

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 fasted for 48 hours; steroid profiles from plasma and urine samples were assessed at baseline, after 24 hours, and 48 hours by liquid and gas chromatography–mass spectrometry.

Results: Fasting did not change overall steroidogenesis, although it increased progesterone production and lowered relative mineralocorticoid, glucocorticoid, and androgen production. The largest decrease in urine metabolites was seen for β -cortol, dehydroepiandrosterone, and androstenediol; higher levels were found for pregnanediol in urine and progesterone and aldosterone in serum. Activity of 17 α -hydroxylase/17,20-lyase (CYP17A1), essential for androgen biosynthesis, was decreased after fasting in healthy women as were 21-hydroxylase (CYP21A2) and 5 α -reductase activities. By contrast, hydroxysteroid 11-beta dehydrogenase 1 (HSD11B1) activity for cortisol inactivation seemed to increase with fasting.

Conclusion: Significant changes in steroid metabolism occurred after 48 hours of fasting in healthy women. 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

Abbreviations: 11OXOET, 11-oxo-etiocholanolone; 11 β OHAT, 11 β -hydroxyandrosterone; 11 β OHET, 11 β -hydroxyetiocholanolone; 16 α OHDEA, 16 α -hydroxy-dehydroepiandrosterone; 17HP, 17-hydroxypregnanolone; 17OHP, 17OH-progesterone; 18OHF, 18-hydroxycortisol; 20 α DHE, 20 α -dihydrocortisone; 20 α DHF, 20 α -dihydrocortisol; 20 β DHE, 20 β -dihydrocortisone; 5PT, pregnenetriol; 5 α THB, 5 α -tetrahydrocorticosterone; 5 α THF, 5 α -tetrahydrocortisol; 6 β OHF, 6 β -hydroxycortisol; A4, androstenedione; AKR1C1, aldo-keto reductase family 1 member C1; AKR1C2, aldo-keto reductase family 1 member C2; AKR1C3, aldo-keto reductase family 1 member C3; AKR1C4, aldo-keto reductase family 1 member C4; ALDO, aldosterone; AT, androsterone; BMI, body mass index; CYP11A1, cholesterol 20-22 desmolase; CYP11B1, 11 β -hydroxylase member 1; CYP11B2, 11 β -hydroxylase member 2; CYP17A1, 17 α -hydroxylase or 17/20 lyase; CYP21A2, 21-hydroxylase; DHANDRO, dihydroandrosterone; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosteronesulfate; DHTST, 5 α -dihydrotestosterone; DOC, 11-deoxycorticosterone; E, cortisone; ERK, extracellular signal-regulated kinase; ESTRADIOL, 17 β -estradiol; ESTRADIOL, estradiol; ET, etiocholanolone; F, cortisol; FSH, follicle stimulating hormone; GC-MS, gas chromatography–mass spectrometry; HOMA-IR, homeostatic model assessment for insulin resistance; HSD11B1, hydroxysteroid 11-beta dehydrogenase 1; HSD11B2, hydroxysteroid 11-beta dehydrogenase 2; HSD17B1, 17 β -hydroxysteroid dehydrogenase type 1; HSD17B3, 17 β -hydroxysteroid dehydrogenase type 3; HSD17B5, 17 β -hydroxysteroid dehydrogenase type 5; HSD17B6, 17 β -hydroxysteroid dehydrogenase type 6; HSD3B2, 3 β -hydroxysteroid dehydrogenase type 2; IGF-1, insulin-like growth factor 1; LC-MS, liquid chromatography–mass spectrometry; MAPK, mitogen-activated protein kinase; NAD, nicotinamide adenine dinucleotide; PCOS, polycystic ovary syndrome; PD, pregnanediol; PREG, pregnenolone; PT, pregnenetriol; PTONE, pregnanetriolone; SDS, standard deviation score; SRD5A1, 5 α -reductase type 1; SRD5A2, 5 α -reductase type 2; SULT2A1, dehydroepiandrosterone sulfotransferase; THA, tetrahydro-11-dehydrocorticosterone; THALDO, tetrahydroaldosterone; THB, tetrahydrocorticosterone; THDOC, tetrahydrodeoxycorticosterone; THE, tetrahydrocortisone; THF, tetrahydrocortisol; THS, tetrahydro-11-deoxycortisol; TST, testosterone; UHPLC, ultra-high-performance liquid chromatography; α C, α -Cortol; α CL, α -Cortolone; β C, β -Cortol; β CL, β -Cortolone; Δ 5diol, androstenediol; Δ 5triol, androstenediol.

The physiological regulation of short-term and long-term fasting comprises a big challenge to the human body for maintaining energy balance [1]. The metabolic response to fasting is characterized by a switch from carbohydrate to fat

metabolism. During fasting, insulin secretion is inhibited, and glucagon promotes glycogenolysis as well as gluconeogenesis and inhibits glyconeogenesis [2]. Fasting can lead to changes in the redox state of nicotinamide adenine dinucleotide

Received: 20 February 2022. Editorial Decision: 25 April 2022. Corrected and Typeset: 26 May 2022

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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 \times 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, 100Å, 1.8 μm , 1 mm \times 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

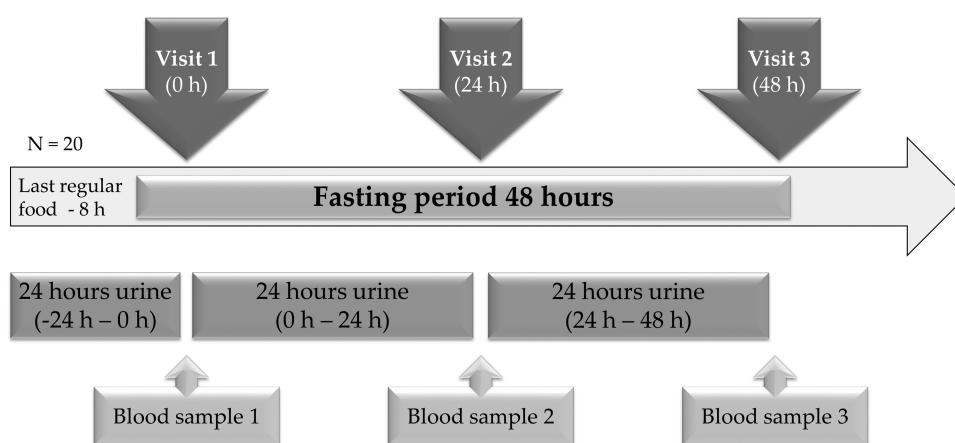


Figure 1. Study design. A total of 20 participants fulfilling the inclusion criteria were recruited, each collecting 3 separate 24-hour urine specimens as instructed and giving blood at each of the 3 corresponding study visits at the given timepoints (0, 24, and 48 hours). Visit 1: baseline. Visit 2: after 24 hours. Visit 3: after 48 hours. After finishing the 48-hour fast, biomaterial collections, and visit 3, all study persons restarted their normal diet.

Table 1. Baseline characteristics of the study population

	N	P25	P50	P75	Range	
					min	max
Age at consultation (years)	20	23.9	26.3	27.9	19.4	34.8
Gestational age (weeks)	17	37	37	38	35	41
Birth weight (kg)	17	3.0	3.5	3.6	2.0	4.0
Birth weight (SDS)	17	0.1	1.0	1.3	-3.4	2.0
Ferriman-Gallwey score	19	1	2	4	0	5
Weight (kg)	20	55.4	60.0	66.4	50.0	89.0
Weight (SDS)	20	-0.2	0.2	0.8	-0.9	2.4
Height (cm)	20	162	169	173	154	182
Height (SDS)	20	-0.3	0.8	1.4	-1.4	2.8
BMI (kg/m ²)	20	20.7	21.7	22.5	18.9	29.6
BMI (SDS)	20	-0.3	0.1	0.3	-0.9	2.0
Waist circumference (cm)	20	66	69	72	65	74
Waist circumference (SDS)	20	-1.4	-1.0	-0.7	-1.5	-0.4
Waist-to-height ratio	20	0.4	0.4	0.4	0.4	0.5
Waist-to-height ratio (SDS)	20	-1.5	-1.2	-0.7	-2.1	-0.2

Data are median, P25 and P75 for each parameter.
Abbreviations: BMI, body mass index; SDS, standard deviation score.

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 progestogens 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 ($\Delta 5$ diol). 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 androsterone (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

Table 2. Changes in serum steroid metabolites in young women fasting for 48 hours

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 25	P 50	P 75	
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
17 α -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
5 α -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
Androstendione	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-Deoxycortisol	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-Deoxycortisol	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	563.3	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	469.7	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
	P 25	P 50	P 75	P 25	P 50	P 75	P 25	P 50	P 75	
Sum of progesterone metabolites	2951	4930	6839	3501	6380	8549	3359	6592	10293	0.116
17-hydroxypregnanolone	331	610	978	306	625	1327	350	576	1113	0.861
Pregnanediol	1167	2226	3093	1618	3016	5199	1517	3096	6933	0.004
Pregnanetriol	1452	1801	2557	1375	2179	3344	1301	1989	3744	1.000
11-oxo-pregnanetriol	20	38	73	23	38	58	23	39	69	0.387
Sum of corticosterone metabolites	1047	1783	2626	1113	1600	2056	1120	1569	2009	0.387
TH-deoxycorticosterone	16	23	44	13	29	47	17	30	45	0.247
TH-11-DH-corticosterone	198	407	618	249	382	437	213	353	487	0.247
TH-corticosterone	295	426	566	269	403	466	258	347	466	0.549
5 α -TH-corticosterone	503	751	1298	498	727	1297	581	786	1035	0.387
Aldosterone metabolites										
TH-Aldosterone	21	37	66	24	35	73	26	35	60	0.350
Sum of androgen metabolites	14761	22018	27619	16020	19430	27048	12834	17630	22151	0.142
Androsterone	3804	5667	8734	3799	6081	8199	3410	5145	6406	0.058
Etiocholanolone	4143	7077	8733	5359	5978	7660	4018	4742	7212	0.058
Dihydroandrosterone	82	121	194	92	116	159	72	94	163	0.157
11-oxo-etiocholanolone	950	1674	3851	1027	1288	3873	1062	1430	4543	0.951
11 β -OH-androsterone	1487	1970	4284	1539	2226	3401	1530	1924	3256	0.259
11 β -OH-etiocholanolone	424	1017	1498	648	1210	1910	684	1310	1844	0.705
Dehydroepiandrosterone	63	229	696	52	89	502	63	119	229	0.024
Androstenediol	125	189	285	100	146	208	76	136	197	0.047
16 α OH-DHEA	318	659	1053	299	550	789	274	511	886	0.449
Androstetriol	381	717	889	406	595	934	399	586	877	0.819
Pregnenetriol	26	59	101	19	41	85	25	36	56	0.247
Testosterone	30	52	79	22	42	72	19	41	51	0.449
5 α -dihydrotestosterone	36	70	108	32	54	99	39	61	105	0.212
Sum of estrogen metabolites	45	76	114	46	73	98	49	69	102	0.638
Estriol	21	42	73	25	41	69	29	47	78	0.142
17 β -estradiol	15	36	47	21	24	37	17	23	31	0.157
11-Deoxycortisol metabolites										
TH-11-deoxycortisol	95	156	198	106	136	170	89	123	177	0.287
Sum of cortisol metabolites	17234	29385	37065	18340	23510	31067	17249	22066	27537	0.142
Cortisone	213	366	460	245	345	381	230	301	382	0.350
TH-cortisone	5589	8155	11455	6031	6599	8439	5107	6872	8885	0.058
β -cortolone	1185	1722	2238	1053	1414	1841	897	1284	1468	0.116
20 α -dihydrocortisone	37	58	100	33	51	65	34	48	63	0.157
20 β -dihydrocortisone	103	226	295	109	156	207	118	153	205	0.157
Cortisol	120	164	284	114	180	213	129	193	246	0.449
TH-cortisol	2406	3991	5128	2963	3432	3992	2545	2935	3554	0.074
5 α -TH-cortisol	1560	2475	4449	1297	2471	3664	1512	2253	3261	0.247
α -cortol	533	806	1219	473	720	942	525	641	748	0.387
β -cortol	737	1321	1527	730	984	1271	651	805	1251	0.001
20 α -dihydrocortisol	49	109	180	44	95	144	63	88	105	0.259
20 β -dihydrocortisol	154	255	359	133	196	233	145	198	244	0.142
6 β -OH-cortisol	114	134	251	94	161	240	100	156	225	0.350
18-OH-cortisol	239	358	678	192	356	495	228	371	524	0.387
α -cortolone	2503	3583	4492	2310	2807	4041	2029	2936	3607	0.142
Sum of 24h urine metabolites	42061	57274	70886	39938	54756	73942	39318	51274	61857	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-.

Table 4. Calculated ratios of serum steroids corresponding to enzyme activities and steroid biosynthetic pathways

Steroid ratios in serum	Visit 1			Visit 2			Visit 3			P value
	P 25	P 50	P 75	P 25	P 50	P 75	P 25	P 50	P 75	
Androgens/Progestagens	1.4	6.0	16.5	1.2	4.1	14.4	1.0	3.0	5.9	<0.001
Mineralocorticoids/Progestagens	1.2	4.1	9.5	0.8	3.2	5.5	0.8	1.7	3.9	<0.001
Glucocorticoids/Progestagens	17.9	77.0	234.8	13.2	76.5	173.8	14.3	45.3	113.3	<0.001
Glucocorticoids/Mineralocorticoids	15.9	22.5	28.9	16.7	22.6	25.0	17.6	22.1	28.5	1.000
Androgens/Mineralocorticoids	1.2	1.7	2.0	1.2	1.5	1.9	1.2	1.4	2.0	0.655
Androgens/Glucocorticoids	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.371
CYP17A1 (17 α -hydroxylase): 17OHP/Progesterone	0.3	3.0	14.2	0.2	1.3	11.1	0.2	0.5	8.0	0.002
CYP17A1 (17,20-lyase): A4/17OHP	1.0	1.7	3.2	0.8	1.6	2.8	0.9	1.4	1.9	0.007
CYP17A1 (17 α -hydroxylase + 17,20-lyase): DHEA/PREG	3.8	6.2	9.0	3.5	5.2	6.5	3.4	4.4	5.6	0.007
CYP21A2: DOC/Progesterone	0.0	0.2	0.8	0.0	0.1	0.3	0.0	0.0	0.2	<0.001
CYP21A2: 11-deoxycortisol/17OHP	0.2	0.4	0.6	0.2	0.3	0.5	0.2	0.3	0.4	0.025
CYP21A2: F/21-deoxycortisol	1096	2179	3573	803	1820	3029	956	1948	2620	0.655
CYP11B1: Corticosterone/DOC	94	130	193	84	121	163	88	115	143	0.074
CYP11B1: F/11-deoxycortisol	282	386	502	258	364	479	283	389	513	0.074
CYP11B2: ALDO/Corticosterone	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.180
HSD3B2: Progesterone/PREG	0.0	0.2	2.9	0.0	0.7	3.1	0.1	1.1	5.1	0.074
HSD3B2: A4/DHEA	0.2	0.3	0.3	0.2	0.3	0.3	0.2	0.3	0.3	0.655
HSD11B1: E/F	0.1	0.1	0.2	0.1	0.2	0.2	0.1	0.2	0.2	0.025
HSD17B: TST/A4	0.1	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.655
SRD5A2: DHTST/TST	0.4	0.5	0.8	0.3	0.5	0.7	0.4	0.6	0.8	0.180
SULT2A1: DHEAS/DHEA	160	219	318	210	237	325	245	271	360	0.025
Backdoor: ET/AT	0.3	0.6	0.8	0.2	0.4	0.7	0.1	0.3	0.7	0.007
Backdoor: A4/AT	4.5	6.7	9.5	4.0	5.8	7.1	4.2	5.3	8.7	0.074
Backdoor: TST/AT	0.9	1.2	1.6	0.7	1.0	1.4	0.7	1.0	1.4	0.180
CYP17 global	8.7	15.6	22.3	7.9	16.4	19.1	7.0	14.6	19.3	0.074
CYP21 global	9.4	11.3	14.2	7.0	11.0	13.7	7.5	10.1	13.0	0.025
HSD3B-global	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.025

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: 17OHP, 17OH-progesterone; A4, androstenedione; ALDO, aldosterone; AT, androsterone; CYP11B1, 11 β -hydroxylase member 1; CYP11B2, 11 β -hydroxylase member 2; CYP17A1, 17 α -hydroxylase or 17/20 lyase; CYP21A2, 21-hydroxylase; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; DHTST, 5 α -dihydrotestosterone; DOC, deoxycorticosterone; E, cortisone; ET, etiocholanolone; F, cortisol; HSD11B1, hydroxy steroid 11-beta dehydrogenase 1; HSD17B, 17 β -hydroxysteroid dehydrogenase; HSD3B2, 3 β -hydroxysteroid dehydrogenase type 2; PREG, pregnenolone; SRD5A2, 5 α -reductase type 2; SULT2A1, dehydroepiandrosterone sulfotransferase; TST, testosterone.

steroid metabolism 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 (5 α -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 synthesis. It comprises 2 distinct activities; the first, 17 α -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.

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 20 α -hydroxysteroid dehydrogenase activity in young patients with anorexia nervosa compared with control subjects. In women with anorexia nervosa, a decrease of 11 β -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)

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
	P 25	P 50	P 75	P 25	P 50	P 75	P 25	P 50	P 75	
21-hydroxylase										
THE/PTONE	107.1	168.4	351.1	148.0	183.1	263.8	97.6	171.9	277.7	0.522
α Cl/PTONE	47.2	93.6	132.1	56.3	88.3	130.2	41.0	73.3	126.7	0.705
(THE + THF + 5 α THF)/(17HP + 5PT + PTONE)	12.2	20.0	26.6	9.4	16.9	27.3	9.4	15.6	24.2	0.705
3β-hydroxysteroid dehydrogenase										
THE/5PT	64.5	120.9	308.0	67.4	194.1	393.1	95.2	217.8	300.1	0.705
(AT + ET)/(DHEA + 16 α OHDHEA + Δ 5triol)	5.3	6.4	12.0	6.0	9.2	13.1	5.2	7.9	11.4	0.549
11β-hydroxylase										
THE/THS	45.8	59.3	67.1	35.9	54.6	68.5	36.7	51.5	70.2	0.350
CYP17 global (17α-hydroxylase and 17,20-lyase)										
(AT + ET)/PD	3.5	6.7	9.0	2.7	4.1	7.6	2.1	3.0	6.7	<0.001
17α-hydroxylase global										
THE/(THA + THB + 5 α THB)	3.5	4.7	6.2	3.4	4.8	6.4	3.0	4.0	5.6	0.116
17α-hydroxylase Δ4-pathway										
17HP/PD	0.2	0.3	0.4	0.2	0.2	0.3	0.1	0.2	0.3	0.001
17,20-lyase global										
(AT + ET)/THE	1.4	1.8	2.1	1.5	1.8	2.3	1.4	1.7	2.2	0.522
17,20-lyase Δ5-pathway										
(DHEA + 16 α OHDHEA)/5PT	12.1	17.4	23.7	10.5	16.1	23.1	14.1	18.4	23.5	0.522
17,20-lyase Δ4-pathway										
(AT + ET)/17HP	11.3	20.8	36.0	10.3	18.7	32.0	10.3	14.7	20.2	0.247
CYP17 global Δ4- vs. Δ5-pathway										
11 β -OHAT/(DHEA + 16 α OHDHEA + Δ 5diol)	1.7	2.1	3.1	1.8	3.1	3.7	1.7	2.5	4.5	0.861
17β-hydroxysteroid dehydrogenase										
(AT + ET)/(THE + THF + 5 α THF)	0.8	0.9	1.2	0.8	1.0	1.2	0.7	0.9	1.2	0.212
Δ 5diol/DHEA	0.3	0.7	1.7	0.4	1.6	2.5	0.5	1.0	2.5	0.247
5α-reductase										
ET/AT	0.8	1.1	1.4	0.8	1.2	1.5	0.8	1.2	1.5	0.001
AT/ET	0.7	0.9	1.3	0.7	0.8	1.2	0.7	0.9	1.3	0.001
5 α THF/THF	0.5	0.7	0.9	0.5	0.7	0.9	0.5	0.7	1.1	0.522
Aromatase (CYP19A1)										
17 β -estradiol/TST	0.4	0.6	1.0	0.5	0.8	1.4	0.5	0.7	1.1	0.387
11β-hydroxysteroid dehydrogenase type 2										
(THE + THF + 5 α THF)/(F + E)	24.8	27.9	30.5	24.0	26.6	32.4	21.6	24.3	28.1	0.091
11β-hydroxysteroid dehydrogenase type 1										
(THF + 5 α THF)/THE	0.7	0.8	1.0	0.6	0.8	1.1	0.7	0.8	1.0	0.350
20α-hydroxysteroid dehydrogenase										
(α C + α Cl)/(THE + THF + 5 α THF)	0.2	0.3	0.3	0.2	0.3	0.3	0.3	0.3	0.3	0.047
20β-hydroxysteroid dehydrogenase										
(β C + β Cl)/(THE + THF + 5 α THF)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.705
20α- vs. 20β-hydroxysteroid dehydrogenase										
(α C + α Cl)/(β C + β Cl)	1.2	1.4	1.8	1.2	1.5	1.9	1.4	1.6	1.9	0.002
3α-hydroxysteroid dehydrogenase										
(THF + 5 α THF)/20 α DHF	53.5	64.3	101.1	51.6	79.7	114.7	59.2	65.6	94.7	0.861

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: 16 α OHDHEA, 16 α -hydroxy- dehydroepiandrosterone; 17HP, 17-hydroxypregnanolone; 5PT, pregnenetriol; 5 α THF, 5 α -tetrahydrocortisol; AT, androsterone; DHEA, dehydroepiandrosterone; ET, etiocholanolone; PD, pregnanediol; PTONE, pregnanetriolone; THE, tetrahydrocortisone; THF, tetrahydrocortisol; α C, α -Cortol; α CL, α -Cortolone; β C, β -Cortol; β CL, β -Cortolone; Δ 5diol, androstenediol; Δ 5triol, androstetriol.

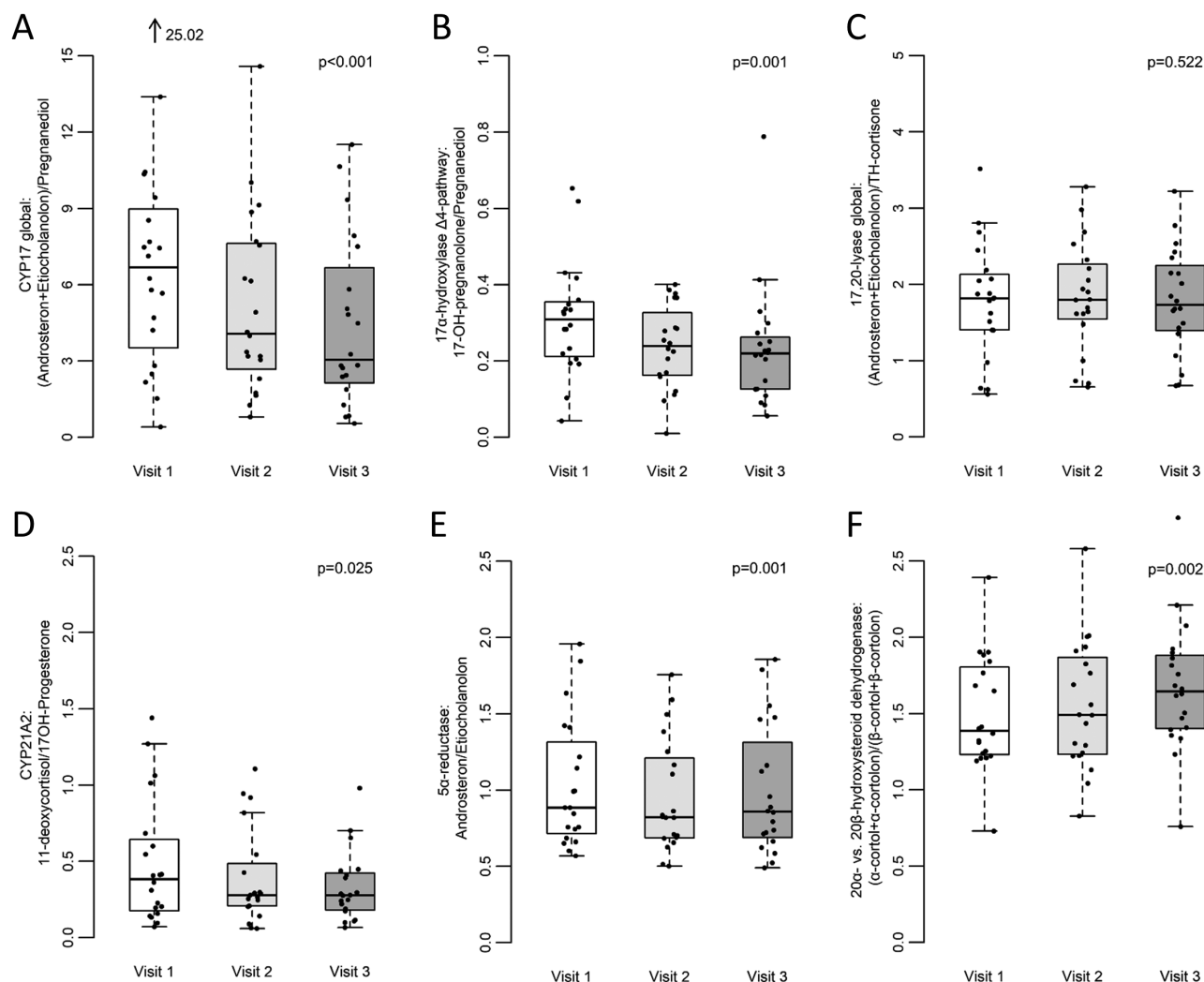


Figure 2. Changes noted in calculated enzyme activities after 48-hour fast in healthy young women. Ratios are given as product over substrate conversion by specific enzymes with lower ratios reflecting lower activity. A-C, CYP17A1 activity. D, CYP21A2 activity. E, 5 α -reductase (SRD5A) activity. F, 20 α - vs 20 β -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.

concentrations in healthy women and stimulates cortisol secretion [23]. Several studies suggested an important role for leptin in the adipo-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 17 α -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 3 β -hydroxysteroid dehydrogenase type II (HSD3B2) was observed responsible for the conversion of pregnenolone (PREG) to progesterone, 17 α -hydroxypregnenolone (17HP) to 17 α -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].

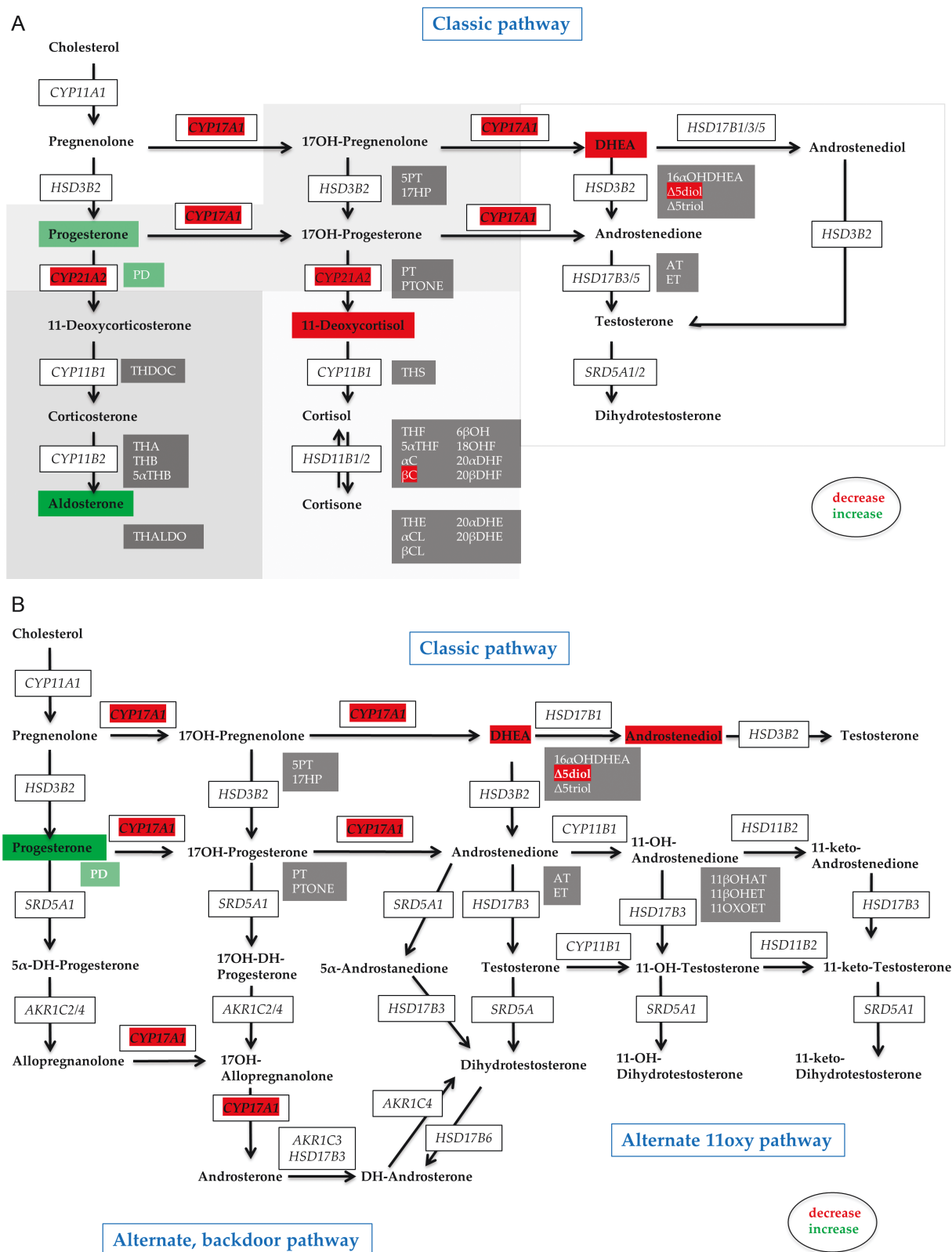


Figure 3. Schematic representation of the steroid biosynthesis pathways studied, and changes observed through fasting. A, The classic pathways leading from progestogen precursors to mineralocorticoids, glucocorticoids, and classic androgens. B, The classic and alternative pathways (backdoor and 11-oxy) 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 was found when calculating with urine metabolites.

There are some limitations of this study. The number of the participants was low (N = 20, respectively N = 19 for analysis) and we did not include covariables such as BMI. However, BMI and waist-to-height ratio were normal in all participants. We did not measure insulin, C-peptide, and leptin after 48 hours of fasting and, thus, we have missed the direct effect of fasting on insulin and leptin secretion. However, as participants were all healthy with normal fasting blood sugar, fasting insulin, and body weight, we assumed that insulin and leptin levels after 48 hours fasting decreased normally.

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.

Acknowledgments

We thank all participants of the study. We also thank especially Barbara Beck for her kind support in all administrative issues.

Funding

This research was partially funded by the “Batzebär – Stiftung der Kinderkliniken Bern” and supported by the PedNet Bern (institutional funds of the University Children’s Hospital Inselspital Berne).

Disclosures/Conflicts of Interest

The authors declare no conflict of interest.

Clinical Trial Information

ClinicalTrials.gov registration no. [NCT03573063](https://clinicaltrials.gov/ct2/show/study/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

- 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](https://doi.org/10.1152/ajpendo.00397.2012)
- Kondoh 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](https://doi.org/10.1098/rsob.200176)
- 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:E1103-E1111. doi:[10.1152/ajpendo.00613.2006](https://doi.org/10.1152/ajpendo.00613.2006)
- 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](https://doi.org/10.1194/jlr.P020867)
- 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;194:105439. doi:[10.1016/j.jsbmb.2019.105439](https://doi.org/10.1016/j.jsbmb.2019.105439)
- 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](https://doi.org/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 I. 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/NEJMra041536
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/dgaa425
12. Giallauria 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, Roduit R, Waeber 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.2015.242859
18. Cook H, Brennan K, Azziz 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, Groessl 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 chimica Slovenica* 2008;55:53-57. <https://pubmed.ncbi.nlm.nih.gov/20098627/>
23. Aschbacher K, Rodriguez-Fernandez M, van Wietmarschen 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. Biason-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/>