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The Effect of Increased Sleep on the Circadian Rhythm of Salivary

Cortisol Concentrations

By

Mariah Jacqueline Scott

Submitted in partial fulfillment of the requirement for the degree of Master of Science in Microbiology from the Department of Biological Science of Seton Hall University October, 2016 © 2016 Mariah Jacqueline Scott

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Abstract

Cortisol is a salivary marker for the hypothalamic-pituitary-adrenal axis (HPA) component of the stress response. The activity of the HPA demonstrates a circadian rhythm. It is well known that sleep deprivation increases cortisol concentrations. In this study, we looked at the effect of an increase of one-hour sleep for one month on the circadian rhythm of the HPA.

Eight college subjects (n=8) collected saliva during their normal sleep wake cycle every 4 hours for 24 hours. Saliva collections were repeated after a month of increase of sleep by 1-hour. The subjects also completed demographic forms that asked for age, sex, time of last meal, smoker, caffeine consumption, time going to sleep, and time waking up. ELISA kits were used to analyze cortisol concentrations.

Salivary cortisol concentrations demonstrated a circadian rhythm, which revealed a significant peak at 0800h, which is consistent with the literature. The study shows that the total concentrations of salivary cortisol secreted over 24 hours as calculated by area under the curve (AUC) reveals that there is a significant decrease with increased sleep.

1. Introduction

1.1 Cortisol as a marker for the hypothalamic-pituitary-adrenal axis

Cortisol has been studied as a salivary marker for the hypothalamic-pituitaryadrenal axis (HPA) component of the stress response. The anatomic substrate of the stress response consists of the HPA and the sympathetic branch of the autonomic nervous system. The stress response stimulates the release of corticotropin releasing hormone (CRH) from the hypothalamus that travels through the hypothalamic-hypophyseal portal vessels to reach the anterior pituitary gland and elicit the release of adrenocorticotropic hormone (ACTH). Within minutes, ACTH stimulates glucocorticosteriods such as cortisol, to be secreted from the adrenal cortex (Sapolsky et al., 2013). Figure 1 describes the HPA.



Figure 1. Beginning with nonspecific high brain centers, signals will increase the release of CRH from the hypothalamus. CRH will stimulate the release of ACTH from the anterior pituitary gland into the bloodstream that will elicit the release of cortisol from the adrenal cortex. Cortisol, a glucocorticosteriod, will circulate in the blood that will cause negative feedback inhibition upon the hypothalamus and pituitary to prevent release of CRH and ACTH respectively.

1.2 Circadian Rhythm of the HPA

The biological clock is within the suprachiasmatic nucleus of the hypothalamus that will elicit a circadian pattern for cortisol concentrations. Previous studies have shown the HPA peaks at 0800h (Krieger et al., 1971; Kudielka et al., 2004).

1.3 The effect of sleep on cortisol

Alteration of the HPA axis can cause physiological and physical malfunctions. These malfunctions include physiological changes from quick frustration to serious depression (Klitzing et al., 2012). A well-studied factor that affects cortisol is sleep deprivation. Sleep deprivation is an acute stressor defined by prolonged wakefulness for more than 12 hours (Maggio et al., 2013). Chronic sleep deprivation will cause an imbalance between anabolic and catabolic hormones mediated by melatonin (Maggio et al., 2013). A decrease in anabolic hormones such as testosterone and GH and an increase in catabolic hormones such as cortisol, ACTH, TSH and thyroid hormones, epinephrine and norepinephrine have been reported (Maggio et al., 2013).

Melatonin is synthesized from serotonin in the pineal gland. This synthesis is controlled by light within the SCN of the hypothalamus (Dubocovich et al., 1997). Hence, melatonin plays a role in the regulation of circadian rhythms due to its being a signal of darkness where melatonin levels increase during darkness (Maggio et al., 2013). Previous studies have shown that sleep has inhibitory effects on cortisol concentrations. Cortisol concentrations are decreased during sleep (Weitzman et al., 1983).

1.4 The effect of sleep on cognition

Studies have shown that total sleep deprivation has caused deficits in simple memory and short-term memory (Lim and Dinges, 2010). A recognized hypothesis called trace reactivation or replay hypothesis states that sleep restores memory by reactivating neuronal activity and transfer of information from temporary hippocampus-dependent storage to long-term hippocampus- independent neocortical storage (Hoffman and McNaughton, 2002). In one study of young adults, almost being awake for 24 hours after routine work or school schedules, visual analog scales (VAS), were significantly lower on concentration and speed of thought correlated with altered cortisol concentrations and hippocampal activation (Klumpers et al., 2015).

1.5 Effect of increased cortisol on cognition

Increased cortisol will affect cognitive abilities and mood changes (Skosnik et al., 2000). In the literature, there have been many longitudinal studies that demonstrate lower cognitive abilities based on irregular high cortisol concentrations. This is due to increased cortisol in the hippocampus being detrimental to memory (Lupien et al., 2005). Studies have shown that a minimum of alteration by 2 hours a day in sleep duration can affect cognition (Devore et al., 2014). Additional studies demonstrated college student cohorts, who show eveningness circadian patterns, have altered subjective alertness, increase of hormonal secretion (Gulec et al., 2013) and have measures of low scores of mental health (Simor et al., 2015). A subject that demonstrates an eveningness sleep/wake cycle tends to wake up and go to bed at a later time in the evening. Thus, eveningness overestimate their sleep needs therefore, they will makes up for lost sleep in the weekends (Taillard et

al., 1999). In addition, sleep duration has a direct effect on cognitive function. High levels of cortisol from eveningness sleep cycle showed a delay in three cognitive tasks: verbal memory, reaction time, and letter search speed (Gaysina et al., 2014). Therefore, cortisol has been shown to be responsible for altered cognition from altered sleep cycles. *1.6 Rationale for the Study*

In this study, we evaluated salivary cortisol concentrations in subjects during a normal sleep wake cycle and then after a month of increasing sleep by one hour each day. We hypothesize that a one-hour difference of increase sleep should decrease cortisol concentrations.

2. Materials and Methods

2.1 Subjects

Eight subjects (n=8) were between 18 and 21 years (5 males and 3 females). Written consent forms were obtained from participating students. Samples were collected from volunteer subjects anonymously after a consent form had been read and any questions pertaining to the study were answered. Within the consent form, subjects were reminded that their participation could stop at any time during the study. Demographic forms were given asking for age, sex, time of last meal, smoker, caffeine consumption, time went to sleep, and time woke up. These forms were given before and after the 4-week period. All of this was performed in accordance with the proposal approved by the Institutional Review Board (IRB) from Seton Hall University (SHU).

2.2 Saliva Collection

Saliva was collected in 15 mL polypropylene centrifuge tubes. Samples were quick frozen in dry ice after each collection and then stored at -70 ° C until assayed. Samples were centrifuged prior to assay (3000 rpm for 15 min at 4° C).

2.3 Biochemical analysis

Saliva samples were assayed by ELISA kits obtained from Salimetrics LLC (State College, PA) for cortisol (cat. # 1-3002). The protocol was followed from the Salimetrics Cortisol ELISA kit. The limit of sensitivity of the cortisol assays is 0.012 μ g/dL. Intrassay CV% for cortisol was 1.18% and interassay CV% was 10.0% respectively.

2.4 Statistical analysis

Paired t test (non-parametric) comparison results from area under the curve data of total n (n=8) subjects was performed. Repeated measures ANOVA (rmANOVA) followed by Tukey-Kramer using GraphPad Prism 6 (2014) was performed (Stewart, J., 2000).

3. Results

Figure 2A. Salivary cortisol concentrations for normal and increased sleep for subject 1. Cortisol concentrations were taken at the following time points: 2400h, 0400h, 0800h, 1200h, 1600h, 2000h.

Figure 2B. Total salivary cortisol concentrations as calculated by area under the curve (AUC) for normal and increased sleep for subject 1.

Figure 3A. Salivary cortisol concentrations for normal and increased sleep for subject 2. Cortisol concentrations were taken at the following time points: 2400h, 0400h, 0800h, 1200h, 1600h, 2000h.

Figure 3B. Total salivary cortisol concentrations area under the curve (AUC) for normal and increased sleep from subject 2.

Figure 4A. Salivary cortisol concentrations for normal and increased sleep for subject 3. Cortisol concentrations were taken at the following time points: 2400h, 0400h, 0800h, 1200h, 1600h, 2000h.

Figure 4B. Total salivary cortisol concentrations area under the curve (AUC) for normal and increased sleep from subject 3.

Figure 5A. Salivary cortisol concentrations for normal and increased sleep for subject 4. Cortisol concentrations were taken at the following time points: 2400h, 0400h, 0800h, 1200h, 1600h, 2000h.

Figure 5B. Total salivary cortisol concentrations area under the curve (AUC) for normal and increased sleep from subject 4.

Figure 6A. Salivary cortisol concentrations for normal and increased sleep for subject 5. Cortisol concentrations were taken at the following time points: 2400h, 0400h, 0800h, 1200h, 1600h, 2000h.

Figure 6B. Total salivary cortisol concentrations area under the curve (AUC) for normal and increased sleep from subject 5.

Figure 7A. Salivary cortisol concentrations for normal and increased sleep for subject 6. Cortisol concentrations were taken at the following time points: 2400h, 0400h, 0800h, 1200h, 1600h, 2000h.

Figure 7B. Total salivary cortisol concentrations area under the curve (AUC) for normal and increased sleep from subject 6.

Figure 8A. Salivary cortisol concentrations for normal and increased sleep for subject 7. Cortisol concentrations were taken at the following time points: 2400h, 0400h, 0800h, 1200h, 1600h, 2000h.

Figure 8B. Total salivary cortisol concentrations area under the curve (AUC) for normal and increased sleep from subject 7.

Figure 9A. Salivary cortisol concentrations for normal and increased sleep for subject 8. Cortisol concentrations were taken at the following time points: 2400h, 0400h, 0800h, 1200h, 1600h, 2000h.

Figure 9B. Total salivary cortisol concentrations area under the curve (AUC) for normal and increased sleep from subject 8.

Cortisol	Time of day**	[F (5,35) = 9.571, p<0.0001]
Cortisol	Treatment	[F(1,7) = 3.886, p = 0.0893]
Cortisol	Time of day x treatment	[F(5,35) = 0.8191, p = 0.5444]

Figure 10A. Mean cortisol concentrations for normal and increased sleep for all 8 subjects.

Figure 10B. Total salivary cortisol concentrations area as calculated by area under the curve (AUC) for normal and increased sleep for all 8 subjects.

3.1 Subject 1

As it can be seen in Figure 2A, salivary cortisol concentrations peaked at 0800h in both the normal and increased sleep samples for this male subject. There was a decrease in total salivary cortisol concentrations as calculated by area under the curve (AUC) secreted over a 24-hour period following an increase in sleep (Figure 2B).

3.2 Subject 2

As it can be seen in Figure 3A, salivary cortisol concentrations peaked at 0800h in both the normal and increased sleep samples for this female subject. There was a decrease in total salivary cortisol concentrations as calculated by area under the curve (AUC) secreted over a 24-hour period following an increase in sleep (Figure 3B).

3.3 Subject 3

As it can be seen in Figure 4A, salivary cortisol concentrations peaked at 0800h in both the normal and increased sleep samples for this female subject. There was a decrease in total salivary cortisol concentrations as calculated by area under the curve (AUC) secreted over a 24-hour period following an increase in sleep (Figure 4B).

3.4 Subject 4

As it can be seen in Figure 5A, salivary cortisol concentrations peaked at 0800h in both the normal and increased sleep samples for this male subject. There was a decrease in total salivary cortisol concentrations as calculated by area under the curve (AUC) secreted over a 24-hour period following an increase in sleep (Figure 5B).

3.5 Subject 5

As it can be seen in Figure 6A, salivary cortisol concentrations peaked at 0800h in both the normal and increased sleep samples for this male subject. There was a decrease in total salivary cortisol concentrations as calculated by area under the curve (AUC) secreted over a 24-hour period following an increase in sleep (Figure 6B).

3.6 Subject 6

As it can be seen in Figure 7A, salivary cortisol concentrations peaked at 1200h in both the normal and increased sleep samples for this male subject. There was a decrease in total salivary cortisol concentrations as calculated by area under the curve (AUC) secreted over a 24-hour period following an increase in sleep (Figure 7B).

3.7 Subject 7

As it can be seen in Figure 8A, during a normal sleep wake cycle the salivary cortisol concentrations peaked at 0400h as compared to after an increase in sleep where it peaked at 1200h for this female subject. There was a decrease in total salivary cortisol concentrations as calculated by area under the curve (AUC) secreted over a 24-hour period following an increase in sleep (Figure 8B).

3.8 Subject 8

As it can be seen in Figure 9A, salivary cortisol concentrations peaked at 0400h and 1200h in the normal circadian samples and 1200h in the increased sleep samples for this male subject. There was an increase in total salivary cortisol concentrations as calculated by area under the curve (AUC) secreted over a 24-hour period following an increase in sleep (Figure 9B).

3.9 Salivary cortisol concentration comparison

As can be seen in Figure 10A, mean salivary cortisol concentrations peaked at 0800h for both normal and increased sleep samples. Using rmANOVA, there was a significant main effect of time of day for s-cortisol, [F (5,35)=9.571, p=0.0001]. The Tukey-Kramer Multiple Comparison Test reports significant differences for the means for s-cortisol between the peak at 0800h and 0400h, 1600h, 2000h and 2400h. There was also a significant difference between 1200h and 2400h.

A non-parametric paired T test was performed for the area under the curve data with the normal circadian and the increased sleep cortisol samples from all subjects because the normal circadian data did not indicate a Gaussian distribution. The results revealed that there is a statistical difference between both groups (p=0.0184) and shows a clear decrease in increased sleep samples (Figure 10B).

4. Discussion

In this study, we examined the effect of increasing the amount of time slept by one hour for 4 weeks on the circadian rhythm of salivary concentrations of cortisol. Salivary cortisol concentrations demonstrated a circadian rhythm, which revealed a significant peak at 0800h, which is consistent with the literature (Morgan et al., 2004; Brown et al., 2008; Hucklebridge et al., 2005) and prior data from our laboratory (Ventre, 2015). The total area under the curve (AUC) of salivary cortisol secreted over 24 hours reveals that there is a significant decrease with increased sleep. Thus far, subjects with a pattern of cortisol peaking at 0800h within a normal circadian have demonstrated a reduction in the amount of total cortisol concentration with an increase of sleep. Our results in a college age population are consistent with decreased cortisol secretion in older adults (Rueggeberg et al., 2012). However, this study did not perform a complete circadian pattern.

Possible confounding variables for this study would include psychological or physical stress, time of going to bed (for example night owls), consuming food or drinking before collecting saliva sample. Therefore, efforts were made to obviate confounding variables. Cortisol secretion is elevated following physiological or physical stress peaking at 20 minutes (Singh et al., 1999). During this study, it was important to verify with subjects that saliva would not be collected before or after any administered exam. We tried not to collect samples during the week of an exam. It should be noted from studies in our laboratory that anticipating taking an exam or actually taking an exam did not trigger an elevation in salivary cortisol or amylase (Smith, 2008, Alfano, 2008, Ventre, 2015). Information on the time the subject went to sleep was collected along with acknowledgement that food or water would not be consumed within 30 minutes of collection of saliva sample.

Eveningness in adolescents occurs due to the requirement of starting your day early and the need to compensate for lost sleep on the weekends. Consequently, a circadian that favors eveningness has been reported to be associated with low scores of health related quality of life (HRQoL), which includes: extreme fatigue in the morning, insomnia symptoms, and behavioral symptoms (Roeser et al., 2012).

In conclusion, the data presented here suggesting that there is a decrease in cortisol concentrations associated with an hour of additional sleep may have implications for the cognitive function. Within just one night of sleep deprivation young adults have shown deleterious neurological signs including intense sleepiness (Louca and Short, 2014). The effects of alertness and cognitive performance have been linked to a decrease in brain activity and function seen in the thalamus (Thomas et al., 2000). According to Hershner and Chervin, in college students, sleep deprivation can affect cognition due to a disruption of their circadian rhythm however, in extreme cases it can cause mood and sleep disorders (Hershner and Chervin, 2014). Studies have shown that napping can be counter active to sleep loss as a way to improve cognitive abilities, such as academic performance, as well as redistribute cortisol secretion within normal limits (Vgontzas et al., 2006, Hershner and Chervin, 2014).

5. Conclusion

In summary, the data supports our hypothesis that an increase in sleep will decrease salivary cortisol concentrations. In this study, we examined the effect of an hour extra sleep on salivary cortisol concentrations and found that there was a decrease in the overall salivary levels over 24 hours in comparison to a normal sleep-wake cycle. Cortisol concentrations contribute to brain cognition, which can be altered from the lack of proper sleep.

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