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Salivary cortisol/DHEA ratio response to acute academic stress and its association with performance in middle-to-late adolescents.

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Abstract

OBJECTIVES: Neuroendocrine coupling across adolescent transition conditions a stage of heightened variability in hypothalamic-pituitary-adrenal axis stress reactivity, likely impacting the adaptive physiological response to psychological stressors during adolescence. This study aimed to assess the salivary cortisol, DHEA, and cortisol/DHEA ratio variation in response to acute academic stress and its association with academic performance.
METHODS: A longitudinal observational study with a combined event- and time-based protocol was conducted. Twenty-two middle-to-late adolescents (mean age 17.6 ± 0.3 years) performed a 60-minute written examination in which saliva samples for cortisol and DHEA determination were collected at four points: before the examination and after its completion, and at the same time points on a control day. Academic performance was assessed based on the examination score determined by the number of marks awarded for each correct response.
RESULTS: Salivary cortisol ($p = 0.012$) and cortisol/DHEA ratio ($p = 0.004$) were significantly higher at pre-examination. When compared to students with low academic performance, high-achieving students exhibited higher cortisol levels ($p = 0.026$) and a higher cortisol/DHEA ratio ($p = 0.017$) at post-examination. Bivariate analysis showed a moderate positive correlation between academic performance and the post-examination cortisol/DHEA ratio ($r_s(20) = 0.44$, $p = 0.039$), which significantly predicted academic performance ($F(1,20) = 4.63$, $\beta = 0.09$, CI 95% [0.003, 0.171], $p = 0.044$).
CONCLUSION: In middle-to-late adolescents, the salivary cortisol/DHEA ratio response to acute academic stress is positively associated with academic performance. The findings of this study provide evidence of the neuroendocrine response to a cognitive demanding stressor as an adaptive mechanism likely mediated by the effect of an acute cortisol surge in cognition enhancement.

ORIGINAL ARTICLE

INTRODUCTION

In states of homeostatic challenge, whether real or perceived, the stress system coordinates an intricate adaptive response to cope with the imposed metabolic and cognitive demands of a social, physiological, or psychological stressor. The variability in the exposure and physiological arousal to stressful stimuli, however, results in distinct states of hypothalamic-pituitary-adrenal (HPA) axis activity that could either benefit or impair adaptation, thereby establishing a stress-response hormesis (Calabrese *et al.* 2023; Lu *et al.* 2021). In contrast to chronic stress, the acute stress response comprises a dynamic process involving goal-directed behaviors followed by a prompt cortisol increase that enhances alertness and cognitive functioning (Russell & Lightman, 2019). In parallel, stress-mediated HPA axis activation stimulates dehydroepiandrosterone (DHEA) adrenocortical secretion which appears to influence the neuropsychological response to stress (Dutheil *et al.* 2021). Although the neurobiological mechanisms are broad, an inverted U-shaped relationship is widely acknowledged, where an acute increase in cortisol upregulates cognitive performance while a sustained increase results in its downregulation (Baldi & Bucherelli, 2005). On the other hand, DHEA acts as a cortisol antagonist potentially modulating its effect on cognitive functioning (Maninger *et al.* 2009). As such, the cortisol/DHEA ratio has been proposed as an indicator to assess HPA axis functionality, and in humans, it has been associated with the behavioral response to distinct types of stressors (Ahmed *et al.* 2023).

Throughout the adolescent transition, gradual neuroendocrine coupling establishes a period of susceptibility to deleterious stressors (Chrousos, 2009; Nicolaides & Kyratzi, 2015), conferring a stage of highly variable HPA axis reactivity associated with stress-induced neurobehavioral dysfunctions and complex psychological responses to the increased demands in social, familial, and educational life domains during adolescence (Eiland & Romeo, 2013; Whelan *et al.* 2021). Particularly, academic stress has been described as impacting psychological well-being in response to exposure to different psychosocial stressors within which examinations constitute a major stress trigger among students (Špiljak *et al.* 2022). However, the extent to which academic stress results in benefits or deleteriousness considerably depends on the context of the stressor and the pattern of induced physiological arousal (Epel *et al.* 2018). It has been shown that the physiological adaptation mediated by adrenal hormones allows an efficient consolidation and coding of relevant inputs to respond to a psychobiological stressor (James *et al.* 2023), likely favoring the response when exposed to an acute stressor with a high cognitive demand such as an academic examination. Nonetheless, the cortisol/DHEA ratio in adolescents has been mainly characterized in the context of psychopathological disorders (Bendezú *et al.* 2021), and while it could be associated with the adaptive response to non-deleterious stress, the neuroendocrine response to acute stress and its relationship to academic performance remains largely unexplored (Flegr & Príplatová, 2010; Pletzer *et al.* 2010; Urwyler *et al.* 2015). The present study assessed the variation in salivary cortisol and DHEA levels, as well as the cortisol/DHEA ratio, in response to a written examination aiming to characterize the physiological response to acute stress in an educational setting and its relationship with academic performance in adolescents.

MATERIALS AND METHODS

Study design and participants

A longitudinal observational study with repeated measures was conducted among high school students attending a private educational institution in the city of Bogotá, Colombia, who were recruited during the first academic trimester of their senior year between August and October 2023. The study protocol was introduced to all students through a conference given by the researchers at the institution, and both male and female adolescents were prospectively enrolled as eligible participants if they were free of psychopathological disorders based on self-reports. Exclusion criteria included individuals with chronic diseases, pregnancy, background of oral, parenteral, or topical contraceptives or corticosteroids use, and a history of traumatic psychological events in the last year. By simple random sampling, 22 middle-to-late adolescents (11 males and 11 females; mean age 17.6 ± 0.3 years) were included in the study. All minors and their legal representatives were informed in detail about the investigation and voluntarily signed an assent and informed consent, respectively. The study was conducted by the ethical principles established in the Declaration of Helsinki and was approved by the ethics committee of the Faculty of Medicine of Universidad Nacional de Colombia according to Assessment Act No. 0005 of March 23, 2023.

To investigate the phasic alteration in HPA axis activity in response to a naturalistic, active, and controllable acute stressor according to the classification of Epel *et al.* (2018), the salivary cortisol/DHEA ratio was assessed by a combined event- and time-based protocol following the recommendations of Stoffel *et al.* (2021). The stressor consisted of a 60-minute written examination, scheduled on a day with no other exams on the previous or subsequent days, which evaluated the knowledge acquired in the calculus course during the first trimester of the senior year. Saliva samples for the measurement of cortisol and DHEA were collected at two time points: [T1] five minutes before the start of the examination (~10:00 AM) and [T2] immediately after the completion of the examination (~11:00 AM). Sampling was carried out at the exact same times (T1 (~10:00 AM) and T2 (~11:00 AM)) on a control day

Tab. 1. Salivary cortisol, DHEA and cortisol/DHEA ratio in examination and baseline conditions

Variable	Sampling day	M_d (IQR)		*p*-value*
		T1 [Pre]	T2 [Post]	
Cortisol (nmol/L)	Baseline	4.02 (2.83)	4.80 (3.84)	0.070
	Examination	4.80 (2.53)	3.21 (2.90)	
DHEA (nmol/L)	Baseline	1.08 (0.78)	0.85 (0.60)	0.154
	Examination	1.01 (0.80)	1.04 (0.59)	
Cortisol/DHEA ratio	Baseline	4.09 (6.58)	4.56 (5.23)	0.014
	Examination	3.98 (3.15)	2.67 (3.69)	

*Friedman's test. T1 [Pre]: Pre-examination. T2 [Post]: Post-examination. M_d: Median. IQR: Interquartile range.

to evaluate HPA axis activity under baseline conditions with daily hassles six weeks after the start of the senior year and six weeks before the exam to minimize cumulative academic stress and anticipatory stress in the days preceding the examination. Thus, each subject corresponded to its own control, reducing the variability of the experimental error as stated by González-Cabrera *et al.* (2014). Between T1 and T2 on the control day, students attended lectures regularly, with no evaluative activities. Academic performance was determined based on the score achieved in the exam where marks were awarded for each correct answer on a total of 100 points, with a passing grade ≥70. Moreover, the hours of sleep on both days and the hours elapsed from awakening to the first saliva sample collection were recorded.

Salivary cortisol and DHEA assays

Salivette® tubes (Sarstedt International, Germany) were used for saliva collection following the protocol established by the commercial house for cortisol measurement. In Corning™ Falcon™ polypropylene tubes (Fisher Scientific, Spain), saliva samples were collected by direct passive salivation for DHEA quantification. Thirty minutes before sample collection, subjects consumed no food or beverages and performed no physical exercise of any kind. Salivette® and polypropylene tubes were centrifuged at 1500 x *g* for 5 min, and subsequently stored at -30ºC for subsequent analysis. Sample processing was performed in duplicate on a Cobas® e 411 analyzer according to the manufacturer's instructions by electrochemiluminescence immunoassays (Elecsys Cortisol II® kit, Roche Diagnostics GmbH) for cortisol measurement, and by enzyme-linked immunosorbent assays (DHEA Saliva ELISA kit, IBL International GmbH) for DHEA determination.

Statistical analysis

Statistical analysis was performed using R Statistical Software (v4.2.3; R Core Team 2021). Data distribution was evaluated using the Shapiro-Wilk test. Descriptive analysis of the variables was performed by calculating the median (M_d) and interquartile range (IQR). Inferential analysis was carried out with non-parametric tests for paired samples using the two-tailed Friedman's test and a *post-hoc* analysis based on the two-tailed Wilcoxon signed-rank test. The non-parametric comparison of independent variables was performed using the two-tailed Mann-Whitney test, while the two-tailed unpaired *t*-test was used for variables with normal distribution. The strength of association between variables was determined by Pearson's correlation coefficient (r) for normally distributed data, and Spearman's correlation coefficient (r_s) for data not normally distributed. Subsequently, a simple linear regression analysis was performed for the variables that exhibited a significant correlation. A logarithmic transformation (ln[x]) of the data in the linear regression model was performed to ensure normality, homoscedasticity, and independence of the residuals, which was evaluated with the Shapiro-Wilk, Levene's, and Durbin-Watson tests, respectively. Differences were considered statistically significant when $p < 0.05$, and when adjustment for multiple comparisons (Bonferroni correction) was conducted, the p value was indicated accordingly.

RESULTS

Hormonal variation in response to examination

The difference in salivary cortisol, DHEA and the cortisol/DHEA ratio between examination and the control day was evaluated using the Friedman's test (Table 1). There was no significant variation in cortisol ($X^2(3) = 7.70$, $p = 0.070$) and DHEA ($X^2(3) = 5.25$, $p = 0.154$) levels. The salivary cortisol/DHEA ratio, on the other hand, differed significantly between examination and control day ($X^2(3) = 10.64$, $p = 0.014$). Notably, sleep hours were lower on the exam day ($M_d = 6.25$ hr (IQR: 0.50)) compared to control day ($M_d = 7.00$ hr (IQR: 0.38)) ($z = -2.83$, $p = 0.005$), however, without a significant difference in time between awakening and first sampling ($z = 0.64$, $p = 0.523$).

A *post-hoc* comparison of salivary levels of cortisol, DHEA, and cortisol/DHEA ratio between T1 and T2 on control and examination day was performed using the Wilcoxon signed-rank test with adjustment for multiple comparisons (Figure 1). On examination day, the salivary cortisol/DHEA ratio at T1 was significantly

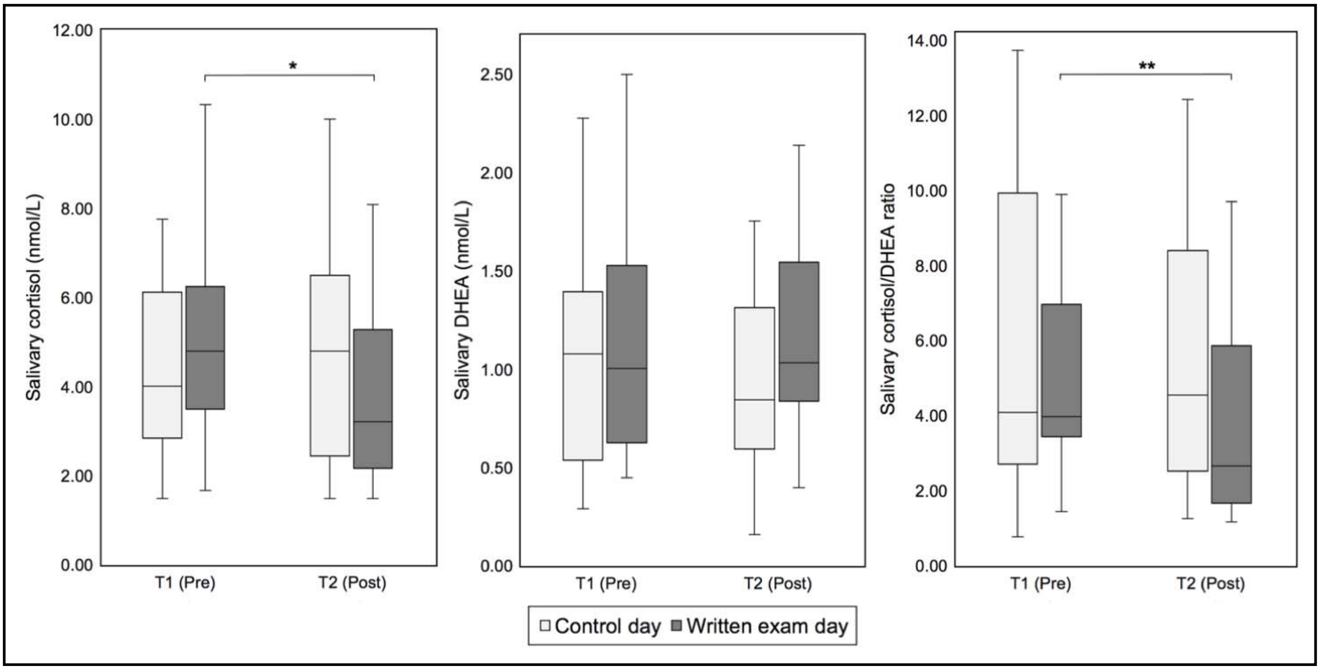

Fig. 1. Salivary cortisol, DHEA and cortisol/DHEA ratio at all sampling points during examination and baseline conditions. T1 (Pre): Pre-examination. T2 (Post): Post-examination. *$p < 0.0125$, **$p < 0.005$; *post-hoc* analysis using the Wilcoxon signed-rank test with adjustment for multiple comparisons (Bonferroni correction) that indicates a significant difference at $p < 0.0125$.

higher compared to T2 (z = -2.87, p = 0.004), however, no significant difference was observed compared to the control day at T1 (z = 0.45, p = 0.656) and T2 (z = -2.10, p = 0.036). Likewise, salivary cortisol concentration at T1 was significantly higher compared to T2 (z = -2.53, p = 0.012) on examination day, with no significant difference at T1 (z = 0.57, p = 0.571) and T2 (z = -1.45, p = 0.146) compared to the control day. Salivary DHEA concentration did not vary between T1 and T2 on the examination day (z = 0.51, p = 0.610), and no significant differences were observed with the control day at T1 (z = 0.26, p = 0.799) and T2 (z = 1.96, p = 0.050). The variation between T1 and T2 on the control day was not significant for any of the variables assessed (cortisol: z = -0.57, p = 0.566; DHEA: z = -0.89, p = 0.372; cortisol/DHEA: z = -0.22, p = 0.824).

Association with academic performance

The median score achieved in the exam was 65 points (IQR: 11.5). Approximately one third of the subjects (n = 7) obtained a passing grade (score ≥70 points). Salivary hormone levels and their change during the exam (Δ = [T2]-[T1]) were compared between subjects who obtained a passing grade and a failing grade using the Mann-Whitney test and the unpaired t-test (Table 2). Salivary cortisol levels at T2 were significantly

Tab. 2. Salivary cortisol, DHEA and cortisol/DHEA ratio in students that approved and failed the exam

Variable	Sampling time	M_d (IQR)		p-value
		Passed (n=7)	Failed (n=15)	
Cortisol (nmol/L)	T1 [Pre]	5.54 (1.30)	4.29 (2.92)	0.679[a]
	T2 [Post]	5.22 (2.42)	2.35 (2.08)	0.026[a]
ΔCortisol (nmol/L)	NA	-0.67 (2.90)	-2.08 (2.97)	0.104[b]
DHEA (nmol/L)	T1 [Pre]	0.66 (0.59)	1.18 (0.84)	0.158[a]
	T2 [Post]	0.98 (0.67)	1.10 (0.50)	0.208[a]
ΔDHEA (nmol/L)	NA	0.22 (0.48)	0.05 (0.76)	0.708[b]
Cortisol/DHEA ratio	T1 [Pre]	6.68 (6.14)	3.93 (2.30)	0.324[a]
	T2 [Post]	6.73 (4.75)	2.25 (1.92)	0.017[a]
ΔCortisol/DHEA ratio	NA	-0.18 (3.53)	-2.00 (1.80)	0.308[b]

[a]Two-tailed Mann-Whitney U test. [b]Two-tailed unpaired t-test. T1 [Pre]: Pre-examination. T2 [Post]: Post-examination. M_d: Median. IQR: Interquartile range.

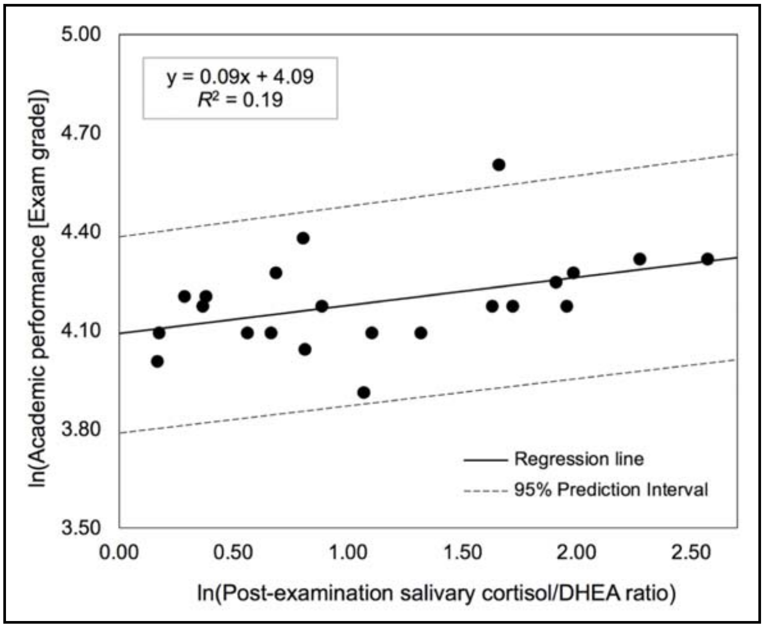

Fig. 2. Scatter plot with linear regression trendline between academic performance and post-examination salivary cortisol/DHEA ratio.

higher in subjects who passed the exam (U = 84.00, p = 0.026), and so was the cortisol/DHEA ratio at T2 (U = 86.00, p = 0.017). Salivary cortisol, DHEA and the cortisol/DHEA ratio of individuals who passed and failed the exam were not significantly different in either group compared to their respective salivary hormone levels on the control day.

Bivariate analysis showed a significant moderate positive correlation between the cortisol/DHEA ratio at T2 and academic performance ($r_s(20) = 0.44$, p = 0.039), however, no correlation was found for the cortisol/DHEA ratio at T1 ($r_s(20) = 0.23$, p = 0.319) and the Δcortisol/DHEA ratio ($r(20) = 0.39$, p = 0.072). Likewise, Δcortisol showed a significant moderate positive correlation with academic performance ($r(20) = 0.44$, p = 0.041) that was not evidenced for cortisol levels at T1 and T2 ($r_s(20) = 0.15$, p = 0.518; $r_s(20) = 0.37$, p = 0.086, respectively). No correlation was found between salivary DHEA levels and its change during the examination with academic performance (T1 DHEA: $r_s(20) = -0.19$, p = 0.401; T2 DHEA: $r_s(20) = -0.22$, p = 0.328; ΔDHEA: $r_s(20) = 0.02$, p = 0.923)). Moreover, further evaluation of the extent to which significantly correlated variables could predict academic performance was performed with a simple linear regression analysis. The cortisol/DHEA ratio at T2 significantly predicted academic performance ($F(1,20) = 4.63$, β = 0.09, CI 95% [0.003, 0.171], p = 0.044) (Figure 2). To ensure the validity of the model, we confirmed residuals compliance with linear regression assumptions including a normal distribution (W = 0.93, p = 0.094), homoscedasticity (Levene's test, p = 0.08), and independence (Durbin-Watson value = 1.91). Conversely, while Δcortisol was significantly correlated with academic performance ($r(20) = 0.44$, p = 0.041), linear regression analysis is not shown as heteroscedasticity of the residuals was present (Levene's test, p = 0.004).

DISCUSSION

This study evaluated the HPA axis response to acute academic stress and its association with academic performance in middle-to-late adolescents. Pre-examination salivary cortisol and the cortisol/DHEA ratio were significantly higher compared to post-examination levels; however, no significant difference was found compared to salivary hormone levels under baseline conditions. When comparing the physiological response based on academic performance, subjects who achieved a passing grade exhibited significantly higher cortisol levels and cortisol/DHEA ratio at post-examination compared to subjects who failed. As for the association with academic performance, post-examination salivary cortisol/DHEA ratio and the change in cortisol levels were found to correlate directly with the score obtained and, in particular, the post-examination cortisol/DHEA ratio significantly predicted academic performance.

In academic settings, studies on the stress response are numerous yet inconsistent due to the varying nature of the stressor and the different levels of academic challenge they represent (Špiljak *et al.* 2022). Oral exams have shown a significant increase in stress biomarkers after completion (Preuß *et al.* 2010), while in written exams there seems to be a higher pre-examination cortisol concentration compared to post-examination (Pletzer *et al.* 2010; Preuß *et al.* 2010; Urwyler *et al.* 2015). The relationship between academic performance and cortisol levels has shown both no correlation (Pletzer *et al.* 2010) and a positive association showing a higher probability of passing grades in individuals with higher cortisol levels (Urwyler *et al.* 2015). Similar to the results of this study, Anjum *et al.* (2021) found that individuals with high academic performance exhibit significantly higher post-examination cortisol levels compared to those with low performance when taking a written test. Moreover, Flegr *et al.* (2010) reported that in college students, subjects with high performance had significantly higher post-examination cortisol levels, and the change in cortisol during the test was negatively correlated with the actual number of errors in the exam. On the other hand, the variation of the cortisol/DHEA ratio has been evaluated to a lesser extent. In the context of anticipatory stress, Irshad *et al.* (2020) found that salivary cortisol levels in the days preceding an examination period were significantly higher compared to baseline levels, whereas DHEA levels did not change significantly and thus the cortisol/DHEA ratio slightly increased. Furthermore, in chronic academic stress, conversely to the direct association between academic performance and the cortisol/DHEA ratio evidenced in this study, Bradi *et al.* (2011) reported an inverse association between the cortisol/DHEA ratio and the cumulative academic achievement; also, subjects who passed the course exhibited significantly higher DHEA levels but without a significant difference in cortisol levels as observed in response to acute stressors.

On a physiological basis, the acute neuroendocrine response to stress converges on adrenocorticotropic hormone (ACTH) elevation followed by an increase in cortisol levels that peak around 20 minutes after the initial exposure to a stressor (Russell & Lightman, 2019). In the central nervous system, an acute surge in cortisol levels results in cognitive enhancement as a result of mineralocorticoid receptors (MRs) activation, whose prompt saturation upon sustained cortisol elevation leads to activation of glucocorticoid receptors (GRs) and consequently to a decrease in cognitive functions (Baldi *et al.* 2005). Accordingly, it has been shown that in humans, blocking MRs impairs memory retrieval, while blocking GRs enhances it (Rimmele *et al.* 2013). Concomitant with adrenal cortisol secretion, ACTH-mediated adrenocortical stimulation results in an augmented release of DHEA and its sulfated form (DHEA-S) (Maninger *et al.* 2009), which peaks at the end of the exposure to a stressor (Dutheil *et al.* 2021). DHEA exerts anti-glucocorticoid effects mediated by the downregulation of GRs translocation and 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1) expression, coupled with competitive inhibition of 11β-HSD1 by its metabolites 7α-hydroxy-DHEA and 7β-hydroxy-DHEA (Pluchino *et al.* 2015). As a neuroactive steroid, DHEA could also antagonize the acute and chronic action of cortisol on cognitive function (Pluchino *et al.* 2015).

As such, the cortisol/DHEA ratio, commonly assessed as an indicator of the net deleterious effects of cortisol, constitute viable an indicator of the HPA axis response to acute stress (Ahmed *et al.* 2023). In this context, physiological adaptation modulated by adrenal hormones and catecholamines allows for effective consolidation and coding of relevant inputs to respond to a stressor demand (James *et al.* 2023). It has been shown that the acute increase in cortisol levels could enhance mnemonic discrimination and tolerance to uncertainty during decision-making (Byrne *et al.* 2020; Jiang *et al.* 2019), potentially favoring the outcome of an examination. Thus, the findings of a positive association between the cortisol/DHEA ratio with academic performance evidenced in this study could be related to acute cognitive enhancement mediated by the acute cortisol surge. Furthermore, while the relationship between psychological and physiological states varies throughout life and affective traits are not specific to a stressor event (Epel *et al.* 2018), the results of this investigation suggest that the cortisol/DHEA ratio may offer insight into psychobiological patterns. Lau *et al.* (2021) described that the variation in cortisol/DHEA ratio after stress exposure might constitute a feature in predicting resilience. Similarly, Lee *et al.* (2024) showed that an imbalance in the cortisol/DHEA ratio could be associated with externalizing problems in adolescents, particularly in boys exposed to early life stress. These findings underscore the potential of the cortisol/DHEA ratio as an index for HPA axis functioning, yet further research is imperative to establish the role of the cortisol/DHEA ratio in predicting behavioral responses or psychological traits.

Among the main limitations of this study, the reliance on self-reports without formally ruling out psychopathological disorders represents a major confounding factor as the absence of psychological screening limits our ability to fully exclude underlying conditions, such as anxiety or depression, that could have influenced the neuroendocrine outcomes as it has been previously described (Ahmed *et al.* 2023). Furthermore, the size of the study population limits the precision of the findings evidenced, as well as a subsequent evaluation of the variation in the cortisol/DHEA ratio response between men and women that has been reported in other stress contexts (Ahmed *et al.* 2023). Also, although variables that could considerably affect the HPA axis response to a stressor were controlled, such as the time between awakening and the first sampling as well as the time of the day at which the sample was taken (Stoffel *et al.* 2021), other variables not considered, including sleep quality and affective/personality traits, could partially influence the pattern of the HPA axis in response to stress. On the other hand, a notable strength of this study is the implementation of a combined event- and time-based protocol, which as highlighted by Stoffel *et al.* 2021, enhances the reliability and validity of the findings.

In conclusion, the present study provides evidence of the induced HPA axis activation, assessed by the salivary cortisol/DHEA ratio, to a natural acute stressor in an academic setting in a middle-to-late adolescent population. In response to a written academic evaluation, pre-examination cortisol and cortisol/DHEA ratio were significantly higher compared to post-examination. The physiological response seemed to vary in relation to academic performance as evidenced by the difference in post-examination salivary cortisol concentration and cortisol/DHEA ratio between low- and high-achieving students. Moreover, the post-examination salivary cortisol/DHEA ratio appears to directly correlate with examination scores and predict academic performance. These findings contribute to the characterization of the HPA axis response to academic stress in adolescents, which in an acute context could be positively associated with academic performance as a result of the net acute action of cortisol surge in cognitive functioning enhancement as part of the physiological adaptations in response to a psychological stressor.

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REFERENCES

1 Ahmed T, Qassem M, Kyriacou PA. Measuring stress: a review of the current cortisol and dehydroepiandrosterone (DHEA) measurement techniques and considerations for the future of mental health monitoring. Stress. 2023; **26**(1): 29–42.
2 Anjum A, Anwar H, Sohail MU, *et al.* The association between serum cortisol, thyroid profile, paraoxonase activity, arylesterase activity and anthropometric parameters of undergraduate students under examination stress. Eur J Inflamm. 2021; **19**: 205873922110008.
3 Baldi E, Bucherelli C. The Inverted "U-Shaped" Dose-Effect Relationships in Learning and Memory: Modulation of Arousal and Consolidation. Nonlinearity Biol Toxicol Med. 2005; B(1): nonlin.003.01.0.
4 Bardi M, Koone T, Mewaldt S, O'Connor K. Behavioral and physiological correlates of stress related to examination performance in college chemistry students. Stress. 2011; **14**(5): 557–566.
5 Bendezú JJ, Howland M, Thai M, *et al.* Adolescent cortisol and DHEA responses to stress as prospective predictors of emotional and behavioral difficulties: A person-centered approach. Psychoneuroendocrinology. 2021; **132**: 105365.
6 Byrne KA, Peters C, Willis HC, Phan D, Cornwall A, Worthy DA. Acute stress enhances tolerance of uncertainty during decision-making. Cognition. 2020; **205**: 104448.
7 Calabrese EJ, Osakabe N, Di Paola R, *et al.* Hormesis defines the limits of lifespan. Ageing Res Rev. 2023; **91**: 102074.
8 Chrousos GP. Stress and disorders of the stress system. Nat Rev Endocrinol. 2009; **5**(7): 374–381.
9 Dutheil F, de Saint Vincent S, Pereira B, *et al.* DHEA as a Biomarker of Stress: A Systematic Review and Meta-Analysis. Front Psychiatry. 2021; **12**.
10 Eiland L, Romeo RD. Stress and the developing adolescent brain. Neuroscience. 2013; **249**: 162–171.
11 Epel ES, Crosswell AD, Mayer SE, *et al.* More than a feeling: A unified view of stress measurement for population science. Front Neuroendocrinol. 2018; **49**: 146–169.
12 Flegr J, Príplatová L. Testosterone and cortisol levels in university students reflect actual rather than estimated number of wrong answers on written exam. Neuro Endocrinol Lett. 2010; **31**(4): 577–581.
13 González-Cabrera J, Fernández-Prada M, Iribar-Ibabe C, Peinado JM. Acute and chronic stress increase salivary cortisol: a study in the real-life setting of a national examination undertaken by medical graduates. Stress. 2014; **17**(2): 149–156.
14 Irshad L, Faustini S, Evans L, Drayson MT, Campbell JP, Heaney JLJ. Salivary free light chains as a new biomarker to measure psychological stress: the impact of a university exam period on salivary immunoglobulins, cortisol, DHEA and symptoms of infection. Psychoneuroendocrinology. 2020; **122**: 104912.
15 James KA, Stromin JI, Steenkamp N, Combrinck MI. Understanding the relationships between physiological and psychosocial stress, cortisol and cognition. Front Endocrinol (Lausanne). 2023;14.
16 Jiang A, Tran TT, Madison FN, Bakker A. Acute stress-induced cortisol elevation during memory consolidation enhances pattern separation. Learning & Memory. 2019; **26**(4): 121–127.
17 Lau WKW, Tai APL, Chan JNM, Lau BWM, Geng X. Integrative psycho-biophysiological markers in predicting psychological resilience. Psychoneuroendocrinology. 2021; **129**: 105267.
18 Lee Y, Donahue GZ, Buthmann JL, Uy JP, Gotlib IH. The cortisol/DHEA ratio mediates the association between early life stress and externalizing problems in adolescent boys. Psychoneuroendocrinology. 2024; **165**: 107034.
19 Lu S, Wei F, Li G. The evolution of the concept of stress and the framework of the stress system. Cell Stress. 2021; **5**(6): 76–85.
20 Maninger N, Wolkowitz OM, Reus VI, Epel ES, Mellon SH. Neurobiological and neuropsychiatric effects of dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS). Front Neuroendocrinol. 2009; **30**(1): 65–91.
21 Nicolaides NC, Kyratzi E, Lamprokostopoulou A, Chrousos GP, Charmandari E. Stress, the Stress System and the Role of Glucocorticoids. Neuroimmunomodulation. 2015; **22**(1–2): 6–19.
22 Pletzer B, Wood G, Moeller K, Nuerk HC, Kerschbaum HH. Predictors of performance in a real-life statistics examination depend on the individual cortisol profile. Biol Psychol. 2010; **85**(3): 410–416.
23 Pluchino N, Drakopoulos P, Bianchi-Demicheli F, Wenger JM, Petignat P, Genazzani AR. Neurobiology of DHEA and effects on sexuality, mood and cognition. J Steroid Biochem Mol Biol. 2015; **145**: 273–280.
24 Preuß D, Schoofs D, Schlotz W, Wolf OT. The stressed student: Influence of written examinations and oral presentations on salivary cortisol concentrations in university students. Stress. 2010; **13**(3): 221–229.
25 Rimmele U, Besedovsky L, Lange T, Born J. Blocking Mineralocorticoid Receptors Impairs, Blocking Glucocorticoid Receptors Enhances Memory Retrieval in Humans. Neuropsychopharmacology. 2013; **38**(5): 884–894.
26 Russell G, Lightman S. The human stress response. Nat Rev Endocrinol. 2019; **15**(9): 525–534.
27 Špiljak B, Vilibić M, Glavina A, Crnković M, Šešerko A, Lugović-Mihić L. A Review of Psychological Stress among Students and Its Assessment Using Salivary Biomarkers. Behavioral Sciences. 2022; **12**(10): 400.
28 Stoffel M, Neubauer AB, Ditzen B. How to assess and interpret everyday life salivary cortisol measures: A tutorial on practical and statistical considerations. Psychoneuroendocrinology. 2021; **133**: 105391.
29 Urwyler SA, Schuetz P, Sailer C, Christ-Crain M. Copeptin as a stress marker prior and after a written examination – the CoEXAM study. Stress. 2015; **18**(1): 134–137.
30 Whelan E, O'Shea J, Hunt E, Dockray S. Evaluating measures of allostatic load in adolescents: A systematic review. Psychoneuroendocrinology. 2021; **131**: 105324.