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# Evaluation of effects of day time napping in college students using salivary endocrinological stress marker Cortisol

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### Abstract

Chronic stress is associated with many disorders such as hypertension, depression, immune suppression, diabetes, and obesity. College students are exposed to constant psychological stress. This research is focused on evaluation of the stress relief effect of day time napping by measuring the levels of sensitive salivary endocrinological stress marker cortisol. Students were divided into three groups according to their napping habits. The first group included students who continued with the usual daily routine, the second group consisted of students who do not take daily naps but were asked to introduce every day nap into their schedule, the third group consisted of students who take daily naps but were asked to remove them from their routine. Saliva samples were collected at the beginning of the experiment and two weeks later. Salivary cortisol levels were evaluated by High Sensitivity Salivary Cortisol Enzyme Immunoassay. There was no statistically significant difference in cortisol concentration in the first two groups. There was a statistically significant decrease of the cortisol level in the third group. These findings suggest that daily napping does not relieve stress, in fact the removal of daily napping from a daily schedule of habitual nappers does significantly lower their stress levels.

**Keywords:** Stress, Daily Napping, Cortisol

### Introduction

College students are exposed to constant psychological stress which has its source in being away from home, high costs of college, working at a job during the school year, having a heavy academic workload, pressure to obtain high grades in connection with career aspirations, and involvement in different scholar activities [1]. College student-athletes are exposed to additional stress from time management between their sport and academic schedules, team peer pressure, trying to retain possession of their scholarship, and missing classes due to traveling. Chronic stress affects the cardiovascular, immune [2], nervous and endocrine [3] systems and may lead, among others, to diabetes [4], hypertension, depression [5], substance abuse, and antisocial personality disorder [6]. Physiological marker of stress, cortisol provides an objective measure of changes in the stress response. Cortisol is the main glucocorticoid hormone in humans and is most commonly associated with stress in the human body. It is released in response to many psychosocial stimuli processed in the hypothalamic-pituitary-adrenal axis. The level of free cortisol in the blood is accurately reflected by the level of cortisol in saliva [7, 8]. Cortisol is secreted more slowly than other adrenal hormones such as

catecholamines (like adrenaline) and remains in the body longer after initial stress. Cortisol is beneficial in times of stress because of its many regulatory functions. It aids in breaking down of glucose and fatty acids for energy, it has anti-inflammatory activity, is involved in fat storage, and immune functioning. However, high and prolonged levels of cortisol have been shown to have negative effects leading to serious medical conditions including diabetes, hypertension, depression, cancer, obesity, improper thyroid functioning, and decreased bone density. Cortisol is well known for its role in storing of abdominal fat and its association with heart attacks, strokes, increased susceptibility to autoimmune disease and infection [9]. Cortisol levels vary with the circadian cycles, peaking during the first hour after awakening [10] and decreasing for the rest of the day. Thus, careful choosing of the testing time is crucial as cortisol levels are dependent on the time of day. Repeated measurement of free cortisol levels within 60 minutes after awakening in the morning is considered a reliable biological marker of adrenocortical activity [10]. Cortisol can be measured in urine, plasma, and saliva. Salivary measures of cortisol are considered valid and reliable and provide distinct advantages including their non-invasive nature [11]. Nonetheless, saliva samples can be affected, among others, by food and caffeine consumption, smoking, and timing of collection, so the protocol compliance is crucial to obtaining valid data. Increasing the validity of the results includes the standardized saliva sampling, consistent collection materials and methods, controlling the effects of food, drinks, and medications [12]. Substantial research has been conducted on methods of lowering stress levels. Suggested methods include exercise (aerobics, yoga, strength training), meditation, listening to music, healthy diet, as well as avoiding alcohol, caffeine, and tobacco. Proper sleep is also known to lower stress. Sleep is a very important aspect to living a healthy life. On average, the human adult needs about 7-8 hours of sleep to restore vital systems in the body. Many college students suffer from sleep deprivation [13] and put themselves at risk for lower grade point averages, increased risk of academic failure, compromised learning, impaired mood, and increased risk of motor vehicle accidents [13]. According to the National Sleep Foundation, short 20-30 minute naps can help improve an individual's mood, awareness, and performance. There are three different types of napping. Planned napping involves the individual taking a nap before they become tired or when they are aware they will stay up later than usual. Emergency napping happens when an individual becomes tired and is struggling to stay awake to complete a task. The last one is habitual napping, which is when an

individual takes a nap at the same time every day. Studies by Mednick [14] showed beneficial effects of napping on enhancing memory and improvement of ability to learn new things. Taub [15] found that taking habitual naps produced immediate positive effects on college student's mood and performance. To our knowledge there are no studies pertaining to the effects of napping on stress in college students. In the present study, we investigated the endocrinological effects of daily napping by measuring the sensitive salivary stress marker cortisol in three groups of college students with different napping habits.

## Material and Methods

For this study, approved by Institutional Review Board, 60 Rogers State University students, 18 to 25 years old were recruited. Participant exclusions included: habitual tobacco use, caffeine dependency, drug use, diagnosed psychological disorders such as depression, anxiety, psychosis, alcohol dependency, endocrine metabolic disorders, autoimmune disorders, severe allergies, major medical conditions. A written informed consent was taken from each participant. Students were assigned to one of the three 20 participant groups, according to their sleeping habits. Group A consisted of students who normally do not take naps but were asked to take naps for two weeks. Group B included students who continued with their normal daily schedule throughout the experiment and served as a control group. Group C consisted of students who normally take daily naps but were asked to remove them from their daily schedule for two weeks. In each of the three groups, half of the participants were student-athletes, and half were non-athletes. Two saliva samples were obtained from each participant, one at the beginning of the experiment and one two weeks later. Saliva samples were collected from all participants at the

same time of the day, within 60 minutes after awakening to minimize the effects of circadian variation. Participants were also asked to wash their mouth before saliva collection and approximately 1 mL of unstimulated saliva was collected in a disposable plastic test tube and immediately stored at  $-20^{\circ}\text{C}$ . All samples were analyzed for the levels of cortisol with Expanded Range High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit [16]. Cortisol concentrations were determined using a 4-Parameter Sigmoid Minus Curve Fit program from MyAssays.com. Subjective stress was also elicited using a 10-point analogue scale in pre and post questionnaires, with 1 marked as "No stress at all" and 10 "the worst stress I can imagine". All data were normally distributed and a single factor ANOVA was performed to detect intergroup differences. Values were considered to be statistically significantly different when  $p < 0.05$ . Student's t-tests were computed for comparison of the means within groups to detect the effects of napping on cortisol levels. The same effect was also studied in subgroups of athletes and non-athletes.

## Results

### Perceived stress questionnaire

In the pre-questionnaire, participants were asked to rate their stress level on a scale from 1-10. Stress was considered high for a number greater than five, low for a number less than five, and moderate for the number five. Out of the 60 participants, 38 were considered to have high stress levels. 9 of these participants were in Group A and after taking naps for two weeks, they stated in their post-questionnaire that napping lowered their stress levels. 12 of the students with high stress levels were in Group C. After removing naps from their daily schedule for two weeks, 6 of them showed higher scores in perceived stress test.

### Salivary cortisol analysis

	Group A	Group B	Group C
Cortisol ( $\mu\text{g}/\text{dL}$ ) Pre-experiment	0.52 $\pm$ 0.34	0.34 $\pm$ 0.22	0.46 $\pm$ 0.32
Cortisol ( $\mu\text{g}/\text{dL}$ ) post experiment	0.47 $\pm$ 0.28	0.32 $\pm$ 0.21	0.33 $\pm$ 0.21

Note: Data shown are means and standard deviations

Table 1. Salivary cortisol average concentrations ( $\mu\text{g}/\text{dL}$ ) for Groups A, B and C in pre and post experiment samples.

Table 1 shows mean concentrations of cortisol in three groups studied in pre and post experiment samples. ANOVA analysis showed no statistically significant difference ( $p=0.13$ ) in cortisol concentrations between groups in pre-experiment samples. Group B included participants who did not make changes to their daily routine and was used as a control to measure whether other than studied factors in the environment of the student population at Rogers State University could cause increase or decrease in the cortisol concentrations. No

statistically significant difference ( $p=0.33$ ) in cortisol concentration was observed in this group. In Group A, introducing daily napping into students schedule for two weeks cause the decrease in cortisol concentration from 0.52 to 0.47  $\mu\text{g}/\text{dL}$ . This difference however, is not statistically significant ( $p=0.17$ ). Surprisingly, a statistically significant decrease in cortisol concentration from 0.46 to 0.33  $\mu\text{g}/\text{dL}$  ( $p=0.004$ ) was observed in Group C indicating that removing daily naps from the routine of habitual nappers lowers their stress levels.

	Group A (athletes)	Group B (athletes)	Group C (athletes)
Cortisol ( $\mu\text{g}/\text{dL}$ ) Pre-experiment	0.58 $\pm$ 0.32	0.32 $\pm$ 0.19	0.44 $\pm$ 0.31
Cortisol ( $\mu\text{g}/\text{dL}$ ) post experiment	0.48 $\pm$ 0.21	0.29 $\pm$ 0.19	0.31 $\pm$ 0.17

Note: Data shown are means and standard deviations

Table 2. Salivary cortisol average concentrations ( $\mu\text{g}/\text{dL}$ ) for Groups A, B and C in pre and post experiment samples for athletes.

ANOVA analysis showed no statistical difference in cortisol concentrations in pre-experiment samples between all three groups. Even though the cortisol concentrations decrease in all three groups

in the post experiment samples, this decrease is not statistically significant ( $p > 0.05$ ).

	Group A (nonathletes)	Group B (nonathletes)	Group C (nonathletes)
Cortisol ( $\mu\text{g/dL}$ ) Pre-experiment	0.47 $\pm$ 0.36	0.35 $\pm$ 0.26	0.49 $\pm$ 0.35
Cortisol ( $\mu\text{g/dL}$ ) post experiment	0.46 $\pm$ 0.34	0.34 $\pm$ 0.22	0.34 $\pm$ 0.26
Note: Data shown are means and standard deviations			

Table 3. Salivary cortisol average concentrations ( $\mu\text{g/dL}$ ) for Groups A, B and C in pre and post experiment samples for nonathletes.

ANOVA analysis showed no statistical difference in cortisol concentrations in pre-experiment samples between all three groups of nonathletes. No statistically significantly different changes in concentrations of cortisol were observed in Groups A or B. In Group C, a statistically significant ( $p=0.018$ ) decrease of cortisol concentration from 0.49 to 0.34  $\mu\text{g/dL}$  was observed.

### Discussion

The purpose of the present study was to examine the effects of daily napping on stress levels in college students measured by a biomarker of stress, cortisol. Chronic stress rather than acute stress usually leads to a number of pathologies. Individual's subjective, perceived stress tests often provide inconsistent results and heavily depend on subject's mood and attitude at the time of testing. Cortisol concentration measurements provide a more objective method in stress evaluation. In this study it was hypothesized that introducing daily naps to student's daily routines will cause a decrease in salivary cortisol concentrations which would be indicative of stress reduction effect. The findings did not support this hypothesis. Salivary cortisol levels did not change significantly in response to introducing daily naps to schedules of the participants indicating that napping does not relieve stress in college students. Interestingly, removing naps from the daily routine of habitual nappers statistically significantly lowers the salivary cortisol concentrations indicating that daily naps are actually adding to the overall stress levels of students. This effect most likely comes from the fact, that removal of daily naps gives students more time during the day to accomplish more tasks which in turn makes them more relaxed, gives them the feeling of accomplishment and lowers the overall stress level. This conclusion is supported by the post-questionnaire, where the participants stated they felt they got a lot more work done having this extra time they would otherwise use for naps. The group of students where this effect was observed consisted of athletes and nonathletes. Further analysis of the two subgroups showed a statistically significant effect of cortisol concentration decrease in group of nonathletes but not in the group of athletes. Our previous studies [17] indicated that cortisol levels in college athletes are statistically significantly lower than nonathletes. Nap removal effect on cortisol levels may be overwhelmed by benefits of exercise in the athlete group. In conclusion, introduction of daily napping routine shows no effect on cortisol concentrations in saliva, so napping may not be a beneficial alternative for relieving stress. Some of the limitations of this study include a small sample size of 20 individuals in each group, and only 10 athletes and 10 nonathletes. Larger groups of participants should be studied in the future and the time of the experiment should be expanded to observe long term effects of napping. This study was also limited by the time of day of sample collections. There are typically individual differences in the diurnal cycle of cortisol. Even though all samples were collected at the same time of the day for all participants, individual differences were not controlled. These findings should be replicated in future studies before being generalized.

**Conflicts of Interest:** The authors declare no conflicts of interest.

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