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# The association of salivary alpha-amylase, heart rate variability, and psychological stress on objectively measured sleep behaviors among college students

**Original article**

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**Abstract:** **Objective:** This study aimed to evaluate sleep behaviors among college students, to assess salivary alpha-amylase (sAA) and heart rate variability (HRV) in association with stress, and to investigate sleep-related factors including sAA, HRV, and stress among them.
**Methods:** Saliva samples for sAA assessment and HRV measurements in the supine position were taken between 3 PM and 6 PM. The level of prolonged psychological stress for the previous week was evaluated using the Korean version of the Global Assessment of Recent Stress (GARS-K), and sleep behaviors were assessed using an actigraphy device.
**Results:** A total of 86 healthy college students participated in this study. Sleep behaviors of the college students were not good, with 84% sleep efficiency (SE) and 62.7 min wake after sleep onset (WASO). The average sAA level was 65.8 U/mL in the participants. Although neither the sAA level nor HRV indices were significantly correlated with prolonged psychological stress, decreased normalized high frequency (nHF) on HRV was independently associated with a higher level of stress when adjusted for age and sex. Higher stress ($r$ = -0.276, $P$ = 0.011) and lower sAA ($r$ = 0.266, $P$ = 0.030) had significant correlations with shorter time in bed; however, it was sAA that was independently associated with time in bed (β = 0.244, $p$ = 0.044). Decreased nHF (β = 0.245, $P$ = 0.027) and higher body mass index (BMI) (β = -0.224, $P$ = 0.043) were independently related to and poorer SE.
**Conclusions:** Poor sleep behaviors were associated with decreased parasympathetic activity, a physiological change to psychological stress, rather than with psychological stress itself among college students. Thus, sAA and HRV should be considered as significant factors for impaired sleep behaviors in relation to psychological stress.

**Keywords:** *alpha-amylase • psychological stress • Autonomic nervous system • heart rate variability • sleep*

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## 1. Introduction

Recently, sleep disturbances are increasing among college students with approximately 48.9% of the college students having a sleep problem.[1] College students could be susceptible to use maladaptive sleep behaviors to adjust increased challenges in terms of personal responsibility, interpersonal relationships, and academic

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pressures. It has been reported that stress and sleep disturbances as important health issues among college students[2] as well as certain types of sleep disturbance such as delayed sleep phase occur more often in young adults than older adults.[3] Since sleep problem contributes to development of mental disorders,[4,5] a major health burden in young people aged 10–24 years,[6] it is needed to investigate sleep and its related factors in college students.

Several studies have shown that psychological stress involves sleep disturbances .[7,8] Although current understanding of the association between stress and sleep is based on the fact that both are subject to temporally and functionally aligned bioregulatory processes, most people experiencing stress do not develop sleep disturbances. In a previous study, individual differences were observed in vulnerability to stress-induced sleep,[9] which may be due to different physiological responses to psychological stress in each individual. Thus, it is important to ascertain the stress-related physiological factors contributing to the development of sleep disturbances. However, there are few such studies in the literature.

Since stress gives rise to the activation of the autonomic nervous system (ANS), heart rate variability (HRV) and salivary alpha-amylase (sAA) have been proposed as surrogate markers of physiological responses to stress. Although the association of HRV with stress is relatively well established,[10] sAA in assessing prolonged psychological stress is still controversial. sAA, the end-product of sympathoadrenal medullary (SAM) activity in the ANS, is one of the major enzymes secreted by the salivary glands under the influence of sympathetic stimuli, peaking 5–10 min after exposure to the acute stressor.[11] Juster et al.[12] reported that chronic stress was not associated with sAA[12]; however, a recent study addressed increased sAA levels in patients with chronic stress.[13] Therefore, it is necessary to evaluate changes in sAA according to the level of psychological stress for a prolonged period.

Furthermore, both sAA and HRV are characterized by 24-h oscillations closely linked with the sleep or sleep-wake cycle. sAA and some indices of HRV show diurnal variations; sAA is lowest at 30 min after awakening and rises steadily throughout the course of the day,[14] while HRV is lowest around noon and rises steadily until midnight.[15] Therefore, alterations in sAA and HRV may play a certain role in the relationship between stress and sleep. Recent studies have addressed the association between poor sleep and increased ANS activity,[16,17] but studies exploring the association among stress, ANS response, and sleep are lacking.

This study aimed to evaluate sleep behaviors among college students to assess sAA and HRV in association with stress and to investigate sleep-related factors including sAA, HRV, and stress among them.

# 2. Methods

## 2.1. Participants

The participants were healthy college students recruited via an online advertisement at a local university in South Korea. Participants diagnosed with any disease and current users of any medication were excluded. Prior to participation, the participants were asked to refrain from consuming any food or drink for 60 min, as well as drinking alcohol, taking any types of nicotine, and consuming caffeine for 12 h. The study needed 85 participants based on G*power 3.1.9 calculation[18] with a power of.80, a medium effect size of 0.15, and four independent variables for multiple linear regression analysis; a total of 86 participants were included.

## 2.2. Sleep behavior measurement

Sleep behaviors were assessed using an actigraphy watch, the Octagonal Motion logger Sleep Watch-L (Ambulatory Monitoring, Ardsley, NY, USA), which was placed on the participants' non-dominant wrist. It recorded activity on zero-crossing mode (ZCM) with a 1-mine epoch for 3–4 consecutive days. The actigraphy has previously shown strong correlations and agreements with polysomnography.[19]

The data recorded with the actigraphy watch were analyzed using Action W-2 software and the Cole-Kripke algorithm for the adult population, which distinguishes sleep and wakefulness with a high degree of accuracy and sensitivity.[20] Average values of time in bed, sleep minutes, wake after sleep onset (WASO), mean wake episodes (MWE), and sleep efficiency (SE) for 3–4 d were utilized as sleep parameters.

## 2.3. Saliva sampling

A Salimetrics oral swab (P/N 5001.02) was used to collect saliva samples. The participants were asked to avoid consuming a major meal or drinks, except water, for 60 min and to rinse their mouths thoroughly with water 10 min before taking a sample. They were instructed to place the absorbent cotton swab simultaneously in the sublingual, submandibular, and parotid salivary gland areas.[21] For accurate results, the absorbent cotton swab was completely saturated before removal. Upon removal, each absorbent cotton swab was sealed in a polypropylene carrier tube. Samples were taken between 3 PM and 6 PM to minimize bias from sAA diurnal variation[22] and immediately stored in a freezer and transferred to –70 °C within 24 h.

We requested the professional clinical laboratory, Green Cross Laboratories, to conduct an analysis of sAA. For analysis, the Salimetrics sAA assay kit (Catalog No. 1-1902, Salimetrics, PA, USA), specifically designed and validated for the kinetic measurement of sAA activity, was used according to the analysis protocol provided by the Salimetrics company.

## 2.4. HRV measurement

HRV was measured using a Heart Rhythm Scanner PE Limited Edition (Biocom Technologies, Poulsbo, WA, USA) in our quiet lab, at a comfortable temperature (22–24 °C), between 3 and 6 PM to minimize the effect of circadian changes in HRV.[23] Participants were instructed to refrain from caffeinated beverages, alcohol, and smoking in the 12 h prior to measurement. After 3-channel ECG electrodes were attached to the inner part of both wrists, the HRV was measured for 5 min in the resting supine position with free breathing after a 5-min stabilization period.[24]

The major indices of HRV, mean heart rate (HR), mean RR, the standard deviation (SD) of the normal RR intervals (SDNN), root mean square of the differences between adjacent RR intervals root mean square of the successive differences (RMSSD), normalized low frequency (nLF), normalized high frequency (nHF), and low frequency (LF)/high frequency (HF) ratio, were then obtained. The mean HR and mean RR indicate mean value and heartbeat interval mean value, respectively. SDNN is the index of the heart's response to changing workloads and was measured in milliseconds.[25] RMSSD is the primary time domain measure used to estimate the vagally mediated changes reflected in HRV.[26] nLF indicates the contribution of the LF in the total power, excluding the contribution of very low frequency (VLF), and reflects sympathetic activity dominantly. nHF indicates the contribution of the HF in the total power, excluding the contribution of VLF, and is mediated almost entirely by the parasympathetic nerve activity. The LF/HF ratio implies the balance between the sympathetic and parasympathetic nerve activities.

## 2.5. Psychological stress evaluation

The eight-item Korean version of the Global Assessment of Recent Stress (GARS-K) was used to evaluate the prolonged psychological stress during the previous week. Scoring of each item ranged from 0 (not stressful) to 9 (the most stressful), with a higher score indicating a higher level of stress.[27] The GARS-K has shown high internal consistency with Cronbach's $\alpha$ of.81 in the Korean population.[28]

## 2.6. Data collection procedure

This study was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committee of I university in South Korea (Approval No. 170228-1A). All the participants provided their written informed consent for participation in the study when they visited our research lab. First, general information including height and weight was obtained. Then, they completed the GARS-K, and their saliva samples were taken. After 5 min of rest, HRV was measured. Thereafter, the participants were equipped with an actigraphy watch; 3 d or 4 d later, they visited our research lab again to return it. The participants were given about $30 to participate in the study.

## 2.7. Statistical analysis

Analyses were performed using IBM SPSS Statistics 25 for Windows (IBM, Armonk, NY, USA), and data were reported as means with SDs or numbers with percentages for continuous and categorical variables, respectively. The normality of the data was assessed by skewness within ±2 and kurtosis within ±10. For variables that do not follow a normal distribution, log transformation was performed to permit approximation of a normal distribution of the data. For univariate analysis, Pearson's correlation was used to investigate relationships among prolonged psychological stress, sAA, HRV, and sleep behaviors. Multiple linear regression was used to determine the factors that were independently associated with sleep behaviors. Our multiple linear regression analysis included age, sex, and factors significant in univariate analysis. To test for multicollinearity, the variance inflation factor was assessed. When it was smaller than 10, multicollinearity was not considered to exist.[29]

# 3. Results

The general characteristics of the 86 participants and descriptive statistics of study variables are shown in Table 1. Their average age was 22.0 years, and 47 (54.7%) were males. The mean body mass index (BMI) was 22.3. Among 86, 42 (48.8%) spent less than 300,000 won for their monthly expenditures, and 78 (90.7%) were non-regular smokers and occasionally drank alcohol.

The average time in bed was 408.5 min, with actual sleep minutes being 325.9 min. WASO, WE, and MWE were 62.7 min, 23.1 times, and 4.5 min, respectively (Table 1). The mean SE was 84.0%. The average score for prolonged psychological stress was 26.1 out of 72

| Variables | Mean ± SD or *n* (%) |
|---|---|
| *Age (years)* | 22.0 ± 1.94 |
| *Sex* | |
| Male | 47 (54.7) |
| Female | 39 (45.3) |
| *BMI* | 22.3 ± 2.42 |
| *Monthly expenditure* | |
| <300 K won | 42 (48.8) |
| 300 K–490 K won | 28 (32.6) |
| >500 K won | 16 (18.6) |
| *Regular smoking* | |
| No | 78 (90.7) |
| Yes | 8 (9.3) |
| *Drinking alcohol* | |
| Everyday | 6 (7.0) |
| Occasionally | 78 (90.7) |
| None | 2 (2.3) |
| *Time in bed (minutes)* | 408.5 ± 81.99 |
| *Sleep minutes (minutes)* | 325.9 ± 77.36 |
| *WASO (minutes)* | 62.7 ± 30.82 |
| *WE (times)* | 23.1 ± 9.05 |
| *MWE (minutes)* | 4.5 ± 4.38 |
| *SE (%)* | 84.0 ± 7.22 |
| *Stress* | 26.1 ± 10.76 |
| *Salivary alpha amylase (U/ml)* | 65.8 ± 45.11 |
| *HRV indices at resting* | |
| Mean HR (beat per minute) | 71.3 ± 11.31 |
| Mean RR interval (millisecond) | 862.5 ± 135.35 |
| SDNN (millisecond) | 51.1 ± 22.0 |
| RMSSD (millisecond) | 45.9 ± 31.3 |
| nLF (%) | 54.6 ± 18.76 |
| nHF (%) | 45.4 ± 18.76 |
| LF/HF (a.u) | 1.8 ± 1.56 |

*Note*: BMI, body mass index; HF, high frequency; HR, heart rate; HRV, heart rate variability; LF, low frequency; MWE, mean wake episodes; nHF, normalized high frequency; nLF, normalized low frequency; RMSSD, root mean square of the successive differences; SDNN, standard deviation of normal RR intervals; SE, sleep efficiency; WASO, wake after sleep onset; WE, wake episodes.

**Table 1.** General characteristics and study variables in the participants ($N$ = 86).

points. The participants' average sAA level was 65.8 U/mL. For HRV indices, the mean HRs and mean RR interval were 71.3 beats/min and 862.5 ms, respectively. The mean SDNN was 51.1 ms, and RMSSD was 45.9 ms. The mean nLF and nHF were 54.6% and 45.4%, respectively. LF/HF was 1.8.

Prolonged psychological stress was not significantly correlated with the levels of sAA and HRV indices in univariate analysis (Table 2). However, prolonged

| Items | Stress | | | |
|---|---|---|---|---|
| | | Adjusted for age and sex | | |
| | r (*p*) | β | SE | *p* |
| nHF on HRV | −0.164 (0.137) | −0.224 | 0.179 | 0.034 |
| Salivary alpha amylase | −0.129 (0.288) | −0.113 | 0.554 | 0.366 |

*Note*: HRV, heart rate variability; nHF, normalized high frequency; SE, sleep efficiency.

**Table 2.** Stress and its association with salivary alpha amylase and HRV ($N$ = 86).

| Variables | Sleep behaviors, *r* (P) | |
|---|---|---|
| | Time in bed | SE |
| Age | 0.225 (0.041) | −0.004 (0.972) |
| Female sex | −0.099 (0.372) | 0.322 (0.003) |
| BMI | 0.121 (0.277) | −0.260 (0.018) |
| Monthly expenditure | 0.138 (0.215) | 0.192 (0.082) |
| Regular smoking | −0.144 (0.192) | −0.066 (0.555) |
| Drinking alcohol | 0.124 (0.269) | −0.083 (0.463) |
| Stress | −0.276 (0.011) | 0.034 (0.760) |
| Salivary alpha amylase | 0.266 (0.030) | 0.097 (0.435) |
| HRV indices at resting | | |
| Mean HR (beat/min) | −0.116 (0.301) | −0.131 (0.240) |
| Mean RR interval (millisecond) | 0.090 (0.420) | 0.118 (0.293) |
| SDNN (millisecond) | 0.061 (0.589) | 0.119 (0.288) |
| LnRMSSD (millisecond) | 0.018 (0.870) | 0.191 (0.086) |
| nLF (%) | −0.076 (0.500) | −0.316 (0.004) |
| nHF (%) | 0.076 (0.500) | 0.316 (0.004) |
| LF/HF (a.u) | −0.129 (0.249) | −0.227 (0.040) |

*Note*: BMI, body mass index; HF, high frequency; HR, heart rate; HRV, heart rate variability; LF, low frequency; nHF, normalized high frequency; nLF, normalized low frequency; RMSSD, root mean square of the successive differences; SD standard deviation; SE, sleep efficiency.

**Table 3.** Correlation among stress, salivary alpha amylase, HRV, and sleep behaviors ($N$ = 86).

psychological stress had a significant relationship with nHF on HRV when adjusted for age and sex (β = –0.224, $P$ = 0.034).

In univariate analysis for sleep behaviors, older age ($r$ = 0.225, $P$ = 0.041), lower stress ($r$ = –0.276, $P$ = 0.011), and higher sAA ($r$ = 0.266, $P$ = 0.030) were significantly correlated with longer time in bed (Table 3). There were no significant correlations between sleep and HRV indices. Increased nLF ($r$ = –0.316, $P$ = 0.004), decreased nHF ($r$ = 0.316, $P$ = 0.004), and increased LF/HF ($r$ = –0.227, $P$ = 0.040) on HRV were correlated with poorer SE. Poorer SE was also correlated with greater BMI ($r$ = –0.260, $P$ = 0.018) and female sex ($r$ = 0.322, $P$ = 0.003).

| Variables | B | SE | β | T | *P* | $R^2$ | *F* |
|---|---|---|---|---|---|---|---|
| *Time in bed* | | | | | 0.040 | 0.147 | 2.670 |
| Constant | 199.751 | 114.318 | | 1.747 | 0.086 | | |
| Age | 9.774 | 4.962 | 0.241 | 1.970 | 0.053 | | |
| Female sex | -1.588 | 19.762 | -0.010 | -0.080 | 0.936 | | |
| Stress | -1.066 | 0.929 | -0.138 | -1.147 | 0.256 | | |
| Salivary alpha amylase | 0.426 | 0.208 | 0.244 | 2.052 | 0.044 | | |
| *SE* | | | | | 0.001 | 0.207 | 5.035 |
| Constant | 83.475 | 10.911 | | 7.651 | 0.000 | | |
| Age | 0.453 | 0.396 | 0.123 | 1.141 | 0.257 | | |
| Female sex | 3.050 | 1.706 | 0.211 | 1.788 | 0.078 | | |
| BMI | -0.677 | 0.329 | -0.224 | -2.057 | 0.043 | | |
| nHF | 0.094 | 0.041 | 0.245 | 2.262 | 0.027 | | |

*Note*: BMI, body mass index; nHF, normalized high frequency; SE, sleep efficiency.

**Table 4.** Factors influencing time in bed and sleep efficiency.

In multiple regression analyses for sleep behaviors, higher sAA was independently associated with a longer time in bed ($\beta = 0.244$, $P = 0.044$), explaining time in bed variance of 14.7% (Table 4). Greater BMI ($\beta = -0.224$, $P = 0.043$) and decreased nHF ($\beta = 0.245$, $p = 0.027$) were independently associated with poorer SE. The explanatory power of the model was 20.7%. There was no multi-collinearity problem between the independent variables with all the variance inflation factor smaller than 10.

# 4. Discussion

We found that sleep behaviors of the subjects were not good, with 84% SE and 62.7 min WASO as SE more than 85% and WASO <20–30 min were considered as good sleep quality.[30] Moreover, our finding was slightly worse than that of the previous results with SE of 86–89% and WASO of 53–54 min in college students.[31,32] We can surmise that sleep behaviors of college students are getting poorer in recent days, which supports urgent necessity for sleep interventions in college students.

In our study, the average level of sAA measured in the late afternoon was 65.8 U/mL, comparable to 94–100 U/mL measured in the evening from a previous study.[33] However, sAA was not significantly correlated with prolonged psychological stress level. There have been contrary findings on the relationship between stress and sAA. Although some researchers posit that sAA could serve as a feasible biomarker of stress[34,35], others report no significant association between stress and sAA.[36,37] Given that the former researchers observed sAA during acute stress, such as tooth extraction and the Trier Social Stress Test, sAA is likely to be more sensitive to acute stress. Second, we cannot help regarding the involvement of sex differences in the association between sAA and prolonged stress. Austin et al. [36] found a positive correlation between perceived stress and sAA in males but a negative correlation in females[36]; in our further analysis (data not shown), we found a similar pattern. Wingenfeld et al.[37] pointed out sex differences in sAA and stress, with only female nurses having significantly increased sAA.[37] Therefore, further study with a larger sample is needed to elucidate sex differences related to sAA and stress. Lastly, sAA may be affected by other factors such as level of stress or coping style. Klaus et al. [38] reported that sAA response was different under a higher stressful condition as compared under a lower stressful condition.[38] Thus, the low level of psychological stress in our participants might have attenuated the relationship between sAA and stress. Since another recent study has suggested that sAA was increased in a group with use of less effective emotion regulation strategy,[39] further studies are needed to investigate stress and sAA along with stress coping style.

Higher psychological stress and lower sAA were significantly correlated with shorter time in bed in our univariate analysis. This finding may indicate that our healthy students with higher psychological stress level and lower sAA shorten their time in bed to take more efficient sleep to adjust to stressful situations, considering shorter time in bed was inclined to be associated with better sleep efficient in our data. On the other hand, it was not psychological stress but higher level of sAA that was independently associated with longer time in bed in the regression analysis. This is in line with a prior study which suggested that increased sAA might represent a measure of increased sleep pressure.[40] Participants with higher sAA levels appear spend longer time in bed, seeking more time for sleep.

On HRV in our study, nLF and nHF were 54.6% and 45.4%, respectively. These were also similar to 41–60% and 45–58% in the previous study among Korean nursing students.[41] Although psychological stress was not significantly correlated with HRV indices at all in the univariate analysis, higher psychological stress was independently associated with decreased nHF when adjusted for age and sex in the regression analysis, which is consistent with a prior finding.[42] Furthermore, lower nHF was independently related to poorer SE even after adjusting age, sex, and BMI; this is also consistent with previous findings suggesting the importance of parasympathetic inhibitory control for sound sleep.[43,44] Therefore, poor sleep may be associated with decreased HF, a physiological change to psychological stress, rather than with psychological stress itself among college students.

Interestingly, greater BMI was independently associated with poorer SE in the multivariate analysis. This is in line with previous studies addressing significant relationship between obesity and sleep quality.[45,46] The association might be due to sleep disordered breathing not only related to obesity[47] but also responsible for worse sleep quality.[48] However, a recent meta-analysis addressed the cause and effect relation between BMI and sleep still remains equivocal.[49] Given that both increased BMI and poor sleep lead to unfavorable health outcomes such as metabolic and cardiovascular diseases,[50,51] healthcare personnel should pay more attention to college students with increased BMI and poor sleep.

Our study has some limitations. First, it may not be possible to generalize our findings because of the small sample recruited through convenience sampling, although the sample size was statistically sufficient. Future studies are needed to investigate sAA and stress regarding sex and coping styles in a larger population with a higher stress level. Second, we could not measure salivary flow, which may influence sAA. However, our findings are assumed to be somewhat acceptable since much variation was not found in the saliva flow rates of similarly aged populations.[52] Third, our participants wore the actigraph for 3–4 d in the study although it is recommended to be worn for 7 d for sleep measurement.[53] Finally, sAA and HRV were measured only once during the daytime and not measured during the nighttime sleep. Ecological momentary assessment (EMA) studies collecting data repeatedly across multiple measurement time points are recommended for further research.

## 5. Conclusions

In our study, sleep behaviors of the college students were not good, with 84% SE and 62.7 min WASO. Higher psychological stress was not independently associated with sAA but with decreased nHF when adjusted for age and sex. Decreased nHF and increased BMI were independently related to poorer SE. It was higher sAA that was independently associated with longer time in bed.

These findings indicate that poor sleep may be associated with decreased parasympathetic activity, physiological change to psychological stress, rather than with psychological stress itself among college students. Although sAA was not related to prolonged psychological stress, higher sAA was associated with longer time in bed. Thus, sAA and HRV should be considered as significant factors for impaired sleep behavior in relation to psychological stress. In addition, our findings provide further insight into the relationship among parasympathetic activity, prolonged psychological stress, and sleep behaviors. In the future, a research study is needed to investigate sAA and psychological stress regarding sex difference and coping styles in a larger population. Further research studies with multiple measurement methods such as EMA would help deepening the understanding of relationships among autonomic functions, psychological stress, and sleep behaviors.

## Ethical approval

Ethical issues are not involved in this paper.

## Conflicts of interest

There are no conflicts of interest.

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