Effects of lisdexamfetamine on plasma steroid concentrations compared with D-amphetamine in healthy subjects: a randomized, double-blind, placebo-controlled study

Running title: D-amphetamine, lisdexamfetamine, and steroids

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Abstract

Lisdexamfetamine is a novel prodrug of D-amphetamine that is used for the treatment of attention-deficit/hyperactivity disorder (ADHD). D-amphetamine releases dopamine and norepinephrine and stimulates the hypothalamic-pituitary-adrenal (HPA) axis, which may contribute to its reinforcing effects and risk of abuse. However, there is currently no data available on the effects of lisdexamfetamine on circulating steroids. The goal of the present study was to assess the effects of lisdexamfetamine on circulating steroids compared with D-amphetamine and placebo. Equimolar doses of D-amphetamine (40 mg) and lisdexamfetamine (100 mg) and placebo were administered in 24 healthy subjects in a randomized, double-blind, placebo-controlled, cross-over study. Plasma concentrations of steroids and D-amphetamine were determined up to 24 h. Plasma D-amphetamine concentrations began to increase and reached peak levels later after lisdexamfetamine administration compared with D-amphetamine administration, but the maximal concentrations and total exposure (area under the curve [AUC]) were similar. Lisdexamfetamine and D-amphetamine significantly increased plasma concentrations of adrenocorticotropic hormone, glucocorticoids (cortisol, cortisone, corticosterone, 11-dehydrocorticosterone, and 11-deoxycortisol), androgens (dehydroepiandrosterone, dehydroepiandrosterone sulfate, and Δ4-androstene-3,17-dione [androstenedione]), and progesterone (only in men) compared with placebo. Steroid concentration-time curves were shifted to later time points because of a non-significantly later onset after lisdexamfetamine administration compared with D-amphetamine, but maximal plasma steroid concentrations and AUCs did not differ between the active treatments. None of the active treatments altered plasma concentrations of the mineralocorticoids aldosterone and 11-deoxycorticosterone or the androgen testosterone compared with placebo. The effects of the amphetamines on glucocorticoid production were similar to those that were previously reported for methylphenidate (60 mg) but weaker than those for the serotonin releaser 3,4-methylenedioxymethamphetamine (MDMA; 125 mg) or direct serotonin receptor agonist lysergic acid diethylamide (LSD; 0.2 mg). Lisdexamfetamine produced comparable HPA axis activation and had similar pharmacokinetics compared with D-amphetamine, with the exception of a later time of onset. Thus, serotonin (MDMA, LSD) may more effectively stimulate the HPA axis than dopamine and norepinephrine (D-amphetamine).

Introduction

Lisdexamfetamine is a prodrug of D-amphetamine [1, 2], and both are used for the treatment of attention-deficit/hyperactivity disorder (ADHD), similar to methylphenidate. In addition to their use as medications, amphetamines and methylphenidate are also misused as recreational drugs or neuroenhancers to induce euphoria or stay awake [3-5]. After oral administration, the conversion of lisdexamfetamine to D-amphetamine is thought to occur gradually in the circulation [6], resulting in a prolonged pharmacokinetic profile with a low peak but sustained plasma amphetamine concentrations [7]. Such a prolonged pharmacokinetic profile is considered to be associated with slower effects on dopamine (DA) release, lower euphoric effects, and a possibly lower risk of misuse [7-9]. Indeed, in rats, a lower peak plasma concentration (C_{max}) of amphetamine was observed after lisdexamfetamine administration, together with a gradual and sustained increase in dopamine efflux and much less locomotor activity compared with D-amphetamine [10]. In humans, 100 mg lisdexamfetamine produced lower subjective "drug liking" than an equivalent dose of 40 mg D-amphetamine in one study, although other subjective effects including euphoria and stimulation did not differ between the two drugs [7]. Moreover, in a recent study of the pharmacokinetics and pharmacodynamics of lisdexamfetamine and D-amphetamine, we found no difference between the two drugs in the maximal plasma concentrations of amphetamine or any of their subjective effects [11].

The goal of the present study was to investigate the effects of lisdexamfetamine and D-amphetamine compared with placebo and with each other on circulating steroids. Amphetamines and methylphenidate enhance subjective mood, concentration, and wakefulness but also act as acute pharmacological stressors that stimulate the hypothalamic-pituitary-adrenal (HPA) axis to elevate concentrations of circulating stress hormones, including adrenocorticotropic hormone (ACTH), cortisol, epinephrine, and norepinephrine (NE) [12-16]. However, the effects of lisdexamfetamine on the HPA axis are unknown. In the US in 2010, lisdexamfetamine was the third-most prescribed drug for ADHD in pediatrics patients [17]. In the US, there was also an increase in lisdexamfetamine misuse cases reported to poison centers between 2007 and 2012, resulting in more cases associated with lisdexamfetamine than extended-release D-amphetamine [18]. Because, disturbances of HPA axis function, *e.g.* the glucocorticoid circadian rhythm, can lead to learning, memory and behavioral deficits, mood disorders such as depression, impaired immune system, and development of metabolic syndrome [19-23], information on the effects of lisdexamfetamine on HPA axis function is of interest. Also unknown is whether lisdexamfetamine produces less HPA axis activation than D-amphetamine based on its reportedly prolonged kinetic characteristics [7-9]. Animal studies indicate that HPA axis stimulation may be associated with a greater risk of drug abuse. Specifically, rats that exhibit greater HPA axis reactivity or were administered corticosterone more likely self-administered D-amphetamine [24]. These observations suggest that lisdexamfetamine may have a lower risk of oral abuse compared with D-amphetamine because of a slowed increase in plasma D-amphetamine concentrations and consequently a lower HPA response. Therefore, we directly compared plasma ACTH and steroid concentrations after administration of equivalent and relatively high doses of D-amphetamine and lisdexamfetamine. The pharmacokinetic, subjective, and cardiovascular effects have been reported in detail elsewhere [11] and selected effects are also shown here. The primary hypothesis of the present study was that lisdexamfetamine would produce a lower C_{max} and longer time to C_{max} (T_{max}) for both D-amphetamine and plasma steroids compared with immediate-release D-amphetamine. Equimolar doses of lisdexamfetamine and D-amphetamine were expected to result in equivalent areas under the plasma concentration-time curve (AUCs) for D-amphetamine and steroids, confirming the use of equivalent doses.

The present study used relatively high doses of lisdexamfetamine and D-amphetamine. D-Amphetamine at low oral doses of 10-20 mg has been repeatedly shown to increase plasma and saliva cortisol concentrations [16, 25-31], with no effect on plasma cortisol levels [32]. Few studies used higher doses of D-amphetamine that would possibly better reflect stimulant misuse. One study reported an increase in plasma cortisol levels compared with baseline after

34 mg D-amphetamine [33]. However, this previous study did not include a placebo control condition. Therefore, the present study investigated the effects of relatively high doses of D-amphetamine (40 mg) and lisdexamfetamine (100 mg) and placebo on plasma concentrations of ACTH and primarily cortisol and other circulating steroids that have not been previously measured.

Both amphetamine and methylphenidate enhance DA and NE neurotransmission [34]. D-amphetamine releases DA and NE from presynaptic terminals and inhibits their reuptake [35]. Methylphenidate only inhibits their reuptake without inducing transporter-mediated release [36]. Although methylphenidate stimulates DA and NE systems similarly to D-amphetamine, methylphenidate produced only moderate stimulating effects on the HPA axis [13, 15]. Specifically, single low oral doses of 10-20 mg methylphenidate had no significant effect on plasma cortisol concentrations compared with placebo [29]. A single intermediate oral dose of 40 mg methylphenidate only moderately increased plasma cortisol levels [14]. A high dose of 60 mg methylphenidate non-significantly increased plasma levels of cortisol, cortisone, corticosterone, and 11-dehydrocorticosterone compared with placebo [13, 15]. Interestingly, the relatively high dose of 60 mg methylphenidate produced at least similar subjective "drug liking" to 30 mg D-amphetamine [15, 37], indicating that methylphenidate may induce lower HPA axis stimulation than D-amphetamine at doses producing similar subjective drug liking. This view is supported by a study that directly compared plasma cortisol concentrations after low 10-20 mg doses of both D-amphetamine and methylphenidate [29], but higher doses of both drugs have not been directly or indirectly compared. Therefore, the present study also indirectly compared the effects of a high dose of 40 mg D-amphetamine with a high dose of 60 mg methylphenidate that was previously tested in the same laboratory in a similar healthy population using the same clinical and analytical methods [13]. Based on previous data [13, 15, 29], the hypothesis was that D-amphetamine would produce greater HPA axis activation than methylphenidate.

A final goal of the present study was to explore the role of different monoamine neurotransmitters in regulating HPA activity. D-amphetamine releases both DA and NE and may release cortisol mainly via NE [38]. In contrast, the amphetamine derivative 3,4-methylenedioxymethamphetamine (MDMA) mainly releases serotonin (5-hydroxytryptamine [5-HT]) and NE [35, 39, 40]. Therefore, MDMA and D-amphetamine may be useful as pharmacological modulators to study the impact of 5-HT *vs*. DA release on HPA axis stimulation. Accordingly, we indirectly compared the effects of D-amphetamine on plasma steroid concentrations with those after 125 mg oral MDMA that was previously tested in the same laboratory using the same clinical and analytical methods [13]. To further study the role of 5-HT *vs*. DA and NE release in psychoactive substance-induced HPA axis stimulation in humans, we also compared the effects of D-amphetamine with similar historical data [41] on the direct 5-HT receptor agonist lysergic acid diethylamide (LSD) [42]. We hypothesized that MDMA and LSD would produce greater increases in cortisol in humans than D-amphetamine. This would indicate a more prominent role for 5-HT compared with DA and NE in stimulating the main human glucocorticoid cortisol by psychoactive substances and further establish cortisol as a marker of acute serotonergic activity [41, 43].

Materials and Methods

Study design

The protocol of the clinical trial (Protocol S1) and the CONSORT checklist (Checklist S1) are available as supporting information. The CONSORT flowchart is depicted in Figure 1. The study used a double-blind (subjects and study personnel), placebo-controlled, cross-over design with three experimental test days (D-amphetamine, lisdexamfetamine, and placebo). The treatment sequence was randomly selected from four blocks of all possible six sequences and all treatments were counterbalanced. The washout periods between sessions were at least 7 days. The study was conducted in accordance with the Declaration of Helsinki and International Conference on Harmonization Guidelines in Good Clinical Practice and approved

by the Ethics Committee northwest/central Switzerland (EKNZ) and Swiss Agency for Therapeutic Products (Swissmedic). The study was registered at ClinicalTrials.gov (NCT02668926). All of the subjects provided written informed consent prior to participating in the study.

Figure 1. CONSORT flowchart. The treatment sequence was randomly selected from four blocks of all possible six sequences and all treatments were counterbalanced.

Participants

Twenty-four healthy subjects (12 men and 12 women; mean age ± SD: 25.3 ± 3.0 years; range: 21-34 years) were included. The allocation to treatment order was conducted by drawing from blocks of six different balanced drug treatment sequences by a pharmacist of the University Hospital Basel not involved in the study. Each code was stored in a sealed envelope until the termination of the study. The sample-size estimation showed that 15 subjects would be needed to detect a meaningful difference of 20% in C_{max} levels between D-amphetamine and lisdexamfetamine with more than 80% power using a within-subjects study design. The inclusion criteria were age between 18 and 45 years, body mass index between 18 and 27 kg/m^2, and birth control for women. The exclusion criteria were chronic or acute medical conditions, including clinically relevant abnormalities on physical exam, laboratory values, or electrocardiography, personal or family (first-degree relative) history of psychotic or major affective disorder, lifetime prevalence of illicit drug use > 5 times (except for tetrahydrocannabinol), illicit drug use within the last 2 months, pregnancy, regular use of medications, smoking (> 10 cigarettes/day), and alcohol consumption (> 10/week). The subjects were asked to abstain from excessive alcohol consumption between test sessions and not drink caffeine-containing drinks after midnight before the study day. Urine drug tests were performed at study inclusion and before each test session using TRIAGE 8 (Biosite, San Diego, CA, USA).

Drugs

Gelatin capsules that contained either lisdexamfetamine dimesylate (100 mg salt; Opopharma, Rümlang, Switzerland) or D-amphetamine sulfate (40.3 mg salt; Hänseler, Herisau, Switzerland), both corresponding to a dose of 29.6 mg D-amphetamine, and placebo capsules (mannitol) were prepared, and randomized by the pharmacy of the University Hospital Basel according to Good Manufacturing Practice. The recommended doses of lisdexamfetamine for the treatment of ADHD are 30-70 mg/day, with an initial dose of 30 mg. The selected dose of 100 mg lisdexamfetamine was relatively high and above the upper recommended daily dose of 70 mg to induce greater subjective drug liking and mimic misuse, and to produce similar plasma concentrations after a single dose to those reached during repeated administration of 70 mg when steady state is reached.

Study procedures

Before the test session, a urine sample was taken to verify abstinence from drugs of abuse, and a pregnancy test was performed in women. The test session began at 8:00 AM by placing an indwelling intravenous catheter in an antecubital vein for blood sampling. At 9:00 AM, a single dose of lisdexamfetamine, D-amphetamine, or placebo was administered orally. During the test session, the subjects did not engage in any physical activity, were resting in hospital beds in a calm standard hospital room, and were served a standardized lunch and dinner at 11:30 AM and 6:30 PM, respectively. For the analysis of hormone and D-amphetamine concentrations in plasma, blood samples were collected in lithium heparin tubes 1 h before and 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, and 24 h after drug administration. The blood samples were immediately centrifuged, and plasma was stored at -20°C. For the determination of ACTH concentrations, blood samples were drawn into ethylenediaminetetraacetic acid-containing tubes 1 h before and 3.5 h after drug

administration. The test session ended at 9:00 PM. The subjects returned home and returned the following day at 9:00 AM to draw the final 24 h blood sample. Subjective, autonomic, and adverse responses were also assessed and have been reported in detail elsewhere [11].

Steroid quantification in plasma

The following plasma steroid hormones with the corresponding lower limit of quantification (LLOQ; values in brackets) were determined using a previously published ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method [41] with minor modifications: cortisol [1.95 nM], cortisone [1.95 nM], corticosterone [0.98 nM], 11-dehydrocorticosterone [0.98 nM], 11-deoxycorticosterone [0.78 nM], aldosterone [0.2 nM], dehydroepiandrosterone (DHEA) [3.91 nM], DHEA sulfate (DHEAS) [19.53 nM], Δ4-androstene-3,17-dione (androstenedione) [0.78 nM], testosterone [0.39 nM], 11-deoxycortisol [0.78 nM], progesterone [0.05 nM], androsterone [3.91 nM], and 17α-hydroxyprogesterone [0.78 nM]. The accuracy was between 85% and 115%, and the coefficient of variation was < 15%, tested at three concentrations for all analytes. The recovery of control samples was in the range of 80-120%. The details of the applied method and its validation were reported previously [41]. Briefly, after protein precipitation, plasma samples that contained deuterium-labeled aldosterone, corticosterone, androstenedione, androsterone, and testosterone as internal standards were solid-phase extracted. After evaporation and reconstitution in methanol, the steroids were separated and quantified by UHPLC-MS/MS using an Agilent 1290 UPLC device coupled to an Agilent 6490 triple quadrupole mass spectrometer equipped with a jet-stream electrospray ionization interface. Analyte separation was achieved using a reverse-phase column (Waters Acquity UPLC BEH C18, 1.7 µm, 2.1 × 150 mm). Mass Hunter software (Agilent Technologies) was used for data acquisition and analysis.

Quantification of adrenocorticotropic hormone in human plasma samples

ACTH was determined by a chemiluminescent immunometric assay (Immulite 2000 ACTH; Siemens, Erlangen, Germany).

Quantification of D-amphetamine concentrations in plasma

Plasma D-amphetamine concentrations were measured using an UHPLC-MS/MS method. Materials, procedures, and method validation are described in detail in the Supplementary Material. The method had a lower limit of detection (LOD) of 0.26 ng/ml and LLOQ of 0.78 ng/ml for D-amphetamine and was validated over the range of 0.78 to 200 ng/ml for D-amphetamine. Plasma D-amphetamine concentrations were primarily measured to confirm the use of bioequivalent lisdexamfetamine and D-amphetamine doses with regard to total D-amphetamine exposure and to assess D-amphetamine-steroid response relationships. The comprehensive pharmacokinetic data from this study have been reported elsewhere [11].

Subjective effects

Visual Analog Scales (VASs) were repeatedly used to assess subjective drug effects over time. The VASs "drug liking", “good drug effects”, “drug high”, and “stimulated” were presented as 100 mm horizontal lines (0 to +100), marked from "not at all" on the left to "extremely" on the right. The VASs were administered 1 h before and 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 24 h after drug administration.

Statistical analyses

C_{max}, E_{max}, and T_{max} were derived directly from the observed data. The time to reach 10% of C_{max} (T_{onset}) and areas under the concentration-time curve from time 0 to 12 h (AUC_{12})

were calculated using the linear trapezoidal method in Phoenix WinNonlin 6.4 software (Pharsight, St. Louis, MO). The statistical analyses were performed using Statistica 12 software (StatSoft, Tulsa, OK, USA) and the computing environment R (R Development Core Team, 2017, Vienna, Austria). Kinetic parameters and subjective effect ratings (E_{max}) were compared using repeated-measures analysis of variance (ANOVA), with drug (D-amphetamine, lisdexamfetamine, and placebo) as the within-subjects factor, followed by the Tukey *post hoc* test. Means of the effect sizes are displayed with confidence intervals of 95%. P-Values of the multiple ANOVAs were Bonferroni-adjusted for the 14 different hormones tested. Additionally, sex differences were assessed by adding sex as a between-subjects factor in addition to drug in complementary ANOVAs. Furthermore, supplementary ANOVAs with order as additional factor were performed to exclude treatment order effects (absence of drug × order interactions). Plasma amphetamine concentration-effect relationships were studied by plotting endocrine responses as a difference from time-matched placebo against the plasma amphetamine concentration for each time point. Selected peak endocrine effects of D-amphetamine and lisdexamfetamine were calculated as differences from placebo and then compared with the effects of 60 mg methylphenidate [13], 125 mg MDMA [13], and 200 µg LSD [41] (also as placebo-corrected responses) using ANOVA, with drug as the between-subjects (between-studies) factor, followed by the Tukey *post hoc* test. The data for methylphenidate, MDMA, and LSD were obtained from previous identical studies in healthy subjects in the same laboratory. The use of placebo-corrected values accounted for between-subject differences in baseline steroid levels and circadian within-subject changes.

Results

Blood could not be drawn from one subject in the D-amphetamine and from one subject in the placebo condition. Therefore, complete datasets were available for D-amphetamine, placebo, and lisdexamfetamine for 23, 23, and 24 subjects, respectively.

Plasma amphetamine levels after administration of D-amphetamine and lisdexamfetamine and subjective effects

The plasma amphetamine concentration-time curves were identical after administration of D-amphetamine and lisdexamfetamine, with the exception of a significantly longer T_{onset} and T_{max} after lisdexamfetamine administration compared with D-amphetamine administration (Fig. 2, Table 1). The C_{max} and AUC_{12} values were similar (Table 1). Subjective drug effects over time are shown in Fig. 2. The effects in "drug liking", “good drug effect”, “drug high”, and “stimulated” between Lisdexamfetamine and D-amphetamine in compared with placebo (Table 1, Fig. 2) did not increase differently. The subjective drug effect-time curves were shifted to the right (Fig. 2) as evidenced by significantly longer time to onset and time to maximal effect values after lisdexamfetamine administration compared with D-amphetamine administration (Table 1), reflecting the pharmacokinetics of the two drugs. However, no differences in E_{max} values were found between lisdexamfetamine and D-amphetamine (Table 1). The pharmacokinetics and additional pharmacodynamic effects are reported in more detail elsewhere [11].

Figure 2. Plasma concentration of amphetamine and subjective drug effects following administration of D-amphetamine, lisdexamfetamine and placebo. D-Amphetamine, lisdexamfetamine, or placebo was administered at t = 0 h. Values for amphetamine (A) are mean ± SEM in 23, 24, and 23 subjects after administration of D-amphetamine, lisdexamfetamine and placebo. Plasma concentration-time curves of amphetamine were similar after administration of lisdexamfetamine compared with D-amphetamine with the exception of a significantly later onset and therefore longer time to reach maximal concentrations. However, maximal concentrations of amphetamine and areas under the concentration-time curves were similar after the two treatments. The effect onset and maximal

response of the subjective effects ("drug liking" (B), “good drug effect” (C), “drug high” (D), and “stimulated” (E)) were significantly delayed after lisdexamfetamine administration compared with D-amphetamine administration but the maximal effects and curve shapes were similar, reflecting the pharmacokinetics of the two substances. The subjective response data are expressed as the mean ± SEM in 24 subjects.

328 **Table 1. Kinetic parameters of plasma steroids and amphetamine after D-amphetamine, lisdexamfetamine, or placebo.**

		D-Amphetamine	Lisdexamfetamine	Placebo	Main effect of drug ($^{a,b,c}F_{2,42}$)	*p* value (non-corrected)	*p* value Bonferroni adjusted	Lisdexamfetamine - D-Amphetamine Mean (CI. low, CI. High)	*p* value	Placebo - D-Amphetamine Mean (CI. low, CI. High)	*p* value	Placebo - Lisdexamfetamine Mean (CI. low, CI. High)	*p* value
Amphetamine	T_{onset}	0.8 ± 0.1	1.4 ± 0.1		33.86	<0.001		0.57 (0.37, 0.78)					
	T_{max}	3.2 ± 0.2	4.4 ± 0.2		16.47	<0.001		1.0 (0.51, 1.58)					
	C_{max}	134 ± 7	128 ± 5		0.88	NS		-3.0 (-13, 7.2)					
	AUC_{12}	1014 ± 47	983 ± 42		0.66	NS		-7.4 (-56, 42)					
Subjective effects													
Drug liking	T_{onset}	0.9 ± 0.1	1.6 ± 0.2		11.1	0.0035							
	T_{max}	2.7 ± 0.4	4.5 ± 0.5		6.66	0.04							
	E_{max}	51.2 ± 5.8	48.0 ± 6.9	3.7 ± 2.6	38.8	<0.001		-3.2 (-17, 11)	NS	-48 (-62, -33)	<0.001	-44 (-58, -44)	<0.001
	AUC_{12}	251 ± 43	260 ± 52	5.2 ± 4.3	17.8	<0.001		9.0 (-105, 122)	NS	-246 (-359, -132)	<0.001	-255 (-368, -255)	<0.001
Good drug effect	T_{onset}	0.9 ± 0.1	1.5 ± 0.1		8.3	0.0092							
	T_{max}	2.8 ± 0.3	4.4 ± 0.5		7.46	0.013							
	E_{max}	48.5 ± 5.6	41.8 ± 6.5	4.0 ± 2.5	30.2	<0.001		-4.4 (-18, 9.6)	NS	-42 (-56, -28)	<0.001	-38 (-52, -38)	<0.001
	AUC_{12}	226 ± 42	236 ± 51	5.4 ± 4.4	14.8	<0.001		9.6 (-103, 122)	NS	-221 (-333, -108)	<0.001	-230 (-343, -230)	<0.001
Drug high	T_{onset}	1.0 ± 0.1	1.9 ± 0.3		8.19	0.01							
	T_{max}	2.4 ± 0.2	3.6 ± 0.4		7.04	0.016							
	E_{max}	35.5 ± 5.6	29.3 ± 6.2	3.3 ± 2.6	16.8	<0.001		-6.2 (-20, 7.7)	NS	-32 (-46, -18)	<0.001	-26 (-40, -26)	<0.001
	AUC_{12}	134 ± 30	125 ± 33	2.5 ± 1.9	10.3	<0.001		-8.8 (-84, 67)	NS	-131 (-207, -56)	<0.001	-123 (-198, -123)	<0.001
Stimulated	T_{onset}	1.0 ± 0.1	1.8 ± 0.3		6.19	0.022							
	T_{max}	2.3 ± 0.2	4.4 ± 1.0		5.88	0.025							
	E_{max}	43.8 ± 5.7	38.0 ± 6.8	2.4 ± 1.7	26	<0.001		-5.8 (-20, 8.8)	NS	-41 (-56, -27)	<0.001	-36 (-50, -36)	<0.001
	AUC_{12}	178 ± 30	152 ± 35	1.7 ± 1.1	16.1	<0.001		-26 (-105, 52)	NS	-176 (-255, -98)	<0.001	-150 (-228, -150)	<0.001
Glucocorticoids													
Cortisol	T_{max}	2.72 ± 0.29	3.17 ± 0.41	1.20 ± 0.41	7.54	0.0016	0.022	0.59 (-0.69, 1.9)	NS	-1.5 (-2.8, -0.19)	0.019	-2.1 (-3.4, -2.1)	<0.001
	C_{max}	534 ± 28.9	519 ± 24.9	417 ± 35.2	17.5	<0.001	<0.001	-26 (-80, 27)	NS	-128 (-181, -74)	<0.001	-102 (-155, -102)	<0.001
	AUC_{12}	4116 ± 286	4207 ± 287	2621 ± 252	62.2	<0.001	<0.001	-56 (-414, 301)	NS	-1501 (-1858, -1143)	<0.001	-1444 (-1801, -1444)	<0.001
Cortisone	T_{max}	4.39 ± 0.3	4.67 ± 0.3	2.80 ± 0.5	6.86	0.0026	0.037	0.32 (-0.91, 1.5)	NS	-1.5 (-2.7, -0.27)	0.012	-1.8 (-3.0, -1.8)	0.0015
	C_{max}	80.6 ± 4.2	82.8 ± 4.3	58.0 ± 3.0	36.3	<0.001	<0.001	0.50 (-7.1, 8.1)	NS	-24 (-31, -16)	<0.001	-24 (-32, -24)	<0.001
	AUC_{12}	707 ± 35.2	739 ± 35.5	503 ± 26.4	47.1	<0.001	<0.001	19 (-41, 78)	NS	-203 (-262, -144)	<0.001	-222 (-281, -222)	<0.001
Corticosterone	T_{max}	2.59 ± 0.47	3.0 ± 0.38	2.24 ± 0.5	1.15	NS	NS	0.59 (-0.75, 1.9)	NS	-0.25 (-1.6, 1.1)	NS	-0.84 (-2.2, -0.84)	NS
	C_{max}	22.6 ± 2.6	20.1 ± 2.0	11.6 ± 1.4	15.8	<0.001	<0.001	-2.3 (-7.4, 2.8)	NS	-12 (-17, -6.5)	<0.001	-9.2 (-14, -9.2)	<0.001
	AUC_{12}	85.1 ± 6.2	83.1 ± 6.0	36.6 ± 3.2	62.7	<0.001	<0.001	-3.0 (-14, 8.4)	NS	-49 (-60, -37)	<0.001	-46 (-57, -46)	<0.001
11-Dehydro-corticosterone	T_{max}	2.96 ± 0.48	2.88 ± 0.38	2.46 ± 0.55	0.4	NS	NS	0.07 (-1.3, 1.4)	NS	-0.41 (-1.8, 0.95)	NS	-0.48 (-1.8, -0.48)	NS
	C_{max}	8.93 ± 0.86	8.82 ± 0.64	5.43 ± 0.53	23.5	<0.001	<0.001	-0.13 (-1.6, 1.4)	NS	-3.8 (-5.3, -2.3)	<0.001	-3.7 (-5.2, -3.7)	<0.001
	AUC_{12}	51.4 ± 4.0	53.7 ± 3.2	28.8 ± 2.2	65.7	<0.001	<0.001	1.6 (-4.1, 7.2)	NS	-23 (-29, -17)	<0.001	-25 (-30, -25)	<0.001
Cortisol + cortisone	T_{max}	2.93 ± 0.3	3.65 ± 0.38	1.20 ± 0.41	9.85	<0.001	0.0043	0.61 (-0.66, 1.9)	NS	-1.7 (-3.0, -0.44)	0.0046	-2.3 (-3.6, -2.3)	<0.001
	C_{max}	601 ± 31	583 ± 26.5	469 ± 36	20.3	<0.001	<0.001	-29 (-85, 27)	NS	-144 (-200, -88)	<0.001	-115 (-171, -115)	<0.001
	AUC_{12}	4824 ± 299	4945 ± 307	3124 ± 266	67.9	<0.001	<0.001	-38 (-429, 354)	NS	-1704 (-2095, -1312)	<0.001	-1666 (-2057, -1666)	<0.001
Ratio cortisol/cortisone	T_{max}	1.83 ± 0.44	3.10 ± 0.61	2.02 ± 0.63	2.79	0.073	NS	1.5 (-0.06, 3.0)	0.064	0.32 (-1.2, 1.9)	NS	-1.2 (-2.7, -1.2)	NS
	C_{max}	9.42 ± 0.57	9.25 ± 0.49	8.81 ± 0.72	0.66	NS	NS	-0.31 (-1.7, 1.0)	NS	-0.66 (-2.0, 0.69)	NS	-0.35 (-1.7, -0.35)	NS
	AUC_{12}	72.3 ± 5.1	69.8 ± 4.0	61.5 ± 5.2	9.93	<0.001	0.0041	-3.9 (-10, 2.4)	NS	-12 (-18, -5.4)	<0.001	-7.8 (-14, -7.8)	0.0097
Corticosterone + 11-dehydro-corticosterone ratio	T_{max}	2.59 ± 0.47	3.0 ± 0.36	2.37 ± 0.54	0.99	NS	NS	0.59 (-0.67, 1.8)	NS	-0.11 (-1.4, 1.1)	NS	-0.70 (-2.0, -0.70)	NS
	C_{max}	31.2 ± 3.3	28.5 ± 2.6	16.9 ± 1.9	18	<0.001	<0.001	-2.5 (-8.9, 3.9)	NS	-15 (-22, -8.8)	<0.001	-13 (-19, -13)	<0.001
	AUC_{12}	136 ± 9.5	137.0 ± 8.6	65.3 ± 5.0	74.8	<0.001	<0.001	-1.2 (-17, 15)	NS	-72 (-87, -56)	<0.001	-70 (-86, -70)	<0.001
	T_{max}	2.59 ± 0.44	2.98 ± 0.43	2.39 ± 0.56	0.28	NS	NS	0.36 (-1.2, 2.0)	NS	-0.14 (-1.7, 1.5)	NS	-0.50 (-2.1, -0.50)	NS

corticosterone/11-	C_{max}	3.01 ± 0.24	2.73 ± 0.14	2.24 ± 0.14	7.48	0.0017	0.023	-0.29 (-0.75, 0.18)	NS	-0.76 (-1.2, -0.29)	<0.001	-0.47 (-0.94, -0.47)	0.045
dehydrocorticosterone	AUC_{12}	18.7 ± 1.1	17.3 ± 0.93	13.9 ± 1.03	18.1	<0.001	<0.001	-1.5 (-3.5, 0.43)	NS	-4.9 (-6.8, -2.9)	<0.001	-3.4 (-5.3, -3.4)	<0.001
11-Deoxycortisol	T_{max}	3.02 ± 0.29	3.83 ± 0.32	4.22 ± 0.67	1.99	NS	NS	0.77 (-0.76, 2.3)	NS	1.3 (-0.23, 2.8)	NS	0.52 (-1.0, 0.52)	NS
	C_{max}	2.70 ± 0.17	2.68 ± 0.17	1.60 ± 0.14	35.3	<0.001	<0.001	-0.02 (-0.39, 0.34)	NS	-1.1 (-1.5, -0.78)	<0.001	-1.1 (-1.5, -1.1)	<0.001
	AUC_{12}	17.7 ± 1.08	18.1 ± 1.2	11.0 ± 1.2	46.3	<0.001	<0.001	0.27 (-1.7, 2.2)	NS	-6.8 (-8.8, -4.9)	<0.001	-7.1 (-9.1, -7.1)	<0.001
Mineralocorticoids													
Aldosterone	T_{max}	4.11 ± 0.84	3.88 ± 0.73	3.43 ± 0.67	0.03	NS	NS	-0.11 (-2.4, 2.2)	NS	-0.25 (-2.5, 2.0)	NS	-0.14 (-2.4, -0.14)	NS
	C_{max}	0.31 ± 0.03	0.34 ± 0.03	0.31 ± 0.03	1.42	NS	NS	0.01 (-0.01, 0.03)	NS	-0.01 (-0.03, 0.01)	NS	-0.02 (-0.04, -0.02)	NS
	AUC_{12}	3.06 ± 0.2	3.19 ± 0.21	3.12 ± 0.24	1.76	NS	NS	0.12 (-0.03, 0.28)	NS	0.06 (-0.09, 0.22)	NS	-0.06 (-0.22, -0.06)	NS
11-Deoxy-	T_{max}	3.43 ± 0.44	3.85 ± 0.48	2.98 ± 0.65	0.56	NS	NS	0.16 (-1.6, 1.9)	NS	-0.59 (-2.3, 1.2)	NS	-0.75 (-2.5, -0.75)	NS
corticosterone	C_{max}	0.53 ± 0.08	0.57 ± 0.07	0.49 ± 0.07	2.29	NS	NS	0.04 (-0.03, 0.11)	NS	-0.02 (-0.09, 0.05)	NS	-0.06 (-0.13, -0.06)	0.088
	AUC_{12}	5.84 ± 0.88	6.10 ± 0.87	5.45 ± 0.86	1.66	NS	NS	0.34 (-0.25, 0.94)	NS	-0.10 (-0.69, 0.50)	NS	-0.44 (-1.0, -0.44)	NS
Androgens													
DHEA	T_{max}	3.50 ± 0.53	4.88 ± 0.53	3.76 ± 0.7	1.58	NS	NS	1.2 (-0.74, 3.2)	NS	-0.11 (-2.1, 1.8)	NS	-1.3 (-3.3, -1.3)	NS
	C_{max}	80.9 ± 7.0	78.5 ± 6.0	57.1 ± 5.3	8.77	<0.001	0.0092	-0.95 (-16, 14)	NS	-24 (-39, -8.7)	<0.001	-23 (-38, -23)	0.0013
	AUC_{12}	609 ± 41.1	608 ± 43.0	455 ± 36.9	20.2	<0.001	<0.001	13 (-51, 77)	NS	-143 (-207, -79)	<0.001	-156 (-220, -156)	<0.001
DHEAS	T_{max}	5.40 ± 0.68	5.80 ± 0.58	4.50 ± 0.64	0.94	NS	NS	0.45 (-1.6, 2.5)	NS	-0.73 (-2.8, 1.3)	NS	-1.2 (-3.2, -1.2)	NS
	C_{max}	13764 ± 1397	14452 ± 1307	11896 ± 1280	11.8	<0.001	0.0012	855 (-522, 2232)	NS	-1932 (-3310, -555)	0.003	-2787 (-4165, -2787)	<0.001
	AUC_{12}	129057 ± 15047	136822 ± 12643	113005 ± 13334	7.05	0.0023	0.032	8277 (-7361, 23915)	NS	-16339 (-31977, -701)	0.038	-24616 (-40254, -24616)	<0.001
Androsterone	T_{max}	4.93 ± 0.89	5.08 ± 0.79	4.74 ± 0.76	0.14	NS	NS	-0.61 (-3.3, 2.1)	NS	-0.30 (-3.0, 2.4)	NS	0.32 (-2.4, 0.32)	NS
	C_{max}	6.93 ± 0.44	6.25 ± 0.46	6.42 ± 0.58	0.12	NS	NS	-0.20 (-1.2, 0.80)	NS	-0.04 (-1.0, 0.96)	NS	0.16 (-0.83, 0.16)	NS
	AUC_{12}	46.6 ± 3.6	44.1 ± 3.5	43.3 ± 4.5	0.21	NS	NS	0.82 (-4.3, 6.0)	NS	-0.61 (-5.8, 4.5)	NS	-1.4 (-6.6, -1.4)	NS
Androstenedione	T_{max}	3.36 ± 0.52	5.13 ± 0.58	2.23 ± 0.81	5.03	0.018	NS	2.0 (-0.23, 4.1)	0.092	-0.95 (-3.1, 1.2)	NS	-2.9 (-5.1, -2.9)	0.0053
in women	C_{max}	3.63 ± 0.40	3.46 ± 0.23	2.59 ± 0.24	11.6	<0.001	0.0082	-0.20 (-0.78, 0.38)	NS	-1.1 (-1.7, -0.53)	<0.001	-0.91 (-1.5, -0.91)	<0.001
	AUC_{12}	31.5 ± 3.4	31.2 ± 2.1	23.6 ± 2.1	8.07	0.0032	0.044	-0.96 (-6.7, 4.8)	NS	-8.9 (-15, -3.2)	<0.001	-8.0 (-14, -8.0)	0.0032
Androstenedione	T_{max}	3.46 ± 0.57	3.96 ± 0.51	2.83 ± 0.82	0.76	NS	NS	0.50 (-1.6, 2.6)	NS	-0.63 (-2.8, 1.5)	NS	-1.1 (-3.3, -1.1)	NS
in men	C_{max}	2.68 ± 0.18	2.52 ± 0.18	2.14 ± 0.19	8.42	0.0019	0.027	-0.16 (-0.47, 0.15)	NS	-0.53 (-0.84, -0.22)	<0.001	-0.37 (-0.69, -0.37)	0.014
	AUC_{12}	22.3 ± 1.2	23.0 ± 1.6	18.7 ± 1.8	8.66	0.0017	0.023	0.64 (-1.9, 3.2)	NS	-3.6 (-6.2, -1.0)	0.0031	-4.2 (-6.8, -4.2)	<0.001
Testosterone	T_{max}	3.32 ± 0.96	6.25 ± 0.83	3.64 ± 1.05	2.36	NS	NS	2.6 (-0.60, 5.7)	NS	0.05 (-3.1, 3.2)	NS	-2.5 (-5.6, -2.5)	NS
in women	C_{max}	0.88 ± 0.1	0.88 ± 0.1	0.87 ± 0.14	0.15	NS	NS	0.01 (-0.26, 0.28)	NS	0.06 (-0.21, 0.33)	NS	0.05 (-0.22, 0.05)	NS
	AUC_{12}	9.25 ± 1.1	9.59 ± 1.2	8.14 ± 0.98	0.77	NS	NS	0.50 (-1.2, 2.2)	NS	-0.42 (-2.2, 1.3)	NS	-0.92 (-2.7, -0.92)	NS
Testosterone	T_{max}	4.21 ± 1.0	4.75 ± 0.66	3.50 ± 0.79	0.64	NS	NS	0.54 (-2.1, 3.1)	NS	-0.71 (-3.3, 1.9)	NS	-1.3 (-3.9, -1.3)	NS
in men	C_{max}	6.23 ± 0.40	6.17 ± 0.4	5.69 ± 0.37	1.46	NS	NS	-0.06 (-0.87, 0.74)	NS	-0.54 (-1.3, 0.27)	NS	-0.47 (-1.3, -0.47)	NS
	AUC_{12}	62.0 ± 4.3	62.0 ± 3.8	57.0 ± 3.6	1.59	NS	NS	0.00 (-7.7, 7.7)	NS	-5.1 (-13, 2.6)	NS	-5.1 (-13, -5.1)	NS
Testosterone +	T_{max}	3.41 ± 0.52	5.13 ± 0.58	2.50 ± 0.78	4.14	0.033	NS	1.9 (-0.29, 4.1)	NS	-0.70 (-2.9, 1.5)	NS	-2.6 (-4.8, -2.6)	0.015
androstenedione	C_{max}	4.45 ± 0.39	4.30 ± 0.22	3.40 ± 0.28	7.74	0.0038	0.053	-0.15 (-0.82, 0.53)	NS	-1.0 (-1.7, -0.37)	<0.001	-0.90 (-1.6, -0.90)	0.0051
in women	AUC_{12}	40.8 ± 3.5	40.8 ± 2.2	31.7 ± 2.4	6.83	0.0062	0.087	-0.46 (-7.2, 6.2)	NS	-9.4 (-16, -2.7)	0.0032	-8.9 (-16, -8.9)	0.0052
Testosterone +	T_{max}	4.38 ± 0.96	4.13 ± 0.62	3.04 ± 0.69	0.84	NS	NS	-0.25 (-2.8, 2.3)	NS	-1.3 (-3.9, 1.2)	NS	-1.1 (-3.6, -1.1)	NS
androstenedione	C_{max}	8.50 ± 0.48	8.54 ± 0.48	7.62 ± 0.51	2.86	0.079	NS	0.05 (-0.97, 1.1)	NS	-0.87 (-1.9, 0.14)	NS	-0.92 (-1.9, -0.92)	0.086
in men	AUC_{12}	84.4 ± 4.7	85.0 ± 4.7	75.7 ± 4.7	3.19	0.061	NS	0.64 (-9.0, 10)	NS	-8.7 (-18, 0.99)	0.089	-9.3 (-19, -9.3)	0.061
Progestins													
Progesterone	T_{max}	5.73 ± 1.3	6.54 ± 0.98	4.73 ± 1.4	0.15	NS	NS	0.45 (-4.0, 4.9)	NS	-0.60 (-5.1, 3.9)	NS	-1.1 (-5.5, -1.1)	NS
in women	C_{max}	3.38 ± 2.4	4.95 ± 3.2	1.94 ± 1.1	0.76	NS	NS	2.2 (-5.0, 9.3)	NS	-1.6 (-8.8, 5.6)	NS	-3.8 (-11, -3.8)	NS
	AUC_{12}	31.8 ± 24.4	41.6 ± 26.7	17.4 ± 11.7	0.57	NS	NS	14 (-52, 81)	NS	-16 (-83, 51)	NS	-30 (-97, -30)	NS
Progesterone	T_{max}	3.25 ± 0.40	3.42 ± 0.6	4.75 ± 0.94	1.53	NS	NS	0.17 (-2.0, 2.4)	NS	1.5 (-0.70, 3.7)	NS	1.3 (-0.87, 1.3)	NS
in men	C_{max}	0.52 ± 0.06	0.50 ± 0.06	0.39 ± 0.06	13.7	<0.001	0.0019	-0.02 (-0.08, 0.04)	NS	-0.13 (-0.19, -0.06)	<0.001	-0.11 (-0.17, -0.11)	<0.001
	AUC_{12}	4.34 ± 0.64	4.47 ± 0.64	3.84 ± 0.68	13.9	<0.001	0.0018	0.13 (-0.16, 0.43)	NS	-0.50 (-0.79, -0.20)	<0.001	-0.63 (-0.93, -0.63)	<0.001
17α-Hydroxy-	T_{max}	4.20 ± 0.58	4.27 ± 0.35	4.24 ± 0.79	0.02	NS	NS	0.00 (-2.0, 2.0)	NS	0.14 (-1.9, 2.2)	NS	0.14 (-1.9, 0.14)	NS

progesterone	C_{max}	4.01 ± 0.47	3.72 ± 0.46	2.91 ± 0.58	2.97	0.062	NS	-0.19 (-1.3, 0.96)	NS	-1.1 (-2.3, 0.03)	0.059	-0.93 (-2.1, -0.93)	NS
	AUC_{12}	31.2 ± 3.8	30.8 ± 4.0	24.5 ± 5.0	1.63	NS	NS	0.48 (-9.9, 11)	NS	-6.7 (-17, 3.7)	NS	-7.2 (-18, -7.2)	NS

Values for amphetamine and steroids are mean ± SEM in 23, 24 and 23 subjects after administration of D-amphetamine, lisdexamfetamine and placebo, respectively. Values for the subjective effects are from 24 subjects (mean ± SEM). CI, Confidence Interval 95%; T_{onset}, time to reach 10% of C_{max} (h); C_{max}, peak plasma concentration (nM); E_{max}, maximal effect on the Visual Analog Scale (%max); NS, not significant (p value > 0.1); T_{max}, time to reach C_{max} (h); AUC_{12}, area under the concentration–time curve to 12 h (ng×h/mL and nM×h and for amphetamine and steroids, respectively); DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; [a]for amphetamine $F_{1,22}$; [b]Subjective effects: $T_{onset/max}$: $F_{1,20}$, E_{max} and AUC_{12}: $F_{2,46}$; [c]only women $F_{2,18}$ or only men $F_{2,22}$. There were no significant differences in the steroid plasma concentrations between D-amphetamine and lisdexamfetamine.

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Effects of D-amphetamine and lisdexamfetamine on plasma steroid and adrenocorticotropic hormone concentrations

The effects of D-amphetamine, lisdexamfetamine, and placebo on plasma steroid hormone concentrations are shown in Fig. 3 and 4. Table 1 shows the corresponding T_{max}, C_{max}, and AUC values, with comparative statistics.

Sex steroid levels were different between males and females when sex was added as additional factor to the ANOVAs (effects of sex: $F_{1,20}$ = 5.34, p = 0.032; 184, p < 0.001; 3.21, p = 0.088;, and 61.7, p < 0.001, for androstenedione, testosterone, progesterone, and testosterone+androstenedione, respectively) but sex did not moderate the drug effects (no relevant sex × drug interactions [Supplementary Table S1]). Therefore, ANOVAs were conducted and results shown for each sex separately for androstenedione, testosterone, progesterone, and testosterone+androstenedione.

Both active treatments significantly and similarly increased the plasma concentrations of the glucocorticoids cortisol, cortisone, corticosterone, 11-dehydrocorticosterone, and 11-deoxycortisol compared with placebo (Fig. 3A-E, Table 1). Elevated glucocorticoid production was reflected by significant increases in the sums of active and inactive glucocorticoids (i.e., cortisol+cortisone, corticosterone+11-dehydrocorticosterone; Table 1) and all glucocorticoids measured (data not shown). The cortisol/cortisone and corticosterone/11-dehydrocorticosterone ratios also increased (Table 1). The pharmacokinetic parameters, such as C_{max}, T_{max}, and AUC_{12}, and the shape of the concentration-time curves for corticosteroids were practically identical following D-amphetamine and lisdexamfetamine administration (Fig. 3, Table 1). The mineralocorticoids aldosterone (Fig. 3F) and 11-deoxycorticosteone (data not shown) and the progestogen 17α-hydroxyprogesterone (Fig. 4C) were unaltered by lisdexamfetamine and D-amphetamine compared with placebo. One exception was plasma progesterone concentrations in men, in which greater C_{max} and AUC values were found compared with placebo (Table 1, Supplementary Fig. S1). Progesterone in women was not significantly altered, although a trend toward an increase was observed (Supplementary Fig. S1). The plasma concentrations of DHEA, DHEAS, and androstenedione (Fig. 4A, B, D, E) were significantly increased by the two active drugs compared with placebo. However, lisdexamfetamine and D-amphetamine had no effect on C_{max} and AUC values for the sum of androstenedione+testosterone neither in women nor in men. Moreover, lisdexamfetamine and D-amphetamine had no effect on the concentrations of testosterone and the androgen degradation metabolite androsterone (Table 1). The plasma concentrations of 11-deoxycorticosterone were above the LOD but below the LLOQ; therefore, the quantification of this steroid was not validly possible.

Figure 3. Plasma concentrations of glucocorticoids and mineralocorticoids (mean and SEM) following administration of D-amphetamine, lisdexamfetamine, and placebo in 23, 24, and 23 subjects, respectively. CYP, cytochrome P450; HSD, hydroxysteroid dehydrogenase. Lisdexamfetamine and D-amphetamine significantly increased the plasma concentrations of the glucocorticoids 11-deoxycortisol (A), 11-dehydrocorticosterone (B), cortisol (C), cortisone (E), and corticosterone (D) compared with placebo. The plasma concentration of aldosterone (F) was unaltered after D-amphetamine and lisdexamfetamine administration compared with placebo.

Figure 4. Plasma concentrations of androgens and one progestogen (mean and SEM) following administration of D-amphetamine, lisdexamfetamine, and placebo in 23, 24, and 23 subjects, respectively. The data in men represent the mean and SEM in 12 subjects. The data in women represent the mean and SEM in 11, 12, and 11 subjects following administration of D-amphetamine, lisdexamfetamine, and placebo, respectively. The plasma concentrations of dehydroepiandrosterone (DHEA) (A), dehydroepiandrosterone sulfate (DHEAS) (B), and androstenedione in women (D) and men (E) were significantly elevated

following administration of D-amphetamine and lisdexamfetamine compared with placebo, whereas no effect on 17α-hydroxyprogesterone (C) was observed.

Plasma concentrations of ACTH are shown in Fig. 5. A main effect of drug was found at the 3.5 h time point ($F_{2,40}$ = 33.83, p < 0.001), and both active drugs resulted in higher plasma ACTH concentrations compared with placebo at 3.5 h (both p < 0.001). There were no relevant order × treatment interactions in the ANOVAs, indicating the absence of confounding by treatment order as expected based on the counter-balanced design (Supplementary Table S1).

Figure 5. Plasma concentration of ACTH measured 1 h before and 3.5 h after drug administration (mean and SEM). Plasma ACTH concentrations increased 3.5 h after D-amphetamine and lisdexamfetamine administration compared with placebo. ***p < 0.001, compared with placebo.

Relationship between plasma amphetamine and steroid concentrations after D-amphetamine and lisdexamfetamine administration

Selected drug exposure-steroid concentration response relationships are shown in Supplementary Fig. S2. Clockwise hysteresis was observed, indicating acute pharmacological tolerance.

Peak endocrine effects following D-amphetamine and lisdexamfetamine administration compared with other prototypical substances

The peak endocrine effects of D-amphetamine, lisdexamfetamine, methylphenidate, MDMA, and LSD are shown in Table 2. The drug effects are presented as within-subject changes from placebo (placebo-corrected responses). D-amphetamine, lisdexamfetamine, and methylphenidate produced comparable increases in cortisol. D-amphetamine increased cortisone and 11-dehydrocorticosterone to greater levels than methylphenidate. MDMA induced higher peak concentrations of cortisol, lower levels of cortisone, but still higher cortisol+cortisone levels than D-amphetamine. LSD produced much higher peak concentrations of cortisol and corticosterone than D-amphetamine, but in contrast to MDMA, the levels of cortisone and 11-dehydrocorticosterone resembled those that were observed after D-amphetamine administration.

Table 2. Peak effects of D-amphetamine, lisdexamfetamine, methylphenidate, MDMA, and LSD on plasma glucocorticoids.

	D-Amphetamine 40 mg (N=22)	Lisdexamfetamine 100 mg (N=23)	Methylphenidate 60 mg (N=16)[a]	MDMA 125 mg (N=16)[a]	LSD 200 µg (N=16)[b]	$F_{4,88}$	p value
Cortisol	314.5 ± 25.7	298.5 ± 17.4	275.7 ± 48.1	513.1 ± 51.9**	690.1 ± 54.3***	20.4	<0.001
Cortisone	38.0 ± 3.3	39.0 ± 3.1	18.1 ± 3.4***	16.4 ± 3.3***	37.5 ± 4.1	10.5	<0.001
Cortisol + cortisone	337.8 ± 27.9	323.5 ± 19.5	288.8 ± 49.1	520.3 ± 54.0*	721.4 ± 55.6***	19.3	<0.001
Corticosterone	19.5 ± 2.7	16.4 ± 2.0	8.9 ± 2.5	27.6 ± 2.4	34.9 ± 3.8**	12.4	<0.001
11-Dehydrocorticosterone	6.5 ± 0.8	6.1 ± 0.6	1.9 ± 0.4***	4.3 ± 0.4	6.0 ± 0.9	7.68	<0.001
Corticosterone + 11-dehydrocorticosterone	26.0 ± 3.4	22.0 ± 2.6	10.3 ± 2.9**	31.6 ± 2.5	40.5 ± 4.6*	10.4	<0.001

Values are mean ± SEM of the peak differences from placebo. *P<0.05, **P<0.01, ***P<0.001 Tukey post hoc test compared with D-amphetamine. MDMA, 3,4-methylenedioxymethamphetamine or ecstasy; LSD, lysergic acid diethylamide. Data were adjusted from [a]Seibert et al. 2014 and [b]Strajhar et al. 2016.

Discussion

The main finding of the present study was that the novel ADHD treatment lisdexamfetamine produced similar HPA axis stimulation and plasma steroid concentration-time curves as the classic immediate-release D-amphetamine. These findings did not support the hypothesis of the study, in which we expected to observe a smaller and more prolonged endocrine response to lisdexamfetamine compared with D-amphetamine. The reason for the identical endocrine responses of the two amphetamine formulations was the unexpected finding of similar peak amphetamine concentrations after lisdexamfetamine and D-amphetamine administration. Lisdexamfetamine had a significantly longer onset and thus also T_{max} but otherwise a very similar plasma amphetamine concentration-time curve shape compared with D-amphetamine. D-Amphetamine administration 1 h later would likely have produced a pharmacokinetic profile that was almost identical to lisdexamfetamine. The steroid concentration-time curves were shifted to the right (delayed in time), similar to the plasma amphetamine concentration-time curve after lisdexamfetamine administration compared with D-amphetamine, but this effect did not reach statistical significance for any of the steroid concentration T_{max} values.

Unexpectedly, the present study found similar C_{max} values for amphetamine and all of the steroids at equivalent doses of lisdexamfetamine and D-amphetamine, which is in contrast to the limited preclinical [10] and clinical [7, 8] data that were used to generate the present study hypotheses. Specifically, a previous study in rats found a lower C_{max} for amphetamine after lisdexamfetamine administration compared with D-amphetamine administration [10], in contrast to the present human data. An initial pharmacokinetic study in humans (referred to in [7]) reportedly found a longer T_{max} and lower C_{max} for plasma amphetamine after lisdexamfetamine administration compared with immediate-release D-amphetamine. However, these data have not been published. The present relatively large study clearly showed comparable C_{max} values for amphetamine after administration of both lisdexamfetamine and D-amphetamine, with equal AUC values, thus demonstrating the equivalence of the drug doses and formulations used (Fig. 2, Table 1, see also [11]). Additionally, in the present study, the subjective and cardiovascular responses to lisdexamfetamine and D-amphetamine did not differ as also reported in detail elsewhere [11]. In contrast, another study in chronic stimulant users found that 100 mg lisdexamfetamine induced lower ratings of subjective “drug liking” than 40 mg D-amphetamine [7]. However, ratings of euphoria, amphetamine-like effects, and stimulant effects did not differ between the two treatments [7]. Altogether, the present findings indicate that the pharmacokinetics and pharmacodynamics of a high dose of the newly marketed medication lisdexamfetamine were practically identical to an equimolar dose of the classic immediate-release D-amphetamine that was administered 1 h later. The present data indicate that the conversion of the prodrug lisdexamfetamine to D-amphetamine only delays the onset of the increase in amphetamine concentrations in the body, without causing relevant alterations in the slope or maximal concentrations. Therefore, lisdexamfetamine unlikely has prolonged clinical effects (aside from the later onset) or a lower abuse potential compared with immediate-release D-amphetamine when used orally (unless the delayed onset is considered to reduce immediate rewarding effects). A lower risk of oral misuse may be expected with a slow elevation of plasma D-amphetamine concentrations and its associated effects, but this was clearly not the case, at least not at the doses tested in the present study. In contrast, extended-release amphetamines may have a lower and delayed C_{max} compared with lisdexamfetamine [44]. Parenteral misuse

of lisdexamfetamine produced effects that were comparable to oral use [45, 46], suggesting an intranasal and intravenous abuse-deterrent property of lisdexamfetamine compared with D-amphetamine.

The effects of lisdexamfetamine on the HPA axis have not been previously studied. In the present study, the effects of lisdexamfetamine and D-amphetamine on HPA axis activation were similar. Both amphetamines increased the concentrations of the active glucocorticoids cortisol and corticosterone and their respective inactive metabolite and precursor cortisone and 11-dehydrocorticosterone, which has been similarly reported for lower doses of D-amphetamine [16, 25, 26, 28-31, 47]. The mineralocorticoids aldosterone and 11-deoxycorticosterone were unaltered by lisdexamfetamine or D-amphetamine, in contrast to increases that were found after MDMA administration [13]. Plasma concentrations of the adrenal androgen precursors DHEA, DHEAS, and androstenedione increased, whereas testosterone and its degradation product androsterone were unaltered by the two D-amphetamine formulations. Plasma progesterone levels increased compared with placebo in men. In women, the absolute increase appeared to be larger but did not reach significance because of high interindividual variability (Supplementary Fig. S1).

In the present study, we statistically compared the endocrine effects and especially cortisol of D-amphetamine with other psychoactive substances that were tested in previous separate studies in our laboratory in healthy subjects under similar conditions [13, 41]. In contrast to our hypothesis, D-amphetamine and lisdexamfetamine produced effects on plasma cortisol and corticosterone concentrations that were comparable to methylphenidate. Although the effects of methylphenidate on these active corticosteroids did not reach significance compared with placebo in our previous smaller study [13], the respective effects of D-amphetamine that were significant compared with placebo in the present study were not significantly greater than those of methylphenidate. However, D-amphetamine produced greater cortisone and 11-dehydrocorticosterone levels than methylphenidate. Nevertheless, the present study indicates that the overall effects of D-amphetamine, lisdexamfetamine, and methylphenidate on plasma steroids at equivalent psychostimulant doses [37] are largely congruent.

Norepinephrine, DA, and 5-HT have all been implicated in mediating HPA axis stimulation [41, 48]. However, the relative contribution of these monoamines to psychotropic-induced HPA axis stimulation in humans is unclear [48]. D-amphetamine may release cortisol mainly via NE [38]. Specifically, D-amphetamine more potently interacts with the NE transporter compared with the DA and 5-HT transporters, and it has a very low potency at the 5-HT transporter [35]. Additionally, the effects of D-amphetamine and methamphetamine on plasma corticosteroids were blocked by α-adrenergic receptor antagonists [49] but not DA receptor antagonists [50]. Purely or predominantly serotonergic substances strongly stimulate the HPA axis [32, 41, 43]. In the present study, we also statistically compared the effects of D-amphetamine with similar historical data on MDMA and LSD that were obtained in the same laboratory using the same clinical and analytical methods [13, 41]. Compared with D-amphetamine and methylphenidate (which stimulate NE and DA), MDMA and LSD (which mainly stimulate 5-HT) produced greater increases in plasma concentrations of the biologically active glucocorticoids cortisol and corticosterone. Additionally, the MDMA-induced elevation of plasma cortisol was shown to be mediated by the release of 5-HT but not DA [51, 52]. These findings support the view that 5-HT activation primarily or more strongly increases plasma cortisol compared with activation of the DA or NE systems [13, 41, 43].

We found other differential effects of the substances studied herein on HPA axis stimulation. Notably, compared with D-amphetamine, MDMA-induced increases in cortisol and corticosterone were paralleled by relatively smaller changes in the respective 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2)-formed metabolite and precursor cortisone and 11-dehydrocorticosterone, indicating impairments in 11β-HSD2 activity that were caused by inhibition or saturation at elevated substrate concentrations by MDMA. In contrast, the LSD-induced increases in cortisol and corticosterone were significantly higher compared with D-amphetamine, whereas the inactive metabolites cortisone and 11-dehydrocorticosterone

induced comparable increases as those after D-amphetamine administration. Both the 5-HT releaser MDMA and 5-HT receptor agonist LSD [42] increased the sum of cortisol+cortisone more than D-amphetamine, indicating greater glucocorticoid production. This finding further supports the critical role of 5-HT in HPA axis stimulation by psychoactive substances and supports the use of cortisol as a marker of acute 5-HT activation [13, 41, 43].

In the present study, the endocrine response to both D-amphetamine formulations showed moderate acute tolerance, reflected by clockwise hysteresis of the amphetamine *vs*. cortisol or corticosterone concentration plots and as reported previously for the subjective response to D-amphetamine [53]. Plasma corticosterone levels normalized more rapidly than D-amphetamine disappeared from plasma (Supplementary Fig. S2). The characteristics of these hysteresis curves were similar after lisdexamfetamine and D-amphetamine administration, thus pointing towards the similarity of these two formulations in humans, in contrast to previous animal data on the pharmacokinetic-pharmacodynamic relationship [10]. Even more pronounced acute tolerance to the cortisol and corticosterone responses was previously reported for the amphetamine derivative MDMA [13, 41] but not for the direct receptor agonist LSD [41]. The effects of lisdexamfetamine and D-amphetamine on plasma concentrations of cortisol and corticosterone lasted 10-12 h in the present study, whereas the effects of MDMA lasted only 4-6 h. These findings may reflect the somewhat longer half-live of D-amphetamine compared with MDMA (11 h *vs*. 8 h, respectively) [54, 55] and likely also more pronounced acute tolerance to the effects of MDMA compared with D-amphetamine.

Activation of the HPA axis by amphetamines may be clinically relevant. This activation reflects a pharmacological stress response and has been shown to include increases in other endocrine markers of stress, including copeptin, oxytocin, epinephrine, and NE in the case of MDMA [13, 15, 40, 56, 57]. In recreational settings, MDMA increased plasma cortisol levels by up to 800% [58]. These marked endocrine responses that are induced by psychostimulants may affect mood, energy metabolism, sleep, and immune function [12, 59]. For example, D-amphetamine, methylphenidate, and MDMA increased natural killer cells in plasma, reflecting activation of innate immune function [12, 60]. Increases in plasma epinephrine concentrations after methylphenidate and MDMA administration were associated with acute increases in circulating natural killer cells [12]. Increases in plasma cortisol following MDMA administration correlated with MDMA's cardiovascular effects and subjective "drug liking" [61]. Steroids may contribute to the mood-enhancing effects of psychostimulants [61-64], enhance the rewarding and reinforcing effects of drugs [24], and increase the risk of misuse. Furthermore, the disruption of circadian rhythms, including steroid secretion, has been associated with impairments in immune function, metabolic disturbances, eating and mood disorders, and cancer progression [65]. Several studies suggest that the chronic misuse of amphetamines interferes with HPA axis function and its circadian rhythms [66-68]. The effects of different chronic stimulant medications on cortisol levels in patients are unclear [69]. Some studies reported elevated morning or bedtime cortisol levels during treatment with methylphenidate and atomoxetine [70], transient increases in cortisol levels during methylphenidate treatment with normalization over 6 months [71], or no effect of methylphenidate [72]. Comparable data on the effects of chronic lisdexamfetamine and D-amphetamine administration on cortisol levels are lacking. Tolerance to subjective and cardiostimulant effects has been observed with chronic lisdexamfetamine use [73-75]. However, whether and to what extent tolerance develops to the neuroendocrine effects of chronic administration of these D-amphetamine formulations and the time-course of such tolerance remain to be determined.

The present study has limitations. We used only single and relatively high doses of lisdexamfetamine and D-amphetamine. The single dose of 100 mg lisdexamfetamine was above the maximal therapeutic dose for the treatment of ADHD of 70 mg. However, the single dose of 100 mg lisdexamfetamine mimics the misuse of lisdexamfetamine and produces plasma D-amphetamine concentrations that were comparable to those of repeated daily administration of 70 mg lisdexamfetamine when steady state is reached. Furthermore, plasma exposure to D-amphetamine would be higher in children compared to adults after the administration of the same dose of lisdexamfetamine [76]. Nevertheless, we cannot exclude

possible differences in the pharmacokinetics and endocrine effects of lisdexamfetamine and D-amphetamine at lower or higher doses than those used in the present study. Additionally, we studied only acute administration. Repeated lisdexamfetamine administration may result in tolerance to its endocrine effects, which has been reported for subjective effects with chronic use [73-75]. Furthermore, the statistical comparisons between the effects of D-amphetamine, methylphenidate, MDMA, and LSD relied on data from different studies within the same laboratory, and thus such comparisons were indirect and not within the same study and subjects. Thus, we cannot exclude that the differences are due to differences between studies rather than drugs. This part of the study was also limited by the use of only one dose for all of the substances.

Conclusion

Lisdexamfetamine and an immediate-release D-amphetamine formulation produced similar peak plasma concentrations of active D-amphetamine and HPA axis stimulation in healthy subjects, suggesting similar pharmacokinetic, endocrine, and likely oral abuse-related properties. Moderate acute pharmacological tolerance to the endocrine response to lisdexamfetamine and D-amphetamine was observed. Whether chronic tolerance develops to the endocrine response of amphetamines requires further study. Comparable HPA axis activation was induced by the noradrenergic/dopaminergic substances lisdexamfetamine, D-amphetamine, and methylphenidate, whereas the serotonergic substances MDMA and LSD induced significantly greater HPA axis activation, supporting a predominant role for 5-HT in HPA axis stimulation by psychoactive substances.

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Supporting Information

Checklist S1.

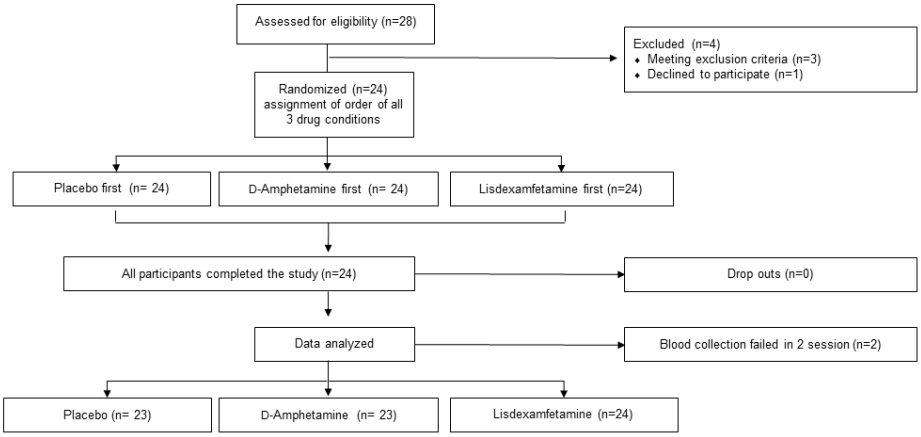
Protocol S1.

S1 File. Experimental section: Quantification of D-amphetamine in human plasma samples.

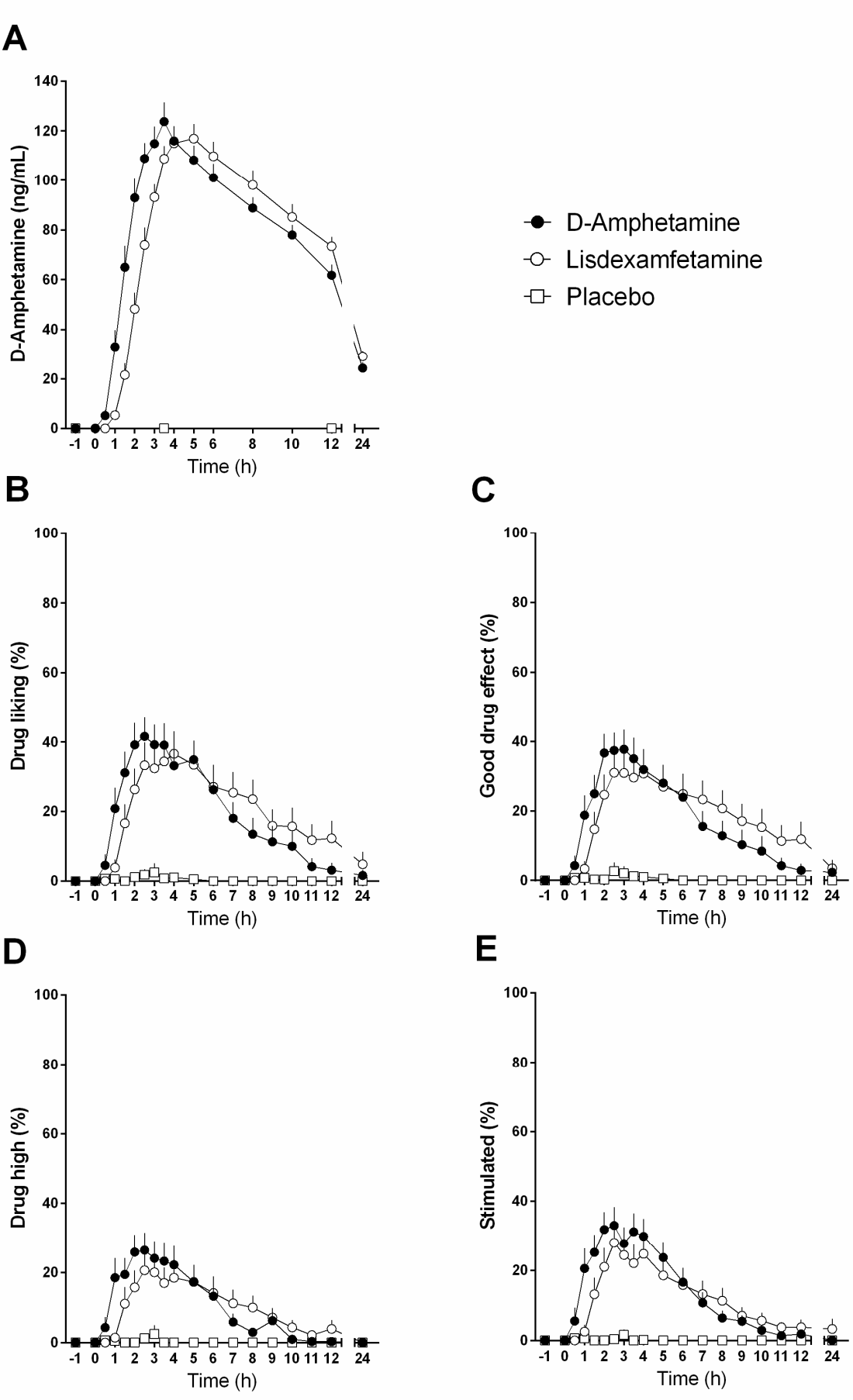
S1 Fig. Plasma concentrations of progesterone. Data in men represent mean and SEM in 12 subjects, whereas data in women represent mean and SEM in 11, 12, and 11 subjects following administration of D-amphetamine, lisdexamfetamine, and placebo, respectively.

S2 Fig. Drug-induced changes in plasma concentrations of cortisol (A) and corticosterone (B) plotted against D-amphetamine concentrations over time (hysteresis curves) after administration of lisdexamfetamine and D-amphetamine in 24 and 23 subjects, respectively. The endocrine response represents the difference from placebo calculated for each time point to account for circadian changes in hormone levels. Lisdexamfetamine and D-amphetamine were administered at t = 0. The time of sampling is noted next to each point. The clockwise hysteresis indicates acute pharmacological tolerance to the endocrine response of amphetamine which was comparable after administration of the two formulations. Data are mean ± SEM.

S1 Table. Interaction analysis of plasma steroids and subjective effects after D-amphetamine, lisdexamfetamine, or placebo with sex and treatment order.

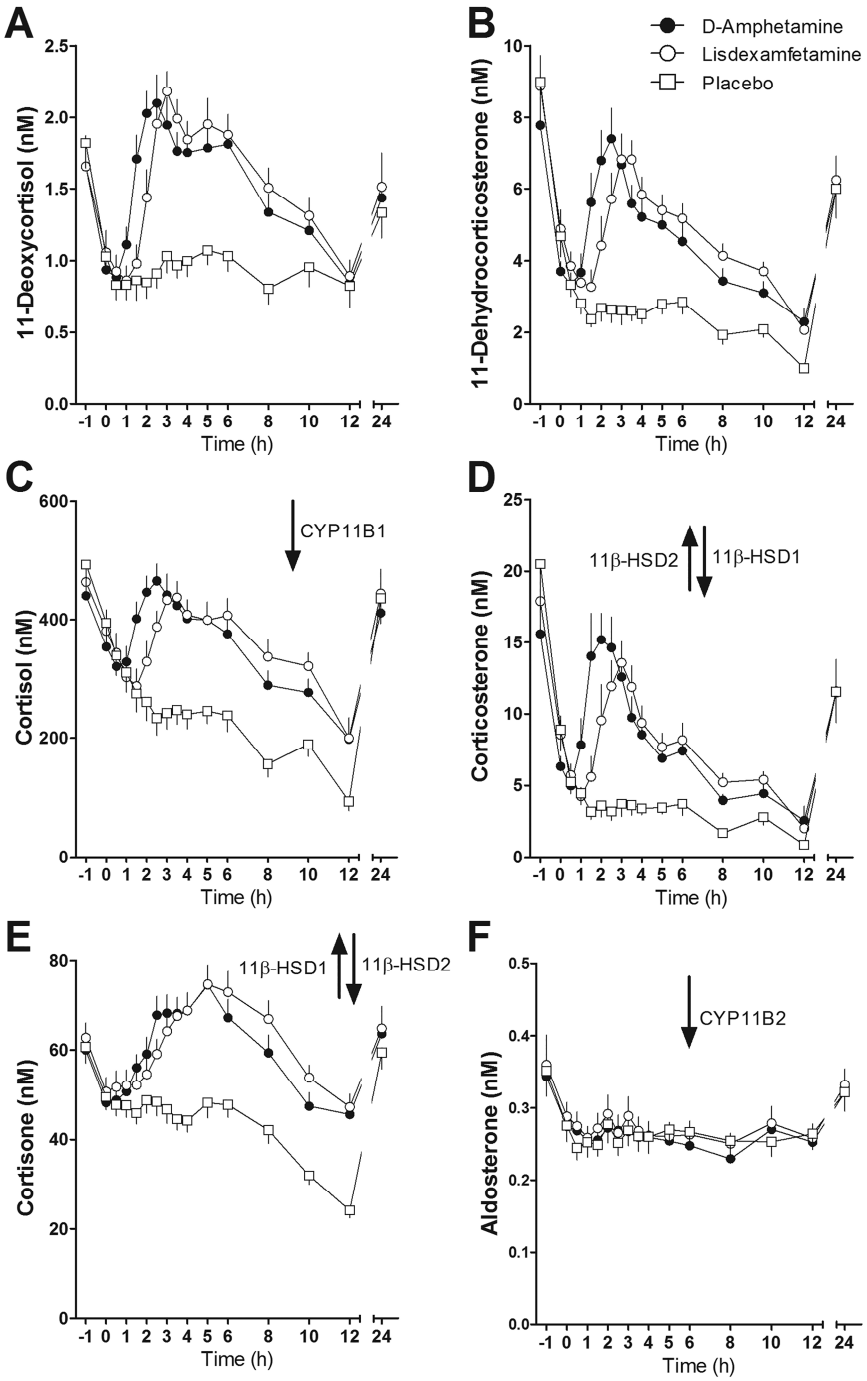

Assessed for eligibility (n=28)
Excluded (n=4)
Meeting exclusion criteria (n=3)
Declined to participate (n=1)
Randomized (n=24) assignment of order of all 3 drug conditions
Placebo first (n= 24)
D-Amphetamine first (n= 24)
Lisdexamfetamine first (n=24)
All participants completed the study (n=24)
Drop outs (n=0)
Data analyzed
Blood collection failed in 2 session (n=2)
Placebo (n= 23)
D-Amphetamine (n= 23)
Lisdexamfetamine (n=24)

878

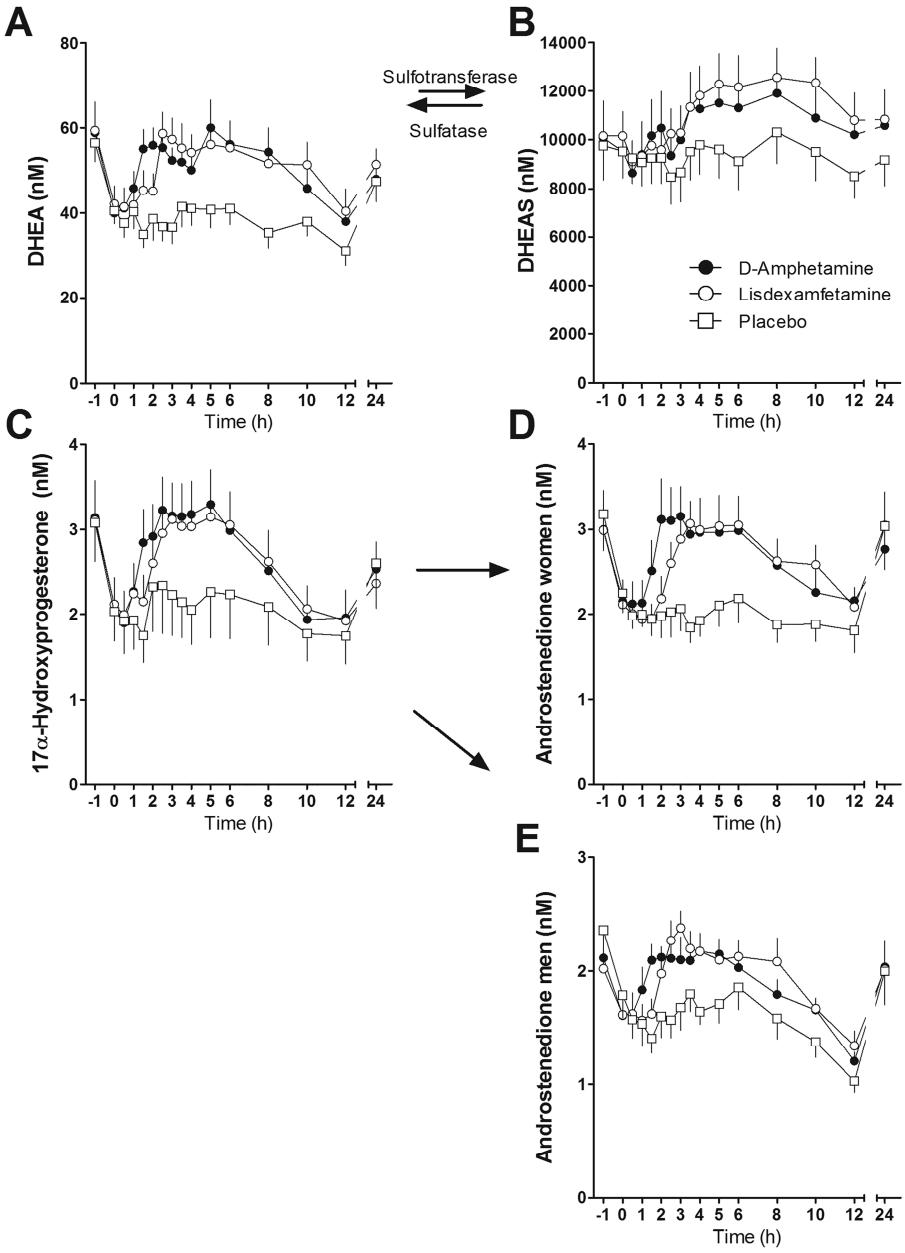

A
D-Amphetamine (ng/mL)
Time (h)
D-Amphetamine
Lisdexamfetamine
Placebo
B
Drug liking (%)
Time (h)
C
Good drug effect (%)
Time (h)
D
Drug high (%)
Time (h)
E
Stimulated (%)
Time (h)

879

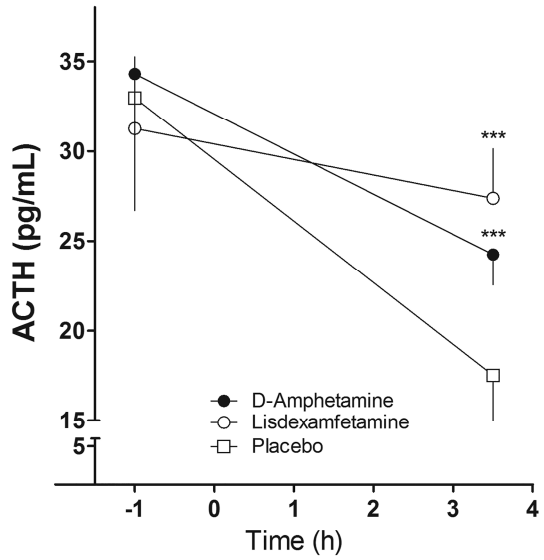
880


A
11-Deoxycortisol (nM)
Time (h)
B
D-Amphetamine
Lisdexamfetamine
Placebo
11-Dehydrocorticosterone (nM)
Time (h)
C
CYP11B1
Cortisol (nM)
Time (h)
D
11β-HSD2
11β-HSD1
Corticosterone (nM)
Time (h)
E
11β-HSD1
11β-HSD2
Cortisone (nM)
Time (h)
F
CYP11B2
Aldosterone (nM)
Time (h)

881
882


A
DHEA (nM)
Sulfotransferase
Sulfatase
B
DHEAS (nM)
D-Amphetamine
Lisdexamfetamine
Placebo
C
17α-Hydroxyprogesterone (nM)
D
Androstenedione women (nM)
E
Androstenedione men (nM)
Time (h)

883
884


35
30
25
20
15
5
ACTH (pg/mL)
***
***
D-Amphetamine
Lisdexamfetamine
Placebo
-1
0
1
2
3
4
Time (h)

885