Short-Term Fasting Attenuates Overall Steroid Hormone Biosynthesis in Healthy Young Women

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Abstract

Context: Fasting is stressful for the human body. It is managed by metabolic adaptations maintaining energy homeostasis and involves steroid hormone biosynthesis, but the exact interplay between energy and steroid metabolism remains elusive. Women with polycystic ovary syndrome (PCOS) suffer from disturbed metabolism and androgen excess, while in women with anorexia nervosa, cortisol and androgen production are decreased. By contrast, starvation of steroidogenic cells shifts adrenal steroid biosynthesis toward enhanced androgen production.

Aim: This study investigated the effect of fasting on steroid production in healthy women.

Methods: Twenty healthy young women were studied after fasting for 48 hours; steroid profiles from plasma and urine samples were assessed at baseline, after fasting, and 48 hours after fasting. Gas chromatography–mass spectrometry (GC-MS) and liquid chromatography–mass spectrometry (LC-MS) were used to quantify steroid substrates.

Results: Fasting did not change overall steroidogenesis, although it increased progestogen production and lowered relative mineralocorticoid, glucocorticoid, androgen production. The decrease in urine metabolites was seen for cortisol and androstenedione, while higher levels were found for pregnanediol in urine and progesterone and aldosterone in serum. Activity of 17β-hydroxycorticosteroids was decreased after fasting in healthy women as were 21-hydroxylase (CYP21A2) and 5α-reductase activities. By contrast, hydroxysteroid 11-beta dehydrogenase 1 (HSD11B1) activity for cortisol inactivation seemed to increase with fasting. Levels of testosterone (TST), tetrahydrocortisone (THE), and tetrahydrocortisol (THF) were decreased. By contrast, starvation of steroidogenic cells shifts adrenal steroid biosynthesis toward enhanced androgen production.

Conclusion: Women with PCOS and healthy women showed significant changes in steroid metabolism after fasting. In contrast to metabolic changes seen at baseline in PCOS women compared to healthy women, and after starving of steroidogenic cells, no androgen excess was observed after short-term fasting in healthy young women.

Key Words: fasting, energy homeostasis, steroid metabolism, androgen metabolism

Acronyms: 

- 11OXOET: 11-oxo-etiocholanolone
- 11βOHAT: 11β-hydroxyandrostenedione
- 11βOHET: 11β-hydroxyetiocholanolone
- 16αOHDHEA: 16α-hydroxydehydroepiandrosterone
- A4: androstenedione
- AKR1C1: aldo-keto reductase family 1 member C1
- AKR1C2: aldo-keto reductase family 1 member C2
- AOR: aldolase
- BMI: body mass index
- CYP1A1: cytochrome P450 1A1
- CYP1B1: cytochrome P450 1B1
- CYP17A1: cytochrome P450 17α-hydroxylase/17,20-lyase
- DHEA: dehydroepiandrosterone
- DHEAS: dehydroepiandrosteronesulfate
- DHT: dihydrotestosterone
- E2: estradiol
- E1: estrone
- E3: estriol
- ERK: extracellular signal-regulated kinase
- FSH: follicle stimulating hormone
- GC-MS: gas chromatography–mass spectrometry
- HOMA-IR: homeostatic model assessment for insulin resistance
- HSD11B1: hydroxysteroid 11-beta dehydrogenase type 1
- IGF-1: insulin-like growth factor 1
- LC-MS: liquid chromatography–mass spectrometry
- MAPK: mitogen-activated protein kinase
- MCR: multiple compared risk
- NAD: nicotinamide adenine dinucleotide
- PCOS: polycystic ovary syndrome
- PD: pregnanediol
- PREG: pregnenolone
- PTONE: pregnanetriol
- PTH: parathyroid hormone
- PTH: parathyroid hormone
- PTONE: pregnanetriol
- SDS: standard deviation score
- SRD5A1, SRD5A2: steroid 5α-reductase type 1, 2
- TST: testosterone
- UHPLC: ultra-high-performance liquid chromatography

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(NAD), a cofactor involved in a variety of oxidation-reduction enzymatic reactions important for glycolysis, fatty acid oxidation, the Krebs cycle, and the function of complex I of the mitochondrial respiratory chain [3, 4]. Likewise, several enzymes of steroid hormone biosynthesis use NAD as an electron source for their catalytic reactions [5]. But the redox state of NAD is also the main regulator of sirtuins, protein deacetylases, and ADP-ribosyltransferases that directly link cellular metabolic signaling to the state of protein posttranslational modifications [6].

Alterations of steroid metabolism have been reported for several disorders affecting energy homeostasis, including anorexia nervosa [7], obesity [8], and thyroid disorders [9]. In particular, it was observed that chronic fasting in young adult women with anorexia nervosa led to a significant decrease of urinary cortisol (F) and total androgen metabolites compared with healthy controls, and these changes were reversible upon refeeding [7]. Women with polycystic ovary syndrome (PCOS) also typically present with an altered steroid profile (eg, hyperandrogenism), and in most cases with obesity and disturbed glucose homeostasis due to insulin resistance [10]. Clinical studies show that moderate weight loss improves both insulin resistance and androgen excess in women with PCOS [11, 12]. However, the exact interrelationship between the steroid hormone biosynthesis, the metabolism, and the energy balance is poorly understood. Metformin has shown an effect on glucose as well as androgen metabolism in several clinical and basic studies [13]. When studying the underlying mechanism of action of this effect in vitro, it was found that complex I of the respiratory chain is targeted by metformin [13]. In addition, steroidogenic adrenocortical NCI-H295R cells cultured in starved condition were found to undergo a marked change in steroid metabolism with a shift toward enhanced androgen production proven by a 50% increase in androstenedione (A4) and nearly doubled activity of the 17,20-lyase activity [14].

Even mechanisms underlying the effect of fasting on steroidogenesis in healthy individuals are incompletely studied. The abovementioned metabolic changes relate to short-term fasting, which is defined by a duration of 2 to 4 days in most studies. Short-term fasting has been suggested to decrease the negative feedback of insulin and leptin on the central nervous system with a decrease of leptin by 75%. This leads to a stimulation of the hypothalamic-pituitary-adrenal axis and thus an increase in F levels by 1.5-fold [9, 15, 16], indicating that there is an interconnection between adrenal steroid biosynthesis and energy homeostasis.

The aim of this study was to investigate the effect of short-term fasting on steroidogenesis in healthy young women to reconcile the differences shown in previous clinical studies of abnormal metabolic states, like anorexia nervosa or PCOS, vs in vitro data. Specifically, we assessed whether short-term fasting in vivo would also lead to an increase in androgen production as observed in vitro. In a prospective study, healthy young women fasted for 48 hours and their urine and plasma steroid metabolomes were assessed at 3 timepoints.

**Methods**

**Subjects**

Informed consent was obtained from all subjects involved in the study. Twenty healthy young women participated in this prospective study. They provided written informed consent. The study was approved by the Ethics Board of the Canton Bern, Switzerland (BASEC ID 2017_00982). Study subjects were recruited between October 2018 and June 2019 in the neighborhood of the University Hospital Berne, Switzerland, through online and blackboard advertisement. In total, 43 women were screened, while 20 women were finally included and completed the study.

**Study Procedures**

Inclusion criteria were a) age between 16 and 35 years; b) normal adult sexual maturation; c) regular menstrual cycles (25-31 days); and d) normal body mass index (BMI; 19.0-30.0 kg/m²). Exclusion criteria were a) chronic diseases; b) ongoing drug treatment and hormonal (including contraception) treatment in the 3 months prior to study participation; c) infertility, pregnancy (current and in the year prior to participation), ongoing breastfeeding; and d) intercurrent illness at or within 1 week before study participation.

After successful screening, each participant was instructed individually to perform the 3-day study during the luteal phase of their regular menstrual cycle (days 14-21). The nutritional intake during the short-term fasting was limited to a maximum of 500 kcal/day (≤ 60 kg) or to a maximum of 800 kcal/day (> 60 kg). A dietary plan was provided. Measures of plasma free fatty acids and diaries with self-reported daily nutritional intake served to monitor compliance of fasting [17]. We instructed participants to collect urine over 24 hours. Each participant collected 3 separate 24-hour urine samples and gave 3 blood samples (at 0, 24, and 48 hours; see also Fig. 1). One participant was partially excluded from analysis due to incomplete sampling.

At visit 1, clinical characteristics of participants were assessed, including height (cm), weight (kg), waist circumference (cm), and the Ferriman-Gallwey score [18] (see Table 1), and a pregnancy test was performed. Additional laboratory tests from serum or plasma were done as detailed in Supplemental Table S1 [19]. All laboratory analyses were assessed in the accredited laboratories of the Institute of Clinical Chemistry and the Steroid Laboratory of Nephrology of the Inselspital (University Hospital of Berne, Switzerland).

**Laboratory Analyses**

Samples were stored at -20 °C before assessing the steroid profiles with in-house methods of gas chromatography–mass spectrometry (GC-MS) and liquid chromatography–mass spectrometry (LC-MS) [20]. In brief, steroid metabolites were extracted from urine using solid phase extraction, followed by enzymatic hydrolysis and derivatization. GC-MS measurements were performed in selected ion monitoring mode (Agilent 7890A gas chromatograph coupled to an Agilent 5977 mass spectrometer; Agilent Technologies, Switzerland) using an Agilent HP1-MS column (15 m × 0.25 mm). Plasma steroid profiles were assessed by LC-MS using a Vanquish UHPLC to a QExactive Orbitrap Plus (both Thermo Fisher Scientific, Switzerland). Plasma samples were purified using solid phase extraction on an OasisPrime HLB 96-Well Plate (Waters, UK). Separation was achieved using an Acquity UPLC HSS T3 Column, 100A, 1.8 µm, 1 mm × 100 mm (Waters, UK). Mobile phases A and B consisted of water + 0.1 % formic acid and methanol + 0.1 % formic acid, respectively (all UPLC...
grade; Sigma-Aldrich, Switzerland). Analytes were eluted using a linear gradient from 46% to 73% B over 8 minutes. The mass spectrometer was operated in positive ion mode using an electrospray ionization source. Serum and urinary steroids are summarized with their respective abbreviation in Supplemental Table S2 [19].

**Statistical Analysis**

Clinical characteristics and steroid metabolite measurements were described with median and the 25th and 75th percentiles. We compared urinary metabolites and metabolite ratios between the 3 visits using Friedman tests. All tests were 2-sided and a *P* value < 0.05 was considered statistically significant. We used the statistical software Stata (Version 16, Stata corporation, Austin, Texas) for all analyses and RStudio (Version 1.1.383, Boston, Massachusetts) for creating the boxplots.

**Institutional Review Board Statement**

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Board of the Canton Bern, Switzerland (BASEC ID 2017_00982 on the 23. March 2018) and enlisted on clinicaltrials.gov (NCT03573063).

**Data Availability Statement**

Data supporting reported results are all given in Tables and Supporting Materials; if anything seems missing, it can be provided upon request.

**Results**

A total of 20 healthy young women were studied. Their median age was 26.3 years (range, 19.4-34.8 years), median BMI was 21.7 kg/m² (range 18.9-29.6 kg/m²). All participants had
normal natural menstrual cycles, were studied during the luteal phase of the cycle, and showed no clinical signs of hirsutism according to the Ferriman-Gallwey score (median score 2 points; range, 0-5). Detailed clinical characteristics describing the study participants are given in Table 1. Note that all subjects had normal glucose and insulin values at baseline and normal parameters reflecting the hypothalamic-pituitary-gonadal axis (Supplementary Table 1 [19]). As an expected result of fasting, plasma pH and free fatty acids increased markedly from baseline to 48 hours of fasting [17].

Results of the serum steroid profiling by LC-MS are shown in Table 2. A significant increase of progesterone and aldosterone after 48 hours of fasting was seen. A decrease of 11-deoxycortisol was also observed with fasting. By contrast, F and androgen metabolites showed no significant changes after 24 and 48 hours fasting. However, an overall significant increase of the sum of androgens was noted, while the sum of androgens showed a nonsignificant tendency to decrease.

Urinary steroid profiles at baseline and after 24 and 48 hours of fasting are summarized in Table 3. Corresponding to the increased plasma progesterone, fasting increased urinary excretion of pregnanediol (PD). By contrast, THE urine metabolite β-cortol, reflecting the glucocorticoid metabolism, decreased after 48 hours of fasting. Fasting also prompted a decrease in urine androgen metabolites including dehydroepiandrosterone (DHEA) and androstenediol (Δ5diol). However, no significant changes were observed when looking at the total sum of urinary steroid metabolites or the subgroups (Table 3).

To assess possible effects of fasting on specific steroid enzyme activities and pathways, we calculated specific substrate to product conversion ratios of metabolites using established formula from literature (Tables 4 and 5) [21]. These calculations revealed for serum and urine that fasting inhibited both activities of CYP17, for example, 17-hydroxylase and 17,20-lyase (Fig. 2A-2C). An inhibitory effect on CYP21A2 activity was also found when calculating from serum metabolites (Fig. 2D), but not from urine. In addition, significant changes in the ratio of etiocholanolone (ET) to androstenedione (AT) were observed indicating a reduced flux through alternative androgen-producing pathways with fasting likely due to inhibition of 5α-reductase activity necessary in all alternative androgen pathways (Fig. 2E). Finally, a change in F metabolism was noted, with fasting favoring the 20α- over the 20β-hydroxysteroid dehydrogenase pathway (Fig. 2F).

Overall, as a result of fasting, a reduced conversion of progestogens into all downstream pathways of mineralocorticoids, glucocorticoids, and androgens was observed (Table 4). Figure 3 provides a graphical summary of all steroids, enzymes, and pathways studied and gives an overview on changes found after 48 hours of fasting in healthy young women with respect to the classic steroid pathways as well as the alternative androgen-producing pathways.

**Discussion**

This prospective study was set up to enhance our understanding of the interplay between energy homeostasis and

| Steroid metabolites in serum (nmol/L) | Visit 1 | | Visit 2 | | Visit 3 | | P value |
|---------------------------------------|---------|---------|---------|---------|---------|---------|
|                                      | P 25    | P 50    | P 75    | P 25    | P 50    | P 75    | P value |
| Pregnenolone                          | 2.8     | 4.3     | 6.3     | 3.7     | 4.7     | 6.8     | 3.3     | 4.8     | 6.5     | 0.074   |
| Progesterone                          | 0.1     | 1.9     | 19.5    | 0.1     | 2.6     | 27.0    | 0.8     | 6.4     | 26.1    | 0.004   |
| 17α-Hydroxyprogesterone               | 1.9     | 3.3     | 6.4     | 2.6     | 3.3     | 5.5     | 2.5     | 4.0     | 5.3     | 0.180   |
| Aldosterone                           | 0.3     | 0.4     | 0.6     | 0.4     | 0.5     | 0.8     | 0.4     | 0.7     | 1.1     | 0.025   |
| Testosterone                          | 0.8     | 1.1     | 1.3     | 0.9     | 1.0     | 1.4     | 0.8     | 1.0     | 1.2     | 0.371   |
| 5α-Dihydrotestosterone                | 0.4     | 0.5     | 0.8     | 0.3     | 0.5     | 0.8     | 0.4     | 0.5     | 0.8     | 0.180   |
| Etiocholanolone                       | 0.3     | 0.6     | 0.7     | 0.2     | 0.5     | 0.7     | 0.1     | 0.4     | 0.7     | 0.655   |
| Dehydroepiandrosterone               | 18.7    | 22.4    | 30.8    | 16.3    | 21.9    | 35.4    | 15.0    | 19.3    | 31.3    | 0.180   |
| Dehydroepiandrosterone sulfate       | 4487    | 5206    | 6753    | 4296    | 5915    | 6891    | 4483    | 6490    | 7251    | 0.180   |
| Androstenedione                       | 4.6     | 6.1     | 7.4     | 4.6     | 5.8     | 7.1     | 4.4     | 5.5     | 6.3     | 0.371   |
| Androsterone                          | 0.7     | 0.9     | 1.3     | 0.7     | 1.0     | 1.8     | 0.8     | 0.9     | 1.4     | 0.180   |
| Cortisone                            | 51.0    | 57.9    | 66.1    | 58.3    | 66.1    | 70.5    | 57.7    | 63.2    | 66.8    | 0.655   |
| Cortisol                             | 354     | 425     | 520     | 298     | 412     | 472     | 309     | 357     | 495     | 0.371   |
| Corticosterone                       | 14.5    | 22.1    | 32.8    | 13.3    | 19.8    | 30.8    | 12.0    | 20.9    | 25.5    | 0.655   |
| 11-Deoxycorticisol                   | 0.8     | 1.1     | 1.9     | 0.8     | 1.1     | 1.5     | 0.7     | 1.0     | 1.6     | 0.025   |
| Deoxycorticosterone                  | 0.1     | 0.2     | 0.3     | 0.1     | 0.2     | 0.2     | 0.2     | 0.2     | 0.3     | 0.180   |
| 21-Deoxycorticosterol                | 0.1     | 0.2     | 0.4     | 0.13    | 0.21    | 0.48    | 0.15    | 0.25    | 0.35    | 0.655   |
| Sum of progestagens                   | 2.0     | 5.2     | 22.9    | 2.8     | 5.9     | 32.5    | 4.3     | 9.8     | 31.3    | 0.007   |
| Sum of mineralocorticoids             | 14.9    | 22.6    | 33.4    | 13.4    | 20.9    | 31.5    | 12.4    | 21.7    | 27.5    | 0.655   |
| Sum of glucocorticoids                | 416.5   | 476.6   | 574.0   | 363.9   | 481.7   | 533.6   | 376.4   | 418.5   | 563.6   | 0.180   |
| Sum of androgens                      | 27.2    | 34.0    | 41.8    | 23.8    | 31.7    | 46.1    | 24.0    | 28.2    | 41.5    | 0.074   |
| Sum of serum steroids                 | 467.9   | 545.0   | 687.8   | 407.5   | 566.5   | 633.9   | 443.8   | 487.1   | 691.5   | 0.180   |

Steroids were measured by LC-MS at baseline (Visit 1) and after 24 hours (Visit 2) and 48 hours fasting (Visit 3). Results are expressed in nmol/L. Data are median (P50) and 25th and 75th percentiles. N = 19. P values derived from Friedman tests comparing the ranks of 3 time points (visit 1, 2, and 3).
Table 3. Steroid metabolites measured in 24-hour urine collections (nmol/24h) before and during the 2 days fasting period by GC-MS

| Urinary steroid metabolites (nmol/24h) | Visit 1 | Visit 2 | Visit 3 | P value |
|----------------------------------------|---------|---------|---------|---------|
| Sum of progesterone metabolites        |         |         |         |         |
| 17-hydroxyprogrenolone                 | 2951    | 4930    | 6839    | 0.116   |
| Pregnanediol                           | 331     | 610     | 978     | 0.861   |
| Pregnanetriol                          | 1167    | 2226    | 3093    | 0.004   |
| 11-oxo-pregnanetriol                   | 1452    | 1801    | 2557    | 1.000   |
| Sum of corticosterone metabolites      | 1047    | 1783    | 2626    | 0.387   |
| TH-deoxycorticosterone                 | 16      | 23      | 44      | 0.247   |
| TH-11-DH-corticosterone                | 198     | 407     | 618     | 1.000   |
| 5α-TH-corticosterone                   | 0       | 0       | 0       | 0.387   |
| Aldosterone metabolites                |         |         |         |         |
| TH-Aldosterone                         | 21      | 37      | 66      | 0.350   |
| Sum of androgen metabolites            | 14761   | 22188   | 27619   | 0.142   |
| Androsterone                           | 3804    | 5667    | 8734    | 0.058   |
| Etiocholanolone                        | 4143    | 7077    | 8733    | 0.058   |
| Dihydroandrosterone                    | 82      | 121     | 194     | 0.157   |
| 11-o xo-ethiocholanolone               | 950     | 1674    | 3851    | 0.951   |
| 11β-OH-androsterone                   | 1487    | 1970    | 4284    | 0.259   |
| 11β-OH-ethiocholanololone             | 424     | 1017    | 1498    | 0.705   |
| Dehydroepiandrostosterone              | 63      | 229     | 696     | 0.024   |
| Androstenediol                         | 125     | 189     | 285     | 0.047   |
| 16αOH-DHEA                             | 318     | 659     | 1053    | 0.350   |
| Androstenediolic                       | 381     | 717     | 889     | 0.157   |
| Pregnenetriol                          | 26      | 59      | 101     | 0.247   |
| Testosterone                           | 30      | 52      | 79      | 0.449   |
| 5α-dihydrotestosterone                | 36      | 70      | 108     | 0.212   |
| Sum of estrogen metabolites            | 45      | 76      | 114     | 0.638   |
| Estradiol                              | 21      | 42      | 73      | 0.142   |
| 17β-estradiol                          | 15      | 36      | 47      | 0.157   |
| 11-Deoxycorticisol metabolites         |         |         |         |         |
| TH-11-deoxycorticisol                 | 95      | 156     | 198     | 0.287   |
| Sum of cortisol metabolites            | 17234   | 29385   | 37065   | 0.142   |
| Cortisone                              | 213     | 366     | 460     | 0.350   |
| TH-cortisone                           | 5389    | 8155    | 11455   | 0.058   |
| β-cortolone                            | 1185    | 1722    | 2238    | 0.116   |
| 20α-dihydrocortisone                   | 37      | 58      | 100     | 0.157   |
| 20β-dihydrocortisone                   | 103     | 226     | 295     | 0.157   |
| Cortisol                               | 120     | 164     | 284     | 0.449   |
| TH-cortisol                            | 2406    | 3991    | 5128    | 0.074   |
| 5α-TH-cortisol                         | 1560    | 2475    | 4449    | 0.247   |
| α-cortol                              | 533     | 806     | 1219    | 0.387   |
| β-cortol                              | 737     | 1321    | 1527    | 0.001   |
| 20α-dihydrocortisol                    | 49      | 109     | 180     | 0.259   |
| 20β-dihydrocortisol                    | 154     | 255     | 359     | 0.142   |
| 6β-OH-cortisol                         | 114     | 134     | 251     | 0.350   |
| 18-OH-cortisol                         | 239     | 358     | 678     | 0.387   |
| α-cortolone                            | 2503    | 3583    | 4492    | 0.142   |
| Sum of 24h urine metabolites           | 42061   | 57274   | 70886   | 0.142   |

N = 19. Data are median (p50) and 25th and 75th percentiles. P values derived from Friedman tests comparing the ranks of 3 time points (visit 1, 2, and 3). Abbreviations: DHEA, dehydroepiandrosterone; TH, tetrahydro-.
CYP17 is a microsomal enzyme for glucocorticoid and sex steroid biosynthesis. It comprises 2 distinct activities; the first, 
17β-hydroxylase or 17/20 lyase; CYP21A2, 21-hydroxylase; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; DHTST, 5α-dihydrotestosterone; DOC, deoxycorticosterone; E, cortisone; ET, etiocholanolone; F, cortisol; HSD11B1, 11β-hydroxysteroid dehydrogenase type 1; HSD17B, 17β-hydroxy steroid 11-beta dehydrogenase; HSD3B2, 3β-hydroxysteroid dehydrogenase type 2; PREG, pregnenolone; SRD5A2, 5α-reductase type 2; SULT2A1, dehydroepiandrosterone sulfotransferase; TST, testosterone.

Fasting comprises a condition of stress through normal energy deprivation and is counteracted by the normal human body with a broad range of physiologic endocrine and metabolic reactions [1-4]. So far, however, the effect of short-term fasting on the steroid metabolome of healthy women was unknown, while some data of chronic fasting have been reported from patients suffering from anorexia nervosa. Wassif et al [7] described lower urinary excretion of total cortisol and androgen metabolites and an increase of 20α-hydroxysteroid dehydrogenase activity in young patients with anorexia nervosa compared with control subjects. In women with anorexia nervosa, a decrease of 11β-hydroxysteroid dehydrogenase type 1 activity for the regeneration of active glucocorticoids was also detected. Thus, both short and long-term fasting seem to affect steroid biosynthesis similarly, although the underlying signaling pathways between acute and chronic fasting may differ [1-4].

Previous studies have shown that short-term fasting suppresses leptin, insulin, and insulin-like growth factor 1 (IGF-1) production in healthy young women during a 48-hour fasting period. Previous studies in women with abnormal metabolic states and in vitro cell model data revealed different changes but led us to hypothesize that a short period of fasting might induce a hyperandrogenic state in healthy women. However, our study did not confirm this hypothesis. After fasting, healthy women produced more steroid precursors but (relatively) less end-products of all steroid pathways, especially androgens. This seemed regulated by an inhibitory effect of fasting on the steroid enzyme activities of CYP17A1 and SRD5A (5α-reductase) essential for glucocorticoid and androgen biosynthesis (Fig. 3); an inhibitory effect on CYP21A2 remained unclear.

CYP17 is a microsomal enzyme for glucocorticoid and sex steroid biosynthesis. It comprises 2 distinct activities; the first, 17α-hydroxylase activity, is key for shifting steroidogenesis beyond mineralocorticoid production, while the second, 17,20-lyase activity, is essential for all sex steroid production [22]. Likewise, SRD5A activity is important for androgen synthesis in all alternative pathways and for the conversion of testosterone to the more potent dihydrotestosterone in the classic pathway.
| Table 5. Calculated ratios of urine steroid metabolites corresponding to enzyme activities and/or steroid biosynthetic pathways |
|---------------------------------------------------------------|
| **Steroid ratios in urine**                                    | **Visit 1** | **Visit 2** | **Visit 3** | **P value** |
|----------------------------------------------------------------|------------|------------|------------|-------------|
| 21-hydroxylase                                               |            |            |            |             |
| THE/PTONE                                                    | 107.1      | 168.4      | 351.1      |              |
| αCl/PTONE                                                    | 47.2       | 93.6       | 132.1      |              |
| (THE + THF + 5αTHF)/(17HP + 5PT + PTONE)                    | 12.2       | 20.0       | 26.6       |              |
| 3β-hydroxysteroid dehydrogenase                              |            |            |            |             |
| THE/SPT                                                      | 64.5       | 120.9      | 308.0      |              |
| (AT + ET)/(DHEA + 16αOHDHEA+Δ5triol)                         | 5.3        | 6.4        | 12.0       |              |
| 11β-hydroxylase                                              |            |            |            |             |
| THE/THS                                                      | 45.8       | 59.3       | 67.1       |              |
| CYP17 global (17α-hydroxylase and 17,20-lyase)               |            |            |            |             |
| (AT + ET)/PD                                                 | 3.5        | 6.7        | 9.0        |              |
| 17α-hydroxylase global                                       |            |            |            |             |
| THE/(THA + THB + 5αTHB)                                      | 3.5        | 4.7        | 6.2        |              |
| 17α-hydroxylase Δ4-pathway                                   | 0.2        | 0.3        | 0.4        |              |
| 17,20-lyase global                                           |            |            |            |             |
| (AT + ET)/THE                                               | 1.4        | 1.8        | 2.1        |              |
| 17,20-lyase Δ5-pathway                                       | 12.1       | 17.4       | 23.7       |              |
| 17,20-lyase Δ4-pathway                                       | 11.3       | 20.8       | 36.0       |              |
| CYP17 global Δ4- vs. Δ5-pathway                              |            |            |            |             |
| 11β-OHAT/DHEA + 16αOHDHEA+Δ5diol                             | 1.7        | 2.1        | 3.1        |              |
| 17β-hydroxysteroid dehydrogenase                            |            |            |            |             |
| (AT + ET)/(THE + THF + 5αTHF)                               | 0.8        | 0.9        | 1.2        |              |
| Δ5diol/DHEA                                                  | 0.3        | 0.7        | 1.7        |              |
| 5α-reductase                                                 |            |            |            |             |
| ET/AT                                                        | 0.8        | 1.1        | 1.4        |              |
| AT/ET                                                        | 0.7        | 0.9        | 1.3        |              |
| 5αTHF/THF                                                   | 0.5        | 0.7        | 0.9        |              |
| Aromatase (CYP19A1)                                          |            |            |            |             |
| 17βestradiol/TST                                             | 0.4        | 0.6        | 1.0        |              |
| 11β-hydroxysteroid dehydrogenase type 2                     |            |            |            |             |
| (THE + THF + 5αTHF)/(F + E)                                  | 24.8       | 27.9       | 30.5       |              |
| 11β-hydroxysteroid dehydrogenase type 1                     |            |            |            |             |
| (THF + 5αTHF)/THE                                           | 0.7        | 0.8        | 1.0        |              |
| 20α-hydroxysteroid dehydrogenase                            |            |            |            |             |
| (αC+αCl)/(THE + THF + 5αTHF)                                | 0.2        | 0.3        | 0.3        |              |
| 20β-hydroxysteroid dehydrogenase                            |            |            |            |             |
| (βC+βCl)/(THE + THF + 5αTHF)                                | 0.2        | 0.2        | 0.2        |              |
| 20α- vs. 20β-hydroxysteroid dehydrogenase                    |            |            |            |             |
| (αC+αCl)/(βC+βCl)                                            | 1.2        | 1.4        | 1.8        |              |
| 3α-hydroxysteroid dehydrogenase                             |            |            |            |             |
| (THF + 5αTHF)/20αDHF                                        | 53.5       | 64.3       | 101.1      |              |

N = 19. Data are median (p50) and 25th and 75th percentiles. A list of abbreviations for all metabolites is given at the end of the article. P values derived from Friedman tests comparing the ranks of 3 time points (Visit 1, 2, and 3). Abbreviations: 16αOHDHEA, 16α-hydroxy-dehydroepiandrosterone; 17HP, 17-hydroxyprogesterone; 5PT, pregnenetriol; 5αTHF, 5α-tetrahydrocortisol; AT, androsterone; DHEA, dehydroepiandrosterone; ET, etiocholanolone; PD, pregnanediol; PTONE, pregnanetriolone; THE, tetrahydrocortisone; THF, tetrahydrocortisol; αC, α-Cortol; αCL, α-Cortolone; βC, β-Cortol; βCL, β-Cortolone; Δ5diol, androstenediol; Δ5triol, androstenetriol.
concentrations in healthy women and stimulates cortisol secretion [23]. Several studies suggested an important role for leptin in the adipose-adrenal interaction and, thus, in the interconnection between energy metabolism and stress regulation in humans [15]. By contrast, the regulation of androgen biosynthesis with fasting remains largely unknown. Studies have shown that leptin treatment of adrenal NCI-H295R cells expressing leptin receptors resulted in increased 17,20-lyase activity, without changing the 17α-hydroxylase activity of CYP17A1, thereby promoting androgen production [24]. In the same line, Kempna et al [14] found a significant increase in androgen production in H295R cells when grown in serum-free starvation medium. Underlying alterations of this shift in the steroid production was enhanced 17,20 lyase activity. However, in the same study, a strong inhibition of 3β-hydroxysteroid dehydrogenase type II (HSD3B2) was observed responsible for the conversion of pregnenolone (PREG) to progesterone, 17α-hydroxyprogrenolone (17HP) to 17α-hydroxyprogesterone (17OHP), and DHEA to androstenedione in the adrenal cortex. Thus, the inhibition of HSD3B2 contributed essentially to the shift of steroid production toward androgens in H295R cells under starvation conditions. In contrast, no significant change of HSD3B2 activity was observed in our fasted women.

So far, multiple studies have tried to find the regulatory network underlying androgen production and energy metabolism, along with their cross-talk at the cellular level, without great success [25]. Most of these efforts have been undertaken to understand and find novel treatment options for PCOS, which manifests most often with marked hyperandrogenism and metabolic disturbances (eg, insulin resistance). Furthermore, the molecular mechanisms that mediate the stress response to fasting are also unclear. Involvement of the MAPK/ERK signaling (mitogen-activated protein kinase/extracellular-signal-regulated kinase) in androgen regulation has been described by several investigators [25, 26]. In vitro, fasting induced the phosphorylation and thus the consequent activation of the MAPK/ERK pathway, which enhanced P450c17 phosphorylation and its 17,20-lyase activity [14, 27]. Alterations in MAPK/ERK signaling have also been described in hyperandrogenic theca cells of PCOS ovaries compared with controls [28].

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**Figure 2.** Changes noted in calculated enzyme activities after 48-hour fast in healthy young women. Ratios are given as product over substrate conversion by specific enzymes with lower ratios reflecting lower activity. A-C, CYP17A1 activity. D, CYP21A2 activity. E, 5α-reductase (SRD5A) activity. F, 20α- vs 20β-hydroxysteroid dehydrogenase activity. Data are shown as boxplots covering the distribution of the data from the first quartile to the third quartile, and the horizontal line inside the box represents the median. Black points within the boxplots correspond to participants, one value in CYP17 global activity was exceeding the scale. Visit 1 was used as baseline, P values are derived from Friedman tests comparing the ranks of the 3 visits.
Figure 3. Schematic representation of the steroid biosynthesis pathways studied, and changes observed through fasting. A, The classic pathways leading from progestogen precursors to mineralocorticoids, glucocorticoids, and classic androgens. B, The classic and alternative pathways (backdoor and 11-oxy) leading to androgens. Steroids found in serum are given in normal script and urinary metabolites are given in italicized script. Enzymes involved are given in boxes. Changes observed with 48-hour fasting in healthy young women are indicated in red when decreasing and in green when increasing through fasting. See article for full list of all abbreviations used.
Another suggested common path leading to the hyperandrogenic and metabolic changes observed with PCOS might be serine phosphorylation of the insulin receptor and of the CYP17A1 enzyme [27-29]. It has been demonstrated that serine phosphorylation of the CYP17A1 enzyme enhances its 17,20-lyase activity for androgen production, and that serine phosphorylation of the insulin receptor weakens its insulin signaling activity and causes insulin resistance. In line with that finding, we reported that starvation of adrenal cells resulted in enhanced serine phosphorylation of CYP17A1 [14].

Yet, another path where energy and steroid metabolism might come together has been revealed by the antidiabetic drug metformin, which also mildly inhibits androgen production. We and others have shown that metformin is able to modulate the activities of complex I and thus NAD+ generation for cellular energy metabolism and energy production [13, 30-32]. Metformin mediated inhibition of complex I resulted in reduced hepatic gluconeogenesis [32] and decreased androgen production of steroidogenic cells through inhibition of HSD3B2 and CYP17A1-17,20 lyase activities.

Our study subjects fasted for 48 hours and increased their free fatty acid levels significantly showing that their metabolism had shifted from glucose to fat burning, their overall steroidogenesis remained unchanged while some androgens decreased. These observed changes are similar to changes reported for patients with anorexia nervosa [7] and for women with PCOS after dieting [11]. Importantly, before and after 48 hours of fasting, the steroid profiles of healthy women differed fundamentally from steroid profiles of women with PCOS, especially in the excretion of androgens (eg, DHEA and androstenediol) [33].

Our results suggested that a short period of fasting can induce a significant increase in serum progesterone and aldosterone. However, the increase was small in magnitude, and its clinical relevance is questionable; study participants did not show any effect of fasting on blood pressure or serum electrolytes. In addition, the urine collection after fasting confirmed only the increase of one progesterone metabolite and showed no effect of fasting on aldosterone metabolite excretion. Likewise, results of calculated estimation for 21-hydroxylase activity from measured serum and urinary steroids were inconclusive. While the calculation from serum metabolites suggested a possible inhibitory effect of fasting on CYP21A2, no effect of fasting on aldosterone metabolite excretion. Yet, another path where energy and steroid metabolism might come together has been revealed by the antidiabetic drug metformin, which also mildly inhibits androgen production.

In conclusion, this is the first prospective study assessing the changes in a large number of urinary and serum steroids in healthy young women during a short-term fasting period of 48 hours. Fasting stressed steroid precursor production without leading to enhanced mineralocorticoid, glucocorticoid, or androgen production. This occurred through inhibition of activities of essential enzymes of steroidogenesis. Steroid changes observed after fasting in healthy women resembled changes reported for anorectic women and differed fundamentally from steroid characteristics seen with PCOS, indicating that the regulation of androgen biosynthesis in normal and abnormal systems are different. Future studies assessing the effect of short-term fasting on steroids in women with PCOS might provide further insight into the interrelationship of hyperandrogenism and energy homeostasis for the pathophysiology of PCOS. We also conclude that regulatory mechanisms underlying (normal) energy homeostasis and steroidogenesis (eg, androgen production) are more complex in vivo and may not follow a simple in vitro cell model.

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Disclosures/Conflicts of Interest

The authors declare no conflict of interest.

Clinical Trial Information

ClinicalTrials.gov registration no. NCT03573063.

Data Availability

Original data generated and analyzed during this study are included in this published article, deposited in BORIS (19), or available upon reasonable request.

References

1. Soeters MR, Soeters PB, Schooneman MG, Houten SM, Romijn JA. Adaptive reciprocity of lipid and glucose metabolism in human short-term starvation. Am J Physiol Endocrinol Metab. 2012;303:E1397-E1407. doi:10.1152/ajpendo.00397.2012
2. Konod H, Teruya T, Yanagida M. Metabolomics of human fasting: new insights about old questions. Open Biol. 2020;10(9):200176. doi:10.1098/rsob.200176
3. Bergman BC, Cornier MA, Horton TJ, Bessesen DH. Effects of fasting on insulin action and glucose kinetics in lean and obese men and women. Am J Physiol Endocrinol Metab. 2007;293:E110-3-E1111. doi:10.1152/ajpendo.00613.2006
4. Browning JD, Baxter J, Satapati S, Burgess SC. The effect of short-term fasting on liver and skeletal muscle lipid, glucose, and energy metabolism in healthy women and men. J Lipid Res. 2012;53:577-586. doi:10.1194/jlr.P020867
5. Schiffer L, Barnard L, Baranowski ES, et al. Human steroid biosynthesis, metabolism and excretion are differentially reflected by serum and urine steroid metabolomes: a comprehensive review. J Steroid Biochem Mol Biol. 2019;194:105-439. doi:10.1016/j.jsbmb.2019.105439
6. Chalkiadaki A, Guarente L. Sirtuins mediate mammalian metabolic responses to nutrient availability. Nat Rev Endocrinol. 2012;8:287-296. doi:10.1038/nrendo.2011.225
7. Wassif WS, McLoughlin DM, Vincent RP, Conroy S, Russell GF, Taylor NF. Steroid metabolism and excretion in severe anorexia nervosa: effects of refeeding. *Am J Clin Nutr.* 2011;93:911-917. doi:10.3945/ajcn.111.012666

8. Deng Y, Zhang Y, Li S, et al. Steroid hormone profiling in obese and nonobese women with polycystic ovary syndrome. *Sci Rep.* 2017;7:14156. doi:10.1038/s41598-017-14534-2

9. Boden G, Chen X, Mozzoli M, Ryan L. Effect of fasting on serum leptin in normal human subjects. *J Clin Endocrinol Metab.* 1996;81:3419-3423. doi:10.1210/jcem.81.9.8784108

10. Ehrmann DA. Polycystic ovary syndrome. *N Engl J Med.* 2005;352:1223-1236. doi:10.1056/NEJMr041536

11. Shang Y, Zhou H, Hu M, Feng H. Effect of diet on insulin resistance in polycystic ovary syndrome. *J Clin Endocrinol Metab.* 2020;105(10):3346-3360. doi:10.1210/clinem/dga425

12. Gallauria F, Palomba S, Vigorito C, et al. Androgens in polycystic ovary syndrome: the role of exercise and diet. *Semin Reprod Med.* 2009;27:306-315. doi:10.1055/s-0029-1225258

13. Hirsch A, Hahn D, Kempna P, et al. Metformin inhibits human androgen production by regulating steroidogenic enzymes HSD3B2 and CYP17A1 and complex I activity of the respiratory chain. *Endocrinology.* 2012;153(9):4354-4366. doi:10.1210/en.2012-1145

14. Kempna P, Hirsch A, Hofer G, Mullis PE, Fluck CE. Impact of differential P450c17 phosphorylation by cAMP stimulation and by starvation conditions on enzyme activities and androgen production in NCI-H295R cells. *Endocrinology.* 2010;151(8):3686-3696. doi:10.1210/en.2010-0093

15. Pralong FP, Rodruit R, Waebber G, et al. Leptin inhibits directly glucocorticoid secretion by normal human and rat adrenal gland. *Endocrinology.* 1998;139(10):4264-4268. doi:10.1210/endo.139.10.6254

16. Bergendahl M, Evans WS, Pastor C, Patel A, Iranmanesh A, Veldhuis JD. Short-term fasting suppresses leptin and (conversely) activates disorderly growth hormone secretion in midluteal phase women—a clinical research center study. *J Clin Endocrinol Metab.* 1999;84(3):883-894. doi:10.1210/jcem.84.3.5536

17. Buttler RM, Martens F, Fanelli F, et al. Comparison of 7 Published LC-MS/MS Methods for the Simultaneous Measurement of Testosterone, Androstenedione, and Dehydroepiandrosterone in Serum. *Clin Chem.* 2015;61:1475-1483. doi:10.1373/clinchem.2014.242839

18. Cook H, Brennan K, Aziz R. Reanalyzing the modified Ferriman-Gallwey score: is there a simpler method for assessing the extent of hirsutism? *Fertil Steril.* 2011;96:1266-70.e1. doi:10.1016/j.fertnstert.2011.08.022

19. Fluck CE. Short-term fasting attenuates overall steroid hormone biosynthesis in healthy young women. *Supplementary material. BORIS portal. Posted April 19, 2022.* https://doi.org/10.48620/56

20. Bileck A, Verouti SN, Escher G, Vogt B, Grosset M. A comprehensive urinary steroid analysis strategy using two-dimensional gas chromatography - time of flight mass spectrometry. *Analyst.* 2018;143:4484-4494. doi:10.1039/c7an01990d

21. Dhayat NA, Dick B, Frey BM, d’Uscio CH, Vogt B, Fluck CE. Androgen biosynthesis during minipuberty favors the backdoor pathway over the classic pathway: Insights into enzyme activities and steroid fluxes in healthy infants during the first year of life from the urinary steroid metabolome. *J Steroid Biochem Mol Biol.* 2017;165:312-322. doi:10.1016/j.jsbmb.2016.07.009

22. Sewer MB, Li D, Dammer EB, Jagarlapudi S, Lucki N. Multiple Signaling Pathways Coordinate CYP17 Gene Expression in the Human Adrenal Cortex. *Acta chima Slovenica* 2008;55:53-57. https://pubmed.ncbi.nlm.nih.gov/20208627/

23. Aschbacher K, Rodriguez-Fernandez M, van Wiertmarschen H, et al. The hypothalamic-pituitary-adrenal-leptin axis and metabolic health: a systems approach to resilience, robustness and control. *Interface focus.* 2014;4:20140020. doi:10.1098/rsfs.2014.0020

24. Biaso-Lauber A, Zachmann M, Schoenle EJ. Effect of leptin on CYP17 enzymatic activities in human adrenal cells: new insight in the onset of adrenarche. *Endocrinology.* 2000;141:1446-1454. doi:10.1210/endo.141.4.7402

25. Udhane SS, Legeza B, Marti N, et al. Combined transcriptome and metabolome analyses of metformin effects reveal novel links between metabolic networks in steroidogenic systems. *Sci Rep.* 2017;7:8652. doi:10.1038/s41598-017-09189-y

26. Nguyen TV, Yao M, Pike CJ. Androgens activate mitogen-activated protein kinase signaling: role in neuroprotection. *J Neurochem.* 2005;94:1639-1651. doi:10.1111/j.1471-4159.2005.03318.x

27. Tee MK, Dong Q, Miller WL. Pathways leading to phosphorylation of p450c17 and to the posttranslational regulation of androgen biosynthesis. *Endocrinology.* 2008;149:2667-2677. doi:10.1210/en.2007-1527

28. Zhang LH, Rodriguez H, Ohno S, Miller WL. Serine phosphorylation of human P450c17 increases 17,20-lyase activity: implications for adrenarche and the polycystic ovary syndrome. *Proc Natl Acad Sci USA.* 1995;92:10619-10623. doi:10.1073/pnas.92.23.10619

29. Hu L, Zhang Y, Chen L, Zhou W, Wang Y, Wen J. MAPK and ERK polymorphisms are associated with PCOS risk in Chinese women. *Oncotarget.* 2017;8:100261-100268. doi:10.18632/oncotarget.22153

30. El-Mir MY, Nogueira V, Fontaine E, Averet N, Rigoulet M, Leverve X. Dimethylbiguanide inhibits cell respiration via an indirect effect targeted on the respiratory chain complex I. *J Biol Chem.* 2000;275:223-228. doi:10.1074/jbc.275.1.223

31. Owen MR, Doran E, Halestrap AP. Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. *Biochem J.* 2000;348(Pt 3):607-614. https://pubmed.ncbi.nlm.nih.gov/10839993/

32. Foretz M, Hebrard S, Leclerc J, et al. Metformin inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state. *J Clin Invest.* 2010;120:2355-2369. doi:10.1172/JCI40671

33. Dhayat NA, Marti N, Kollmann Z, et al; members of the SSG. Urinary steroid profiling in women hints at a diagnostic signature of the polycystic ovary syndrome: A pilot study considering neglected steroid metabolites. *PLoS One.* 2018;13:e0203903 https://pubmed.ncbi.nlm.nih.gov/30308019/