11-Oxyandrogens in Adolescents with Polycystic Ovary Syndrome (PCOS)

Anya E. Taylor¹, Meredith A. Ware¹, Emily Breslow¹, Laura Pyle²³, Cameron Severn²³, Kristen J. Nadeau¹⁴, Christine L. Chan¹, Megan M. Kelsey¹⁴, Melanie Cree-Green¹⁴

¹-Department of Pediatrics, Division of Pediatric Endocrinology, University of Colorado Anschutz Medical Campus, Aurora, CO
²-Department of Pediatrics, University of Colorado Anschutz Medical Campus, Aurora, CO
³-Department of Biostatistics and Informatics, Colorado School of Public Health, Aurora, CO
⁴-Center for Women’s Health Research, Aurora, CO, USA

To whom correspondence should be addressed:
Melanie Cree-Green, MD, PhD
Children’s Hospital Colorado
University of Colorado Anschutz Medical Campus
PO Box 265, 13123 E 16th Ave, Aurora, CO 80045
Phone: 720-777-5743 Fax: 720 777-7301
Email: Melanie.Green@childrenscolorado.org

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Abstract:

**Background/context:** Polycystic ovary syndrome (PCOS) is common and diagnosis requires an elevated testosterone. The clinical importance of adrenal 11-oxyandrogens in PCOS is unclear. We sought to determine if 11-oxyandrogens: 1) better identify PCOS diagnosis compared to testosterone 2) predict clinical comorbidities of PCOS, 3) are altered with combined oral contraceptive pill (COCP) or metformin therapy.

**Methods:** Data from 200 adolescent females aged 12-21 years, most with obesity, enrolled across 6 studies in pediatric endocrinology were included: 70 non-PCOS controls, 115 untreated PCOS, 9 PCOS+obesity treated with COCP, 6 PCOS+obesity treated with metformin. 11-hydroxyandrostenedione (11OHA4), 11-hydroxytestosterone (11OHT), 11-ketotestosterone (11KT) and testosterone were measured with LC/MSMS. Data between 1) untreated PCOS and controls and 2) untreated PCOS and the 2 treatment groups were compared.

**Results:** Untreated girls with PCOS had higher 11OHA4 (p=0.003) and 11OHT (p=0.005) compared to controls, but not 11KT (p=0.745). Elevated 11OHA4 remained significant after controlling for obesity. Testosterone better predicted PCOS status compared to 11-oxyandrogens (ROC analysis: 11OHA4 AUC=0.620, 11OHT AUC=0.638; testosterone AUC=0.840). Among untreated PCOS, all three 11-oxyandrogens correlated with hirsutism severity. 11KT (p=0.039) and testosterone (p<0.006) was lower in those on COCP treatment compared to untreated PCOS. Metformin treatment had no effect on 11-oxyandrogens, although testosterone was lower (p=0.01).

**Conclusions/Relevance:** While 11-oxyandrogens do not aid in the diagnosis of PCOS, they relate to excess hair growth. COCP treatment may related to 11-KT, however, further work is needed to determine causality, relationship with metabolic outcomes, and the clinical utility of measuring these androgens in PCOS.

**Keywords:** 11-oxyandrogens, polycystic ovary Syndrome, adolescents
Introduction

Polycystic ovary syndrome (PCOS) is common, clinically presents during adolescence, and is associated with an increased risk for obesity. Diagnostic criteria have changed in recent years in acknowledgement of the heterogeneity of the clinical phenotype\(^1\). In adolescents, an elevated serum testosterone or clinical signs consistent with excess testosterone are required for diagnosis under both the classic and latest diagnostic criteria\(^2,3\). Clinical phenotypic characteristics of androgen excess in PCOS include hirsutism, acne, and androgenic alopecia. PCOS is also associated with obesity and the metabolic syndrome which may manifest as dysglycemia, hyperlipidemia, hepatic steatosis and cardiovascular disease\(^2\). The severity of metabolic disease is thought to relate to testosterone concentrations and presence of and degree of obesity\(^4\).

Recently, there has been a renewed interest in quantifying the adrenal derived 11-oxygenated androgens (11-oxyandrogens for short) in hyperandrogenic disorders due to their newly realized potency and potential to serve as biomarkers\(^5\). Most adrenal androgens are synthesized in the zona reticularis, with dehydroepiandrosterone (DHEA) and its sulfate, DHEAS, being the most abundant\(^6\). The enzyme 3β-hydroxysteroid dehydrogenase type 2 (HSD3B2) converts DHEA to androstenedione, followed by aldo-keto reductase 1C3 (AKR1C3) modulation of androstenedione to testosterone. Androstenedione and testosterone emerge as substrates for the adrenal enzyme cytochrome P450 11β-hydroxylase to yield 11-hydroxyandrostenedione (11OHA4) and 11-hydroxytestosterone (11OHT)\(^6\). Additionally, 11OHA4 can be oxidized to 11-ketoandrostenedione (11KA4), and 11OHT can be oxidized to 11-ketotestosterone (11KT) primarily in peripheral tissue such as in the skin and to a lesser extent in the adrenal glands\(^6\). These C19 steroids have emerged as major compounds in several androgen excess disorders\(^6\). Androgen excess often manifests during adrenarche, between 6-8 years of age, secondary to expansion of the zona reticularis; this results in increased synthesis of adrenal androgens, which allows overproduction (hyperandrogenemia) if enzymatic regulators become inhibited\(^7\). For instance, 11KT has been found to be
elevated in girls with premature adrenarche, indicating it plays a role in clinical symptoms of hyperandrogenism in females.\textsuperscript{7}

The ovaries have traditionally been considered the primary source of androgen excess in females with PCOS,\textsuperscript{6} with minimal adrenal contribution; however, limited studies have been conducted to determine the contribution of 11-oxyandrogens.\textsuperscript{8-11} Rege et al. found that 11KT is the dominant circulating bioactive androgen during normal and premature adrenarche and suggested that this androgen may be responsible for a subset of girls with premature adrenarche being more susceptible to the development of PCOS and insulin resistance.\textsuperscript{7} Wen et al. measured 11OHA4 concentrations in plasma and follicular fluid from the ovary and found that raised plasma concentrations may reflect extra-adrenal contribution to hyperandrogenism due to follicular fluid concentrations being lower in women with PCOS than normally cycling women.\textsuperscript{13} Another potential cause of the adrenal androgen excess could be ovarian metabolism of androstenedione into testosterone, and they conclude that 11OHA4 cannot be regarded as a biomarker for PCOS.\textsuperscript{13} Due to 11KT having the same relative androgenicity as testosterone and suggestions that 11OHA4 concentrations may be elevated due to adrenal contributions, further examination into the role 11-oxyandrogens play in adolescents with PCOS is needed.\textsuperscript{6}

The aims of this project were primarily to assess if 11-oxyandrogens better identified PCOS diagnosis compared to testosterone, as well as to assess the relationship between 11-oxyandrogens and clinical symptoms and co-morbidities, and to measure the effects of common pharmaceutical treatments for PCOS on 11-oxyandrogens hormone concentrations in adolescents with PCOS.

**Materials and Methods**

Participants included were enrolled in one of six previously conducted pediatric endocrine studies at Children’s Hospital Colorado who had stored frozen serum samples and consented to blood banking for future endocrine testing. Three studies were focused on understanding metabolic disease in PCOS: Androgens and Insulin Resistance Study (AIRS, prior to NCT, N=76, PI: M Cree-Green); Liver and Fat Regulation in Overweight
Adolescent Girls (APPLE, NCT02157974, N=92, PI: M Cree-Green\textsuperscript{15}) and Post-Prandial Liver Glucose Metabolism in PCOS (PLUM, NCT03041129, N=17, PI: M Cree-Green\textsuperscript{16}). All studies recruited females between the ages of 12 and 21 years, described as having a sedentary activity level (<3 hours of physical activity a week), and had a BMI $\geq$90 percentile or between the 10\textsuperscript{th}-85\textsuperscript{th} percentile. PCOS status was met based on the NIH definition: irregular menstrual cycles, at least 1.5 years post-menarche, and either clinical (cystic acne or hirsutism) or biochemical (elevated testosterone) markers of hyperandrogenism\textsuperscript{2}. Other causes of oligomenorrhea (thyroid, prolactin, ovarian failure) and hyperandrogenism (congenital adrenal hyperplasia and adrenal tumor) were ruled out with standard laboratory tests. Exclusion criteria included currently pregnant or breastfeeding, implanted medical devices not compatible with MRI, evidence of diabetes, and use of medication such as combined oral contraceptive pills (COCPs) and those known to affect insulin sensitivity for both PLUM and AIRS; however, for the PCOS group in APPLE, COCPs and treatment with metformin was allowed. Additional healthy controls without PCOS were included from Characterization of Glucose Variability by Continuous Glucose Monitoring (NCT02211235, PI: C. Chan\textsuperscript{17,18}), Combined Influence of Puberty and Obesity on Insulin Resistance in Adolescents (HIPS, NCT01775813, PI: MM Kelsey\textsuperscript{19}), and Microbiome Insulin Sensitivity Study (MISS, NCT03120871, PI: MM Kelsey\textsuperscript{20}).

For each of the studies, participants had at least 2 visits: one for consent and screening (eligibility labs and history) and one for a metabolic and hormonal visit. A physical was performed by a pediatric endocrinologist (MCG, CLC, MMK or KJN). Hirsutism was assessed with the modified Ferriman-Gallwey score (FGS) and acne graded in severity from none, mild (comedones or small pustules), moderate (pustules and some inflammation) or severe (nodulcytic). Blood pressure was measured after at least 5 minutes of rest with the appropriately sized cuff. Weight was measured without shoes in light clothes and height
assessed with a calibrated stadiometer. Fasting laboratory measures included hormones (estradiol, progesterone, testosterone, free testosterone, sex hormone binding-globulin (SHBG), DHEA-S) and metabolic measures (glucose, insulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lipid panel, HbA1c). Insulin and glucose were also frequently sampled during a 2-hour oral glucose tolerance test in AIRS, PLUM and APPLE. Hepatic fat percentage was assessed via MRI proton density fat fraction in AIRS, PLUM and APPLE participants, as previously described.\textsuperscript{4,14}

Eleven-oxyandrogen concentrations were measured from frozen serum samples by LabCorp Esoterix (Calabasas, CA) via liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis. Other measurements were performed as previously described.\textsuperscript{4,14}

Briefly, total testosterone was analyzed using liquid chromatography–tandem mass spectrometry and free testosterone with equilibrium dialysis (Esoterix, Calabasas Hills, CA); DHEA-S and SHBG by electrochemiluminescence immunoassay by Esoterix (Calabasas Hills, CA); estradiol and progesterone via chemiluminescent immunoassay (Beckman Coulter, Brea, CA); AST and ALT were measured on a Vitros® 5600 (Ortho Clinical Diagnostics, Rochester, NY). Serum insulin was analyzed with RIA (Millipore, Billerica, MA); FFA (Wako Chemicals, Inc., Richmond, VA) were assessed enzymatically. HbA1c was measured by DCCT-calibrated ion-exchange HPLC (Bio-Rad Laboratories, Hercules, Calif).

Total cholesterol, high density lipoprotein cholesterol (HDL-C), and triglyceride assays were performed enzymatically on a Hitachi 917 autoanalyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN). Low density lipoprotein cholesterol (LDL-C) concentrations were calculated by the Friedewald equation.

Calculations performed included the free androgen index, calculated from total testosterone and SHBG\textsuperscript{2}. This formula was also used to calculate a “free 11-oxyandrogen index” for each of the 3 metabolites. Measures of insulin sensitivity included Homeostatic
Model Assessment for Insulin Resistance (HOMA-IR) calculated as fasting plasma glucose [mg/dL] x fasting plasma insulin [mg/dL] / 405\textsuperscript{14}, the Matsuda index as 10000/(sqrt(fasting glucose*fasting insulin*mean glucose*mean insulin))\textsuperscript{21}, and the oral minimal model calculated with a 2-hour exponential model utilizing SAAM II software (SAAM II software v 2.2, The Epsilon group, Charlottesville, VA, USA)\textsuperscript{22}.

The distributions of all variables were examined prior to analysis. Descriptive statistics included means and standard deviations if normally distributed, or medians and 25\textsuperscript{th}, 75\textsuperscript{th} percentile for non-normally distributed data. T-tests or Wilcoxon rank-sum tests were performed to compare groups, and multiple linear regression was performed to control for the effect of obesity in the androgens only. Associations were evaluated via simple linear regression. Logistic regression and Receiver Operator Curve (ROC) analysis was used to evaluate sensitivity and specificity of androgens for PCOS status using the Youden Index as a cutoff (sensitivity+ Sensitivity -1). ANOVA was utilized to compare differences in outcomes between untreated PCOS and the two treatment groups, and not controlled for BMI, as it was similar between the groups. All analysis was performed in GraphPad Prism 9.1.0 (San Diego, CA).

Results

Two-hundred participants had samples available for analysis. This included 115 with untreated PCOS (13 lean [BMI = 22.6 ± 2.2 kg/m\textsuperscript{2}] and 102 with overweight/obesity [BMI = 35.4 ± 5.3]), 70 controls (18 normal weight [BMI = 20.2 ± 1.4], 52 with obesity [BMI=35.5 ± 10.4]), 9 girls with PCOS and obesity (BMI=33.7 ± 5.7) treated with COCPs, and 6 girls with PCOS and obesity (BMI=36.4 ± 5.3) treated with metformin. Demographic and physical exam data for the untreated PCOS and control groups are shown in Table 1. The untreated PCOS group was older and had higher BMI percentiles (p<0.001), as well as more participants in the category of obesity (p=0.24). As
expected, FGS hirsutism scores were significantly higher in the PCOS cohort (p<0.001). Laboratory data for the untreated PCOS and control groups are shown in Table 2. The medians of total testosterone, (p<0.001), free androgen index (p<0.001), and free testosterone (p<0.001) were significantly higher in those with untreated PCOS. Two of the 11-oxyandrogens, 11OHA4 (p=0.003) and 11OHT (p<0.001) were significantly higher in PCOS, with data graphed in Figure 1. When these comparisons were adjusted for obesity, the following results were found: total testosterone, (p<0.001 for PCOS, β=18.4±3.3 ng/dL), free testosterone (p<0.001 for PCOS, β=5.38±0.78, pg/mL), FAI (p<0.001 for PCOS, β=6.1±1.0, no units), 11KT (p=0.262 for PCOS, β=3.2±2.8 ng/dL), 11OHA (p=0.018 for PCOS, β=28.5±12.0, ng/dL), 11OHT (p=0.118 for PCOS, β=2.0±1.3, ng/DL). However, there was no difference between PCOS and controls for 11KT. Table 2 also shows descriptive data for other comorbidity measures that are associated with PCOS. Significant findings between the two groups include SHBG, FAI, cholesterol, LDL, triglycerides, HbA1c, 2-hour glucose, fasting insulin, and 2-hours insulin, ALT, AST. There were no significant differences in hypertension measures.

ROC analysis was performed to determine sensitivity and specificity of the various androgen measures for diagnosing PCOS and are shown in Figure 2. As expected, total testosterone (Figure 2A, sensitivity 79%, specificity, at Youden index, AUC=0.840), free testosterone (Figure 2C, sensitivity 85%, specificity 78%, AUC=0.869) and calculated free androgen index (Figure 2E, sensitivity 83%, specificity 78%, AUC=0.865) performed well for diagnosing PCOS (AUC>0.84 for all). 11KT did not perform well at identifying individuals with PCOS (AUC=0.519). The Youden index for 11OHA4 for PCOS (Figure 2D, sensitivity 75%, specificity 31%, AUC=0.620) and 11OHT (Figure 2F, sensitivity 69%, specificity 51%, AUC=0.638), respectively, were not as strong as testosterone or free androgen index.

Since FGS hirsutism score and insulin sensitivity (HOMA-IR), were highly different between PCOS and controls and of clinical importance, we examined the relationship between these two measures with 11-oxyandrogens and free testosterone (Figure 3). There was a significant correlation.
between all three 11-oxyandrogens ([11OHA4, p=0.030], [11OHT, p=0.013], [11KT, p=0.008], free testosterone (p=0.007) and FGS score. Whereas there was a strong relationship between higher free testosterone and worse HOMA-IR, there was no relationship between HOMA-IR and any of the 11-oxyandrogens. We also calculated a “free 11-oxyandrogen index” for each metabolite, to determine if there was role of SHBG in altering the measurement of the androgens and affecting correlations. We did not find that any of the correlations improved with a calculated free index (data not shown).

Demographic information for participants on pharmaceutical medical treatment (COCPs or metformin) for PCOS are shown in Table 3. Hirsutism and SHBG scores were statistically different (p=0.007 and p<0.001), which was expected as COCPs are known for improving excess hair growth and increasing SHBG to bind testosterone. Figure 4 demonstrates the differences between the untreated group and the two treatment arms. If initial overall ANOVA was significant, then pairwise comparisons are shown on the graph. Between the untreated PCOS group and the COCP-treated group, there was a statistical decrease in total testosterone (Median difference = -17.0 ng/dL, p=0.006), FAI (Median difference = -6.3 units, p<0.001), free testosterone (Median difference = -5.6 units, p<0.001), and 11KT (Median difference = -7.4 units, p=0.040). Statistical differences between the untreated PCOS group and the metformin-treated group were seen in total testosterone (Median difference = -15.0 ng/dL, p=0.011), and in FAI between metformin and COCPs (Mean difference = -7.6, p=0.002).

Discussion:

We found that adolescent girls with PCOS had higher 11OHA4 and 11OHT values than non-PCOS controls. However, 11-oxyandrogens did not appear to have any clinical utility when it came to aiding in the diagnosis of PCOS compared to serum testosterone, determined by the statistical relationship shown in the ROC analysis. While 11-oxyandrogens correlated with hirsutism severity, they did not correlate well with many measures of dysmetabolism. An interesting finding was that COCPs did lower androgens and testosterone concentrations, but not androstenedione (Figure 3).
These findings further strengthen the argument that ovarian testosterone, not androgenic testosterone derivatives, are the primary source of excess androgens in PCOS. The data in this study suggest that 11-oxyandrogen production does correlate with excess hair growth in PCOS but does not seem to be a driving force behind PCOS or aid in diagnosis.

Results from existing studies that look at 11-oxyandrogen concentrations in PCOS vary greatly, especially between patient populations. Our data are consistent with Pretorius et al. in regards to 11OHA4 being elevated in PCOS and relating to hyperandrogenic signs associated with PCOS. The association of 11-oxyandrogens and dermatological manifestations of androgen excess, namely hirsutism, are consistent with findings from Hudson et al on the clinical role of 11-oxyandrogens. Yoshida et al. found that, in a cohort of 28 participants with PCOS, 4 had increased serum concentrations of 11-oxyandrogens but normal levels of testosterone, and 20 had increased serum concentrations of testosterone, indicating that both play a significant role in the phenotype of PCOS. These combined results suggest that 11-oxygenated steroids are unlikely to be of high utility as clinical biomarkers in PCOS.

As PCOS first manifests during puberty, it is important to understand at what age and stage in development 11-oxyandrogens begin to increase, and in what settings these may be altered. Holownia et al. found that the rise in concentrations of 11OHA4 in children appears to reflect three phenomena: the maturation of the adrenal gland in adrenarche, progression through puberty shown by steadily increasing levels of DHEAS with a sharp rise in estradiol in girls, and, lastly, stress which is likely the cause of the wide variation of concentrations with chronological age. Abnormalities are noted with premature adrenarche, including a 3.5-fold elevation in 11OHA4, 11OHT and 11KT than testosterone in girls with premature adrenarche. Davio et al. found that 11KT is the dominant circulating bioactive androgen during normal and premature adrenarche and that concentrations do not seem to decline in women as they age. Ovarian-derived testosterone is higher in peripubertal girls with obesity. However, in premenarchal daughters of women with
PCOS, 11-oxyandrogens were not elevated relative to age-matched controls\(^9\). Our results in older adolescents were in direct contrast to O’Reilly et al. who found that serum concentrations of 11KT were significantly higher than those of testosterone in women with PCOS and in female controls\(^9\). We speculate that 11KT concentrations may be lower in adolescents with PCOS due to incomplete zona reticularis growth with submaximal adrenal hormone release.

Some of the discrepancy in findings regarding 11-oxyandrogens in health and disease may be due to measurement methodology. LC-MS/MS allows for accurate and simultaneous measurement of multiple adrenal and gonadal steroids\(^26\). However, even with an accurate and reliable assay, the interpretation of androgens can be confounded by alterations in SHBG concentrations and diurnal variation. As androgens peak in the morning, morning measurement is preferable and is standard for our protocols (6-8 AM). Some of the variability in results across studies may be explained by lack of standardization in timing of the androgen blood draw. Of greater importance is the effect of SHBG on total testosterone concentrations — roughly 65% of circulating testosterone is bound to SHBG\(^27\). Women with low SHBG and PCOS can have normal total testosterone levels, but elevated bioavailable circulating testosterone, and this can lead to a PCOS diagnosis potentially being missed\(^27\). Indeed, the calculated FAI, or free testosterone, measured by equilibrium dialysis in our study had greater associations with PCOS clinical findings, hirsutism and metabolic measures\(^26\). However, unlike testosterone, when we calculated a free 11-oxyandrogen index for each of the three 11-oxyandrogen metabolites, it did not change the diagnostic utility or relationship to metabolic disease. Further, in pre-pubertal girls, 11KT concentrations did not relate to SHBG\(^9\). The combination of these findings suggests that SHBG may not be as important for these androgen metabolites, and that uncorrected “total” values can be measured.

While the role of COCPs and metformin in treating hormonal and metabolic abnormalities in PCOS is well established, knowledge surrounding the effect of treatments on adrenal and 11-oxyandrogens is limited. Kjøtrød et al. found that during 12-14 weeks of metformin pretreatment for
women with PCOS about to undergo fertility treatments, DHEAS levels increased in the metformin group compared to controls, whereas other adrenal androgens such as DHEA and androstenedione were unaffected. In our small group of participants on treatment, we found a slight decrease in total and free testosterone, but no changes in other measures with metformin. It is known that COCPs decrease testosterone production and also decrease bioavailable testosterone by increasing SHBG, consistent with our results. We were unable to find any published information on COCPs and 11-oxyandrogens, although COCPs have been shown to decrease other adrenal androgens, such as DHEAS. We did find that 11KT was significantly lower and 11OHA4 and 11OHT were non-significantly slightly lower, in those treated with a COCP. These findings support the data in this study that while COCPs are of further interest in studying to determine their exact relationship with 11-oxyandrogens in perhaps adrenal disease, particularly 11-KT, metformin has little effect on 11-oxyandrogens, where adrenal DHEAS was similarly unaffected by metformin.

It is estimated that 20-30% of women with PCOS have increased adrenal androgens. For example, androgen levels remain higher in PCOS patients compared to healthy women even after ovarian suppression with GnRH agonists. However, Moran et al. report that in normal adult women, testosterone is derived 25% from ovaries, 25% from the adrenals, and 50% from peripheral conversion of androstenedione, and in hirsute women with PCOS the predominant source of androgen overproduction is primarily ovarian. This is further supported by findings that adrenal and ovarian stimulation data do not fully account for endogenous testosterone or 11-KT observed in girls with hyperandrogenism and suggests the possibility of peripheral production via AKR1c3 in adipose tissue. Alpañés et al. also noted that adrenal hyperandrogenism in patients with PCOS ranged from 20-60% in early studies depending on the definition used, age, BMI, and race. Our results support that the primary source of androgens that affect adolescents with PCOS is the ovaries, but a more diverse group of participants may lead to different conclusions.
We found a relationship between adrenal androgens and hirsutism, but not acne nor acanthosis (Figure 4). This finding is consistent with the argument by Hudson et al. that 11OHA4 can be used as a marker to assess the adrenal contribution to hirsutism. However, the 11-oxyandrogens correlated with so few metabolic measures that they likely do not have a role in the manifestation of dysmetabolism in PCOS.

While this study did have many strengths, further investigation is needed on the topic of 11-oxyandrogens and their importance in PCOS. Highlights of this study include a large adolescent sample size of varied weight, with limited data on the role of the two most common treatments for PCOS, metformin and COCPs. Although 11-oxyandrogens were not better than testosterone in diagnosing PCOS, this is confounded by the fact that testosterone is part of the diagnosis of PCOS. This study was cross-sectional and longitudinal data are needed. Furthermore, our racial and ethnic demographics reflect that of the local community of Colorado, and a broader, more diverse sample would be needed to determine if heritage affects 11-oxyandrogen concentrations.

Understanding the role 11-oxyandrogens play in androgen disorders is complex. We did not find that 11-oxyandrogens aid in diagnosing of PCOS, and they did not relate to measures of metabolic disease. However, they do relate to clinical signs of hyperandrogenism, and should perhaps be considered in the work-up of hirsutism. Metformin does not seem to have a significant effect on these adrenal androgens and effects of COCPs appear to be modest, though longitudinal randomized studies would be needed to confirm this hypothesis. Finally, these results confirm that ovarian androgen excess is the prevalent contributor to clinical features of PCOS, even in adolescents with pubertal adrenal stimulation.
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Data Availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.
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Figure titles

Figure 1: Androgen concentration distributions in untreated PCOS compared to non-PCOS controls.

Figure 2: Predictive value of different androgen concentrations for PCOS diagnosis

Figure 3: Correlations for androgen, metabolic and dermatological measures

Figure 4: Androgen concentration distributions in untreated PCOS compared PCOS treated with metformin and COCPs

Figure legends

Figure 1. Individual points, means and 25th and 75th percentile bars are shown for each of the androgen concentrations. Statistical analysis is controlled for obesity status.

Figure 2: Receiver operator curves of each of the androgen concentrations as relates to PCOS diagnosis are shown. P-values are from unadjusted comparisons.

Figure 3: Individual points, lines of best fit of each of the androgen concentrations related to FGS score and HOMA-IR calculated value.

Figure 4: Individual points, means and 25th and 75th percentile bars are shown for each of the androgen concentrations. The overall ANOVA is reported if non-significant, and if significant, only significant pairwise p-values are shown.
Table 1. Demographic variables and physical exam findings for participants with and without PCOS

|                      | Untreated PCOS (n=115) | Controls (n=70) | P-value |
|----------------------|------------------------|-----------------|---------|
| **Age (years)**      | 16 (15, 17)            | 14 (13, 16)     | <0.001  |
| **Race, n(%)**       |                        |                 |         |
| Caucasian            | 98 (85)                | 46 (73)         |         |
| Black                | 15 (13)                | 15 (23)         | 0.146   |
| Asian                | 2 (2)                  | 1 (2)           |         |
| More than one race   | 0                      | 1 (2)           |         |
| **Hispanic Ethnicity, n(%)** |                |                 | 0.447   |
|                      | 61 (53)                | 31 (46)         |         |
| **BMI (kg/m²)**      | 34.3 (30.2, 37.8)      | 30.9 (22.4, 36.1)| 0.071   |
| **BMI%ile**          | 98.1 (96.0, 99.0)      | 96.7 (67.0, 98.6)| <0.001  |
| Normal BMI (BMI%ile <90, N (%) ) |        |                 | 0.024   |
|                      | 13 (11)                | 18 (26)         |         |
| **Menarche**         | 12 (11, 12)            | 11 (11, 12)     | 0.172   |
| **Frequency of menses n (%)** |                |                 |         |
| 4-6 weeks            | 3 (3)                  | 34 (92)         |         |
| 1-2 months           | 33 (30)                | 1 (3)           |         |
| 3-6 months           | 41 (37)                | 2 (5)           | <0.001  |
| 7-12 months          | 22 (20)                | 0               |         |
| >1year               | 11 (10)                | 0               |         |
| **Hirsutism FGS Score** | 6.0 (3.0, 11.0)        | 0 (0, 3.0)      | <0.001  |
| **Acne Severity n(%)** |                    |                 |         |
|     |     |     |
|-----|-----|-----|
| none | 8 (7)| 18 (50) |
| mild | 52 (46)| 11 (31) |
| moderate | 45 (40)| 6 (17) |
| severe | 8 (7)| 1 (2) |

Data are expressed as Median (25th, 75th percentile); mean ± standard deviation; or number (% of total); Includes all study controls; \(^2\)Missing CF study values, n=6; \(^3\)Missing HIP study values, n=12; \(^4\)Missing MISS data, n=17, \(^5\)Missing CF study values, HIP values, and MISS values, n=35.
Table 2. Biochemical measures for participants with and without PCOS

|                      | Untreated PCOS (n=115) | Controls (n=70) | P value |
|----------------------|------------------------|-----------------|---------|
| **Androgens**        |                        |                 |         |
| Total Testosterone (ng/dL) | 43.0 (33.0, 56.0) | 25.0 (20.0, 34.5) | <0.001  |
| Free Testosterone (pg/mL) | 7.8 (5.4, 11.0)  | 3.6 (2.9, 4.7)  | <0.001  |
| DHEAS (ug/dL)        | 196 (125, 311)         | 147 (75, 263)   | 0.061   |
| Progesterone (ng/mL) | 0.80 (0.50, 1.20)      | 0.80 (0.60, 1.27)| 0.007   |
| Estradiol (pg/mL)    | 45.0 (35.0, 56.0)      | 53.0 (31.5, 91.5)| 0.002   |
| SHBG (nmol/L)        | 19.0 (13.4, 27.1)      | 27.2 (20.9, 40.5)| 0.001   |
| FAI                   | 7.3 (5.1, 11.5)         | 3.4 (2.6, 4.5)  | <0.001  |
| **11-OH Androgens**  |                        |                 |         |
| 11-KT (ng/dL)        | 25.5 (17.8, 42.5)      | 26.3 (17.1, 36.6) | 0.745   |
| 11-OHA4 (ng/dL)      | 117 (68, 179)          | 88 (62, 123)    | 0.003   |
| 11-OHT (ng/dL)       | 12.7 (7.8, 16.9)       | 8.9 (6.3, 13.2) | 0.001   |
| **Lipids**           |                        |                 |         |
| Cholesterol (mg/dL)  | 151 (129, 175)         | 133 (121, 148)  | 0.003   |
| LDL (mg/dL)          | 88.0 (69.5, 116.5)     | 78.6 (67.0, 89.8)| 0.008   |
| HDL (mg/dL)          | 37.0 (32.0, 44.0)      | 38.0 (32.6, 47.0)| 0.052   |
| Triglycerides (mg/dL)| 109 (77, 150)          | 89 (70, 118)    | 0.016   |
| **Glycemia**         |                        |                 |         |
| HbA1c (%)            | 5.3 (5.2, 5.6)         | 5.2 (5.1, 5.5)  | 0.060   |
| Fasting Glucose (mg/dL)† | 88 ± 8                | 87 ± 5          | 0.977   |
| 2-hour Glucose (mg/dL)‡ | 132 ± 25              | 114 ± 29        | 0.002   |

**Insulin Resistance**
|                        | Median 25th, 75th percentile | Mean ± Standard Deviation | Number (%) |
|------------------------|-----------------------------|----------------------------|------------|
| Fasting Insulin (mIU/L)†‡ | 21 (15, 31)                  | 16 (10, 22)                 | 0.009      |
| 2-hour Insulin (mIU/L)†‡ | 172 (98, 338)               | 105 (21, 189)               | 0.001      |
| Peak Insulin(mIU/L)†‡    | 250 (112, 427)              | 210 (142, 330)              | 0.294      |
| HOMA-IR‡                 | 5.0 (3.3, 7.1)              | 4.2 (3.4, 5.2)              | 0.033      |
| Matsuda‡                 | 1.6 (0.9, 2.1)              | 1.8 (1.5, 2.1)              | 0.343      |
| OMM‡                    | 1.5x10^{-4} (8.4x10^{-5}, 3.3x10^{-4}) | 2.2x10^{-4} (1.4x10^{-4}, 4.0x10^{-4}) | 0.543      |
| Hypertension            |                             |                            |            |
| Systolic BP (mmHg)‡      | 121 ± 12                    | 121 ± 12                    | 0.859      |
| Diastolic BP (mmHg)‡     | 66 (63, 72)                 | 66 (61, 72)                 | 0.652      |
| Fatty Liver Disease      |                             |                            |            |
| ALT (IU/L)‡              | 34 (28, 42)                 | 28 (24, 35)                 | 0.002      |
| AST (IU/L)‡              | 38 (32, 46)                 | 34 (29, 40)                 | 0.017      |
| % Liver Fat‡             | 4.5 (2.5, 8.7)              | 3.2 (2.6, 5.8)              | 0.102      |
| Hepatic Steatosis n (%)‡ | 53 (47)                     | 9 (25)                      | 0.0182     |

Data are expressed as Median (25th, 75th percentile); mean ± standard deviation; or number(% of total); †=10 exenatide participants excluded; ‡Has all study controls; †‡Missing CF study values, n=6; §Missing HIP study values, n=12; ¶Missing MISS data, n=17; §‡Missing CF study values, HIP values, and MISS values n=35
Table 3. Demographic variables and physical exam findings for participants with untreated and pharmaceutically treated PCOS

|                      | Untreated PCOS (N=115) | COCP (N=9) | Metformin (N=6) | P-value |
|----------------------|-------------------------|------------|-----------------|---------|
| **Age (years)**      | 15 ± 2                  | 15 ± 1     | 15 ± 1          | 0.845   |
| **Race, n(%)**       |                         |            |                 |         |
| Caucasian            | 98 (85)                 | 9 (100)    | 6 (100)         | 0.636   |
| Black                | 15 (13)                 | 0          | 0               |         |
| Asian                | 2 (2)                   | 0          | 0               |         |
| More than one race   | 0                       | 0          | 0               |         |
| **Hispanic Ethnicity, n(%)** |                 |            |                 |         |
|                      | 61 (53)                 | 6 (67)     | 4 (67)          | 0.723   |
| **BMI (kg/m²)**      | 33.9 ± 6.4              | 33.7 ± 5.7 | 36.4 ± 5.3      | 0.621   |
| **BMI%ile**          | 94.1 ± 12.2             | 97.1 ± 1.5 | 98.5 ± 0.6      | 0.454   |
| **Menarche (years)** | 12 ± 2                  | 12 ± 1     | 11± 1           | 0.291   |
| **Duration of menses** |                         |            |                 | <0.001  |
| Normal               | 3 (3)                   | 8 (88)     | 1 (17)          |         |
| 1-2 months           | 33 (30)                 | 0          | 3 (50)          |         |
| 3-6 months           | 41 (37)                 | 0          | 1 (17)          |         |
| 7-12 months          | 22 (20)                 | 1 (13)     | 1 (17)          |         |
| >1year               | 11 (10)                 | 0          | 0               |         |
| **Hirsutism**        | 6.0 (3.0, 11.0)         | 3.0 (0.5, 5.5) | 1.5 (0.8, 5.5) | 0.007   |
| **Acne Severity n(%)** |                         |            |                 |         |
| none                 | 8 (7)                   | 2 (22)     | 1 (17)          | 0.245   |
| mild                 | 52 (46)                 | 4 (45)     | 5 (83)          |         |
| moderate             | 45 (40)                 | 3 (33)     | 0               |         |
| severe               | 8 (7)                   | 0          | 0               |         |
|                   | Median (25\(^{th}\), 75\(^{th}\) percentile) | Mean ± Standard deviation | Number (% of total) |
|-------------------|---------------------------------------------|--------------------------|---------------------|
| **DHEA-S**        | 226.0 ± 135.4                               | 209.6 ± 60.1             | 142.0 ± 60.8        | 0.263              |
| **Progesterone**  | 0.99 ± 0.86                                 | 1.16 ± 0.61              | 1.77 ± 3.06         | 0.240              |
| **Estradiol**     | 50.7 ± 30.8                                 | 46.4 ± 49.4              | 72.3 ± 55.2         | 0.153              |
| **SHBG**          | 19.0 (13.4, 27.1)                           | 102.2 (65.5, 123.3)      | 12.0 (8.2, 16.4)    | <0.001             |

Data are expressed as Median (25\(^{th}\), 75\(^{th}\) percentile); mean ± Standard deviation; or number (% of total); \(^x\)

Missing MISS PCOS values, n=2.
Figure 1

- Total Testosterone
  - PCOS
  - Controls
  - p<0.001

- 11-Ketotestosterone
  - PCOS
  - Controls
  - p=0.745

- Free Testosterone
  - PCOS
  - Controls
  - p<0.001

- 11-Hydroxyandrostenedione
  - PCOS
  - Controls
  - p=0.003

- Free Androgen Index
  - PCOS
  - Controls
  - p<0.001

- 11-Hydroxytestosterone
  - PCOS
  - Controls
  - p=0.005
Figure 2

- **Total Testosterone**
  - AUC = 0.840
  - p < 0.001

- **11-Ketotestosterone**
  - AUC = 0.519
  - p = 0.67

- **Free Testosterone**
  - AUC = 0.869
  - p < 0.001

- **11-Hydroxyandrostenedione**
  - AUC = 0.620
  - p = 0.006

- **Free Androgen Index**
  - AUC = 0.865
  - p < 0.001

- **11-Hydroxytestosterone**
  - AUC = 0.638
  - p = 0.002
Figure 3

1. FGS vs. Free Testosterone
   - \( p = 0.007 \)
   - \( r^2 = 0.066 \)

2. FGS vs. 11-Hydroxyandrostenedione
   - \( p = 0.042 \)
   - \( r^2 = 0.030 \)

3. FGS vs. 11-Ketotestosterone
   - \( p = 0.007 \)
   - \( r^2 = 0.063 \)

4. FGS vs. 11-Hydroxytestosterone
   - \( p = 0.013 \)
   - \( r^2 = 0.054 \)

5. HOMA-IR vs. Free Testosterone
   - \( p < 0.001 \)
   - \( r^2 = 0.271 \)

6. HOMA-IR vs. 11-Hydroxyandrostenedione
   - \( p = 0.120 \)
   - \( r^2 = 0.024 \)

7. HOMA-IR vs. 11-Ketotestosterone
   - \( p = 0.030 \)
   - \( r^2 = 0.042 \)

8. HOMA-IR vs. 11-Hydroxytestosterone
   - \( p = 0.314 \)
   - \( r^2 = 0.020 \)
