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Investigation of Salivary cAMP Response Element-Binding Protein (CREB) and Cortisol Hormone Responses According to Metacongestion Skills: Bayburt University Example

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Abstract
The aim of this study is to investigate the salivary cAMP Response Element Binding Protein (CREB) and cortisol hormone responses of university students according to their metacongestion skills. The study was conducted on 80 university students (40 female, 40 male) of different genders who study at Bayburt University Faculty of Theology and do not have any health problems. Participants were asked to fill out the demographic data questionnaire and the Metacognition Scale-30 questionnaire to evaluate their Metacognition Skills functions through face-to-face interviews. CREB and Cortisol hormone levels in saliva samples taken from the students participating in the study were examined using the ELISA technique. Number, percentage, mean, chi-square, T-Test, Pearson Correlation and ANOVA tests were used to evaluate the data. In all analyses, the significance value was taken as $p<0.05$. The average salivary cortisol hormone levels of the participants were determined as 2.045 ng/ml in women and 2.103 ng/ml in men, and salivary CREB levels were determined as .994 ng/ml in women and 1.049 ng/ml in men. In addition, a strong negative relationship was found between cortisol and CREB, and a positive relationship was found between cortisol and the metacognition questionnaire and its sub-dimensions. Stress is an important factor in the management of metacognitive skill functions in university students. Stress level is important for the biological, psychological and spiritual health of students and their academic success. As a result, it is thought that our current study will contribute to and benefit strategies for stress management and the development of metacognitive skills in university students.

Keywords: Metacognitive skills functions, cAMP Response Element-Binding Protein (CREB), Cortisol, Hormone, University Students

Introduction

Students, Many factors such as adapting to a new environment, academic pressure, social relationships, financial concerns and uncertainties about the future can cause stress in university students. In addition, during their university life, they often come together with economic concerns, academic issues, social relationships, career problems and many stressful situations related to business life. Therefore, stress management skills are of critical importance for university students. It is called the ability to monitor and regulate their own cognitive processes along with being aware of their own cognitive structure and learning characteristics (Gagne et al. 1988). Students learn to think about their own thinking processes and can apply learning strategies that will enable them to overcome difficult learning. Metacognition skill function is the ability of an individual to monitor, regulate and control their cognitive processes. Metacognition skill functions not only increase the academic success of university students, but also are an important factor in raising them as more equipped, self-confident and lifelong learners. Thanks to these skills, they make a significant contribution to coping with the difficulties of university life, maximizing their learning potential and being successful in their future careers. For this reason, it is of great importance for universities to include activities aimed at developing metacognition skills in their educational programs.

CREB (Cyclic AMP-Response Element Binding Protein) is a protein that plays an important role in the brain's memory structure and which memories are stored for a long time and which are lost instantly, and is even essential for this process (Johannessen et al., 2004). Metacognitive skills significantly affect memory performance by allowing individuals to be aware of their own memory processes, use effective memory strategies, organize their learning and remembering processes, and accurately assess their confidence in their memories (Schneider, 2010;Bayraktar, 2019). Individuals with advanced metacognitive skills learn more effectively, can remember information for longer periods of time, and can more successfully cope with the

difficulties they encounter during the remembering process.

Stress is the emotional, mental and physical reaction caused by anxiety resulting from an event or thought that occurs momentarily, makes one feel in danger or requires a struggle. Cortisol, called the stress hormone, is a corticosteroid hormone synthesized in the zona fasciculus region of the adrenal cortex (Bayraktar, 2020). Cortisol level in saliva reflects free cortisol in the blood and is used to determine the stress level (Kudielka et al., 2009; Orkun Erkılıç et al., 2024; Ozcan Böyük et al., 2024; Orkun Erkılıç and Bayraktar, 2025a; Orkun Erkılıç and Bayraktar, 2025b; Orkun Erkılıç et al., 2025). Cortisol, Chronic stress, prolonged cortisol secretion, and chronic inflammation, along with serotonin depletion, cause symptoms of depression (Hannibal and Bishop, 2014). High stress level, metacognitive skills are reported to be associated with high stress level (Drigas and Mitsea, 2021). In this context, it is aimed to examine the effect of salivary cAMP Response Element-Binding Protein (CREB) and cortisol hormone response

Methods

Participants and Procedures

The universe of the study consisted of 80 university students (40 female, 40 male) of different genders who were studying at the Faculty of Theology and had no health problems (Figure 1). Ethics committee approval (2025/Decision no: 121) and institutional permission were obtained before the study. Participants were informed about the study in accordance with the Declaration of Helsinki and their consent was obtained to fill out the Informed Consent Form. Volunteer participants were included in the study. The sample size of the study was calculated using the G*Power 3.1.9.7 analysis program; It was determined as 80 with a 95% confidence interval, 5% margin of error and 80% power. Data were collected using the general information form for university students and the Metacognition 30 Questionnaire (BFQ) via face-to-face interviews in an average of 15 minutes. Saliva samples were taken to determine the salivary CREB and cortisol hormone levels of the participants.

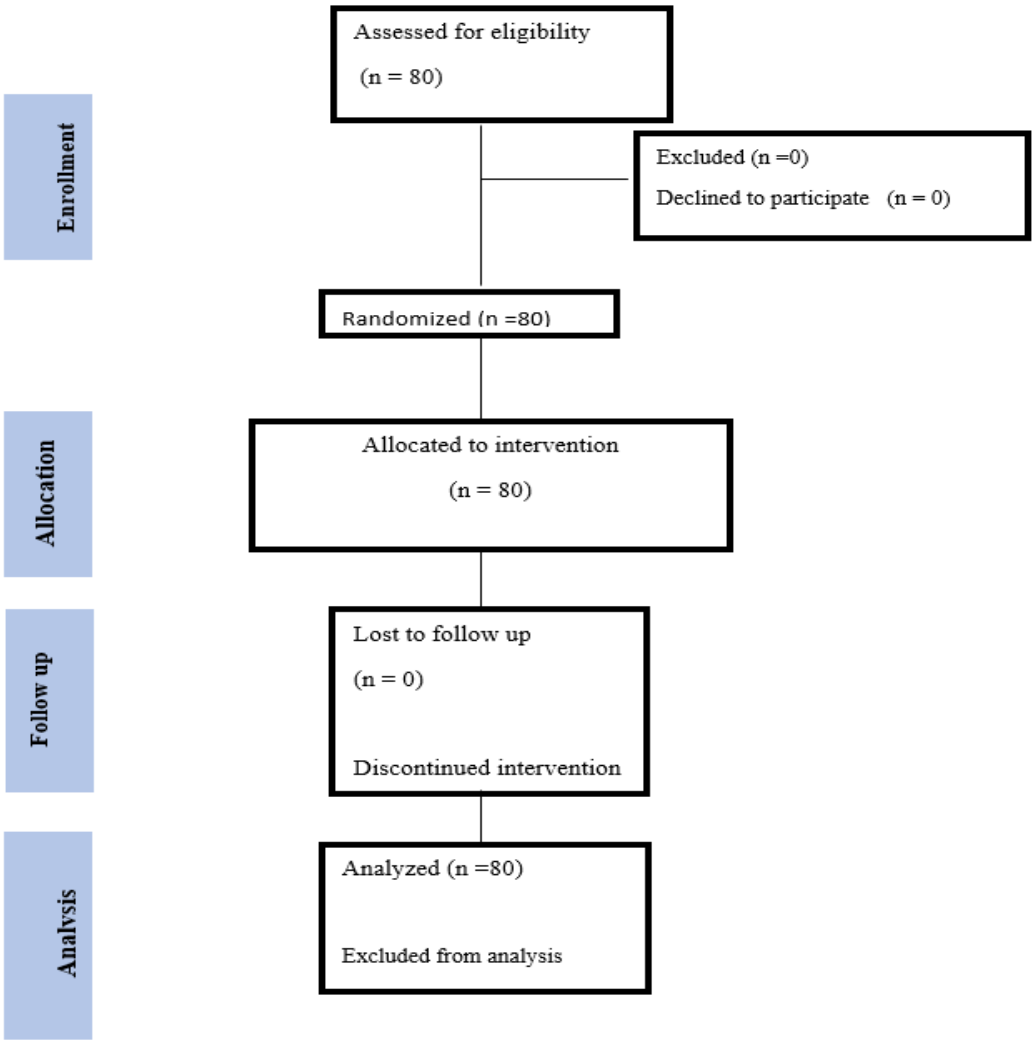

Figure 1. Study cohort flow chart.

The collection of research data: The collection of research data: The data were collected face-to-face with the Personal Information Form and Metacognition Scale-30 in university students (18-24 years) in an average of 15 minutes. Metacognition Scale-30 is used to determine the functions of metacognition skills (Tosun and Irak, 2008).

Collection of Saliva Samples: Saliva samples were collected from participants. Saliva samples were collected at a time period of 08:00-09:00 in the morning using the passive salivation method in Salivette tubes (Sarstedt, GERMANY) at a rate of 5 cc. After centrifuging at 2000 g for 20 minutes in a refrigerated centrifuge (NF 1200R, NUVE, Ankara, TÜRKİYE) in the laboratory, saliva samples were stored at -80°C until analyzes for cortisol hormone levels were performed.

Measurement of salivary cortisol hormone and CREB levels: The study utilized the Human Cortisol ELISA Kit (BT LAB, Cat.No E 1 003Hu, China) and Human CREB ELISA (BT LAB, Cat.No Kit SG-11988, China) to quantify the CREB and cortisol hormone amounts in saliva samples. The ELISA kit determined concentrations ranging from 31.25 to 2000 pg/mL. The intra-assay coefficients were 8.0% and the inter-assay coefficients were 10.0%. The protocol followed was as indicated in the manufacturer's catalog.

The Metacognition Questionnaire (MCQ-30): The scale developed by Wells and Cartwright-Hatton (2004) was adapted into Turkish by Tosun and Irak. The scale consists of five conceptually distinct but interrelated factors. These five factors are: (1) positive beliefs, (2) cognitive confidence, (3) uncontrollability and danger, (4) cognitive self-consciousness, and (5) need to control thoughts. All factors include two common components: positive and negative metacognitive beliefs (structures) and metacognitive processes (selective attention, monitoring of internal cognitive processes). The statements in each item offer responses on a four-point Likert-type rating scale: "definitely disagree", "partly disagree", "partly agree", and "definitely agree". The overall internal consistency (Cronbach's Alpha) value of the scale is .930.

Statistical analysis:

Statistical analyses were performed using the SPSS (Statistical Package for the Social Sciences) version 26.0 software package. Numerical data were presented as mean ± standard deviation (SD). As a result of the normality test, the data were found to have a normal distribution and parametric tests along with one-way ANOVA were used. The analysis of correlations between numerical parameters was performed using the Pearson correlation test. All analyses were conducted at a 95% confidence interval, and a p-value of <0.05 was considered statistically significant.

Results

Table 1 shows the data of the Metacognition questionnaire scale and its sub-dimensions used in the study, as well as the mean, standard deviation, minimum-maximum and skewness-kurtosis values of the salivary cortisol and CREB results.

Table 1. Descriptive Statistics

Value	Min.	Maks.	$\bar{X}$	sd	Skewness	Kurtosis
Cortisol (ng/ml)	1.76	2.56	2.074	.170	.517	.162
CREB (ng/ml)	.41	2.02	1.022	.528	.425	-1.319
The Metacognition Questionnaire	30.00	108.00	76.25	13.721	-.864	1.143
Positive Beliefs	6.00	23,00	13.45	4.206	-.063	-.820
Cognitive Confidence	6.00	20,00	13.01	4.410	.049	-1.234
Uncontrollability and Danger	6.00	23,00	15.80	3.262	-.775	.943
Cognitive self-Consciousness	6.00	23,00	18.250	3.612	-.908	.269
Need to Control Thoughts	6.00	23,00	15.737	3.983	-.472	-.116

The Skewness and Kurtosis values in the table are generally within the acceptable range of -2 to +2. This shows that the distributions of the analyzed variables have a normal distribution.

The average value for the salivary cortisol level taken from the participants was calculated as 2.074 ng/ml. According to the standard deviation (0.170), it is seen that the data is not very scattered around the mean, that is, the participants' cortisol levels are relatively close to each other. In the salivary CREB level, it was determined that the average was 1.022 and the standard deviation (0.528) was higher than cortisol. In other words, unlike the cortisol data, there is more variability among the participants in CREB levels.

The mean score on the Metacognition Questionnaire

Scale is 76.25. This value is slightly above the theoretical midpoint of the scale, which is 75. Scores range from 30 to 108. This may suggest that the sample generally tends to have slightly above-average metacognitive activity (potentially dysfunctional metacognitions). When the theoretical range of the scale (30-120) is considered, it is seen that participants scored in a wide range. The mean score on the positive beliefs subscale of the metacognition questionnaire scale is 13.45. This score is slightly below the mean point of the positive beliefs subscale. This shows that participants have a moderate level of positive belief about the benefits of worrying. The mean score on the cognitive confidence subscale is 13.01. Since high scores on this subscale indicate low confidence, this result indicates that participants tend to report a moderate level of general distrust in their memory and attention abilities. The mean score on the uncontrollability and danger subscale is 15.80, which is slightly above average. This shows that the participants' belief that their thoughts are uncontrollable and dangerous is slightly above average. The mean score for cognitive self-consciousness is 18.25, which is well above average. This means that the participants in the sample are relatively preoccupied with their own thought processes or monitor them. Finally, the mean score for the need to control thoughts subscale is 15.73, which is slightly above average. This shows that the participants' need to control their thoughts is slightly above average.

Table 2: Comparison of data by gender (Independent Sample T-Test)

Value	Gender	N	$\bar{X}$	sd	t	p
Cortisol (ng/ml)	Female	40	2.045	.144	-.459	.647
	Male	40	2.103	.191		
CREB (ng/ml)	Female	40	.994	.550	-1.522	.132
	Male	40	1.049	.512		
The Metacognition Questionnaire	Female	40	77.85	13.98	1.044	.300
	Male	40	74.65	13.43		
Positive Beliefs	Female	40	13.50	4.320	.106	.916
	Male	40	13.40	4.143		
Cognitive Confidence	Female	40	13.22	4.714	.429	.669
	Male	40	12.80	4.133		
Uncontrollability and Danger	Female	40	15.50	3.426	-.821	.414
	Male	40	16.10	3.103		
Cognitive self-Consciousness	Female	40	19.10	2.985	2.152	**.034***
	Male	40	17.40	4.005		
Need to Control Thoughts	Female	40	16.52	4.349	1.793	.077
	Male	40	14.95	3.456		

Table 2 examines whether there is a statistically significant difference between the total score of cortisol, CREB, Metacognition Questionnaire-30 and the mean scores ($\bar{X}$) of the 5 sub-dimensions of this scale for female (N=40) and male (N=40) participants. It was determined that there was no statistically significant difference between genders in CREB, cortisol, The metacognition questionnaire total score and the positive beliefs, cognitive confidence, uncontrollability and danger and need to control thoughts sub-dimensions of the scale. However, a statistically significant difference was found between the mean score of females (19.10) and the mean score of males (17.40) in the cognitive self-consciousness sub-dimension of the metacognition questionnaire scale (t=2.152, p=0.034). The fact that females had higher cognitive awareness scores than males may mean that female participants tend to be more preoccupied with their own thought processes or monitor them than males.

Table 3. Relationships between participants' salivary cortisol, CREB, the metacognition questionnaire scale and its sub-dimensions (Correlation Analysis)

		1	2	3	4	5	6	7	8
1.Cortisol	r	1							
	p								
2.CREB	r	-.492**	1						
	p	.000							
3.The Metacognition Questionnaire	r	.28*	-.200	1					
	p	.042	.075						
4.Positive Beliefs	r	.151	-.220*	.711**	1				
	p	.782	.050	.000					
5.Cognitive Confidence	r	.173	-.183	.736**	.584**	1			
	p	.125	.103	.000	.000				
6.Uncontrollability and Danger	r	.020	-.168	.673**	.270*	.298**	1		
	p	.859	.136	.000	.016	.007			
7.Cognitive self-Consciousness	r	.193	-.056	.558**	.137	.420**	.053	1	
	p	.086	.622	.000	.224	.000	.640		
8.Need to Control Thoughts	r	.242*	-.065	.822**	.401*	.503**	.517**	.466**	1
	p	.031	.569	.000	.000	.000	.000	.000	

**: Correlation is significant at 1% level
*: Correlation is significant at 5% level

Table 3 shows the results of the Pearson correlation analysis examining the relationships between the participants' salivary cortisol and CREB levels and the metacognition questionnaire total score and its sub-dimensions. According to these results, there is a strong, negative relationship between cortisol and CREB (r = -0.492, p = 0.000), a weak, positive relationship with the metacognition questionnaire (r = 0.228, p = 0.042), and a weak, positive and statistically significant relationship with the metacognition questionnaire need to control thoughts sub-dimension (r = 0.242, p = 0.031). There is a positive relationship between cortisol and other scale sub-dimensions, but it is not statistically significant (p>0.05). There is a weak, negative and statistically significant relationship between the CREB metacognition questionnaire positive beliefs sub-dimension (r = -0.220, p = 0.050). There is a negative relationship between the scale community score and other sub-dimensions and CREB, but it is not statistically significant (p>0.05). There appear to be strong or very strong, positive and statistically significant (p=0.000) relationships between the total score of the Metacognition questionnaire and all its sub-dimensions.

Discussion

Memory is critical for academic success in university students, and various stress factors negatively affect this important cognitive function. It is of great importance for students to be aware of these stress factors and develop strategies to cope with them, both for their general health and academic success. If universities provide mechanisms to help students with stress management, time management, healthy lifestyle habits, and psychological support, this will contribute to students having a healthier and more successful university life.

Cyclic AMP Regulatory Element Binding Protein (CREB) is a transcription factor involved in the development, maintenance and neuronal plasticity of the nervous system, as well as learning and memory (Wang et al., 2016). CREB plays a role in increasing synaptic efficacy in adaptive processes such as learning and memory (Barco et al. 2003). CREB has a physiological role in both the adaptive response to stress and the regulation of the BDNF response (Alfonso et al., 2006). CREB activity in the hippocampus increases in the presence of stress (Böer et al., 2007), and depressive behaviors caused by inflammatory stress depend on CREB in the hippocampus (Ni et al., 2019). Stress causes a wide range of molecular effects that produce structural, functional, molecular and behavioral changes in the brain, especially in the hippocampus (Sapolsky, 2003; Bayraktar, 2020).Xu et al. (2006) reported that chronic stress procedure caused downregulation of BDNF level and decreased phosphorylated cAMP response element binding protein (pCREB)/CREB levels and (pCREB/CREB) ratio in the hippocampus and frontal cortex of stressed rats. Qi et al. (2008) reported that chronic forced swimming stress induced depressive-like behaviors and decreased P-ERK2, P-CREB, ERK1/2 and CREB levels in the hippocampus and prefrontal cortex. In our current study, when the correlation between salivary cortisol and CREB levels is examined, a strong negative relationship is observed (r=-0.492). The results of our current study are consistent with research results reporting that stress potentially negatively

affects CREB-mediated processes (Xu et al., 2006; Qi et al., 2008;Lu et al., 2013). Our current results differ from the research findings indicating that CREB functions as a key upstream integrator of neuroprotective signaling against oxidative stress-induced neuronal cell death (Lee et al., 2009). We believe that this situation is due to the decrease in CREB levels in the CREB/BDNF pathway due to increased stress in studies that are consistent with the reason.When the relationship between cortisol levels and metacognitive processes was examined, it was found that cortisol showed a weak but significant positive relationship with the MCQ-30 total score and especially the "Need to Control Thoughts" sub-dimension. This can be interpreted as higher stress levels (high cortisol) may lead individuals to think more about their own thoughts (general metacognition) and especially to feel the need to control their thoughts. This finding partially overlaps with studies in the literature reporting a relationship between high stress levels and metacognitive skills (Drigas & Mitsea, 2021). However, the fact that the relationship is weak and limited to only certain dimensions suggests that the connection between stress and metacognition may not be linear or may be affected by other factors. No significant relationship was found between CREB levels and metacognitive skills in general. A significant ($p=0.050$) weak negative relationship was found only with the "Positive Beliefs" sub-dimension. This suggests that the measured CREB levels may be related to basic memory mechanisms. Potential indirect relationships between CREB and metacognition (e.g., both are affected by stress or learning processes) need to be examined in further studies. In terms of gender differences, the most striking finding of the study is that women scored significantly higher than men in the "Cognitive Awareness" sub-dimension. This suggests that female students at the Faculty of Theology tend to monitor their own thought processes more or engage in these processes more than male students. The reasons for this difference may be cultural influences, gender-specific thinking or coping styles, and other differences. However, the fact that there was no significant difference between genders in the levels of cortisol and CREB, which are biological markers measured in this study, suggests that this gender difference in cognitive awareness may not be directly related to these biological stress or memory markers. The relationship between gender and metacognition exhibits a complex structure in terms of different sub-dimensions and contexts. The relationships among the MCQ-30 sub-dimensions show that the scale has a generally consistent structure. Particularly, the strong relationship between the "Need to Control Thoughts" and many sub-dimensions suggests that this theme may play a central role in the metacognitive profile.

Limitations

There are some limitations to this study. First, the use of self-report measurement tools in this study may cause response bias. In addition, the cross-sectional design does not allow for the establishment of a causal relationship between variables. For example, it cannot be determined whether high cortisol affects metacognition or whether certain metacognitive styles increase stress levels. Second, the results cannot be generalized to all students in Türkiye because this study was conducted in a province in the Eastern Black Sea Region of Türkiye. Despite these limitations, the study also had some strengths. This study is valuable because it is the first study to examine salivary cAMP Response Element Binding Protein (CREB) and Cortisol Hormone responses according to Metacongestion Skills in terms of some variables.

Conclusion

University life is an important development process in which students experience many new academic, personal and social lives. In university students, physical and psychological health is negatively affected due to physical, psycho-social and psychological stress factors, and their metacognition skills are negatively affected. In conclusion, this study revealed the effect of salivary CREB and cortisol hormone levels on university students' metacognition skills. Thus, it is thought that it will contribute and benefit to the strategies for developing interventions to protect students' health and support their academic success.

Declarations

Ethical considerations

The research was approved by the Bayburt University Research Ethics Committee (2025/Decision no: 121). Before the data were collected by the researchers, participants were informed about the study in accordance with the Declaration of Helsinki and their written/verbal consent was obtained. All methods were conducted in accordance with relevant guidelines and regulations.

Authors' Contribution

S.O. and B.B. dsigned the study. S.O. and B.B. collected data. S.O. analyzed the data. S.O. and B.B. prepared the draft plan. All authors contributed to writing the manuscript. All authors read and approved the final manuscript.

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Data Availability Statement
The corresponding author upon reasonable request will provide data supporting the findings of this study.

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Disclosure statement
No potential conflict of interest was reported by the author(s).

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