Investigation of the Effect of the Exam Period on the Nutritional Status and Salivary Cortisol Levels of University Students

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Abstract

The aim of this study is to investigate the effects of the examination period on the nutritional status and salivary cortisol levels of university students. The study was conducted on 60 university students (30 female, 30 male) of different genders who did not have any health problems and were studying at Bayburt University Faculty of Health Sciences. Participants filled out the demographic data questionnaire and Food Frequency Questionnaire (FFQ) during a face-to-face interview. Cortisol hormone levels were examined in saliva samples taken from students participating in the study 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 mean age of the participants in the sample was 20.45±1.11 years; mean height was 171.30±8.83 cm; mean weight was 66.85±10.04 kg and mean BMI was 22.73±2.63. According to the findings obtained from the study, a strong positive relationship (.682*) was observed between salivary cortisol and BMI. The mean salivary cortisol hormone levels of the participants were determined as 6.393 ng/ml in women and 2.979 ng/ml in men ($p<0.05$). Examining the effects of the exam period on the nutritional status and salivary cortisol levels of university students is important to protect the health of students and support their academic success. As a result, it is thought that our current study will contribute and benefit students who are in the exam period to develop healthy nutrition and stress management strategies.

Keywords: Exam Period, Nutritional Status, Cortisol, Hormone, University Students

Introduction

An exam is a method used to measure a person's knowledge and experience on a particular subject (Goldsmith et al., 1991). It aims to measure the knowledge, skills, talents, aptitudes, abilities, physical fitness or classification of the person taking the exam on many other subjects (Jacobi, 1987; Alam and Mohanty, 2023). For this reason, exams are considered a stress factor for individuals (Sung and Chao, 2015). Exam stress is intense anxiety that prevents the effective use of previously learned information during the exam and leads to a

decrease in success (Orkun Erkılıç et al., 2024). High levels of ongoing stress cause anxiety, emotional well-being, and deterioration of academic performance (Lyndon et al., 2014; Gebhart et. al., 2020). Acute exam stress also causes changes in the nervous system, physiologically in the cardiovascular system, and in the endocrine and immune systems (Gebhart et al., 2020).

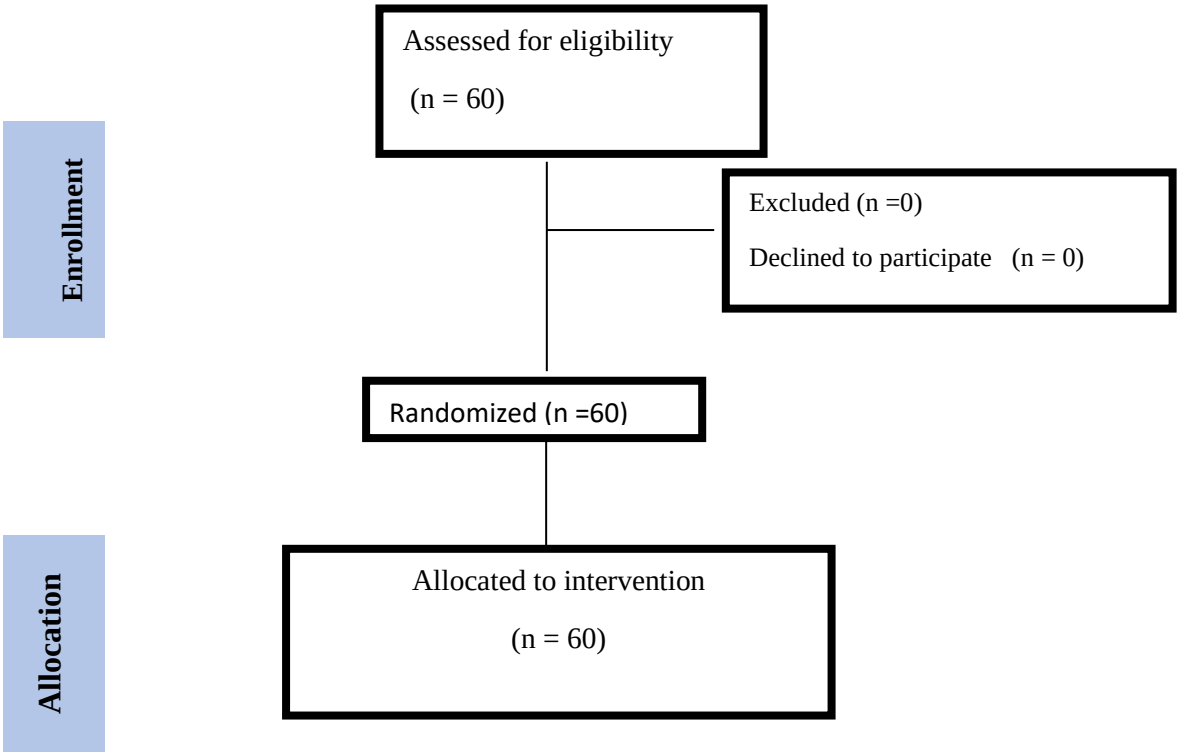
Stress is the physical and psychological response of an individual to the threatening, pressured or challenging situations he or she encounters. Stress is the emotional, mental and physical reaction caused by anxiety resulting from an event or thought that occurs momentarily, makes one feel in danger or requires a struggle. Stress causes mood changes along with emotional fluctuations (Kirbaş et al., 2024; Okur, 2024). Cortisol is a corticosteroid hormone associated with stress due to its role in regulating the body's response to stress, synthesized in the zona fasciculus of the adrenal cortex (Bayraktar, 2020). Cortisol levels gradually increase within a few minutes (usually less than 10 minutes) after the onset of stimulation and reach peak concentrations 10–30 minutes after the stressor has ended (Foley and Kirschbaum, 2010). Cortisol level in saliva reflects free cortisol in the blood (Kudielka et al., 2009; Orkun Erkılıç et al., 2024; Ozcan Böyük et al., 2024). In this context, it is aimed to examine the effect of the exam period on the nutritional status and salivary cortisol levels of university students.

Methods

Participants and Procedures

The population of the study consisted of 60 university students (30 female, 30 male) of different genders who were studying at the Faculty of Health Sciences and did not have any health problems (Figure 1). Ethics committee approval (2024/ Decision no: 98/10) and institutional permission were obtained before the study. The participants were informed about the study in accordance with the Declaration of Helsinki and their consent was obtained for the Informed Consent Form. Volunteer participants were included in the study. The study's sample size was

calculated using the G*Power 3.1.9.7 analysis program; It was determined as 60 with 95% confidence interval, 5% margin of error, and 80% power. The data was collected face to face in an average of 15 minutes using a form containing general information and the Food Frequency Questionnaire (FFQ) for university students. Saliva samples were taken to determine the salivary cortisol hormone levels of the participants.



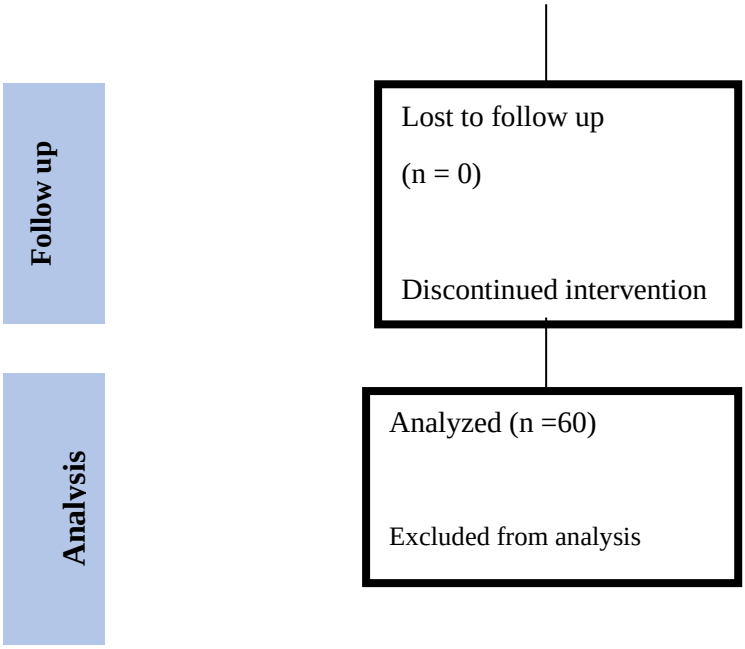


Figure 1. Study cohort flow chart.

The collection of research data: The data were collected face-to-face with the Personal Information Form and Food Frequency Questionnaire in university students (18-24 years) in an average of 15 minutes. Food frequency questionnaire is used to determine the frequency of food or food groups consumed per day, week or month and, if desired, in quantity. The food consumption frequency method is a method frequently used to determine the relationship between nutrition and disease risk. The FFQ can be prepared in different ways depending on the purpose (Orkun Erkılıç and Rakıcıoğlu, 2020; Orkun Erkılıç and Pekcan, 2020).

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 levels: The study utilized the Human Cortisol ELISA Kit (BT LAB, Cat.No E 1 003Hu, China) to quantify the 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.

Statistical analysis:

The data obtained through survey forms in the study were processed and analyzed by the researcher using the SPSS 26.0 package program. As a result of the analysis performed for the normality test of the data, the skewness and kurtosis (Skewness and Kurtosis) values were found to be between -2 and +2 and it was assumed that the normality assumption was accepted. In the analyses for the variables of gender and BMI status from the demographic questions, T-test, which is a parametric test for two-group comparisons, and ANOVA tests were applied for comparisons of more than two groups. In this context, independent sample T-Test was applied for the variables with two groups, and ANOVA analyses were applied for the variable with more than two groups. The groups that caused the significant difference as a result of the comparison of more than two groups were determined with Tukey HSD, which is a Post-Hoc test. Pearson Correlation Analysis was applied to examine the relationships between the FFQ used in the study and the cortisol measurements with age, height, weight and BMI. In all analyses, the significance (p) value was taken as 0.05. In the results of the applied tests, when $p<0.05$, the difference was considered statistically significant, and when $p>0.05$, the differences were considered statistically insignificant.

Results

In Table 1, where the demographic information of the research participants is examined, it is seen that there are 30 female (50.0%) and 30 male (50.0%) participants by gender; 1

underweight (1.7%), 50 normal (83.3%) and 9 overweight (15%) participants by BMI groups. The average age of the participants was calculated as 20.45±1.11 years; average height as 171.30±8.83 cm; average weight as 66.85±10.04 kg and average BMI as 22.73±2.63.

Table 1: Demographic variables

Variables	Groups	f	%
Gender	Female	30	50
	Male	30	50
BMI Groups	Underweight	1	1,7
	Normal	50	83,3
	Overweight	9	15
	Obese	-	-

	Min.	Max.	Mean	SD.
Age	19	24	20,45	1,111
Height(cm)	156	187	171,30	8,838
Weight(kg)	50	90	66,85	10,043
BMI	18,12	29,30	22,73	2,631

Table 2 shows the number of meals consumed (per day) and food groups in the food frequency questionnaire used in the study, as well as the mean, standard deviation, minimum-maximum and skewness-kurtosis values of salivary cortisol results. If the skewness and kurtosis values are between -2 and +2, the assumption of normality is accepted.

Table 2: Descriptive statistics

	Min.	Max.	$\bar{X}$	sd	Skewness	Kurtosis
Number of meals consumed (per day)	1	4	2,58	,743	,345	-,409
Dairy products	2	5	3,32	,930	,102	-,852
Meat products	2	5	3,82	,624	-,288	,473
Bread and cereals	1	5	3,18	,873	,102	-,092
Vegetables	1	5	3,18	,892	-,227	-,016
Fruits	1	5	2,75	1,188	,004	-,822
Fats and oils	1	5	3,13	1,033	-,753	-,017
Sweets	1	5	3,07	1,388	-,319	-1,260

Cortisol(ng/ml)	2,03	9,91	4,686	2,204	,814	-,346

In this case, parametric tests were used for the FFQ where the normality assumption was provided. It was observed that the data had a normal distribution and the mean value in the salivary cortisol level taken from the participants was calculated as 4.686±2.20 ng/ml.

Table 3: Comparison of number of meals consumed (per day), food grops and salivary cortisol level by gender

	Gender	**N**	$\bar{X}$	**sd**	**t**	**p**
Number of meals consumed (per day)	Female	30	2,60	,621	,172	,864
	Male	30	2,57	,858		
Dairy products	Female	30	3,30	1,022	-,138	,891
	Male	30	3,33	,844		
Meat products	Female	30	3,97	,615	1,902	,062
	Male	30	3,67	,606		
Bread and cereals	Female	30	3,07	,868	-1,036	,305
	Male	30	3,30	,877		
Vegetables	Female	30	3,10	,960	-,720	,474
	Male	30	3,27	,828		
Fruits	Female	30	2,70	1,264	-,324	,747
	Male	30	2,80	1,126		
Fats and oils	Female	30	2,83	1,053	-2,333	**,023***
	Male	30	3,43	,935		
Sweets	Female	30	3,00	1,438	-,369	,713
	Male	30	3,13	1,358		
Cortisol (ng/ml)	Female	30	6,393	1,884	9,520	**,000***
	Male	30	2,979	,555		

According to the T-Tests examining the statistical differences in the data obtained from the participants and the cortisol measurements according to gender groups; significance was determined in the cortisol and fats and oils consumption data ($p<.05$). It is seen that the average fats and oil consumption score of the male participants is higher than the females. On the other hand, the average salivary cortisol levels of the female participants were higher than the males.

Table 4: Comparison of number of meals consumed (per day), food groups and salivary cortisol level by BMI (ANOVA)

	BMI	N	x̄	sd	F	p	Post Hoc (Tukey)
Number of meals consumed (per day)	Underweight[a]	1	4,00				
	Normal[b]	50	2,56	,733	1,905	,158	-
	Overweight[c]	9	2,56	,726			
Dairy products	Underweight[a]	1	3,00				
	Normal[b]	50	3,40	,948	1,221	,303	-
	Overweight[c]	9	2,89	,782			
Meat products	Underweight[a]	1	3,00				
	Normal[b]	50	3,84	,618	,906	,410	-
	Overweight[c]	9	3,78	,667			
Bread and cereals	Underweight[a]	1	3,00				
	Normal[b]	50	3,20	,926	,060	,942	-
	Overweight[c]	9	3,11	,601			
Vegetables	Underweight[a]	1	3,00				
	Normal[b]	50	3,26	,828	1,140	,327	-
	Overweight[c]	9	2,78	1,202			
Fruits	Underweight[a]	1	4,00				
	Normal[b]	50	2,70	1,216	,652	,525	-
	Overweight[c]	9	2,89	1,054			
Fats and Oils	Underweight[a]	1	4,00				
	Normal[b]	50	3,14	1,107	,420	,659	-
	Overweight[c]	9	3,00	,500			
Sweets	Underweight[a]	1	4,00				
	Normal[b]	50	2,98	1,421	,649	,527	-
	Overweight[c]	9	3,44	1,236			
Cortisol(ng/ml)	Underweight[a]	1	2,140				
	Normal[b]	50	4,688	2,220	,728	,487	-
	Overweight[c]	9	4,956	2,181			

*Post hoc tests cannot be performed due to less than two values in at least one group.

According to one-way ANOVA analyses examining the statistical differences in the data obtained from the participants and cortisol measurements according to BMI groups, it was

determined that there was no significant difference in the results of salivary cortisol and other data according to BMI groups (p<.05).

Table 6: Relationships between continuous demographic variables of the participants, food consumption frequencies during the exam period and cortisol levels

		1	2	3	4	5	6	7	8	9	10	11	12	13
1.Age	r	1												
2.Height(cm)	r	,530**	1											
3.Weight(kg)	r	,348**	,649**	1										
4.BMI	r	-,024	-,044	,727**	1									
5.Number of meal cons.	r	,026	,048	,053	,057	1								
6.Dairy p.	r	,106	-,022	-,124	-,155	-,149	1							
7.Meat p.	r	-,001	-,208	-,123	,008	,052	,365**	1						
8. Bread and cereals	r	,123	-,009	-,028	-,022	-,027	,178	,094	1					
9.Vegetables	r	,121	,012	-,141	-,197	-,241	,317*	,031	,478**	1				
10.Fruits	r	,061	,119	,032	-,072	-,120	,088	,166	,290*	,412**	1			
11.Fats and oils	r	,095	,105	-,023	-,102	,118	,273*	,065	,405**	,286*	,069	1		
12.Sweets	r	-,031	-,014	,003	,026	-,285*	-,109	-,103	,269*	,181	,247	,266*	1	
13.Cortisol(ng/ml)	r	,-,153	-,113	-,179	,682**	,001	,016	,229	-,068	-,168	-,090	-,226	-,039	1

a. Cannot be calculated because all values are the same in at least one of the variables
** : Correlation is significant at the 1% level
* : Correlation is significant at the 5% level

When the relationships between the continuous demographic variables of the participants, the frequency of food group consumption during the exam period and cortisol levels were examined; It was seen that there was a positive moderate relationship between the frequency of meat products and dairy products consumption (.365**), between the frequency

of vegetables and bread and cereal products consumption (.478**), between the frequency of fruit and vegetable consumption (.412**); between the frequency of fats and oil and bread and cereal products consumption (.405**). It was seen that there was a positive strong relationship between cortisol and BMI (.682**).

Discussion

Exam anxiety is a combination of physiological overarousal, tension, and somatic symptoms as well as worry, fear, fear of failure, and catastrophizing that occur before or during examination situations (Tharu, 2023; Demir, 2023; Oflaz and Demir, 2024). The hypothalamic-pituitary-adrenal (HPA) axis is a pathway involved in the release of hypothalamic corticotropin-releasing hormone (CRH), whose increased activity following stress exposure leads to increased blood levels of cortisol, a glucocorticoid (GC) hormone that affects sympathetic nervous responses (Oyola and Handa, 2017; Hinds and Sanchez, 2022). Research results reporting changes in cortisol hormone levels vary. When a person is faced with a stressor such as an exam, cortisol secretion is activated via the hypothalamic pituitary adrenal axis (HPA axis) (Foley and Kirschbaum, 2010). While some studies report that cortisol hormone levels are higher immediately before an exam and decrease after the exam is completed (Dickerson and Kemeny, 2004; Verschoor and Markus, 2011), several studies have found no change in cortisol concentrations or only a decrease after the exam (Vedhara et al., 2000, reported that cortisol increased further after the exam (Schoofs et al., 2008; Preuß et al., 2010).

Cortisol hormone levels also vary according to stress response and food intake. Low blood glucose levels prevent stress-induced HPA activation (Kirschbaum et al., 1997). During stress, food choices often shift toward sweet and fatty foods because they are perceived as rewarding (Zellner et al., 2006; Born et al., 2010). Carbohydrates, compared to water, have been reported to increase serum cortisol concentrations, whereas fat and protein do not (Martens

et al., 2010). Vicennati et al. (2002) reported that a high-carbohydrate meal significantly increased plasma cortisol levels in visceral obese individuals. Lemmens et al. (2011) reported that consuming a high-protein meal and a high-carbohydrate meal did not affect the physiological cortisol response and psychological mood response differently, with men showing a higher meal-induced salivary cortisol response than women. Lacroix et al (2004) showed that high protein/high fat diets significantly reduced cortisol levels. Our current study is similar to research results reporting that cortisol and fat type consumption increases salivary cortisol hormone levels in female participants compared to male participants (Larsson et al., 2009; Epel et al., 2010; Pearlmutter et al., 2020), while it differs from some research results (Lemmens et al., 2011).

Conclusion

The exam period can increase students' stress levels, which can increase the secretion of the cortisol hormone. Changes in eating habits and high cortisol levels during the exam period can negatively affect students' physical and psychological health. In conclusion, this study has revealed the effects of the exam period on university students' nutritional status and salivary cortisol hormone levels. Examining the effects of the exam period on the nutritional status and salivary cortisol levels of university students will contribute to and benefit strategies for developing more effective health programs and interventions for students in terms of protecting their health and supporting their academic success.

Declarations

Ethical considerations

The research was approved by the Bayburt University Research Ethics Committee (2024/ Decision no: 98/10). 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

T.O.E., B.B. and F.G designed the study. T.O.E; B.B and F.G collected data. T.O.E and B.B. analyzed the data. T.O.E 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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