The negative simple correlation between adiposity markers and concentrations of 17-OHPE in HA-PCOS groups strongly indicates that adiposity may diminish its secretion.

Otherwise, VAI, and LAP were reported to be positively correlated with 17-OHPE in NA-PCOS patients [34].

The regulation of the steroidogenic enzyme activities in adrenal and ovaries are complex and not completely understood. The adipose tissue products impact on corticosteroidogenic enzymes need to be characterized. A relationship between plasma concentrations of adipokines and body fat distribution was recently demonstrated [57]. Despite the current study did not measure directly any adipokine products, most of the anthropometric measured in the current study are proven surrogates of the presence of abdominal visceral adiposity. It is known that the adipose tissue is involved in the regulation of glucocorticoid metabolism, via 11 β -hydroxysteroid dehydrogenase [54, 58]. In addition, adipocyte products control the ovarian function [59], via a bidirectional communication between certain adipokines and granulosa and theca cells [60–62]. In fact, it was already demonstrated that receptors for leptin, adiponectin, TNF α , and IL-6, are expressed both in theca and granulosa ovarian cells [63].

Abdominal anthropometric parameters have already been associated with adipocyte dysfunction and abnormal secretion of adipokines and inflammatory markers [57, 64–69]. The positive correlation between WHR and 17,20 lyase (Δ 5) in HA-PCOS indicated the influence of central obesity in the activity of this enzyme. However, in an early study the activity of this enzyme was higher in NA-PCOS and controls [35]. Taking into account both studies, 17,20 lyase (Δ 5) activity was lower in both groups of PCOS when compared to controls [3]. The mechanism explaining the positive correlation between WHR and 17,20 lyase (Δ 5) activity is not clear. It is possible that local estradiol, higher in hyperandrogenic states, may activate this enzyme [70]. Further leptin, and IL-6 may also increase 17,20 lyase activity [71, 72]. The hyperinsulinemic state may also activate the expression of 17,20 lyase [73].

The negative correlation between 17,20 lyase, via Δ 5, and CI, VAI, and LAP in both NA-PCOS and HA-PCOS groups may reflect the down-regulated effect of leptin in adrenal cells [47, 74]. In non-

PCOS controls, 17,20 lyase activity was not influenced by any anthropometric parameter in the current study. In HA-PCOS, women the increased 17,20 lyase activity in the $\Delta 4$ pathway corroborates previously in vitro and in vivo studies [35]. The positive correlations between 17,20 lyase ($\Delta 4$ pathway) activity with adipose tissue in NA-PCOS and HA-PCOS support a previous observation [34] and suggest that an undefined adipocyte marker activates this enzyme in this pathway. Interestingly, there were positive correlation of 17,20 lyase ($\Delta 4$) with CI, VAI, and LAP in normal cycling women.

The positive correlation of WHR with 17-hydroxylase activity in HA-PCOS may reflect the resistin action on the activity of this enzyme [72, 75]. This effect was already demonstrated in ovarian and theca cells of polycystic ovaries [75]. Resistin was also associated with BMI in PCOS women [76]. Similar activity of 17-hydroxylase in PCOS and non-PCOS subjects was also reported [75]. The negative correlation of 17-hydroxylase with CI in normal cycling women may reflect the leptin action on the activity of this enzyme [73]. As to the 3β -HSD activity, it was found that it tended to be higher in the HA-PCOS group. On the other hand, decreased activity of this enzyme in both NA-PCOS and HA-PCOS was reported by others [77, 78]. In the conversion of 17-OHPE into 17-OHP4 the activity of 3β -HSD is inversely correlated with androgen production [75]. In the present study WHR showed positive correlation with 3β -HSD activity in HA-PCOS. On the other hand the activity 3β -HSD was negatively correlated with VAI in both NA-PCOS and HA-PCOS, probably by the inhibitory action of leptin in adrenal gland [55]. The WHR was positively correlated with 3β -HSD activity in the conversion of DHEA into A4 in HA-PCOS but, in controls, this correlation was negative. A possible role of resistin in this enzyme action was reported [75].

The activity of 21-hydroxylase did not correlate with any anthropometric parameter in both PCOS groups, supporting previous observation [34, 41]. The finding that the activity of 21-hydroxylase positively correlated with adiposity indicators in normal cycling controls is novel but still unclear. No anthropometric parameter influenced the combined 21-hydroxylase and 11-hydroxylase activities in PCOS patients in the current study, and no study reporting on this matter was found for comparisons. The negative correlation of 11 β -hydroxylase activity with abdominal obesity markers in normal controls in the present study partially corroborates previous findings [34].

After multiple regression analysis BMI and LAP were predictive of 17,20 lyase ($\Delta 5$) activity in the NA-PCOS group, indicating that the hyperandrogenemic state itself does not modulate the action of this enzyme. However, total body mass and the lipid accumulation may increase its activity. The adipocyte mediator which could be involved in 17,20 lyase activity ($\Delta 5$ pathway) is not clear at the moment. In the $\Delta 4$ pathway, 17,20 lyase activity was influenced by LAP in non-PCOS women, and by FM in the HA-PCOS group. Interestingly, WHR predicted the 3β -HSD activity in HA-PCOS, conversion of 17-OHPE into 17-OHP4. Though the results of the present study could not be compared to any previous report, they, even that indirectly, indicate that the steroidogenic enzymes activities are largely modulated by adiposity in PCOS women. Mainly, enabling a higher and undesirable androgen production. Even though the clinical implications are not clear, these findings support the knowledge lounge that in PCOS women, abnormal fat mass distribution facilitates androgen production, worsening their prognoses.

Conclusions

Finally, and in addition to the actual knowledge, the present study demonstrated that in HA-PCOS women the 17,20 lyase ($\Delta 4$ pathway) activity is positively associated with FM, and FM/LBM ratio, but not related with the conicity index. This observation suggests that fat mass as a whole and not its distribution in the body influences 17,20 lyase ($\Delta 4$) activity; yet in HA-PCOS, $\Delta 5$ pathway, the activity 17,20 lyase was associated with WHR and negatively with IC, VAI, and LAP. The 3β -HSD activity, both in the conversion of 17-OHPE into 17-OHP4 and DHEA into A4, was predicted by WHR and total BW.

In NA-PCOS group, 17,20 lyase ($\Delta 5$) was negatively associated with IC, VAI, and LAP and the 3β -HSD is negatively influenced by VAI. Therefore, anthropometric parameters, in both HA-PCOS and NA-PCOS, have different impact on steroidogenic enzyme activities. Nevertheless, the clinical implications of those findings need further investigation. Future studies aiming to analyze the intimate relationship between adipocyte-steroidogenic cells using either basic or clinical designs are needed. Based on this scenery, it is not possible at this time, to tailor any new measures to benefit the overweight or obese PCOS women in addition to lifestyle modification.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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