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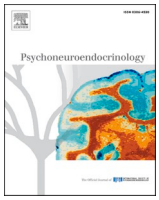

Associations of psychoactive substances and steroid hormones in hair: Findings relevant to stress research from a large cohort of young adults

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ABSTRACT

Objective: Epidemiological studies increasingly use hair samples to assess people's cumulative exposure to steroid hormones, but how the use of different psychoactive substances may affect steroid hormone levels in hair is, so far, largely unknown. The current study addresses this gap by establishing the substance exposure correlates of cortisol, cortisone, and testosterone in hair, while also accounting for a number of relevant covariates.

Method: Data came from a large urban community-sample of young adults with a high prevalence of substance use (N = 1002, mean age=20.6 years, 50.2% female), who provided 3 cm of hair samples. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) quantified cortisol, cortisone, and testosterone, as well as delta-9-tetrahydrocannabinol (THC), 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy"), cocaine, several opioids, and their respective metabolites. Multiple linear regression models with covariates were used to predict steroid hormone levels from substance exposure in a four-step approach: In the full sample, low and high substance hair concentrations (median split) were first tested against no use for each substance individually (step 1) and for all substances together (step 2). Then, within the participants with any substance in hair only, the continuous hair concentration of each substance in pg/mg (step 3) and finally of all substances together, were regressed (step 4).

Results: Low, high, and continuous levels of THC in hair were robustly associated with higher levels of cortisol (sig. in step 1 low THC: $\beta = 0.29$, $p = .021$; high THC: $\beta = 0.42$, $p = .001$; step 2: low THC: $\beta = 0.27$, $p = 0.036$, and high THC: $\beta = 0.40$, $p = .004$, and step 4: $\beta = 0.12$, $p = .041$). Participants with high MDMA levels had higher levels of cortisone without adjusting for other substances (step 1: $\beta = 0.34$, $p = .026$), but this effect was not significant in the other models. While high THC levels were associated with lower levels of testosterone in step 2 ($\beta = -0.35$, $p = .018$), MDMA concentration was positively related to testosterone concentration with and without adjusting for other substances (step 3: $\beta = 0.24$, $p = .041$; step 4: $\beta = 0.17$, 95%, $p = .015$) in male participants.

Conclusion: The use of psychoactive substances, especially of cannabis and ecstasy, should be considered in studies investigating steroid hormones in hair.

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1. Introduction

Psychological and psychiatric research increasingly relies on the use of hair samples as a non-invasive procedure for obtaining cumulative and reliable measures of steroid hormones such as cortisol, cortisone, and testosterone. Specifically, glucocorticosteroids (cortisol, cortisone) are used to index how individuals respond to internal and external stressors, while testosterone is examined as a correlate of behavior and metabolic health. Past research has identified key covariates of steroid hormones in hair, including sex, age, hair color, and use of contraceptives in females (Binz et al., 2018; Carillo Vázquez et al., 2022; Stalder and Kirschbaum, 2012; Stalder et al., 2017; Wosu et al., 2015); these covariates are now routinely taken into account in analyses of steroid hormones in hair. Past work measuring steroids in hair typically did not consider the use of psychoactive substances as a covariate, but preliminary evidence suggests that 3,4-methylenedioxymethamphetamine (MDMA, also known as "Ecstasy") and cocaine, for example, are directly linked with changes in steroid hormone levels in hair (Parrott and Downey, 2017; Voegel et al., 2022). Furthermore, cannabis and opioid use have both been associated with changes in the psychological and physiological stress response – reflected in glucocorticosteroids, but also with changes in sex hormones (King et al., 2011; Massaccesi et al., 2022); however, their associations with measures of hair steroids have not been clarified yet.

1.1. Steroid hormone measurement

Despite their considerable variation with the circadian rhythm (Dabbs Jr, 1990; Oster et al., 2017), salivary and blood profiles of steroid hormones are commonly used in psychological and psychiatric research, because they constitute a non-intrusive and reliable method of obtaining hormone data across a short time frame. However, with a growing body of work on allostatic load, i.e., "*the cost of chronic exposure to fluctuating or heightened neural or neuroendocrine response resulting from repeated or chronic environmental challenge*" (McEwen and Stellar, 1993, p. 2093), researchers' interest in measuring average hormones levels over longer periods of time has increased. Hair analyses represent a suitable opportunity to measure cumulative steroid hormone exposure, with each centimeter of scalp hair reflecting approximately 1 month of exposure, which eliminates the consideration of circadian rhythm (Stalder and Kirschbaum, 2012). As higher levels of hair cortisol have been associated with more stress-related events, hair cortisol has been suggested as a measure of cumulative psychological stress (Stalder and Kirschbaum, 2012). Similarly, the cortisol metabolite cortisone has been suggested as an additional endogenous stress marker that can be measured in hair (Pittner et al., 2020a; Staufenbiel et al., 2015; Voegel et al., 2020). In addition to the glucocorticoids, testosterone can also be determined in hair (Dettenborn et al., 2016; Hildebrandt et al., 2016; Shields et al., 2019), for example to investigate the relation of its cumulative load with social behavior and cognition (Dettenborn et al., 2013; Duell et al., 2021; Grotzinger et al., 2018; Shields et al., 2019).

1.2. Psychoactive substance measurement

Adolescents and adults can report their own substance use, but such self-reports are prone to misremembering and underreporting; they also do not result in exact quantifications of substance use severity and exposure (Steinhoff et al., 2023). When exact quantification of substance exposures is needed (e.g., in forensic tests), hair analysis allows for more accurate detection and quantification of psychoactive substances (Cooper et al., 2012). These quantifications can reliably detect infrequent and frequent stimulant use (e.g., cocaine and MDMA) and opioid use (Scholz et al., 2022), and weekly to daily cannabis use (Steinhoff et al., 2023; Taylor et al., 2017).

1.2.1. Psychoactive substances and glucocorticosteroids

Previous work has shown that substance use–including cocaine (Kexel et al., 2022; Manetti et al., 2014; Schote et al., 2019; Sinha, 2008), MDMA (Hysek et al., 2014; Parrott et al., 2014a), cannabis (King et al., 2011), and opioids (Kroll et al., 2019; Massaccesi et al., 2022), is associated with hypothalamic-pituitary-adrenal (HPA) axis functioning. However, previous studies typically did not measure both substances *and* hormones *in hair*. Parrot and colleagues found that recent MDMA use was associated with a four-fold increase in hair cortisol concentrations (Parrott et al., 2014b), but the study relied on self-reported MDMA use only. Voegel and colleagues found increased hair cortisone concentrations in a sample of chronic cocaine users (Voegel et al., 2022), which was most pronounced in a subgroup with cocaine dependence. Additionally, cocaine hair concentrations were correlated with cortisol and cortisone in hair, while MDMA was correlated with cortisone hair concentrations only (Voegel et al., 2022). However, given the high selectivity of clinical samples, these findings cannot be generalized to community-samples. Furthermore, polysubstance use in the community is common (Steinhoff et al., 2022), meaning that studies quantifying substances and their metabolites in hair would ideally measure the concentrations of multiple psychoactive substances in order to identify which substance is, in fact, driving associations with steroid hormones.

1.2.2. Psychoactive substances and testosterone

Little is known about the associations of substance use and testosterone in hair. Some work reported increased plasma testosterone levels following acute cocaine (Heesch et al., 1996) and MDMA administration (Parrott et al., 2014a). A Danish study of male military conscripts found elevated serum testosterone levels in those using cannabis (Gundersen et al., 2015), while a study of men in the US found no differences in testosterone between those using versus not using cannabis (Thistle et al., 2017). A recent review stated that the evidence is, so far, inconclusive (Payne et al., 2019). For opioids, a meta-analysis of seventeen studies reported lower serum and plasma testosterone levels in males who used opioids compared to those who did not (Bawor et al., 2015), but such associations have not been replicated using hair samples, which may yield more precise results about long-term associations.

1.3. The current study

In light of the increased use of hair samples to measure steroid hormones, and in light of the high prevalence of substance use amongst young people (Steinhoff et al., 2023), it is imperative to assess whether, and to what extent, the use of psychoactive substances is associated with altered glucocorticosteroids and testosterone levels in hair. Yet, the largest meta-analysis to date investigating covariates of hair cortisol concentrations did not include measures of psychoactive substances, which has been criticized (Parrott and Downey, 2017; Stalder et al., 2017). Furthermore, whilst many covariates of hair cortisol concentrations have been systematically documented, less is known about covariates of cortisone and testosterone concentrations in hair. Here, we address these gaps in research by examining associations of psychoactive substances and hair cortisol, cortisone, and testosterone in a community sample of 20-year-olds ($N = 1002$) with a high prevalence of substance use (Quednow et al., 2022), while first identifying and then adjusting for relevant covariates (e.g., hair color, hair treatment, BMI). Building on previous evidence (Voegel et al., 2022), we hypothesized that participants with cocaine exposure would display higher levels of cortisol and cortisone than those without exposure. Additionally, we expected MDMA to be positively associated with cortisone hair concentrations (Parrott et al., 2014b). Moreover, we hypothesized that participants with opioid exposure would have lower levels of testosterone. Given that previous evidence for cannabis with regard to testosterone was mixed, no a priori hypotheses were defined here. The remaining associations between substances and steroids were investigated at an exploratory level given the lack of previous studies.

2. Methods

2.1. Participants

Data came from the prospective-longitudinal *Zurich Project on the Social Development from Childhood to Adulthood* (z-proso). In 2004, 1675 children from 56 primary schools were selected from the 90 public schools in Zurich using a cluster-stratified randomized sampling approach, of which $n = 1583$ (94.5%) have participated at least once throughout the data collection waves. In total, nine waves of data were collected, every two to three years. Participants attended 1st grade (and beyond) in Switzerland, but, consistent with Zurich's diverse population, their parents came from more than 80 different countries. A detailed description of the z-proso sample is available elsewhere (Ribeaud et al., 2022). The data analyzed here were collected between 10th April and 23rd September 2018, when participants were 20 years old on average (M=20.57, SD=0.38). All participants who responded to the regular z-proso assessment were invited to donate a hair sample; $n = 1002$ (i.e., 85% of age 20 participants) provided a sample which could be analysed.

2.2. Hair analysis

Trained interviewers collected data from participants, who gave at least 3 cm of hair which was proximal to the scalp/body. Scalp hair was collected when possible (91.3%); when not possible, leg (5.6%) or arm hair (3.1%) were collected. Substances were quantified using liquid chromatography-tandem mass spectrometry. The analytical approaches used for analyzing substances and cortisol, cortisone, and testosterone are described elsewhere (Scholz et al., 2022).

2.3. Measures

Potential *covariates* were chosen because they have been investigated as covariates of hair steroid hormones for at least one of the investigated steroid hormones (Staufenbiel et al., 2015; Stalder et al., 2017), including hair treatment factors (i.e., hair bleaching, hair tint, hair color, hair washing frequency, hair straightening, sweating intensity), time of year of hair collection (collection calendar week between Spring and Fall 2018), other pharmacological factors potentially affecting HPA functioning (self-reported frequent tobacco smoking, self-reported frequent alcohol consumption, self-reported contraceptives use), Body-Mass-Index (BMI), and stressors exposure (self-reported stressful life events in the past 3 years, self-reported hours of sport per week). Information about hair collection covariates which may affect the quality of the samples (sample weight, length of analyzed sample) were collected, but not included as covariates in the main analyses, because previous analyses in this sample indicated adequate quality (Steinhoff et al., 2023). Some participants omitted some answers in the self-report questionnaire. The range of missing information across covariates was 1 (hair color) - 13 (hair washing frequency). Due to the large number of covariates, a threshold for including covariates into multiple regression models was set to an alpha-level of $p < 0.1$. In the multiple regression models, only the psychoactive substances, which were the predictors of interest, were interpreted.

We did not investigate age as a covariate, as the age range in our sample was narrow (range: 19–22, SD=0.38). For detailed information on how the covariates were assessed, see **supplement S1.**

Independent variables included the hair concentrations of total delta-9-tetrahydrocannabinol (THC), total cocaine, total 3,4-methylenedioxymethamphetamine (MDMA), and morphine equivalents of several opioids according to Kroll and colleagues (Kroll et al., 2018). Total THC was composed of the sum of THC and cannabinol (CBN). Total cocaine was composed of the sum of cocaine, benzoylecgonine, cocaethylene, and norcocaine. Total MDMA was composed of the sum of 3,4-methylenedioxymethamphetamine, 3,4-methylenedioxyamphetamine (MDA), and 3,4-methylenedioxyethylamphetamine (MDEA, analogue substance to MDMA). For opioids, hair concentrations were converted into morphine equivalents (Kroll et al., 2018). See **supplement S2.1–2.** for a full list of metabolites and description of morphine equivalents and **supplement S2.3.** for the number of participants testing positive for each opioid. Ketamine (positive hair samples: $n = 23$), benzodiazepines ($n = 14$), and amphetamines ($n = 19$) were not included in the analyses due to lack of statistical power.

All hair substance concentrations below the substance-specific limit of detection were set to 0. This threshold was determined by a forensic hair analyst, who was not involved in the conducting of statistical analyses, for each main substance found in hair. This allowed us to confidentially assume that someone had been exposed to a substance, voluntarily or not (for more details see **supplement S2.4**).

2.4. Analytic strategy

Preprocessing: One extreme outlier on the testosterone variable was removed, as it clearly indicated a non-normal condition (testosterone=8156 pg/mg). Steroid hormone concentrations were log-transformed due to positive skew (for details about range, skew and kurtosis before and after transformation, see **supplement S3.1**), in line with previous research (Feller et al., 2014; Pittner et al., 2020b; Rippe et al., 2016; Staufenbiel et al., 2015). We used the formula $log(x + 1)$.

Covariate identification: Each covariate was regressed on cortisol and cortisone, adjusting only for sex. Because testosterone is the primary sex steroid hormone in males and carries out different biological functions in both sexes, models investigating testosterone were estimated separately in male and female participants.

Multiple linear regression models were conducted in four steps:

The first and the second steps were conducted on the whole sample. The substance concentrations were highly right-skewed, as most participants had no detectable concentration of a given compound in their hair (e.g., for n = 909 participants MDMA was not detected in their hair). To address this lack of variance in the predictor variables, two dichotomous variables resulting from median splits were used in models that were estimated in the whole sample. (see Table 1 for descriptive characteristics of the hair samples). Total cocaine, total THC, total MDMA, and morphine equivalents were regressed on each hormone using dummy-coding (reference=substance not detected in hair, low=participants in the low median split, high=participants in the high median split). In the **first (whole sample) step**, models were estimated separately for each psychoactive substance (e.g., low and high cannabis included in the model predicting cortisol). To control for polysubstance use, in the **second (whole sample) step,** one model for each steroid hormone, respectively, included variables coding the median splits of all four psychoactive substances.

In the third and fourth steps, the sample was restricted to participants who had at least one of the four substance groups in their hair, using continuous concentration of substances in pg/mg as predictors. These analyses tested linear associations between substance and steroid hormone concentrations to see whether we would find a dose-response effect. In the **third (subsample) step**, each model was computed separately for each substance within any participants who tested positive for that substance in hair (e.g., total MDMA in pg/mg and covariates estimating cortisol in participants who had any MDMA in hair, $n = 93$). In the **fourth (subsample) step**, models were estimated in a sample of any participants who showed any psychoactive substance concentration in hair, with all substance concentrations included ($n = 286$).

All models were estimated in R version 4.2.2 with the lm() function. Partial eta squared (equivalent to Cohen's partial f^2) was calculated using the etaSquared() function. Models were run using complete case analysis.

Table 1
Descriptive statistics for steroid hormone concentrations, psychoactive substances concentrations, demographic characteristics and covariates.

		Total sample	Male	Female	Any psychoactive substance in hair
		$n = 1002$	$n = 499$	$n = 503$	$n = 286$
Steroid hormones					
Cortisol	M, SD	5.48 (6.40)	5.38 (7.43)	5.59 (5.19)	5.81 (5.01)
	Median	3.75	3.50	4.10	4.4
Cortisone	M, SD	26.1 (19.8)	28.3 (22.1)	23.9 (17)	29.6 (22.4)
	Median	21.5	22.6	20.0	23.4
Testosterone	M, SD	1.25 (2.31)	2.01 (2.89)	0.49 (1.09)	1.43 (1.66)
	Median	0.60	1.40	0.20	0.9
Psychoactive substances					
Total THC (THC + CBN)	*n* any THC	132	95	37	–
	M, SD within participants with THC	226 (407)	246 (467)	127 (137)	–
	Median within participants with THC	94.0	104	87.0	–
Total cocaine (cocaine + benzoylecgonine + cocaethylene + norcocaine)	*n* any cocaine	93	66	27	–
	M, SD within participants with any cocaine	3210 (10,600)	4100 (12,400)	929 (1290)	–
	Median within participants with any cocaine	352	366	336	–
Total MDMA (MDMA + MDA + MDEA)	*n* any MDMA	93	60	33	–
	M, SD within participants with any MDMA	1120 (2410)	980 (2068)	1381 (2950)	–
	Median within participants with any MDMA	143	152	143	–
Morphine equivalents (opioids)*	*n* any opioids within participants with any opioids	125	69	56	–
	M, SD within participants with any opioids	58.8 (206)	82.8 (272)	28 (55)	–
	Median within participants with any opioids	10.15	16.0	9.06	–
Hair factors					
Hair bleaching %		19.6	2.4	36.6	14.3
Hair colour					
	Blonde hair (%)	23.6	19.5	27.6	16.9
	Brown hair (%)	54.0	53.6	54.5	51.2
	Black hair (%)	22.4	26.9	17.9	31.9
Hair colouring %		25.7	< 5[c]	48.1	19.6
Hair tint %		14.5	< 5[c]	27.0	13.6
Hair washing frequency (weekly) (M, SD)		4.49 (2.18)	5.55 (2.14)	3.43 (1.63)	4.88 (2.32)
Hair straightening %		17.9	< 5[c]	34.6	14.3
Sweating intensity (1–10) (M, SD)		4.1 (2.07)	4.53 (1.99)	3.67 (2.07)	4.35 (2.1)
Sociodemographic factors					
Education status					
	NEET %	3.7	< 5[c]	3.4	9.1
	Medium education %	69.6	68.7	70.4	73.4
	Higher education	26.7	27.2	26.2	17.5
Socio-economic status (ISEI) (M, SD)		47.1 (19.8)	48.0 (20.4)	46.1 (19.2)	44.3 (19.3)
Migration background (both parents born abroad) %		47.3	46.6	48.1	53.4
Other substances (self-report)					
Frequent alcohol use (3 months) %		86.0	88.2	83.9	87.0
Frequent smoking (3 months) %		33.2	34.3	32.1	53.1
Contraceptives					
Estrogen-progestin n		–	–	160	36
Progestin-only n		–	–	43	7
Stress and lifestyle related					
Stressful life events (3 years) (M, SD)		3.79 (2.41)	3.95 (2.50)	3.71 (2.32)	4.58 (2.66)
Sport (hours per week) (M, SD)		4.72 (2.59)	5.22 (2.64)	4.23 (2.45)	4.51 (2.64)
BMI (M, SD)		23.4 (4.24)	24.1 (4.38)	22.7 (3.97)	23.6 (4.41)
Hair quality variables					
Hair type					
Scalp %		91.3	82.7	99.8	85.2
Arm hair %		3.1	6.1	< 5[c]	4.9
Leg hair %		5.6	11.2	0	9.9
Hair length that was analysed in cm (M, SD)		2.82 (0.52)	2.65 (0.68)	2.99 (0.12)	2.7 (0.66)
Weight of hair sample in pg/mg (M, SD)		12.8 (4.74)	12.9 (4.69)	12.6 (4.79)	12.4 (4.35)

Note: hair concentrations shown in pg/mg. NEET: not in education, employment, or training ISEI=International Socioeconomic Index of occupational status. * For a full list of opioids and *n* testing positive for each opioid, see **supplement.** [c] counts and percentages for n < 5 not stated due to confidentiality. MDMA = 3,4-methylenedioxymethamphetamine, MDA = 3,4-methylenedioxyamphetamine, MDEA = 3,4-Methylenedioxy-N-ethylamphetamine.

3. Results

3.1. Descriptive statistics

Table 1 shows the sample characteristics. Over a quarter of the sample (28.5%) had at least one of the four psychoactive substances in hair examined here. Of these, about a third (33.9%) of participants had more than one psychoactive substance in hair. Wilcoxon rank sum test and Pearson's $\chi 2$ tests were calculated to estimate sex differences for substances, steroid hormones, and covariates, as well as differences between participants who had no psychoactive substance in hair compared to those who had any of the four detected in hair (see **supplement S4**).

3.2. Associations of covariates and psychoactive substances with steroid outcomes

3.2.1. Cortisol and cortisone

Cortisol was positively associated with darker hair colour, sweating intensity, number of stressful life events, BMI, hours of exercise per week, and later data collection (i.e., toward Fall as opposed to Spring). Higher cortisone was associated with darker hair colour, lower hair washing frequency, sweating intensity, stressful life events, frequent tobacco smoking, and earlier data collection (Table 2.1).

3.2.2. Testosterone

Darker hair colour was associated with higher levels of testosterone in both males and females. Higher sweating intensity, higher BMI and frequent alcohol use was associated with higher levels of testosterone in females. Number of stressful life events and later collection were associated with higher testosterone concentrations in male participants (Table 2.2). Contraceptives use was generally associated with lower levels of cortisol, cortisone and testosterone, as shown previously with this dataset (Carillo Vázquez et al., 2022).

Table 2.2 shows model results.

3.3. Regression models predicting glucocorticosteroids with covariate adjustment

Results are presented separately for each of the three steroid hormones.

3.3.1. Cortisol

Step 1: In models estimated separately for each psychoactive substance, conducted in the whole sample, being in the high cocaine median split group was associated with higher levels of cortisol ($\beta = 0.36$, 95% CI = 0.06–0.65, p = .017, $\eta_p^2 = 0.005$). Being in either the low ($\beta = 0.29$, 95% CI = 0.04–0.53, p = .021, $\eta_p^2 = 0.005$) or high THC ($\beta = 0.42$, 95% CI = 0.17–0.67, p = .001, $\eta_p^2 = 0.011$) median split group was associated with higher levels of cortisol.

Step 2: In the model which included all four psychoactive substances, conducted in the whole sample, both being in the low ($\beta = 0.27$, 95% CI = 0.02–0.52, p = 0.036, η_p^2 =0.005) and high THC ($\beta = 0.40$, 95% CI = 0.13–0.66, p = .004, η_p^2 = 0.008) group was associated with higher levels of cortisol. Being in the high cocaine median split group was no longer a significant predictor of cortisol.

Step 3: In the model conducted in participants who had THC in hair, we did not find a linear association between total THC concentration and cortisol.

Step 4: However, in the sample of participants with any psychoactive substance in hair, we found a linear association between total THC concentration and cortisol ($\beta = 0.12$, 95% CI = 0.01–0.24, p = .041, η_p^2 = 0.015).

3.3.2. Cortisone

Step 1: Being in the high cocaine median split group was a significant predictor of cortisone ($\beta = 0.30$, 95% CI = 0.01–0.60, p = .048, η_p^2 = 0.005). Being in the high MDMA median split group was also a significant predictor of cortisone ($\beta = 0.34$, 95% CI = 0.04–0.64, p = .026 η_p^2 = 0.005).

Models from steps 2, 3 and 4 did not show significant associations between psychoactive substances and cortisone (association between high MDMA and cortisone in step 2: $\beta = 0.27$, 95% CI= −0.08 to 0.63, p = .133 association between high cocaine and cortisone in step 2: $\beta = 0.11$, 95% CI=−0.24 to 0.46, p = .542). Nevertheless, *step 4* revealed a positive association at an alpha level of p = .057 between total MDMA concentration and cortisone ($\beta = 0.11$, 95%CI = 0.00–0.23, $\eta_p^2 = 0.013$).

3.3.3. Testosterone in male participants

Step 1: We found no significant associations.

Step 2: When all substance median splits were included in one model, being in the high cannabis median split group was associated with lower levels of testosterone in male participants ($\beta = -0.35$, 95% CI=- 0.64 to −0.06, p = .018, η_p^2 =0.011).

Step 3 and 4: We found positive linear associations between total MDMA concentration and testosterone concentration (*step 3*: $\beta = 0.24$, 95% CI = 0.01–0.46, p = .041, $\eta_p^2 = 0.083$; *step 4:* $\beta = 0.17$, 95% CI = 0.03–0.31, p = .015, $\eta_p^2 = 0.036$).

Table 2.1
Linear regression models predicting glucocorticoids, adjusting for sex.

	Cortisol (log)			**Cortisone (log)**		
	β	CI	p	β	CI	p
Hair factors						
Hair bleaching	0.06	-0.01–0.12	.109	-0.02	-0.09–0.04	.494
Hair colour (reference: brown hair)						
Blonde hair	**-0.12*****	-0.19–− 0.06	< .001	-0.03	-0.09–0.04	.406
Black hair	**0.12*****	0.05–0.18	< .001	**0.13*****	0.07–0.19	< .001
Hair colouring	0.05	-0.03–0.12	.217	-0.03	-0.10–0.04	.412
Hair tint	0.04	-0.03–0.11	.228	0.00	-0.07–0.06	.973
Hair washing frequency	-0.05	-0.13–0.02	.137	**-0.08***	-0.15–− 0.01	.029
Hair straightening	0.02	-0.04–0.09	.492	-0.04	-0.11–0.02	.211
Sweating intensity	**0.08***	0.01–0.14	.019	**0.06**[a]	-0.01–0.12	.082
Collection calendar week	**0.15*****	0.09–0.22	< .001	**-0.09****	-0.15–− 0.02	.007
Other substances (self-report)						
Frequent alcohol use (3 months)	0.01	-0.05–0.07	.741	0.01	-0.05–0.07	.759
Frequent smoking (3 months)	0.03	-0.03–0.09	.366	**0.07***	0.01–0.13	.020
Contraceptives (reference: no contraceptive)						
progestin	**-0.10*****	-0.17–− 0.04	< .001	-0.05	-0.11–0.02	.140
estrogen-progestin	**-0.12*****	-0.19–− 0.05	< .001	**-0.11****	-0.18–− 0.04	.002
Stress and lifestyle related						
Stressful life events (3 years)	**0.07***	0.01–0.13	.033	**0.07***	0.01–0.13	.033
Sport (hours per week)	**0.06**[a]	0.00–0.13	.055	0.01	-0.06–0.07	.829
BMI	**0.07***	0.00–0.13	.042	0.04	-0.02–0.11	.166

Note: Dummy variables were entered into the model simultaneously. [a] p < .1, *p < .05, ** p < .01 *** p < .001, CI= 95% confidence intervals. β = standardized beta coefficients, with bold values significant at p < .1.

Table 2.2
Bivariate linear regression models predicting testosterone, estimated separately for males and females.

	Testosterone (log) in males			Testosterone (log) in females		
	β	CI	p	β	CI	p
Hair type and treatment						
Hair bleaching	0.04	-0.05–0.12	.430	-0.04	-0.12–0.05	.414
Hair colour (reference: brown hair)						
Blonde hair	**-0.22*****	-0.30–− 0.13	< .001	**-0.14*****	-0.23–− 0.05	< .001
Black hair	**0.31*****	0.23–0.39	< .001	**0.12***	0.03–0.21	.011
Hair colouring	0.02	-0.07–0.11	.671	-0.05	-0.14–0.04	.256
Hair tint	0.05	-0.04–0.14	.265	-0.01	-0.09–0.08	.893
Hair washing frequency	0.03	-0.06–0.12	.481	-0.06	-0.15–0.02	.157
Hair straightening	0.03	-0.06–0.12	.542	0.02	-0.06–0.11	.588
Sweating intensity	0.00	-0.09–0.09	.966	**0.08**[a]	-0.01–0.17	.070
Collection calendar week	**0.12****	0.03–0.21	.008	0.02	-0.07–0.10	.717
Other substances						
Frequent alcohol use (3 months)	0.01	-0.08–0.10	.840	**-0.13****	-0.22–− 0.05	.002
Frequent smoking (3 months)	0.01	-0.08–0.09	.885	-0.01	-0.10–0.08	.810
Contraceptives (reference: no contraceptive)						
progestin	–	–	–	-0.05	-0.13– 0.04	.310
Estrogen-progestin	–	–	–	**-0.19*****	-0.28–− 0.10	< .001
Stress and lifestyle related						
Stressful life events (3 years)	**0.11***	0.02–0.20	.014	0.03	-0.06–0.12	.470
Sport (hours per week)	0.06	-0.03–0.15	.179	-0.07	-0.15–0.02	.147
BMI	-0.01	-0.10–0.08	.778	**0.17*****	0.09–0.26	< .001

Note: Dummy variables were entered into the model simultaneously. [a] p < .1 *p < .05, ** p < .01 *** p < .001, CI= 95% confidence intervals. β = standardized beta coefficients, with bold values significant at p < .1.

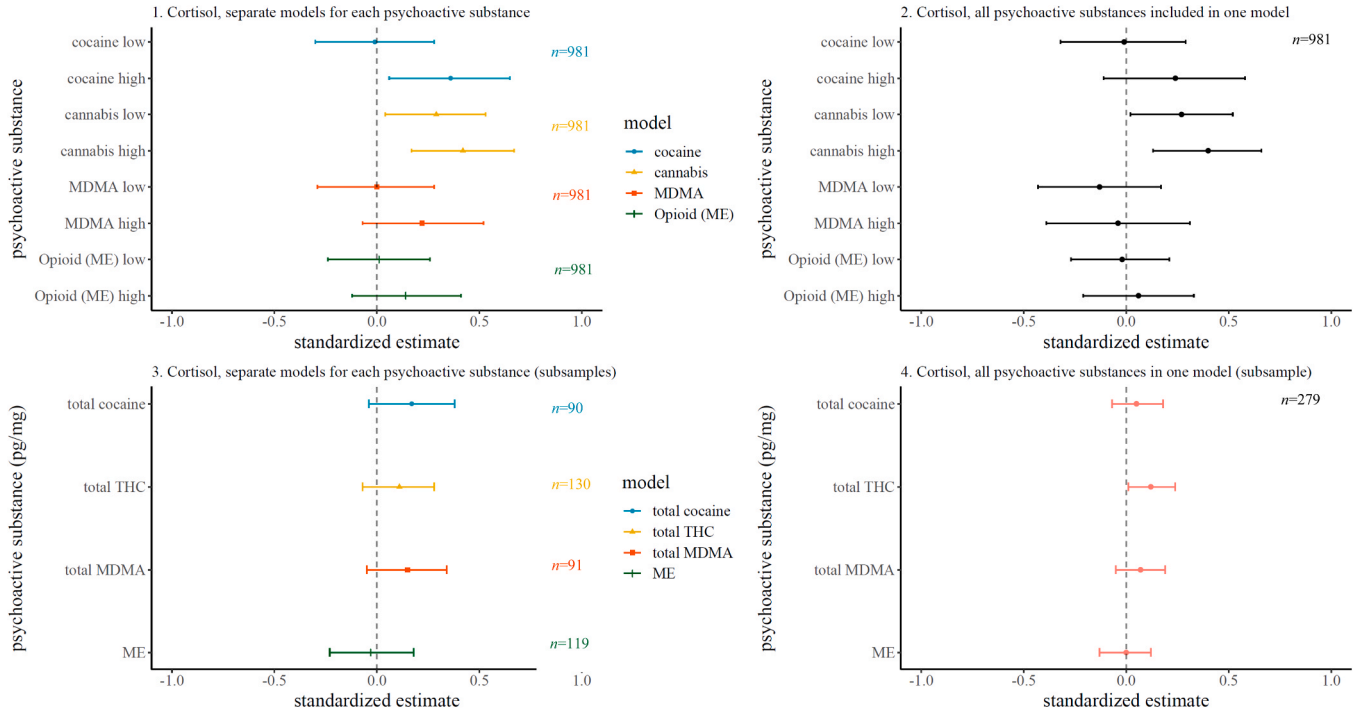

Fig. 1. Four analytic steps, all models estimating cortisol (log). Figures show standardised estimates for psychoactive substances as predictors, with 95% confidence intervals. 1. four models estimated in the whole sample, one for each psychoactive substance. 2. One model estimated in the whole sample, including all median splits as predictors. 3. Four models estimated within participants who showed each of the psychoactive substances in hair. 4. One model estimated within participants with any of the four psychoactive substances in hair. ME=morphine equivalent.

We found no other significant linear associations in *step 3 and 4* between substance concentrations and testosterone levels in male participants.

3.3.4. Testosterone in female participants

We found no significant associations between psychoactive substances and testosterone in female participants in any model step.

3.3.5. Sensitivity analyses

3.3.5.1. Self-reported cannabis use. Because we consistently identified an association of total THC exposure and cortisol, we investigated whether self-reported cannabis use explained as much variance as total

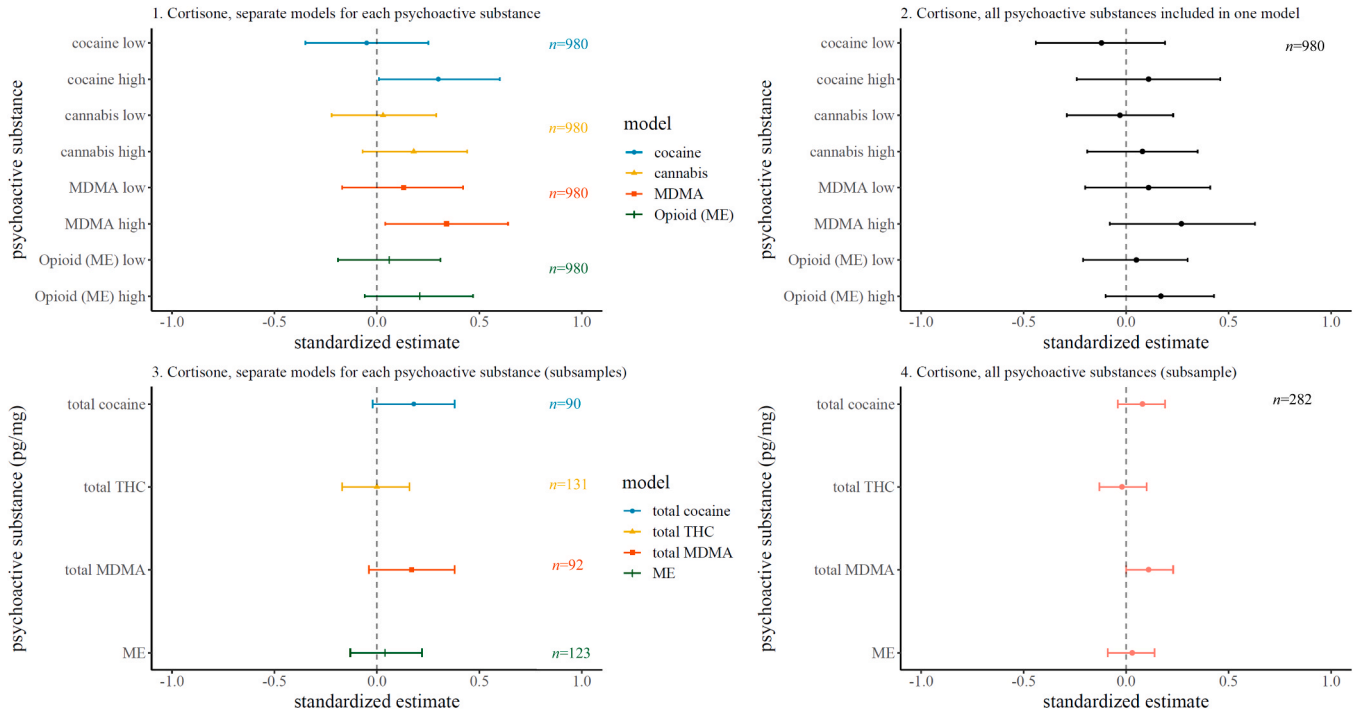

Fig. 2. Four steps, all models estimating cortisone (log). Figures show standardised estimates for psychoactive substances as predictors, with 95% confidence intervals. 1. four models estimated in the whole sample, one for each psychoactive substance. 2. One model estimated in the whole sample, including all median splits as predictors. 3. Four models estimated within participants who showed each of the psychoactive substances in hair. 4. One model estimated within participants with any of the four psychoactive substances in hair. ME=morphine equivalent.

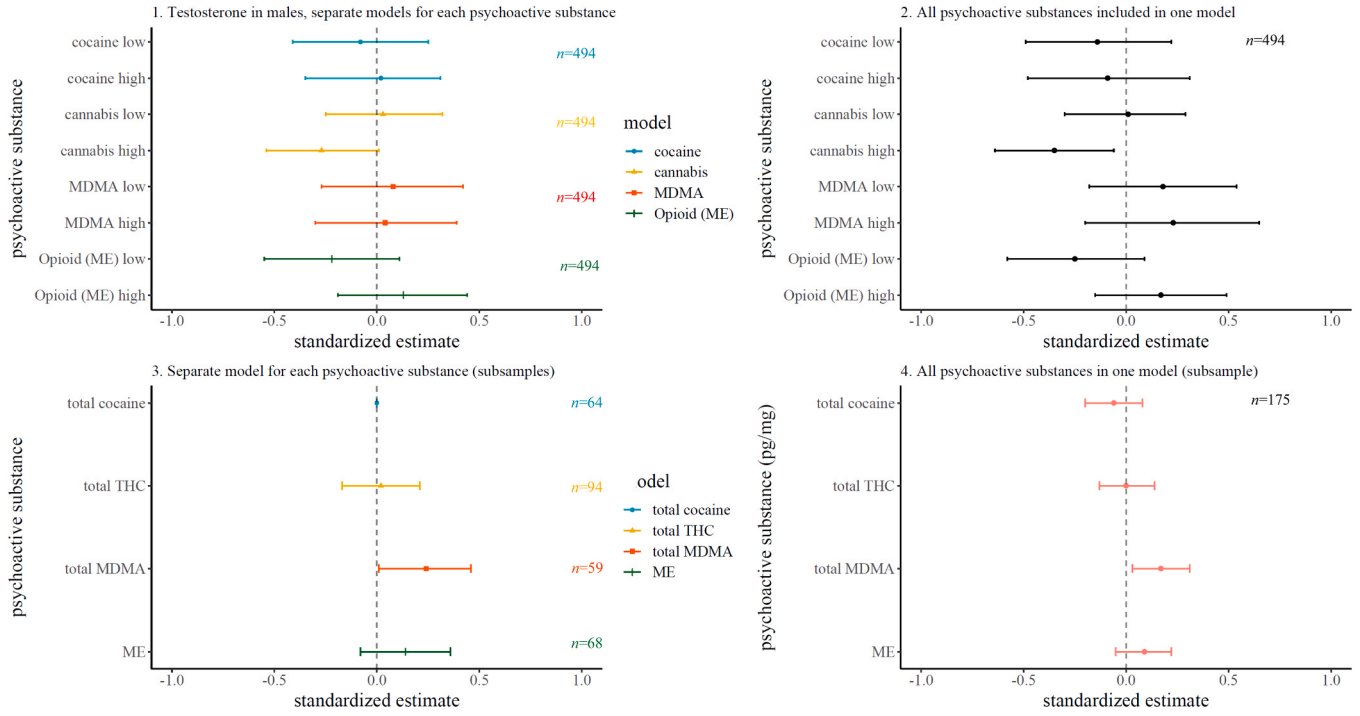

Fig. 3. Four steps, all models estimating testosterone (log) in male participants. Figures show standardised estimates for psychoactive substances as predictors, with 95% confidence intervals. 1. Four models estimated in the whole male sample, one for each psychoactive substance. 2. One model estimated in the whole sample, including all median splits as predictors. 3. Four models estimated within participants who showed each of the psychoactive substances in hair. 4. One model estimated within participants with any of the four psychoactive substances in hair. ME=morphine equivalent.

THC concentration in hair. In a regression model including weekly to daily self-reported cannabis use (frequent cannabis use) and less than weekly self-reported use (infrequent cannabis use) as predictors along with covariates, the infrequent cannabis use predictor was not associated with cortisol, while the self-reported frequent cannabis use variable was associated at an alpha level of $p = .063$ ($\beta = 0.08$, 95% CI=0–0.17).

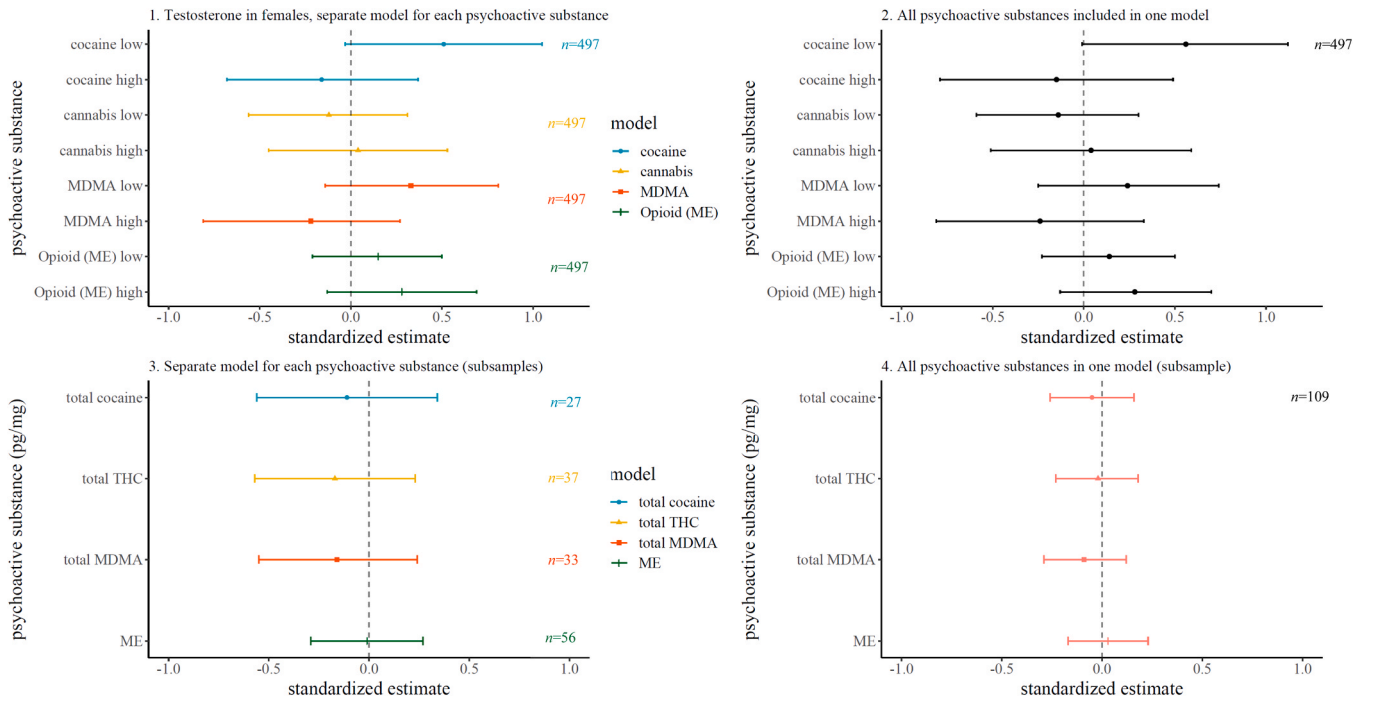

Fig. 4. Four steps, all models estimating testosterone (log) in female participants. Figures show standardised estimates for psychoactive substances as predictors, with 95% confidence intervals. 1. Four models estimated in the whole female sample, one for each psychoactive substance. 2. One model estimated in the whole sample, including all median splits as predictors. 3. Four models estimated within participants who showed each of the psychoactive substances in hair. 4. One model estimated within participants with any of the four psychoactive substances in hair. ME=morphine equivalent.

Fig. S4 **in the supplement** depicts levels of cortisol and self-reported cannabis use.

3.3.5.2. Migration background and socio-economic status. Main analyses were estimated using data collected when participants were 20 years old. Information on migration background and socio-economic status (SES) were collected in previous collection waves ($n = 62$ missing cases for either/or SES and migration background in participants who provided hair samples when they were 20 years old). For information about these covariates, see **supplement S1**. We ran models with significant psychoactive substance predictors adjusting for socio-economic status and/or migration background if these covariates were predictive of the steroid hormones while controlling only sex (analogue to the covariate identification step). Results remained significant in analyses conducted in the whole sample. However, in the analyses restricted to any positive substance in hair (*step 4*), continuous total THC concentration was no longer a significant predictor of cortisol ($\beta = 0.11$, 95% CI=−0.01 to 0.23, $p = .084$) and continuous total MDMA concentration was no longer a significant predictor of testosterone in male participants ($\beta = 0.11$, 95% CI=0.01–0.23, $p = .075$), after adjusting for socio-economic status. In those models, that included other identified covariates, SES was not a significant predictor of these steroid hormones, and the confidence intervals for psychoactive substance predictors increased. These models were estimated in ~10% fewer participants (cortisol $n = 264$ and testosterone $n = 162$).

3.3.5.3. Scalp vs. body hair. Our main analyses included participants who gave both scalp and body hair. We ran models adjusting for hair type by adding the variable *body hair* (reference = scalp hair) to our models. The resulting effect sizes were almost unchanged.

3.3.5.4. Stressful life events. The questionnaire regarding stressful life events covered the past 3 years whereas the hair sample analysis was performed on the proximal 3 cm (i.e., reflecting approximately the last 3 months of exposure). Therefore, we recoded the life events variable to include only events that occurred 4–9 months prior to data collection. Again, this hardly affected the ensuing estimates.

4. Discussion

The number of studies utilizing measures of steroid hormone concentrations in hair has increased tremendously. Although psychoactive substances are a putative correlate of hair steroid concentrations, work examining associations of psychoactive substances with steroids, with both measured in hair, is scarce. Considering high prevalence rates of substance use in adolescents and young adults (European Monitoring Centre for Drugs and Drug Addiction, 2020), this may result in biased estimates of hair steroids. Our study examined these associations, using a large community sample of 20-year olds with a low risk of selection bias and high levels of substance use (Quednow et al., 2022). Findings revealed robust associations of hair-measured THC and cortisol, specifically in the full models taking all substances into account. MDMA showed a positive correlation with cortisone (step 1), but this effect was not significant in the other models. In male participants, MDMA and THC were both associated with testosterone, albeit in opposite directions. These findings suggest that THC and MDMA, ideally measured in hair, should, when possible, be assessed in studies investigating steroids in hair. In the following section, we discuss the findings for each individual substance.

4.1. Cannabis

Our study documented associations of hair THC and cortisol, by revealing associations of low, high, and continuous total THC concentration and cortisol, indicating a possible dose-response effect.

Ample evidence from animal studies demonstrates that THC administration acutely activates the HPA axis (Steiner and Wotjak, 2008). Previous human evidence from studies using serum and saliva samples also indicated altered endocrine functioning in cannabis users, but the picture was surprisingly mixed: One study found higher salivary

cortisol concentrations in cannabis users than non-users (King et al., 2011), while another study found increased plasma cortisol in response to acute THC administration in infrequent cannabis users, but a blunted response in frequent cannabis users (Ranganathan et al., 2009). Huizink and colleagues found evidence of altered circadian rhythm functioning in cannabis users, with lower cortisol awakening response in early-onset cannabis users but higher levels of cortisol in the evenings for all cannabis users (Huizink et al., 2006). All these previous findings were limited to small samples and small time-windows of the body's exposure to cortisol; our findings utilizing hair data extend the picture by providing evidence of longer-term, higher cumulative cortisol concentrations in participants that show both low and high THC exposure compared to participants that show no exposure.

Interestingly, male participants in our sample with high total THC concentration had lower testosterone, but this association was not seen when using the continuous variable within the subsample exposed to substances. Our findings might be explained by a non-linear relationship between cannabis and testosterone, or a confounding characteristic of individuals with high cannabis exposure that we did not control for, such as a lifestyle. Our result supports findings from animal models that showed lower testosterone levels and also impaired sexual function following chronic cannabis administration, even though previous results in humans have been mixed (Gundersen et al., 2015; Nassan et al., 2019; Thistle et al., 2017). Additional studies are thus needed to elucidate associations of cannabis consumption and cumulative testosterone concentrations in males (Meah et al., 2022).

About 13% of the sample tested positive for THC. It is important to note that this number does not capture participants who report infrequent cannabis use, as hair analysis does not detect occasional use patterns but mainly highly regular use (Taylor et al., 2017), as confirmed in a previous study conducted in this sample (Steinhoff et al., 2023). Nevertheless, our sensitivity analysis indicated that low, high, and continuous total THC concentrations in hair were better predictors of cortisol than self-reported cannabis use.

4.2. Cocaine

The association of being in the high cocaine group with higher cortisol and cortisone ceased to be significant when adjusting for other psychoactive substance use, notably cannabis. This finding differs from recent findings that hair cortisone in participants with chronic cocaine use was elevated compared to participants showing recreational or no use, and that cocaine was significantly correlated with cortisol and cortisone in hair (Voegel et al., 2022). However, the previous study included more severe cocaine users showing on average 3-fold higher total cocaine concentrations in hair (average concentration = 9674 pg/mg) than in the present sample of cocaine users (3210 pg/mg). Additionally, Voegel and colleagues only assessed and controlled for self-reported cannabis use, which was shown to be a less robust predictor in the present study. Moreover, craving, a core symptom of cocaine use disorder, is associated with elevated stress susceptibility (Kexel et al., 2022). On top of acute stimulating effects of cocaine exposure on the HPA axis (Wemm and Sinha, 2019), cocaine craving may itself lead to increased glucocorticoid levels, creating a feedback loop. This would, however, not have been detected in our sample with relatively low consumption levels. In sum, our findings indicate that, in a community-sample, the predominantly recreational use of cocaine is not associated with altered steroid hormones in hair after adjusting for other substances.

4.3. MDMA

Experimental work shows that the acute administration of MDMA strongly activates the HPA axis and this work is consistent with the bioenergetic stress model of MDMA (Parrott et al., 2014b; Voegel et al., 2022). In our study, we found that participants in the high MDMA group did have higher cortisone levels, which is in line with previous research (Voegel et al., 2022). However, this effect was weaker when all substances were included in the model. Furthermore, within the subsample of participants testing positive for any of the studied psychoactive substances, the total MDMA-concentration was not a significant predictor of cortisone. Interestingly, the MDMA users in the present sample were on average about 11 years younger, but showed higher total MDMA hair concentrations (average 1120 pg/mg) when compared to the cocaine users of Voegel and colleagues (2022, 746 pg/mg). As higher age has been shown to correlate with lower hair cortisol levels previously (Garcia-Leon et al., 2018), a possible explanation could be that MDMA-driven changes in hair cortisol become more detectable in the mid-adult age range. In addition, it is likely that our well-powered statistical model, which included more relevant covariates (e.g., stressful life events, hair washing frequency, sweating, hair color, contraceptives use) and more substances led to more realistic effect size estimations in comparison to Voegel and colleagues (2022).

Our findings also revealed a linear association between total MDMA concentration and testosterone concentration in hair in male participants exposed to any psychoactive substances. This supports evidence from a study that found elevated saliva testosterone levels in participants after self-administering MDMA in a club compared to another session where they were not taking the substance (Parrott et al., 2008). A more recent study in a controlled laboratory setting, however, did not find any effects of a low dose of the potent serotonin releaser MDMA on acute testosterone levels (Schmid et al., 2015). Given the complex interplay between serotonin and testosterone (Birger et al., 2003) it is too early to specuate whether repeated serotonin releases induced by MDMA or a subsequent phase of serotonin depletion with down-regulation of certain serotonin receptors (Roberts et al., 2016) is responsible for elevated testosterone levels in more intense MDMA users. Alternatively, lifestyle differences or neurobiological predispositions of these MDMA users may also account for their alterations in hair androgens.

4.4. Opioids

We did not find associations between standardized opioid concentrations (morphine equivalents) and steroid hormone concentrations. This contradicts pervious findings from a meta-analysis indicating testosterone suppression in opioid-using men (Bawor et al., 2015). However, the meta-analysis included studies conducted in participants who were on average 10 years older and showed mostly heroin dependence or participation in opioid maintenance treatments (e.g., methadone or buprenorphine). In contrast, our sample, which showed more than 10% of participants testing positive for opioids in hair, comprised young participants who mostly consumed codeine, a low potency opioid in comparison to heroin and opioid maintenance drugs (see supplement Table S2.3.), in a recreational or occasional use pattern. In Switzerland, codeine is available in the form of an over-the counter cough syrup, and increasingly used for non-medical reasons by adolescents (Quednow et al., 2022). It is likely that our sample did not show high enough levels of opioid use to impact hair testosterone levels. Importantly, this does not rule out that regular opioid use may eventually lead to testosterone suppression in men assessed here, and this should be monitored in future studies.

4.5. Sample characteristics and covariates

In contrast to previous meta-analysis findings (Stalder et al., 2017), which had indicated 20% higher cortisol in males than in females, females from our sample had significantly higher levels of cortisol than their male counterparts. The previous meta-analysis mostly comprised mid-adult samples, however, who may differ from the young adults in our sample in many ways. Indeed, Binz and colleagues (2018) found higher hair cortisol concentration in adult males, but not adolescent

males compared to females. Thus, sex differences for glucocorticosteroids may change across the lifespan, and future meta-analyses should assess age as a potential moderator of sex differences in steroid hormones.

While early studies did not show associations of steroid hormones and hair color (Dettenborn et al., 2012), our results support evidence from more recent investigations carried out in larger sample sizes showing that darker hair was associated with higher levels of cortisol and cortisone (Binz et al., 2018). Furthermore, our results reveal that this is also true for testosterone. It has been suggested that, while steroid hormones such as cortisol may not bind strongly to melanin, hydrogen bridge bonding or van der Waals linkage may be responsible for this association and also explain associations between psychoactive substances and hair color found in other studies (Borges et al., 2003). Thus, it is imperative to control for hair color in studies investigating steroid hormones and psychoactive substances in hair, as this poses a serious threat of confounding.

Stressful life events in the previous 3 months were associated with higher glucocorticosteroid levels in hair. Maybe unsurprisingly, participants who had any psychoactive substances detected in hair reported, on average, a higher number of stressful life events, making it an important covariate to account for. Indeed, Grassi-Oliviera and colleagues found a positive association between negative life events three months prior and admission for crack-cocaine treatment and hair cortisol concentrations in 23 crack-dependent women (Grassi-Oliveira et al., 2012). For testosterone, we found an association with stressful life events effect in male participants. Testosterone secretion has been observed in MRI scanning environments, described as stressful, in children and adolescents using saliva sampling (Eatough et al., 2009). A study conducted in incarcerated men suggests that HPA and hypothalamic-pituitary-gonodal (HPG) axis may function dually in response to acute and chronic stressful settings (Dismukes et al., 2015). However, studies also show reductions in testosterone following mental stress (Zitzmann and Nieschlag, 2001). Overall, the evidence is mixed, and further studies are needed to test testosterone response to stressful life experiences in young adults.

We found opposite directions for the associations of cortisone and cortisol and week of data collection, with increasing levels of cortisol and decreasing levels of cortisone between April and September. In a Dutch sample, cortisol concentrations were also higher in spring and summer compared to autumn and winter Staufenbiel and colleagues (2015), but there were no seasonal effects on cortisone. Wester and colleagues found that natural sunlight exposure led to reductions in cortisol and cortisone concentrations found in hair samples (Wester et al., 2016), however, our findings only found this for cortisone. Overall, our findings suggest that, even when issues of circadian rhythm no longer have to be considered in hair data collection, the time of year may be important to assess.

We found relatively small effect sizes, as our complex models explained about 10–20% of the variance in steroid hormones. As a twin study of Rietschel and colleagues found 72% heritability in hair cortisol concentrations (Rietschel et al., 2017), this might be explained by a strong genetic component. While we assessed cumulative stressful life events in the previous three years, other adversity factors such as burden with psychiatric symptoms–which were not extensively assessed in the present study–may explain additional variance. However, it is important to state that small effect sizes are to be expected when investigating complex psychological phenomena, such as stress (Götz et al., 2022).

4.6. Strengths and limitations

This study has many strengths, notably the large community sample, which is ethnically and socioeconomically diverse. The study is also the first to systematically investigate associations of four major substance groups with steroid hormones in hair, allowing the identification of unique variance contributions of each substance while adjusting for the others. It is also among the first to directly investigate covariates of hair testosterone concentrations, which were accounted for in analyses, such as hair color, smoking, alcohol consumption, stressful life events, and physiological correlates such as BMI.

Nevertheless, this study comes with several limitations. First, we did not investigate other psychoactive substances such as amphetamines, ketamine, 2 C drugs, benzodiazepines, z-drugs, as only a small number of participants had quantifiable concentrations of these in their hair. Additionally, psychedelics such as LSD and psilocybin were not quantified because they currently cannot be detected in hair.

Second, due to the strong variance in substance hair concentrations and zero-inflation in the controls, we used median splits to create substance exposure groups in the whole sample. Binary groups are often used in substance use research, for example to reflect recreational vs. chronic use (Voegel et al., 2022). However, a drawback of using median splits is that they are sample-dependent, limiting the generalizability of group differences.

Third, the use of local and systemic corticosteroids (e.g., cortisone inhalers, injections, or skin creams) was not assessed. Both local and systemic corticosteroids have been associated with changes in hair glucocorticoid concentrations (Abell et al., 2016; Wester et al., 2017); this may have been a source of confounding. Another possible source of confounding comes from psychological stress. While we were able to collect information on stressful life events during 4–9 months prior to data collection, we did not have individual information from the same period as represented by hair analyses (3 months). Thus, this time-window may have been too wide to detect a confounding effect of stressful life events on the association between substance use and glucocorticoid concentrations.

Finally, a limitation of our cross-sectional analyses is the lack of ability to make inferences about directionality of effects.

5. Conclusion

Total THC concentration in hair was a robust predictor of cortisol and should be assessed in studies that investigate cortisol as an index of chronic stress. In the recent decade, the policy landscape of cannabis legalization has changed dynamically, bringing with it changes in consumption patterns. In the US, cannabis consumption is now at its highest levels among young people (Knopf, 2022). Accordingly, assessing cannabis exposure in studies of cortisol is more important than ever, and in fact research into their association may be warranted beyond understanding the role of cannabis as a confounder in associations of cortisol with other variables. Our cross-sectional analyses do not allow any assumptions about directionality of effect; however, our results provide a foundation for future longitudinal investigations of associations between cannabis and steroid hormone function. Our study also establishes a basis for further investigations of the effects of MDMA exposure on cortisone and testosterone concentrations.

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Declaration of Competing Interest

The authors report no conflicts of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2023.106369.

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