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Beyond Adrenal and Ovarian Androgen Generation: Increased Peripheral 5α-Reductase Activity in Women with Polycystic Ovary Syndrome

MARTIN FASSNACHT, NADINE SCHLENZ, SUSANNE B. SCHNEIDER, STEFAN A. WUDY, BRUNO ALLOLIO, AND WIEBKE ARLT

Department of Medicine, Endocrine and Diabetes Unit, University of Würzburg (M.F., N.S., S.B.S., B.A., W.A.), 97080 Würzburg; and Steroid Research Unit, Center of Pediatrics and Adolescent Medicine, Justus Liebig University (S.A.W.), 35392 Giessen, Germany

Hyperandrogenism, a main clinical feature of polycystic ovary syndrome (PCOS), is thought to result from enhanced ovarian and adrenal androgen generation. To investigate the contribution of peripheral steroidogenesis, we used an oral challenge with dehydroepiandrosterone (DHEA) and analyzed its downstream conversion toward androgens in eight women with PCOS (age, 20–32 yr; body mass index, 20–41 kg/m^2) and eight healthy women matched for age and body mass index. They underwent frequent serum sampling and urine collection for 8 h on three occasions: at baseline, and after 4 d of dexamethasone (Dex; 4 × 0.5 mg/d), followed by ingestion of 100 mg DHEA or placebo. Dex induced similar significant suppression of circulating steroids in both groups. The oral DHEA challenge led to similar significant increases in the area under the concentration-time curve (0–8 h after Dex) of serum DHEA, DHEA sulfate, androstenedione, and testosterone. However, after oral DHEA, PCOS women had significantly higher increases in serum 5α-dihydrotestosterone ($P < 0.01$), its main metabolite androstanediol glucuronide ($P < 0.05$), and the 5α-reduced urinary androgen metabolite androsterone ($P < 0.05$). PCOS women also had significantly higher baseline excretion of 5α-reduced glucocorticoid ($P < 0.01$) and mineralocorticoid metabolites ($P < 0.05$). Taken together, these data indicate enhanced peripheral 5α-reductase activity in PCOS. Thus, not only ovary and adrenal, but also liver and peripheral target tissues, significantly contribute to steroid alterations in PCOS. (*J Clin Endocrinol Metab* 88: 2760–2766, 2003)

HYPERANDROGENISM IS THE principal biochemical abnormality in women affected by the polycystic ovary syndrome (PCOS), the most common endocrine disorder in women of reproductive age (1, 2). However, the origin of hyperandrogenemia in PCOS still remains controversial (3). Acute ovarian stimulation with GnRH agonists elicits higher androgen responses in PCOS women (4, 5). Ovarian suppression with long-acting GnRH agonists leads to a significant decline in circulating androgens in both healthy and PCOS women (6–8), but androgen levels remain higher in PCOS. Likewise, PCOS women show an exaggerated androgen response after adrenal stimulation with ACTH (9–11); and, after adrenal suppression by dexamethasone (Dex), PCOS women still have higher circulating androgen levels than controls (12). Thus, it is generally accepted that both ovary and adrenal significantly contribute to hyperandrogenemia in PCOS. It has also been suggested that enhanced activity of the steroidogenic enzyme P450c17, which is expressed both in adrenal and ovary (13), may be responsible for hyperandrogenemia in PCOS (14, 15). The 17,20 lyase activity of P450c17 represents the crucial step in the biosynthesis of dehydroepiandrosterone (DHEA) (16), which is the mandatory precursor of human androgen synthesis. *In vitro* studies found increased androgen secretion by ovarian theca cells from PCOS patients (17, 18) and reported enhanced 5α-reductase activity, *i.e.* conversion of testosterone (T) to 5α-dihydrotestosterone (DHT), in ovarian follicles from PCOS women (18).

However, beyond increased adrenal and ovarian androgen synthesis, there may be an important contribution of peripheral androgen synthesis to hyperandrogenism in PCOS. Stewart *et al.* (19) reported a significantly increased urinary baseline excretion of 5α-reduced androgen and glucocorticoid metabolites in women with PCOS, thereby providing indirect evidence for increased peripheral 5α-reductase activity. We have previously shown that orally administered DHEA is readily converted to androgens (Fig. 1) in women, looking both at healthy women with transient adrenal suppression by Dex (20) and at women with chronic adrenal insufficiency (21). Therefore, we used an oral challenge with DHEA as a diagnostic tool to clarify whether downstream conversion of DHEA toward androgens differs between healthy women and women with PCOS.

Abbreviations: A'dione, 4-Androstene-3,17-dione; ADG, 5α-androstane-3α,17β-diol-17-glucuronide; An, androsterone; $AUC_{(0-8\ h)}$, area under the curve (0–8 h); Dex, dexamethasone; DHEA, dehydroepiandrosterone; DHEAS, DHEA sulfate; DHT, 5α-dihydrotestosterone; E2, 17β-estradiol; Et, etiocholanolone; GC-MS, gas chromatographic-mass spectrometric; PCOS, polycystic ovary syndrome; T, testosterone; THA, tetrahydro-11-dehydrocorticosterone; THB, tetrahydrocorticosterone; THE, tetrahydrocortisone; THF, tetrahydrocortisol.

Subjects and Methods

Subjects

Eight women with PCOS (age range, 20–32 yr; median body mass index, 25.7 kg/m^2; range, 20.3–41.3 kg/m^2) were recruited from the outpatient clinic of a secondary/tertiary care referral unit. In all patients, diagnosis of PCOS was established by fulfillment of the following two

criteria: first, clinical evidence of hirsutism (seven of eight patients presented with a Ferriman-Gallwey score more than 8) and/or oligo-/ameneorrhea (5/8); second, hyperandrogenemia [*i.e.* serum concentrations of DHEA sulfate (DHEAS) (5/8), androstenedione (7/8), and/or T (6/8) above the normal range for females]. In addition, all patients showed an elevated LH/FSH ratio (*i.e.* more than 1.5 at baseline and/or more than 3.0 after stimulation with 100 μg GnRH iv). Eight healthy control subjects were recruited via local advertising and were matched for sex, age, and body mass index (median, 24.5 kg/m^2; range, 19.8–37.8 kg/m^2). All controls had regular menstrual cycles; normal serum concentrations of DHEAS, androstenedione, and T; and no evidence of hirsutism. Further inclusion criteria for both groups were: normal blood cell counts, and normal hepatic and renal function parameters. Exclusion criteria for both patients and controls were: evidence of 21-hydroxylase deficiency or 3β-hydroxysteroid dehydrogenase deficiency, hyperprolactinemia, hypo- or hyperthyroidism, diabetes mellitus, pregnancy, current or previous intake of antiandrogenic drugs, current or previous long-term glucocorticoid treatment, current intake of drugs known to induce hepatic P450 enzymes, and current intake of oral contraceptives. Before the initiation of the study, the protocol had been approved by the Ethics Committee of the University of Wuerzburg, and written informed consent was obtained from all study participants.

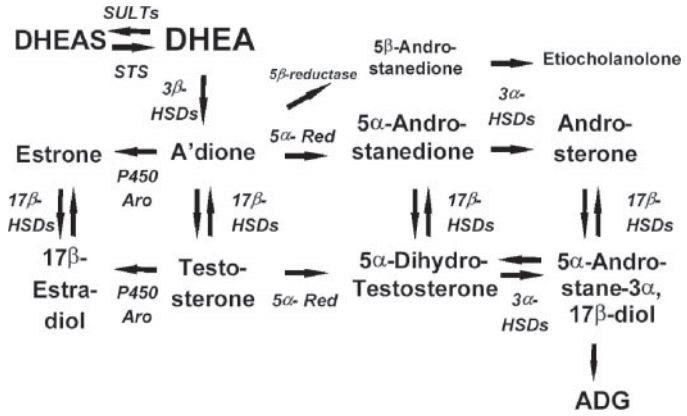

FIG. 1. Schematic figure of downstream conversion of DHEA toward androgens (and estrogens) by steroidogenic enzymes. 3α-HSDs, isozymes 1–4 of 3α-hydroxysteroid dehydrogenase; 3β-HSDs, isozymes 1 and 2 of 3β-hydroxysteroid dehydrogenase; 17β-HSDs, isozymes 1–5 and 7 of 17β-hydroxysteroid dehydrogenase; 5α-Red, isozymes 1 and 2 of 5α-reductase; P450 Aro, P450 aromatase; STS, steroid sulfatase; SULTs, hydroxysteroid sulfotransferases SULT2A1 and SULT2B1; ADG, 5α-androstane-3α,17β-diol-17-glucuronide.

Study protocol

The study was performed in a single-dose, randomized, cross-over design. All participants were studied during the early follicular phase of three subsequent cycles; in oligo-/amenorrhoic subjects, study days were separated by 4 wk. Cycle 1 served as baseline. Preceding the study days during cycles 2 and 3, all subjects were pretreated with oral Dex (4 × 0.5 mg daily for 4 d). On study d 2 and 3, either placebo or 100 mg DHEA (25-mg capsules, Natrol) were administered orally at 0900 h in a randomized order. On all three study days, frequent blood sampling was performed, starting after an overnight fast at 0830 h (−30 min), followed by sampling at 0, 30, 60, 90, 120, 150, 180, 240, 300, 360, and 480 min. In addition, all participants collected their urine from 0900 h till 1700 h. Standardized meals were served at 1030 h and 1500 h.

Measurements

Serum steroid hormone concentrations were determined by established specific RIAs; cortisol (Diagnostic Systems Laboratories, Inc., Sinsheim, Germany) [cross-reactivity to DHEA, 0.02%; T, 0.14%; and 17β-estradiol (E2), 0.02%]; DHEA (Diagnostic Systems Laboratories, Inc.) [cross-reactivity to DHEAS, 0.04%; 4-androstene-3,17-dione (A'dione), 0.46%; and T, 0.03%]; DHEA sulfate (DHEAS) (DPC Biermann, Bad Nauheim, Germany) [cross-reactivity to DHEA, 0.08%; androstenedione, 0.12%; T, 0.10%; E2, 0.03%; and estriol, 0.03%]; A'dione

TABLE 2. Effects of Dex pretreatment (4 × 0.5 mg/d) on AUCs of eight sampling hours (AUC 0–8 h) for measured steroids [median, range (min-max)] in PCOS women (n = 8) and in controls (n = 8)

Steroid hormone	Group	Median suppression after Dex (% of AUC 0–8 h at baseline)	Range of suppression after Dex (min-max) (% of AUC 0–8 h at baseline)
DHEA	Controls	26	14–39
	PCOS	16[a]	9–28
DHEAS	Controls	18	12–25
	PCOS	16	3–23
Androstenedione	Controls	41	37–72
	PCOS	41	20–105
Testosterone	Controls	23	9–49
	PCOS	28	11–103
Dihydrotestosterone	Controls	32	14–48
	PCOS	38	7–63
Androstanediol glucuronide	Controls	21	11–34
	PCOS	40	9–66

[a] $P < 0.05$ for the comparison PCOS *vs.* controls.

TABLE 1. AUCs of eight sampling hours (AUC 0–8 h) for measured steroids (mean ± SEM) in PCOS women (n = 8) and in controls (n = 8) at baseline, after 4 d of Dex (4 × 0.5 mg/d), after 4 d of Dex, followed by a single oral dose of 100 mg DHEA (Dex + DHEA), and after correction for the Dex-suppressed baseline: [Δ (Dex + DHEA) − Dex]

Steroid hormone	Group	Baseline	Dex	Dex + DHEA	Δ (Dex + DHEA) − Dex
AUC 0–8 h	Controls	254 ± 39	58 ± 13[b]	278 ± 28	246 ± 28
DHEA (nmol/h)	PCOS	481 ± 84[d]	76 ± 14[c]	317 ± 57	241 ± 54
AUC 0–8 h	Controls	50 ± 7	9 ± 1[c]	87 ± 6[a]	79 ± 6
DHEAS (μmol/liter·h)	PCOS	75 ± 11	12 ± 3[c]	88 ± 12	76 ± 10
AUC 0–8 h	Controls	60 ± 5	27 ± 2[c]	116 ± 11[b]	89 ± 12
A'dione (nmol/liter·h)	PCOS	91 ± 10[d]	42 ± 8[b]	103 ± 22	62 ± 20
AUC 0–8 h	Controls	5.8 ± 0.9	1.5 ± 0.4[c]	5.2 ± 1.2	3.6 ± 1.2
T (nmol/liter·h)	PCOS	16.9 ± 2.1[f]	8.2 ± 2.8[c,d]	11.8 ± 2.5[b,d]	3.6 ± 1.1
AUC 0–8 h	Controls	4.2 ± 0.6	1.2 ± 0.1[b]	5.1 ± 0.7	3.9 ± 0.6
Dihydrotestosterone (nmol/liter·h)	PCOS	6.5 ± 1.0	2.5 ± 0.8[c]	10.5 ± 1.6[b,e]	8.0 ± 1.0[f]
AUC 0–8 h	Controls	91 ± 31	18 ± 5[a]	91 ± 13	73 ± 14
Androstanediol glucuronide (nmol/liter·h)	PCOS	123 ± 23	38 ± 7[b,d]	160 ± 13[f]	121 ± 13[c]

To convert from nM × h to ng/ml·h, divide DHEA by 3.467, A'dione by 3.492, T by 3.467, DHT by 3.467, and ADG by 2.13. To convert from μM × h to μg/dl·h, divide DHEAS by 0.02714.

[a] $P < 0.05$, [b] $P < 0.01$, and [c] $P < 0.001$ for the comparison *vs.* baseline. [d] $P < 0.05$, [f] $P < 0.001$, and [e] $P < 0.01$ for the comparison PCOS *vs.* controls.

(DPC Biermann) [cross-reactivity to DHEA, 0.02%; DHT, 0.05%; and estrone, 0.08%]; T (DPC Biermann) [cross-reactivity to A'dione, 0.5%; DHT, 3.1%; E2, 0.02%]; DHT (Diagnostic Systems Laboratories, Inc.) [cross-reactivity to T, 0.02%; A'dione, 1.90%; ADG, 0.19%; and E2, 1.41%]; and 5α-androstane-3α,17β-diol-17-glucuronide (ADG) (Diagnostic Systems Laboratories, Inc.) [cross-reactivity to DHT-glucuronide, 1.2%; no cross-reactivity to 5α-androstane-3β,17β-diol or 5α-androstane-3α,17β-diol-3-glucuronide]. Cross-reactivities to other steroids relevant to this study were less than 0.01%. For all assays, the intra- and interassay coefficients of variation were less than 8% and less than 12%, respectively.

Urinary steroid profiles were determined using quantitative data produced by gas chromatographic-mass spectrometric (GC-MS) analysis according to the method described by Shackleton (22). In brief, free and conjugated urinary steroids were extracted by solid-phase extraction, and the conjugates were enzymatically hydrolyzed, followed by recovery of the hydrolyzed steroids by Sep-Pak (Waters, Milford, MA) extraction. Known amounts of three internal standards (5α-androstane-3α,17α-diol; stigmasterol; and cholesteryl butyrate) were added to a portion of each extract before formation of methyloxime-trimethylsilyl ethers. Gas chromatography was performed using an Optima-1 fused silica column. Helium was used as carrier gas. The gas chromatograph (Agilent 6890 Series GC; Agilent 7683 Series Injector, Agilent Technologies, Boehlingen, Germany) was directly interfaced to a mass selective detector (Agilent 5973N MSD) operated in selected ion-monitoring mode. Calibration of the GC-MS was achieved by analysis of a reference mixture containing known amounts of all of the separation compounds. The injections took place with an 80 C (2 min) gas chromatography oven; the temperature was then increased by 20 C/min to 190 C (1 min). Then, for separation of steroids, it was increased by 2.5 C/min to 272 C. After calibration, values for the excretion of individual steroids were determined by measuring the selected ion peak areas against the internal standards.

Statistics

All data are reported as mean ± SEM. The area under the concentration-time curve, 0–8 h [$AUC_{(0-8\ h)}$] for the measured serum steroid hormones was calculated by means of trapezoidal integration. The normal distribution of results was ascertained by using the Kolmogorov-Smirnov-Lilliefors test. Thus, comparisons between PCOS and control groups were carried out by *t* test for unpaired samples, and comparisons of results at baseline and after DHEA, within each group, were performed by *t* test for paired samples. Significance was defined as $P < 0.05$.

Results

Serum hormones at baseline

As expected, women with PCOS had significantly higher baseline concentrations of serum DHEA, A'dione, and T; serum DHEAS, DHT, and ADG showed a trend toward significant difference (all $P < 0.1$) (Table 1). By contrast,

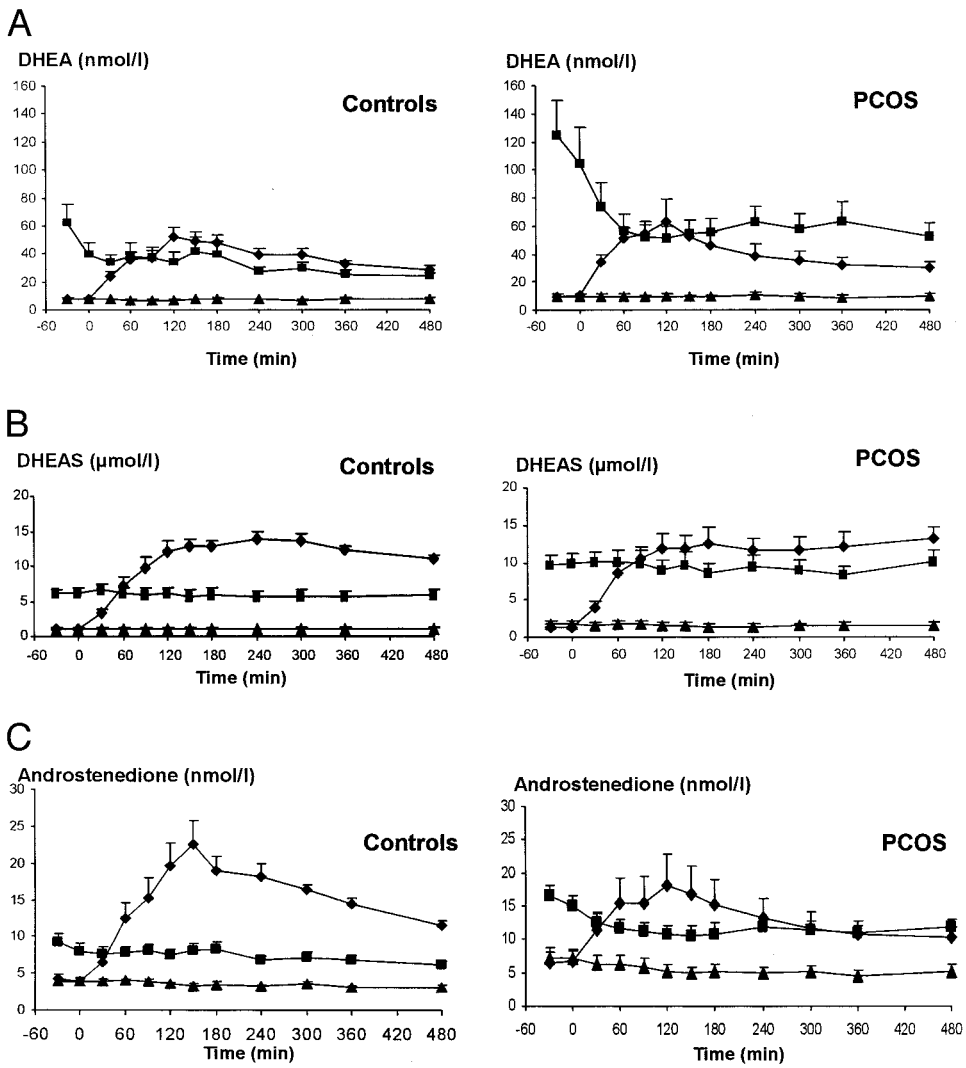

FIG. 2. Mean serum concentrations (±SEM) of DHEA (A), DHEAS (B), and androstenedione (C) in women with PCOS (n = 8) and in controls (n = 8) at baseline (-■-), after 4 d of Dex (4 × 0.5 mg/d) (-▲-), and after 4 d of Dex followed by the ingestion of a single dose of 100 mg DHEA (-♦-). To convert the values for DHEA to ng/ml, divide nM by 3.467; to convert values for DHEAS to µg/dl, divide µM by 0.02714; to convert values for androstenedione to ng/ml, divide nM by 3.492.

serum cortisol was nearly identical in the two groups (PCOS *vs.* controls: $AUC_{(0-8\ h)}$ 2611 ± 156 *vs.* 2403 ± 214 nM × h, not significant; to convert nM × h to μg/dl × h, divide by 27.59).

Serum hormones after Dex

Pretreatment with Dex led to a lasting and pronounced suppression of serum DHEA, DHEAS, A'dione, T, DHT, and ADG, both in women with PCOS and in healthy controls (Table 1). Comparing the changes in mean $AUC_{(0-8\ h)}$ values, there was a slightly higher suppression of serum DHEA in PCOS than in controls ($P < 0.05$) but a similar suppression of serum DHEAS and A'dione (Table 2). PCOS women showed somewhat less suppression of serum T, DHT, and ADG; however, this was not significant, because of a considerable amount of interindividual variability in response to Dex (in particular, within the PCOS group) (Table 2). This may also explain why there was no significant difference between PCOS and controls with regard to absolute levels of serum hormones after Dex, with the exception of serum T [$AUC_{(0-8\ h)}$ PCOS *vs.* controls: 8.2 ± 2.8 *vs.* 1.5 ± 0.4 nM × h, $P < 0.05$; to convert nM × h to ng/ml × h, divide by 3.467] and the DHT main metabolite ADG (38 ± 7 *vs.* 18 ± 5 nM × h, $P < 0.05$; to convert nM × h to ng/ml × h, divide by 2.13), which both remained significantly higher in PCOS (Table 1).

Serum hormones after DHEA

After oral administration of 100 mg DHEA, serum levels of DHEA, DHEAS, and A'dione rose to a similar extent in PCOS and controls (Fig. 2), with no significant difference in $AUC_{(0-8\ h)}$ between the groups after correction for the Dex-suppressed baseline (Table 1). Also, the expected increase in serum T was nearly identical between PCOS and controls (Fig. 3). By contrast, PCOS women exhibited significantly higher increases in serum DHT ($P < 0.01$) and in the major DHT metabolite ADG ($P < 0.05$) after DHEA ingestion (Fig. 3). Correction for Dex-suppressed baselines (Fig. 4) further illustrates this increase in the androgenic pool downstream from the catalytic activity of 5α-reductase, the enzyme that converts T to DHT.

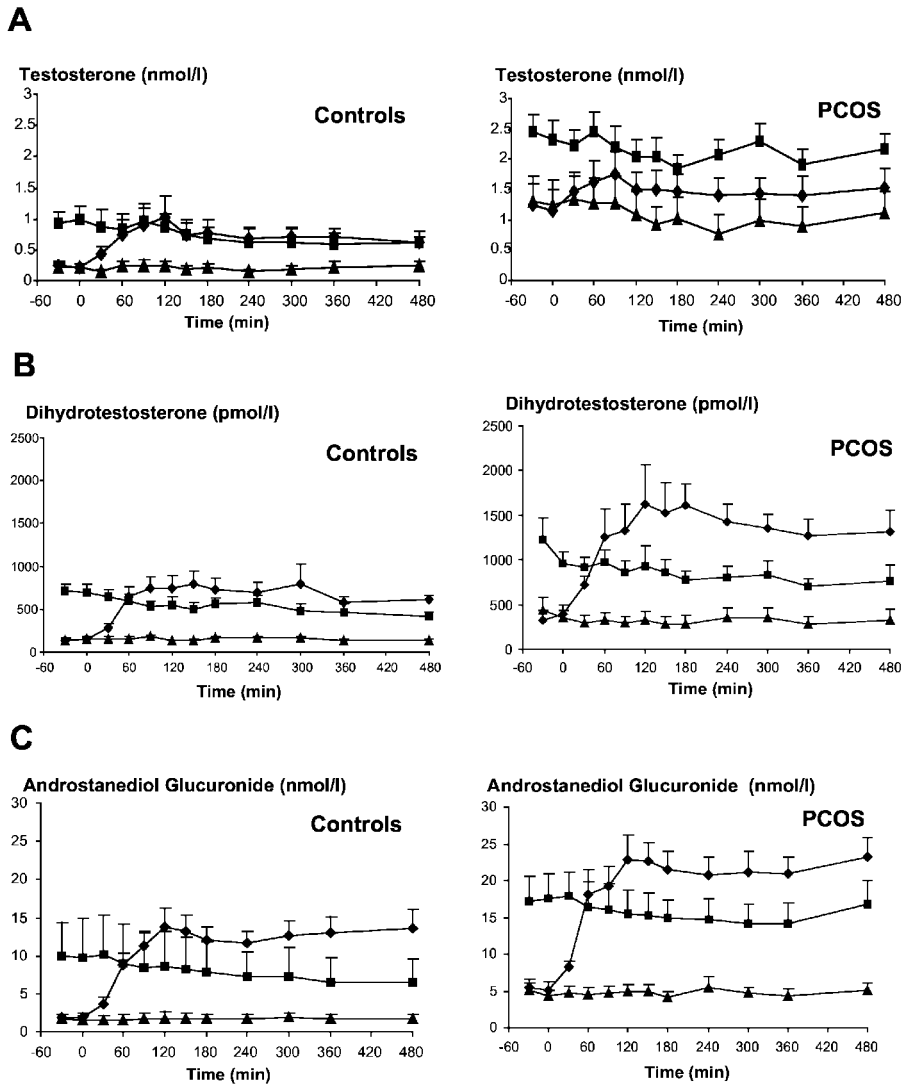

FIG. 3. Mean serum concentrations (±SEM) of T (A), DHT (B), and 5α-androstane-3α,17β-diol glucuronide (C) in women with PCOS (n = 8) and in controls (n = 8) at baseline (-■-), after 4 d of Dex (4 × 0.5 mg/d) (-▲-), and after 4 d of Dex followed by the ingestion of a single dose of 100 mg DHEA (-◆-). To convert the values for T to ng/ml, divide nM by 3.467; to convert values for DHT to ng/ml, divide nM by 3.467; to convert values for 5α-androstane-3α,17β-diol-17-glucuronide to ng/ml, divide nM by 2.13.

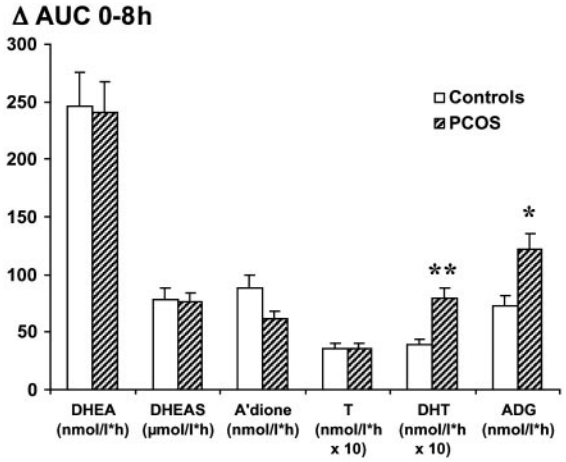

FIG. 4. Mean $AUC_{(0-8\ h)}$ (±SEM) for serum steroid concentrations in women with PCOS (n = 8) and in healthy controls (n = 8) after 4 d of Dex + 100 mg DHEA (after subtraction of the Dex-suppressed baseline). *, $P < 0.05$; **, $P < 0.01$ for the comparison PCOS *vs.* controls.

Urinary steroid metabolites

As expected, PCOS women had a significantly higher baseline excretion of androgen metabolites than did controls [androsterone (An) + etiocholanolone (Et): 5940 ± 1161 *vs.* 2030 ± 385 μg/8 h, $P < 0.05$], reflecting the significantly higher levels of serum androgens in PCOS. However, despite similar $AUC_{(0-8\ h)}$ values for serum cortisol in both groups, baseline excretion of glucocorticoid metabolites [sum of tetrahydrocortisone (THE), tetrahydrocortisol (THF), 5α-THF, cortoles, and cortolones] and mineralocorticoid metabolites [sum of tetrahydro-11-dehydrocorticosterone (THA), tetrahydrocorticosterone (THB), and 5α-THB] was significantly increased in PCOS women (Fig. 5). After Dex pretreatment, glucocorticoid and mineralocorticoid metabolites were suppressed down to around 10% of baseline levels, and androgen metabolites decreased to 15–30% of baseline levels in both groups (Table 3). After the oral DHEA challenge, the 5α-reduced androgen metabolite An increase was significantly higher in PCOS than in controls (5308 ± 1385 *vs.* 2633 ± 253 μg/8 h, $P < 0.05$), whereas the increase in the 5β-reduced androgen metabolite Et failed to become significant (Table 3). However, because of considerable interindividual variations in urinary steroid excretion, the calculation of the ratios of 5α-THF to 5β-THF and of An to Et, which are considered to be approximate measures of net 5α-reductase activity, did not reveal a significant difference between PCOS and controls.

Discussion

We successfully used an oral challenge with DHEA as a tool to unmask significant differences in peripheral androgen activation between women with PCOS and healthy controls. Our results intriguingly suggest an increased 5α-reductase activity in women with PCOS, as schematically illustrated in Fig. 6. Exogenous DHEA administration resulted in enhanced generation of serum DHT, which is generated from T by 5α-reductase, and of its major metabolite ADG. Concurrently, urinary excretion of the 5α-reduced androgen metabolite An after DHEA administration was significantly higher in PCOS. All these changes are readily explained by increased peripheral 5α-reductase activity. Interestingly, this difference became more readily apparent after the oral DHEA challenge. This may indicate the superiority of this dynamic intracrine test over the measurement of baseline concentrations, which only represent a net measure of enzymatic activities.

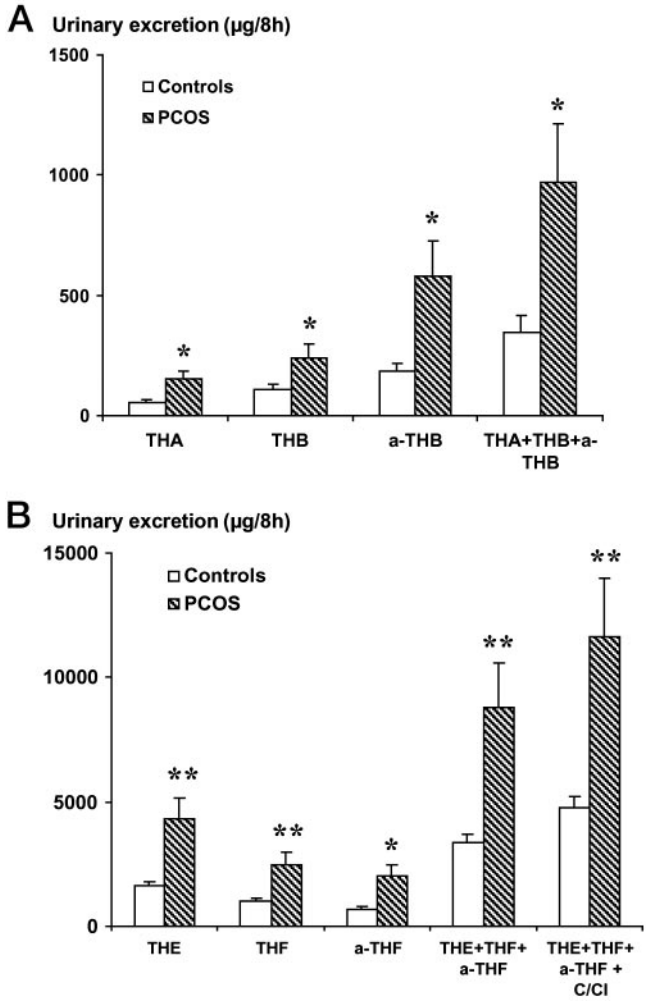

FIG. 5. Urinary excretion of glucocorticoid (A) and mineralocorticoid (B) metabolites (mean ± SEM) at baseline in women with PCOS (n = 8) and in healthy controls (n = 8). *, $P < 0.05$; **, $P < 0.01$ for the comparison PCOS *vs.* controls. Glucocorticoid metabolites: a-THF, 5α-THF; C, controls; Cl, cortolones. Mineralocorticoid metabolites: a-THB, 5α-THB.

PCOS women also had a significantly higher baseline excretion of 5α-reduced glucocorticoid and mineralocorticoid metabolites. Though the ratios of 5α- to 5β-reduced steroid metabolites failed to become significantly different in our study, this may have been mainly attributable to the generally considerable interindividual variation in urinary steroid excretion rates and the relatively low number of subjects in our study (n = 8). In keeping with our findings, another recent study in only 9 patients with PCOS also found a significantly increased excretion of total glucocorticoid metabolites, whereas the differences in the ratios of 5α- to 5β-

TABLE 3. Urinary excretion of steroid metabolites (mean ± SEM) in PCOS women (n = 8) and in controls (n = 8) at baseline, after 4 d of Dex (4 × 0.5 mg/d) and after 4 d of Dex, followed by a single oral dose of 100 mg DHEA (Dex + DHEA)

Steroid hormone	Group	Baseline	Dex	Dex + DHEA
An (μg/8 h)	Controls	967 ± 173	276 ± 70	2633 ± 253
	PCOS	3258 ± 799[a]	490 ± 77	5308 ± 1385[a]
Et (μg/8 h)	Controls	1063 ± 119	342 ± 102	3614 ± 369
	PCOS	2682 ± 465[b]	423 ± 45	5828 ± 1702
THE (μg/8 h)	Controls	1649 ± 210	138 ± 30	148 ± 29
	PCOS	4288 ± 672[b]	286 ± 39[b]	297 ± 47[a]
THF (μg/8 h)	Controls	994 ± 89	111 ± 29	121 ± 30
	PCOS	2490 ± 418[b]	251 ± 34[b]	260 ± 45[a]
a-THF (μg/8 h)	Controls	697 ± 111	97 ± 29	107 ± 31
	PCOS	2032 ± 482[a]	239 ± 33[b]	246 ± 45[a]
All GC (μg/8 h)	Controls	4752 ± 431	570 ± 152	628 ± 157
	PCOS	11644 ± 1924[b]	1304 ± 179[b]	1362 ± 238[a]
THA (μg/8 h)	Controls	55 ± 11	5.1 ± 1.6	7.6 ± 2.4
	PCOS	149 ± 35[a]	6.0 ± 3.9	4.9 ± 3.1
THB (μg/8 h)	Controls	111 ± 18	13 ± 3	13 ± 3
	PCOS	240 ± 50[a]	21 ± 2	23 ± 4[a]
a-THB (μg/8 h)	Controls	182 ± 33	13 ± 3	16 ± 2
	PCOS	582 ± 164[a]	25 ± 3[a]	27 ± 3[a]
THA + THB + aTHB (μg/8 h)	Controls	348 ± 56	32 ± 5.4	36 ± 4
	PCOS	971 ± 243[a]	52 ± 7[a]	55 ± 7[a]

a-THF, 5α-Tetrahydrocortisol; all GC, all glucocorticoid metabolites (= sum of THE, THF, a-THF, cortols, and cortolones); a-THB, 5α-tetrahydrocorticosterone.

[a] $P < 0.05$; [b] $P < 0.01$ for the comparison PCOS *vs.* controls.

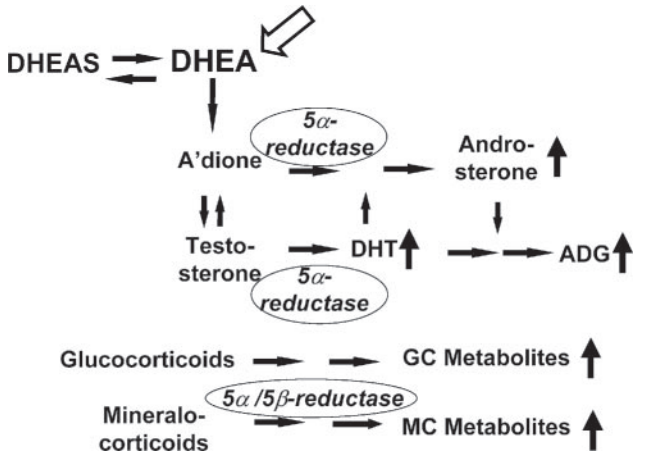

FIG. 6. Schematic summary of the observed differences between PCOS and control women (serum steroids after Dex pretreatment followed by oral DHEA challenge; urinary steroid excretion at baseline). GC, Glucocorticoid; MC, mineralocorticoid.

reduced steroids failed to become significant (23). Stewart *et al.* (19) analyzed urinary steroid excretion in 11 patients and were the first to report an increased ratio of 5α- to 5β-reduced glucocorticoid and androgen metabolites in PCOS. The validity of these findings was confirmed by the results of a recent (and so far, largest) study on urinary steroid excretion in 98 women with PCOS (24). However, our study is the first to report significantly increased excretion of 5α- and 5β-reduced mineralocorticoid metabolites. The pathophysiological consequences of enhanced glucocorticoid and mineralocorticoid metabolism in PCOS remain elusive. Because serum cortisol did not differ between PCOS and controls, an enhanced generation of glucocorticoids, and possibly also of mineralocorticoids, in PCOS may try to compensate for the faster metabolism. Whether excess generation of gluco- and mineralocorticoids also leads to a biologically relevant increase in receptor binding (with a potential impact on blood pressure and glucose tolerance) remains to be speculated. Stewart *et al.* (19) suggested that enhanced clearance of cortisol caused by increased 5α-reductase activity could lead to a counterregulatory activation of the HPA axis and thus to enhanced adrenal androgen generation. However, they failed to demonstrate an increase in ACTH secretion or pulsatility in a follow-up study (25).

An increase in 5α-reductase activity will inevitably lead to enhanced androgen activation by conversion of T to DHT in peripheral target cells of androgen action. This clearly points toward the importance of the relative contributions of liver and peripheral target tissues to enhanced androgen generation in PCOS. In our study, adrenal androgen generation was suppressed by Dex; and, though DHEAS has been suggested to be a precursor for ovarian steroidogenesis (26), our findings are unlikely to be explained by increased ovarian uptake of DHEA. More probable, they are the result of enhanced androgen synthesis outside ovary and adrenal, *e.g.* liver, skin, and other peripheral target tissues of androgen action. It is conceivable that enhanced 5α-reductase activity and increased androgen synthesis are interrelated events, *e.g.* by transcriptional regulation of 5α-reductase expression by androgens and their precursors.

The importance of the periphery is underlined by the significant increase in the major DHT metabolite ADG (27) after the DHEA challenge in our PCOS women. Serum ADG concentrations are a good measure of intracrine androgen activation (28), *i.e.* the generation and subsequent metabolism of DHT within one-and-the-same peripheral target cell. This does not become readily apparent by an increase in circulating DHT but rather by an increase in serum ADG (29), because DHT will only reenter the bloodstream after it has been metabolized to ADG. The concept of enhanced peripheral androgen generation by 5α-reductase is further supported by results from previous studies showing increased

5α-reductase activity in skin biopsies from hirsute women (30, 31), which correlates with increased ADG production (32).

In conclusion, our results are highly suggestive of an increased peripheral 5α-reductase activity in women with PCOS and thus of a significant contribution of peripheral and intrahepatic steroidogenesis to the steroid changes observed in PCOS. This may shift our view on PCOS, beyond ovary and adrenal, to the modulation of steroidogenesis at the prereceptor level in most, if not all, peripheral target cells of steroid action.

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Address all correspondence and requests for reprints to: Wiebke Arlt, M.D., Division of Medical Sciences, University of Birmingham, Queen Elizabeth Hospital, Edgbaston, Birmingham B15 2TH, United Kingdom. E-mail: w.arlt@bham.ac.uk.

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