

Effect of increase in cortisol level due to stress in healthy young individuals on dynamic and static balance scores

 **Mahmut Cay,**¹  **Cihat Ucar,**²  **Deniz Senol,**³  **Furkan Cevirgen,**³
 **Davut Ozbag,**³  **Zuhal Altay,**⁴  **Sedat Yildiz**⁵

¹Department of Anatomy, Usak University Faculty of Medicine, Usak, Turkey

²Department of Physiology, Adiyaman University Faculty of Medicine, Adiyaman, Turkey

³Department of Anatomy, Inonu University Faculty of Medicine, Malatya, Turkey

⁴Department of Physical Medicine and Rehabilitation, Inonu University Faculty of Medicine, Malatya, Turkey

⁵Department of Physiology, Inonu University Faculty of Medicine, Malatya, Turkey

ABSTRACT

OBJECTIVE: Stress is a condition caused by various factors and characterized by imbalance in body functioning, impair in nervous system, and tension. The purpose of this study was to examine the effects of cortisol level, which increases in healthy young individuals due to stress, on dynamic and static balance scores as well as to present the results caused by high levels of stress.

METHODS: In this study, 107 healthy medicine faculty students in their second year (who will take the same committee exam) aged between 19 and 23 years were included. The first balance measurements and saliva samples were taken 40 days before the committee exam, and this period was acknowledged as the relaxed period. The same students were considered for balance measurements again on the day of committee exam; saliva samples were collected, and cortisol concentration was determined. This period was acknowledged as the stressful period. The State-Trait Anxiety Inventory (STAI) was given to the participants in their relaxed and stressful periods. Dynamic balance scores were measured with Star Excursion Balance Test (SEBT). Static balance scores were measured with One Leg Standing Balance Test (OLSBT).

RESULTS: The mean cortisol level was found to increase approximately 9 times in stressful periods compared with that in relaxed periods. STAI, which shows state anxiety, showed an increase supporting this increase. In stressful periods, dynamic balance scores showed obvious decrease in all directions. In addition, in stressful periods, an obvious decrease was observed in static balance scores compared with those in relaxed periods.

CONCLUSION: This study showed that stress negatively affected dynamic and static balance, even for short periods of time. We believe that our study will form a positive source and basis when correlated with long terms stress and balance measurements.

Keywords: Cortisol; dynamic balance; static balance; stress.

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Correspondence: Dr. Deniz SENOL. Inonu Universitesi Tip Fakultesi, Anatomi Anabilim Dalı, Malatya, Turkey
Phone: +90 506 447 06 86 e-mail: deniz.senol@inonu.edu.tr

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Stress is a situation caused by various factors and characterized by an imbalance in body functioning, impair in the nervous system and tension [1]. An individual who is stressed because of an unpleasant event responds to this situation with physiological and emotional changes as well as changes in perception and behavior. Even though people may think that they are not very much affected by events, they may develop reactions without realizing [2]. Stress most frequently occurs in uncontrollable, unwanted situations and when a person has more workload than he can handle [3]. A chemical or physical imbalance that occurs in cells or tissue fluid as a result of a change in the body or in the external environment is called physiological stress. There are 3 components of physiological stress. These are exogenous or endogenous stress factors and chemical or physical imbalance caused by stress factors and the adaptation response of the body to these conditions [4]. The results of the researchers examined the association between stress and immune system and monoaminergic system show that 2 stress sensitive endocrine response systems are hypothalamic–pituitary–adrenal (HPA) axis and sympathetic adrenal medullary system [5]. When a person is faced with a stressor that he cannot control with existing cope mechanisms, HPA axis is activated through the association cortex, amygdala, and hippocampus, which causes the cortisol blood level to rise and brain functions to be affected through the neurons in the brain and glucocorticoid receptors in glial cells [6]. Cortisol is a steroid structured hormone released from the outer part of the cortex of suprarenal gland and exhibits glucocorticoid effect [7, 8]. Due to its small, fat-soluble structure (MW~362 Da), it passes from capillary vessels to cells primarily through passive diffusion [8, 9] and is distributed to body parts such as saliva, cerebrospinal fluid, sweat, hair, and urine [10]. Cortisol has circadian oscillation rhythm. While cortisol level in blood is high in the early hours of the morning, it drops to its lowest level at midnight [8–10]. While a major amount of the cortisol in blood is bound to corticosteroid-binding protein (CBG or transcortin) with high affinity, a small amount is carried as bound to albumin. Free cortisol is found in blood in a low rate (3–10% of the total cortisol) [8, 9]. Cortisol concentration in saliva has been reported to reflect the free cortisol in blood [8–11]. Salivary cortisol is used in psychoneuroendocrinological monitoring as an important parameter with reasons such as measuring the free cortisol level noninvasively, not causing pain, and allowing a person to get sample alone easily whenever wanted [12]. Maintaining balance and

a stabilized posture is an indispensable part of a great number of movement practices [13]. Balance control is a complex motor skill that includes planning and applying flexible movement figures as well as the integration of sensory input [14]. The integration of the information coming from sensory system gives information to a person about his/her orientation to maintain posture control in space that allows for regulatory reflexive movements [15]. However, sensory inputs are not responsible alone for the maintenance of postural control. Postural stability is bound to complete neural pathways for the integrity of muscle mass and efficiency of the muscles in the central nervous system for motor control [16]. Environmental components on balance include somatosensory, visual, and vestibular systems. The central nervous system combines the environmental inputs coming from these systems and chooses the responses of many suitable muscles in order to control body position and posture on support basis [17, 18]. The purpose of this study is to examine the effects of cortisol level, which increases in healthy young individuals as a result of stress, on dynamic and static balance scores and to present the factors that caused by high levels of stress.

MATERIALS AND METHODS

This study was conducted with the 2016/45 numbered permission of the Clinical Researches Ethical Board. In this study, 107 medicine faculty students in their second year aged between 19 and 23 years, who did not drink or smoke, did not have any orthopedic diseases or surgical intervention, did not participate in any sportive exercises, and did not use any psychiatric drugs during the period measurements were made were included in the study voluntarily. Consent forms were taken from all the students. The first balance measurements and saliva samples were taken 40 days before the committee exam and this period was acknowledged as the relaxed period. The same students were taken for balance measurements again on the day of committee exam and saliva samples were collected and cortisol concentration was determined. This period was acknowledged as the stressful period. STAI was given to students in their relaxed or stressful periods. STAI is a scale that is frequently used to assess STAI anxiety [19].

Analysis of cortisol in saliva

The passive drool method as described by Granger et al. [20] (2007) was used to collect saliva samples. The sam-

ples taken were kept at -20°C in a laboratory freezer. First, they were stored at thawing, then they were centrifuged for 10 min at $4000\times g$, and supernatants were analyzed with ELISA. Assay buffer was used to dilute each sample 1:5 and all samples were assayed in triplicate. ELISA procedure; carbonate buffer, pH 9.6 was used to dilute cortisol-BSA stock solution (1 mg/mL) and at $200\ \mu\text{L}/\text{well}$ it was added to a 96-well microtiter plate. The plate was incubated overnight at 4°C and using an 8-channel pipette, it was washed 5 times with wash buffer. The blocking buffer ($200\ \mu\text{L}/\text{well}$) blocked some binding sites that were not occupied by the coating antigen 2 h at 37°C . After washing steps (5 times), standard solutions or samples ($40\ \mu\text{L}/\text{well}$) and diluted 1st Ab (antiserum) ($40\ \mu\text{L}/\text{well}$) were added in duplicate and incubated for 45 min at 37°C . Biotinylated anti-rabbit antibody was added ($100\ \mu\text{L}/\text{well}$) after washing 5 times, and the plate was incubated for 30 min at 37°C . After the plate was washed 5 times, streptavidin peroxidase solution ($100\ \mu\text{L}/\text{well}$) was added and the plate was incubated at 4°C for 15 min. Following this, the plate was washed again for 5 times, and then the substrate solution ($150\ \mu\text{L}/\text{well}$) was added and incubated for 10 min in dark. Stop solution ($50\ \mu\text{L}/\text{well}$) was added after incubation, and then using a microplate reader, the absorbance was measured at 450 nm. Intra-assay variation (CV) was found to be 5.6%, while inter-assay variation was found to be 7.8%.

Balance measurements

Dynamic balance scores were measured with SEBT. SEBT is a frequently used simple and reliable measurement method used in the assessment of dynamic postural control [21–23]. SEBT has been reported to have enough sensitivity to find out balance deficits associated with musculoskeletal injuries such as ankle instability by previous studies [24–26]. SEBT was used to assess dynamic balance. With the subjects standing in the middle of a grid constructed by 8 measure tapes extending out at 45° from each other, SEBT was performed. Along each of the 8 measure tapes, the subject had to reach as far as possible, touch the tape lightly, and then while maintaining a single-leg stance with the other leg in the center of the grid, he had to return the reaching leg back to the center. In order to complete the task, the subjects had to reach behind the stance leg while reaching in lateral and posterolateral directions. The subjects began with the anterior direction and continued clockwise around the grid. All subjects started in the center of the grid with a

TABLE 1. Relaxed and stressed period cortisol levels, STAI average \pm SD and results of Wilcoxon paired 2 sample test

Parameter	Relaxed	Stressed	p
Cortisol	18.05 \pm 18.98	159.83 \pm 460.36	<0.001
STAI	38.79 \pm 3.31	42.71 \pm 4.23	<0.001

STAI: The State-Trait Anxiety Inventory; SD: Standard deviation.

right stance leg. Following the completion of the 3 trials in the 8 directions, a 5-min rest was given, after this rest, the test continued with a left stance leg. With a mark on the tape, each of the reach distances was recorded as the distance from the center of the grid to point of maximum excursion by the reach leg [27–30]. OLSBT was used to measure static balance. It was measured on stable platform with both eyes open for 60 s and closed for 30 s (EOSB; eyes open static balance, ECSB; eyes closed static balance), and the subjects were told to maintain their balance for maximum duration. When the stance foot shifted in any way or the non-stance foot touched the ground, the measurement was stopped [30–32].

Statistical analysis

Shapiro–Wilk test was used to find out whether the data were normally distributed. They were found to be abnormally distributed. Wilcoxon paired 2 sample test was used for the analyzes of data. Correlations were calculated with Spearman Rho coefficient. A level of $p < 0.05$ was considered as statistically significant. IBM SPSS Statistics 22.0 for Windows package program was used for statistical analysis.

RESULTS

The average age of the 107 people who participated in the study was calculated as 20.5 ± 1.36 . Cortisol analyses in the saliva samples taken in both relaxed and stressful days and averages and \pm standard deviation of STAI were given in Table 1. Average cortisol level was found to increase approximately 9 times in stressful periods compared with that in relaxed periods. STAI, which shows state anxiety, showed an increase supporting this increase. In order to assess whether the difference between relaxed and stressful period cortisol and STAI scale increase was significant, Wilcoxon Paired 2 sample test was conducted on the data. According to analysis results, a statistically signifi-

TABLE 2. Averages and \pm SD of relaxed and stressed period SEBT and Wilcoxon paired 2 sample test results

SEBT	Right leg			Left leg		
	Relaxed	Stressed	p	Relaxed	Stressed	p
A	57.41 \pm 12.07	46.49 \pm 10.93	<0.001	57.85 \pm 13.94	45.29 \pm 11.17	<0.001
AM	57.74 \pm 13.67	45.00 \pm 11.56	<0.001	56.01 \pm 13.28	43.67 \pm 11.78	<0.001
M	46.55 \pm 15.43	33.75 \pm 12.38	<0.001	49.39 \pm 14.50	35.17 \pm 11.92	<0.001
PM	55.18 \pm 14.57	43.39 \pm 12.22	<0.001	55.00 \pm 15.47	43.60 \pm 12.39	<0.001
P	55.97 \pm 13.43	44.65 \pm 11.95	<0.001	55.72 \pm 15.19	44.54 \pm 11.83	<0.001
PL	55.50 \pm 13.50	46.40 \pm 11.53	<0.001	55.21 \pm 14.48	44.84 \pm 11.07	<0.001
L	56.31 \pm 14.22	47.11 \pm 11.87	<0.001	56.01 \pm 15.81	46.78 \pm 10.87	<0.001
AL	57.81 \pm 13.77	46.22 \pm 11.98	<0.001	57.62 \pm 14.06	45.28 \pm 11.62	<0.001

SD: Standard deviation; SEBT: Star Excursion Balance Test; A: Anterior; AM: Anteromedial; M: Medial; PM: Posteromedial; P: Posterior; PL: Posterolateral; L: Lateral; AL: Anterolateral.

TABLE 3. Average and \pm SD of static balance scores measured by OLSBT

OLSBT	Right leg			Left leg		
	Relaxed	Stressed	p	Relaxed	Stressed	p
EOSB	54.14 \pm 10.89	42.67 \pm 14.66	<0.001	53.94 \pm 9.80	39.75 \pm 14.12	<0.001
ECSB	18.37 \pm 7.69	9.20 \pm 5.42	<0.001	17.72 \pm 8.21	9.43 \pm 6.11	<0.001

SD: Standard deviation; OLSBT: One Leg Standing Balance Test; EOSB: Eyes open static balance; ECSB: Eyes closed static balance.

cant ($p < 0.05$) increase was found in cortisol and STAI scores during relaxed and stressful periods (Table 1).

Table 2 gives the average \pm standard deviation of measurements conducted in relaxed and stressed periods in terms of right and left foot SEBT anterior (A), anteromedial (AM), medial (M), posteromedial (PM), posterior (P), posterolateral (PL), lateral (L), anterolateral (AL) directions.

When SEBT was examined, it was found that for the right foot, dynamic balance scores showed 19% anterior, 22% anteromedial, 28% medial, 21% posteromedial, 20% posterior, 16% posterolateral, 12% lateral, and 20% anterolateral decreases in stressful periods compared with those in relaxed periods. For the left foot, dynamic balance scores showed 22% anterior, 22% anteromedial, 29% medial, 21% posteromedial, 20% posterior, 19% posterolateral, 17% lateral, and 21% anterolateral decreases in stressed periods compared with those in relaxed periods. According to Wilcoxon paired 2 sample test results, dynamic balance scores measured by SEBT

were found to be statistically significant for the right and left feet between relaxed and stressed periods ($p < 0.05$). When Table 2 was examined, it was found that stress had a negative effect on individuals' dynamic balance scores. In stressed periods, dynamic balance scores showed significant decreases in all directions.

Table 3 gives the results of OLSBT and Wilcoxon paired 2 sample test results conducted showed that opening and closing of the eyes in relaxed and stressed periods for right and left foot. Eyes open stressed period right foot static balance scores showed 21% decrease when compared with relaxed period, while left foot static balance scores showed 26% decrease. Eyes closed stressed period right foot static balance scores showed 50% decrease when compared with relaxed period, while left foot static balance scores showed 47% decrease. According to Wilcoxon paired 2 sample test results, static balance scores measured by OLSBT were found to be statistically significant for the right and left feet between relaxed and stressed periods ($p < 0.05$). When Table 3 was examined, it was found that exam stress had a nega-

TABLE 4. Spearman Rho correlation analysis results (dynamic balance scores - static balance scores)

Test		Right leg		Left leg		
		Cortisol	STAI	Cortisol	STAI	
Dynamic balance	A	r	-0.366	0.222	-0.304	0.245
		p	<0.001	0.001	<0.001	<0.001
	AM	r	-0.373	0.213	-0.308	0.213
		p	<0.001	0.234	<0.001	0.253
	M	r	-0.299	0.001	-0.295	0.000
		p	<0.001	0.213	<0.001	0.213
	PM	r	-0.355	0.251	-0.373	0.251
		p	<0.001	<0.001	<0.001	<0.001
	P	r	-0.400	0.213	-0.304	0.213
		p	<0.001	0.255	<0.001	0.286
	PL	r	-0.364	0.000	-0.308	0.000
		p	<0.001	0.213	<0.001	0.213
L	r	-0.252	0.256	-0.269	0.225	
	p	<0.001	<0.001	<0.001	0.001	
AL	r	-0.286	0.213	-0.352	0.213	
	p	<0.001	0.213	<0.001	0.252	
Static balance	EO	r	-0.186	0.328	-0.322	0.365
		p	0.006	<0.001	<0.001	<0.001
	EC	r	-0.107	0.336	-0.173	0.343
		p	0.117	<0.001	0.011	<0.001

STAI: The State-Trait Anxiety Inventory; A: Anterior; AM: Anteromedial; M: Medial; PM: Posteromedial; P: Posterior; PL: Posterolateral; L: Lateral; AL: Anterolateral.

tive effect on students' static balance scores. When static balance scores in stressed periods were compared with those in relaxed periods, an obvious decrease was found.

Table 4 gives the dynamic balance scores measured by SEBT and Spearman Rho correlation analysis results connected on static balance scores measured by OLSBT. According to the results of the analysis, cortisol increase, which increased with stress, was found to correlate negatively with balance tests. It was found that cortisol increase due to stress affected balance negatively. Correlation analysis conducted with STAI state anxiety questionnaire was found to support this result.

DISCUSSION

Human body tries to adjust to stressful events with various responses. Individuals who face the effect of a stress stimulus, respond to this by taking a physiologi-

cal or psychological defense [33]. Stimulating messages from internal and external environments change a person's balance and the organism tries to rebalance and maintain adjustment. If a person's efforts are insufficient, his/her adjustment will be disrupted. The factors that disrupt adjustment put the organism in a difficult situation [34]. As long as a person adjusts to changes, he/she can keep balance, order, happiness, and health and tries consciously or unconsciously to maintain this balance and order. Sometimes he/she will be successful in these efforts, keeps his/her old balance and order and maintain adjustment [35]. Maintaining balance and a stable posture is an indispensable part of many movement practices [13]. Balance control is a motor skill that includes planning and practicing flexible movements as well as integrating sensory inputs [14]. The integration of the information coming from perceptive systems gives information to a person about orientation to maintain posture control in space that allows regulatory reflexive movements [15]. However, sensory inputs alone are not responsible for maintaining postural control. Postural stability depends on the integrity of muscle mass, the efficiency of the systems in the central nervous system, and the complete neural pathways for motor control [16]. As a response to stress, the endocrine system begins to release cortisol, and a sudden increase in cortisol levels is an adaptive function [36]. In our study, we compared the cortisol concentration of individuals in their relaxed and stressed periods. In their study conducted with 35 medicine faculty students, Singh et al. [37] (2012) compared cortisol levels in relaxed and stressed periods and made measurements on exam stress. They found cortisol levels as 2.48 ± 1.5 ng/ml in male students and 2.92 ± 1.9 ng/ml in female students during relaxed periods, while the levels were 5.02 ± 3.1 ng/ml in male students and 5.19 ± 3.1 ng/ml in female students during stressed periods. In a previous study, Schoofs et al. [38] (2008) found that academic exams caused very high increases in salivary cortisol levels. In our study, cortisol levels in stressed period were found to increase about 9 times compared with relaxed periods. This result was expected and our values were in line with the literature.

Some of the physical responses to stress are: shaking, muscle spasm, myotonia, cramps, numbness in fingers and toes [39–41]. Frequent physical symptoms and complaints during stress are pain in neck, nape, waist and back, spasm and arthralgia, imbalance and swaying while standing, sitting and walking [42]. In recent study with cyclists, Filho et al. [43] (2015) stated that stress factors

differed in short-term period and these changes affected performance. Bali [44] (2015) stated that sportive performance was not just a biomechanical and physiological event, psychological factors also affected performance significantly and in addition more than optimum stress affected performance negatively. In previous study on young (22.3 ± 3.6 years) and old (82.3 ± 9.6 years) individuals, Sarabon and Rosker (2015) found dynamic balance scores they measured with SEBT as 76.2 ± 6.78 cm in young individuals and 37.3 ± 7.26 cm in old individuals in anterior direction; as 65.1 ± 8.63 cm in young individuals, 30.3 ± 7.36 cm in old individuals in lateral direction, as 77.9 ± 11.0 cm in young individuals, and as 36.1 ± 8.05 cm in old individuals in posterior direction [45]. The results of this study are in line with that by Sarabon and Rosker (2015) on young individuals. In literature, we could not find any studies similar to our study that is on static and dynamic balance test in stressed and relaxed periods. The results of our study showed that static and dynamic balances of individuals were worse in stressed period when compared with relaxed period. The results of static and dynamic balance test showed unstableness and swaying in postural balances in stressed periods of individuals and also it was seen that they could not stand for long periods of time. When SEBT scores were analyzed, 12% and 28% decrease was found in all directions in dynamic balance scores of stressed period for right foot when compared with relaxed period. For left foot, a decrease between 17% and 29% was found in SEBT scores of stressed period when compared with relaxed period. In addition, significant differences were found in OLSBT results we conducted eyes open and closed in relaxed and stressed periods. Static balance scores measured with OLSBT were found to have lower values in stressed period when compared with relaxed period. As a conclusion; dynamic and static balance scores between stressed period and relaxed period in healthy individuals seem to be a response for stress. It is not known whether cortisol increase in stressed period has an effect on balance center. Cortisol increase and balance disruption were not in the same rates in this period. Thus, it should be researched whether cortisol increase has a positive effect on balance centers. Does the disruption of balance trigger cortisol increase or does cortisol increase contribute to the disruption of balance? In addition, it has been reported in literature that extreme cortisol released in healthy individuals due to long-term stress can cause negative effects such as increase in blood pressure, atherosclerosis, diabetes, immune suppression, osteolysis,

and myolysis [36]. As a result of the levels short-term stress affected static and dynamic balance in our study, we predict that long-term stress may cause permanent disruption of postural balance in young individuals. Further studies will present more obviously how long-term stress can affect postural balance in healthy individuals. We believe that our study will form a positive source and basis to new studies and contribute to literature.

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