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THE EFFECTS OF SLEEP RESTRICTION ON URINARY AND SALIVARY CORTISOL LEVELS

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ABSTRACT

Sleep deprivation is a serious issue which affects many individuals in an increasingly hectic day and age. Not prioritizing sleep can lead to host of negative health outcomes, including hormonal dysregulation. Excessively increased or decreased levels of cortisol, in particular, can have adverse effects on the body through changes to the hypothalamic adrenal axis. Consequently, this study sought to understand how sleep restriction could impact the levels of urinary and salivary cortisol. This was accomplished by recruiting subjects for an 11-day inpatient study performed at the Pennsylvania State University Clinical Research Center. Three sleep conditions were observed: three days of baseline (10 hours of sleep opportunity), five days of sleep restriction (5 hours of sleep opportunity) and one day of recovery (10 hours of sleep opportunity). Hourly saliva samples and 24-hour urine samples were taken throughout and analyzed via the DRG Urinary Cortisol Assay Kit and the Salimetrics Cortisol Assay Kit respectively. The results for both urinary and salivary cortisol showed flat and blunted lines not indicative of sleep restriction. Comparing the two measures against each other, there was no association seen across most of the subjects and a slight negative association for a few. Limitations such as the small sample size and interference from other procedures could be remedied in the future while implementing new methods of measuring cortisol.

TABLE OF CONTENTS

LIST OF FIGURES

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This thesis would not have been possible without the opportunities provided to me by Dr. Buxton. As a thesis advisor and mentor, he has provided invaluable advice about how to make the most of my undergraduate research experience. Also, I want to thank Dr. Francis for helping to revise this project, Dr. Gyekis for providing initial ideas and input as I started writing, and the Biobehavioral Health Biomarker Core Lab for analyzing the samples (supported in part by a grant from the Pennsylvania State University Clinical and Translational Science Institute, Dr. Anne-Marie Chang, Principal Investigator). I also want to thank the College of Health and Human Development for their support through the summer research grant and the Edith Pitt Chace Award. Additionally, I want to thank the other members of the lab who helped collect the data and ensure that the study ran smoothly, and the study participants for their patience. Finally, I want to thank my parents, my sister, and all my friends who offered support and guidance throughout the last few years.

Chapter 1

Introduction

As an essential biological function vital to recovery, energy conservation, and survival, sleep should occupy an important role in everyone's life. Short (<7 hours) as well as long (>8 hours) habitual sleep patterns have been associated with increased risk of mortality, morbidity, and development of disease promoting risk factors. To reduce risk of chronic diseases and attain optimal health outcomes, adults should obtain 7-8 hours of sleep per night. However, surrounded by the demands of an increasingly busy society, many people are having difficulty finding the time to sleep.

Sleep time reaches a minimum between 45-54 years when compensated work time is also at a maximum (Basner et al., 2007). Additionally, a negative relation has been observed between technology use and sleep for adolescents, providing further indication of how the changing, digitalized times could precipitate lower levels of societal sleep (Hysing et al., 2015). In fact, approximately 35% of US adults fail to meet the minimum recommendation of seven hours' sleep each night. As the levels of sleep have drastically decreased across the last few decades, the effects of sleep restriction are having long-lasting mental and physiological changes.

This study will particularly seek to explore the hormonal dysregulation of cortisol production across an eleven-day inpatient sleep restriction study. This will be accomplished through collecting a 24-hour urine sample within three separate sleep conditions of baseline (10 hours of sleep), sleep restriction (5 hours of sleep), and recovery (10 hours of sleep). Analysis of the samples through the DRG ELISA assay should provide a better understanding of how consecutive nights of sleep restriction can impact the production of cortisol.

The implications for this study are far-reaching, as many adults continue to prioritize work, commute time, or leisure activities above sleep. Consequently, as chronic sleep deprivation becomes more

widespread, an excess or deficit of cortisol could result in a variety of negative health outcomes through damage to the HPA (hypothalamic adrenal) axis. Additionally, methods of analyzing cortisol in future studies will also be discussed, as the need for more research becomes evident.

1.1 Consequences of Sleep Deprivation

The importance of sleep cannot be understated as it plays a vital role in cognitive performance, memory consolidation, pain regulation, and clearance of toxins (Lichtenstein et al., 2015). In examining cardiovascular outcomes, short sleep is associated with an overall increase in the incidence of cardiovascular disease and hypertension. As biological pathways such as cardiovascular autonomic control, inflammatory responses, and endothelial function are affected, individuals experience sleep deprivation become more susceptible to congestive heart failure, coronary artery disease and arrhythmias (Tobalidini et al., 2017).

Experimental as well as observational studies have also shown that sleep deprivation reduces insulin sensitivity and lowers glucose tolerance (Leproult, 2012). If these effects continue for prolonged periods of time, the pancreas could lose β-cell function, resulting in the development of type 2 diabetes. Additionally, sleep restriction has also been associated with higher levels of the hormone ghrelin and lower levels of leptin, resulting in an increase in food intake and decrease in satiety. In particular, sleep restriction leads people to gravitate towards fat, sweet, and salty snacks, ultimately leading to increased body-mass index as well as obesity (Watson et al., 2015).

Also, although the direction of the relationship between short sleep duration and mental health is unclear, it has been demonstrated that mental health suffers. Insufficient sleep has been associated with depression, obsessive-compulsive disorder and suicidal thoughts. Additionally, the pattern of weekend bedtime delay and oversleep were also found to impact behavior, aggression, and mental health disorders in young children and adolescents in particular.

In examining immunological issues, it seems that short sleep places individuals at higher susceptibility for developing infectious diseases such as upper respiratory problems and pneumonia. The weakened nature of the immune system following short sleep has also been linked to decreased natural killer cell function and vaccine response (Watson et al., 2015). Although fewer studies have linked sleep habits to cancer, researchers did find that sleeping less than 6 hours per night was associated with an almost 50% increased risk of developing a colorectal adenoma (Zhang et al., 2013). Affected individuals were also more likely to have been diagnosed with sleep apnea or worked irregular sleep shifts, further discouraging the advent of irregular or disrupted sleep schedules (Thompson et al., 2010).

Pain and sleep are also linked in what appears to be a bidirectional relationship. Sleeping less than five hours was associated with increased pain in many studies. Yet increased pain was also related to obtaining less sleep in following nights, hinting at the cyclical nature of the two factors (Watson et al., 2015). Several longitudinal studies have also shown that insomnia symptoms strongly predict the development of chronic pain disorders whereas previously existing pain does not strongly predict the development of insomnia. Consequently, short sleep duration has temporally been related to a resulting increase in pain (Finan et al., 2013).

Lapses in attention can lead to disturbances in the daily lives of individuals, as well. Drowsy driving was responsible for 2.5% of fatal motor crashes and 2% of all nonfatal crashes (Connor, 2001). Additionally, excessive daytime sleepiness due to sleep deprivation can lead to greater incidence of work-related injuries (Mukherjee et al., 2015). Sleep disorders such as insomnia, sleep apnea, narcolepsy, and circadian disruption can also place a burden on the health of individuals as well as seriously endanger the safety of others around them. The costs of healthcare continue to be impacted by these increasingly prevalent sleep disorders.

Faced with the plethora of negative outcomes surrounding short sleep, it becomes evident that sleep is a crucial part of daily life. However, this study will aim to focus on the effects of sleep on levels of cortisol. As an important hormone in the hypothalamic pituitary (HPA) axis, cortisol acts as a

mediating factor precipitating many of the chronic diseases and health states mentioned above. Consequently, understanding how sleep restriction in a laboratory setting impacts fluctuations in cortisol levels can also shed light on how chronic short sleep can negatively impact the body for individuals in society.

1.2 The Hypothalamic-Pituitary-Adrenal Axis & Stress

Stress, or any state that disturbs the body's internal balance, is resolved by the body's self-regulating processes. One such component of the stress response is the hypothalamic-pituitary-adrenal (HPA) axis. Cells in the paraventricular nucleus of the hypothalamus are stimulated to release corticotropin-releasing factor (CRF) and arginine vasopressin (AVP) into the hypophyseal portal. This in turn stimulates the anterior pituitary to secrete adrenocorticotropic hormone (ACTH) which consequently promotes the synthesis and release of glucocorticoids from adrenal glands. Activation of the HPA axis is regulated by the release of inhibitory and excitatory neurotransmitters as well as negative feedback loops to maintain hormone levels at predetermined set points.

Additionally, the mineralocorticoid (type-I) and glucocorticoid (type-II) receptors also contribute to the negative feedback system. Although cortisol binds with greater affinity to the mineral receptors, during times of great stress it also binds with low affinity to the type-II receptors. This activation of the glucocorticoid receptors usually results in the termination of the stress response. The maintenance of cortisol levels within a specific bandwidth of acceptable levels is important because chronically high or low levels of cortisol can have long-term negative effects.

1.3 The Effects of Cortisol on the Body

Cortisol is the main glucocorticoid in humans and plays a vital role in maintaining various functions of the body. For one, cortisol regulates glucose levels by triggering enzymes that promote gluconeogenesis while also sustaining glycogen synthesis in the liver. Cortisol also keeps the body at an appropriate pH by preventing cells from losing sodium while accelerating the loss of potassium. The immune response is weakened when cortisol prevents T-cells from proliferating and stifles inflammation by inhibiting the secretion of histamines. Excess cortisol can also impact memory by overwhelming the receptors in the hippocampus, an area of the brain where memories are stored.

Differences in basal cortisol levels as well as the HPA axis response can depend on a variety of factors. Heritable influences, for one, account for 62% of the differences in resting glucocorticoid levels. This can occur through polymorphisms in certain genes that encode for different receptors and binding proteins. Additionally, early life experiences such as prenatal ethanol exposure, maternal stress, and childhood trauma can have prolonged effects on the responsiveness of the HPA axis (Stephens et al., 2012). Finally, ongoing everyday sources of stress such as work, family, neighborhood violence or exposure to adversity can lead to differences in cortisol levels, as well. In particular, chronic long-term stress can lead to a permanently increased allostatic load. This, in turn, can create a shift in the circadian rhythm of cortisol release and cause an elevation in baseline cortisol levels. Consequently, the HPA axis becomes more sensitive, necessitating a greater cortisol burden following a stressful event (Stephens et al., 2012).

When an individual experiences a stressful situation, a normal response consists of a rapid rise in cortisol levels followed by a decline when the stressor is removed or terminated. Some types of stress are beneficial and can motivate a person to achieve their goals through heightened activation. However, other types of stress that continue for long periods of time can result in negative outcomes such as prolonged healing times, vulnerability to infections, memory problems, increased abdominal fat, and reduced thyroid function.

Scientists have coined the term “adrenal fatigue” to describe the effects of reduced cortisol production in response to chronic stress. Without enough cortisol in the bloodstream, the body has difficulty mounting an appropriate fight-or-flight response. Studies show that children and adolescents who are victims of severe recent trauma have elevated levels of non-stress cortisol compared to the control group. However, adults who report traumatic life experiences or exposure to prolonged stressful situations exhibit a deficit of non-stress cortisol concentrations. This effect can be seen across a variety of scenarios involving retrospective reports of chronic stress (Trickett et al., 2010). Attenuation of the cortisol response has been observed in adults who have experienced domestic violence, combat, the Holocaust, and rape.

The “attenuation hypothesis” explains that the HPA axis copes with hypersecretion of cortisol in times of chronic stress through the downregulation of cortisol secretion. (Trickett et al., 2010). Reducing the levels of cortisol has an adaptive function in that prolonged exposure to the hormone can have negative effects on brain structures as well as cardiovascular and immunological functions. Hypercortisolism can lead to dysfunction in aging, deficiencies of related hormones, and development of diseases. However, hypocortisolism can have its own maladaptive effects on the body. Many clinical syndromes such as “burnout”, fibromyalgia, posttraumatic stress disorder, chronic fatigue syndrome, allergies, autoimmunity, and inflammation are related to HPA changes causing hypocortisolism.

As sleep is also a chronic, recurring stressor, dysregulation of the HPA axis can occur, resulting in abnormal levels of cortisol. Consequently, understanding how sleep deprivation impacts cortisol levels is important as the hormone plays a vital role in the body. Previous research has also sought to quantify and describe this relationship between sleep restriction and cortisol concentrations.

1.4 Previous Studies on Sleep and Cortisol

Previous sleep research has indicated that short sleep results in definite hormone dysregulation. Sleep deprivation in healthy young men, for instance, was followed by an elevation in evening cortisol levels (Leproult et al. 1997). Whereas cortisol normally declines across the day, it was found that this occurred at a slower rate following sleep deprivation. Additionally, following a night of complete sleep deprivation, the sleep-to-wake spike in morning cortisol levels was found to be absent due to the lack of transition between sleep and wake states. However, a night of partial sleep deprivation led to an amplified cortisol response upon waking (Leproult et al., 1997). Similar results were also found in women, as they exhibited lower levels of morning peak cortisol and less cortisol decline from the morning to the evening. This resulted in the same evening elevation of cortisol observed in the study of (Omisade et al., 2010)

Consequently, while sleep loss does not function as stressor per se, it does hinder the HPA axis in recovering from the overstimulation of sleep deprivation and results in the reduced response to circadian rhythms. Chronically elevated levels of evening cortisol due to prolonged sleep deprivation could create disturbances in the central and peripheral nervous system. An excess of glucocorticoids could result in memory problems due to impaired hippocampal function as well as insulin resistance. Additionally, aging is associated with increased evening plasma levels as well as decreased HPA axis resiliency. Consequently, long term stress exposure speeds up the aging process through increased exposure to glucocorticoids (Leproult et al., 1997).

Increased levels of cortisol in the evening following sleep loss has also been associated with a decrease in the appetite suppressing hormone leptin and a simultaneous increase in the hunger-promoting hormone ghrelin. Sleep deprived individuals with higher subjective ratings of hunger tend to gravitate towards calorie-dense and carbohydrate-rich foods. Chronic sleep deprivation is consequently linked with higher body mass index (BMI), larger waist to hip ratios, and ultimately higher instances of obesity (Omisade et al., 2010).

Faced with the numerous negative outcomes associated with chronic stress due to sleep deprivation, it is necessary to examine how cortisol levels change accordingly, as abnormal levels of cortisol can impact many processes in the body. Consequently, this project will seek to add to the existing sleep literature by examining the possible changes in cortisol levels associated with sleep restriction. Through collecting both 24-hour urine samples and hourly saliva samples across the study, we can relate the measures to sleep condition as well as compare the measures to each other to determine the efficacy and downfalls of both in the context of sleep research.

Chapter 2

Methods

This project was carried out within the context of a larger 11-day inpatient study at the Pennsylvania State University Clinical Research Center by the Sleep, Health, and Society Lab. The purpose of the study was to examine the effects of sleep deprivation on a variety of factors such as overall health, cardio-metabolic disease risk, cognition, and the epigenome. However, this project focuses specifically on the levels of urinary cortisol across the conditions of baseline, sleep restriction, and recovery.

2.1 Recruitment and Screening

Subjects were recruited for the study via flyers posted throughout State College and advertisements posted online on Craigslist and the Penn State research portal. Healthy, non-smoking and right-handed men between the ages of 20 and 35 were sought for an eleven-day study with a total compensation amount of up to $2,250 (if all screening and study procedures were completed). Men who responded to this advertisement were then given a thorough explanation of procedures and risks involved in the sleep restriction study, and if still interested, provided written, informed consent to be screened. A staged screening process followed, wherein potential subjects were administered questionnaires about sleep, psychological, and medical history, among other questionnaires. If the potential subject remained eligible, they were asked to provide urine and blood samples, as well as have a physical examination, meet with a clinical psychologist, and meet with research staff to provide written informed consent for the research portion of the study.

Additionally, prior to the commencement of the study and for at least one week subjects were obliged to spend 10 hours in bed each night trying to sleep, calling a time-stamped voicemail system to verify sleep/wake times. Additionally, subjects were also expected to keep a journal of food intake and wear a Spectrum Pro (Philips-Respironics, Murrysville PA), or a wrist watch that measures wrist activity from which sleep patterns can be estimated using validated algorithms (Marino et al., SLEEP, 2013). Also, three weeks prior to the study and during the eleven days of the study, subjects must consent to refrain from using alcohol, caffeine, nicotine, prescription or non-prescription drugs, recreational or street drugs, herbal supplements, vitamin supplements, and other foreign substances.

2.2 Inpatient Study

For the entirety of eleven days and nights, the subject was obliged to live at the Clinical Research Center, Pennsylvania State University. This project was able to include the data from five subjects (n=5), one from each month through the period of May 2016 to August 2016. Most of the subjects' time was spent inside of the assigned room kept at specified light levels and temperatures except for certain tests. Procedures outside the scope of the current thesis occurred outside of the room but within walking distance to the Clinical Research Center.

Subjects in the study followed a structured schedule detailing everything from wake and sleep time, meals, and sample collections. Three nights of baseline sleep (10 hours/night) are followed by five nights of sleep restriction (5 hours/night) and concluded with two nights of recovery (10 hours/night). Throughout the study, the subject is monitored closely by nurses, technicians and research assistants to ensure that he is not falling asleep or dozing during the day. Emphasis was placed on only allowing sleep at night with the lights off in the subject's room to obtain valid results.

2.3 Urine Collection and Assay

Urine was collected across the inpatient study beginning on Day 2 following an 08:00 void. The subject was then instructed to urinate only into the collection jugs following the first void. At 08:00 on the subsequent day (Day 3), the subject was instructed to urinate one final time before the collection jug was closed. The volume of the 24-hour sample was recorded and a 10-mL aliquot was performed. The aliquot was then stored and frozen upright to prevent the sample from freezing to the top. This pattern was carried out for the remainder of the study.

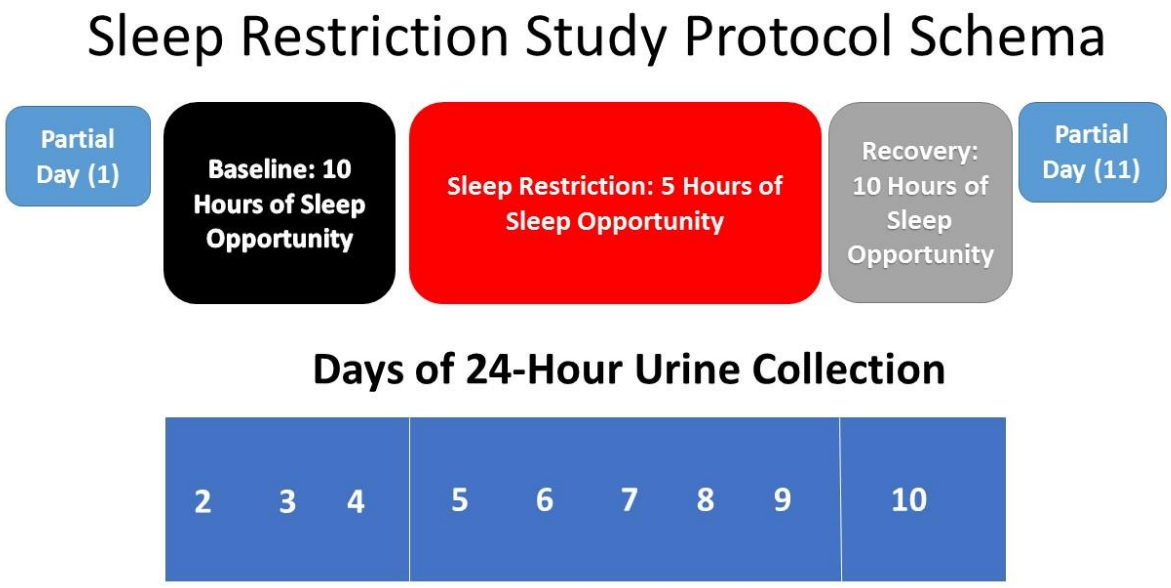


Figure 1: Sleep Restriction Study Protocol Schema Relating to 24-Hour Urine Collection

The urine assay was performed by the Biomarker Core Lab using the DRG Urinary Cortisol Assay Kit. In a laboratory setting, this kit measures free cortisol concentration in samples of urine through the competitive immunoenzymatic colorimetric method. Cortisol is mainly excreted in urine in an unbound (free) form whereas cortisol is bound in plasma by corticosteroid-binding globulin and albumin. The cortisol in the sample competes with the cortisol conjugated with horseradish peroxidase (HRP) for

binding of a limited number of antibodies in the solid phase. After incubation, separation is performed through solid-phase washing. The enzyme HRP interacts with the substrate (H_2O_2) to develop a blue color that changes to yellow when the stop solution (H_2SO_4) is added. The intensity of the yellow color is inversely proportional to the concentration of cortisol in the sample and can be calculated through a calibration curve. The resulting cortisol concentrations for the 10 mL aliquots were then adjusted to account for the daily volume of the individual 24-hour samples. This produced the final amount of free (unbound) μg of cortisol produced across each 24-hour period of sample collection (08:00-0:800).

2.4 Saliva Collection and Assay

Saliva samples were collected across the length of the sleep restriction study at hourly intervals barring certain circumstances. Following the first hour of wake time, samples were taken every fifteen minutes to quantify the morning cortisol spike. Additionally, certain procedures which took place outside of the Clinical Research Center or more time-consuming procedures such as blood draws or fat biopsies resulted in an inability to collect hourly samples. Researchers were also asked to omit or delay the collection of a sample if they noticed that the subject had recently drank water or consumed food (15-20 minutes prior).

The samples were then analyzed by the Biomarker Core Lab using the Salimetrics Cortisol Assay Kit. This kit operates via similar principles as the DRG Urinary Cortisol Assay Kit by measuring the free cortisol concentration via the competitive immunoenzymatic colorimetric method. As explained above (Section 2.3) in relation to the DRG Assay, the cortisol in the saliva sample also competes with horseradish peroxidase (HRP) to bind with the cortisol antibodies in the solid phase. Again, a separation is completed through solid-phase washing followed by a color change mechanism identical to the one explained above. The intensity of the color change is related to a calibration curve to obtain the cortisol concentration (μg/dL) at each time point in which the sample was taken.

Chapter 3

Results

The levels of cortisol for five subjects (n=5) were assessed through the measures of 24-hour urine collection and hourly saliva samples. After assays for cortisol concentrations, levels were visually compared by plotting the results across 10 days. The first three days constituted the baseline condition (10 hours/sleep each night) while the subsequent five days comprised the sleep restriction condition (5 hours/sleep a night) and the final day served as a recovery night (10 hours of sleep). Each sleep restriction study is identified by a label at the top consisting of the year of the study (16), the subject number (1-5) and sleep restriction (SR).

3.1 Urine Cortisol Results

These figures (Figure 2) depict the levels of free (unbound) urinary cortisol each day across nine complete days of the sleep restriction study. A 24-hour urine collection was started on Day 2 at 08:00 and continued until 08:00 on the subsequent day (Day 3). This pattern was continued across all complete inpatient days of the study for all voids. Aliquots were saved from known volumes of 24-hour urine samples. These 10 mL aliquots were then analyzed for cortisol content using the DRG Urinary cortisol ELISA Assay (see Methods). Three conditions were observed on the night before the 24 collection: Baseline (10 hrs/sleep opportunity) from Days 2-4, Sleep Restriction (5 hrs/sleep opportunity) from Days 5-9, and Recovery (10 hrs/sleep opportunity) on Day 10. These sleep conditions were represented by filled black, red, and grey data points respectively. Figure 2 shows the average level of free cortisol (± SD) across participants.

Figure 2: Subjects 1-5 Free Urinary Cortisol Vs. Day of Study.

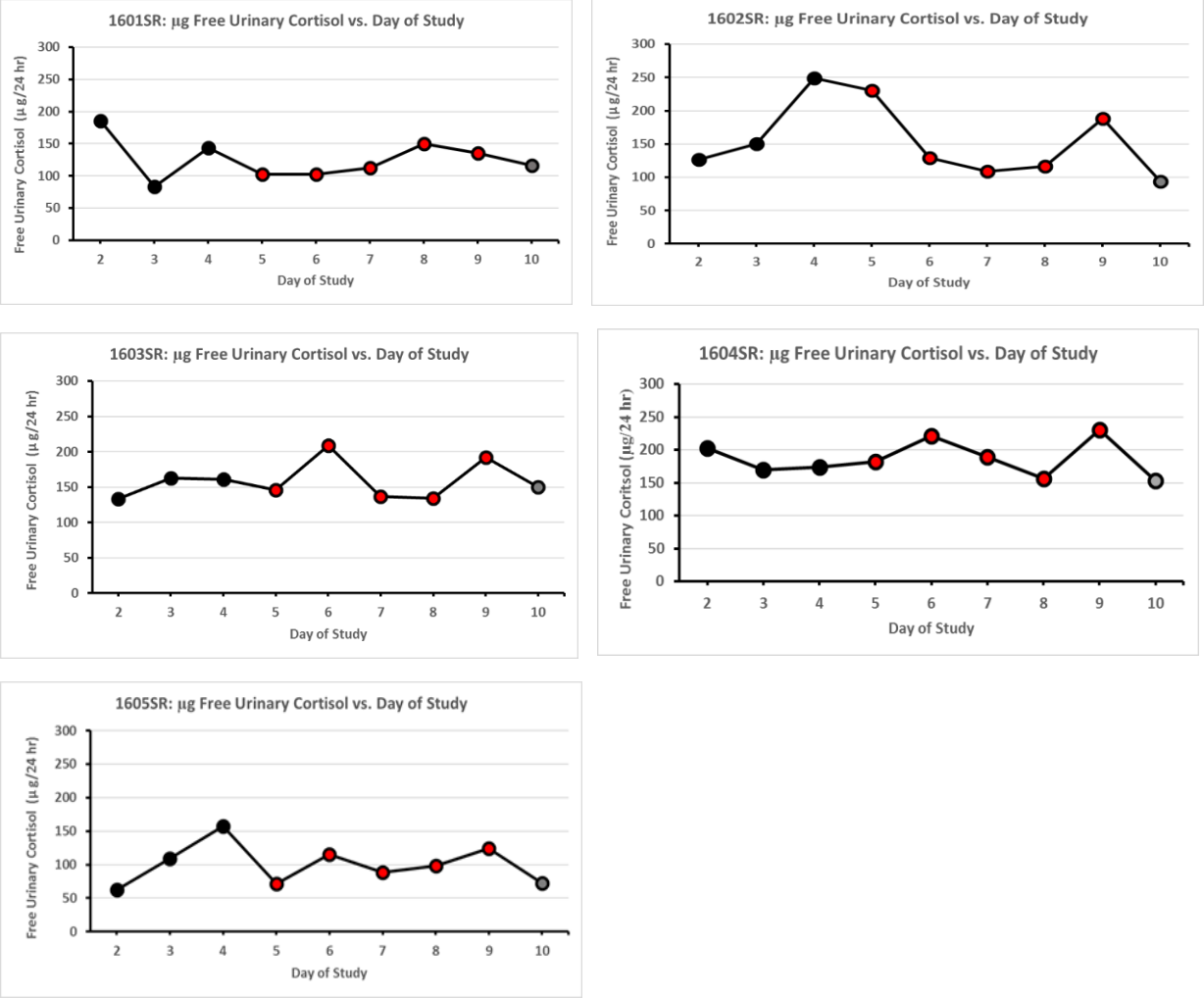


Figure 3 below represents the average free urinary cortisol levels for each day across each of the five studies. Additionally, the standard deviation is also depicted with the inclusion of error bars. Figure 4 shows the normalized urinary cortisol levels, or the percent of the study mean across each of the study days. Standard deviation is, again, shown through the inclusion of the error bars. The sleep condition, as described previously, is also shown through filled black points for baseline, filled red points for sleep restriction, and filled grey points for recovery.

Figure 3: Average Across All Studies: µg Free Urinary Cortisol Vs. Day of Study

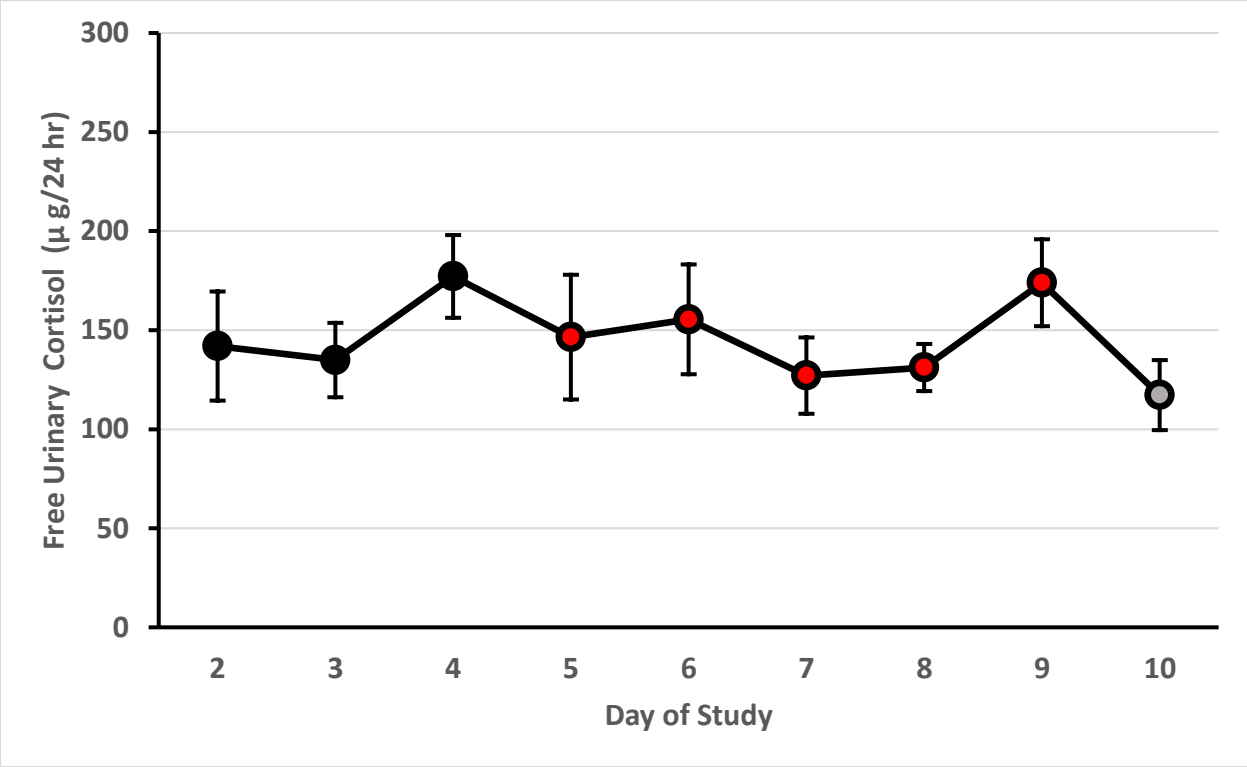
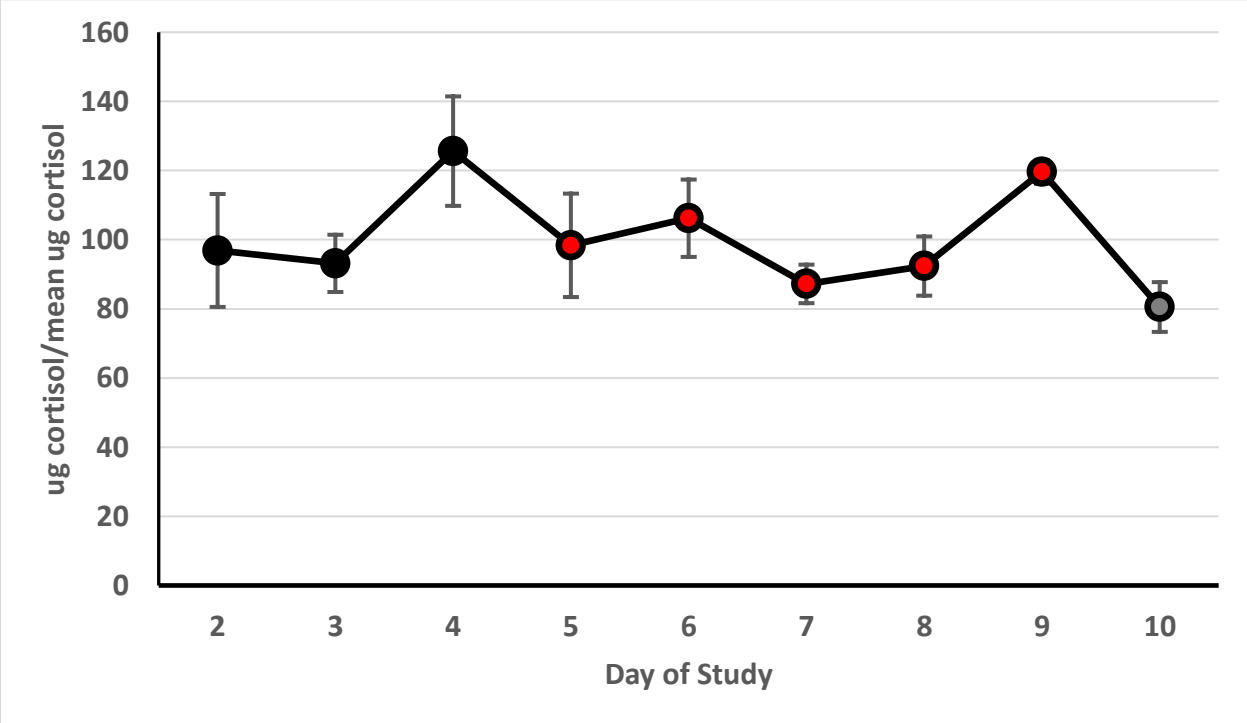


Figure 4: Normalized Urinary Cortisol (% Study Mean) Vs. Day of Study



3.2 Saliva Cortisol Results

These figures (Figure 5) depict the levels of free (unbound) salivary cortisol across nine consecutive days of the sleep restriction study. Saliva samples were taken hourly barring procedures or recent consumption of food or drink. Consequently, the average of the cortisol levels from samples taken between 08:00-22:00 was calculated and used as the daily mean for each subject. Three conditions observed on the night before the collection of that day's saliva samples were: Baseline (10 hrs/sleep opportunity) from Days 2-4, Sleep Restriction (5 hrs/sleep opportunity) from Days 5-9, and Recovery (10 hrs/sleep opportunity) on Day 10. These sleep conditions were represented by filled black, red, and grey data points respectively.

Figure 5: Subjects 1-5 Mean Salivary Cortisol (µg/DL) Vs. Day of Study

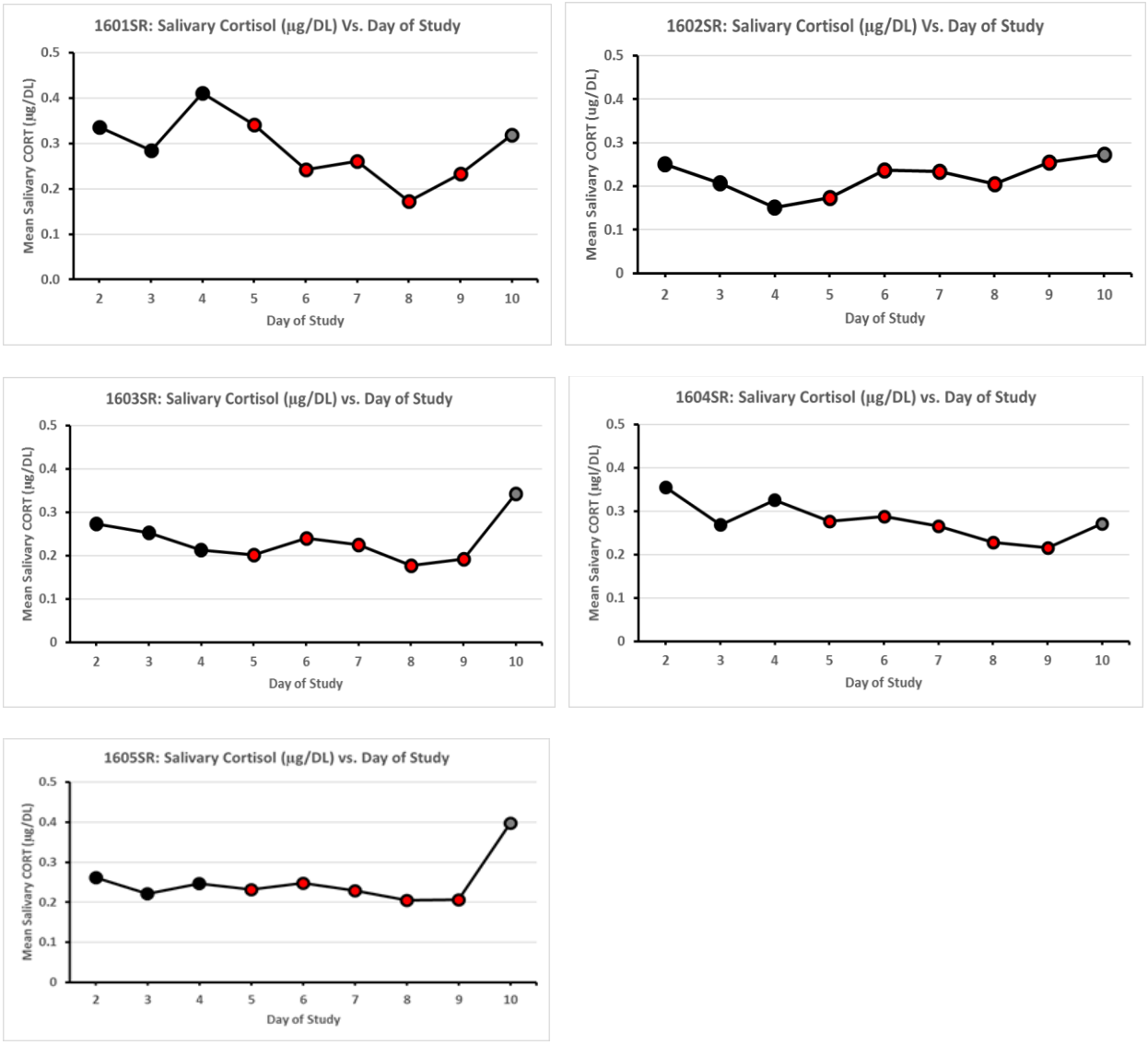


Figure 6 below represents the average of the individual salivary data for each day of the study. Figure 7 shows the normalized salivary cortisol levels, or the percent of the study mean across each of the study days. Standard deviation is, again, shown through the inclusion of the error bars. The sleep condition, as described previously, is demonstrated by filled black points for baseline, filled red points for sleep restriction, and filled grey points for recovery.

Figure 6: Mean Salivary Cortisol (µg/DL) Vs. Day of Study Across All Subjects

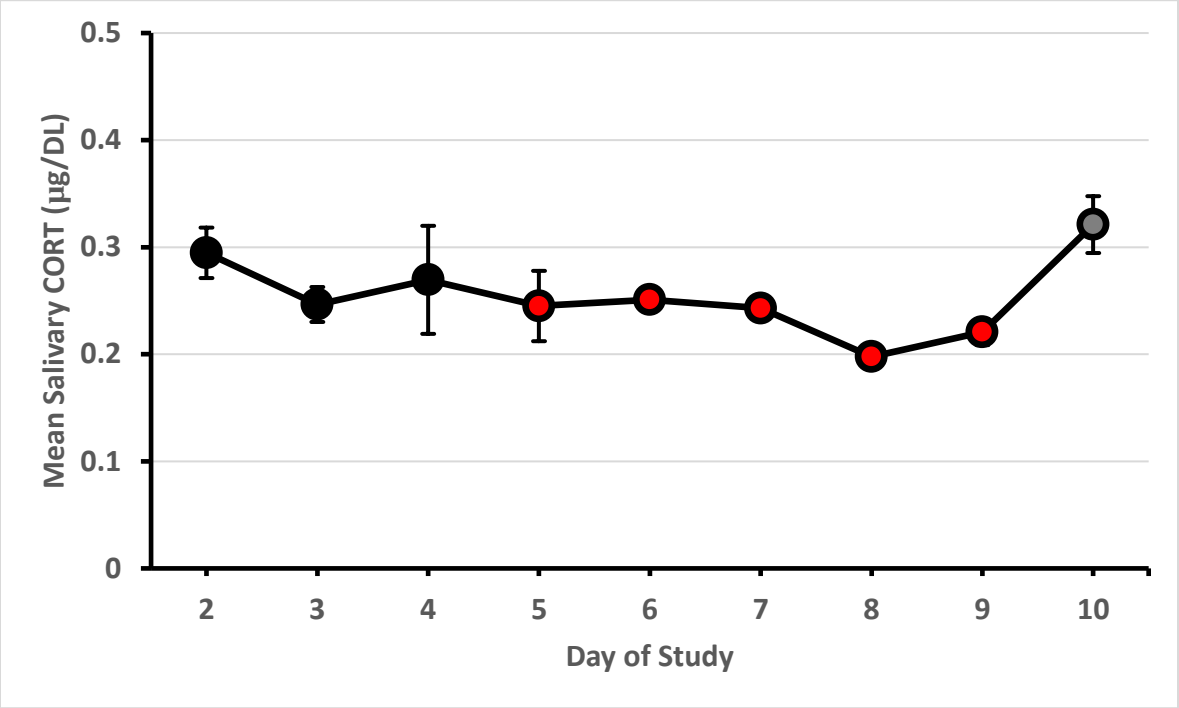
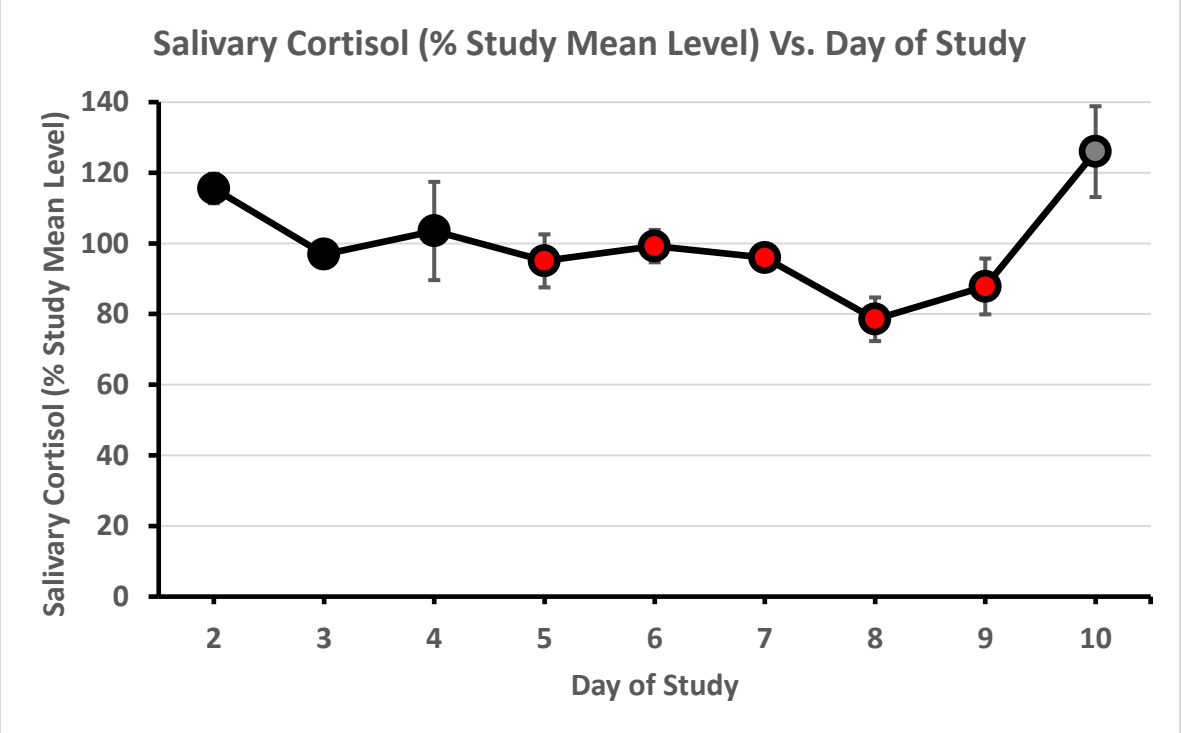


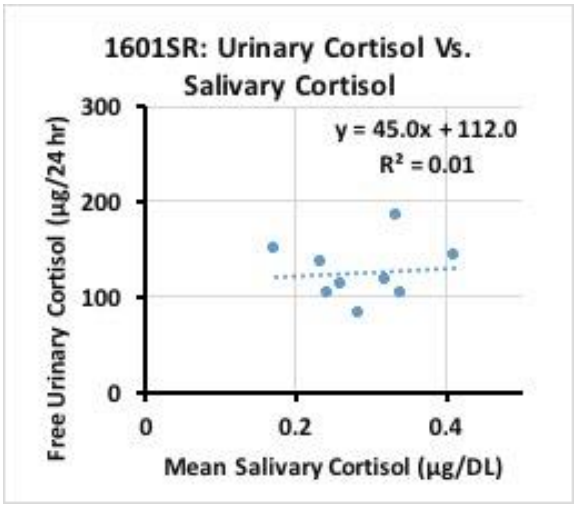
Figure 7: Normalized Salivary Cortisol (% Study Mean Level) Vs. Day of Study

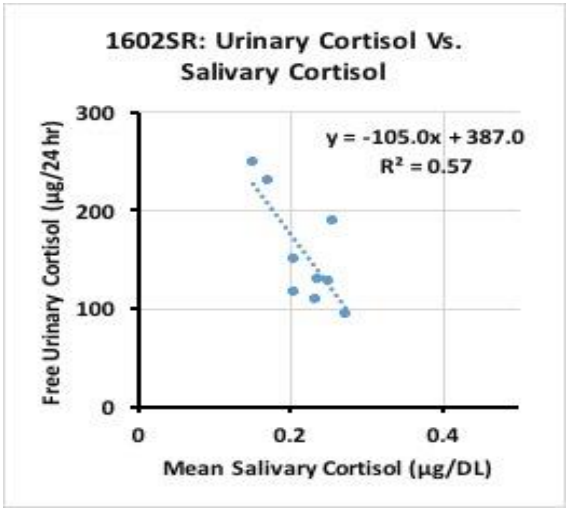


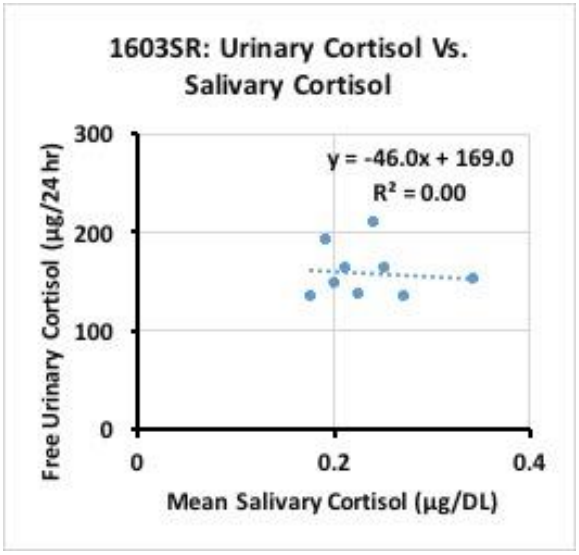
3.3 Urine Vs. Saliva

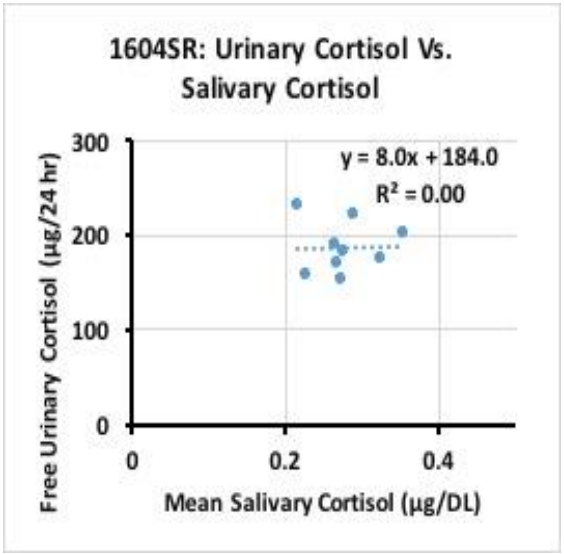
The figure below shows plots of urinary cortisol (μg/24 hr) vs. salivary cortisol (μg/DL) across each of the five studies. Additionally, the linear regression lines and r squared values are listed in the top right corners to quantify relationships between the two measures used.

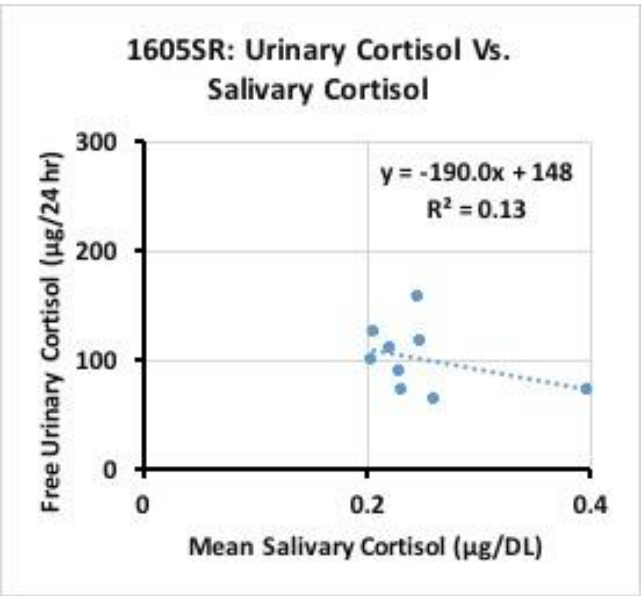
Figure 8: Urinary Cortisol Vs. Salivary Cortisol Across Each Study










1605SR: Urinary Cortisol Vs. Salivary Cortisol
y = -190.0x + 148
R² = 0.13
Free Urinary Cortisol (µg/24 hr)
300
200
100
0
0
0.2
0.4
Mean Salivary Cortisol (µg/DL)

Chapter 4

Discussion

In this study of 5 nights of sleep restriction, we collected data on both 24-hr (integrated) cortisol levels and 30-minute interval saliva samples for determining free cortisol levels across the waking period. Contrary to most prior literature on the effects of sleep deprivation and sleep restriction on cortisol levels, we observed flat, blunted responses providing no support for an effect of sleep restriction on free cortisol levels. We also noted that some elevations of cortisol levels occurred on days with Intravenous Glucose Tolerance Test (IVGTT) due to the HPA axis response to increased glucose and insulin levels inherent to that metabolic challenge. It is also necessary to take into the account the impact which other procedures occurring on these days may have on cortisol fluctuations. Additionally, examining the findings against one another shows the differences in the portion of each day and the way that the methods detect cortisol levels. Suggestions for future analyses are also provided.

4. 1 Urinary Cortisol Analysis

Examining the levels of free cortisol from the 24-hour urine samples, it is interesting to note how much the overall range varies between individuals. Subject 1, for example, has cortisol levels of less than 150 µg/24 hr for nine out of the ten days, including sleep restriction. In contrast, Subject 5 also has cortisol levels less than 150 µg/24 hr for nine out of the ten days, with a maximum cortisol level of 157.46 µg on Day 4. However, Subject 2 reaches a maximum cortisol level of almost 250 µg on Day 4, which is almost 100 µg more than Subject 5's highest cortisol concentration. Subjects 3 and 4, however, presented with intermediate levels of cortisol, showing maximum concentrations of 209 µg and 221 µg, respectively.

Additionally, Subjects 1 and 5 fluctuate approximately 100 µg across the days of the study whereas Subjects 2 and 4 span a maximum range of 150 µg. However, Subject 3 shows the most stability with a range spanning only 75 µg, further demonstrating the individual differences in the response to sleep restriction. Different individuals cope with challenging situations differently and thus present with varying physiological responses. Whereas some remain at lower levels of cortisol across the length of the study, others were not able to regulate their stress levels as well. These individual differences are important to consider while examining the relationship between cortisol levels and sleep restriction, as not everyone will exhibit the same magnitude of stress response.

Another factor to consider in the analysis is the existence of possible outliers. Day 7 of 1604SR, for example, showed a dip to 58 µg of cortisol following a peak at approximately 221 µg on the previous day. Representing only 34% of the study mean for Subject 4, 58 µg of cortisol appeared to be a dramatic decline following a day of sleep restriction when the cortisol level was 128% of the study average. Additionally, with a reduced 24-hour volume (1980 mL) recorded in the study logs, it seems that there could have been some loss of urine samples if the subject did not follow protocol and urinate only in the provided jugs, particularly in the period between wake time and lunch. To remediate the effect that such a drastic outlier had on the analysis, we removed the point and replaced with a linearly interpolated value, the average of Day 6 and Day 8.

However, even after fixing this mistake, many of the graphs show flat or null findings with respect to the relationship between cortisol and sleep restriction. Examining Figure 8, it appears that cortisol levels tend to show a modest decrease from the study mean going from Day 2 (97%) to 3 (93%). This initial elevation could be attributed to the novelty of the subject's first full day in a sleep restriction study as they begin partaking in procedures and are introduced to protocol. However, on Day 4, which is still included within the Baseline condition, it seems that cortisol levels rise to 125% of the study mean level, closely mirroring the elevation that occurs on Day 9, a sleep restriction day. This leads to the question of whether this increase is due to a sleep condition or another factor entirely.

As this sleep restriction study examines many other outcomes, it is necessary to consider that the cortisol concentration could be due to other study factors influencing the study. For example, on Day 4 and 9, the Intravenous Glucose Tolerance Test (IVGTT) takes place. This procedure involves an intravenous infusion of a glucose load (followed by a dose of exogenous insulin 20 minutes later) to test insulin secretion and insulin sensitivity. Cortisol, however, inhibits insulin production to keep glucose from being stored, favoring its immediate use as the stress hormone, thus explaining the increase to 126% of the study mean level on Day 4 as well as the 120% increase on Day 9. Interestingly, however, there is a greater standard deviation found on Day 4 (29.8%) versus Day 9 (9.5%). This could be indicative of the effects of sleep deprivation, as the subjects responded to the IVGTT with varied levels of elevated cortisol on Day 4 following 10 hours of sleep but showed more stable signs of increased cortisol and stress physiology on Day 9, following only 5 hours of sleep.

In fact, examining the individual data, it seems that Subject 1, 2, and 5 experience modest increases in cortisol levels from Day 3 to 4 (at least 50 µg), when the IVGTT is performed. However, Subject 4 shows a very minute increase in cortisol levels from Day 3 (170.01 µg) to Day 4 (174.13 µg) while Subject 3 presents with a small decrease in cortisol levels from Day 3 (162.79 µg) to Day 4 (161.36 µg). During sleep restricted days however, 4 subjects showed an increase in cortisol levels from Day 8 to Day 9 when the IVGTT takes place. Again, 3 out of the 5 subjects demonstrate increases of greater than 50 µg while Subject 5 showed an increase of approximately 25 µg. Consequently, it seems that there was a smaller cortisol response to the insulin in the blood following a baseline night (10 hours of sleep) compared to a sleep restricted night (5/hours of sleep).

Consequently, the decrease in average cortisol levels seen from Day 4 (177 µg) to Day 5 (147 µg) could be attributed to sleep restriction, or the removal of the IVGTT procedure. The effects of sleep restriction could play a role in the increase of average cortisol levels from Day 5 (146.5 µg) to Day 6 (155.48 µg), or an 8% increase when comparing the values to the mean study level. Continuing across the study, there is a slight decrease in the average levels of cortisol on Day 7 (87% of the study mean level. Day 8, however, is only slightly decreased (92%) from the study mean level, and then Day 9 exhibits a

characteristic increase in cortisol levels due to the IVGTT procedure. Hence, it remains difficult to determine an effect of sleep deprivation with the interference of IVGTT stress effect combined with the impact of the possible outlier.

However, Day 10, or the recovery day (10 hours of sleep), presents with a decrease in cortisol levels across every single subject. Again, this decrease could be attributed to the removal of the stressor or the IVGTT as the same relationship of decreased cortisol was observed from Day 4 to 5. However, there was a 27% decrease from the mean study level observed from Day 4 to 5, there is an almost 40% decrease from Day 9 to 10. Consequently, it seems that recovering from the impacts of sleep deprivation could have also played a role in this magnified decrease of cortisol levels, as well.

4.2 Salivary Cortisol Analysis

Examining the saliva cortisol levels, many of the plots again show flat lines or blunted lines. The individual variation for the subjects in this measure is much less than for urine cortisol, with only Subject 1 showing an range of values larger than 0.3 µg/DL. In fact, for most of the subjects, their daily averages fell between the range of 0.2-0.3 µg/DL. Across all the subjects from Day 2 to 3, there is a decrease of approximately 20% of the study mean level. Speculating, this could again be indicative of the adjustment to the sleep restriction study on Day 2 followed by a subsequent increase in comfort levels with study staff and protocol.

From Day 3 to 4 and Day 8 to 9 there is a slight increase in cortisol levels as the insulin-inducing IVGTT procedure is introduced. An increase of approximately 6% of the study mean level occurs from Day 3 to 4 whereas an increase of 10% is noted from Day 8 to 9. This could be due to the effects of sleep restriction, as a greater increase in the percent increase of the mean levels of cortisol is observed across sleep restricted days than baseline. The standard deviation seen in the percent mean of the study level is also much lower on Day 9 (8%) than on Day 4 (28%). This could also be indicative of sleep restriction, as there is greater variability in how the subjects cope with the introduction of the IVGTT on Day 4, a

baseline day. However, on Day 9, there is a smaller standard deviation observed with an increased concentration of cortisol, implying that sleep restriction (5 hours of sleep) could be resulting in a more unified increase in cortisol.

From Day 4 to 5, there is the characteristic drop in cortisol levels which was also present in the urine cortisol plot. Again, this could be due to the removal of the stressor IVGTT as the percent of the study mean levels drop from 103% to 95%. Across Days 5, 6, and 7, cortisol levels remain stable, despite the onset of sleep restriction (5 hours of sleep a night). Following a slight increase from the mean study level from Day 5 to 6 (4.5%), there is a subsequent decrease from Day 6 to 7 (5%), indicating that sleep restriction is possibly having no significant effect on the daily fluctuations of cortisol. However, from Day 7 to Day 8, there is a drastic decrease in cortisol levels as it dips from 95% to 78% of the mean study levels. Why this happens remains unclear, as there is no obvious stressor such as the IVGTT being removed and other conditions remain controlled.

From Day 9 to 10, there is a drastic increase of cortisol constituting 38% of the mean study level. However, this goes against expectations as Day 9 is when the IVGTT procedure is performed and the final day of sleep restriction. Going from 5 hours of sleep a night to 10 hours of sleep coupled with the removal of a stressor should lead to a decrease in cortisol levels. These findings with saliva cortisol also go against the urine cortisol ones, which demonstrated a modest decrease in cortisol levels going from Day 9 to 10. However, just as nearly every subject showed a decrease in urine cortisol levels from Day 8 to Day 9, every subject showed an increase in saliva cortisol levels across those days.

However, as a method, saliva cortisol shows less of a standard deviation in the values across the days than urine cortisol does. The largest standard deviations in the percent of the study mean level occur on Day 4 (28%) and Day 10 (26%). In the urine cortisol results, however, approximately 5 of the 10 days present with standard deviations greater than 20%, whereas only the two aforementioned days had comparable standard deviations in the saliva samples. Although they showed drastically different results on Day 10, most of the other trends observed with urine cortisol were also seen in the saliva plots, as well.

4. 3 Comparing Urinary and Salivary Cortisol

The levels of urinary and salivary cortisol were compared by plotting the values for each day of the study and determining if there was any correlation at the individual level (Figure 8). We hypothesized that they would be positively correlated since urinary levels are integrated free cortisol levels for the day, and saliva, though only during the daytime, is the majority of the higher-cortisol hours across the 24-h day. Subjects 3 and 4 showed no level of association with r squared values of 0.00 and r squared of 0.01 for Subject 1. The data from Subject 5, however, showed some level of association with an r squared value of 0.13 tied to a negative relationship between urinary and salivary cortisol. The results from Subject 2 again demonstrate a negative association between the two variables supported by an r squared of 0.57. Consequently, while three of the five subjects do not show any significant relationship, the other two bring into question whether there is a negative association between the two measures of cortisol.

While we expected cortisol levels to be similar across measures and represent the physiologic response to sleep restriction, other researchers have found similar findings relating the lack of association between salivary and urinary cortisol. One such study found that peak levels of salivary cortisol occurred at morning collection and acted as a much better estimate of 24-hour urine cortisol than multiple samples taken across the day. However, this study also showed that the mean was positively correlated with free urinary cortisol, although to a lesser extent (Yehuda et al., 2013). Consequently, the negative association observed between the two measures could be related to the added variable of sleep restriction in our study.

Whereas urinary cortisol is an integrated, 24-hour measure, salivary cortisol can only be measured during waking times, which could explain why salivary cortisol decreased when urinary cortisol increased. Another study which considered the effects of sleep deprivation across several weeks showed that although 24 hour urinary catecholamines (norepinephrine and epinephrine) were significantly elevated, salivary cortisol showed no difference or a slight decrease across subjects (Zhang et al., 2011).

This could lead future research, as 24-hour measures seem to be more effective in examining the effects of sleep restriction as demonstrated by this study and several others.

Chapter 5

Limitations and Future Directions

Although it was hypothesized that sleep restriction would lead to an increase in cortisol concentration, this was not supported by these preliminary findings in the first 5 subject of the project. This section will aim to understand possible reasons why this association was not observed as well as discuss implications for the future regarding sleep research and cortisol quantification.

5.1 Limitations

One main limitation of this study is the sample size. With an n of only 5, even one outlier could influence the results. With more subjects contributing to the average, the results will be able to show significant findings and cancel out the effects of outliers or errors. Consequently, as the study continues, more data can be collected and added to this analysis. Another limitation is that limitation of this study is that the procedures and protocols relating to other aspects of the sleep restriction study are affecting the levels of cortisol. This effect is clearly seen with the increases in cortisol levels occurring in both urine and salivary cortisol concentrations on Day 4 and Day 9.

On both days, the IVGTT takes place, measures glucose tolerance by intravenously introducing the molecule into the blood stream. As insulin increases in response, cortisol levels incease as well to counteract the effect of the storage-promoting hormone. Additionally, with many blood draws associated with the procedure, it could also be characterized as a stressful experience. Similar increases in cortisol levels on Day 4 and 9 bring into question if other procedures such as fMRI, FLIPI, or other blood draws and fat biopsies could also be influencing the results. In the future, it could be beneficial to restructure the study so that these other factors influencing cortisol levels could be controlled for or removed. As long as such factors remain, it would be difficult to separate the effects of sleep restriction from responses to other procedures.

5.2 Future Directions

Sleep deprivation remains a significant problem for many members of today's busy society. Short sleep has been associated with a host of negative health outcomes, prompting the need for better sleep habits and hygiene. Following a regular sleep schedule, creating a comfortable sleeping space, and reducing the amount of technology used prior to bedtime can all help in creating a more effective sleep time. For adults, prioritizing the target 7-9 hours of sleep could lead to overall improved health while also reducing the incidence of many chronic conditions.

Looking forward, one way to improve upcoming studies linking chronic sleep deprivation to cortisol would be to find new technologies of measuring cortisol concentration across time. As an essential adrenal hormone necessary for the maintenance of homeostasis, cortisol is a substance that should be carefully quantified within the human body. Chronically elevated or consistently decreased levels of cortisol over time could lead increasingly negative outcomes. Consequently, finding a standardized method of measuring cortisol would prove invaluable from the standpoint of a health provider as well as a scientific researcher.

After being used in research studies as well as in the fields of psychiatry and endocrinology, salivary cortisol presents an easy, non-invasive method of measuring an acute response to stressors. Finger-prick blood spot samples represent another quick, minimally invasive and easily storable method of measuring acute cortisol levels. However, due to the somewhat painful nature of this collection procedure, many subjects or patients can be deterred from submitting to such testing. However, to assess chronic measures of stress, Urinary cortisol is an established method which measures the total free cortisol excreted over 24 hours. However, collecting 24-hour urine samples can prove to be tedious and difficult, while also shedding little light on the acute stress response due to the integrated nature of the results, as was seen in this study.

With cortisol released in a constantly changing circadian rhythm, it is still necessary to find a convenient, easily stored method of measuring the long-term excretion of cortisol. The advent of hair

cortisol marks a significant change in how the hormone can be quantified by researchers and health professionals alike. Collection occurs through cutting a small, often barely noticeable sample of hair from the vertex of the skull. Although slightly uncomfortable, this process is completed quickly while also being minimally invasive. The sample itself can be stored at any temperature in a sterile, sealed container. Additionally, hair samples do not decompose like body fluids or a tissue biopsy.

Also, analysis of the hair sample through assays would allow for a retrospective timeline of cortisol levels. This is important as exposure to cortisol can be compared to development of common conditions such as diabetes, heart disease, depression, and many more. With hair growing at a rate of one centimeter per month, researchers could map the cortisol levels obtained from hair samples against major and minor life events that occurred across the last few months. Findings suggest that there are also high levels of intraindividual stability in hair cortisol concentrations, further supporting the utility of this method (Stalder et al., 2012). Consequently, using hair cortisol to track the changes over time in a chronically sleep deprived population could possibly better inform researchers about the long-term effects of short sleep.

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PROFESSIONAL EXPERIENCE

Morgan Academic Center for Student Athletes January 2016-Present

Employed as a tutor for student athletes focusing on helping them to learn information from a variety of Penn State classes.

EXTRACURRICULARS

Patient Ambassador May 14th – August 20th 2014

Served as a link of communication between the hospital staff and the patients by ensuring that the patients had all amenities that they needed and were finding their hospital experience to be satisfactory.

Gamma Sigma Sigma

Gamma Sigma Sigma is a service sorority that requires sisters to participate in at least thirty hours of sponsored service events per semester such as volunteering at assisted living homes, working at soup kitchens, and babysitting children in the community.

Mortar Board National Honor Society March 2016-Present

Initiated into the Archousi chapter of Mortar Board at Penn State, a national leadership and academic honor society.

RESEARCH

Buxton-Chang Sleep Lab September 2015-Present

As a research assistant at the Sleep Lab, I collaborate with the other assistants to help further the work of Dr. Anne Marie Chang and Dr. Orfeu Buxton.

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Paoli Hospital Key Volunteer Award September 2012

Received the Key Volunteer Award for commitment to service as a volunteer at Paoli Hospital.

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