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Clinical Research Article

Interplay of Cortisol, Testosterone, and Abdominal Fat Mass in Normal-weight Women With Polycystic Ovary Syndrome

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Abstract

Context: Ovarian and adrenal steroidogenesis underlie endocrine-metabolic dysfunction in polycystic ovary syndrome (PCOS). Adipocytes express aldo-keto reductase 1C3 and type 1 11β-hydroxysteroid dehydrogenase, which modulate peripheral androgen and cortisol production.

Objectives: To compare serum adrenal steroids, including 11-oxygenated androgens (11-oxyandrogens), cortisol, and cortisone between normal-weight women with PCOS and body mass index- and age-matched ovulatory women with normal-androgenic profiles (controls), and assess whether adrenal steroids associate with abdominal adipose deposition.

Design: Prospective, cross-sectional, cohort study.

Setting: Academic medical center.

Patients: Twenty normal-weight women with PCOS and 20 body mass index-/age-matched controls.

Intervention(s): Blood sampling, IV glucose tolerance testing, and total-body dual-energy x-ray absorptiometry.

Main Outcome Measure(s): Clinical characteristics, hormonal concentrations, and body fat distribution.

Results: Women with PCOS had higher serum total/free testosterone (T) and androstenedione (A4) levels and a greater android/gynoid fat mass than controls (androgens $P < .001$; android/gynoid fat mass ratio, $P = .026$). Serum total/free T and A4 levels correlated positively with android/gynoid fat mass ratio in all women combined ($P < .025$, all values). Serum 11ß-hydroxyA4, 11-ketoA4, 11ß-hydroxyT, 11-ketoT, cortisol, and cortisone levels were comparable between female types and unrelated to body fat distribution. Serum 11-oxyandrogens correlated negatively with % total body fat, but lost significance adjusting for cortisol. Serum cortisol levels, however, correlated inversely with android fat mass ($P = .021$), with a trend toward reduced serum cortisol to cortisone ratio in women with PCOS vs controls ($P = .075$), suggesting diminished 11β-hydroxysteroid dehydrogenase activity.

Conclusion: Reduced cortisol may protect against preferential abdominal fat mass in normal-weight PCOS women with normal serum 11-oxyandrogens.

Key Words: adipose, polycystic ovary syndrome, adipocyte, AKR1C3, testosterone, androstenedione, androgens

Abbreviations: 11KA4, 11-ketoA4; 11KT, 11-ketoT; 11OHA4, 11ß-hydroxyA4; 11OHT, 11ß-hydroxyT; 11-oxyandogen, 11-oxygenated androgen; 17OHP4, 17-hydroxyprogesterone; A4, androstenedione; adipose-IR, adipose insulin resistance; AIRg, acute response to glucose; AKR1C3, aldo-keto reductase 1C3; BMI, body mass index; CV, coefficient of variation; DHEAS, dehydroepiandrosterone sulfate; E1, estrone; E2, estradiol; FFA, free fatty acid; FSIVGTT, frequently sampled IV glucose tolerance testing; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NIH, National Institutes of Health; PCOS, polycystic ovary syndrome; Si, insulin sensitivity index; T, testosterone; TG, triglyceride; SHBG, sex hormone binding globulin.

Ovarian and adrenal steroidogenesis are interwoven into the endocrine-metabolic pathophysiology of polycystic ovary syndrome (PCOS) [1-5]. Within the adrenal, cytochrome P450 11ß-hydroxylase catalyzes the hydroxylation of androstenedione (A4) and testosterone (T) to 11ß-hydroxyandrostendione (11OHA4) and 11ß-hydroxytestosterone (11OHT), respectively [4-6]. 11β-hydroxysteroid dehydrogenase type 2 then converts 11OHA4 and 11OHT to their respective ketosteroids, 11-ketoandrostendione (11KA4) and 11-ketotestosterone (11KT), with 11KT also originating from reduction of 11KA4 via aldo-keto reductase 1C3 (AKR1C3; also known as 17β-hydroxysteroid dehydrogenase type 5). Although 11OHA4 is quantitatively the dominant 11-oxygenated androgen (11-oxyandrogen), its bioactivity is negligible, but it serves as substrate for 11KT, a potent androgen with bioactivity comparable to that of T [4-6].

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Elevated serum 11OHA4 levels have been reported in one-half of National Institutes of Health (NIH)-defined women with PCOS who are overweight [1]. More recently, serum 11-oxyandrogen levels have been shown to be elevated in 40% of PCOS women by Rotterdam criteria, and to positively correlate with body mass index (BMI), fasting circulating insulin levels, and insulin resistance in some [7, 8], but not all [9], studies.

We have previously shown that normal-weight hyperandrogenic women with PCOS exhibit low-normal insulin sensitivity and metabolic alterations that accompany preferential abdominal fat accumulation [10-14]. It is unclear, however, whether adrenal steroidogenesis interacts with an elevated androgens pathway in normal-weight women with PCOS to further affect endocrine-metabolic pathophysiology related to altered body fat distribution [15]. It is also unclear whether adipocyte expression of AKR1C3 and types 1 or 2 11βHSD in these women with PCOS modulate peripheral androgen and cortisol activation/inactivation. Therefore, the present study compares serum adrenal steroids, including 11-oxyandrogens, cortisol, and cortisone, between normal-weight women with PCOS and BMI- and age-matched ovulatory women with normal-androgenic profiles (controls), and assesses whether adrenal steroids associate with preferential abdominal adipose accumulation.

Materials and Methods

Study Participants

Twenty normal-weight women with PCOS and 20 control women (19-35 years; 19-25 kg/m^2) who had previously been enrolled by the principal investigator (D.A.D.) of our NIH-funded study (P50 HD071836; 4/1/2013-3/30/2023) examining adipose dysfunction in PCOS were included in this study [10-13, 15, 16]. Each woman with PCOS was individually age-/BMI-matched to a normoandrogenic ovulatory (control) woman who was similarly enrolled for comparison; all women were healthy individuals, as published previously [10-13, 15, 16] except for 1 woman with PCOS with hypertriglyceridemia and severely reduced insulin sensitivity index (Si) below the level of the general population [17].

PCOS was diagnosed by 1990 NIH criteria and biochemical hyperandrogenism, as previously defined by an elevated mean serum total or free T level from 2 separate blood samples >2 SD above the normal ranges of the age- and BMI- matched control group [10-13, 15, 16]. Sixteen subjects with PCOS had polycystic ovarian morphology in either ovary, defined by transvaginal ultrasonography as a follicle number per ovary ≥20 and/or an ovarian volume ≥10 mL (phenotype A), whereas 4 subjects with PCOS did not have polycystic ovaries (phenotype B) [18, 19]. Control women had normal menstrual cycles at 21- to 35-day intervals and a luteal phase progesterone (P4) level without evidence of androgen excess [3]. Exclusion criteria, including late-onset congenital adrenal hyperplasia, thyroid dysfunction, and hyperprolactinemia, have previously been reported [10-13, 15, 16]. All studies were performed according to the Declaration of Helsinki after approval by the UCLA institutional review board and signed informed consent by each subject.

Body Fat Distribution

Waist and hip measurements were determined in all subjects [10-13, 15, 16]. In 18 women with PCOS and age-/BMI-matched controls, a total body dual-energy x-ray absorptiometry scan was performed with a Hologic QDR Discovery A densitometer (Hologic, Inc, Bedford, MA) as previously reported [10-13, 15, 16]. During the COVID-19 pandemic, dual-energy x-ray absorptiometry scan also was used with a Hologic Horizon A densitometer (Hologic, Inc.) in 3 women with PCOS using the same calibrations, range-of-interest definitions, and analysis methods. Android and gynoid fat regions were from the first lumbar vertebra to the top of pelvis and from the femoral head to the mid-thigh, respectively.

Blood Sampling

Blood sampling was performed during the follicular phase in control women and during an anovulatory interval in women with PCOS. Absent luteal function in women with PCOS was confirmed by low serum P4 levels. Fasting blood samples were collected at 10 AM and obtained immediately before frequently sampled IV glucose tolerance testing (FSIVGTT) for adrenal and ovarian steroids (ie, 11OHA4, 11KA4, 11OHT, 11KT, 17-hydroxyprogesterone [17OHP4], cortisol, cortisone, dehydroepiandrosterone sulfate [DHEAS], total and free T, A4, estrone [E1], estradiol [E2]), gonadotropins, glucose, free fatty acid [FFA], insulin, sex hormone binding globulin (SHBG), and lipids (total cholesterol, high-density lipoprotein [HDL], low-density lipoprotein [LDL], triglyceride [TG]). Fasting blood values were used to calculate adipose insulin resistance (adipose-IR, defined by the product of fasting circulating FFA [mmol/L] and insulin levels [pmol/L]).

The FSIVGTT was performed in all women using the modified minimal model of Bergman [20], except in 1 control who declined the study. Briefly, glucose in 50% concentration (0.3 g/kg) and regular human insulin (0.03 units/kg) were injected IV under fasting conditions at 0 and 20 minutes, respectively, and blood was collected at −20, −15, −5, 0, 2, 4, 8, 19, 22, 30, 40, 50, 70, 90, and 180 minutes for glucose and insulin determinations. Mathematical modeling of circulating glucose and insulin levels defined: Si (ie, insulin action to accelerate glucose uptake and suppress glucose production) and acute response to glucose (AIR g [ie, pancreatic β-cell response to glucose infusion]).

Hormonal and Metabolic Assays

Quantification of serum 11-oxyandrogens, cortisol, cortisone, T, and A4 was performed in a single run by liquid chromatography-tandem mass spectrometry at the University of Michigan, Ann Arbor, as previously described [21, 22]. The intra-assay coefficients of variation (CVs) for all steroids were <3.5% and all detection limits were >2.4 ng/dL.

Serum levels of DHEAS and E1 were measured by liquid chromatography-tandem mass spectrometry (Quest Diagnostics Nichols Institute, San Juan Capistrano, CA), as previously described [10-13, 15, 16]. The intra-assay CVs were DHEAS, 2.6% and E1, 10.2%. The inter-assay CVs were DHEAS, 4.4% and E1, 9.5%. The detection limits were DHEAS, 2 μg/dL and E1, 10 pg/mL. Free T was calculated from the concentrations of total T, SHBG (Beckman Coulter Cat# A48617, RRID:AB_2893035 [http://antibodyregistry.org/AB_2893035]), and albumin. The intra-assay CV for free T was 1.6% and the detection limit was 0.03 pg/mL.

Serum steroid product to precursor ratios were used as indirect markers of enzymatic activity (ie, 11OHA4/A4 and

11OHT/T ratios as CYP11ß1 activity, 11KA4/11OHA4 and 11KT/11OHT ratios as HSD11ß2 activity, T/A4 ratio and 11KT/11KA4 ratios as AKR1C3 activity, and cortisol to cortisone ratio as HSD11ß1 activity).

Serum measurements of insulin (Roche Cat# 12017547, RRID:AB_2756877 [http://antibodyregistry.org/AB_2756877]), LH (Roche Cat# 11732234, RRID:AB_2800498 [http://antibodyregistry.org/AB_2800498]), FSH (Roche Cat# 11775863, RRID:AB_2800499 [http://antibodyregistry.org/AB_2800499]), and E2 (Roche, Cat# 03000079, RRID:AB_2893079 [http://antibodyregistry.org/AB_2893079]) by electrochemiluminescence; glucose by a hexokinase method; and fasting lipids by spectrophotometry were performed at the UCLA Center for Pathology Research Services, as previously described [10-13, 15, 16]. The intra-assays CVs were insulin, 0.6%; LH, 1.0%; FSH, 2.1%; E2, 7.0%; glucose, 0.8%; total cholesterol, 0.7%; LDL, 0.5%; HDL 0.6%; and TG, 0.6%. The inter-assays CVs were insulin, 2.6%; LH, 2.3%; FSH, 2.8%; E2, 10.7%; glucose, 0.9%; total cholesterol, 1.0%; LDL, 1.2%; HDL, 0.9%; and TG, 0.7%. Detection limits were insulin, <1 µU/mL; LH, <0.3 mIU/mL; FSH, <0.3 mIU/mL; E2, <12 pg/mL; glucose, <10 mg/dL; total cholesterol, <11 mg/dL; LDL, <10 mg/dL; HDL, <4 mg/dL; and TG, <9 mg/dL.

Serum FFAs were measured by quantitative spectrophotometry (ARUP Laboratories, Salt Lake City, UT). The intra- and inter-assay CVs for FFAs were 1.8% and 1.2%, respectively, as previously reported [10-13, 15, 16]. The detection limit for FFA was 0.01 mmol/L.

Statistical Analysis

The primary outcome measure was a difference in serum 11OHA4 levels, as the major 11-oxyandrogen, between women with PCOS and controls. Based on previously reported differences between nonobese women with PCOS and controls in serum 11OHA4 levels [8], a sample size of 20 per group (women with PCOS vs controls) provided adequate power (>80%) to detect standardized effect sizes as small as 0.91 between groups using a 2-sample *t*-test ($\alpha = .05$, 2-tailed). Additional exploratory analysis were female-type differences in serum cortisol and cortisone levels as well as serum steroid product to precursor ratios as indirect enzymatic markers of CYP11ß1, HSD11ß2, AKR1C3, and HSD11ß1 activities.

An unpaired Student *t*-test compared patient characteristics and clinical hormone/metabolic values between PCOS and age and BMI pair-matched control subjects. Results were presented with mean ± SD unless otherwise noted. Pearson correlation coefficients examined associations of serum adrenal steroid levels with clinical outcomes [23]. As a sensitivity analysis, partial correlations were examined with the same associations after adjusting for adrenal cortisol, to determine whether cortisol was confounding the adrenal androgen findings. For significant associations, we also fit linear regression models and the prediction lines were superimposed on the scatterplots for Fig. 1. Because of potential distributional assumption violations (eg, nonnormality), a log transformation was applied to some measures before analysis (eg, LH, AIRg, TG, adipose IR). Statistical analyses were run using IBM SPSS V27 (Armonk, NY) and *P* values <.05 were considered statistically significant.

Results

Patient Characteristics and Hormone/Metabolic Levels

Consistent with our previous studies [10-13, 15, 16], age, BMI, waist, and hip measurements were comparable between women with PCOS and controls. Total body mass, total body fat, percent body fat, and android as well as gynoid fat masses were similar between female types (Table 1). The android to gynoid fat mass ratio, however, was significantly greater in women with PCOS than controls ($P = .026$) because of a shift away from percent gynoid fat ($P = .026$) to percent android fat mass ($P = .062$).

Serum log LH, total/free T, A4, and 17OHP4 levels were greater in PCOS women than controls (log LH, $P = .004$; androgens, $P < .001$; 17OHP4, $P = .004$) (Table 1) and were accompanied by a trend for reduced SHBG levels ($P = .054$). Log adipose-IR values also were greater in PCOS women than controls ($P = .017$), because in part to a trend for increased fasting circulating insulin levels ($P = .075$). Low-normal Si values in PCOS women were accompanied by high-normal log AIRg values, as published previously [10-13, 15, 16]. There were no female-type differences in serum FSH, estrogen, DHEAS, or fasting glucose and cholesterol levels, although log TG levels were higher in PCOS women than controls ($P = .035$).

Serum 11-oxyandrogens, cortisol, and cortisone were comparable between women with PCOS and controls (Table 1). Serum 11OHA4/A4 and 11OHT/T ratios, however, were significantly reduced in women with PCOS compared with controls, reflecting a predominant ovarian source for T and A4 in women with PCOS (both ratios, $P < .01$) (Table 2). Serum 11KA4/11OHA4 and 11KT/11OHT ratios, as indirect enzymatic markers of HSD11ß2 activity, were similar between the 2 female groups, as were serum T/A4 ratio and 11KT/11KA4 ratios, as indirect enzymatic markers of AKR1C3 activity. Of interest, the cortisol to cortisone ratio as an indirect enzymatic marker of HSD11ß1 activity tended to be reduced in women with PCOS vs controls ($P = .075$).

Clinical Correlations

In all women combined, serum total/free T, A4, and 17OHP4 levels correlated positively with log LH levels (total T: $R = +0.55$, $P < .001$; free T: $R = +0.62$, $P < .001$; A4: $R = +0.61$, $P < .001$; 17OHP4: $R = +0.60$, $P < .001$) (Table 3). Serum T, A4, and17OHP4 also correlated positively with BMI (total T: $R = +0.42$, $P = .007$; A4: $R = +0.34$, $P = .029$; 17OHP4: $R = +0.41$, $P = .009$), whereas serum free T and A4 correlated positively with waist to hip ratio (free T: $R = +0.44$, $P = .005$; A4: $R = +0.33$, $P = .035$).

Serum total/free T, A4, and 17OHP4 levels were unrelated to total body mass, total body fat, or % total body fat (P = NS, all values). Nevertheless, serum total/free T and A4 levels, but not 17OHP4 levels, correlated positively with the android to gynoid fat mass ratio (total T: $R = +0.41$, $P = .013$; free T: $R = +0.49$, $P = .003$; A4: $R = +0.45$, $P = .006$; 17OHP4: $R = +0.19$, $P = .276$). This was due to positive relationships of serum total/free T, and to a lesser degree serum A4, with android fat mass (total T: $R = +0.34$, $P = .044$; free T: $R = +0.35$, $P = .038$; A4: $R = +0.28$, $P = .095$) but not with gynoid fat mass (P = NS, all values) (Fig. 1).

Serum total/free T, A4, and 17OHP4 levels also correlated positively with log adipose-IR (total T: $R = +0.34$, $P = .037$;

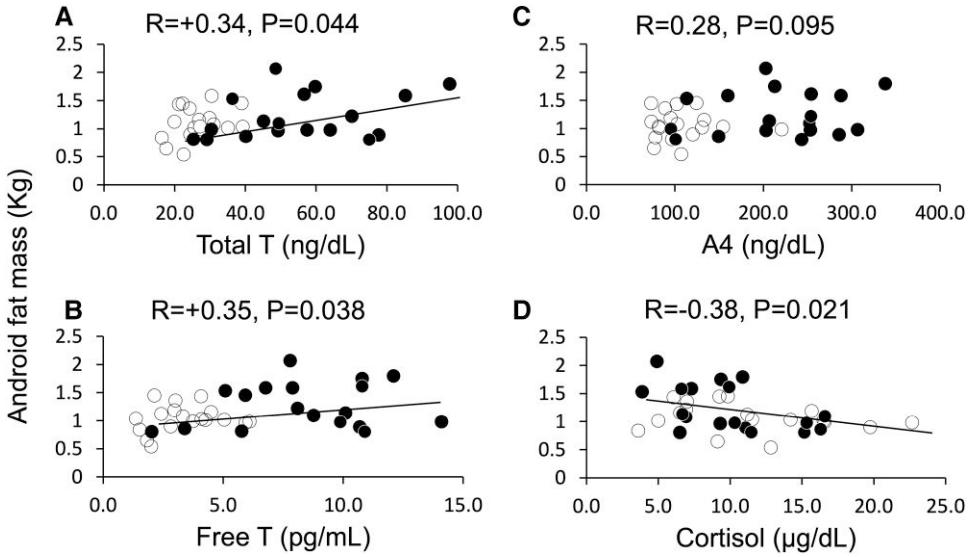

Figure 1. Correlations of android fat mass with serum (A) total T, (B) free T, (C) A4, and (D) cortisol levels in normal-weight women with PCOS and body mass index-/age-matched controls. Filled circles, women with PCOS; open circles, controls. All y-axes for android fat mass are identical in scale. A4, androstenedione; PCOS, polycystic ovary syndrome; T, testosterone.

free T: $R = +0.40$, $P = .011$; A4: $R = +0.39$, $P = .013$; 17OHP4: $R = +0.37$, $P = .018$), but not with Si values (all steroids, P = NS). In addition, serum free T levels correlated positively with circulating fasting insulin ($R = +0.44$, $P = .005$) and log TG ($R = +0.35$, $P = .025$) levels, while correlating negatively with SHBG ($R = -0.57$, $P < .0001$). During FSIVGTT, serum free T levels also correlated positively with log AIRg ($R = +0.39$, $P = .015$) and tended to correlate negatively with Si ($R = -0.27$, $P = .098$).

Serum 11OHT, 11KT, and 11KA4 levels also correlated positively with BMI (11OHT: $R = +0.36$, $P = .023$; 11KT: $R = +0.49$, $P = .001$; 11KA4: $R = +0.34$, $P = .034$), but were unrelated to body fat distribution (Table 3). Serum 11-oxyandrogen levels, however, correlated negatively with % total body fat (11OHA4: $R = -0.42$, $P = .011$; 11KA4: $R = -0.34$, $P = .046$; 11OHT: $R = -0.36$, $P = .031$; 11KT: $R = -0.37$, $P = .027$). In addition, serum 11KA4 levels correlated positively with circulating fasting insulin ($R = +0.33$, $P = .041$), whereas serum 11OHT levels correlated negatively with SHBG ($R = -0.32$, $P = .047$) levels. None of the serum 11-oxyandrogens was related to log LH, log adipose-IR, log TG, Si, or log AIRg.

Serum cortisol and cortisone levels correlated inversely with % total body fat (cortisol: $R = -0.45$, $P = .006$; cortisone: $R = -0.49$, $P = .002$) (Table 3). In addition, serum cortisol and cortisone levels correlated negatively with android fat mass (cortisol: $R = -0.38$, $P = .021$; cortisone: $R = -0.35$, $P = .035$) (Fig. 1) but were unrelated to any other metabolic outcome. Because both serum cortisol and 11-oxyandrogen levels were correlated negatively with % total body fat, partial correlations for serum 11-oxyandrogens were examined after adjusting for serum cortisol to determine whether glucocorticoid action was confounding the findings. Adjusting for serum cortisol, serum 11-oxyandrogen correlations with % total body fat were no longer significant.

All 4 serum 11-oxyandrogen and cortisol levels correlated positively with serum DHEAS levels (11OHA4: $R = +0.47$, $P = .002$; 11KA4: $R = +0.33$, $P = .038$; 11OHT: $R = +0.47$, $P = .002$; 11KT: $R = +0.36$, $P = .021$; cortisol: $R = +0.33$, $P = .040$) (Table 3).

Discussion

The present study confirms an increased android to gynoid fat mass ratio in normal-weight women with NIH-defined PCOS, as previously described [10-13, 15, 16], but without a concomitant elevation in serum 11-oxyandrogen levels, suggesting a predominant ovarian contribution to androgen excess in normal-weight women with NIH-defined PCOS. Serum 11OHA4/A4 and 11OHT/T ratios were reduced in these women with PCOS and resembled the diminished serum 11OHA4/A4 ratio reported in normal-weight women with PCOS by the Rotterdam criteria [7].

In our study, as in others, 11OHA4 was the most abundant 11-oxyandrogen [7, 8]. Serum 11OHT, 11KT, and 11KA4 levels positively correlated with BMI in all women combined, agreeing with positive relationships of serum 11OHT and 11KT levels with BMI in some [7], but not all [9] women with Rotterdam-defined PCOS. Serum 11-oxyandrogen levels, however, were normal in our women with PCOS with low-normal insulin sensitivity, as they are in more than one-half of overweight/obese women with PCOS [7, 8]. That serum 11KA4 correlated positively with circulating fasting insulin levels in our women with PCOS agrees with a similar relationship of serum 11KA4 levels with insulin resistance in overweight/obese PCOS women [8], implicating adrenal insulin receptor signaling in this interaction, as seen in insulin-resistant women with lipodystrophy [24]. Serum DHEAS levels in our women with PCOS also were normal, as previously reported [7, 8], and were closely linked with serum 11-oxyandrogen and cortisol levels, confirming the common adrenal origin and ACTH governance of these steroids.

Serum 11-oxyandrogen levels correlated negatively with % total body fat, as did serum cortisol and cortisone levels. Serum 11-oxyandrogen levels, however, were no longer significant when adjusting for serum cortisol levels. Therefore,

Table 1. Patient characteristics and serum hormone and metabolic levels in normal-weight control vs women with PCOS[a,b]

Patient characteristics	Controls (N = 18)	PCOS (N = 18)	*P* value
Age (y)	27.7 ± 4.8	24.8 ± 4.4	.069
BMI (kg/m^2)	21.7 ± 1.6	22.1 ± 1.9	.495
Waist (cm)	75.2 ± 5.1	76.1 ± 4.9	.572
Hip (cm)	89.3 ± 6.0	87.9 ± 5.5	.483
Total body mass (kg)	60.7 ± 7.8	60.9 ± 7.2	.945
Total body fat (kg)	19.3 ± 3.2	19.9 ± 3.2	.620
Percent body fat (%)	31.8 ± 2.9	32.8 ± 4.1	.429
Android fat (kg)	1.1 ± 0.3	1.2 ± 0.4	.126
Percent android fat (%)	5.5 ± 0.7	6.2 ± 1.3	.062
Gynoid fat (kg)	4.1 ± 0.8	4.0 ± 0.6	.663
Percent gynoid fat (%)	**21.4 ± 1.6**	**20.3 ± 1.0**	**.026**
Android/gynoid fat ratio	**0.26 ± 0.04**	**0.31 ± 0.07**	**.026**
Hormone/metabolic levels	**Controls (N = 20)**	**PCOS (N = 20)**	***P* value**
Log LH (mIU/mL)	**0.89 ± 0.2**	**1.1 ± 0.2**	**.004**
FSH (mIU/mL)	6.0 ± 2.3	5.3 ± 1.4	.257
E1 (pg/mL)	63.0 ± 32.4	67.4 ± 28.1	.655
E2 (pg/mL)	100.4 ± 106.7	72.0 ± 56.1	.301
Total T (ng/dL)[c]	**27.4 ± 6.6**	**53.2 ± 20.4**	**<.001**
Free T (pg/mL)[c]	**3.4 ± 1.4**	**8.0 ± 3.2**	**<.001**
A4 (ng/dL)[c]	**106.9 ± 34.9**	**208.4 ± 73.3**	**<.001**
11KT (ng/dL)[c]	30.3 ± 13.0	35.2 ± 18.5	.333
11OHT (ng/dL)[c]	11.9 ± 7.9	12.3 ± 5.5	.866
11KA4 (ng/dL)[c]	17.4 ± 7.4	17.5 ± 7.5	.960
11OHA4 (ng/dL)[c]	107.9 ± 61.8	111.3 ± 59.1	.858
17OHP4 (ng/dL)[c]	**35.8 ± 15.3**	**62.7 ± 34.2**	**.004**
DHEAS (µg/dL)	181.4 ± 97.0	221.8 ± 69.6	.139
Cortisol (ng/dL)[c]	11199.7 ± 5189.7	9740.4 ± 3781.9	.314
Cortisone (ng/dL)[c]	2122.5 ± 619.9	2278.4 ± 652.0	.440
Fasting glucose (mg/dL)[d]	85.2 ± 6.0	85.8 ± 6.5	.796
Fasting insulin (µU/mL)[d]	4.6 ± 1.9	5.8 ± 2.1	.075
Si (×10^{-4}/min/µU/mL)[d]	6.0 ± 5.1	4.2 ± 2.0	.143
Log AIRg (µU/mL)[d]	2.4 ± 0.2	2.5 ± 0.2	.150
SHBG (nmol/L)	68.2 ± 33.2	49.2 ± 26.8	.054
Log adipose-IR[d]	**1.2 ± 0.2**	**1.4 ± 0.2**	**.017**
Log triglyceride (mg/dL)	**1.7 ± 0.1**	**1.8 ± 0.2**	**.035**
HDL-C (mg/dL)	64.8 ± 11.9	62.2 ± 12.5	.513
Non-HDL-C (mg/dL)	89.2 ± 25.6	93.6 ± 26.6	.592
LDL-C (mg/dL)	78.6 ± 23.7	78.2 ± 24.9	.960
Total cholesterol (mg/dL)	154.8 ± 29.4	155.9 ± 29.0	.913

Mean ± SD. Boldface values represent significant differences between female groups.
Conversion to SI Units: T (× 0.0347 nmol/L), free T (× 3.47 pmol/L), A4 (× 0.0349 nmol/L), KT (× 0.0331 nmol/L), OHT (× 0.0329 nmol/L), KA4 (× 0.0322 nmol/L), OHA4 (× 0.0331 nmol/L), 17OHP4 (× 0.0303 nmol/L), DHEAS (× 0.0271 µmol/L), cortisol (× 0.0276 nmol/L), cortisone (× 0.0277 nmol/L), E1 (× 3.699 pmol/L), E2 (× 3.67 pmol/L), LH (× 1.0 IU/L), FSH (× 1.0 IU/L), glucose (× 0.0555 mmol/L), insulin (× 7.175 pmol/L, total cholesterol (× 0.0259 mmol/L), HDL-cholesterol (× 0.0259 mmol/L), LDL-cholesterol (× 0.0259 mmol/L), non-HDL-cholesterol (× 0.0259 mmol/L), triglycerides (× 0.0113 mmol/L).
Abbreviations: 11KA4, 11-ketoA4; 11KT, 11-ketoT; 11OHA4, 11ß-hydroxyA4; 11OHT, 11ß-hydroxyT; A4, androstenedione; AIRg, acute response to glucose; BMI, body mass index; DHEAS, dehydroepiandrosterone sulfate; E1, estrone; E2, estradiol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PCOS, polycystic ovary syndrome; Si, insulin sensitivity index; T, testosterone.
[a]Modified from references previously reported for these subjects [10-13, 16].
[b]Total body dual-energy x-ray absorptiometry studies (controls = 18, PCOS = 18).
[c]New measurements as determined by liquid chromatography-tandem mass spectrometry at the University of Michigan, Ann Arbor [21, 22].
[d]Frequently sampled IV glucose tolerance testing, and fasting glucose/insulin as well as log adipose insulin resistance studies (controls = 19, PCOS = 20).

Table 2. Steroid ratios as indirect enzymatic markers of AKR1C3, CYP11ß1, HSD11ß2, and HSD11ß1 in normal-weight women with PCOS vs controls

Ratio	Controls (N = 20)	PCOS (N = 20)	*P* value
AKR1C3			
T/A4	0.265 ± 0.067	0.255 ± 0.051	.599
11KT/11KA4	1.835 ± 0.520	2.100 ± 0.736	.198
CYP11ß1			
11OHA4/A4	**1.040 ± 0.480**	**0.560 ± 0.284**	**<.001**
11OHT/T	**0.460 ± 0.300**	**0.240 ± 0.147**	**.006**
HSD11ß2			
11KA4/11OHA4	0.170 ± 0.057	0.170 ± 0.066	.999
11KT/11OHT	2.810 ± 0.818	2.965 ± 0.835	.559
HSD11ß1			
Cortisol/cortisone	5.245 ± 1.938	4.295 ± 1.251	.075

Mean ± SD. Boldface values represent significant differences between female groups.
Abbreviations: 11KA4, 11-ketoandrostenedione; 11KT, 11-ketotestosterone; 11OHA4, 11ß-hydroxyandrostenedione; 11OHT, 11ß-hydroxytestosterone; A4, androstenedione; AKR1C3, aldo-keto reductase family 1 member C3 (also known as 17ß-hydroxysteroid dehydrogenase type 5); CYP11ß1, adrenal-specific cytochrome P450 11ß-hydroxylase; HSD11ß1, 11ß-hydroxysteroid dehydrogenase type 1; HSD11ß2, 11ß-hydroxysteroid dehydrogenase type 2; PCOS, polycystic ovary syndrome; T, testosterone.

the inverse relationships between serum 11-oxyandrogens and % total body fat were most likely driven by cortisol, probably through diminished glucocorticoid-induced lipolysis, because cortisol infusion in normal-weight human volunteers increases plasma palmitate concentration [25, 26], whereas cortisol, not adrenal androgens, provides the predominant negative feedback regulation of the hypothalamus-pituitary-adrenal axis. In addition, exposure of adipocytes from women or male rats to dexamethasone *in vitro* enhances catecholamine-stimulated lipolysis, with glucocorticoid-enhanced lipolysis in rats accompanied by upregulation of adenylate cyclase activity and β-adrenergic receptor number [27, 28]. In normal-weight women with PCOS, therefore, glucocorticoid-enhanced lipolysis may interact with androgen inhibition of catecholamine-induced lipolysis, as seen in subcutaneous abdominal PCOS adipose [29-32], to determine fat accretion [15].

Serum cortisol and cortisone levels also correlated negatively with android fat mass in all women combined. Moreover, a trend toward reduced serum cortisol to cortisone ratio in the present women with PCOS agrees with decreased HSD11ß1 activity in other normal-weight women with PCOS [33], and with increased urinary excretion of 11-oxo to 11-hydroxy metabolites of cortisol in nonobese women with PCOS [34]. Importantly, HSD11ß1 activity correlates inversely with increased abdominal adiposity in individuals with normal serum glucocorticoid levels, as evidenced by a reduced ratio of urinary cortisol to cortisone metabolites and an impaired cortisone to cortisol conversion after oral cortisone acetate [35]. Therefore, in normal-weight women with NIH-defined PCOS, a trend toward reduced adipose/hepatic HSD11ß1 and cortisol activities could compensate for the positive relationship of ovarian hyperandrogenism with increased intra-abdominal fat mass, while modifying hypothalamic steroid negative feedback to maintain normal circulating cortisol and 11-oxyandrogen levels [10, 29, 30, 32, 36, 37].

If so, reduced adipose/hepatic HSD11ß1 and cortisol activities of greater magnitude in overweight/obese women with PCOS would likely promote 11-oxyandrogen production [8, 9, 34], because increased abdominal adiposity in women impairs BMI suppression of the hypothalamo-corticotropic-adrenal axis [36]. This mechanism, however, may be insufficient to compensate for HSD11ß1 mRNA upregulation in visceral adipose as a correlate with omental fat hypertrophy in obese women [38], agreeing with increased HSD11ß1 mRNA expression in visceral adipose as a predictor of metabolic dysfunction through increased adiposity in women with NIH-defined PCOS [39].

Our results differ from previous reports in several ways. First, the serum 11KA4/11OHA4 ratio, as an indirect enzymatic marker of HSD11ß2, was normal our normal-weight women with PCOS, despite being elevated in normal-weight Japanese women with PCOS by the Rotterdam criteria [7], perhaps because of ethnic differences. Second, the serum T/A4 ratio, as an indirect enzymatic marker of AKR1C3, also was normal in the present women with PCOS, although elevated in the same individuals by different steroid quantification via mass spectrometry [16]. In women with PCOS, however, an elevation in the T/A4 ratio is greater in adipose than blood [40], so that using serum to estimate abdominal adipose AKR1C3 activity likely underestimates our previous finding of AKR1C3 mRNA overexpression in PCOS adipocytes matured *in vitro* [16]. Equally important, the proportional contribution of 11-oxyandrogen levels to total androgens in normal-weight women with PCOS is lower than in the obese PCOS phenotype [8], in agreement with findings in Japanese women [7].

Important strengths of this study were the use of healthy, normal-weight women with PCOS by NIH criteria with a mild PCOS phenotype [41, 42] and who were age- and BMI-matched to controls to eliminate the confounding effects of age and obesity on study outcomes [7, 8, 22, 43-46]. Our experimental design also required collection of fasting blood samples at 10 AM to avoid diurnal variation of adrenal steroidogenesis, allowing us to study the interrelationships of serum 11-oxyandrogens with glucocorticoids, adjusting for the confounding effects of cortisol.

An important limitation of our study, however, was the use of morning serum, rather than 24-hour urinary, collections to measure steroid levels, which may have underestimated our ability to detect enhanced daily urinary excretion of glucocorticoid and androgenic metabolites in women with PCOS [33, 34]. Moreover, we did not measure 5a-reductase activity, as others have done [34], nor did we examine tissue-specific steroid metabolism, which could have influenced the interactions examined. The small number of subjects with PCOS also diminished statistical power to examine subtle interactions between adrenal steroids and clinical outcomes, and limited applicability of our data to women of different PCOS phenotypes, ethnicity, or adiposity. Finally, our assessment of body fat distribution and function did not investigate tissue-specific regulation of HSD11ß1 activity by cytokines [47], given that greater HSD11ß1 oxoreductase activity in omental than in subcutaneous fat cells accompanies increased visceral adipocyte size, enhanced visceral fat accumulation, and reduced insulin sensitivity [48, 49].

Nevertheless, our findings suggest that glucocorticoids play an important role in the health of normal-weight women with PCOS. An interplay between adrenal and ovarian steroidogenesis appears to serve as a metabolic adaptation in these

Table 3. Correlations of serum steroid levels with clinical, hormonal, and metabolic measurements

Parameter	11-OHT[a] R, P	11-KT[a] R, P	11-OHA4[a] R, P	11-KA4[a] R, P	Cortisol[a] R, P	Cortisone[a] R, P	Total T[a] R, P	Free T[a] R, P	A4[a] R, P	E1, [b] R, P	E2, [b] R, P
BMI	**R = +0.36 P = .023**	**R = +0.49 P = .001**	R = +0.43 P = .058	**R = +0.34 P = .034**	R = −0.01 P = .973	**R** = +0.15 P = 0.353	**R = +0.42 P = .007**	R = +0.33 P = 0.051	**R = +0.34 P = .029**	R = +0.12 P = .456	R = +0.06 P = .702
WHR	R = +0.22 P = .181	R = +0.11 P = .501	R = +0.15 P = .347	R = −0.02 P = .908	R = −0.03 P = .838	R = +0.15 P = 0.344	R = +0.28 P = .075	**R = +0.44 P = .005**	**R = +0.33 P = .035**	R = +0.10 P = .539	R = −0.03 P = .836
Total body mass	R = +0.25 P = .148	R = +0.19 P = .276	R = +0.12 P = .481	R = +0.10 P = .575	R = +0.04 P = .836	R = +0.04 P = .797	R = +0.18 P = .281	R = +0.08 P = .634	R = +0.10 P = .572	R = +0.07 P = .688	R = +0.06 P = .732
Total body fat	R = −0.08 P = .660	R = −0.13 P = .447	R = −0.21 P = .219	R = −0.15 P = .382	R = −0.30 P = .079	R = −0.33 P = .052	R = +0.14 P = .409	R = +0.09 P = .604	R = +0.02 P = .912	R = +0.09 P = .591	R = −0.07 P = .699
% total body fat	**R = −0.36 P = .031**	**R = −0.37 P = .027**	**R = −0.42 P = .011**	**R = −0.34 P = .046**	**R = −0.45 P = .006**	**R = −0.49 P = .002**	R = +0.02 P = .887	R = +0.05 P = 0.758	R = −0.07 P = .693	R = +0.07 P = .690	R = −0.18 P = .305
Android fat	R = −0.14 P = .426	R = −0.10 P = .553	R = −0.24 P = .161	R = −0.14 P = .402	**R = −0.38 P = .021**	**R = −0.35 P = .035**	**R = +0.34 P = .044**	**R = +0.35 P = .038**	R = +0.28 P = .095	R = +0.26 P = .128	R = −0.17 P = .318
Gynoid fat	R = −0.12 P = .497	R = −0.18 P = .290	R = −0.26 P = .133	R = −0.17 P = .329	R = −0.24 P = .151	R = −0.29 P = .087	R = +0.04 P = 0.807	R = −0.03 P = .858	R = −0.10 P = .548	R = 0.00 P = .997	R = +0.07 P = .674
Android/gynoid fat ratio	R = −0.09 P = .589	R = −0.02 P = .908	R = −0.14 P = .401	R = −0.09 P = .601	R = −0.30 P = .075	R = −0.23 P = .174	**R = +0.41 P = .013**	**R = +0.49 P = .003**	**R = +0.45 P = .006**	R = +0.32 P = .058	R = −0.21 P = .209
Log adipose-IR	R = +0.05 P = .748	R = +0.25 P = .120	R = +0.16 P = 0.325	R = +0.24 P = .140	R = +0.02 P = .918	R = +0.22 P = 0.179	**R = +0.34 P = .037**	**R = +0.40 P = .011**	**R = +0.39 P = .013**	R = +0.03 P = .852	R = +0.05 P = .747
Log LH	R = −0.15 P = .361	R = −0.04 P = .818	R = +0.05 P = .765	R = −0.12 P = .470	R = +0.06 P = .719	R = +0.01 P = 0.958	**R = +0.55 P < .001**	**R = +0.62 P < .001**	**R = +0.61 P < .001**	**R = +0.40 P = .010**	R = +0.15 P = .356
Si	R = −0.02 P = .913	R = −0.05 P = .766	R = −0.04 P = .826	R = −0.02 P = .916	R = +0.16 P = .338	R = +0.01 P = .960	R = −0.12 P = .481	R = −0.27 P = .098	R = −0.21 P = .201	R = +0.04 P = .808	R = +0.03 P = .841
Log AIRg	R = −0.05 P = .781	R = −0.09 P = .591	R = −0.09 P = .583	R = +0.10 P = .562	R = −0.27 P = .091	R = −0.17 P = .308	R = +0.16 P = .328	**R = +0.39 P = .015**	R = +0.26 P = .105	R = −0.07 P = .683	R = +0.23 P = .161
Log triglyceride	R = −0.02 P = .886	R = +0.02 P = .924	R = +0.06 P = .721	R = +0.10 P = .540	R = −0.06 P = .701	R = −0.02 P = .280	R = +0.23 P = .144	**R = +0.35 P = .025**	R = +0.31 P = .054	R = +0.09 P = .601	R = +0.14 P = .404
Fasting glucose	R = +0.27 P = .091	R = +0.23 P = .152	R = +0.25 P = .126	R = +0.23 P = .161	R = +0.25 P = .119	R = +0.19 P = 0.251	R = +0.00 P = .990	R = +0.05 P = 0.747	R = +0.07 P = .679	R = +0.02 P = .886	R = +0.08 P = .587
Fasting insulin	R = +0.16 P = .318	R = +0.21 P = .193	R = +0.13 P = .420	**R = +0.33 P = .041**	R = −0.05 P = 0.766	R = +0.27 P = .095	R = +0.25 P = .130	**R = +0.44 P = .005**	**R = +0.37 P = .020**	R = −0.15 P = 0.376	R = +0.08 P = .619
SHBG	**R = −0.32 P = .047**	R = −0.20 P = .210	R = −0.27 P = .088	R = −0.27 P = .088	R = −0.02 P = .890	R = −0.10 P = .533	R = −0.17 P = .303	**R = −0.57 P < .0001**	**R = −0.35 P = .025**	R = −0.05 P = .743	R = 0.00 P = .987
DHEAS	**R = +0.47 P = .002**	**R = +0.36 P = .021**	**R = +0.47 P = .002**	**R = +0.33 P = .038**	**R = +0.33 P = .040**	R = +0.31 P = .050	R = +0.14 P = .388	R = +0.29 P = .073	**R = +0.34 P = .031**	R = +0.04 P = .804	R = −0.22 P = .182

Total body dual-energy x-ray absorptiometry studies (controls = 18; PCOS = 18); frequently sampled IV glucose tolerance testing, and fasting glucose/insulin as well as log adipose insulin resistance (adipose-IR) studies (controls = 19; PCOS = 20). Boldface values represent significant correlations.

Abbreviations: 11OHA4, 11ß-hydroxyandrostendione; 11OHT, 11ß-hydroxytestosterone; 11KA4, 11-ketoandrostendione; 11KT, 11-ketotestosterone; A4, androstenedione; AIRg, acute response to glucose; BMI, body mass index; DHEAS, dehydroepiandrosterone sulfate; E1, estrone; E2, estradiol; Si, insulin sensitivity; T, testosterone; WHR, waist to hip ratio.

[a]New measurements as determined by liquid chromatography-tandem mass spectrometry at the University of Michigan, Ann Arbor [21, 22].

[b]E1 and E2 modified from references previously reported for these subjects [10-13, 16].

women with PCOS to enhance subcutaneous fat storage, while promoting circulating glucose as well as FFA availability as energy substrate for crucial target tissues. Such a metabolic adaptation, however, could predispose women with PCOS to excess weight gain in today's obesogenic environment, potentially increasing their risk of developing lipotoxicity [15, 50-52].

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Data Availability

Original data generated and analyzed during this study are included in this published article or in the data repositories listed in References.

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