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Salivary Cortisol Profile Under Different Stressful Situations in Female College Students: Moderating Role of Anxiety and Sleep

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ABSTRACT

Objectives: This study investigated the level of cortisol under different stressful situations and its relationship with sleep and anxiety in female college students. **Methods:** Salivary cortisol was measured 6 times a day during a routine period free of examination stress and a stressful period. Area under the curve (AUC) was calculated for cortisol level for awakening response (AUC_{AG}) and during the day (AUC_{TG}). Sleep characteristics and anxiety were measured using an actigraph and the Spielberger State-Trait Anxiety Inventory, respectively, during the different periods. **Results:** Thirty-six people participated in the study. During the stressful period, anxiety had a positive correlation with sleep efficiency ($P = .020$), wake after sleep onset ($P = .023$), and mean wake episodes during sleep ($P = .048$). Poorer sleep efficiency ($P = .014$), greater wake after sleep onset ($P = .008$), and mean wake episodes during sleep ($P = .044$) were significantly associated with less AUC_{AG}. Trait anxiety was significantly higher in participants with greater AUC_{TG} ($P = .008$). **Conclusions:** Female college students with increased anxiety under the stressful situation slept better. Those with poor sleep showed attenuated morning cortisol secretion. Those with higher trait anxiety had greater cortisol during the day.

Keywords: anxiety, area under curve, cortisol, sleep, stress

College students are known to experience a variety of stress as they faced high academic burden and uncertainties of their future.[1] Their stress leads to sleep problems,[2] although cause-and-effect relation remains unclear. Moreover, female students could be more vulnerable to the effect of stress and sleep problems due to cycling hormones.[3]

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Stress is generally defined as a threat to the physiological or psychological integrity of an individual, which results in a physiological and/or behavioral response.[4] The physiological stress response is believed to be manifested through the hypothalamic-pituitary-adrenal (HPA) system.[5] Thus, cortisol, the end product of the HPA axis, has been widely used as a biomarker of stress. Nevertheless, there is a lack of studies using cortisol to investigate stress of female college students.

Furthermore, contrary results on altered cortisol level and perceived stress have been reported.[6,7] Instability of cortisol measurement from a single measurement of cortisol is thought to be responsible for this discrepancy. As cortisol is released in a diurnal cycle—peaking around 30 minutes after awakening followed by a gradual decline throughout the day and reaching a nadir during nocturnal sleep[8]—cortisol awakening response and diurnal slope have been studied to indicate physical and mental conditions in various populations.[6,9] However, values of cortisol taken from measuring for a day or two may have low stability.[10] Recently, the computation of the area under the curve (AUC) has been suggested as a stable marker comprising information that is contained in repeated measurement over time and is believed to be more reliable.[11]

In addition, possible moderating effects of other factors on cortisol secretion could exist. Because the hypothalamus, the highest system in the HPA axis, has a complex set of interactions with suprachiasmatic nuclei and cortical limbic area that are liked with sleep and emotions, including anxiety,[12] cortisol secretion might be associated with sleep and anxiety. Several studies have suggested the involvement of and anxiety in cortisol secretion,[13,14] but few have investigated psychological factors, sleep behavior, and their influences on cortisol secretion.

The purpose of the study was to evaluate the AUC for the diurnal salivary cortisol profile under situations with different stress levels in female college students. We also investigated the associations among sleep, anxiety, and salivary cortisol profile. Findings from this study will add to a knowledge base about cortisol secretion and stress in female college students and will provide information on the role of anxiety and sleep for cortisol secretion.

Methods

The study was a descriptive and cross-sectional study exploring associations of salivary cortisol with sleep and anxiety under situations with different stress levels in female college students.

With institutional review board approval, the participants were recruited from a university located in South Korea. The inclusion criteria were female students aged 20 to 30 years, healthy without any inflammatory conditions. Participants who were unable to follow the study's instructions and those currently taking any medications including hormonal contraceptives were excluded.

Measurement of Salivary Cortisol Profile

The saliva specimens were collected 6 times a day in situations with different stress levels. The saliva specimens were taken upon wakening, 30 minutes after awakening, 1 hour after awakening, between 11 AM and 12 PM, between 3 and 4 PM, and between 9 and 10 PM on a day during routine period (in the routine situation) and again on another day just before or during an examination period (in the stressful situation) in the semester. The saliva specimens were collected by participants according to the sampling protocol using collection tubes. Saliva samples were kept in a portable cooler during sampling and then, once returned, were stored at $-70°C$. A commercially available enzyme immunoassay kit (Salimetrics, State College, Pennsylvania) was used to analyze salivary cortisol.

The AUC from repeated measurements of salivary cortisol across the day was computed to derive the total cortisol level during the awakening response and

Increased anxiety may help people adjust to stressful situations by improving sleep quality.

the total cortisol level during the day. Total cortisol level during awakening response (AUC_{AG}) was taken as the AUC from the 3 morning cortisol samples within 1 hour after awakening. Total cortisol level during the day (AUC_{TG}) was taken as the AUC from the other cortisol samples. The formulas are as follows:[15]

Total cortisol level during awakening response

$$(AUC_{AG}) = \sum_{i=0}^{i=1} \frac{(S_i + S_{i+1}) \times (t_{i+1} - t_1)}{2}$$

Total cortisol level during the day (AUC_{TG})

$$= \sum_{k=1}^{k-1} \frac{(S_k + S_{k+1}) \times (t_{k+1} - t_k)}{2}$$

Note that s denotes cortisol sample, t denotes time sample taken in hours, i denotes cortisol samples within 1 hour after awakening, and k denotes all samples taken excluding cortisol samples within 1 hour after awakening.

Perceived Stress

To assess current stress perception, we used the Korean version of the Global Assessment of Recent Stress Scale that measures the amount of stress or change with stressful events that occurred over the past 1 or 2 weeks.[16] It consists of 8 items, and each item is rated on a 10-point Likert scale ranging from 0 to 9. The total score is 72, with higher scores indicating a greater level of stress. The Cronbach's α for the Global Assessment of Recent Stress in this study was .82.

Anxiety

Anxiety was evaluated with the Korean version of the Spielberger State-Trait Anxiety Inventory.[17] It contains 40 items—20 items are used to measure trait anxiety asking how they generally feel and 20 to measure state anxiety asking how they feel currently—rated on a 4-point Likert scale. Score range from 20 to 80, with higher scores indicating a greater level of anxiety. The Cronbach's α for the State-Trait Anxiety Inventory in this study was .94.

Sleep Characteristics

Sleep characteristics were measured using an actigraphy watch, the Octagonal Motionlogger Sleep Watch-L (Ambulatory Monitoring Inc, Ardsley,

New York), for 2 consecutive nights during the time that salivary specimens were collected. It was worn on the participants' nondominant wrist to derive movement-based estimates of sleep-wake characteristics. The actigraph unit was set to 1-minute epoch and zero-crossing mode. We used the Action W-2 software and the Cole-Kripke algorithm (Ambulatory Monitoring Inc, Ardsley, New York) to analyze the sleep data. A previous study has shown good reliability and validity with a reasonable sensitivity higher than 0.60 in polysomnography-actigraphy comparison.[18]

For sleep characteristics, time in bed, sleep efficiency (SE), wake after sleep onset (WASO), and mean wake episode during sleep (MWE) were obtained indicating total minutes in bed for nocturnal sleep, percentage of actual sleep at night, total wake minutes during nocturnal sleep, and mean duration of wakes during sleep, respectively. Average values of the 2 nights were used for all the sleep characteristics.

Data Collection Procedure

During a routine period in the semester, an initial meeting with participants took place where the researcher explained the course of the study and a research assistant obtained informed consent. The participants completed demographic and psychometric questionnaires and were then instructed about the standardized collection of saliva samples according to the study protocol.[19] Participants were asked not to brush their teeth or eat at least 20 minutes before sampling and to store the samples taken in the portable cooling bag during the day or in refrigerators. They also wore an actigraphy unit on their nondominant wrist for 48 hours. Two days later, they visited our research office to return the saliva samples taken and the actigraphy unit. The participants were then given about $15 for participating in the study.

The entire procedure was repeated when the participants made the second visit to the research team to obtain data during a stressful period just before or in the middle of an examination period.

Data Analysis

All analyses were performed using SPSS software version 21 (Chicago, Illinois). Descriptive statistics were used to summarize demographic and study variables. Paired t tests were used for comparison of study variables under different stressful situations. Independent t tests were used to analyze sleep and anxiety according to salivary cortisol level. As time in bed, SE, and WASO did not follow normal distribution, Wilcoxon signed rank tests and Mann-Whitney U tests were used for statistics regarding those variables. The level of statistical significance was set at $P < .05$.

Results

Thirty-six people participated in the study, and 72 cases were analyzed. The demographic characteristics of the participants are shown in Table 1. The mean age was 21 years, and the mean weight was 55 kg. Nine (25.0%) were smokers, and 30 (83.3%) were occasional drinkers. Seventeen (47.2%) and 23 (63.9%) were on the luteal phase in normal condition and in stressful condition, respectively. There were no significant relationships among hormonal periods, AUC, and sleep parameters.

The perceived stress level reported in the stressful situation was 33.1, significantly higher than 25.1 in the nonstressful situation ($P < .001$; Table 2). However, other study variables including total cortisol level, sleep, and anxiety were not significantly different between the situations.

There were no correlations between anxiety, perceived stress, and sleep in the nonstressful situation (Supplemental Digital Content 1, available at http://links.lww.com/JNN/A138). However, higher anxiety was correlated with better SE ($P = .020$, $P = .026$), greater WASO ($P = .023$, $P = .027$), and wake episodes during sleep ($P = .048$, $P = .037$) in the stressful situation.

In the routine situation, the MWEs were significantly longer in participants with higher total cortisol levels during awakening response (AUC$_{AG}$; $P = .044$), whereas there were no significant differences between sleep and total cortisol levels during the day (AUC$_{TG}$; Supplemental Digital Content 2, available at http://links.lww.com/JNN/A139). In the stressful situation, SE was significantly poorer in the participants with less AUC$_{AG}$ ($P = .014$). WASO ($P = .008$) and MWEs ($P = .044$) were significantly greater in the participants with less AUC$_{AG}$. Trait anxiety was significantly higher in the participants with greater AUC$_{TG}$ ($P = .008$).

Discussion

This is the first study to explore the diurnal cortisol response and its relationship with sleep and anxiety in different stressful situations in female college students. In our study, cortisol AUC and anxiety tended to be greater in the stressful situation compared with the nonstressful situation, but this finding was not significant. There have been some controversies about the relationship between cortisol AUC and stress. McGregor et al[20] reported that the AUC of cortisol was slightly reduced during the period just before qualifying examinations at the end of academic year in university students, contrary to our finding. The discrepancy may come from the period of stress or emotional instability. It has been shown that chronic

TABLE 1. Correlation of Anxiety, Perceived Stress, and Sleep in the Different Stressful Conditions (N = 36)

	At Normal Condition, rho (p)			At Stressful Condition, rho (p)		
	Perceived Stress	State Anxiety	Trait Anxiety	Perceived Stress	State Anxiety	Trait Anxiety
Sleep duration, min	0.097 (.578)	0.162 (.353)	0.170 (.328)	0.228 (.187)	0.099 (.583)	−0.152 (.407)
Sleep efficiency, %	−0.116 (.505)	−0.021 (.904)	−0.083 (.636)	0.100 (.569)	0.404 (.020)	0.393 (.026)
WASO, min	0.150 (.390)	0.058 (.740)	0.122 (.485)	−0.028 (.873)	−0.394 (.023)	−0.391 (.027)
Wake episodes during sleep, times	0.134 (.444)	0.093 (.596)	0.094 (.593)	−0.013 (.943)	−0.347 (.048)	−0.370 (.037)
Mean wake episodes during sleep, min	0.096 (.583)	−0.041 (.815)	0.068 (.699)	−0.082 (.640)	−0.342 (.052)	−0.249 (.169)

stress response can show either a blunted cortisol level or a prolonged elevation without recovery to baseline in the afternoon.[21] Responses to a short-term stress test, on the other hand, have manifested as increased cortisol AUC.[22] Although there is no consensus on an exact distinction between acute and chronic stress in terms of duration of stress response, it is likely that a routine examination and perceived stress from it lasting for less than 1 month could be considered as an acute type of stress. Because our participants perceived midterm or final examinations as acute stress rather than chronic stress, they might have demonstrated slightly increased cortisol AUC in the stressful situation. In addition, the difference in baseline cortisol AUC levels should be noted. The average level of cortisol AUC throughout the entire day in the routine situation was about 2.0 μg/dL, much lower than the 8.95 μg/dL (89.46 ng/mL) in the study of McGregor et al.[20] This may indicate different cortisol responses in the stressful situation.

Interestingly, a higher anxiety level was significantly correlated with better SE and less disturbed sleep only in the stressful situation. This is contrary to previous findings that showed a significant association between higher anxiety and poor sleep in liver transplantation patients[23] and in patients with cancer.[24] A possible explanation for the difference is that our healthy participants with a higher anxiety level in the stressful situation modified their sleep behavior to adjust to the situation, whereas the participants in the previous studies might have failed to modify their sleep behavior. A higher level of perceived stress was accompanied by increased anxiety,[25] which induces adaptive responses such as sleep modification.[26] However, in the previous studies, the participants could not modify their sleep because of other factors leading to poor sleep such as symptoms of disease and hospitalization. Therefore, further research is needed to investigate stress, cortisol AUC, anxiety, and related behavioral responses in the population with illness.

Furthermore, poorer SE and greater disturbed sleep were associated with a lower level of cortisol AUC_{AG} under the stressful situation in the study, indicating that poorer sleep may lead to a less amount of morning cortisol secretion. This is concordant with a previous study.[27] It seems that a poorer quality of sleep at night can decrease morning cortisol secretion to attenuate the ability to fight against a stressful challenge.[28]

On the other hand, a higher level of cortisol AUC_{TG} was found in the participants with greater trait anxiety in the stressful situation, although AUC_{AG} had little relevance to any type of anxiety, consistent with a previous study.[29] It is likely that anxiety generated

TABLE 2. Anxiety, Perceived Stress, and Sleep by Total Salivary Cortisol Concentration

		Total Salivary Cortisol Concentration					
		AUC_{AG}			AUC_{TG}		
		Less (n = 19)	More (n = 17)	t or U	Less (n = 22)	More (n = 14)	t or U
At normal condition	Sleep duration, min	501.9 ± 261.42	411.7 ± 71.40	116.500[a]	463.2 ± 252.02	435.2 ± 77.78	111.500[a]
	Sleep efficiency, %	97.9 ± 2.67	96.7 ± 2.47	95.500[a]	97.4 ± 2.60	97.2 ± 2.89	128.500[a]
	WASO, min	9.3 ± 11.13	14.3 ± 11.80	106.000[a]	10.9 ± 10.60	12.7 ± 13.92	129.000[a]
	Mean wake episodes during sleep, min	1.6 ± 1.35	2.8 ± 2.05	−2.095[b]	2.3 ± 1.91	2.2 ± 1.82	0.046
	State anxiety	46.1 ± 10.39	42.8 ± 11.70	0.889	43.2 ± 10.47	46.1 ± 11.60	−0.750
	Trait anxiety	47.2 ± 10.21	42.0 ± 10.40	1.521	43.8 ± 10.15	45.2 ± 10.19	−0.409
	Perceived stress	26.2 ± 9.65	24.0 ± 10.78	0.635	26.0 ± 11.08	24.0 ± 9.37	0.528
At stressful condition	Sleep duration, min	413.1 ± 95.08	362.3 ± 74.99	97.000[a]	401.0 ± 88.65	373.2 ± 90.26	116.000[a]
	Sleep efficiency, %	95.6 ± 4.04	98.5 ± 1.18	78.000[a,b]	97.3 ± 2.55	96.4 ± 4.37	145.000[a]
	WASO, min	19.3 ± 18.86	5.6 ± 4.70	73.500[a,c]	11.6 ± 13.76	15.1 ± 18.55	141.000[a]
	Mean wake episodes during sleep, min	3.3 ± 2.72	1.8 ± 1.27	2.118[b]	2.7 ± 2.64	2.4 ± 1.67	0.379
	State anxiety	47.1 ± 10.30	47.9 ± 8.41	−0.248	46.0 ± 9.17	49.7 ± 9.65	−1.119
	Trait anxiety	45.4 ± 11.33	47.9 ± 9.57	−0.670	43.0 ± 9.63	52.7 ± 9.27	−2.810[c]
	Perceived stress	34.3 ± 10.74	31.6 ± 11.26	0.744	33.0 ± 9.90	33.1 ± 12.71	−0.026

Abbreviations: AUC_{AG} = area under the curve from the 3 morning cortisol samples within 1 hour after awakening; AUC_{TG} = area under the curve from the other cortisol samples; WASO = wake after sleep onset.
[a]Mann-Whitney U test. [b]$P < .05$. [c]$P < .01$.

from personal attributes is involved in a higher level of afternoon cortisol amount, which was associated with chronic stress.[21] Thus, female college students with a higher level of trait anxiety may be vulnerable to having chronic stress when they have increased perceived stress in the short term.

Our study has limitations. One limitation of this study is that our findings may not be generalized to all female college students because of the small convenience sample. Another limitation is that we used salivary cortisol data from a single day, although we collected the saliva samples from 6 time points per day and analyzed the cortisol level in the AUC as AUC is known to be stable.[11]

Conclusion

In summary, no significant difference was found in cortisol amount between the nonstressful and stressful situations, although perceived level of stress was significantly different. However, in the stressful situation, higher anxiety levels were significantly correlated with better SE and less disturbed sleep in the female college students. In addition, poorer sleep was associated with less cortisol AUC_{AG}. Increased cortisol AUC_{TG} was related to trait anxiety, not to state anxiety in the stressful situation.

The finding from this study suggests that female college students with increased anxiety in the stressful condition had better sleep; thus, nurses should consider that the slightly increased anxiety could be suitable to adjust to a stressful situation by improving sleep pattern when caring for those with increased stress levels.

In the stressful condition, female college students with poor sleep had attenuated morning cortisol secretion. Those with higher trait anxiety also had greater cortisol during the day. Because altered cortisol secretion is associated with health problems,[30] as well as with vulnerable stress responses, an assessment of cortisol level is needed for female college students with increased perceived stress especially when it is accompanied by sleep problems or an increased level of trait anxiety.

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