

Subjective Stress and Diurnal Salivary Cortisol in Adults with Frequent Cannabis Use

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ABSTRACT

Objective: The hypothalamic-pituitary-adrenal (HPA) axis, the major neuroendocrine system that responds to stress, may be dysregulated by substance use. The rise of cortisol in the morning, known as the cortisol awakening response (CAR), is believed to be a reliable marker for individual differences in HPA axis activity. The purpose of this study was to investigate differences in the CAR between individuals with frequent cannabis use (CU) and healthy controls (HC) and determine whether cannabis use status moderates the relationship between subjective stress and cortisol levels. **Method:** 39 CU and 43 HC reported subjective stress levels and collected daily saliva samples at awakening, 30 minutes later, and in the evening, on two consecutive weekdays. We examined group differences in the CAR (30 min post awakening – awakening cortisol), and the association between stress and cortisol levels at each collection time with group entered as a moderator in regression analyses. **Results:** There were no significant group differences in the CAR ($p = .23$). At awakening, subjective stress was positively related to cortisol levels ($p = .001$) and CU had higher cortisol than HC ($p = .047$), which was significantly related to consequences of cannabis use ($p = .007$). **Conclusions:** While the rise of the CAR did not differ between groups, individuals with frequent cannabis use exhibited significantly higher basal cortisol levels at awakening. This elevation was positively correlated with cannabis-related consequences, suggesting that awakening cortisol may serve as a neurobiological marker of problematic cannabis use and warrants further investigation.

Key words: = cannabis; hypothalamic-pituitary-adrenal axis; stress; cortisol awakening response

In the United States, 29% of adults (ages 19-30) report past 30-day cannabis use, a figure that has nearly doubled over the past 15 years (Patrick et al., 2025). Further, 10.8% of this age group now reports daily cannabis use (≥ 20 occasions in the past 30 days), which has more than doubled in that same timeframe (Patrick et al., 2025), indicating a rise in frequent cannabis use. In line with the stress-coping theory of substance use (Wills et al., 1990), coping with stress is a primary motivation to use cannabis and is linked with

problematic use (Casajuana et al., 2021; Scarfe et al., 2022; Spradlin & Cuttler, 2019). The relationship between stress and cannabis use is complex and bidirectional, such that stress may increase risk for cannabis use and cannabis can alter long-term stress responses (Al'Absi & Allen, 2021). While acutely, cannabis may provide relief from perceived stress, its chronic use may dysregulate stress-response systems over time (Al'Absi & Allen, 2021). Thus, understanding the relationship between frequent cannabis use and

stress physiology is essential for clarifying its potential health risks, as altered response of the stress neuroaxis may be related to the maintenance of substance use (Hyman & Sinha, 2009; Sinha, 2008).

Cannabis and Hypothalamic-Pituitary-Adrenal Axis Functioning

The hypothalamic–pituitary–adrenal (HPA) axis is the body’s central neuroendocrine stress-response system, which coordinates the release of corticotropin-releasing hormone (CRH), adrenocorticotrophic hormone (ACTH), and ultimately cortisol (Clow et al., 2010; Russel & Lightman, 2019). Acute activation of the HPA axis mobilizes energy resources to meet environmental demands, but repeated activation from chronic stress or heavy substance use can lead to allostatic load, which produces physiological wear-and-tear and long-term dysregulation of stress systems (McEwen, 1998). HPA axis dysregulation has also been observed with commonly used substances. For example, both nicotine (Bruijnzeel 2012; Rohleder & Kirschbaum, 2006) and alcohol (Blaine & Sinha, 2017; Boschloo et al., 2011; Lovallo et al., 2000; Stephens & Wand, 2012) use are associated with dysregulation of the HPA axis, as measured via levels of cortisol. Similarly, evidence for alterations in HPA axis functioning have been reported for both the acute and chronic effects of cannabis, with repeated use being related to reduced cortisol levels in response to acute stress and acute cannabis administration (Cservenka et al., 2018; Glodosky et al., 2021). In contrast, typical diurnal cortisol levels as a function of chronic cannabis use are less understood, furthering the need to investigate potential alterations of the HPA axis in the absence of acute substance use or stress induction (Cservenka et al., 2018; Glodosky et al., 2021).

Cannabis and the Cortisol Awakening Response

While multiple indices can be used to assess HPA axis functioning, one marker is the cortisol awakening response (CAR), which reflects both state and trait characteristics of an individual (Hellhammer et al., 2007). The CAR refers to the sharp rise in cortisol that occurs within the first 30 minutes after waking, is considered a marker

of HPA axis functioning and daily stress regulation (Clow et al., 2010; Pruessner et al., 1997), and may signal the body’s ability to prepare for the upcoming demands of the day (Clow et al., 2010; Powell & Schlotz, 2012). Disruptions in the CAR have been associated with depression, anxiety, and impaired executive functioning (Dedovic & Ngiam, 2015; Stalder et al., 2025). Heavy alcohol use has also been related to altered HPA axis functioning, with frequent drinking and high perceived stress being correlated with a blunted CAR among emerging adults (Obasi et al., 2015). While several studies have investigated the acute response to cannabis and stress among individuals with frequent cannabis use (Cservenka et al., 2018; Glodosky et al., 2021), to our knowledge, only one recent study has examined the CAR in adults with chronic cannabis use (Glodosky et al., 2026). The study found that individuals with frequent cannabis use demonstrated a blunted cortisol awakening response (CAR), a flatter diurnal cortisol slope, and elevated afternoon/evening cortisol compared to healthy controls. Similar patterns of HPA axis disruption have been observed in populations characterized by heightened stress vulnerability or cannabis-related risk. For instance, adolescents who initiated cannabis use at an early age exhibited lower cortisol levels 30 minutes after awakening compared to those with later onset cannabis use (Huizink et al., 2006). Likewise, among individuals with schizophrenia, those whose illness onset followed cannabis exposure showed higher baseline cortisol alongside a flattened CAR, indicating dysregulation of stress reactivity (Monteleone et al., 2014). In particular, inefficient stress coping may be associated with a blunted CAR, which could indicate impairments in the body’s ability to mount a typical preparatory stress response for the demands of the day (Clow et al., 2010; Dedovic & Ngiam, 2015). Therefore, examining whether cannabis use is associated with alterations in the CAR provides a window into how cannabis use may translate into dysregulation of individuals’ stress systems.

Current Study

Apart from one study (Glodosky et al., 2026), the current literature has not examined the CAR in an adult sample of individuals with frequent

cannabis use. Additionally, this naturalistic study builds upon previous laboratory-based research to measure diurnal cortisol response by having participants collect saliva samples in an at-home environment. The current study expands upon recent findings by Glodosky et al. (2026) and addresses some limitations. For instance, previous findings were limited by a single-day sampling protocol. We utilized a two-consecutive-day sampling protocol to enhance reliability by examining cortisol averages across collection timepoints. In addition, while Glodosky et al. (2026) included participants with elevated levels of depression and anxiety, we explicitly excluded participants for any self-reported lifetime diagnosis of a psychiatric disorder in an effort to isolate the effects of frequent cannabis use on the outcomes of interest. In sum, the study aims to replicate and extend recent findings by 1) determining whether there are group differences in the CAR in adults with frequent cannabis use and healthy controls and 2) for the first time, assessing the association between subjective stress and diurnal cortisol levels. In particular, the current study extends previous literature by investigating the correspondence between subjective vs. physiological stress levels among adults with frequent cannabis use. Based on prior findings of a blunted CAR in individuals with chronic cannabis use (Glodosky et al., 2026), we hypothesized individuals with frequent cannabis use would exhibit a blunted CAR relative to healthy controls. In addition, previous research has indicated a significant association between momentary stress and cortisol release in daily life in the absence of acute stress induction (Jacobs et al., 2007; Joseph et al., 2021; Smyth et al., 1998). Thus, we conducted exploratory analyses to test the interaction between substance use group and subjective stress in relation to diurnal cortisol levels in individuals with frequent cannabis use relative to healthy controls.

METHODS

Participants and Demographics

Participants were recruited through community postings, such as flyers in coffee shops and cannabis dispensaries, as well as online advertisements on Facebook and Craigslist. After obtaining informed consent, eligibility interviews

took place. Participants were excluded for self-reported neurological or severe medical conditions that required treatment, lifetime psychiatric disorder diagnosis, prenatal exposure to alcohol or drugs, pregnancy, and current use of psychotropic or steroid medications. The use of these medications was excluded to ensure that there would be no confounding effects on HPA axis functioning, including basal cortisol, the CAR, and diurnal cortisol rhythm (Meador-Woodruff & Greden, 1988).

A total of 82 individuals participated in the current study, including 39 who used cannabis frequently (CU) and 43 who were healthy controls (HC). Individuals with frequent cannabis use were defined as those who used cannabis ≥ 3 days per week in the past year, reported cannabis use in the past month, and had a combined ≤ 15 lifetime uses of illicit substances other than cannabis. Individuals who had more than 15 lifetime uses combined of other illicit substances were excluded to avoid potential confounds of polysubstance use in the sample (Casey & Cservenka, 2020; Lahanas & Cservenka, 2019; Vele et al., 2022). In contrast, healthy controls reported ≤ 1 day/month cannabis use in the past year, no past month use, and < 50 lifetime uses. Healthy controls were excluded if they had > 90 days of lifetime cigarette use, any lifetime illicit substance use, or reported drinking five or more alcoholic drinks for males and four or more for females on any occasion (Vele et al., 2022). All study procedures were approved by and in accordance with the ethical guidelines of the Oregon State University Institutional Review Board (Study #8699).

Self-report Measures

Once eligible, participants were invited to a study visit and asked to complete self-report questionnaires on sleep, stress, depression, and anxiety. To characterize the sample on sleep quality, the Pittsburgh Sleep Quality Index (PSQI) was used, with higher scores reflecting poorer sleep quality (Buysse et al., 1989).

The Perceived Stress Scale (PSS) is a 14-item self-report inventory that measures the severity of perceived stress in the past month (Cohen et al., 1983), with questions answered on a Likert scale (0 = Never, 1 = Almost Never, 2 = Sometimes, 3 = Fairly Often, 4 = Very Often). Higher scores

indicate greater perceived prevalence of stress over the past month.

The sample was also characterized for self-reported depression and anxiety, via the Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI), respectively. The BDI is a self-report inventory measuring the severity of depressive symptoms, consisting of 21 items (Beck et al., 1961). These describe symptoms of depression, including sadness, loss of pleasure, and suicidal ideation. Participants completing this measure were instructed to select the statement that best described the way they have felt in the past two weeks. Scores could range from 0 to 63, with higher scores reflecting greater depressive symptoms. The BAI is a 21-item self-report inventory for measuring the severity of anxiety (Beck et al., 1988). The scale contains items describing both psychological and physiological symptoms of anxiety (e.g., nervousness, fear, difficulty breathing, heart pounding, etc.) where a total score of 0-21 is considered low anxiety, a score of 22-35 is moderate anxiety, and a score of 36 and above is high anxiety. Participants were asked to report on their anxiety in the past month.

The Timeline Followback (TLFB) survey, a calendar assessment that asks participants to recall their substance use over the past 30 days (Sobell & Sobell, 1992), was completed by all participants to determine their past month cannabis and alcohol use. Participants with any cannabis use in the past six months completed the 21-item Brief Marijuana Consequences Questionnaire (B-MACQ) to assess recent problems (e.g., cognitive, social, risk-related, academic/occupational, and health-related consequences) related to cannabis use (Simons et al., 2012; $\alpha = 0.95$). Participants answered yes or no to indicate whether the problem occurred to them in the past six months and a total score was calculated (0-21), with higher scores reflecting more cannabis-related consequences.

In addition to frequency of cannabis use in the past month, participants were asked about forms of use and methods of administration using the Daily Sessions, Frequency, Age of Onset, and Quantity of Cannabis Use Inventory (DFAQ-CU; Cuttler & Spradlin, 2017). As frequency of cannabis use is a commonly used metric for defining recent cannabis use (Carlini & Schauer, 2022; Substance Abuse and Mental Health

Services Administration, 2024), we chose to use this variable to define cannabis use criteria for the CU group. Due to limitations in statistical power, we did not consider form or method of administration as additional moderators in the current sample, but primary form and method of cannabis use are reported in Table 4.

Procedures

Upon arriving at the lab for the study visit, participants completed a breathalyzer test to assess for the absence of acute intoxication by alcohol and provided a urine sample to assess for recent substance use. All participants had a breathalyzer reading of 0.00 and were able to continue with the study visit. To evaluate recent substance use, the Rapid Drug Test Cup-12 panel urinary analysis was used (CLIA Waived, Inc.).

To determine diurnal cortisol levels, participants were instructed to collect saliva samples at the time of awakening (no more than 5 minutes after waking up), 30 minutes later, and in the evening (at 9:00 PM) on two consecutive weekdays (Berger et al., 2017; Bublitz et al. 2012, Rosnick et al., 2016; Simon et al., 2016). Collecting samples at awakening and 30 minutes later is a commonly used approach to capture the elevated levels that define the awakening diurnal cortisol rhythm (Adam & Kumari, 2009; Dong et al., 2024). Additionally, given prior research indicating greater basal evening cortisol between individuals who later used cannabis (Huizink et al., 2006) as well as those with frequent cannabis use (Glodosky et al., 2026), relative to healthy controls, evening saliva samples were also collected from all participants.

During their visit, participants received instructions on how to collect saliva samples and were provided with kits containing oral Salivette swabs from Salimetrics' Passive Drool Method (Salimetrics, LLC, 2025). Participants were instructed to avoid eating, drinking, consuming caffeine, alcohol, tobacco, cannabis, or illegal substances prior to morning sample collection, and at least one hour prior to evening collection. In addition, they were instructed not to brush their teeth for 30 minutes before collecting the sample (Butler et al., 2017; Glodosky et al., 2026).

Participants were given a pamphlet to record their subjective stress ratings (0 = no stress, 10 = high stress), the time of day, and date of sampling.

To collect the sample, participants were instructed to collect a minimum of 0.75 mL and no more than 1.5 mL of saliva into the respective tube (Salimetrics LLC, 2025). In order to ensure compliance, participants were sent reminders the day before each of their scheduled saliva collection days using Mosio, a research-focused text messaging software (Mosio, Seattle, WA). Once saliva collection was completed, participants were asked to store samples at home in their freezer prior to returning them to the lab.

After participants returned samples to the lab, they were stored in a laboratory freezer at -20 degrees Celsius for no longer than six months and then shipped on dry ice overnight to UC Irvine Institute for Interdisciplinary Salivary Bioscience Research for cortisol immunoassay analysis. The assay detection ranges were 0.007 µg/dL (lower limit) to 3.000 µg/dL (upper limit), with a coefficient of variation of 4.85%. A total of 492 samples (82 participants, 6 samples each) were sent for analysis, but two evening samples could not be pipetted due to thick mucins, resulting in a total of 490 assayed samples.

Data Analyses

Cortisol levels were quantified using immunoassays, and data were analyzed using the IBM Statistical Package for the Social Sciences (version 29). Prior to primary analyses, the normality of model residuals was assessed using Shapiro-Wilk tests and residual plots. To correct for positive skewness and meet the assumption of normally distributed residuals, the evening cortisol dependent variable was log-transformed prior to regression analyses. Residuals for awakening and 30 minutes post-awakening cortisol values met assumptions of normality.

Demographic, sleep, mental health, and substance use characteristics were compared between groups with chi-square and independent samples *t*-tests. For the first aim, an independent samples *t*-test assessed group differences in CAR (the mean 30 min post-awakening across two days of sampling minus mean awakening cortisol across two days of sampling) between CU and HC. For the second aim, multiple linear regressions examined associations between average subjective stress (mean stress across two days at each collection time) and average cortisol (mean cortisol across two days at each collection time) with group (CU vs. HC) as the moderator. A significance threshold of $p < 0.05$ was applied for all analyses.

RESULTS

Demographic and Substance Use Characteristics

Participants significantly differed in age such that CU were older than HC (Table 1). Although there were significant differences between the two groups, age was not related to cortisol levels at any of the collection times. There were no group differences in sex assigned at birth ($p = .55$), racial ($p = .51$), or ethnic ($p = .82$) identity. PSQI total scores, BDI, BAI, and PSS were all significantly higher in CU relative to HC. Importantly, groups did not differ on the average total hours of sleep they were getting in the past month ($p = .58$), nor the average time of awakening ($p = .12$) on the two collection days (Table 2). Groups did not differ on average self-reported stress at any of the collection time points (at awakening: $p = .35$, 30 minutes post-awakening: $p = .75$, evening: $p = .07$).

Table 1. *Demographics*

Baseline characteristic	CU ($N = 39$)		HC ($N = 43$)		p
	<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>	
Age	25.74	10.46	21.21	2.44	.01
Sex (M/F)	21/18		26/17		.55
Race (%)					.51
Asian	15.38		9.30		
Black/African American	0.00		2.33		
Native Hawaiian/Pacific Islander	2.56		0.00		

White	74.36	72.09	
More than one	5.13	13.95	
Unknown	2.56	2.33	
Ethnicity (%)			.82
Hispanic	7.89 ^a	9.30	
Not Hispanic	92.11	90.70	

Note. Bold *p* values reflect significant group differences. ^a*n* = 1 missing for CU.

Table 2. *Sleep and Mental Health Characteristics*

	CU (<i>N</i> = 39)		HC (<i>N</i> = 43)		<i>p</i>
	<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>	
PSQI Total Score	4.90	2.52	3.65	1.80	.01
PSQI Total Hours Slept	7.25	1.02	7.37	0.86	.58
Time of Awakening	7.97	1.50	7.48	1.31	.12
BDI	8.05	6.35	3.44	2.95	<.001
BAI	5.74	5.22	3.40 ^a	4.29	.03
PSS	35.18	7.99	30.26 ^a	5.92	.002

Note. Bold *p* values reflect significant group differences. PSQI = Pittsburgh Sleep Quality Index; BDI = Beck Depression Inventory; BAI = Beck Anxiety Inventory; PSS = Perceived Stress Scale. ^a*n* = 1 missing for HC

As expected, past month cannabis use days, assessed via the TLFB, was significantly higher in CU relative to HC (Table 3). Two participants in the HC group reported using cannabis once in the month prior to their study visit, despite being eligible at screening. Urine

toxicology screening indicated all HC urine samples were THC-negative, and 33/39 CU samples were THC-positive. When examining alcohol use, CU reported significantly more alcohol use days in the past month relative to HC (Table 3).

Table 3. *Recent Substance Use*

	CU (<i>N</i> = 39)		HC (<i>N</i> = 43)		<i>p</i>
	<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>	
TLFB (Past 30-day Cannabis Use Days)	20.56	8.66	0.05 ^a	0.21	< .001
TLFB (Past 30-day Alcohol Use Days)	5.10	4.62	2.53	2.96	.004

Note. Bold *p* values reflect significant group differences. TLFB = Timeline Followback. ^a*n* = 2 HC reported 1 day of past month cannabis use at time of study visit despite screening eligible.

Participants were asked about forms and methods of administration on the DFAQ-CU. Marijuana flower was the primary form of

cannabis used (81.6%) while bongs (water pipes) were the primary method of administration reported (40.5%) in the current sample (Table 4).

Table 4. *Methods and Forms of Cannabis Use in the CU Group*

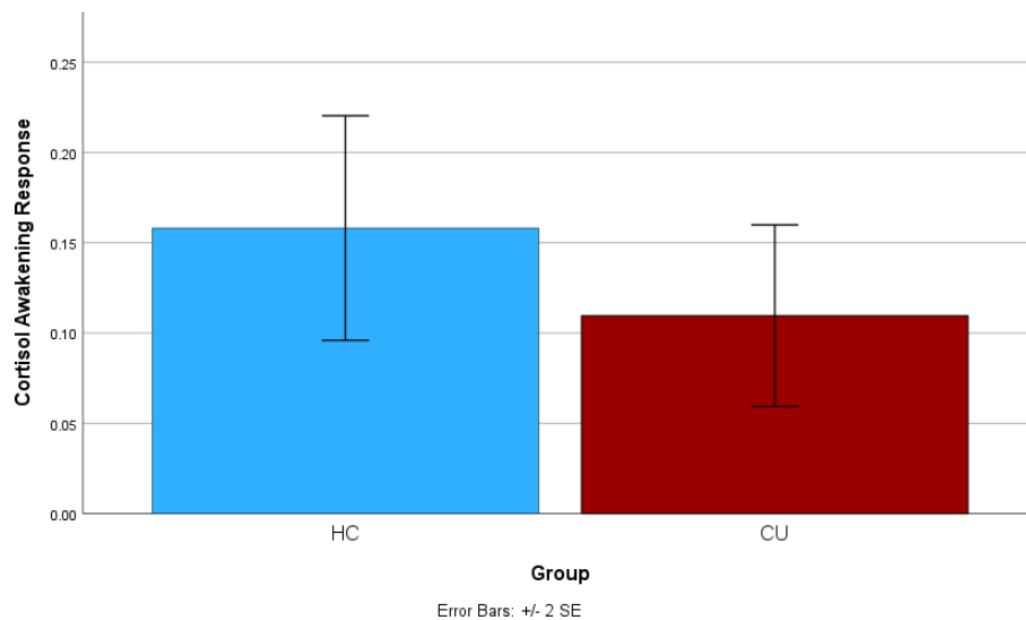
Cannabis Administration	Frequency	Percent
Method ^a		
Joints	7.00	18.90
Blunts	1.00	2.70
Hand pipe	7.00	18.90
Bong (water pipe)	15.00	40.50
Vaporizer	4.00	10.80
Edibles	3.00	8.10
Total	37.00	100.00
Form ^b		
Marijuana Flower	31.00	81.60
Concentrates	5.00	13.20
Edibles	2.00	5.30
Total	38.00	100.00

Note. ^a $n = 2$ missing; ^b $n = 1$ missing.

Group Differences in the Cortisol Awakening Response

To test the first aim, the CAR was compared between CU and HC using an independent

samples t -test. Figure 1 shows that, contrary to hypotheses, there was no significant group difference in the CAR, $t(80) = -1.20$, $p = .23$, Cohen's $d = 0.27$.

Figure 1. *Group Differences in the Cortisol Awakening Response*

Note. Mean cortisol awakening response (CAR) for healthy controls (HC) and individuals with frequent cannabis use (CU). There were no group differences in the CAR ($p = .23$).

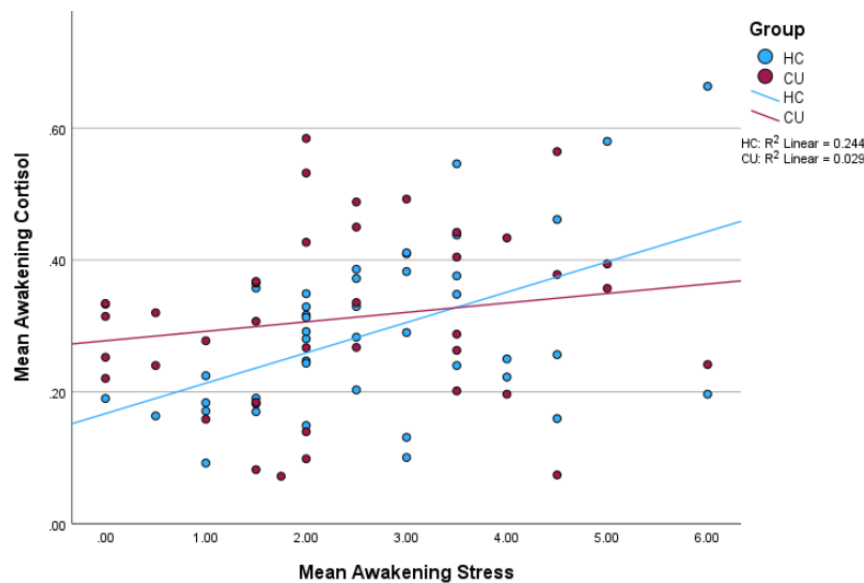
Subjective Stress and Cortisol Associations

For the second aim, multiple regression analyses were used to examine the interactions

between stress and group status on cortisol levels at each time of collection. The model examining the effect of subjective stress, group, and their interaction on morning cortisol levels was significant, $F(3, 78) = 4.31$, $R^2 = 0.14$, $p = .007$; Figure 2. There was a significant main effect of subjective stress, such that higher stress was related to higher cortisol levels ($\beta = 0.52$, $t = 3.32$,

$p = .001$), and a main effect of group, such that CU had higher cortisol at awakening relative to HC ($\beta = 0.43$, $t = 2.02$, $p = .047$). A trend-level interaction was also observed between the two variables ($\beta = -0.39$, $t = -1.70$, $p = .093$), suggesting a trend towards a stronger association between subjective and physiological stress in HC relative to CU at awakening.

Figure 2. *Subjective Stress and Cortisol at Awakening*



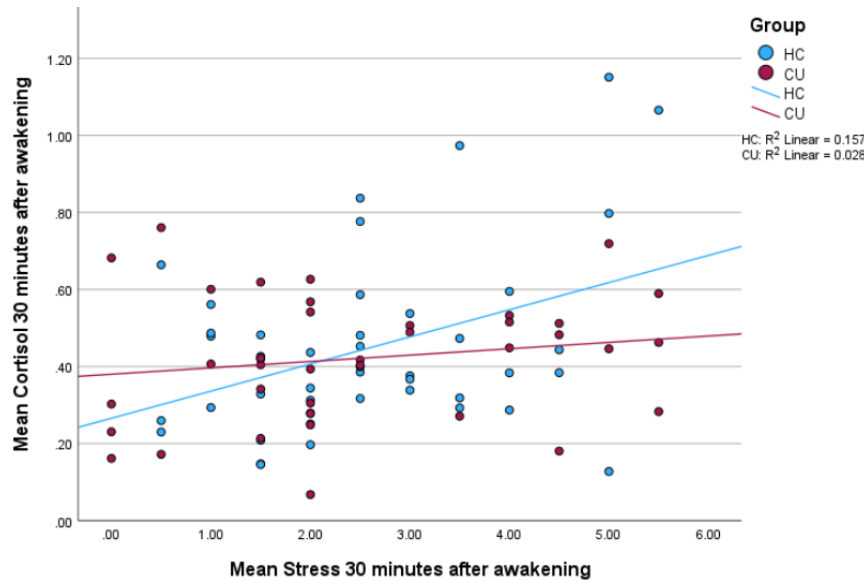
Note. Associations between subjective stress and cortisol levels at awakening in healthy controls (HC) and individuals with frequent cannabis use (CU). The regression model was significant ($R^2 = 0.14$, $p = .007$), with subjective stress positively related to cortisol ($p = .001$). CU exhibited higher awakening cortisol than HC ($p = .047$). A trend-level interaction ($p = .093$) suggested a stronger alignment between subjective stress and cortisol in HC compared to CU.

The model examining the effect of subjective stress, group, and their interaction on cortisol levels 30 minutes after awakening was significant, $F(3, 78) = 3.63$, $R^2 = 0.12$, $p = .016$; Figure 3. There was a significant main effect of subjective stress, such that higher stress was related to higher cortisol levels ($\beta = 0.51$, $t = 3.12$, $p = .003$), but no main effect of group ($\beta = 0.28$, $t = 1.31$, $p = .193$). A trend-level interaction was

present between group and stress ($\beta = -0.44$, $t = -1.82$, $p = .072$), suggesting a trend towards a stronger association between subjective and physiological stress in HC relative to CU 30 minutes after awakening.

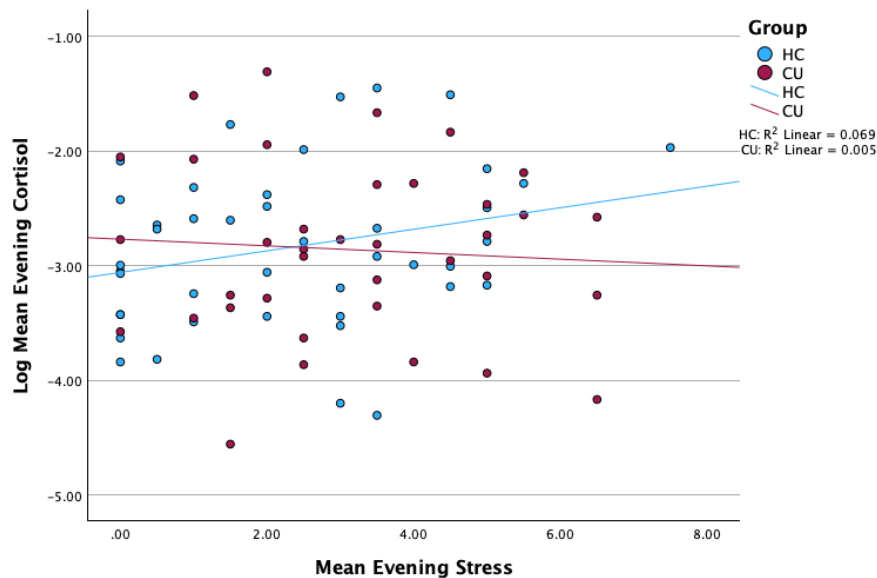
The model examining the average subjective evening stress, group, and their interaction on log-transformed evening cortisol levels was not significant, $F(3, 76) = 1.27$, $R^2 = 0.04$, $p = .41$; Figure 4.

Figure 3. *Subjective Stress and Cortisol 30 Minutes After Awakening*



Note. Associations between subjective stress and cortisol levels 30 minutes post-awakening in healthy controls (HC) and individuals with frequent cannabis use (CU). The regression model was significant ($R^2 = 0.12$, $p = .016$), with subjective stress positively related to cortisol levels ($p = .003$). There was no significant main effect of group ($p = .193$). A trend-level interaction ($p = .072$) suggested a stronger alignment between subjective stress and cortisol in HC compared to CU.

Figure 4. *Subjective Stress and Evening Cortisol*



Note. Evening subjective stress and log-transformed cortisol levels in healthy controls (HC) and individuals with frequent cannabis use (CU). The regression model including evening stress, group, and their interaction was not significant.

Post-hoc Analyses

In order to test whether the greater proportion of males relative to females in our sample may

have explained the current findings, an exploratory post-hoc t -test and regressions stratified by sex were conducted to test group differences in the CAR and examine the effects of

subjective stress, group, and their interaction on diurnal cortisol. There were no significant group differences in the CAR for CU vs. HC in males: $t(45) = -1.45$, $p = .15$, Cohen's $d = 0.43$, or females: $t(45) = -0.67$, $p = .51$, Cohen's $d = 0.23$. Stratification revealed significant models for males at awakening, $F(3, 43) = 2.91$, $R^2 = .17$, $p = .045$, and at 30 minutes post-awakening, $F(3, 43) = 5.92$, $R^2 = .29$, $p = .002$. For males, higher subjective stress was related to higher cortisol at both time points ($\beta = 0.52$, $t = 2.67$, $p = .01$ and $\beta = 0.73$, $t = 3.86$, $p < .001$, respectively). The main effect of group at awakening observed in the full sample was no longer significant for males: $\beta = 0.48$, $t = 1.67$, $p = .10$, potentially due to reduced statistical power. In contrast, the models examining awakening and 30 minutes post-awakening for females were not significant (both p 's $> .240$). Models examining evening cortisol remained nonsignificant for both males and females, consistent with earlier findings.

Given the significant effect of group (CU > HC) on awakening cortisol, we examined whether cannabis use metrics (number of cannabis use days in the past 30 days) and consequences of cannabis use via the B-MACQ were related to awakening cortisol levels among CU. Past 30-day cannabis use days were not related to average awakening cortisol among CU, $r(37) = 0.09$, $p = .57$. However, there was a significant positive correlation between B-MACQ total scores and awakening cortisol, $r(36) = 0.43$, $p = .007$, among CU (missing B-MACQ for one participant).

DISCUSSION

The present study examined diurnal cortisol patterns and subjective stress among adults with frequent cannabis use compared to healthy controls. Contrary to hypotheses and previous work (Glodosky et al., 2026), there were no significant group differences in the cortisol awakening response (CAR). Despite similar sample ($N = 82$) and substance use characteristics (average of ≥ 20 days of cannabis use among CU) to the study by Glodosky et al. (2026), we did not replicate their findings. Results indicate PSQI total scores, BDI, BAI, and PSS were all significantly higher in CU relative to HC, such that individuals who used cannabis frequently reported poorer sleep quality in the past month and higher levels of self-reported depression,

anxiety, and perceived stress, as seen in previous research (Glodosky et al., 2026). The previous study included participants with higher mean BDI scores than the current sample, later waking times in those who used cannabis frequently relative to healthy controls, and differences in statistical analyses for modeling the CAR, which may have contributed to differences in findings; namely, the CAR was modeled separately among individuals with cannabis use relative to the non-use group (Glodosky et al., 2026), whereas the current study compared group differences in the CAR. Furthermore, exploratory sex-stratified analyses in the current sample were consistent with the findings indicating lack of group differences for the CAR. This suggests that the greater proportion of males relative to females in the current study relative to Glodosky et al. (2026) may not explain differences between the studies' findings, although sex-stratified analyses were limited by statistical power. Thus, sample characteristics and statistical approaches are important points to consider when comparing the current findings to comparable previous research.

For the second aim of the study, CU had greater cortisol levels at the time of awakening relative to HC, and average awakening cortisol was significantly related to self-reported cannabis use consequences. Prior research has found greater basal salivary cortisol in individuals with frequent cannabis use relative to controls during morning collection times (King et al., 2011), and when collapsing multiple collection times between the morning and early afternoon (Carol et al., 2017). In addition, one study found that individuals with frequent cannabis use and schizophrenia had higher baseline morning cortisol for the first at-home collection time point relative to healthy controls (Monteleone et al., 2014), in line with the current findings. This elevated level of morning cortisol could reflect basal hypersecretion of cortisol in individuals with frequent cannabis use and could be a marker of HPA axis dysregulation. Importantly, there was a significant association between these levels and cannabis-related consequences, suggesting that the severity of cannabis use may be associated with HPA axis impairment. Second, greater subjective stress was associated with higher cortisol levels across participants during morning collection times, which supports prior research of self-reported stress and cortisol

associations (Jacobs et al., 2007; Joseph et al., 2021; Smyth et al., 1998). Finally, although only a trend interaction between subjective stress and group was present during the morning collection times, these findings indicated that individuals with frequent cannabis use may show less alignment between their self-reported stress and their physiological cortisol responses, which may reflect altered stress-response functioning. While speculative, repeated cannabis use may weaken the alignment between subjective stress and physiological cortisol signaling, consistent with the stress-coping framework, where coping behaviors influence both perceived and physiological stress (Lazarus & Folkman, 1984; Wills et al., 1990). Chronic cannabis use may also increase allostatic load, producing wear-and-tear on stress-regulatory systems and blunting adaptive cortisol responses throughout the day (McEwen, 1998). These effects mirror patterns observed with alcohol and nicotine, which are associated with dysregulated HPA axis functioning following repeated use (Boschloo et al., 2011; Stephens & Wand, 2012). Elevated anxiety, depressive symptoms, and poorer sleep quality among CU may further exacerbate HPA axis dysregulation, creating a feedback loop in which cannabis use intended to reduce stress may become increasingly maladaptive.

These results contribute to a growing literature on the complex relationship between substance use and stress. While acute cannabis use may alleviate stress, chronic use may disrupt the body's ability to form adaptive stress responses, highlighting the potential long-term consequences of using cannabis as a coping strategy (al'Absi et al., 2021). Our findings also underscore the importance of assessing both subjective and physiological stress markers.

Strengths and Limitations

The current study has important strengths that should be highlighted. To our knowledge this is the only the second study to examine the CAR in individuals with frequent cannabis use (Glodosky et al., 2026) in the absence of other co-occurring psychopathology, such as schizophrenia (Monteleone et al., 2014). We excluded participants with any self-reported lifetime diagnosis of a psychiatric disorder to prevent the confounding effects of co-occurring

psychopathology on the measures of interest. In addition, groups did not differ in average waking times, which is beneficial as previous research found that waking times were significantly related to diurnal cortisol (Glodosky et al., 2026). There are some limitations to be considered. The cross-sectional design prevents causal interpretations of the data. Thus, it is not possible to know the directionality between cannabis and cortisol relationships in the current study. Additionally, participants were asked to abstain from substance use prior to sampling, but short-term effects from abstinence could have potentially influenced measurements of stress and cortisol. While the current study had a relatively small sample size ($N = 82$), it is noteworthy that this is comparable to the two previous studies of adults with frequent cannabis use that examined the CAR ($N = 82$, Glodosky et al., 2026; $N = 65$, Monteleone et al., 2014). Future research should attempt to not only collect larger sample sizes but also examine the roles of long-term cannabis abstinence on the CAR in addition to investigating diurnal cortisol rhythm among individuals with current frequent cannabis use. Lastly, to reduce participant burden, we only asked participants to collect three saliva samples over two consecutive days, similar to prior research (Berger et al., 2017; Bublitz et al. 2012, Rosnick et al., 2016; Simon et al., 2016). While this is sufficient to capture the CAR and basal levels of evening cortisol, more frequent sampling could provide detailed insights of HPA axis functioning, such as area under the curve and diurnal cortisol slope (Dong et al., 2024; Glodosky et al., 2026; Hoyt et al., 2021; Segerstrom et al., 2014).

Future Directions

Future research should aim to clarify the long-term effects of frequent cannabis use on the individual's stress system through longitudinal designs. More frequent cortisol sampling over extended periods, combined with objective (e.g., actigraphy) verification of awakening and saliva collection times, would improve accuracy and reliability of CAR measurements. Examining the effects of cannabis frequency, quantity, potency, and age of cannabis use onset on stress responses is critical to understanding the relationship between cannabis use metrics and stress

neuroaxis functioning as this is currently unknown. Additional studies with larger samples should also investigate individual differences, such as sex (Kirschbaum et al., 1992) and psychological factors such as resilience (Aizpura-Perez et al., 2023), that may moderate the CAR. Lastly, exploring longitudinal associations between cortisol and health outcomes could clarify whether the observed effects on HPA axis functioning are adaptive or pose significant long-term health risks.

Conclusions

In the current study, we did not find significant group differences in the CAR between individuals with frequent cannabis use and healthy controls. Frequent cannabis use may be associated with morning hypersecretion of cortisol at awakening, which could serve as a marker of HPA axis dysregulation. Given the rise in adult cannabis use and the limited research examining the relationship between cannabis use and stress neuroaxis functioning, future studies should investigate whether HPA axis functioning represents a modifiable biological marker that can be altered by reductions in cannabis use.

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