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EFFECT OF SLEEP RESTRICTION ON POSTPRANDIAL SALIVARY CORTISOL

NATALIYA PERKHALYUK

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Reviewed and approved* by the following:

Orfeu M. Buxton
Professor of Biobehavioral Health
Thesis Supervisor

Helen Kamens-Horton
Assistant Professor of Biobehavioral Health
Honors Adviser

* Electronic Signatures are on file in the Schreyer Honors College.

ABSTRACT

Restricting sleep to meet the demands of increasingly hectic work schedules is becoming common place in the United States. Sleeping less than the recommended number of hours per night disrupts the circadian rhythm of hormone secretion leading to metabolic impairment. Sleep restriction, defined as sleeping 5 hours/night has been linked to increased risk of developing cardiometabolic disease. Previous studies found that one week of sleep restriction significantly reduces insulin sensitivity (Buxton et al., 2010). In addition, restricting sleep for just 5 nights impairs the metabolism of glucose and lipids (Ness et.al., 2019). The aim of the current analysis is to determine whether postprandial salivary cortisol levels following an evening meal (dinner) are altered by prior sleep restriction conditions compared to a sleep replete (well-rested) condition. This study separately analyzed data from two similar sleep restriction studies. Each study investigated the effects of sleep restriction on metabolic response across three sleep conditions: baseline sleep, restricted sleep and recovery sleep. In Study 1, the protocol included three nights of baseline sleep (10 hours/ night), seven nights of sleep restriction (5 hours/ night) followed by one night of sleep recovery (10 hours/ night). In Study 2, the protocol included three nights of baseline sleep (10 hours/ night), five nights of sleep restriction (5 hours/ night) followed by two nights of sleep recovery (10 hours/ night). In Study 1, all meals were equicaloric with a controlled macronutrient composition regarded as a standard meal (STD). Study 1 had no high fat meals. Study 2 contained two identical high fat meals (HFD) for two nights of the study whereas standard meals (STD) were served the remaining days but differed in macronutrient composition. Hourly saliva samples were collected for cortisol assays to determine differences

in pre-and postprandial levels for baseline and sleep restriction conditions. We hypothesized that, compared to baseline extended sleep, restricted sleep will result in an increased level of postprandial salivary (free) cortisol. Our results indicated a significant increase in pre- to postprandial salivary cortisol in the sleep restriction condition compared to baseline sleep in one pair of nights but not the other in Study 1, and no significant difference in pre- to postprandial cortisol in the sleep restriction compared to baseline sleep condition in Study 2. A mixed effect model indicated sleep condition as the significant driver of increased cortisol concentrations in Study 1, whereas in Study 2, the significant driver was meal type. Sleep restriction (5h/ night) for 1 week significantly increased pre to postprandial salivary cortisol following a dinner meal. Pre to postprandial salivary cortisol was also significantly increased after the consumption of a high fat meal regardless of sleep condition. These findings raise concerns regarding sleep restriction and diet and their role in metabolic dysregulation due to increased levels of cortisol.

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Chapter 1

Introduction

Sleep plays a regulatory role in maintaining the homeostasis of various interconnected physiological processes. The Suprachiasmatic Nucleus (SCN), a bundle of nerve cells located within the hypothalamus, produces a central circadian rhythm that dictates the timing of the sleep-wake cycle as well as other circadian rhythms. The SCN is also a central regulator of the timing of hypothalamic- pituitary-adrenal axis (HPA) activity. The hypothalamus initially releases corticotropin releasing hormone (CRH) which acts on the anterior pituitary gland to cause the release adrenocorticotropic hormone (ACTH; Chung et. al., 2011). ACTH then acts on the adrenal gland to release various glucocorticoids (Chung et. al., 2011), such as cortisol, which exhibits a 24-hr rhythm in secretory patterns (Liyanarachchi, et.al., 2016). Physiological changes in response to cortisol include insulin suppression, mobilization of energy stores via gluconeogenesis (generation of glucose from non-carbohydrate carbon sources) and glycogenolysis (break down of glycogen), and immune system suppression.

Most bodily fluids provide accurate circulating cortisol concentrations, including saliva. Circadian rhythms, defined as endogenous processes responsible for regulating the sleep- wake cycle exhibited by humans in a 24-hour period, are controlled by circadian oscillators which coordinate changes in the environment. In people with healthy sleep habits, the circadian rhythm of cortisol follows a steady rise during the latter part of the night with a peak an hour after

awaking to prepare the body for the day's metabolic activity. Following its peak, cortisol steadily declines throughout the day apart from a slight rise following a mid-day meal to reach a trough about 12 hours after awakening (Omisade, et.al, 2010). Ultradian rhythms are physiological changes that occur multiple times within a 24-hour time period whereas a circadian rhythm is a cycle that occurs once in a 24-hour time period (Spiga, et. al., 2017). In addition to following a 24-hour rhythm, cortisol secretion exhibits an ultradian rhythm with a pulse production every 1-2 hours (McMaster et.al., 2011). Studies suggest that the ultradian rhythm of cortisol plays a crucial physiological role in activating glucocorticoid and mineralocorticoid receptors that in turn activate the transcriptional response of glucocorticoid-responsive genes (Spiga, et. al., 2017). The current study tracks cortisol levels over an afternoon-evening time period (1500h to 2100h), as cortisol levels exhibit a circadian-driven decline, to distinguish variations in cortisol levels due to the ultradian rhythm in cortisol from a meal-induced change in cortisol levels under sleep-restricted conditions.

Sleep restriction, defined as sleeping ≤5 hours/night, leads to physiological stress and the mobilization of fuel sources via reactivity of the HPA axis. The mobilization of fuel sources via gluconeogenesis and glycogenolysis increases the level of glucose in circulation. In addition, restricting sleep significantly reduces insulin sensitivity which impedes the body’s ability to regulate glucose levels (Buxton et. al., 2010). A reduction in insulin sensitivity increases the chance of developing diabetes. Several studies found that short sleep has been linked to an increased risk in hypertension, cardiovascular disease, and early mortality (Buxton et. al., 2010). Because cortisol is also a stress response hormone, it also potentiates a sympathetic response from the nervous system that increases blood pressure which, under chronic conditions, can lead to early cardiometabolic disorders and early mortality (Nagai et. al., 2010). Sleep restriction also

contributes to a perceived psychological stress that influences food choice (Lemmens et. al., 2011). Under stress, individuals are more inclined towards sweets and fat food most likely because sweets and fat foods are seen as rewarding (Lemmens et. al., 2011). Consumption of sweets and high fat foods may influence physiological stress by increasing reactivity of the HPA axis and elevating cortisol concentrations (Lemmens et. al., 2011).

This study aims to determine whether post-prandial salivary cortisol levels following an evening meal (dinner) are altered by prior sleep restriction conditions (5 hours of sleep/ night) compared to a well-rested condition (10 hours of sleep/night). The two studies used in this analysis both demonstrated significantly elevated salivary cortisol levels under the sleep restriction condition compared to the well-rested condition during evening hours when cortisol should be declining (Buxton et. al., 2010 & Ness et. al., 2019). We hypothesized that the difference in pre to postprandial cortisol would be greater under sleep restriction conditions when compared to the well-rested condition. Salivary cortisol samples were collected from participants hourly across two sleep restriction studies (N= 35). To assess the effect of sleep condition on postprandial cortisol, salivary cortisol samples were assayed and analyzed based on sleep condition and evening meal.

1.1 Cortisol Circadian Rhythm

Glucocorticoids are essential regulators of metabolic feedback associated with short- and long-term effects on the equilibrium between anabolism and catabolism in peripheral tissue (Stimson, et.al., 2014). In the short term, glucocorticoids stimulate catabolism, the breakdown of energy stores to liberate energy substrates such as glucose, amino acids, and fatty

acids via gluconeogenesis in the liver (Geer et. al., 2014). In addition, they decrease glucose uptake and utilization in skeletal muscle and white adipose tissue by inhibiting the insulin response (Kuo et. al., 2015). As a result of the inhibitory effect of glucocorticoids on insulin, excessively elevated cortisol can result in hyperglycemia and insulin resistance in the long term (Kuo et. al., 2015).

Cortisol is a type of glucocorticoid that is secreted by the adrenal gland. The secretion of cortisol by the adrenal gland follows a circadian rhythm dictated by the SCN located in the hypothalamus. The SCN, the central circadian pacemaker consists of 10,000 neurons subdivided into a ventral core that receives information from the brain stem and retina (Chan & Debono, 2010). The dorsal region is responsible for the rhythmic response. Predominantly, circadian rhythms synchronize to sunlight exposure, which is why humans naturally exhibit diurnal sleeping behaviors, meaning they are awake during day light and asleep during the night.

Studies found that cortisol follows a distinct rhythm of peak levels at around 8:30 am (11,500 ng/dl) and a low of <14ngl/dL at around midnight during a 24 hr cycle (Chan & Debono, 2010). The secretion of cortisol is regulated by the HPA axis and levels across the day are modulated by the circadian rhythm signaled by the SCN. In the HPA axis, neurochemical signals act on the hypothalamus and neurons in the paraventricular nucleus causing the release of corticotrophin-releasing hormone (CRH) and arginine vasopressin which then stimulates adrenocorticotropin hormone (ACTH) synthesis and secretion via the pituitary gland (Chung et. al., 2011). The release of ACTH from the corticotrophin cells of the anterior pituitary gland promotes cortisol secretion from the adrenal gland. Cortisol then acts as a feedback loop to

inhibit its production both on the pituitary and hypothalamic level but not on the SCN (Herman, et al, 2016).

1.2 Cortisol response to sleep restriction

Several studies found that sleep loss from total sleep deprivation (Leproult et al., 1997), partial acute sleep deprivation (Leproult et. al., 1997), or sleep restriction for 7 nights (Buxton et. al., 2010) result in an elevated cortisol concentration in the evening. Measurements of ACTH and cortisol in frequent intervals throughout the day elucidate the role of the HPA axis in elevation of evening cortisol. A sleep restriction study consisting of 2 nights of 4 hours in bed found spontaneous HPA axis hyperactivity due to an overall increase of 19% in daytime ACTH and a total cortisol increase of 21% (Guyon, et.al., 2014). Reduced duration in sleep induced a mild physiological stress response with no psychological change in perceived stress (Guyon, et.al., 2014). Furthermore, the fluctuations in daily cortisol levels under restricted sleep conditions are characterized by a total evening increase of 30% (Guyon et. al., 2014). The significant elevations in evening cortisol may partially explain the adverse effects of sleep restriction in terms of metabolic deficits. The mechanism through which elevated cortisol causes metabolic disturbances is by reducing insulin sensitivity. Sleep restriction of 5 hours/ night for one week resulted in increased afternoon and evening free cortisol concentrations (Buxton et. al., 2010). Elevated evening cortisol likely result in decreased insulin sensitivity assessed the following morning (Van Cauter et. al., 1997). A decrease in insulin sensitivity in turn reduces glucose metabolism, which can lead to metabolic disorders such as diabetes.

1.3 Postprandial cortisol response to meals

The relationship between macronutrient composition of a meal and postprandial cortisol has been studied with a range of findings. Lemmens and colleagues studied the influence of a high carbohydrate versus high protein meal on the change in postprandial cortisol under conditions of high stress, induced by means of a psychological computer test, and rest (Lemmens et.al., 2011). The study found that male participants exhibited increased postprandial cortisol levels in all conditions (high stress- protein, high stress- carbohydrate, rest-protein and rest - carbohydrate) while female participants only exhibited increased cortisol under the rest-protein and stress-protein conditions (Lemmens et. al., 2011). This demonstrated that within the context of this study, men show a higher meal induced postprandial salivary cortisol response compared to women (Lemmens et. al., 2011). Martens and colleagues studied the effect of single macronutrient on cortisol concentration in blood serum among healthy men. They found that postprandial cortisol concentrations were reduced after a protein and fat meal, in the form of a shake, which did not differ from the control meal in total calories while no decrease was present after a carbohydrate meal (Martens et. al., 2012). The control used in this study was water. Furthermore, a study conducted by Alleman and Bloomer investigated the hormonal response to lipid and carbohydrate meals during the postprandial period. The study found no influence of meal type on postprandial cortisol response (Alleman and Bloomer et. al., 2011). These studies assessed postprandial cortisol during a morning and afternoon meal with no sleep restriction variable. No studies have been conducted to assess the influence of macronutrient meal composition on postprandial cortisol under varying sleep conditions.

1.4 Previous studies on sleep restriction and cortisol

In 2010, a study in young adult men collected hourly salivary cortisol levels and reported elevations of mean afternoon and evening cortisol levels following a week of sleep restriction (5hours/night) compared to a sleep replete (10 hours/night) baseline (Buxton, et.al., 2010). Visually, a marked increase in cortisol also followed the evening meal following a week of sleep restriction, however, this same apparent trend was not present under baseline sleep replete conditions. A separate study looking at the impact of sleep restriction on cortisol and leptin levels in young adult women demonstrated the same trend upon visual inspection, but that was not the focus of that paper and meal responses were not statistically evaluated in the paper (Omisade, et.al., 2010). A third study focusing on sleep as a modulator of neuroendocrine function reported elevated postprandial cortisol after a week of sleep restriction (Speigel, et.al., 2004). Recently, a study completed at Penn State by Dr. Chang looked at sleep restriction and postprandial lipemic response (Ness, et.al., 2019). During this study salivary cortisol was collected and assayed but had not yet been analyzed to evaluate this potential alteration of the HPA salivary cortisol following the evening meal will be higher under sleep restriction conditions when compared to sleep replete conditions across 3-4 different studies.

Chapter 2

Study 1 methods

The protocol of study 1 is outlined in Figure 1. All procedures of the randomized, clinical study were approved by the Human Research Committee of the Brigham and Woman's Hospital located in Boston, Massachusetts. The protocol upheld the principles of ethical human research expressed in the Declaration of Helsinki. All participants gave written informed consent.

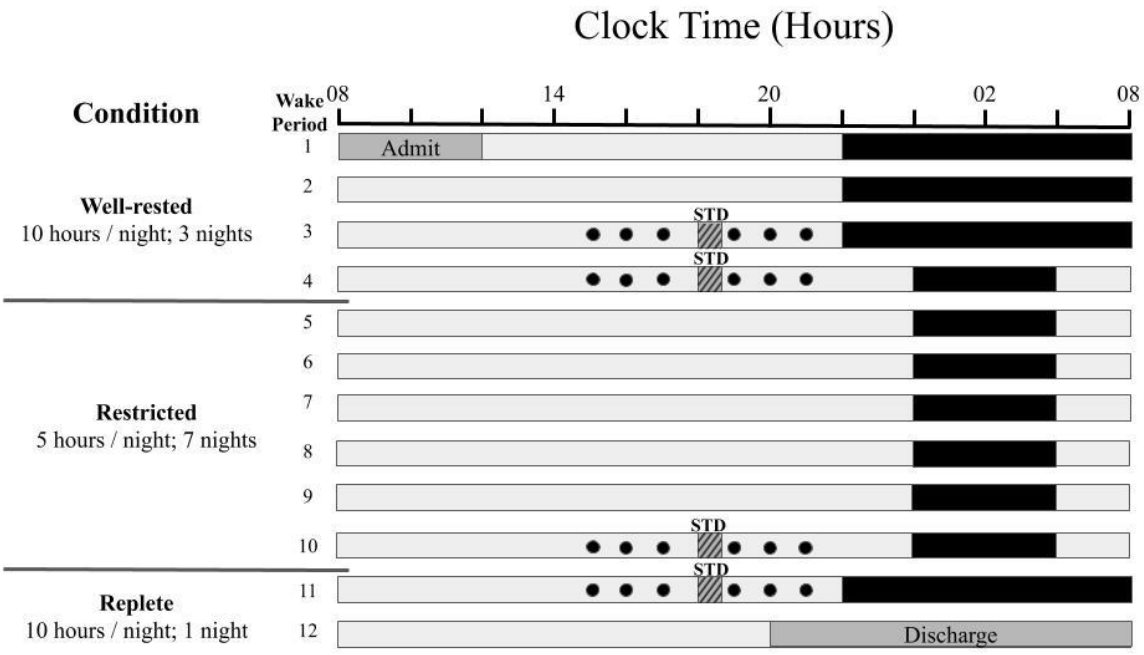

Figure 1. Study 1 Protocol.

The light grey shading indicates the time during which participants were awake (Wake Period, WP). Black shading indicates the time during which participants spent in bed. Black circles indicate the time of salivary cortisol sample collection used for data analysis. Diagonally shaded boxes indicate the dinner meal which was a standard (STD) meal.

2.1 Participant recruitment and screening

Healthy male participants were recruited using flyers, newspaper and website advertisements. Participants were screened for sleep patterns, medical and psychological history. A physical exam conducted by licensed physician ensured all participants were in good health. Urine and blood samples were collected for hematology and serum chemistry testing. All samples came back within normal levels including thyroid and metabolic panels. All urine toxicology screenings came back negative (Buxton, et.al., 2010). Health problems indicated by abnormal physical exam, urine or blood samples results excluded potential participants from the study.

2.2 Pre-study conditions

Prior to starting the experiment, participants were instructed to sleep at home for at least 5 days (mean 8.9 days [range 5-21]) for 10 h per night of time in bed (TIB) from 10PM to 8AM (±1 h) to ensure a controlled sleep replete condition upon entrance to experimental portion of study with minimal sleep debt. TIB was monitored by calling into a time-stamped answering phone answering system, wore wrist activity monitors (Minimitter, Bend, OR) and kept a sleep diary (Buxton, et.al., 2010).

2.3 In study laboratory conditions

Participants were admitted to the General Clinical Research Center at Brigham and Women's Hospital for a 12-day inpatient stay; with procedures occurring when participants were awake **(**defined as a Wake Period, WP). The initial phase of the study started with a 3-day well-rested sleep period of 10/h night TIB with baseline sample collection. Participants were then switched to the sleep restriction schedule (5h/night TIB) for the subsequent 7 nights. This sleep period was constantly centered at 0300 h. During awake hours participants were allowed to read, write, work on their computer, play board or card games, listen to or play music, do arts and crafts and lightly stretch. Participants were continuously monitored by research technicians either directly via in person interaction or remotely by video. Light levels were kept at <1 Lux (complete darkness) during sleep periods. Subjects were randomly selected to receive either modafinil (100 mg per tablet) or placebo (two tablets at 0600h and one tablet at 1300 h) during the seven-day sleep restriction protocol. This study reported no significant difference between the modafinil group and placebo group in terms of evening salivary cortisol and so in this thesis all participants were grouped together (Buxton et. al., 2010).

2.4 Controlled diet

Participants received an equicaloric, controlled macronutrient diet composed of 58-60% carbohydrates, 15-17% protein, 25-27% ± 1% fat, 800-1,000 mg calcium, 100 ±2 mEq potassium, and 200±2 mEq sodium throughout the inpatient protocol. The macronutrient

composition was regarded as a standard meal (STD), i.e., high carbohydrate meal, not a high fat meal as for one of the meals in Study 2. Meals were evaluated for the postprandial cortisol response on dinners provided during wake periods 3 and 10, and 4 and 11 (pairs of wake periods selected for having identical procedures but differing in prior amounts of sleep opportunity).

2.5 Saliva sampling

Pre- and post-prandial saliva samples used for analysis were collected hourly from 15:00 to 21:00 around the meal window of 18:00 to determine free cortisol concentrations on the last two days of the well-rested sleep and sleep restriction.

2.6 Assays

The free cortisol level in each saliva sample was measured using solid-phase radioimmunoassay (Coat-A-Count; DPC, Los Angeles, CA) with sensitivity <0.02 µg/dl and precision 4-5%.

2.7 Statistical analyses

Mean salivary cortisol concentrations were calculated across all subjects by hour from 1500 to 2100 and wake period (time during which the participants were awake). Baseline differences in salivary cortisol were assessed by sleep condition. A paired t-test was run to

indicate the presence of a significant difference in overall mean salivary cortisol concentrations between well-rested sleep and restricted sleep conditions. This was done by taking the average salivary cortisol concentration across all participants for every hour (1500h- 2100h). The second paired t-test was conducted to find evidence of a significant difference in mean salivary cortisol levels at the postprandial collection time of 1900h.

Pre-prandial salivary cortisol was assessed by the 1800h sample, while postprandial salivary cortisol was assessed by the 1900h sample. Pre- to postprandial difference in cortisol concentration was calculated for each sleep condition by subtracting the pre-prandial salivary cortisol concentration (taken at 1800h) from the postprandial salivary cortisol concentration (collected at 1900h) for every participant. The difference in change from pre- to postprandial salivary cortisol was compared between the sleep restriction condition and the well-rested sleep condition for wake periods 3 and 10 (WP3 and WP10) and for wake periods 4 and 11 (WP4 and WP11). A third paired t-test was conducted to determine whether the difference in pre- to postprandial salivary cortisol concentrations varied between sleep restriction and well-rested sleep.

Chapter 3

Study 2 Methods

The protocol of Study 2 is outlined in Figure 2. All experimental procedures were reviewed and approved by the Institutional Review Board at The Pennsylvania State University. The protocol followed the principles established in the Declaration of Helsinki. Informed consent for procedural protocol was obtained in two phases. In the first phase, participants granted written informed consent for screening procedures (described below). After screening procedures, eligible participants met with a senior study investigator and underwent the second phase to grant written, informed consent for the in-laboratory study protocol.

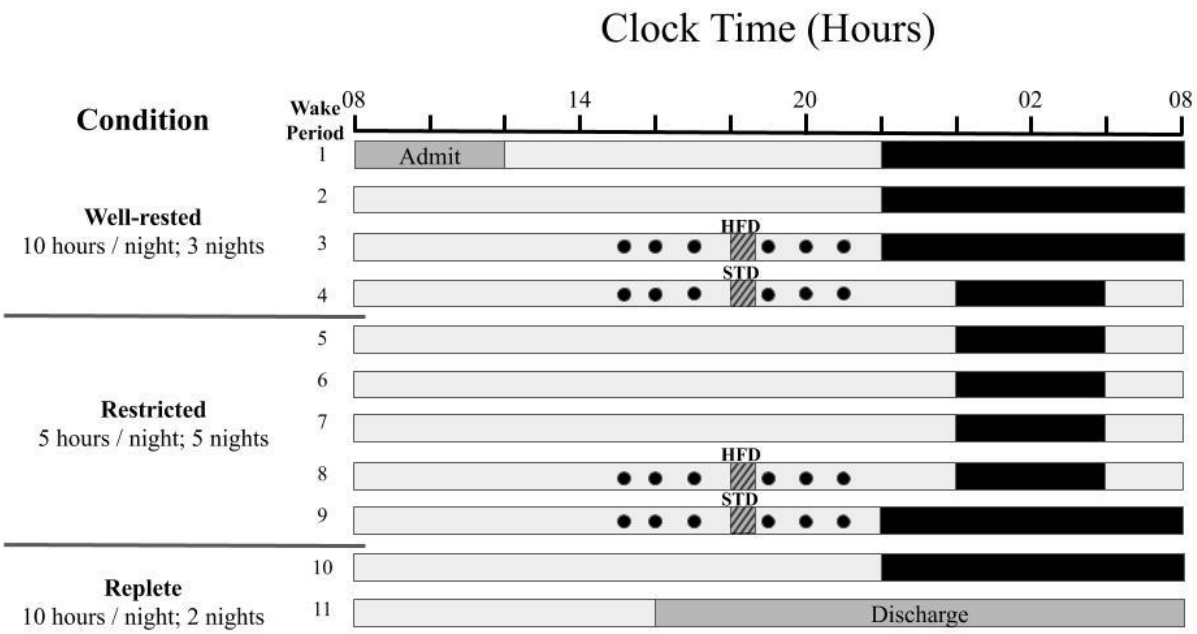

Figure 2. Study 2 Protocol.

The light grey shading indicates the time during which participants were awake (Wake Period, WP). Black shading indicates the time during which participants spent in bed. Black circles indicate the time of salivary cortisol sample collection used for data analysis. Diagonally shaded boxes indicate the dinner meal which was either a standard (STD) meal or a high fat (HFD) meal

3.1 Participant recruitment and screening

Participants were recruited using electronic and posted advertisements proceeded by the completion of a secure online screening questionnaire (Qualtrics, Seattle, WA). Indication of recent medicine and drug/tobacco use, shift work or recent travel across time zones, and current medical disorders, diseases and conditions qualified as exclusion criteria. Once written, informed consent for screening procedures was obtained, eligible participants underwent a physical exam conducted by a study clinician. Exclusion criteria included poor cardiometabolic health [waist circumference >102 cm, body mass index (BMI) ≤ 18 kg/m_2 , seated systolic blood pressure >

130 mmHg or diastolic blood pressure >85 mmHg, $HbA_{1c} \geq 5.7\%$, HDL cholesterol < 40mg/dl, LDL cholesterol ≥ 145 mg/dl, fasting plasma triglycerides ≥ 150 mg/dl, fasting glucose > 100mg/dl]. Participants with a waist circumference > 102 cm were screened for central adiposity/abdominal obesity (a result of the metabolic syndrome related to increased risk of cardiometabolic dysfunction in adults) and excluded. Screening questionnaires on sleep disorders and medical history were also completed in addition to the submission of a urine sample for toxicology screening. A sleep-wake log and actigraphy recording (Spectrum, Philips-Respironics, Murrysville, PA) were completed for 1 wk. prior to the experimental protocol by all participants to determine habitual sleep. All participants were screened by a clinical psychologist, to ensure they were free of psychological disorders and to determine their ability to participate in the in-laboratory protocol as well as their readiness to comply with the experimental protocol.

3.2 Pre-study condition

Prior to starting the in-laboratory protocol, participants were required to maintain a 10-h TIB routine every night for at least 1 week (≥ 6 nights) (22:00-08:00), deviating no more than ±1 h in TIB. Three simultaneous methods assessed sleep protocol: every night participants went to bed and woke up every morning, they called a messaging system to record the exact time; participants kept a sleep-wake log to record bed times and wake times; participants wore and altigraph during the pre-study conditions to record movement, ambient light exposure, and wear/non-wear status. Participants were asked to abstain from alcohol, drugs, tobacco, and

caffeine (coffee, energy drinks, tea, chocolate, etc.). A second urine sample was collected for toxicological screening once participants were admitted to the in-laboratory protocol.

3.3 In laboratory study conditions

The study protocol consisted of 3 conditions (Fig. 2): 3 well-rested sleep nights (days 1-4), with a sleep opportunity (TIB) of 10 h/night; 5 nights of restricted sleep (days 5-9) with 5-h TIB/night; and 2 nights of recovery sleep (days 10-11). Study staff instructed participants when to go to bed (22:00 well-rested and recovery conditions: 00:30 restriction condition) and when to wake up (08:00 well-rested and recovery conditions; 05:30 restricted condition) at designated times to maintain protocol. Under the sleep restriction condition, sleep was reduced evenly from bedtime and wake time to ensure a constant nocturnal midpoint in all conditions.

This reduced phase shifting of the circadian pacemaker and controlled for circadian misalignment as a moderator for sleep loss on metabolism. The participants stayed in their own private room at the Clinical Research Center (Pennsylvania State University) throughout the duration of the study. Wakefulness was monitored constantly (excluding showering time or bathroom use) during wake periods. Light exposure was measured and controlled at 100 lux during wake periods and 0 lux (complete darkness) during sleep periods. Participants were limited to light stretching as their exercise and were instructed to remain upright during wake periods. Sitting or reclining on the bed during wake periods was prohibited.

3.4 Controlled diet

A certified dietician prepared all meals compliant with predetermined macro- and micronutrient content for a specific controlled diet based on protocol. All meals were prepared in the Clinical Research Center's metabolic kitchen. Meal contents were composed accordingly: 55-60% of calories from carbohydrate, 15-17% of calories from protein, 25-30% of calories from fat, 200-1,00 mg calcium/day, 100 2-milliequivalent (meq) K, and 200 2 meq Na. Food quantities were modified based on each participant estimated total daily energy expenditure calculated as an average of the Harris-Benedict and Mifflin-St. Joer equations with low-activity factors 1.1 and 1.5, respectively (Frankenfield et. al., 2005, Harris & Benedict, 1918). All meals (breakfast, lunch, and dinner) were consumed within 30 minutes. During wake periods 3 and 8 (days selected for having identical procedures but differing in prior amounts of sleep opportunity) participants ate a high fat dinner meal (HFD) that was equicaloric to the standard dinner meal (STD) served the remaining days but differed in macronutrient composition. The HFD meal consisted of 1041 kcal composed of 48.9 g of fat, 110.5 g of carbohydrate, and 46.6 g of protein (Ness et. al., 2019b).

3.5 Saliva sampling

Pre- and post-prandial saliva samples were collected hourly from 15:00 to 21:00 around the meal window of 18:00 to determine free cortisol concentrations on the last two days of well-rested sleep and sleep restriction.

3.6 Assays

The free cortisol level in each saliva sample was measured using a cortisol enzyme immunoassay kit (Salimetrics ELISA Kit; State College, PA) with a lower limit of sensitivity of 0.007 µg/dL.

3.7 Statistical analyses

Mean salivary cortisol concentrations were calculated across all subjects by hour from 1500 to 2100 and wake period (time during which the participants were awake). Baseline differences in salivary cortisol were assessed by sleep condition. A paired sample t-test was run to indicate the presence of a significant difference in overall mean salivary cortisol concentrations from 1500h to 1900h between well-rested and restricted sleep conditions. A second T-test was conducted to find evidence of a significant difference in mean salivary cortisol levels at the postprandial collection time of 1900h.

Pre-prandial salivary cortisol was quantified by the 1800h sample, while postprandial salivary cortisol was quantified by the 1900h sample. Pre- to postprandial difference in cortisol concentration was calculated for each sleep condition by subtracting the pre-prandial salivary cortisol concentration (taken at 1800h) from the postprandial salivary cortisol concentration (collected at 1900h) for every participant. The difference in change from pre- to postprandial salivary cortisol was compared between the sleep restriction condition and the well-rested sleep condition for wake periods with the same daily protocol.

A third T-test was conducted to determine whether the difference in pre- to postprandial salivary cortisol concentrations varied between sleep restriction and the well-rested sleep

condition A mixed effects model was utilized to determine whether fixed effects of sleep condition, diet, or both contributed to significant differences in pre-post salivary cortisol concentrations. This model also included random effects for individual intercepts.

Chapter 4

Results

4.1 Participants

Twenty healthy men (mean ± SD: age 26.8 ± 5.2 years; BMI 23.3±3.1 kg/m2) completed Study 1 (see Table 1). Fifteen healthy men (mean ± SD: age 22.33 ± 2.82 years; BMI 24.69 ±2.99 kg/m2) completed Study 2 (see Table 1). Salivary cortisol concentrations were analyzed respective to the participant's study.

Table 1. Participant characteristics

	Study 1	**Study 2**
Mean Age (± SD)	26.8±5.2	22.33±2.82
Mean BMI (± SD)	23.2±3.1	24.69±2.99
Participants	N=20	N=15

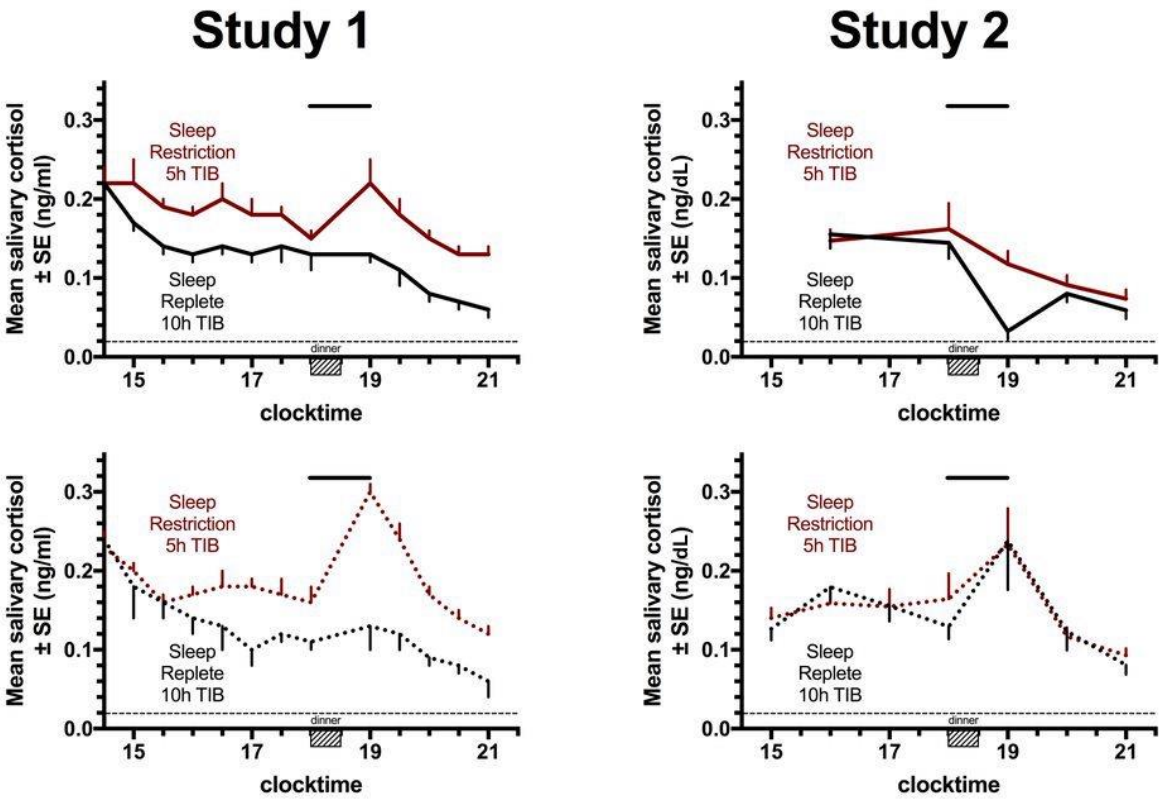

Figure 3. Time series of salivary cortisol concentrations

Salivary cortisol levels were assessed under well-rested sleep (10 h/night TIB [black lines]) and restricted (5 h/night TIB [red lines]). Panels A (WP3&10) and B (WP4&11) depict mean salivary cortisol concentrations (ng/ml)± SEM from Study 1 (wake periods paired based on identical procedural protocol and meal type). Panels C (WP3&8) and D (WP4&9) depict mean salivary cortisol concentrations (ng/dL) ± SEM from Study 2 (wake periods paired based on identical procedural protocol and meal type). Black horizontal line above data depicts the pre to postprandial salivary cortisol concentration difference.

4.2 Study 1 Salivary Cortisol Concentrations

In Study 1, there were no baseline differences in pre to postprandial salivary cortisol within the well-rested sleep condition (WP3 and WP4: $t(38)= 0.999$, $p=0.392$). However, there were baseline differences in pre to postprandial salivary cortisol concentrations in the sleep restriction condition (WP10 and WP11: $t(38)= 3.51$, $p=0.002$).

Figure 3A, B shows a visual timeline of mean salivary cortisol concentrations (ng/ml) with the standard error of measure from hours 1500 to 2100 under sleep restriction (red) and well-rested sleep conditions (black). Concentrations from samples taken before or after the scheduled time were interpolated to appropriately align with the hourly schedule. In Study 1, salivary cortisol concentrations were elevated under the sleep restriction condition compared to the well-rested sleep across both pairs of nights (assessed between 1500-2100 h) (Figure 3A,B). When comparing WP3 to WP10, postprandial salivary cortisol under sleep restriction (WP10) had a mean ($\pm$ SEM) concentration of 0.217 ng/ml $\pm$ 0.029 and a mean concentration of 0.132 ng/ml $\pm$ 0.062 under well-rested sleep condition (WP3) ($t (38)= 4.17$, $p <0.001$) (Figure 3A). When comparing WP4 to WP11, postprandial salivary cortisol under sleep restriction (WP11) had a mean ($\pm$ SEM) concentration of 0.287ng/ml$\pm$ 0.049 and a mean concentration of 0.128 ng/ml $\pm$0.096 under well-rested sleep (WP4) ($t (38)=4.29$, $p < 0.01$) (Figure 3B). Postprandial salivary cortisol was significantly increased in the sleep restriction condition compared to the well-rested sleep condition for both sleep restriction nights (WP3 & WP10: $t(38) = -2.61$, $p= 0.013$, WP4 & WP11: $t(38) = -2.96$, $p= 0.005$) (Figure 3A, B).

4.3 Study 2 Salivary Cortisol Concentrations

In Study 2, the pair of wake periods 3 and 4 (WP3 and WP4) occurred within the well-rested sleep condition (10 hours of sleep per night) while the pair of wake periods 8 and 9 occurred during restricted sleep (5 hours of sleep per night). In Study 2, there were no baseline differences in pre to postprandial salivary cortisol concentrations in response to identical meals compared across both the well-rested sleep condition (WP3 and WP4) and sleep restriction condition (WP8 and WP9) (WP3 and WP4: $t(27)= 0.963$, $p=0.344$, WP8 and WP9: $t(27)= 0.272$, $p=0.654$).

Figure 3C, D depicts mean salivary cortisol concentrations (ng/ml) with the standard error of measure from hours 1500 to 2100 under sleep restriction (red) and sleep conditions (black). Concentrations from samples taken before or after their scheduled time were interpolated to align with the scheduled hourly timeline. When comparing WP3 to WP8, salivary cortisol concentrations were elevated in the sleep restriction condition (WP8) compared to well-rested sleep (WP3) ($t (27)= 1.50$, $p= 0.5$) (Figure 3C). When comparing WP4 to WP9, salivary cortisol concentrations did not differ between sleep restriction (WP9) and well-rested sleep (WP4) ($t (27)= .278$, $p= 0.6$) (Figure 3D). For WP3 and WP8, postprandial salivary cortisol under sleep restriction (WP8) had a mean ($\pm$ SEM) concentration of 0.096 ng/dL $\pm$0.012 and a mean concentration of 0.118 ng/dL $\pm$0.017 under well-rested sleep (WP3) (Figure 3C). The mean postprandial salivary cortisol did not differ significantly between WP3 and WP8, $t(27) = -1.13$, $p = .267$. For WP4 and WP9, postprandial salivary cortisol under sleep restriction (WP9) had a mean ($\pm$ SEM) concentration of 0.238 ng/dL $\pm$0.062 and a mean concentration of 0.233

±0.046 under well-rested sleep (WP4) (Figure 3D). The mean postprandial salivary cortisol did not differ significantly between WP4 and WP9, t(27) = 0.06, p = .952(Figure 3D).

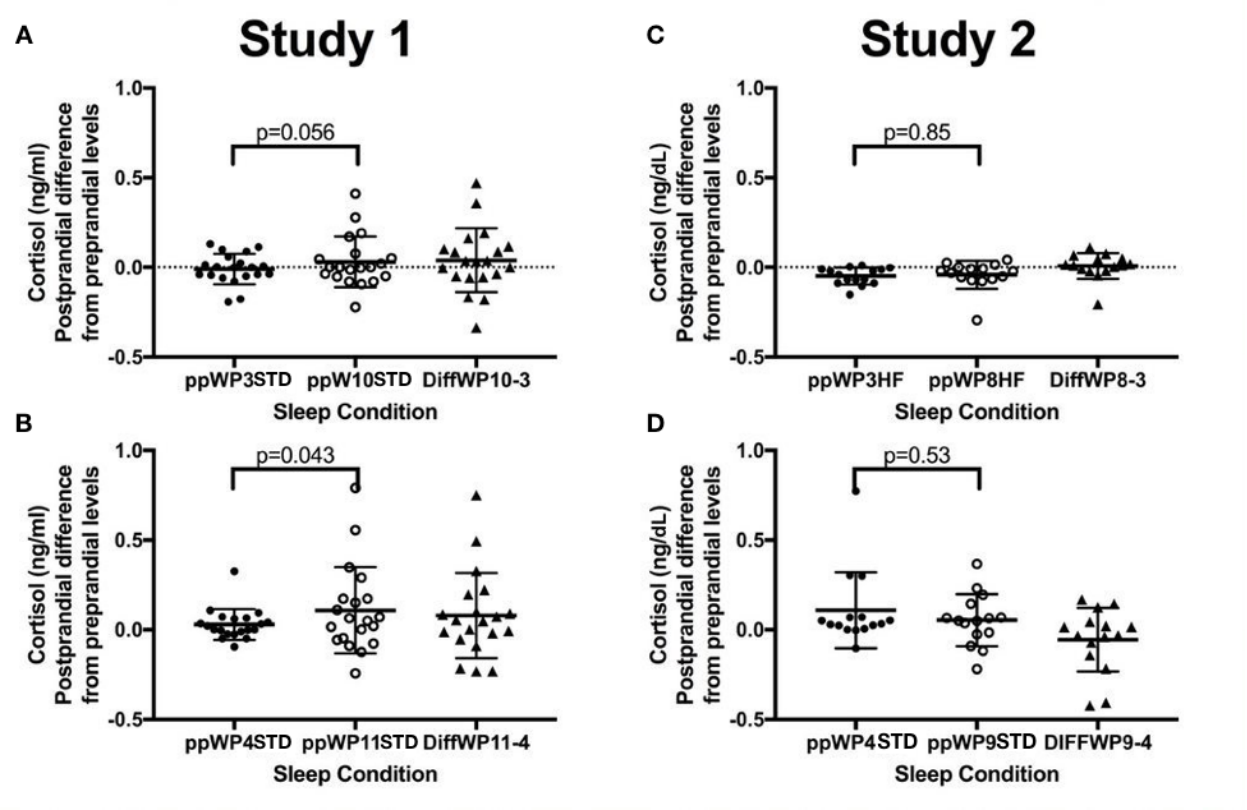

Figure 4. Pre to postprandial differences in salivary cortisol.

Left panels depict Study 1, right panels depict Study 2, for mean differences (on paired wake periods [WP] with identical procedures and meals) in postprandial (pp) salivary cortisol ±SD, as well as individual values. STD: standard dinner meal (panels A, B, and C). HF: high fat dinner meal (panel D). The differences in postprandial differences (Diff) is depicted by the right most column within each panel (triangles). P-values shown are the results of mixed model analyses.

4.4 Study 1 Postprandial differences in salivary cortisol concentrations

Figure 4A shows pre to postprandial differences in salivary cortisol for each subject by wake period (ppWP3STD and ppWP10STD) along with the difference between wake periods (DiffWP10-3). The pre to postprandial salivary cortisol difference for the sleep restriction condition in WP10 was not significantly different from the difference in pre to postprandial cortisol in the well-rested sleep condition, WP3 ($t(38) = -1.97$, $p = 0.056$) (Figure 4A). Figure 4B shows pre to postprandial differences in salivary cortisol for each subject by wake period (ppWP4STD and ppWP11STD) along with the difference between wake periods (DiffWP11-4). The pre to postprandial salivary cortisol difference for the sleep restriction condition in WP11 was significantly different from the difference in pre to post prandial cortisol in the well-rested sleep condition, WP4 ($t(23.5) = -2.15$, $p = 0.043$) (Figure 4B).

4.5 Study 2 Postprandial differences in salivary cortisol concentrations

Figures 4C shows pre- to postprandial differences in salivary cortisol for each subject by wake period (ppWP3HF and ppWP8HF) along with differences between wake periods (DIFFWP8-3) with identical meals. The pre- to postprandial salivary cortisol difference for the sleep restriction condition in WP8 was not significantly different from the pre- to postprandial difference in the well-rested sleep condition, WP3 ($t(27) = -0.19$, $p = 0.851$) (Figure 4C). Figure 4D shows pre to postprandial differences in salivary cortisol for each subject by wake period (ppWP4STD and ppWP9STD) along with the difference between wake periods (DIFFWP9-4). The pre- to postprandial difference for the sleep restriction condition in WP9 was not

significantly different from the pre- to postprandial difference in the well-rested sleep condition, WP4 (t (27) =0.64, p= 0.529) (Figure 4D).

4.5 Mixed model analysis of study 2

A mixed model analysis was used to determine whether meal type and/or sleep condition had a significant impact on fluctuations in salivary pre- to postprandial cortisol level differences. In Study 2, the meal had a significant effect on the change in pre- to postprandial salivary cortisol levels $F(1, 43) = 13.02$, $p < 0.001$, whereas sleep condition did not $F(1, 43) = 0.47$, $p= 0.058$ (Table 2, Figure 4).

Table 2. Study 2 mixed effects model results

Effect	Numerator DF	Denominator DF	F value	P value
Sleep	1	43	0.47	0.058
Meal	1	43	13.20	0.0008

Chapter 5

Discussion

This study evaluated salivary cortisol levels from two separate cohorts of healthy men each undergoing a distinct clinical sleep restriction study. This study expanded on previously reported findings of disruptions in cortisol circadian rhythm by examining cortisol concentrations after a dinner meal in baseline and sleep restriction conditions. We demonstrated that postprandial salivary cortisol levels are affected by both sleep restriction conditions and meal type. In study 1, sleep condition was the variable associated with an elevated postprandial salivary cortisol concentration whereas, in study 2 meal type was associated with an elevated postprandial salivary cortisol concentration. These finding support the initial hypothesis that sleep restriction affects postprandial salivary cortisol concentrations but also introduces a novel finding that meal type too affects postprandial salivary cortisol concentrations after a dinner meal.

5.1 Comparing salivary cortisol concentrations in Study 1 and Study 2

Previous studies have reported elevated levels of cortisol under sleep restriction conditions compared to baseline sleep (Buxton et al, 2010). This study found significantly higher

levels of cortisol under the sleep restriction condition compared to baseline sleep during the 1500h to 2100h time frame in Study 1 but not in Study 2. Although both studies contained a similar participant pool with comparable characteristics, the protocol of the two studies differed slightly. The two studies varied in the number of nights of sleep restriction with Study 1 consisting of 7 nights of 5h TIB and Study 2 consisting of 5 nights of 5h TIB. The extra two nights of sleep restriction could explain why an elevated cortisol concentration was seen in the evening hours from 1500h to 2100h in Study 1 but not in Study 2. Guyon and colleagues conducted a study that looked at two nights of sleep restriction and two nights of baseline sleep found significantly elevated overall concentrations of free cortisol, however, this was mostly attributed to elevations in morning cortisol since evening cortisol concentrations were unchanged (Guyon et. al., 2014). Furthermore, in the study conducted by Guyon and colleagues, the protocol for sleep restriction was defined as 4h TIB and baseline sleep as 10h TIB (Guyon, et.al., 2014). A study conducted by Spiegel and colleagues found elevations in evening cortisol in the sleep debt condition, defined as six nights of 4 hours of sleep per night (Spiegel et. al., 1999). However, this was compared to recovery sleep, defined as six nights of 12 hours in bed per night, following the sleep debt and so the protocol was different from that of Study 1 and Study 2 where the extended sleep condition was prior to sleep restriction (Spiegel et. al., 1999). Reynolds and colleagues reported elevated cortisol during a night sleep restriction protocol, defined as 4 hours of sleep per night, however this finding was attributed to significant increases in afternoon cortisol concentrations and not evening (Reynolds et. al., 2012). In this current analysis of Study 1 and Study 2, aside from the difference in the number of sleep restriction nights the protocols were essentially identical.

The salivary cortisol concentration following the dinner meal, at 1900h, was significantly higher under the sleep restriction condition compared to baseline sleep in both pairs of nights for Study 1 (WP3+10 and WP4+11). In Study 2, postprandial salivary cortisol concentration following the dinner meal at 1900h was not significantly different in the sleep restriction condition compared to baseline sleep in either pairs of nights (WP3+8 and WP4+9).

5.2 Differences in pre to postprandial salivary cortisol between sleep conditions

The pre to postprandial differences in salivary cortisol were compared in sleep restriction and in baseline sleep conditions as well as between conditions for both studies (difference in pre- to post-prandial delta's between sleep restriction and baseline sleep). Study 1 showed a significantly larger increase in pre to postprandial cortisol in the sleep restriction condition compared to the baseline condition but only for the second pair of nights (WP4 and WP11). The differences in postprandial responses between sleep conditions were not significant in the first pair of nights (WP3&10) for Study 1 or either pairs of nights in Study 2 (WP3&8 and WP4&9).

The significant difference between pre and postprandial salivary cortisol between sleep conditions is crucial in understanding the effect that sleep restriction can have on metabolic health. An increased level of cortisol, regardless of time, poses dangerous effects on metabolic health. Several sleep restrictions studies found that elevations in cortisol impairs glucose metabolism (Reynold et.al., 2012, Buxton et. al., 2010, Spiegel et. al., 1999). Elevated cortisol has been observed in numerous studies that also observed decreased insulin sensitivity in numerous studies, although the cortisol increase, and insulin sensitivity reduction were

uncorrelated (Buxton et. al., 2010, Nedeltcheva et. al., 2009, Spiegel et. al., 1999). The literature indicates that the activation of the hypothalamic-pituitary-adrenal axis is responsible for neuroendocrine dysregulation caused by sleep restriction (Hirotsu et. al., 2015). Increased levels of cortisol have various effects on metabolism such as activation of gluconeogenesis in the liver which results in the production of glucose from non-carbohydrate carbon sources for energy expenditure (Kuo et. al., 2015). In addition to increased levels of circulating glucose, the inhibitory effect of cortisol on insulin in white adipose tissue and skeletal muscle explains the dangers of chronic elevation in cortisol levels (Kuo et. al., 2015). Hyperglycemia combined with decreased insulin sensitivity increased the chances of developing obesity and diabetes (Buxton et. al., 2010). Further studies should be done to assess whether these results are able to be replicated.

5.3 Novel Finding

In Study 2, baseline measures in pre to postprandial salivary cortisol did not differ significantly within the well-rested sleep condition or within the sleep restriction condition. Participants were given a high fat dinner (HFD) opposed to a standard dinner (STD) during the second pair of nights. In Study 2, WP4 and WP9 which had an identical high fat dinner (HFD) meal composed of 48.9 g of fat, 110.5 g of carbohydrate, and 46.6 g of protein, both showed an increase in pre to postprandial salivary cortisol even though the increase was not significantly different between sleep conditions. A mixed effect model found that meal type was the driving factor in pre to postprandial change in salivary cortisol while sleep condition had no significant effect. The dinner meal during these wake periods was a standard meal. This novel finding

shows that not only can sleep condition affect the metabolic response to food, as indicated by the findings from Study 1, but also, meal type influences metabolic body function.

A study looking at how macronutrient composition of a meal affects cortisol levels found that a high protein meal causes the greatest increase in cortisol 30 min after a 1200h and 1600h meal compared to a high carbohydrate, high fat and standard meal (Slag, et.al., 1981). However, other studies report conflicting findings with a high carbohydrate meal resulting in the greatest increase in cortisol compared to a high fat and high protein meal as well as a high fat meal as resulting in the greatest increase in cortisol (Martens et. al., 2010, Stachowicz & Lebiedzińska, 2016). There are no definitive findings in the literature regarding macronutrient composition of a meal and its effect on cortisol levels. Prior work on macronutrient composition of meals on changes in postprandial cortisol failed to assess evening cortisol, with most studies looking at changes following a morning meal, an afternoon meal or both (Martens et. al., 2010, Stachowicz & Lebiedzińska, 2016). Furthermore, no prior work studied the influence of macronutrient composition of a meal on postprandial cortisol under various sleep conditions (well-rested sleep vs. restricted sleep).

Chapter 6

Limitations and future directions

This study analyzed data from two sleep restriction studies with similar protocols to investigate whether sleep restriction affected postprandial salivary cortisol levels. Although there were significant findings, there are a lack of reference studies to explain the findings. This section will discuss limitations of this study as well as propose future studies.

6.1 Overall Limitations

The first limitation of this study was that it analyzed data from a relatively small pool of participants, N=35. In addition to the small pool, all participants were males and within a young age range with a mean age of 26.5 years old. In order to achieve a more representative result, the participant pool should be expanded and diversified in terms of gender and age. The protocols of the two studies compared were very similar but did differ in the number of sleep restriction nights. Study 1 saw significantly elevated pre- to post-prandial salivary cortisol concentrations on days 6 and 7 of sleep restriction. Study 2 however, only had a five-night sleep restriction

protocol and did not show any significantly elevated pre- to postprandial cortisol levels on days 4 and 5 of sleep restriction.

6.2 Future directions

The behavior of restricting sleep remains prevalent in today's society. Studies have found that cortisol contributes to reduced insulin sensitivity and is associated with decreased insulin secretion. The metabolic disfunction resulting from cortisol can have long term effects on human health such as weight gain, the development of obesity and diabetes.

To investigate the effect of meal type on postprandial cortisol, novel sleep restriction studies should include a wider range of meal types for comparison. For example, such a study protocol would include days with a high fat, high carbohydrate and high protein meal during sleep restriction conditions and well-rested sleep conditions for comparison. By determining the effect meal type has on postprandial salivary cortisol concentration under sleep restriction conditions, new dietary guidelines can be introduced to both the general public and those who are most susceptible to developing metabolic disorders as a result of sleep behaviors.

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ACADEMIC VITA

NATALIYA PERKHALYUK

nataliyaperks@gmail.com

PROFESSIONAL SUMMARY

Motivated student currently working towards a bachelor's degree in Biological Sciences with an indented graduation date in May 2020. Excellent administrative and logistical skills. Presently working as an undergraduate researcher in a clinical sleep laboratory while writing a thesis in fulfillment of an honors diploma at the Pennsylvania State University. Adept at following experimental protocol, collecting and organizing data.

EDUCATION

Bachelor of Science: Biology, 2020

Pennsylvania State University - University Park, PA

- Schreyer Honors College Scholar

Relevant Coursework: Cellular biology, molecular biology, physiology, cadaver anatomy, organic chemistry, inorganic chemistry, English technical writing, research application writing

Senior Thesis: The effect of sleep restriction on post-prandial cortisol

EXPERIENCE

Research Assistant 08/2018 to Current

Pennsylvania State University – State College, PA

- Worked with principal investigators to coordinate qualitative research aimed to enhance sleep quality with sound. Carried out approved protocols on human subjects and recorded and analyzed data for reoccurring trends.

Learning Assistant 08/2019 to Current

Pennsylvania State University- State College, PA

- Facilitated learning among undergraduate students in organic chemistry. Assisted with homework, in class discussions and led weekly workshops outside of the classroom.

Secretary- Vaccinate America 09/2018 to Current

Pennsylvania State University- State College, PA

- Spoke to parents at local schools about vaccination timelines for children and helped clarify myths about vaccines. Developed advertising strategies aimed at college students to increase seasonal flu vaccination rates on campus.

English Tutor 01/2019 to Current

Mid-State Literacy Council- State College, PA

Taught English grammar, vocabulary and conversation to recent immigrants wishing to improve their English. Compiled lesson plans and assignments to enrich student learning.

Doctor Shadowing 01/2019 to 05/2019

Milton S. Hershey Medical Center – Hershey, PA

- Observed firsthand medical procedures in the radiology department and learned about various medical imaging techniques.

LEADERSHIP

- Troop leader for Plast Ukrainian Scouting Organization in New York City
- Youth Leadership Counselor for summer camps in New York and Germany
- Executive Board member- Vice President of Ukrainian club

SKILLS

Decision making, critical thinking, problem solving, organization, communication, teamwork, creativity, adaptability

Microsoft software, SPSS, scientific writing, data analysis, specimen handling