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RESEARCH ARTICLE

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Examination-related anticipatory levels of dehydroepiandrosterone and cortisol predict positive affect, examination marks and support-seeking in college students

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ABSTRACT

Dehydroepiandrosterone (DHEA) and cortisol release appear to have contrasting effects on stress perception during stressful tasks. This study aimed to investigate anticipatory examination stress in college students by considering DHEA, cortisol, psycho-emotional aspects and examination performance. Seventy-six students (66 females, 10 males; age range 18–25 years) provided saliva samples and completed questionnaires in two sessions 48 hours apart. During the second session, the students performed the examination. The questionnaires used were the State-Trait Anxiety Inventory, the Positive and Negative Affect Scale, and the Brief-Coping Orientation to Problems Experienced Inventory. DHEA, cortisol, anxiety and negative affect showed an anticipatory rise before the examination (all $ps < 0.001$). This rise of DHEA and cortisol was associated with lower positive affect ($p = 0.001$ and $p = 0.043$, respectively). However, only the DHEA anticipatory levels were linked to poorer examination marks ($p = 0.020$). Higher levels of the DHEA/cortisol ratio in anticipation of the examination were related to lower scores on the support-seeking strategy ($p = 0.022$). There was no association between DHEA and cortisol levels and anxiety, negative affect, active and avoidant coping strategies, or academic record. These results suggest that how DHEA and cortisol respond in anticipation of examination stress significantly impacts students' emotional well-being during examination periods and how they cope with stress. They also suggest that levels of DHEA in anticipation of an academic stressor have detrimental effects on stress management.

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Dehydroepiandrosterone; cortisol; affect; state anxiety; coping strategies; examination stress

Introduction

Academic examinations can cause stress and activate the Hypothalamic-Pituitary-Adrenal (HPA) axis, which releases cortisol and dehydroepiandrosterone (DHEA) into the bloodstream (Dutheil et al., 2021; Ulrich-Lai & Herman, 2009). Cortisol and DHEA mutually regulate each other, influencing the effects of stress (Kalimi et al., 1994; Maninger et al., 2009). DHEA plays a crucial protective role against the harmful effects of glucocorticoids under acute stress (Morgan et al., 2004). In contrast to cortisol's catabolic effects, DHEA is anabolic, providing protection and regeneration during stress (Maninger et al., 2009; Theorell, 2009).

Cortisol levels have been the primary focus of research on the psychobiological response to examination stress in the academic context (Garces-Arilla et al., 2023; Helbig & Backhaus, 2017; Preuss et al., 2010; Ringeisen et al., 2019). Although cortisol levels have received more attention, DHEA levels associated with standardized psychological stressors have also been studied, with mixed results. Some studies indicate an increase in DHEA concentrations (Izawa et al., 2008; Shirotsuki et al., 2009), whereas others report no changes in salivary DHEA in response to these stressors (Hidalgo et al., 2020; Smith et al., 2020). According to a meta-analysis (Dutheil et al., 2021), DHEA levels temporarily increase during stressful situations and gradually decrease after the stress has ended. The meta-analysis also concludes that DHEA levels increase in response to acute mental stress, regardless of the stressor type or duration.

Research has shown that cortisol levels during stress are negatively associated with positive psychological states or traits (for a review, see Chida & Hamer, 2008). Conversely, studies suggest that DHEA plays a role in improving stress tolerance (Morgan et al., 2009), which may facilitate cognitive aspects necessary for successful stress management (Smith et al., 2020). Sripada et al. (2013) explored the potential protective function of exogenous DHEA in the emotional

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appraisal of male participants who performed a laboratory task. The participants who received DHEA exhibited better emotion regulation during the task. Additionaly, DHEA reduced activity in regions of the brain that generate emotions and enhanced activity in areas associated with emotion regulation in this study.

Coping strategies' relationship with HPA reactivity has also been studied (Biondi & Picardi, 1999), but research has revealed a disparity in results. van Eck et al. (1996) found no correlation between HPA response and coping styles. However, other authors have shown that ineffective coping strategies such as rumination, self-blame, and avoidance can lead to increased cortisol levels (Höhne et al., 2014; Janson & Rohleder, 2017). Distraction coping has shown potential in reducing the cortisol response of female students during a laboratory stressor (Zoccola et al., 2014). The stressor consisted of a verbal task. The students who used a distraction strategy during the task had lower post-stress cortisol levels than those who used rumination. These findings suggest that coping strategies must be considered when examining the stress response, as some coping strategies may affect hormone response levels.

The association between DHEA concentrations, academic performance and perceived coping strategies was investigated in students who performed a mental stress-inducing task in the laboratory (Wemm et al., 2010). Students who remained calm during the stressful task exhibited a lower cortisol/DHEA ratio and lower risk of class failure (Wemm et al., 2010). However, these students' perceived coping strategies did not play a predictive role in the studied variables (Wemm et al., 2010).

Exploring the relationship between DHEA and cortisol levels, psychological variables, coping strategies and college students' academic performance during real-life examinations is essential for gaining insights into stress resilience and academic success.

This study investigates college students' examination stress, considering DHEA, cortisol, psycho-emotional aspects, and examination performance. Firstly, we examined the hormonal (i.e. DHEA, cortisol, and DHEA/cortisol ratio levels) and psychological (i.e. anxiety, negative and positive affect) response to an academic examination, along with the examination performance and use of coping strategies in college students. Secondly, we examined the associations between hormonal anticipatory responses and psychological response, examination performance and the use of coping strategies.

Considering that both DHEA and cortisol levels are dependent on HPA activation, we expected an increase in both hormones during the stressful session. Given that DHEA plays a protective role against the harmful effects of cortisol in stressful situations, we expected that DHEA levels would be positively associated with positive psychological states and better examination performance, whereas cortisol levels would have a negative relationship with these variables. Regarding the associations between hormonal levels and coping strategies, we hypothesized that cortisol levels would be positively associated with inefficient coping strategies, and DHEA levels would be positively associated with active coping strategies.

Material and methods

Participants

The inclusion criteria for the study were as follows: (1) being an undergraduate student of Psychology at the University of Zaragoza and (2) being enrolled in the subject Basic Biology I. The exclusion criteria comprised: (1) the presence of cardiovascular, endocrine, neurological (e.g. epilepsy), or psychiatric disorders (e.g. depression or anxiety disorders); (2) a history of general anesthesia within the last year; (3) smoking more than five cigarettes per day; (4) self-reported daily alcohol and drug use; (5) visual or hearing impairment; and (6) undetectable levels of DHEA and cortisol according to the test kit used (described in the Biochemical analyses section).

Thus, the sample comprised 66 women (87%) and 10 men (13%) aged 18 to 25 (women: $M = 18.62$, $SD = 1.11$; men: $M = 18.40$, $SD = 0.70$).

Participants provided written informed consent before participation in the study. The study followed the European Directive 2001/20/EC and the Declaration of Helsinki for biomedical research involving human subjects. The research protocol was approved by the Research Ethics Committee of Aragon (CEICA, PI20/074).

Procedure

Participants attended two individual sessions in the educational facilities where they studied. Sessions started at 11 a.m., lasting no more than 110 minutes each, and followed a pre-post experimental design (T1-T2). Figure 1 shows a schematic diagram of the procedure described below. The first session (i.e. non-stressful session) occurred 48 hours before an official examination (i.e. stressor), while the second session (i.e. stressful session) took place on the day of the official examination. Also, at the beginning of both sessions, participants had a 5-minute acclimatization period. Participants were asked to maintain their general habits, sleep as usual, not consume alcohol and avoid vigorous physical activity the day before each session. They were also instructed not to smoke or take any stimulating substance, eat, or drink anything except water for two hours before each session and not to brush their teeth for at least one hour before the sessions.

Session 1 (non-stressful session)

In the non-stressful session, participants completed the following self-assessment questionnaires, which are described in the Questionnaires and Measures section: a sociodemographic questionnaire (e.g. weight, height, age, educational and economic level, among others), the Brief-COPE, the Positive and Negative Affect Scale (PANAS; T1), and the State-Anxiety Inventory of the State-Trait Anxiety Inventory (STAI-S; T1). After completing these questionnaires, participants completed a memory task (participants viewed a set of 60 emotional pictures, including 20 positive, 20 negative, and 20 neutral pictures) consisting of a visual recognition memory task (these results were not considered in the purpose of this research). Immediately after that, they again completed the

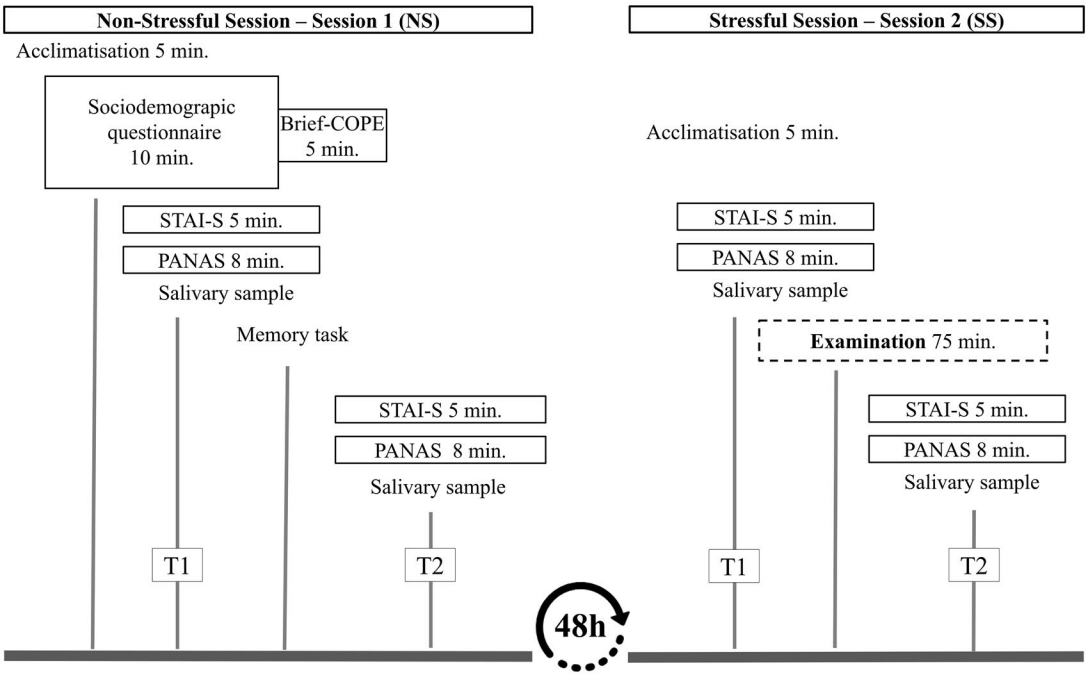

Figure 1. Timeline of the study procedure. In the non-stressful session (NS), an acclimatization period preceded a sociodemographic questionnaire and a coping strategies questionnaire (Brief-COPE). Following this, participants completed the State-Trait Anxiety Inventory (STAI-S) and the Positive and Negative Affect Scale (PANAS), and a saliva sample (T1) was also collected. After a memory task, participants again completed the STAI-S and PANAS, providing another saliva sample (T2). Subsequently, 48 hours later, participants faced the stressful session (SS), which began with an acclimatization period. During this session, participants completed the STAI-S and PANAS, followed by a saliva sample (T1). Then, an official examination took place, leading to post-stressor assessments of the STAI-S and PANAS, accompanied by the collection of another salivary sample (T2).

PANAS (T2) and the STAI-S (T2). Also, we collected two saliva samples to measure cortisol and DHEA levels across the session. Specifically, the first saliva sample (T1) was taken at the beginning of the session while completing PANAS and STAI-S for the first time (T1), and the second saliva sample (T2) was collected at the end of the session while completing the PANAS and the STAI-S for the second time (T2) (Figure 1). This session, conducted in the same classroom as the stressful examination session, ensured a comparable setting with neutral cognitive demand and followed a similar timing for recording hormonal and psychological variables. The visual recognition memory task, chosen for its neutrality, involved participants in a cognitive activity without performance assessment or impact on academic achievement.

Session 2 (stressful session)

The stressful session occurred 48 hours later. At the onset of this session, participants completed the PANAS and the STAI-S (T1) and provided the first salivary sample (T1). Then, the examination stressor occurred, consisting of the official examination of a subject of the psychology degree. Finally, participants completed the PANAS and the STAI-S questionnaires (T2) again and provided the second salivary sample (T2) (Figure 1).

Questionnaires and measures

Anxiety

To determine the levels of state anxiety in the participants, the Spanish version (Guillén-Riquelme & Buela-Casal, 2011) of the State Anxiety Inventory was used (STAI-S, Spielberger, 1989). This questionnaire assesses anxiety as an emotional state through 20 items. Participants completed this questionnaire twice for each session, before (T1) and immediately after the memory task or the examination (T2). Items were answered rated on a 3-point Likert scale to evaluate how they felt at that time, from 0 (*not at all*), to 3 (*extremely*). In the present study, Cronbach's Alpha coefficient was good and excellet in all four assesments (NS-T1: α = .91, 95% CI [0.87, 0.94]; NS-T2: α = .91, 95% CI [0.88, 0.94]; SS-T1: α = .88, 95% CI [0.84, 0.92]; SS-T2: α = .91, 95% CI [0.87, 0.94]).

Affect

Affect was evaluated with the Spanish version (Sandín et al., 1999) of the Positive and Negative Affect Schedule (PANAS, Watson et al., 1988). This 20-item questionnaire measures mood using two dimensions: positive affect (PA) and negative affect (NA), with 10 items each. Participants completed this questionnaire twice for each session, before (T1) and immediately after the memory task or the examination stress (T2). Participants responded using a 5-point Likert scale, from 1 (*not at all*) to 5 (*extremely*). In this study, Cronbach's alpha values were good in all four assesments for PA (NS-T1: α = .80, 95% CI [0.73, 0.86]; NS-T2: α = .85, 95% CI [0.80, 0.90]; SS-T1: α = .86, 95% CI [0.80, 0.90]; SS-T2: α = .88, 95% CI [0.84, 0.92]) and for NA (NS-T1: α = .88, 95% CI [0.84, 0.92]; NS-T2: α = .78, 95% CI [0.70, 0.85]; SS-T1: α = .85, 95% CI [0.80, 0.90]; SS-T2: α = .89, 95% CI [0.85, 0.92]).

Coping strategies

The Brief-Coping Orientation to Problems Experienced Inventory (Brief-COPE; Carver, 1997), translated into Spanish

by Morán et al. (2010), was used to measure coping strategies in stressful situations. The instrument consists of 28 items rated on a Likert scale ranging from 0 (*never*) to 3 (*very often*) that measures how participants cope with a stressor. The 28 items are divided into 14 subscales of two items each. Three scales including active coping, avoidance coping, and support-seeking were derived from the study of Andrew et al. (2013). In this study, Brief-COPE showed an acceptable and good Cronbach's Alpha coefficient for all three subscales (active coping: α = .73, 95% CI [0.63, 0.81]; avoidance coping: α = .67, 95% CI [0.55, 0.77]; support-seeking: α = .85, 95% CI [0.79, 0.90]).

Examination performance

The official examination of the Basics Biology I subject comprised 50 three-choice questions, lasted 75 minutes, and represented the participants' first official examination for their university degree. It was the natural acute stressor in this study. The mark obtained for the examination was used to measure performance in the stressful task.

General academic performance

The overall academic grade at the end of the academic year, ranging from 0 to 10, represented the student's comprehensive annual academic performance across all course subjects and served as a long-term measure of academic success.

Biochemical analyses

Participants provided two saliva samples at each session to assess cortisol and DHEA levels by passive drooling. Participants deposited approximately 5 ml of saliva in plastic vials for each sample. After collection, all saliva samples were frozen at −20 °C until the biochemical analyses were performed in the Laboratory of Social Cognitive Neuroscience at the University of Valencia using Salimetrics Cortisol Enzyme Immunoassay kits (Salimetrics, State College, PA, USA). Assay sensitivity was 0.007 µg/dl. All four samples from each participant were analyzed using the same kit and in duplicate. Intra- and inter-assay coefficients of variation were all below 10%.

Statistical analyses and data management

Cortisol and DHEA values were logarithmically transformed as they did not have a normal distribution after the Kolmogorov-Smirnov test. However, raw values are represented to facilitate the interpretation of the figures.

Menstrual cycle phase (MCP) and body mass index (BMI) were considered to assess their potential influence. Repeated-measures ANOVAs were conducted separately to explore MCP differences in DHEA and cortisol levels. This involved using Session (non-stressful vs. stressful) and Time (T1 vs. T2) as within-subject variables, and MCP as the between-subject variable. The same analytical approach was employed to investigate potential variations in DHEA and cortisol levels based on BMI, with BMI as the between-subject variable. Post hoc comparisons were conducted with Bonferroni correction.

Separate repeated-measures ANOVAs, with Session (non-stressful session vs. stressful session) and Time (T1 vs. T2) as within-subject variable, were performed for hormonal—DHEA, cortisol, and DHEA/cortisol ratio—and psychological—anxiety, negative and positive affect—responses to stress. Post hoc comparisons were conducted with Bonferroni correction.

Separate regression analyses were conducted to explore the association between pre-examination hormonal levels—DHEA, cortisol, and DHEA/cortisol ratio—and pre-examination psychological scores—anxiety, and negative and positive affect—. The pre-examination time point was considered because was when higher values were recorded within the stressful session. Sex, age and body mass index (BMI) were included as covariates, given their established impacts on HPA-axis activity, as evidenced by prior studies (sex and age: Kudielka et al., 2009; BMI: Hewagalamulage et al., 2016). Additionally, hormonal and psychological levels registered at the onset of the non-stressful session, specifically targeting the homornal and psychological variable used in each analysis, were also included as covariates.

Furthermore, separate regression analyses were performed to explore the association between pre-examination hormonal levels—DHEA, cortisol, and DHEA/cortisol ratio—, and examination performance scores—examination mark, and general academic performance—, and coping strategies—active coping, avoidance coping, and support-seeking—. Covariates included in the analyses comprised sex, age, BMI and hormonal levels registered at the onset of the non-stressfull session.

Separate regression analyses were conducted to explore the association between coping strategies—active coping, avoidance coping, and support-seeking—and examination performance scores—examination mark, and general academic performance—. To address potential confounding effects, covariates including sex, age, and BMI were incorporated into the analyses.

All regression models were checked for multicollinearity, all the variables had a variance inflaction factor <10 and a tolerance > 0.1.

The significance level was < .05. Statistical analyses were performed with SPSS 25.0.

Results

Preliminary analyses: characteristics of the sample

Table 1 presents the characteristics of the sample and the descriptive statistics for the variables examined in the study. The variables MCP or BMI did not significantly affect hormonal levels ($p \geq 0.059$); additional details, such as statistical analyses and visual representations, are available in Supplementary Figure 1 and Supplementary Figure 2, respectively.

Salivary DHEA levels

There was a significant main effect for Session ($F(1,75) = 15.531$, $p < 0.001$, $\eta^2 = 0.172$), Time ($F(1,75) = 34.511$, $p < 0.001$,

η^2 = 0.315), and the Session×Time interaction ($F(1,75)$ = 4.317, $p = 0.041$, η^2 = 0.054). In general, participants exhibited higher DHEA levels in the stressful session compared to the non-stressful session ($p < 0.001$) and at T1 compared to T2 ($p < 0.001$). Post hoc analyses revealed that participants showed higher DHEA levels at T1 in the stressful session than in the non-stressful session ($p < 0.001$), but these levels did not differ between sessions at T2 ($p = 0.099$). Also, participants showed higher DHEA concentrations at T1 than at T2 in both sessions (both *p*s < 0.001) (see Figure 2A).

Table 1. Characteristics of the sample and descriptive statistics for the variables examined in the study.

Variables			
	n (%)		*M* (*SD*)
Characteristics		**Psychological variables**	
MCP		Anxiety	
Menstrual	19 (29)	NS-T1	23.72 (10.95)
Follicular	10 (15)	NS-T2	22.58 (10.94)
Ovulatory	10 (15)	SS-T1	37.05 (8.91)
Luteal	16 (24)	SS-T2	26.14 (10.51)
Premenstrual	11 (17)	Positive affect	
BMI		NS-T1	29.50 (5.94)
Underweight	3 (4)	NS-T2	27.64 (7.14)
Normal weight	64 (84)	SS-T1	26.92 (6.83)
Overweight	9 (12)	SS-T2	26.14 (7.87)
	M (*SD*)	Negative affect	
Hormonal variables		NS-T1	21.91 (7.77)
DHEA		NS-T2	21.24 (7.62)
NS-T1	191.48 (131.73)	SS-T1	30.50 (7.03)
NS-T2	155.47 (96.61)	SS-T2	23.01 (6.74)
SS-T1	246.95 (175.09)	Coping strategies	
SS-T2	163.56 (90.03)	Active	17.30 (3.66)
Cortisol		Avoidance	13.17 (4.75)
NS-T1	5.93 (4.66)	S. Seeking	9.01 (2.64)
NS-T2	3.33 (2.11)	Academic performance	
SS-T1	7.51 (5.32)	Examination	5.31 (1.91)
SS-T2	3.50 (1.64)	General	6.66 (1.58)
D/C ratio			
NS-T1	40.86 (25.20)		
NS-T2	57.38 (37.12)		
SS-T1	38.75 (23.03)		
SS-T2	54.81 (33.50)		

Note: M = mean; SD = standard deviation; MCP = Menstrual Cycle Phase; BMI = Body Mass Index; DHEA = Salivary DHEA concentrations; Cortisol = salivary cortisol concentrations; D/C ratio = DHEA/cortisol ratio; Anxiety = Anxiety score; Positive affect = Positive affect score; Negative affect = Negative affect score; Active = Problem-solving and cognitive restructuring coping strategies score; Avoidance = Negative coping aspects, behavioral disengagement and denial strategies score; S. Seeking = Support-seeking coping strategy score; Examination = examination mark; General = General academic performance; NS-T1 = non-stressful session immediately before the memory task; NS-T2 = non-stressful session immediately after the memory task; SS-T1 = stressful session immediately before the examination; SS-T2 = stressful session immediately after the examination.

Salivary cortisol levels

A significant main effect was observed for Session ($F(1,75)$ = 17.130, $p < 0.001$, η^2 = 0.186), Time ($F(1,75)$ = 175.031, $p < 0.001$, η^2 = 0.700), and the Session×Time interaction ($F(1,75)$ = 4.188, $p = 0.044$, η^2 = 0.053). Overall, participants exhibited higher cortisol levels during the stressful session compared to the non-stressful session ($p < 0.001$) and at the onset than at the end of sessions (T1 vs. T2: $p < 0.001$). Post hoc analyses revealed that the cortisol concentrations were higher at T1 and at T2 in the stressful session compared to the non-stressful session (T1: $p < 0.001$, and T2: $p = 0.035$). Furthermore, participants showed higher cortisol concentrations at T1 than at T2 in both sessions (both *p*s < 0.001) (Figure 2B).

DHEA/cortisol ratio

No significant main effects were observed for Session ($F(1,75)$ = 0.256, $p = 0.614$, η^2 = 0.003), Time ($F(1,75)$ = 0.101, $p = 0.751$, η^2 = 0.001), and the Session×Time interaction ($F(1,75)$ = 1.265, $p = 0.264$, η^2 = 0.017) (Figure 2C).

State anxiety

A significant main effect was observed for Session ($F(1,75)$ = 60.529, $p < 0.001$, η^2 = 0.447), Time ($F(1,75)$ = 72.533, $p < 0.001$, η^2 = 0.492), as well as Session×Time interaction ($F(1,75)$ = 39.555, $p < 0.001$, η^2 = 0.345). Overall, participants reported higher anxiety scores during the stressful session compared to the non-stressful session ($p < 0.001$) and at T1 compared to T2 ($p < 0.001$). Post hoc analyses revealed elevated state anxiety levels for participants at both times (T1: $p < 0.001$, and T2: $p = 0.007$) in the stressful session compared to the non-stressful

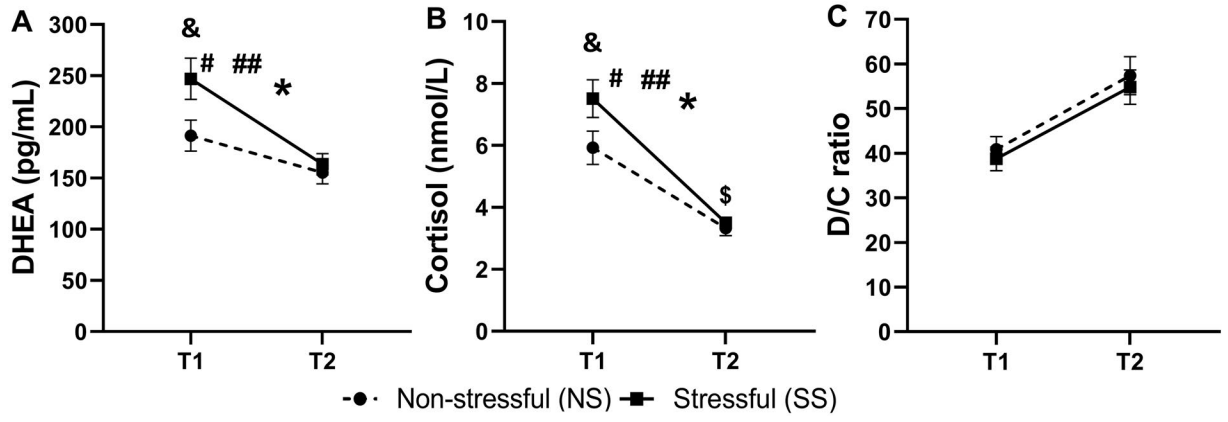

Figure 2. DHEA (A), cortisol (B) and DHEA/cortisol ratio (C) concentration levels at T1 and at T2 in non-stressful session (NS) and stressful session (SS).
Note: The depicted values are means, and error bars represent the standard error of the mean. *$p < 0.001$ (main effect: SS vs. NS); #$p < 0.001$ (main effect: T1 vs. T2); &$p < 0.001$ (SS vs. NS at T1); $$p = 0.035$ (SS vs. NS at T2); ##$p < 0.001$ (T1 vs. T2 within each of the two sessions).

session. In addition, participants in the stressful session showed higher anxiety scores at T1 than at T2 ($p < 0.001$), but these scores did not differ between T1 and T2 in the non-stressful session ($p = 0.207$) (Figure 3A).

Affect

Main Session effects were observed for both positive ($F(1,75) = 9.836$, $p = 0.002$, $\eta^2 = 0.116$) and negative ($F(1,75) = 43.009$, $p < 0.001$, $\eta^2 = 0.364$) affect. The Time factor was also significant for positive ($F(1,75) = 12.718$, $p = 0.001$, $\eta^2 = 0.145$) and negative ($F(1,75) = 66.503$, $p < 0.001$, $\eta^2 = 0.470$) affect. Overall, participants had higher scores in positive affect ($p = 0.002$) and lower scores in negative affect ($p < 0.001$) in the non-stressful session compared to the stressful session. Scores were also lower at T2 than at T1 in both positive ($p = 0.001$) and negative affect ($p < 0.001$). Moreover, the Session×Time interaction was significant for negative affect ($F(1,75) = 40.691$, $p < 0.001$, $\eta^2 = 0.352$), but not for positive affect ($F(1,75) = 2.193$, $p = 0.143$, $\eta^2 = 0.028$). Thus, post hoc analyses revealed that participants had higher negative affect at T1 and T2 in the stressful session compared to the non-stressful session (T1: $p < 0.001$, and T2: $p = 0.049$). Furthermore, participants had higher negative affect scores at T1 than at T2 in the stressful session ($p < 0.001$), whereas their negative affect scores did not differ between the two times in the non-stressful session (T1 vs. T2: $p = 0.297$) (Figure 3B and 3C).

Relationship between pre-examination hormonal levels and pre-examination psychological scores

Table 2 shows the results of the regression analyses, with positive affect scores as the criterion variable, and includes standardized betas, their significance, and the model's general statistics. The significant associations are described below. The pre-examination levels of both DHEA and cortisol were predictive of pre-examination scores on positive affect ($p = 0.001$ and $p = 0.043$, respectively). The higher the levels of these two hormones, the lower the scores on positive affect. However, the DHEA/cortisol ratio was not associated with pre-examination scores on positive affect ($p = 0.958$). Also, the levels of DHEA, cortisol, or the DHEA/cortisol ratio did not predict scores on state anxiety or negative affect ($ps \geq 0.077$), as illustrated in Supplementary Table 1.

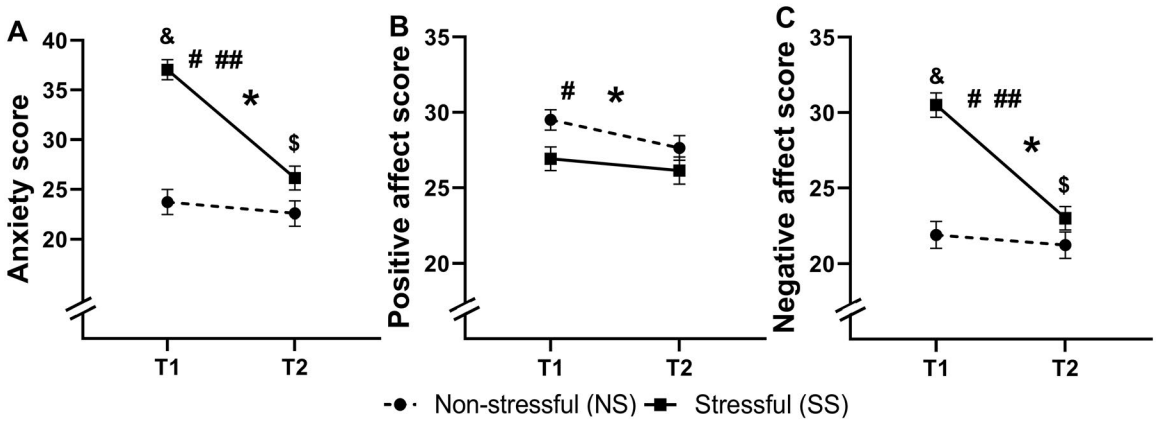

Figure 3. Anxiety (A), positive (B) and negative (C) affect scores at T1 and at T2 across sessions.
Note: The depicted values are means, and error bars represent the standard error of the mean. *$p \leq 0.002$ (main effect: SS vs. NS); #$p \leq 0.001$ (main effect: T1 vs. T2); &$p < 0.001$ (SS vs. NS at T1); $$p \leq 0.049$ (SS vs. NS at T2); ##$p < 0.001$ (T1 vs. T2 within the SS).

Table 2. Relationship between pre-examination hormonal levels and pre-examination scores in positive affect.

Positive affect scores								
Model 1	**R^2 = 0.294; Adj R^2 = 0.244 $p < 0.001$**		Model 1	**R^2 = 0.297; Adj R^2 = 0.247 $p < 0.001$**		Model 1	**R^2 =0.298; Adj R^2 = 0.247 $p < 0.001$**	
	β (CI 95%)	*p*		β (CI 95%)	*p*		β (CI 95%)	*p*
Sex	−0.224 (−8.56; −0.43)	0.031	Sex	−0.213 (−8.38; −0.17)	0.042	Sex	−0.217 (-8.43; −0.28)	0.036
Age	−0.065 (−1.73; 0.90)	0.528	Age	−0.069 (−1.76; 0.87)	0.504	Age	−0.070 (-1.76; 0.87)	0.498
BMI	0.081 (−0.30; 0.70)	0.432	BMI	0.080 (−0.30; 0.69)	0.431	BMI	0.084 (-0.29; 0.70)	0.411
DHEA (NS-T1)	−0.035 (−6.52; 4.60)	0.731	Cortisol (NS-T1)	0.066 (−3.24; 6.35)	0.519	D/C ratio (NS-T1)	−0.068 (-0.19; 0.09)	0.506
PA (NS-T1)	0.503 (0.34; 0.81)	<0.001	PA (NS-T1)	0.514 (0.36; 0.82)	<0.001	PA (NS-T1)	0.507 (0.36; 0.82)	<0.001
Model 2	R^2 = 0.403; Adj R^2 = 0.351; ΔR^2 = 0.109; p = 0.001		Model 2	R^2 = 0.338; Adj R^2 = 0.281; ΔR^2 = 0.041; p=0.043		Model 2	R^2 = 0.298; Adj R^2 = 0.237; ΔR^2 < 0.001; p = 0.958	
	β (CI 95%)	*p*		β (CI 95%)	*p*		β (CI 95%)	*p*
DHEA (SS-T1)	−0.504* (−19.29; −5.40)	0.001	Cortisol (SS-T1)	−0.281* (−13.46; −0.24)	0.043	D/C ratio (SS-T1)	0.006 (-1.05; 1.11)	0.958

Note: Model 2: *$p < 0.05$. β shows standardized values. Adj R^2 = Adjusted *R*-squared; ΔR^2 = *R*-squared change; CI 95% = Confidence interval; BMI = Body mass index; DHEA (NS-T1) = DHEA concentration levels at T1 in non-stressful session; DHEA (SS-T1) = DHEA concentration levels at T1 in stressful session; Cortisol (NS-T1) = Cortisol concentration levels at T1 in non-stressful session; Cortisol (SS-T1) = Cortisol concentration levels at T1 in stressful session; D/C ratio (NS-T1) = DHEA/Cortisol ratio at T1 in non-stressful session; D/C ratio (SS-T1) = DHEA/Cortisol ratio at T1 in stressful session; PA (NS-T1) = Positive affect score at T1 in non-stressful session.

Table 3. Relationship between pre-examination hormonal levels and examination performance.

	Examination mark								
Model 1	**R^2 = 0.039; Adj R^2 = −= .0 p=0.583**		Model 1	**R^2 = 0.024; Adj R^2 = −= .0 p=0.782**		Model 1	**R^2 =0.026; Adj R^2 = −= 02 p=0.758**	
	β (CI 95%)	p		β (CI 95%)	p		β (CI 95%)	P
Sex	0.020 (−1.20; 1.42)	0.867	Sex	0.013 (−1.26; 1.41)	0.912	Sex	0.024 (−1.21; 1.49)	0.840
Age	−0.121 (−0.64; 0.21)	0.309	Age	−0.127 (−0.66; 0.20)	0.293	Age	−0.138 (−0.69; 0.19)	0.262
BMI	−0.079 (−0.22; 0.11)	0.507	BMI	−0.072 (−0.21; 0.11)	0.545	BMI	−0.073 (−0.21; 0.12)	0.546
DHEA (NS-T1)	0.123 (−0.84; 2.72)	0.299	Cortisol (NS-T1)	−0.002 (−1.58; 1.55)	0.984	D/C ratio (NS-T1)	−0.040 (−0.05; 0.04)	0.736
Model 2	R^2 = 0.111; Adj R^2 = 0.048; ΔR^2 = 0.072; p = 0.020		Model 2	R^2 = 0.036; Adj R^2 = −0.033; ΔR^2 = 0.012; p=0.360		Model 2	R^2 = 0.027; Adj R^2 = −0.042; ΔR^2 = 0.002; p = 0.740	
	β (CI 95%)	p		β (CI 95%)	p		β (CI 95%)	p
DHEA (SS-T1)	−0.408* (−5.13; −0.46)	0.020	Cortisol (SS-T1)	−0.150 (-3.25; 1.20)	0.360	D/C ratio (SS-T1)	−0.041 (-0.41; 0.29)	0.740

Note: Model 2: * p<0.05. β shows standardized values. Adj R^2 = Adjusted R-squared; ΔR^2 = R-squared change; CI 95% = Confidence interval; BMI = Body mass index; DHEA (NS-T1) = DHEA concentration levels at T1 in non-stressful session; DHEA (SS-T1) = DHEA concentration levels at T1 in stressful session; Cortisol (NS-T1) = Cortisol concentration levels at T1 in non-stressful session; Cortisol (SS-T1) = Cortisol concentration levels at T1 in stressful session; D/C ratio (NS-T1) = DHEA/Cortisol ratio at T1 in non-stressful session; D/C ratio (SS-T1) = DHEA/Cortisol ratio at T1 in stressful session.

Table 4. Relationship between pre-examination hormonal levels and support-seeking strategy.

	Support-seeking coping strategy scores							
Model 1	**R^2 = 0.127; Adj R^2 = 0.078 p=0.044**		Model 1	**R^2 = 0.142; Adj R^2 = 0.094 p=0.026**		Model 1	**R^2 =0.129; Adj R^2 = 0.080 p=0.041**	
	β (CI 95%)	p		β (CI 95%)	p		β (CI 95%)	p
Sex	0.328 (0.82; 4.27)	0.004	Sex	0.348 (0.97; 4.43)	0.003	Sex	0.324 (0.79; 4.24)	0.005
Age	0.097 (−0.32; .80)	0.392	Age	0.085 (−0.34; 0.77)	0.449	Age	0.101 (−0.31; 0.81)	0.373
BMI	−0.116 (−0.32; 0.10)	0.305	BMI	−0.113 (−0.32; 0.10)	0.315	BMI	−0.120 (−0.33; 0.10)	0.291
DHEA (NS-T1)	−0.003 (−2.37; 2.32)	0.981	Cortisol (NS-T1)	0.124(−0.90; 3.15)	0.272	D/C ratio (NS-T1)	0.043 (−0.05; 0.07)	0.703
Model 2	R^2 = 0.133; Adj R^2 = 0.071; ΔR^2 = 0.005; p = 0.514		Model 2	R^2 = 0.154; Adj R^2 = 0.094; ΔR^2 = 0.012; p=0.319		Model 2	R^2 = 0.193; Adj R^2 = 0.135; ΔR^2 = 0.064; p = 0.022	
	β (CI 95%)	p		β (CI 95%)	p		β (CI 95%)	p
DHEA (SS-T1)	−0.111 (−4.23; 2.14)	0.514	Cortisol (SS-T1)	0.153 (−1.43; 4.31)	0.319	D/C ratio (SS-T1)	−0.263* (−0.97; −0.79)	0.022

Note: Model 2: *p<0.05. β shows standardized values. Adj R^2 = Adjusted R-squared; ΔR^2 = R-squared change; CI 95% = Confidence interval; BMI = Body mass index; DHEA (NS-T1) = DHEA concentration levels at T1 in non-stressful session; DHEA (SS-T1) = DHEA concentration levels at T1 in stressful session; Cortisol (NS-T1) = Cortisol concentration levels at T1 in non-stressful session; Cortisol (SS-T1) = Cortisol concentration levels at T1 in stressful session; D/C ratio (NS-T1) = DHEA/Cortisol ratio at T1 in non-stressful session; D/C ratio (SS-T1) = DHEA/Cortisol ratio at T1 in stressful session.

Relationship between pre-examination hormonal levels and academic performance

Table 3 presents the outcomes of the regression analyses and their main statistics, with examination mark as the criterion variable. The pre-examination levels of DHEA were predictive of marks in the official examination (p=0.020). The participants with higher DHEA levels exhibited lower examination marks. Neither pre-examination cortisol levels nor the DHEA/cortisol ratio were associated with the examination mark ($p \geq 0.360$). Also, there were no associations between participants' pre-examination hormonal levels of their general academic performance ($p \geq 0.229$), as illustrated in Supplementary Table 2.

Relationship between pre-examination hormonal levels and coping strategies

Table 4 shows the results of the regression analyses and their main statistics, with the Support-seeking coping strategy as the criterion variable. The participants' pre-examination levels of DHEA/cortisol ratio were predictive of their use of support-seeking strategy to cope with stressors (p=0.022). The higher the pre-examination levels of DHEA/cortisol ratio, the lower the scores on the support-seeking strategy. The pre-examination levels of both DHEA and cortisol failed to predict support-seeking strategy ($p \geq 0.319$). Also, there were no associations between pre-examination hormonal levels and either active or avoidance coping strategies ($p \geq 0.079$), as illustrated in Supplementary Table 3.

Relationship between coping strategies and academic performance

No associations were found between the participants' scores in active coping, avoidance coping, or support-seeking coping strategies and their examination marks ($p \geq 0.084$) or their general academic performance ($p \geq 0.203$). Supplementary Table 4 presents the results of these regressions analyses and main statistics.

Discussion

This study analyzed the DHEA, cortisol and psycho-emotional responses of college students to academic examination stress.

The stressor was an official examination. The study considered the students' performance on the examination, their academic record and coping strategies. The study also investigated the associations between DHEA and cortisol anticipatory responses and psychological scores (i.e. affect and state anxiety), academic performance and coping strategies. The results indicated that students exhibited elevated levels of DHEA and cortisol in anticipation of the examination. There was also a pre-examination rise in anxiety and negative affect, and an overall decrease in positive affect on the day of the examination. After controlling for the hormonal levels reached at the beginning of the non-stressful session, both DHEA and cortisol levels were associated with a reduction in positive affect just before the examination. The pre-examination DHEA levels were negatively associated with examination performance, whereas cortisol levels showed no such association. The heightened anticipatory DHEA response relative to cortisol (i.e. DHEA/cortisol ratio) correlated with a decreased tendency to seek support in response to stressful situations. Perceived coping strategies by students did not correlate with their performance in the official examination or their academic record.

The levels of DHEA and cortisol were higher in anticipation of the examination. Previous research has reported elevated levels of salivary cortisol at the pre-examination time in the context of written (Garces-Arilla et al., 2023; Preuss et al., 2010; Verschoor & Markus, 2011) and oral examinations (Helbig & Backhaus, 2017; Ringeisen et al., 2019). Considering DHEA levels in response to academic stressors, one study registered students' DHEA and cortisol levels over an examination period and compared them with the levels of a non-examination period (Irshad et al., 2020). Results from this study showed that cortisol levels increased in the examination period, but DHEA levels did not show any significant change. Some studies have found that DHEA rises after acute stressor-tasks performed in laboratory settings (Izawa et al., 2008; Shields et al., 2016; Shirotsuki et al., 2009). These results contrast with the anticipatory rise of DHEA before the examination found in our study. Therefore, further research is necessary to determine how DHEA reacts to different stressors based on their duration and nature.

The current study indicates that both hormones exhibit a similar response pattern. This finding is consistent with the results reported by Izawa et al. (2008), who also observed that DHEA and cortisol secretion followed a comparable response to an acute laboratory stressor. However, the peak of DHEA secretion occurred about 10 minutes before the peak of cortisol. This suggests that the anticipation and preparation phase may trigger a sequential hormonal response, with an initial increase in DHEA followed by cortisol activation. In our study, samples were collected before and after the examination, so the time interval between them was 75 minutes. This timeframe may not precisely capture the hormonal peak, as glucocorticoids typically peak around 20 to 40 minutes after stressor onset, returning to baseline levels approximately one hour later (Ulrich-Lai & Herman, 2009). The post-examination measurement likely represents the stabilization of DHEA and cortisol levels following the stressor-induced peak.

In line with the DHEA and cortisol changes, the students anticipated emotional distress during the examination. This was manifested in high anxiety levels and negative affect before the examination task. Previous research on acute natural stressors supports these findings regarding negative affect (Verschoor & Markus, 2011) and anxiety (Merz et al., 2019; Merz & Wolf, 2015; Ringeisen et al., 2019).

Contrary to our initial expectations, the anticipatory rise of DHEA levels correlated negatively with heightened positive affect, mirroring the pattern observed with cortisol. In addition, there was no association between anxiety and negative affect and variations in the levels of the two hormones. It is difficult to separate the individual effects of the two hormones in stressful situations, given their concurrent activation. Izawa et al. (2008) found that lower DHEA levels were linked to higher negative affect scores during the recovery period of an acute laboratory stressor. The type of stressor used could explain this discrepancy. With their anticipation and prolonged psychological stress, academic examinations may elicit different DHEA responses from the more controlled conditions of laboratory stressors. Therefore, extrapolating results is challenging due to the methodological differences. Regarding academic stressors, oral examinations (Merz & Wolf, 2015) or presentations (Merz et al., 2019) triggered anticipatory hormonal and emotional responses. Anticipatory processes can affect the stress response, including the release of DHEA. This means that stress responses can be influenced not only by the immediate stressor but also by prior psychological preparation. This anticipatory phase could play a key role in shaping subsequent physiological and emotional reactions during the stressful event.

The lack of associations between anxiety and negative affect scores and levels of DHEA and cortisol may be due to the pressure associated with the examination that all the students experienced. These scores were particularly elevated compared to positive affect scores. Poor performance on the examination would have significant implications for students' academic progress. Also, scheduling the non-stressful session two days before the official exam and in the same classroom may have facilitated the increase in anticipatory levels of anxiety and negative affect.

The natural stressor of the study elicited an anticipatory response in both DHEA and cortisol. However, only the DHEA response was associated with ineffective performance during the examination task, predicting lower examination marks. The literature suggests that DHEA plays a cognitive-enhancing role (do Vale et al., 2014; Smith et al., 2020). However, our results are incongruent with that idea. The difference in results may be due to the type of performance variable and context. The examination mark reflects aspects related to long-term preparation for the official examination of the subject rather than representing a pure cognitive ability assessed under laboratory conditions. Previous research has suggested that the DHEA response to acute stressors is attenuated in prolonged stressful situations (Lennartsson et al., 2013). Students who have engaged in more extensive examination preparation may have lower DHEA levels before the examination, which could lead to achieving higher marks.

When considered separately, neither pre-examination cortisol levels nor pre-examination DHEA levels showed

significant associations with the coping strategies assessed in this study. A significant association was observed when there was a combination of high DHEA and low cortisol levels (i.e. DHEA/cortisol ratio). Students with this hormonal pattern reported using support-seeking coping strategies less frequently. Previous research has shown that cortisol reactivity to a laboratory stressor is associated with negative coping strategies (Biondi & Picardi, 1999; Höhne et al., 2014; Janson & Rohleder, 2017). Moreover, studies suggest that individuals with reduced cortisol/DHEA ratios are more likely to adapt their behavior to meet the requirements of challenging tasks within an academic context (Wemm et al., 2010). The emotional intensity of a stressor may influence the relationship between hormonal response and coping strategies. Our stressor was a real examination conducted in university facilities well-known to the participants. While there was an anticipatory response of negative emotions, their intensity might be comparatively low compared to other acute stressors. Other authors suggest that cortisol response is linked to the use of stress-coping strategies in situations of intense negative emotionality (Langer et al., 2022). Research on the support-seeking strategy has found no association with cortisol response to a cognitive stressor (Bohnen et al., 1991) or has found a negative association with cortisol response to a social stressor (Sladek et al., 2017). The study by Ota et al. (2015) found that daily DHEA levels were negatively associated with social support received at work, whereas the cortisol/DHEA ratio did not show a significant relationship. Although a support-seeking strategy is potentially advantageous in the long term, it did not show any association with academic performance in the sample studied or with any other coping strategy evaluated.

The study presents several limitations that should be considered when interpreting the results. Firstly, the investigation included a relatively small sample of undergraduate students, limiting the extrapolation of findings to larger or older populations. Furthermore, the proportion of participants of both genders is not balanced, and the number of males is insufficient to analyze the influence of sex on the studied variables. The gender imbalance resulted from a higher proportion of female enrollment in the course and, overall, in the degree program. Secondly, additional physiological indicators commonly associated with the stress response were not investigated (i.e. heart rate and blood pressure). Moreover, including a measure of trait anxiety could have provided valuable insights. This is especially important, given the propensity of women in the studied age range to experience elevated anxiety levels. Additionally, incorporating a measure of the participants' perceived stress levels during the official examination or memory task would have provided more direct insights into their psychological state when confronted with these tasks. Thirdly, the hormonal levels in the control condition may reflect anticipation of the examination, as they were measured 48 hours before the examination. Finally, students were in the examination period during their first year of university, which could lead to notably elevated stress levels (Abouserie, 1994). This aspect requires special attention when generalizing the outcomes to other acute stressors in real-life situations.

Conclusions

An increase in both DHEA and cortisol levels before an examination was linked to a decrease in positive emotions, but only an increase in DHEA levels was associated with poorer performance. This study challenges the presumed positive association between DHEA levels and successful stress management. Moreover, it provides empirical evidence of how DHEA levels may interact with emotional and coping responses to a naturalistic acute stressor. These findings have implications for a variety of fields, including psychology, physiology, and education. Understanding how hormonal responses interact with psychological and cognitive processes during stress may provide a basis for interventions to improve stress management strategies and academic performance. In addition, the study explores the potential role of DHEA in mood regulation, raising interesting questions about its broader implications beyond the stress response.

Disclosure statement

The authors report no conflicts of interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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