IMPACT OF MENSTRUAL CYCLE PHASE ON METABOLIC EFFECTS OF SLEEP RESTRICTION

by

Amanda L. LeRoux

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ABSTRACT

The effects of one night of 3h sleep on cortisol levels were assessed in two groups of women at different points in their menstrual cycles: mid-follicular and mid-luteal. Eighteen women (age: 21.8 ± 0.54; BMI: 22.6 ± 0.63, mean ± SEM) were studied. Salivary samples were collected at six times during two consecutive days: first after a 10 h overnight sleep opportunity and then after a night with a 3 h sleep opportunity. Secondary analysis examined the impact of sleep restriction on self-reports of hunger ratings and mood. Women in the follicular phase showed a significant decrease ($p = 0.004$) in their cortisol awakening responses after sleep restriction and an elevation in afternoon/evening cortisol levels ($p = 0.008$), whereas women in the luteal phase showed no change. Overall group increases in hunger and deterioration in fatigue and vigour were observed. Menstrual cycle phase dramatically altered the responses of women to a single night of sleep restriction.

LIST OF ABBREVIATIONS USED

ACTH	Adrenocorticotropic Hormone
ALLO	Allopregnanolone
ANOVA	Analysis of Variance
AUCg	Area Under the Curve with Respect to Ground
AUCi	Area Under the Curve with Respect to Increase
BMI	Body Mass Index
CAR	Cortisol Awakening Response
CBT	Core Body Temperature
CRH	Cortisol Releasing Hormone
CTRL	Control
ELISA	Enzyme-Linked Immunosorbent Assay
FP	Follicular Phase
HAM-A	Hamilton Anxiety Rating Scale
HDRS	Hamilton Depression Rating Scale
HPA	Hypothalamic-Pituitary-Adrenal
IWK	Izaak Walton Killam
LH	Luteinizing Hormone
LL	Late Luteal
LLPDD	Late Luteal Phase Dysphoric Disorder
LP	Luteal Phase
LSC	Life Sciences Centre\
MEQ	Morningness-Eveningness Questionnaire
MetS	Metabolic Syndrome
MF	Mid-Follicular
PAI	Personality Assessment Inventory
PMDD	Premenstrual Dysphoric Disorder
PMS	Premenstrual Syndrome
POMS	Profile of Mood States
PSD	Partial Sleep Deprivation
PSG	Polysomnography
REM	Rapid Eye Movement
SCN	Suprachiasmatic Nucleus
SE	Sleep Efficiency
SOL	Sleep Onset Latency
SWS	Slow-wave Sleep
TMD	Total Mood Disturbance
Ultra-SSW	Ultra Short Sleep Wake

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CHAPTER 1

INTRODUCTION

1.1 Overview

There is extensive evidence that spontaneous short sleep and imposed sleep restriction are linked to altered metabolic and endocrine function. Sleep restriction has been associated, for example, with increased cortisol and decreased leptin levels and increased appetite, especially for carbohydrate- and fat-rich foods (Brondel et al., 2010; Taheri et al., 2004; Spiegel et al., 2004). While most of these studies on this topic have included only male participants, a few recent studies have confirmed some of these findings in women (Omisade et al., 2010; Patel et al., 2006). One such laboratory study involving females showed effects of sleep restriction on cortisol and leptin levels, but a retrospective analysis of the data also suggested that menstrual cycle phase was a strong modulator of these effects of sleep loss. These preliminary results suggest that reproductive hormones may have a significant impact on how sleep loss affects the metabolic endocrine system in women (Omisade et al., 2010). The object of the work presented in this thesis is an attempt to systematically address the issue of whether menstrual phase affects endocrine and behavioural responses to acute sleep loss.

1.2 Cortisol and Circadian Function

Cortisol is a steroid hormone secreted by the adrenal cortex that has very broad physiological actions, including effects on metabolism, glucose utilization and the secretion of other hormones. Although cortisol is released acutely in response to stress, it

also demonstrates prominent spontaneous circadian variation in the absence of stressors. The daily cortisol pattern is largely unaffected by age, weight, or time of waking (Edwards et al., 2001; Van Cauter et al., 1997). Under normal sleep-wake conditions, individuals with healthy Body Mass Indices (BMIs) and without disease of the hypothalamic-pituitary-adrenal (HPA) axis show rising cortisol levels late in the night, a further peak within 30-45 min after waking (the cortisol awakening response; CAR, Clow et al., 2010) and a steady decrease throughout the day, with a nadir at approximately 12 h after waking (Edwards et al., 2001; Wust et al., 2000; Taheri et al., 2004). Figure 1 depicts the daily pattern of salivary cortisol in humans, along with the CAR. In addition, there is evidence in both humans and animals that the circadian oscillator (pacemaker) in the hypothalamic suprachiasmatic nucleus (SCN) is responsible for controlling this daily cortisol rhythm. It is for this reason that cortisol is sometimes used as an indicator of the integrity of the human circadian system (Clow et al., 2010; Buijs et al., 2003).

Cortisol can be measured from most bodily fluids, but salivary levels are often studied because they are easy to obtain and include only the free (unbound) fraction of cortisol that is bioavailable, and are highly correlated with cortisol plasma levels. A large proportion of cortisol in the body is bound to large proteins and carried throughout the body in the blood. However, only a small fraction of cortisol is thought to be biologically active. Due to its low molecular weight and lipophilic nature, unbound cortisol is able to passively diffuse into cells, making it feasible to measure the free cortisol levels in all bodily fluids, including saliva (Kalman & Grahn, 2004).

Sleep loss has repeatedly shown to alter the daily pattern of cortisol levels in men, principally by elevating afternoon cortisol levels. This effect has been found in studies that use either a single night of total or partial sleep deprivation (Leproult et al., 1997) or several nights of continuous partial sleep loss (Spiegel et al., 1999; Spiegel et al., 2004: Wu et al., 2008). It is likely that women have been specifically excluded from laboratory studies looking at the impact of sleep loss on endocrine rhythms due to the added complication of their reproductive monthly menstrual cycles. However, in a recent large-scale epidemiological study, self-reports of shorter sleep duration were correlated with lower morning cortisol levels in both men and women (Kumari et al., 2009). A recent study involving only women reported an elevation of cortisol levels in the afternoon and a decrease in morning cortisol levels after a single night of sleep restriction (3 h sleep opportunity), and a depression of the morning cortisol peak (Omisade et al., 2010). The changes in morning cortisol values have not been discussed in prior studies performed in men.

Many studies have shown the importance of sleep for the regulation of metabolic processes, and sleep restriction is accompanied with sustained elevation of cortisol during the day following sleep loss. This sustained elevation of cortisol has serious metabolic consequences as it mimics the effects of chronic stress by altering glucose and lipid metabolism. Elevated cortisol concentrations have also been correlated to metabolic disorders, such as the Metabolic Syndrome (MetS) and insulin resistance (Plat et al., 1999; Whitworth et al., 2005; Anagnostis et al., 2009). MetS is a disorder characterized by abdominal obesity, dyslipidemia, hyperglycemia, and hypertension (Cornier et al.,

2009). Elevated cortisol has also been shown to induce insulin resistance through inhibiting the peripheral utilization of glucose and by decreasing the translocation of glucose to the cell membrane of white and red blood cells, and rat hippocampal cells (Rizza et al., 1982; Piroli et al., 2007). Both insulin resistance and metabolic syndrome have also been associated with sleep loss (Prinz, 2004; Gottlieb et al., 2005; Stranges et al., 2010). Sufferers of Cushing's syndrome- a disorder characterized by high cortisol levels- also display symptoms similar to that of MetS, such as elevated waist circumference, and increases in lipid and glucose levels (Chanson, 2010).

1.3 Sleep/Circadian Variation Across the Menstrual Cycle

The spontaneous menstrual cycle of women in their reproductive years is characterized by rhythmic changes in ovarian hormone levels with a periodicity of approximately 22-36 days. Levels of the steroid hormones estradiol (the principal estrogen in non-pregnant women) and progesterone, the main progestin, change dramatically during the menstrual cycle. Estradiol levels are high during the **follicular phase** (FP) of the menstrual cycle during which an ovum develops, and peaks just before ovulation. Following ovulation, the ovarian follicle forms a corpus luteum that secretes high levels of progesterone, characteristic of the **luteal phase** (LP) of the cycle. When fertilization and implantation of the ovum do not occur, hormone levels fall and the uterine lining begins to shed (menses) (Jabbour et al., 2006). While these hormonal cycles are principally involved in the development of the ovum and preparation for implantation and pregnancy, they also have a number of effects on sleep patterns, body temperature, appetite, behaviour and mood (Baker et al., 2007).

There is some evidence supporting the interaction between menstrual phase and sleep. Manber & Bootzin (1997) studied the changes of sleep across the menstrual cycle in healthy women (n=32). A variety of different measures were recorded twice a day in diaries, such as bedtime, wake-up time, time to sleep onset, sleep quality, number of mid-sleep awakenings, sleep onset after mid-sleep awakenings, difficulty waking up in the morning, and feeling alert and refreshed in the morning. They also reported sleep quality on a 1 to 5-likert scale: 1 (very poor) to 5 (very sound). They found no change in sleep duration, but sleep disturbances increased, with poorer sleep quality in the late luteal phase as compared with the mid-follicular phase (Manber & Bootzin, 1997). A more recent study by Baker & Driver (2004) showed similar results. Ovulatory cycles were confirmed by measuring the surge of luteinizing hormone (LH) that occurs right before ovulation. They found that young women (n= 26) without significant menstrual-associated complaints reported poorer sleep quality 3 to 6 days premenstrually and during the first 4 days of menstruation. There was also a delay in sleep onset and an increased number of awakenings premenstrually.

However, the findings of some research have failed to provide evidence for an impact of menstrual cycle on sleep. Laessle et al. 1990, investigated changes in mood and autonomic variables from 30 women across a single menstrual cycle. The women reported the length of their sleep and sleep quality on a likert scale of 1 to 5, similar to the measure used by Manber & Bootzin (1997). They found no change in sleep quality or sleep duration in young women who had normal menstrual cycles. However no measures

other than sleep duration and quality were used to measure sleep. Thus, although these two measures are reliable, they are broad and might have missed some sleep disturbances that were detected in the other studies that assessed more aspects of sleep such as sleep disturbances, sleep onset latency and sleep efficiency. Overall, from these results, the studies that measured more sleep features reported that sleep quality was lowest premenstrually, along with some sleep complaints being carried over to early menses during the late luteal and early follicular phases.

Experimental studies documenting nocturnal polysomnographic (PSG) sleep can demonstrate whether or not the neurophysiology of sleep in women differs across the menstrual cycle. Sleep is typically divided two broad types: non-rapid eye movement (NREM), which is further divided into 3 stages, and rapid eye movement (REM). The results of these PSG studies in women indicate sleep onset latency (SOL), sleep efficiency (SE), and NREM slow wave sleep (SWS) are stable across the menstrual cycle, whereas decreases in REM sleep are observed during LP compared to FP (Schechter et al., 2010; Baker & Driver, 2007). There are also reports of higher amounts of NREM Stage 2 sleep during the LP, increasing from 49.6% during the FP to 54.7% in the LP (Driver et al., 1996). Nevertheless, PSG findings are not unequivocal, and inconsistencies persist regarding the variation of SWS (Ito et al., 1993; Baker et al., 2002) and REM sleep across the menstrual cycle (Shechter et al., 2010; Lamarche et al., 2007; Baker et al. 2007; Baker et al., 2001; Ito et al., 1993). A review article by Baker & Driver (2007) states the only consistent theme across the literature is a robust increase in spindle frequency activity during stage 2 sleep and a minor decrease in REM sleep during the LP.

Menstrual phase has also been reported to have an effect on daily rhythms of body temperature and hormones such as cortisol and melatonin (Baker & Driver, 2007). Compared to the FP, body temperature is elevated and the amplitude of the temperature rhythm is reduced during the LP (Baker et al., 2002). There is also evidence that the amplitude of the melatonin rhythm may be blunted during the LP (Brun et al., 1987). Results related to cortisol rhythms have been inconsistent and are limited in scope. Studies using different methods have found delays (Parry et al., 1994), advances (Parry et al., 2000) or no change in the timing and amplitude of cortisol concentrations between the follicular and luteal phases of the cycle (Bloch et al., 1998; Steiner et al., 1984).

Parry et al. (1994) studied 11 healthy female controls and 20 women with late luteal phase dysphoric disorder (LLDD: also called premenstrual dysphoric disorder, PMDD). PMDD is a more severe form of premenstrual syndrome (PMS), which is characterized by a collection of physiological symptoms with or without emotional symptoms. Cortisol levels were measured every 30 min from 18:00 to 09:00 h during the mid-follicular (MF) and late luteal (LL) phases. They found that the cortisol peak was significantly *delayed* in the LL compared with the MF phase in normal control, but not in LLPDD, subjects (Parry et al., 1994). In a separate study of 15 women with PMDD and 15 normal control subjects, Parry et al. (2000) again observed altered timing but not altered levels of cortisol in PMDD. However, in this study, the cortisol acrophase was *advanced* by ~1 h in the LL versus MF phase in normal control, but not in PMDD, subjects. Thus, the same group of researchers reported either a delay or advance of the cortisol peak in the luteal

phase of healthy controls, but neither change was reported in women with PMDD. Therefore it is hard to conclude whether a phase change actually does exist across the menstrual cycle.

Shibui et al. (2010) reported a significant reduction in the amplitude, but no change in the circadian timing, of cortisol rhythms in the luteal compared with the follicular menstrual cycle phase in eight healthy women undergoing ultra-short sleep-wake (ultra-SSW) cycles for 26 h. In each 30 min period, they had a 10 min nap in darkness, and were kept awake in dim light for the remaining 20 min. The authors also reported an increase in the mean value and reduction in the amplitude of the 24 h rhythm of body temperature, and a reduced melatonin secretion in the luteal phase compared with the follicular phase. They suggested that reduced oscillation amplitude of the circadian pacemaker in the luteal phase could account for these results. However, participants were kept on an ultra-SSW cycle. Therefore these results may not be applicable to normal sleep in women and may instead represent how high-frequency sleep-wake cycles are affected by menstrual phase. In summary, few studies have examined cortisol circadian rhythms in healthy women during the menstrual cycle and those that have done so have reported either no phase difference (Bloch et al., 1998; Steiner et al., 1984), a decrease in cortisol amplitude during the LP (Shibui et al., 2000), and a cortisol phase advance (Parry et al., 2000) or delay (Parry et al., 1994) during the late LP.

1.4 Effects of Sleep Loss on Appetite Regulation and Hunger, and Influence of Menstrual Phase

Sleep loss has previously been reported to modulate hormones involved in appetite regulation; leptin and ghrelin. Leptin is a peptide hormone produced by fat cells; it communicates the status of energy balance to the central nervous system and acts as an appetite suppressor (Ahima et al., 2000). Ghrelin is a peptide hormone that comes principally from the stomach lining and acts on the hypothalamus to promote food intake and fat deposition (van der Lely, 2009). In addition to their opposing roles in regulation of appetite, leptin and ghrelin have been reported to have a variety of other physiological effects.

Both these hormones are important components in energy balance regulation in the body. Under normal conditions, in healthy individuals, leptin and ghrelin follow a 24 h cycle modulated by the sleep/wake cycle. Leptin shows increasing levels during the daytime, reaching a nocturnal maximum (Schoeller et al., 1997; Figure 2). Ghrelin follows a 24 h pattern of nocturnal elevation, that peaks during the sleep period and declines towards the morning (Cummings et al., 2001; Figure 2). Recent research has examined the effects of sleep loss on the patterns of these hormones. Spiegel et al. (1999; 2004) found that sleep restriction in men decreased leptin levels. However since then, several studies have failed to reproduce this effect. In one study, leptin levels were measured in women at only two daily time points and a significant increase rather than decrease in morning levels was found (Omisade et al., 2010). These findings, along with some of the other studies (Schmid et al., 2008; Pejovic et al., 2010) that have failed to

reproduce the effect of the earlier findings from Speigel et al. (1999; 2004), may be a result of differences in study design.

Firstly, the study by Spiegel et al. (2004) showed that sleep loss was associated with the activation of what they described as the 'stress system': elevation of cortisol levels reflective of HPA activity, and an increase of sympathovagal balance (derived from heart rate variability recordings). In contrast, studies that have failed to show an effect did not report on the status of stress via HPA activity, or if they did measure sympathovagal balance, found no difference (Pejovic et al., 2010). Activations of the HPA axis have previously been reported to cause changes in leptin levels (Dallman et al., 2003, Rayner and Trayhurn, 2001). Therefore, the changes seen in appetite regulating hormones may not be caused by sleep loss on its own, but rather by activation of the stress system alongside sleep restriction.

Another difference could be the lack of a standard protocol for mealtime. The diurnal rhythm of leptin is heavily influenced by prior food intake (Schoeller et al., 1997). All the studies showing an increase in leptin after sleep deprivation have controlled for food intake prior to sampling, however many of the studies showing no effect did not. In addition, these studies did not control for gender. It is well established that leptin levels are higher in women (Kennedy et al., 1997), and leptin levels have also been reported to vary across the menstrual cycle, being elevated in the LP (Einollahi et al., 2009; Groschl et al., 2002). Of note, studies finding lower or no changes in leptin have enrolled men exclusively, whereas those finding increased levels have enrolled exclusively women, or

both men and women. Therefore responses of leptin to sleep loss may depend on several different factors like sex, prior meal intake and activation of the stress system.

In addition to these changes in hunger regulating hormones, sleep loss has been associated with increases in appetite under conditions during which food intake is controlled . Spiegel et al. (2004) were the first to report a rise in subjective feelings of hunger after two days of restricted sleep to 4 h, and since then, five studies have reported similar results, whereas five studies have reported no effect of sleep loss on hunger. Several study-related differences could account for these discrepancies. Studies that have reported hunger ratings at only one time point (Schmid et al., 2008) or before each meal (Pejovic et al., 2010) have found hunger ratings to decrease, and studies that have reported hunger throughout the day (St. Onge et al. 2011), or once before dinner (Omisade et al., 2010) reported no change after sleep loss. This suggests that hunger ratings are sensitive to time of day and time since previous meal.

In addition, studies that have reported that sleep restriction increases hunger ratings have been performed exclusively in men (Spiegel et al., 2004, Schimd et al. 2008; Brondel et al., 2010), whereas those that show no effect have included women. In fact, in the study by St.Onge et al. (2011), there was a sex by sleep interaction in rating of fullness in the short-sleep compared to the normal-sleep group. Men reported feeling less full after short sleep, whereas women did not. Of the studies looking exclusively at women, no change in hunger was reported after one night of 3 h restricted sleep (Omisade et al., 2010) or 4 nights of progressive sleep loss (Bosy-Westphal et al., 2008).

It is possible that sleep restriction could influence ratings of hunger in men and women differently and the potential effect of menstrual phase in this sex difference that could be modulating these effects, has yet to be explored.

Although differences in 'hunger ratings' across the menstrual cycle have never been assessed, there is research looking at changes in 'food cravings' (Dye & Blundell, 1997). It appears that specific food cravings for carbohydrate rich foods do show a cycle-related pattern, peaking premenstrually (luteal phase) and declining after menstruation and further postmenstrually (follicular phase) (Davidson et al., 2007). However, a study looking at women in the luteal phase with and without PMS found that food cravings only occurred in the PMS group, with no change in cravings in the control group (Reed at al., 2008). This suggests that symptoms associated with PMS in some women may contribute to observed changes in cravings among women as a group. Since there are reports of menstrual phase affecting ratings of both appetite and mood, there is a need to explore this relationship in association with sleep loss to try to isolate any confounding effects of mood and menstrual phase on sleep loss and hunger.

1.5 Effect of Sleep Loss on Mood in Healthy Controls

In addition to these metabolic effects, sleep appears to be associated with changes in mood (Kahne-Greene et al., 2007, Bernier et al., 2009, Björntorp, 2001; Leproult et al., 1997; Cadwell & LeDuc 1998, Orton et al., 1989). Studies that have experimentally restricted the length of sleep among healthy participants have found a relationship between sleep-deprivation and increased depressive symptoms. In a study by Kahn-

Greene et al., (2002), 25 healthy military participants, both male and female, were asked to initially complete a Personality Assessment Inventory (PAI; Morey, 1991), which evaluates symptoms of various psychological disorders. They were then required to stay awake for 56 h before completing the PAI again. Following sleep deprivation, scores on the affective psychological disorder components of the inventory were significantly increased.

In another study, by Bernier et al. (2009), 13 healthy women and women experiencing unipolar depression were restricted to 2.5 hours in bed for 1 night. Participants completed the Profile of Mood States assessment at baseline and following sleep restriction. Healthy women experienced significant decreases in vigour and increases in fatigue, confusion and tension suggesting that sleep restriction in healthy women may increase the likelihood of them developing depressive-like symptoms.

An early meta-analysis of 18 studies done by Pilcher & Huffcat (1996) identified three general types of sleep deprivation protocols: (1) long term total sleep loss (>45 hours), (2) Short-term total sleep loss (<45 hours), and (3) partial sleep restriction (<7 hours every 24 hours). They found that virtually all forms of sleep deprivation resulted in increased negative mood states, especially feelings of fatigue, confusion and decrease in positive mood states such as vigour compared to changes in cognition and physical strength. Interestingly, these effects were strongest for studies using partial sleep restriction protocols, and the effect was seen in both males and females.

One study compared the effects of sleep loss on healthy male and female pilots and found that on mood tests, women reported feeling less tense and more energetic than men following sleep loss; however, the sample size was very small (n=6) and they did not control for the women's menstrual cycle (Caldwell & LeDuc, 1998). Therefore, there is no way of knowing whether or not the timing of women's cycle impacts the way in which she reports changes in her mood in relationship to sleep loss.

1.6 Mood and the Menstrual Cycle

There is extensive research examining how mood varies across women's menstrual cycles independent of experimentally manipulating sleep duration. Much of this research focuses around the premenstrual syndrome (PMS), which is characterized by a number of physiological and emotional symptoms that occur during the last part of the luteal phase of the menstrual cycle (Richardson, 1995). It is believed that at least 75% of women experience PMS at some point during adulthood (Richardson, 1995). Although there is a broad popular belief among the general public and scientists alike that there are mood changes associated with menstrual phase, there is still debate in the scientific literature regarding their existence.

A systematic review was published looking at the history of over 40 quality research studies published on the changes in mood across the menstrual cycle. Of these studies, 38% found no association between mood with any menstrual cycle phase, 40% found an association between negative mood in the premenstrual phase combined with beginning of menses. Only 15% of the studies found an association between negative

mood and the premenstrual phase and 8% found an association between negative mood and a non-premenstrual phase (Romans et al., 2012).

The largest percentage amongst these studies show negative mood to be highest in the premenstrual and early menses phase. However, it is striking that over 40% of these studies have failed to find any connection, yet it is still of popular belief in the media and with physicians according to the American Congress of Obstetricians and Gynecologists (2011), that mood changes often occur during the menstrual cycle, and only during the pre-menstrual period, despite only 15% of studies supporting this idea.

The inconsistencies among these results suggest a need for future research to be conducted using highly controlled experimental conditions. Perhaps the most important need is controlling for women's prior belief of PMS or when they are most likely to have mood symptoms during their cycle. This will prevent women from stereotyping their changes in mood to the menstrual cycle. Research focusing on menstrual phase difference would also be strengthened if there were endocrine evidence of menstrual phase, rather than relying solely on self-reports. This could potentially prevent studying women during an incorrect phase. There is also a need to use a standard assessment tool for measuring mood changes during the menstrual cycle. A lot of the assessment tools used only focus on changes in negative mood without counterbalancing for changes in positive mood.

Women showing late-luteal (premenstrual) mood changes may also report increased food cravings, including preferences for high-carbohydrate and high-calorie food (Both-

Orthman et al., 1988; Dye & Blundell, 1997). Since there are also reports of luteal changes in sleep quality and since sleep loss has been reported to have similar effects, there is the possibility that sleep changes may mediate these effects of menstrual phase on appetite. There have, however, been few controlled studies that compared mood and appetite changes in different menstrual phases and there is little information about the potential impact of sleep loss on these features.

1.7 Statement of Research Questions and Rationale

This study examined whether menstrual cycle phase affects the changes in metabolic profiles of women after one night of partial sleep restriction (3 h sleep opportunity). Women were studied at two distinct menstrual cycle phases: mid-luteal and mid-follicular.

Partial sleep loss was chosen over total sleep deprivation for three reasons: (1) It more closely matches the partial sleep loss seen most frequently in society; (2) It is a relatively easy protocol for the participants to tolerate; and (3) It is the most frequently used approach so the results would be most readily comparable to existing findings.

The hormone cortisol was studied because previous findings have demonstrated alterations in its circadian cycle resulting from partial or total overnight sleep deprivation, and because cortisol is an important regulatory hormone that has significant effects on metabolic processes in regulating fat breakdown and inhibiting glucose uptake in adipose tissue. The protocol for this study also included acquiring additional saliva samples

intended for analysis of other hormones that have been reported to be affected by sleep loss, such as ghrelin and leptin, but these analyses have not yet been conducted and do not form part of this thesis.

Hypotheses:

a) The circadian levels of cortisol in the morning at baseline will be higher for the follicular phase compared to the luteal phase.

b) There will be alterations in the circadian patterns of cortisol after sleep loss and these alterations will differ between the follicular and luteal phase groups.

These hypotheses were based on the preliminary findings from Omisade et al. (2010), where they saw a trend towards higher cortisol levels during the morning at baseline and a significant increase in cortisol after sleep loss during the follicular phase, but no difference during the luteal phase (Figure 3).

A secondary goal of the experiment was to examine if sleep loss affects self-reports of mood and hunger, and if menstrual cycle phase modulates these effects. Since previous research doesn't make for strong predictions, this analysis was mostly exploratory.

Chapter 2

METHODS

2.1 Participants Selection

All participants had to meet the following inclusion criteria: female, 19-25 years of age, non-smoker, habitual daytime activity (i.e. no shift work), no travel over three or more time zones within the last 6 weeks prior to the study, habitual continuous sleep duration of 7-10 h without frequent naps, no history of eating disorders, affective disorders (including post-traumatic stress disorder (PTSD), chronic stress, anxiety or depression symptoms in the clinical range), no sleep disorders, and no ongoing hormone treatments, specifically hormonal birth control of any form for at least three months prior to participating in the study. Women on hormonal birth control were excluded specifically because morning cortisol levels have been shown to be lower in women using oral contraceptives compared to non-users (Pruessner et al., 1997). Professional or semi-professional athletes were excluded due to potential disruptions in their menstrual cycles resulting from their training regimes. Participants were recruited using advertisements on notice boards at Dalhousie University, the Isaaz Walton Killam (IWK) Health Centre, and online via Kijiji. The Capital District Health Authority research ethics board in conformity with the Canadian Tricouncil research ethics guidelines approved all procedures.

Participants underwent initial screening that included measurements of weight, height, hip and waist circumference and completed questionnaires on the Hamilton

Depression Rating Scale (HDRS), Hamilton Anxiety Rating Scale (HAM-A), MINI International neuropsychiatric interview for major Axis I psychiatric disorders in the DMS-IV (for exclusion purposes), Morningness-Eveningess Questionnaire (MEQ, Horne and Ostberg, 1976) and Profile of Mood States (POMS). Some of these inventories are commonly used to assess severity of depression and anxiety in psychiatric patients, because they have been found to discriminate between healthy individuals and those with these disorders (Hamilton, 1959; Hamilton , 1960). Although not originally designed as screening tools, they are regularly used as such. Elevated scores on these inventories do not necessarily predict diagnosis of depression or anxiety disorders or any other psychiatric disorder, but they do indicate psychological maladjustment. The MEQ was used to ensure that participants were not extreme morning or evening types. Anyone with MEQ scores greater than 69 (extreme Morningness) or less than 31 (extreme Eveningness) were not included in the study, as cortisol rhythms have been reported to differ between these extreme Morning and Evening types (Randler & Schaal, 2010).

Participants were randomly assigned to two groups (mid-follicular & mid-luteal) to achieve similar numbers in the two groups, using a random group generator system (www.randomizer.org). During the course of the study some women dropped out and therefore replacement participants had to be 'pseudo-randomly' assigned to that same phase group. During screening, self-reports were obtained for their first day of their last menstrual cycle. The first day of menstruation was defined as Day 1. After screening, they were asked to contact the research co-coordinator and report when the first day of their next cycle occurred. Participants in the mid-follicular group started their

participation in the study within two weeks after the first day of menses, and participants in the mid-luteal group started the study within two weeks of the first day of their next predicted onset of menses (Figure 4). Menstrual phase was confirmed three ways: (1) self-reports of first day of last menstrual period start of cycle before participating in the experiment, (2) self-reports of first day of next menstrual period after they took part in the experiment, and (3) measured salivary progesterone levels. Progesterone levels <200 pg/mL are considered typical of the follicular phase and levels >200 pg/mL are typical of the luteal phase (Gandara et al., 2007).

Twenty healthy female participants of reproductive age were recruited to participate in the study; one withdrew from the study due to illness and one was excluded because she was not able to sleep at least 6 h during the first (baseline) night of the experiment. Demographic characteristics for all included participants (n=18) are reported in Appendix 1, including age, BMI, menstrual phase, MEQ score, typical sleep duration, and sleep duration during the experimental nights.

2.2 Materials/Procedures

Participants who met all inclusion/exclusion criteria were selected and equal numbers of participants were pseudo-randomly assigned to one of two different phases of their menstrual cycles, aiming for approximately the midpoints of their follicular and luteal phases, based on their previous cycles. A minimum of two full cycles was required before taking part in the study. However, some women's cycles were tracked for as long as 6 months due to scheduling their mid-phase with availability in their schedule and with

availability of bedrooms in the Chronobiology lab. Therefore, some women's cycles were studied for a maximum of 6 months.

The timing of the follicular and luteal midpoints for participants was established carefully based on their individual average menstrual cycle length of their previous tracked cycles. Mid-follicular phase is typically described as day 8-10 and mid-luteal is typically described as day 20-22 in a 28-day menstrual cycle (Stricker et al., 2006). However, research has shown considerable variation in menstrual cycle length between and within women. Between women, normal cycles range from 22-36 days (Fehring et al., 2006), and therefore women were included in the study if their cycle length averaged somewhere within this range.

Several studies have concluded that the follicular phase is the source of variation in menstrual cycle length, while the luteal phase remains constant between and within menstrual cycles of women (Lenton et al., 1984; Wilcox et al., 2000; Cabral and de Medeiros, 2007). The follicular phase can range from 12-24 days, with 12 days for shorter cycles and 24 days for longer cycles, while the luteal phase remains relatively stable within and between women at 12-14 days (Harlow and Ephross, 1995; Cole et al., 2009). Therefore, women with shorter cycles will inevitably have shorter follicular phases and earlier luteal phases relative to menses. Thus, their phase midpoints will be earlier compared to a woman with a longer average cycle.

Therefore, timing assignments for the current study were determined taking this information into account. We scheduled to study women with a cycle length that averaged 22 days on cycle Days 4-6 (with onset of menses defined as Day 1) as their mid-follicular phase and cycle Days 15-16 as their mid-luteal phase. Women with a cycle length that averaged 36 days were scheduled to be studied on cycle Days 18-20 as their mid-follicular phase and cycle Days 29-31 as their mid-luteal phase. If cycles averaged somewhere in between 22 and 36 days, then midpoints were adjusted accordingly to the difference (Table 1).

One week prior to the laboratory component of the study, each participant was given a food diary to record her eating habits, a sleep diary and a wrist actigraph (Motionlogger; Ambulatory Monitoring Inc., Ardsley, NY) to document their usual sleep patterns and ensure that typical sleep duration fell within the normal range (6-9 h; The Canadian Sleep Society (CSS), 2013). Actigraphy is the continuous measurement of activity or movement and can be used as an indirect measure of sleep/wake cycles in young healthy males and females over an extended period of time. Periods of wakefulness result in a bar, while quiet times are represented as a flat line and are taken to mean sleep (Ancoli-Israel et al, 2003).

The typical sleep duration of each participant was measured using actigraph data, along with their self-reports of bedtime and wake-times from their sleep diaries to confirm the actigraph data. Sleep logs and actigraphy have been shown to yield similar data for sleep timing, duration and onset and offset but not for number and duration of

night awakenings or number of naps (Lockley et al., 1999). Therefore participants were asked to record in their diaries if/when they napped each day and for how long.

All participants spent 3 nights and 2 full days in the Chronobiology Laboratory at the Queen Elizabeth II Health Sciences Centre during the months of January-April 2013. On the first night (Night 1), participants came to the laboratory at approximately 22:00; they were weighed on a standard medical scale and their height and waist and hip circumferences were measured using a tape measure in order to accurately determine their body-mass indices (BMIs) and waist-hip ratios prior to taking part in the study. A single salivary sample was also taken at approximately 22:00 for measurement of progesterone levels in order to confirm their menstrual phase. Participants then went to a darkened bedroom in the laboratory and were allowed to sleep from 22:00 to 08:00 in order to habituate them to the lab environment. The next day, they were allowed to return to their usual schedule but cautioned against napping or drinking alcohol. Naps were not allowed during participation in the study. Actigraphy data were analyzed during this day by the research coordinator to confirm that participants had a normal sleep pattern during the week and that they had an adequate amount of sleep during the habituation night.

On the second night (Night 2) in the laboratory, participants were allowed to sleep on the same schedule, but remained in the laboratory and adjacent hospital environment during the following day (Day 1; Baseline). Salivary samples were obtained immediately after awakening (08:00) and every 3 h until 20:00 on Day 1 in order to establish baseline endocrine patterns after a full night's sleep (5 samples). A sixth saliva sample was taken

30 min after awakening in order to measure the peak level of cortisol associated with the CAR that is normally observed at that time (Pruessner et al., 1997).

Saliva was collected using the passive drool collection method into two 2.0 mL vials. Participants were instructed to pool saliva in their mouths and use a straw to drool into each vial. After 5 min, saliva was collected and frozen immediately at -20^{o} C and stored in the Chronobiology Lab of the Queen Elizabeth II Health Sciences Centre for two days, then transported in a cooler to Dr. Tara Perrot's laboratory in the Life Sciences Centre (LSC) of Dalhousie University and stored at -20 ^{o} C for up to 6 months before being thawed and assayed.

On the third night (Night 3), participants were kept awake during the first part of the night in the company of a research assistant, and allowed to sleep in the same bedroom only from 05:00 to 08:00. During the wake period up to 05:00, participants watched movies, went for walks around the hospital and read books to stay awake. They were not allowed to eat during this period and were only permitted to drink water. Salivary sampling on an identical schedule was repeated in the laboratory during the following day (Day 2; Post-sleep restriction). Figure 5 depicts a schematic of the three-night and two-day protocol along with times of cortisol and behavioural measurements. Because of limited availability of the Chronobiology bedrooms, participants were in the same environment on all three nights, but did not necessarily sleep in the same bedroom during the adaptation night (Night) 1 as during Nights 2 and 3. Each participant, however, slept in the same bedroom during Nights 2 and 3 and was studied in the same laboratory area

during Days 1 and 2.

During the two-day laboratory portion of the study, participants were also asked to complete behavioural questionnaires every time they provided a saliva sample. They were asked to rate their levels of hunger using 10 cm scales, where 0 represented 'not at all' and 10 represented 'extremely' hungry. These scales were shown previously to be sensitive to effects of sleep loss on hunger in men. The food types varied in caloric and fat content and were divided into the following categories; sweets, salty foods (e.g. chips, salted nuts), starchy foods (e.g. potatoes), fruits, vegetables, meat, fish, eggs, and dairy (Spiegel et al., 2004). The hunger and craving scales are not formal assessment instruments, so their psychometric properties have not been evaluated. These scales provided information regarding the participants' subjective evaluations of their appetite states, and the results were used for descriptive and qualitative analyses only. Participants were also asked to rate their mood states using the Profile of Mood States (POMS) questionnaire. The POMS contains 65 items comprising 6 factors that reflect various mood domains (Tension-Anxiety, Depression-Dejection, Anger-Hostility, Fatigue-Inertia, Vigor-Activity, and Confusion-Bewilderment). It was designed to measure general psychological distress in healthy, psychiatric, and physically ill populations (McNair, Lorr & Droppelman, 1971). The schedule of procedures during the laboratory portion of the study is summarized in a schematic in Figure 5.

All participants were provided with regular meals that they selected from a predetermined menu. Menu items were selected with choices that were consisted with

participants' usual diets and with Canada Food Guide recommendations. They were not allowed any caffeine, sugar or junk food, or foods high in saturated fats. Food intake for both days was closely matched. Participants were provided with the same menu items in the same amounts on both days and required to finish each meal. The only differences in meals allowed were different types of fruit or vegetables or fruit juice type, but amount of food consumed was identical.

Participants stayed in the controlled laboratory environment during the two test days and were allowed to engage in non-strenuous activities, such as reading, playing games, listening to music, watching video recordings, or working on a computer. The study ended after the salivary sampling and final questionnaires at 19:45-19:55 in the evening of Day 2. Participants were then sent home either by an arranged ride or by taxi (no driving allowed following sleep restriction). Participants were contacted 24-48 h later to inquire as to their status and successful recovery from the sleep restriction procedure. In addition, participants emailed the researcher as to when their next menstrual cycle started to confirm the cycle during which they were studied.

Participants were informed during the consent procedure prior to the commencement of the study that they were free to withdraw from the study at any time for any reason. They were also informed that they need not discuss the reasons with the experimenter if they did not wish to do so, and that their choices to withdraw and to discuss or not to discuss the reasons for their decision with the experimenter would not result in any negative consequences to them personally, financially, or in terms of their

healthcare. The two participants who withdrew from the study prematurely were compensated for their time according to a pre-determined scale and any samples obtained were used for the assay optimization procedure but not included in the final data analyses.

After all 18 participants successfully completed the study; saliva samples were thawed and used for assay. The concentrations of cortisol and progesterone in saliva were measured using commercially available enzyme linked immunosorbent assays (ELISA) for each hormone. All samples were analyzed as duplicates. The cortisol concentration in saliva was determined with a kit specifically designed to measure cortisol in saliva (High Sensitivity Salivary Cortisol ELISA, no. 1-3002; Salimetrics™ , USA) based on the competitive binding ELISA technique. Cortisol standard curves were constructed in the range of 0.012-3.0 μg/dL using standards supplied by the manufacturer. The inter-assay coefficient of variability (CV) was 4.3 %. The full procedures were performed in accordance to the salivary cortisol EIA kit insert no. 1-3002 supplied by the manufacturer (Salimetrics, 2012). Progesterone concentrations in saliva were determined using a similar commercially available ELISA from the same manufacturer (Salivary progesterone ELISA, no. 1-1502; Salimetrics™ , USA) based on the same competitive binding technique. Progesterone standards were constructed in the range of 10-2430 pg/mL using standards supplied by the manufacturer. The inter-assay variability CV was 7.8 %. The full procedures were performed in accordance to the salivary progesterone EIA kit insert no. 1-1502 supplied by the manufactorers (Salimetrics, 2010).

2.3 General Approach to Statistical Analyses

All statistical analyses were performed with the program SPSS for Windows, version 17. The threshold for statistical significance was set at .05 unless otherwise specified, and all tests were two tailed.

Box-plot graphs were created for each level of the variables for all the raw scores and difference scores used in the statistical analyses, in order to identify outlier values. In a box plot, the middle 50% of the data are contained within the box itself, and the horizontal line inside the box represents the median value. The 95% confidence intervals of the data are represented by the ends of the vertical lines on each side of the box. Individual points outside the ends represent extreme values (circle). These extreme values were carefully assessed in order to insure that these data had been correctly entered and/or calculated, and that no experimental notes indicated any problem or unusual event in relation to the acquisition of that data point. All the extreme values were found to be accurate and unrelated to any problem in the data acquisition process; these values were attributed to biological variability and were retained in the statistical analyses.

The following general approach to statistical analysis was taken. Normality of data distribution was assessed for each level of the analyzed variables. If the assumption of normality of distribution was violated, a non-parametric statistical model was used. Equality of error variances between the two groups was also tested for each level of the analyzed variables prior to conducting between-group comparisons. When the latter assumption was violated, a test with 'equality of variances not assumed' was used.

The main outcome measures were the patterns of cortisol measured on Day 1 (Baseline) and Day 2 (Post-sleep restriction). In particular, we compared levels of these hormones in the first samples taken in the morning, and during the afternoon/evening, using area-under-the-curve with respect to ground measurement (Figure 6 and 7; Pruessner et al., 2003). Baseline values on Day 1 were compared across groups to determine whether women under normal sleep conditions show similar cortisol patterns at different stages of their menstrual cycles. We then examined the main effects of Day and of menstrual cycle phase and their interactions on these measures using two 2 x 2 mixed ANOVAs. The between-subjects factor was Menstrual Phase group with two levels (follicular and luteal phase), and the within-subjects factor was Sleep Condition with two levels (Baseline day and Post-sleep restriction day). The effect size was calculated for all results that demonstrated statistical significance using Cohen's *d* calculation. The effect size measures the strength of the phenomenon and Cohen's *d* is defined as the difference between two means divided by a standard deviation for the data.

Secondary analyses examined the effect of Sleep Condition and its interaction with menstrual cycle phase on the self-report ratings of mood-state variables (POMS scores), and hunger ratings using 2 x 2 mixed ANOVAs. We also examined the correlations between cortisol variables on Day 1 and hunger and progesterone values across the groups. Similar correlations were examined using difference scores between the Baseline and Post-sleep restriction days.

Chapter 3

RESULTS

3.1 Characteristics of Participants

Typical sleep patterns prior to the experiment were measured using actigraphy data and daily sleep diaries. Diaries were used as a guide to score the actigraphy data to ensure that sleep/wake times, naps and times spent not wearing the actigraph were matched between the self-report data and actigraphy. There were no discrepancies noted between the two measures for any participant. Averaging sleep parameters over 7 nights assessed typical sleep patterns. Five participants: 3 from the Follicular group and 2 from the Luteal group, wore their actigraphs for fewer nights, so their sleep parameters were calculated based on data averaged over 4-6 nights. Participants also wore the actigraph during each night of the experiment, so data from these days were used to calculate the average time slept for the Baseline and Sleep-restriction nights of the experiment.

Participant's normal food consumption patterns during the week before the study were assessed through their food diaries. All participants typically ate 3 meals a day and snacked in between, although some participants skipped a few meals over the course of the week. No other unusual dietary habits were noted.

Menstrual phase was established three ways: (1) Day 1 of their cycle (based on their self-reports over 2 to 6 cycles) was used to schedule the participants for their laboratory experiment. (2) After they participated in the experiment, they reported the

timing of Day 1 of their next cycle to the experimenter. (3) Progesterone levels were assayed based on a saliva sample obtained during the first night of the laboratory phase of the study. Progesterone levels of each participant are presented in Appendix 1. Four women, two from the Follicular and two from the Luteal phase group had progesterone levels that fell outside the expected range (< or > 200 pg/mL) for their assigned phase. These expected ranges were provided by the assay manufacturer, and have been confirmed by other studies (Gandara et al., 2007). For each of these participants, the timing of prior menstrual cycles and their subsequent report of onset of menses after participation in the study were consistent with their group assignment. In addition, removing their data had no effect on the outcome of the results. Therefore, their data were retained in their assigned group.

One participant started her subsequent menstrual cycle 12 days earlier than expected, which would suggest that she was in a later stage of her assigned cycle than anticipated. Her progesterone data were consistent with her assigned follicular menstrual phase and her cortisol data were characteristic of this group as well. Removing her values from the cortisol analysis did not change anything in terms of outcomes and her data were retained in the analysis.

Means, standard deviations and sample size of the following variables: age at the time of study, BMI, progesterone values, average typical sleep assessed by actigraphy before the study (h), and average sleep duration (h) during the two experimental nights, are presented in Appendix 1 as a function of Group. Normality of distribution was tested

for each variable within each Group using the Shapiro-Wilk's test for normality (Appendix 2); the test was not significant for any of the variables. The Levene's test for equality of error variances was conducted for each variable (Appendix 2); variances did not differ significantly between the groups. Six one-way analyses of variance (ANOVAs) were computed for each of the variables comparing mean scores between the Follicular and Luteal phase groups. No between-group differences were found for Age, BMI, MEQ scores, average typical sleep assessed by actigraphy before the study, and average sleep duration during the two experimental nights during the Follicular and Luteal phases (Table 2).

3.2 Cortisol Values at Different Phases of the Menstrual Cycle

3.2.1 Baseline Cortisol Values:

Six one-way ANOVAs were conducted to evaluate whether there were baseline differences in cortisol levels between the two menstrual phase groups during Day 1 of the experiment. The six dependent variables were the mean cortisol values on Day 1 at six time points: 08:00, 08:30. 11:00, 14:00, 17:00, 20:00 h. ANOVAs revealed no significant difference in the six baseline cortisol values between the two menstrual phase groups (Table 3).

Boxplots were created for the raw cortisol values at each time point on Day 1 in order to visualize the data distributions and check for extreme values (Appendix 3). Means, standard deviations and sample sizes for the cortisol values are presented in Appendix 4 as a function of Time of Day and Group. Normality of data distribution was tested at each level of the variables using Shapiro-Wilk's test for normality (Appendix 5);

all variables were normally distributed. Equality of variances between the two groups was assessed with the Levene's test (Appendix 5); variances did not differ significantly between groups.

3.2.2 CAR and Afternoon/Evening Cortisol Levels at Different Menstrual Phases

I. Cortisol Area Under the Curve to Ground

Two 2X2 mixed ANOVAs were conducted to evaluate the effects of sleep deprivation on the change in the CAR and afternoon/evening cortisol levels throughout the day. The two dependent variables were the 'Area under the curve with respect to ground' (AUCg) for the morning CAR (M-AUCg) and for the afternoon/evening (E-AUCg) cortisol values. This formula can be seen in Figure 8 and has been used previously to measure the change in cortisol values across the day (Pruessner et al., 1997; Fekedulegn et al., 2003; Omisade et al., 2010). To capture the morning CAR, M-AUCg was calculated from 08:00 to 08:30 (Figure 6 and Figure 7). To capture change in cortisol in the afternoon/evening, E-AUCg was calculated from 14:00 to 20:00 (Figure 6 and Figure 7). The within-subject factor with two levels was Sleep Condition (Baseline, Post-sleep restriction). The between-subject factor was Phase with two levels (Follicular and Luteal phases).

ANOVAs revealed significant main effects of within subject factor Sleep Condition for both dependent variables. A significant decrease ($p= 0.01$) in mean cortisol M-AUCg was observed after sleep restriction, and a significant increase ($p=0.001$) in mean cortisol E-AUCg was also observed after sleep restriction (Table 4). ANOVAs also revealed

significant Group X Day interaction for both dependent variables: CAR (p=0.005) and evening cortisol (p=0.006) values (Table 4).

Boxplots were created for M-AUCg and E-AUCg values, using the raw AUCg data from each participant in order to visualize the distributions (Appendix 6). Means, standard deviations and samples sizes for these cortisol values are presented in Appendix 7. Equality of variances between the two groups was assessed with the Levene's test (Appendix 8); for one variable, the Post-sleep Restriction E-AUCg cortisol value was not equally varied around the mean. Normality of data distribution was tested at each level of the variables using Shapiro-Wilk's test for normality (Appendix 9); one variable, the Post-sleep Restriction E-AUCg cortisol value, was not distributed normally.

To follow-up the Phase x Day interaction and main effect of Sleep Condition for the M-AUCg and E-AUCg cortisol, three paired-sample t-tests, as well as three Wilcoxon signed-rank tests were performed for each variable for the overall group and as a function of menstrual phase group. This was done to identify specific changes in the M-AUCg and E-AUCg cortisol levels in the two phases across the two Sleep Conditions. Results revealed no difference for the M-AUCg and E-AUCg cortisol values in the Luteal phase group. However, there was a significant decrease in M-AUCg (p=0.005; d=1.33) and an increase in E-AUCg (p=0.008; d=1.472) in the Follicular phase group (Table 5). Therefore, the main effects of Sleep Condition revealed by the initial two 2 x 2 mixed ANOVAs, indicating a decrease in the M-AUCg and increase in E-AUCg cortisol, were entirely attributable to the sleep-related differences in the Follicular phase group.

Line graphs were created to show the changes in cortisol values across the six measured time points (08:00, 08:30, 11:00, 14:00, 17:00, 20:00) throughout the day as a function of both menstrual phase and sleep condition (Figure 6 & Figure 7). Bar graphs were also created to show the difference in M-AUCg (Figure 9) and E-AUCg Cortisol (Figure 10) values in the Follicular and Luteal phase groups.

II. Cortisol Area Under the Curve in Relation to Baseline

Area under the curve with respect to ground is an ideal way to measure overall changes in absolute cortisol values over a time period, and provides important information about the underlying physiology (Fekedulegn et al., 2003). However, for a dynamic measure like the CAR, which represents a quick rise in cortisol 30 min after awakening, another approach is to examine the degree to which there is a change from baseline levels in response to the two Sleep Conditions. The increase in the CAR can be calculated using an alternative 'Area under the curve with respect to increase' (M-AUCi) calculation (Pruessner et al., 1997; Figure 8).

One 2 x 2 mixed ANOVA was conducted to evaluate the effects of sleep restriction on the M-AUCi cortisol value. The within-subject factor was Sleep Condition with two levels (Baseline, Post-sleep Restriction). The between-subject factor was Group with two levels (Follicular and Luteal phases).

The results revealed no significant differences in the effects of sleep deprivation on the M-AUCi for either group (Table 6). Cortisol levels on the Post-sleep restriction day did not rise to as high a peak at 08:30 as on the Baseline day, but they started at a lower

level at 08:00, so the increases were parallel on the two days, and did not differ significantly.

Means, standard deviations and samples sizes for M-AUCi cortisol values are presented in Appendix 10. Normality of data distribution was tested at each level of the variables using the Shapiro-Wilk's test for normality (Appendix 11); all variables were normally distributed. Equality of variances between the two groups was assessed with the Levene's test (Appendix 11); variances did not differ significantly between groups.

3.3 Hunger Self-Report Scores

Self-report repeated measures of hunger were obtained from each participant. The five daily self-report scores for hunger were averaged to generate an overall hunger score for each day of the study (Baseline and Post-sleep Restriction day) for each group.

Daily average hunger scores were analyzed using a one-way ANOVA. Table 7 shows that there was no significant effect of menstrual cycle phase on average hunger on Day 1 (equal variance assumed; Appendix 12). A 2 x 2 mixed ANOVA was conducted to evaluate the effects of sleep deprivation on average hunger ratings. The within-subject factor with two levels was Sleep Condition (Baseline and Post-sleep restriction). The between-subject factor with two levels was Group (follicular phase and luteal phase). The ANOVA revealed a significant main effect of Sleep Condition for hunger ratings r ($p= 0.04$), with no Sleep Condition X Phase interaction (Table 8).

To follow-up the main effect of Sleep Condition on hunger ratings, three paired-sample t-tests were performed to show the effects of sleep deprivation on hunger ratings in the overall group, the follicular phase and the luteal phase. Results (Table 9) showed a statistically significant effect of sleep condition across all participants ($p=0.000$), and for both the follicular group ($p=0.018$) and the luteal group ($p=0.003$). Therefore, both groups showed similar increases in hunger ratings after one night of sleep restriction (Figure 11).

Additional follow-up tests were performed to determine whether hunger ratings were affected by time of day. Three hunger scores were created from the raw data to create a morning average score (08:00 and 11:00), an afternoon score (14:00), and an evening average score (17:00 and 20:00).

A 3 x 2 mixed ANOVA was conducted to analyze the effects of time of day on the effects of sleep deprivation in self-report ratings of hunger across the two menstrual phases. The within-subject factor with two levels was Sleep Condition (Baseline & Post-sleep restriction). The within-subject factor with three levels was time of day (Morning, Afternoon, and Evening). The between-subject variable was menstrual phase with two levels (Follicular and Luteal).

The ANOVA revealed significant main effects of time, a main effect of sleep condition (as was reported previously), and no interaction between Time and Phase (Table 10). To follow-up these results, three repeated-measures non-parametric tests

(Friedman tests) evaluating differences in overall group median scores across Time levels were conducted. In addition three paired-sample t-tests, evaluating differences in overall group mean scores over the Time levels were performed. The results of these analyses are presented in Table 11. Tests for the effects of Time were conducted using Bonferonni adjusted alpha levels of 0.017 per test (0.05/3). Results indicated that there was a significant effect of sleep deprivation on evening ($p=0.012$; $d=0.718$) hunger scores, but not on morning or afternoon hunger scores across the entire group ($n=17$). Figure 12 presents the effects of sleep deprivation across the three different time points.

Boxplots were created for each level of the variables from the raw average hunger scores to visualize the data distribution (Appendix 13). Means, standard deviations and sample sizes for the hunger rating scores are presented in Appendix 14 as a function of Sleep Condition and Phase. Means, standard deviations and samples sizes of hunger scores are presented in Appendix 15 as a function of Sleep Condition and Time. Normality of data distribution was tested for each level of the variables, using the Shapiro-Wilk's test for normality (Appendix 16); all variables were normality distributed in both groups. Equality of error variances between the two groups was assessed with the Levene's test (Appendix 12); all variables were equally distributed.

3.4 Mood Self-Report Scores

Six subscales of the POMS were used: tension, depression, anger, vigour, fatigue, and confusion. Five of these reflect negative mood states and one (vigour) reflects a positive mood state and was therefore scored inversely. Scores on these six subscales

were combined into a single variable reflecting Total mood disturbance (TMD). The five daily self-reports on the six subscales and the five daily variable scores were averaged separately to create seven daily scores for each day of the study (Baseline and Post-sleep Restriction) that were analyzed statistically.

Seven one-way ANOVAs were conducted to evaluate the baseline differences in all variables of the POMS across the first day of the experiment in the two menstrual phase groups. The seven dependent variables were the self-report measures of mood states (Anger, Depression, Anger, Vigour, Fatigue, Confusion, and Total mood disturbance) for each menstrual phase groups: Follicular and Luteal. ANOVAs revealed no baseline differences between groups (Table 12, equal variance assumed; Appendix 17).

Seven 2 x 2 mixed ANOVAs were performed in order to evaluate the effects of sleep deprivation on the mood variables, and whether these effects differed by menstrual phase. The seven dependent variables were the self-report measures of mood states (Anger, Depression, Anger, Vigour, Fatigue, Confusion, and Total mood disturbance). The within-subject factor with two levels was Sleep Condition (Baseline and Post-sleep restriction. The between-subject factor with two levels was Phase (Follicular phase and Luteal phase).

The statistical significance of effects on mood variables was assessed using Bonferonni-adjusted alpha levels of 0.008 per test (0.05/6). The ANOVAs revealed that sleep restriction had significant effects on three variables: Fatigue scores increased; Total

mood disturbance scores increased; and Vigour scores decreased (Table 13). Scores on one variable (Confusion) appeared to increase after sleep restriction, but the p value (0.011) did not reach significance after a Bonferonni correction. The interaction effect of Sleep Condition by Phase was not significant (Table 13).

To follow-up these results, repeated-measure non-parametric tests (Friedman tests) evaluating differences in overall group median scores across the levels of the Sleep Condition was performed for the Fatigue variable, because the data were not normally distributed. Paired-sample t-tests to evaluate differences in overall group mean scores across Sleep Condition levels were performed for Vigour and Total mood disturbance scores, which were normally distributed. These tests were performed separately for all participants (n=16), the follicular group (n=8) and the luteal group (n=8) using a Bonferroni-corrected p value of 0.008. Results showed that there were significant effects of sleep deprivation on Fatigue (p=0.000; d=0.467), Vitality (p=0. 000; d= 1.106) and Total mood disturbance (p=0.002; d=0.988) scores for the entire group and for each menstrual phase group separately (Table 14). Therefore, the main effects of Sleep Condition on Fatigue, Total mood disturbance and Vigour were observed in both groups (Figure 13).

Boxplots were created at each time point using mean mood scores from the baseline condition for each participant in both groups in order to visualize the data distributions and check for extreme values (Appendix 18). Means, standard deviations and sample sizes of the mood scores are presented in Appendix 19, as a function of Mood, Sleep

Condition and Group. Normality of data distribution was tested at each level of the variables as a function of Phase using Shapiro-Wilk's test for normality (Appendix 20). The test revealed that for four variables, mood scores were not normally distributed: (1) Luteal phase anger scores, (2) Luteal phase fatigue scores, (3) Follicular phase depression scores, and (4) Luteal phase tension scores. Equality of variances between the two groups was assessed with the Levene's test (Appendix 16); variances did not differ significantly between groups.

Normality of data distribution for the follow up tests was tested for each variable as a function of Sleep Condition using the Shapiro-Wilk's test for normality (Appendix 21). The test revealed that 3 variables did not have normal distribution: (1) Anger baseline scores, (2) Fatigue baseline scores, and (3) Depression baseline scores. Equality of variances between the two conditions was assessed with the Levene's test (Appendix 17); variances did not differ significantly between groups.

3.5 Association Between Hunger Scores and Cortisol Values

Bivariate correlations were computed between each cortisol value from the five time points on Day 1 (Baseline) of the experiment with the corresponding self reports of Hunger obtained at the same times. These correlations were conducted for the overall group since Phase did not affect hunger scores differently on each day of the experiment. In addition, the change in cortisol values at each time point between Day 1 and Day 2 (ex. Day 2 08:00 cortisol value minus Day 1 08:00 cortisol value) were correlated with the analogous Hunger difference scores between days. Pearson correlations were used

when scores from both variables being assessed were normally distributed. Spearman correlations were used with rank values when data for one or both variables were not normally distributed. The Shapiro-Wilk's test was used to assess normality of distribution for these variables (Appendix 22).

Correlations for baseline and difference scores are presented in Table 15. The baseline and difference cortisol values showed no significant correlations with their corresponding baseline and difference hunger values.

3.6 Association Between Progesterone and Cortisol Values

Bivariate correlations were computed between progesterone levels and the M-AUCg and E-AUCg values for cortisol on Day 1 to determine whether progesterone levels correlated with baseline cortisol measures. In addition, the change in cortisol values for M-AUCg and E-AUCg between Day 1 and Day 2 was correlated with progesterone values to determine whether progesterone values correlated with the impact of sleep restriction on cortisol patterns. Pearson correlations were used when scores from both variables being assessed were normally distributed. Spearman correlations were used with rank scores when data for one or both variables were not normally distributed. The Shapiro-Wilk's test was used to assess normality of distribution for these variables (Appendix 22).

Correlations for baseline and difference scores for cortisol M-AUCg and E-AUCg scores with progesterone are presented in Table 16. Progesterone levels were not

significantly correlated with these cortisol measures at baseline. There were also no significant correlations between progesterone levels and difference cortisol values.

3.7 Associations Between Mood and Hunger Ratings

Bivariate correlations were computed between the baseline average hunger score and baseline scores in mood states Fatigue and Vigour. Only these two mood states were chosen because they were the only variables of the POMS that showed any significant difference between the baseline and sleep deprived condition. In addition, difference scores were derived from day 1 and day 2 of both the average hunger rating and the two mood states: fatigue and vigour. Pearson correlations were used when scores from both variables being assessed were normally distributed. Spearman correlations were used with rank scores when data for one, or both variables were not normally distributed. The Shapiro-Wilk's test was used to assess normality of distribution for these variables as well (Appendix 22).

Correlations for baseline and difference scores are presented in Table 17. No baseline correlations reached the threshold of significance. After sleep deprivation, vigour did not correlate with differences in hunger scores; however fatigue was positively correlated with difference hunger scores ($r(16)=0.68$, $p=0.007$). This indicates that after sleep deprivation, these two variables were strongly correlated. A scatter plot summarizes these results in Figure 14. Overall, there was a strong positive correlation between fatigue and hunger. The greater the increase in self-report ratings of fatigue, the greater increase in self-report ratings of hunger.

CHAPTER 4

DISCUSSION

4.1 Cortisol Values at Different Menstrual Cycle Phases

4.1.1 Baseline Daily Cortisol Patterns

The CAR was measured using the AUCg calculation (Figure 8). Upon awakening at 08:00, women during the FP show an elevated level of cortisol, followed by a 30-70% increase at 08:30 representative of the CAR. This is consistent with what is know about the diurnal rhythm of cortisol in humans, which rises during the late evening into the early morning and then peaks 30-45 minutes after awakening (Clow et al., 2009).

Women in the LP showed a similar baseline 08:00 morning cortisol concentration and CAR compared to the FP group. In the present study, women from both groups appeared to be starting from similar baseline morning values, with a similar value in the CAR. Upon closer investigation of the data in Figure 7, there is a smaller peak in the LP compared to the FP consistent with Omisade et al. (2010), with more limited data (Figure 3), yet the difference is not significant. This may mean that there is no difference or both studies just lacked the power necessary to detect the small difference.

Studies that have investigated the CAR across the menstrual cycle have failed to detect a difference related to menstrual cycle phase (Kudielka and Kirschbaum 2003; Bouma et al., 2009; Wilfram et al., 2011). Wilfram et al. (2011) investigated whether the CAR is significantly modulated by the menstrual cycle using a within subject design across several cycles. They found that cortisol increases after awakening did not differ

significantly between the luteal and follicular phase. What these results suggest, along with the results from the present study, is that there does not seem to be a difference in the CAR between the luteal and follicular phases.

The cortisol afternoon-evening values were calculated using the area under the curve to ground calculation (Figure 8). During this time, women during the follicular and luteal phases showed a rapid decline of cortisol levels into the evening consistent with the cortisol diurnal rhythm of healthy men and women (Clow et al., 2009). There was no difference between follicular and luteal phase groups in the level of cortisol during the afternoon at baseline.

A small number of studies have looked at the rhythms of cortisol across the menstrual phases. In healthy women, there have been reports of a phase delay (Parry et al., 1994), phase advance (Parry et al., 2000) or decreased amplitude (Shibui et al., 2000) during the LP compared to the FP or no effect at all (Steiner et al., 1984; Bloch et al., 1998). The current study shows no difference, but it could be that any differences that do exist cannot be detected through only one day of sampling. There is a limited number of studies and inconsistencies that currently characterize the knowledge on circadian variation in cortisol in women, therefore it is important to replicate these studies under highly controlled experimental conditions with adequate sample sizes, within-subject design, consistent menstrual phase assignment and multiple sampling throughout both phases.

4.1.2 Effects of Sleep Restriction on Cortisol

Women during their FP showed a significant change in the CAR after sleep restriction, whereas the CAR of women during the LP did not change. This resulted in lower morning values of cortisol in the FP after sleep loss, because the increase from the lower baseline (M-AUCi) was identical before and after sleep loss. Therefore sleep loss affects the morning level of cortisol, presumably altering the overnight rise, but not the CAR in the FP.

The most likely interpretation of these results is that sleep restriction has an impact on the rate at which cortisol rises throughout the night in women in the follicular phase, but not in women in the luteal phase. One limitation of this study, inherent in the use of salivary sampling, is that cortisol was not measured from 20:00 to 08:00. It would be useful in future to sample during this period (using indwelling catheters) to assess how cortisol levels change during overnight waking or sleep restriction at different menstrual cycle phases. Although few previous studies have reported on the effects of sleep deprivation on morning cortisol levels, published figures do suggest a slower rate of cortisol rise throughout the morning after either a night of partial sleep deprivation, or six consecutive nights of partial sleep deprivation (Figure 1 in Leproult et al., 1997; Figure 1B in Spiegel et al., 2004). They were able to successfully capture the morning rise by collecting blood every 30 minutes intravenously over the course of the late night and early morning.

These results suggest that the reduced rise in cortisol reflects a reduction in the amplitude of the circadian oscillation of cortisol levels during the FP. Both a high peak in the morning and a steep decline in the afternoon are normal features of the human daily cortisol rhythm. Our results indicate that sleep loss disturbs this presumably adaptive feature of cortisol rhythm during the FP, but not the LP. One previous study that demonstrated lowered morning cortisol values following sleep loss used insomniac patients (Backhaus et al., 2004), which could have potentially confounded results. The other studies failed to control for menstrual phase (Waye et al., 2003; Greifahn et al., 2008; Kumar et al., 2009), so it has yet to be determined whether the phase difference in the cortisol response is a consistent finding. Future research should be done using sleep restriction protocols similar to the one used in the current study with healthy participants that have no history of sleeping disorders. In addition, they need to take menstrual phase into account.

Lower morning cortisol levels have been correlated with increased body weight and an increase risk for the risk factors associated with metabolic syndrome (Praveen et al., 2011). Thus, the dampening of cortisol levels in the morning associated with sleep deprivation could be the factor driving the link between sleep loss and an increased risk for weight gain, obesity and other metabolic disorders. Future research can look at the correlation between sleep loss, morning cortisol and weight to determine whether or not a relationship exists.

Previous research looking at the effects of sleep deprivation on cortisol circadian rhythms in men has primarily focused on evening cortisol levels (Spiegel et al., 2004; Leproult et al., 1997). In the present study, evening cortisol values were assessed using E-AUCg scores (see calculations in Appendix 6). We found that afternoon cortisol values were significantly increased during the follicular phase compared to the luteal phase after sleep restriction. This is the first experiment to explore the relationship between menstrual phase and cortisol alterations following sleep loss. Previous research on both men (Leproult et al., 1997; Spiegel et al., 2004) and women (Omisade et al., 2010) found evening cortisol increases after sleep loss, similar to those we observed in women during their mid-follicular phase. It is believed that the increase in cortisol observed after sleep loss is due to an impairment of the negative-feedback control of the HPA axis (Spiegel et al., 2004).

Elevated afternoon cortisol levels have been found to be associated with metabolic alterations that could promote weight gain through alterations in glucose metabolism. Elevated cortisol levels, as a result of prolonged activation of the HPA axis has been shown to be related to the development of visceral obesity and other related features of the metabolic syndrome (Plat et al., 1999; Whitworth et al., 2005; Anagnostis et al., 2009). The elevation in cortisol levels during the afternoon seen in women during the follicular phase could partially explain the link between sleep deprivation and weight regulation and obesity.

In contrast to women studied during the follicular phase, those studied during the luteal phase did not show significant increases in cortisol in the afternoon/evening. Vgontzas et al. (2004) studied cortisol secretion following chronic, low-grade sleep deprivation (8 h for 4 nights, followed by 6 h for 8 nights) in 12 healthy males and 13 healthy females. After one week, cortisol levels were elevated during the afternoon and these effects were stronger in males than they were in females. The study did not assess nor control menstrual phase in the women, so it is possible that a lack of response in some women during their luteal phase contributed to the smaller response of women as a group.

The observational design of this study makes it hard to speculate why these two groups are responding so differently in the morning and during the evening, but it could be due to the variation of sex hormones -progesterone and estrogens- between the menstrual phases. Therefore, one can speculate that these sex hormone differences contribute to the different response to sleep loss. However, in the absence of any hormonal manipulations, it cannot be concluded that these hormones are involved.

4.1.3 Interaction between Sex Hormones and Sleep

The results demonstrate that a single night of sleep restriction has a different impact on women's daily cortisol rhythms at different menstrual cycle phases. The results suggest that menstrual phase may alter the way that women respond to sleep loss. The menstrual phase is characterized by distinct alterations in reproductive hormones.

Therefore, there is a potential role of the hypothalamic-gonadal axis interacting with the HPA axis.

The circadian cycle of cortisol in women may be affected by sleep loss differently during the menstrual cycle due to its associated variations in sex hormones. There is extensive evidence supporting the interaction between menstrual phases and specific circadian cycles of hormones such as melatonin and physiological processes such as body temperature (Baker & Driver, 2007). Body temperature has a circadian rhythm of 0.8-1 ^{o}C oscillations between daytime maximum and nighttime minimum (Moore et al., 1982). In women, this rhythm is increased by 0.4 ^{o}C during the luteal phase, which is thought to be due to the thermogenic effects of progesterone dominating during this phase (Baker et al., 2002). Melatonin levels are higher during the nighttime compared to daytime (Morris et al., 1990) and there is evidence that melatonin rhythms are blunted during the luteal phase (Brun et al., 1987). Evidence suggests that these hormones, as well as body temperature are important for sleep organization, and can be affected by lack of sleep (Zeitzer et al., 2007). Therefore, the changes in sex hormones across the menstrual cycle may be responsible for the different effects of sleep loss seen in each menstrual phase group.

Anderson et al. (2009) studied changes in sexual behavior, corticosterone and progesterone levels in rats between different phases of the estrous cycle in response to sleep loss. Figure 4 shows the hormonal fluctuations of the rat estrous cycle compared to the human menstrual cycle. Corticosterone is the dominant adrenal corticosteroid in rats

just as cortisol is in humans. Female rats were either subject to partial sleep deprivation (PSD), or maintained and used as controls (CTRL). After the experiment, rats were decapitated and blood was taken for progesterone and corticosterone analysis. Corticosterone and progesterone levels were compared between the PSD and CTRL groups. They found that sleep loss caused a significant decrease in concentrations of corticosterone during the diestrous phase, which had the lowest level of progesterone compared to all other phases. Levels of corticosterone were almost identical in the PSD and CTRL conditions during the proestrous phase that had the highest baseline level of progesterone. However, progesterone levels were lower in the PSD group compared to the CTRL group during the proestrous phase, but not any other phase. This suggests that cortiscosterone and progesterone levels may also be impacted through sleep loss.

The rat estrous cycle does have distinct phases with surges in progesterone and estrogen at times during their reproductive cycle just like in humans. However, rat estrous cycles are typically very short and they do not have a functional LP similar to humans in that they do not develop a fully functioning corpus luteum unless they receive coital stimulation (Marcondes et al., 2002). Therefore, it is hard to compare changes during rat estrous cycle to the human menstrual phases. However, this study does provide some evidence supporting the idea that high progesterone levels in rats are correlated with no change in corticosterone level following sleep loss. This is analogous to the high progesterone levels during the luteal phase being correlated with a lack of afternoon/evening cortisol elevation after sleep loss in humans.

The interaction between female gonadal hormones and the response to stress has been shown to occur at several different levels of the HPA axis, both centrally and peripherally, and this interaction is bidirectional. Stress has been shown to disrupt reproduction in both human and animal models (For review see Dobson & Smith, 2000). In animal models, progesterone has been shown to be a potent modulator of HPA axis stress regulation. Progesterone can function as: (1) a direct glucocorticoid receptor agonist (Arriza et al. 1987) (2) can bind to glucocorticoid receptors at a different site than other glucocorticoids (Svec, 1988), (3) can increase rate of dissociation of glucocorticoids from the receptor (Rousseau et al. 1972), and (4) can diminish the effectiveness of cortisol feedback on stress responsiveness (Keller-Wood et al. 1988). Unfortunately, evidence on the impact of progesterone on the HPA axis in humans is sparse.

Recent research in humans has shown a relationship between progesterone, allopregnanolone (ALLO), which is a metabolite of progesterone, and HPA activity. Genazzani et al. (1998) showed increases in plasma concentrations of progesterone and ALLO after an intravenous bolus of corticotrophin releasing hormone (CRH) or adrenocorticotropic hormone (ACTH). Following stressor exposure, progesterone and ALLO levels have been shown to increase (Girdler et al., 2001; Klatzkin et al., 2006). These results suggest that progesterone increases in response to stress, but does not provide evidence regarding HPA or cortisol response to fluctuating levels of progesterone. It has also been shown that the positive correlation between progesterone

and cortisol is the strongest in men and women taking oral contraceptives, but not in healthy cycling women (Wirth et al., 2007).

We did not find any significant correlations between progesterone levels and changes in cortisol levels in either the morning or evening after sleep restriction. This lack of correlation has to be interpreted cautiously because there are large individual differences in progesterone levels (Fujmoto et al., 1990), and the absolute values used in these correlations may be less relevant than the changes in levels across menstrual phases in individual women. This possibility could be addressed by a study in which responses to sleep loss and changes in progesterone levels are assessed in individual women at both follicular and luteal phases.

In conclusion, sleep deprivation may modulate cortisol release in a progesterone hormonal-neurochemical mechanism. The decrease of melatonin and the delayed circadian core-body temperature (CBT) rhythm in LP and the lack of circadian alterations in cortisol following sleep loss are events that appear to be linked to progesterone.

4.2 Impact of Menstrual Phase and Sleep Loss on Hunger

4.2.1 Baseline Hunger at Different Menstrual Phases

There was no difference in hunger during the baseline day between the luteal and follicular phase. This is consistent with the majority of the literature, which suggests that women consume more food during the premenstrual period or the late LP as compared to the mid-LP or FP (Barr et al., 1995; Li et al., 1999; Pelkman et al., 2000; Davidson et al.,

2007). The present study only measured baseline hunger ratings at two time points in the women's cycle: mid-follicular and mid-luteal. Studies that have shown a relationship between increase in food intake and menstrual phase have generally reported this to occur premenstrually. The pre-menstrual period, as stated previously usually begins 5 days prior to menses where progesterone levels are decreasing steeply, whereas the current study measured hunger approximately 10 days prior to menses. The mid-luteal phase is not the phase at which these changes have been reported to occur. This particular time of phase was chosen because it has the highest progesterone levels.

4.2.2 Effects of Sleep Loss on Hunger

A single night of sleep deprivation was associated with a statistically significant increase in hunger among all participants, and this increase did not differ between menstrual cycle phases. Specifically, increases in hunger were observed at all time points, but were only statistically significant during the evening. These results were as predicted and support previous findings that insufficient sleep is associated with weight gain and obesity through altering metabolic pathways and influencing energy metabolism (Watanabe, M et al., 2010; Lyytikainen et al., 2011). Although research has been mixed with regards to sleep loss effects on 'hunger ratings', there is evidence supporting the link between insufficient sleep and increases in the hunger-stimulating hormone ghrelin, and increases in appetite when food intake is controlled for (Spiegel et al., 1999; Schmid et al., 2008; Pejovic et al., 2010).

Markwald et al. (2013) studied 16 adults across 5 nights of insufficient sleep and found that energy expenditure increased during the course of the study, however energy intake, specifically during the evening, was in excess of energy needed in order to maintain a proper energy balance. This excess led to almost a kilogram of weight gain after the 5 days, despite increases in leptin and decreases in ghrelin and no change in hunger ratings. However, food during their study was available for participants at any time, whereas in the current study, food intake on the post sleep deprivation day was identical to their baseline day. It is possible that since the participants from the current study were not able to actively increase their food intake ad libitum, then increases in hunger ratings may be reflective of their increase in energy need.

One interpretation of these results and our own data is that increases in hunger after sleep loss are a physiological and behavioural adaptation to provide the body with extra energy needed to sustain activities during the extended hours of wakefulness. This argument is strengthened by the fact that hunger seems to be highest during the evening, when the participant has been without adequate amounts of sleep for the longest. Sleep has been proposed to be a physiological mechanism to conserve energy (Berger and Phillips, 1995). Energy expenditure is lower following sleep onset compared to pre-sleep wakefulness (Krieder et al., 1958, Bonnet et al., 1991). Recently, Jung et al. (2011) were able to quantify the energy saved during sleep compared to being awake. They found that during the nighttime, energy was increased by approximately 32% during a single night of total sleep deprivation and significantly decreased by 4% after the sleep recovery night of 8 hours. This works out to be approximately 562 Kj of energy lost by missing a single

night of sleep. Therefore, without sleep, it is possible that increases in hunger may reflect a need to take more energy to make up for the loss of energy associated with being awake. As Markwald et al. (2010) suggests this intake may be more than needed to offset the energy cost of sleep loss when food is readily available. Therefore, the weight gain and obesity that is shown to be associated with decreased sleep duration (Watanabe, M et al., 2010; Lyytikainen et al., 2011) may be from overeating during prolonged periods of wakefulness.

It is also possible that sleep loss alters brain mechanisms involved in non-homeostatic food intake such as mood and comfort or reduced eating restraint. Future research can examine the impact that differences in these factors can have on changes in hunger following sleep deprivation.

4.3 Impact of Menstrual Phase and Sleep Loss on Mood

4.3.1 Baseline Mood at Different Menstrual Phases

In the present study, we observed no significant changes in subjective mood across the different cycle phases. Although a link between the menstrual cycle phases and mood seem to be well established (Richardson, 1995; Steiner, 1997; Yonkers et al., 2008), the current results are not surprising since most of these mood changes are thought to occur during the late-luteal phase. The participants from this study were studied only during the mid-follicular and mid-luteal phases.

Romans et al. (2013) collected data from Canadian women across their menstrual cycles for 6 months. They reported that only half of their individual tested mood items showed any menstrual cycle correlation, and those mood changes that were detected occurred either in the menses phase alone or during menses plus the premenstrual phase. This is consistent with the literature suggesting that mood changes only occur during the 'perimenstrual' period. Therefore, the current results are in agreement with earlier studies in that there were no spontaneous changes in the mid-follicular or mid-luteal phase at which these women were studied.

4.3.2 Effects of Sleep Loss on Mood

A single night of sleep deprivation was associated with a statistically significant decrease in self-reports of the positive mood state Vigour, and a significant increase in self-reports of Fatigue and Total Mood Disturbance (TMD); however this did not differ across the menstrual cycle phases (Figure 13). It is important to note that the changes in mood states of the POMS both have to do with energy levels (i.e. Vigour and Fatigue). The TMD is calculated through the addition of all negative mood states and the reverse score of the positive mood states. Therefore the increase observed in the overall group merely reflects changes in Vigour and Fatigue.

The changes detected by the POMS after sleep deprivation are not related to traditional mood variables, such as sadness or anger; rather they reflect changes in energy level (decreased vigour and increased fatigue). The increase seen in the women during the current study still fell within the normal range of the general population. These findings are consistent with previous studies that have reported detrimental effects of

sleep restriction in healthy participants. Orton et al. (1989) measured the changes in mood following 31 consecutive hours of work with reduced sleep in young physicians. Deterioration in self-report scores following sleep loss was observed in the POMS subscale of Fatigue and Vigour. However, they also observed significant changes in depression, anger and confusion. This may reflect the effect of laboratory versus workplace on sleep deprivation studies. There are no responsibilities in the lab compared to the workplace and this added pressure/stress may more strongly affect negative mood.

This is the first study that examined the effects of menstrual cycle phase on the mood effects of sleep deprivation, and no phase-related differences were found in any of the mood states assessed by the POMS. A few studies have measured women's mood in response to sleep deprivation, but they never controlled for or reported on menstrual cycle phase. Within these studies, gender differences in response to sleep loss were reported. Reynolds et al., (1986) found that women had higher TMD scores on the POMS compared to men in an elderly population following sleep loss. Caldwell & LeDuc (1998) also reported that female aviators had higher scores of tension and anxiety and lower vitality compared to male aviators following 56 hours of wakefulness.

It could be speculated that the menstrual cycle is playing a role in these gender differences. While the current study found no effect of menstrual cycle on mood following 3 h of sleep restriction, this could be because of the exploratory nature of this research design for this particular question. The present study recorded mood from one day in the menstrual cycle using only one measure of mood (POMS). It is conceivable,

that a more elaborate measure would be necessary to assess subtle mood changes across the cycle following sleep loss. The POMS is not routinely used to measure mood states during the menstrual cycle and it may not be able to detect menstrual related mood changes. This scale places more emphasis on negative mood states than on positive moods such as happiness. This limits a complete description of mood experience. For example, a woman may have changes in amplitude for both positive and negative mood in a certain cycle phase. If only negative mood is studied, then it puts a bias in assuming that only negative mood changes.

A well-designed menstrual mood study should also collect data from all phases on the menstrual cycle (early and late follicular, ovulation and early and late luteal), using multiple measures of mood. In addition, these studies should use prospective rating and blind the menstrual cycle focus of the study to participants in order to minimize the effects of possible PMS labeling from prior beliefs. This could help distinguish if the differences in how men and women respond to sleep loss could be partially due to the physiological changes that occur across the menstrual cycle.

4.4 Summary and Conclusion

Sleep loss has repeatedly been reported to be a risk factor for weight gain, obesity and other metabolic dysfunctions (Watanabe, M et al., 2010; Lyytikainen et al., 2011), but the underlying mechanisms involved and whether they are similar in men and women have not been determined. This study provided new information about how normal

hormonal changes during the menstrual cycle in women affect their endocrine and other responses to sleep loss.

We found a significant change in morning and evening cortisol levels following sleep loss during the FP and no change at all during the LP. During the FP, women who were sleep-restricted showed a decrease in amplitude of cortisol rhythm across the circadian cycle with a decrease in morning values and an increase in evening values, whereas women during the LP show no difference at all. A potential explanation for this phenomenon is that progesterone, which is higher during the LP, may modulate the effects of sleep loss on the circadian pattern of cortisol. Future research on women taking oral contraceptives, those on hormone replacement therapy or those who are postmenopausal and have low levels of estrogen and progesterone could help clarify the role of sex hormones in modulating the response of cortisol to sleep loss. As mentioned previously, no correlation was reported between progesterone and the change in cortisol in response to sleep loss. This may mean that progesterone is not a relevant factor or that variability in progesterone levels among women obscured any potential effects. In addition, following cortisol across more sample times overnight would provide us with more information regarding the circadian response of cortisol following sleep loss in both of these groups.

Secondary analyses in this study examined how hunger and mood were affected by a single night of sleep deprivation and whether these changes were modulated by menstrual phase. In both cases, there was no influence of the particular menstrual phases studied on

responses to sleep loss. Future research could explore whether there are modulatory effects related to other phases of the menstrual cycle, in particular during menses and the premenstrual phase. There is no research that has explored this relationship between menstrual phase and sleep loss effects on hunger and mood.

In terms of the how the behavioural variables responded to sleep loss, women at both menstrual phases reported increased hunger, increased fatigue and decreased vigour, consistent with previous findings. One interpretation of these behavioural results is that feelings of less energy (increased fatigue and decreased vigour) are associated with the need for more energy, expressed as increased hunger. We observed a significant correlation between changes in fatigue and hunger, but not vigour and hunger. Previous research demonstrated the link between sleep loss and hunger by showing that sleep loss was associated with increases in energy expenditure and over-compensating increases in energy intake, leading to weight gain (Markwald et al. 2013).

In conclusion, this study demonstrated that menstrual phase affects physiological responses to sleep loss. This result supports the need to integrate research on women and their physiology, including aspects that differ radically from the physiology of men, into metabolism-related research. Further research into the physiological changes in women following sleep loss may ultimately provide a better understanding of how reduced hours of sleep affects risks for weight gain and hormonal disruption, as well as the development of associated chronic diseases in women, and ultimately improve diagnosis and treatment of women's health problems

Figure 1

The diurnal rhythm of salivary cortisol in healthy individuals under normal sleep/wake conditions. Independent of stress, cortisol rises in the early morning and peaks roughly 30-45 minutes after awakening, known as the cortisol awakening response. This is followed by a steady decrease throughout the day, with a nadir at approximately 12 hours after awakening. Adapted from Salimetrics (2012).

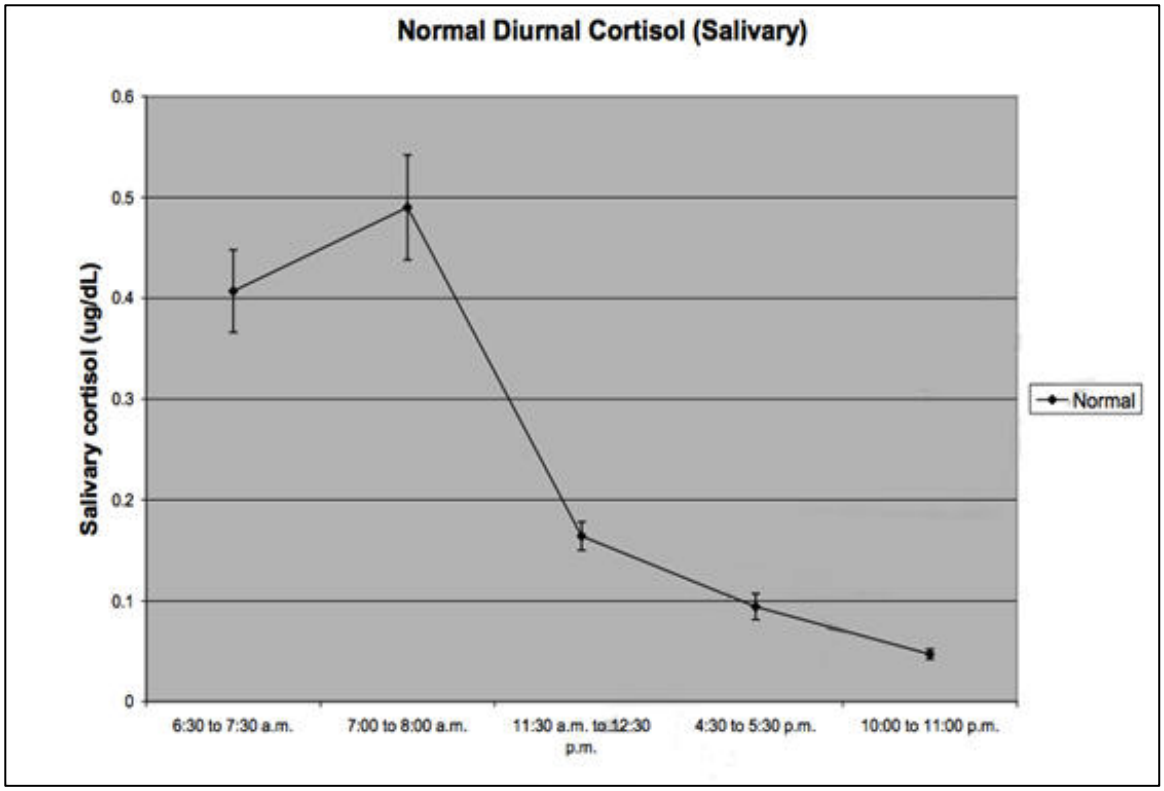


Figure 2

Average plasma ghrelin (A) and leptin (B) concentrations during a 24-h period in 10 human subjects consuming breakfast (B), lunch (L), and dinner (D) at the times indicated (0800, 1200, and 1730, respectively). Bedtime was at 2200 and wake-up time was at approximately 0800. Adapted from the American Diabetes Association (2011).

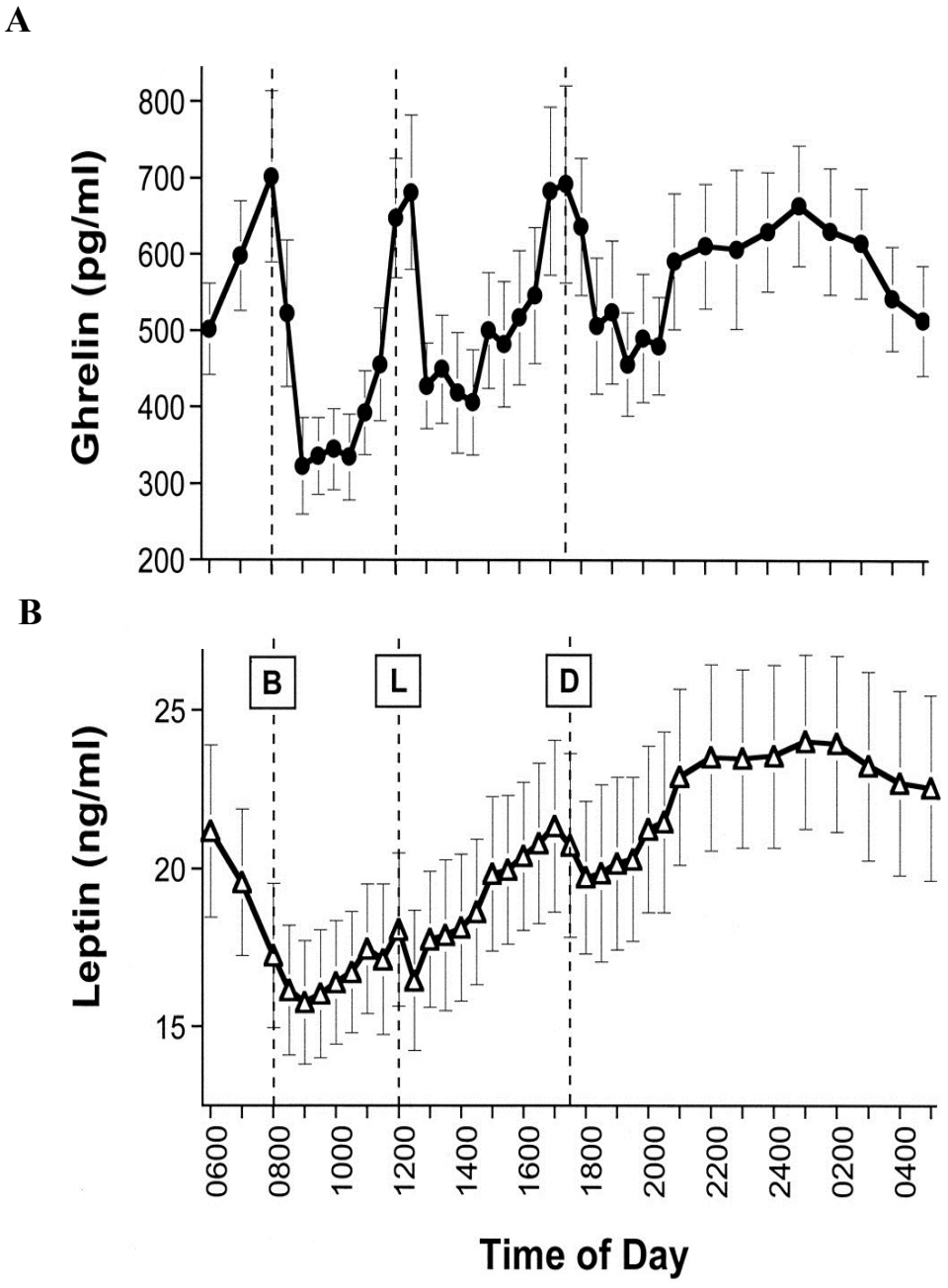


Figure 3

Mean cortisol values (ug/dL) collected 30 minutes after awakening in Follicular, Luteal and Oral Contraceptive users (O.C.) group. Morning 1 represents the Baseline day and Morning 2 represents the morning after the sleep restriction. Adapted from Omisade et al. (2010)

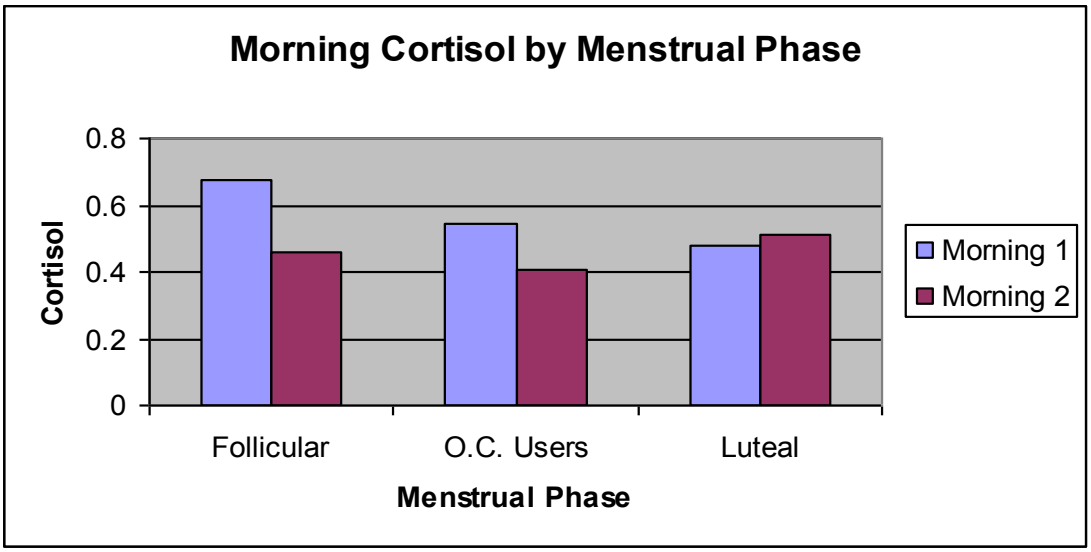


Figure 4

Estradiol and progesterone levels during the human menstrual cycle and the rat estrous cycle. (A) A Schematic of the 28 day human menstrual cycle is shown. Red lines indicate the approximate timing of sampling for the Follicular phase group. Blue lines indicate the approximate timing of sampling for the Luteal phase group. (B) A Schematic of the 4-day estrous cycle in rats. Adapted from Scharfman and MacLusky (2006).

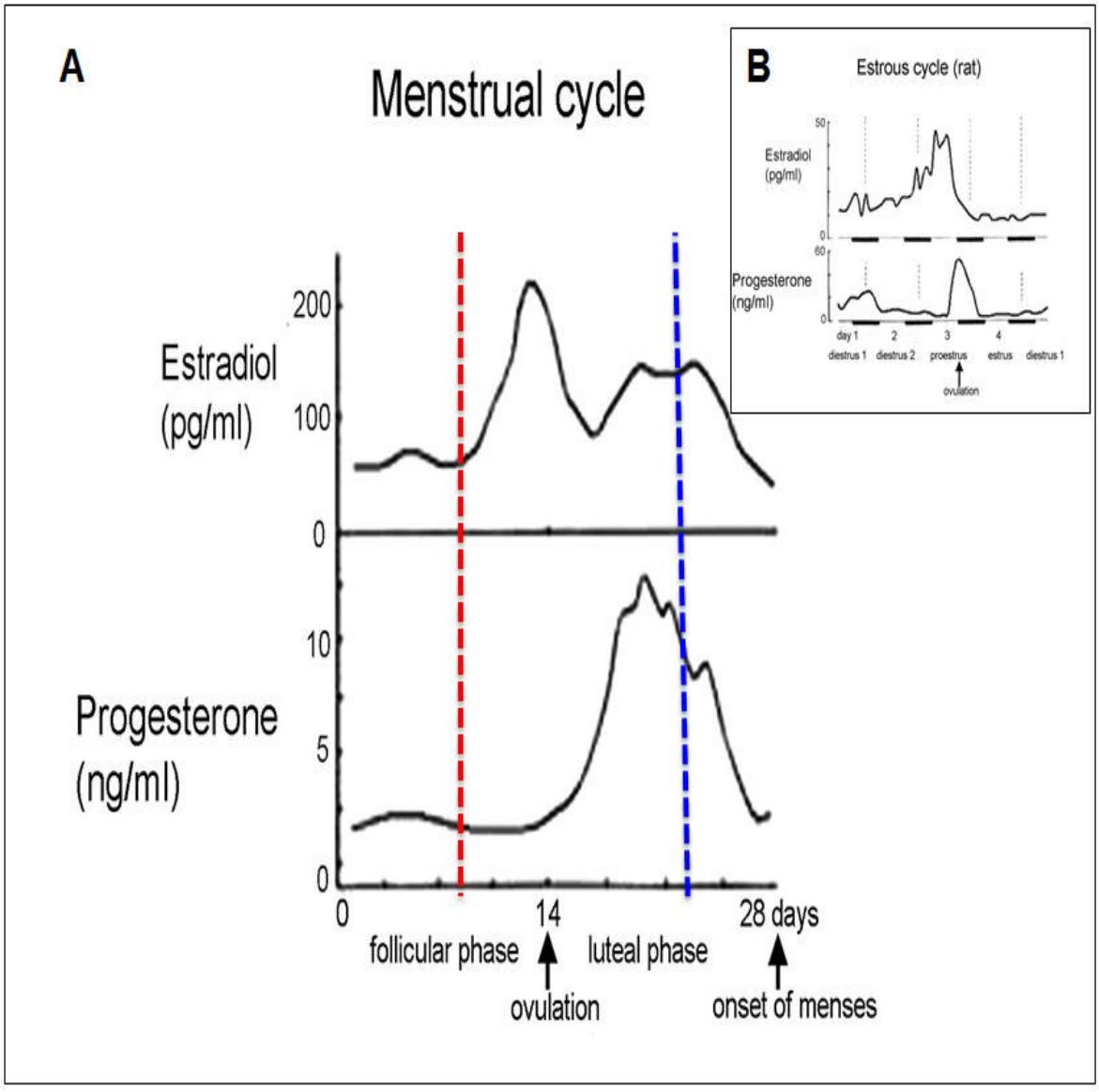


Figure 5

Schedule of procedures during the laboratory study. Filled rectangles represent the sleep opportunity for participants. Salivary samples for cortisol were obtained at the times indicated by arrows. Red arrows at 08:00 indicate first morning sample at wake-up and black arrows indicate the following saliva samples with 3 h time intervals. Adapted from Omisade et al. (2010).

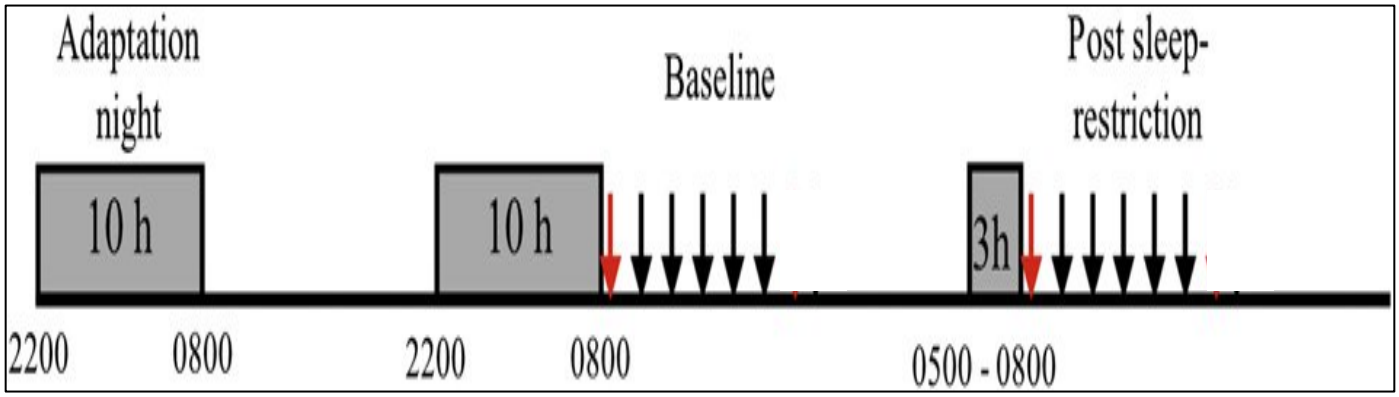


Figure 6

Mean salivary cortisol concentration across time of day for Baseline (circles) and Post sleep-restriction (triangles) days for the Follicular phase group (n=9). The blue dashed line indicates the morning period (08:00-08:30) for which the area under the curve of cortisol was calculated (M-AUCg). The red dashed line indicates the afternoon-evening period (14:00-20:00) for which the area under the curve of cortisol was calculated (E-AUCg).

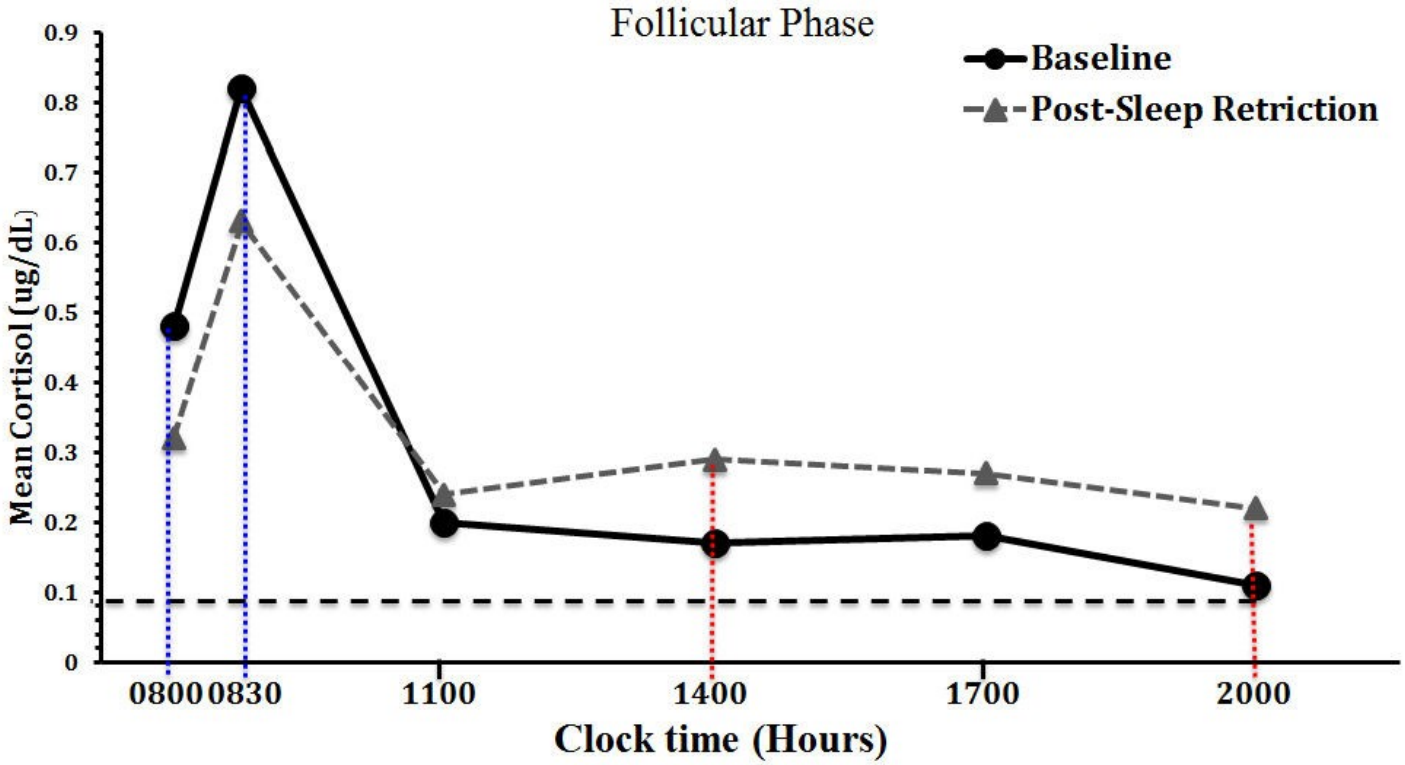


Figure 7
Mean salivary cortisol concentration across time of time for Baseline (circles) and Post sleep-restriction (triangles) days for the Luteal phase group (n=9). The blue dashed line indicates the morning period (08:00-08:30) for which the area under the curve of cortisol was calculated (M-AUCg). The red dashed line indicates the afternoon-evening period (14:00-20:00) for which the area under the curve of cortisol was calculated (E-AUCg).

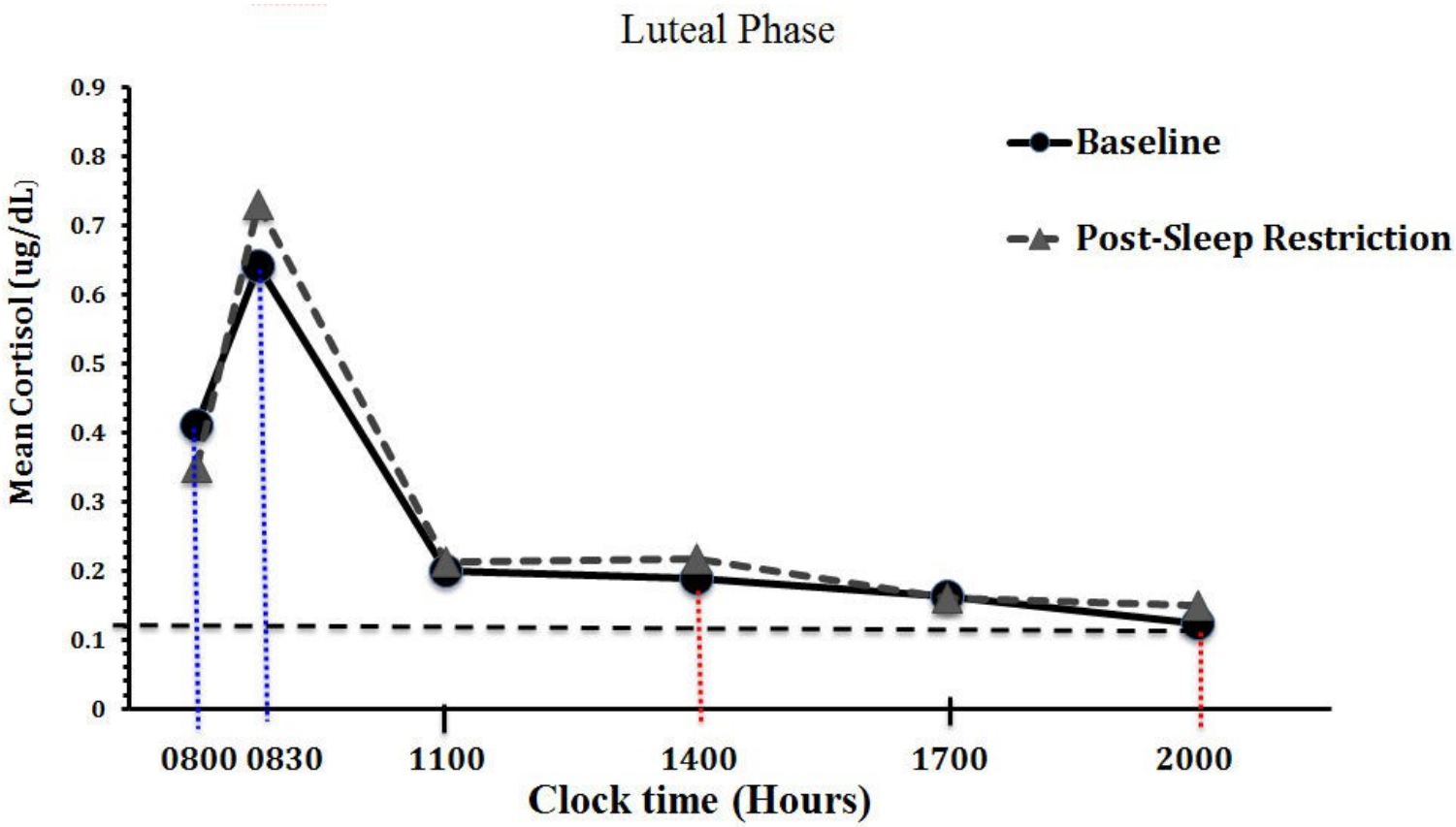
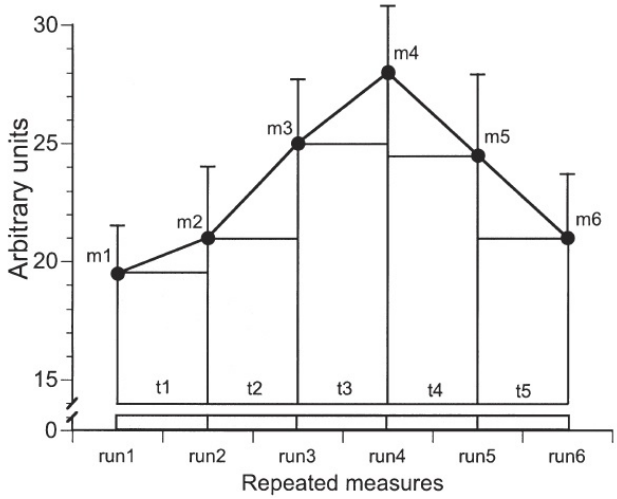


Figure 8

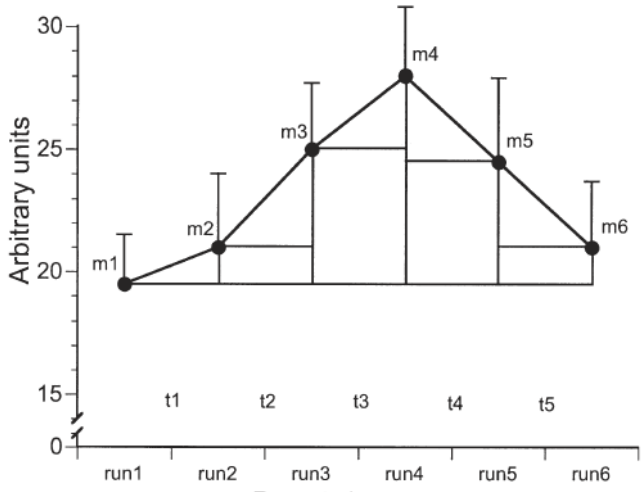
Representation of time course over the six arbitrary measurements used for Area Under the Curve calculations. (A) The triangles and rectangles illustrate the composition of the area under the curve with respect to the ground (AUCg). m1 to m6 denote the single measurements, and t1 to t5 denote the time interval between the measurements. The formula is the summation of the rectangles and triangles illustrated in the figure (B) The triangles and rectangles illustrate the composition of the area under the curve with respect to increase (AUCi)

A.



$$AUC_G = \frac{(m_2 + m_1)\cdot t_1}{2} + \frac{(m_3 + m_2)\cdot t_2}{2} + \frac{(m_4 + m_3)\cdot t_3}{2} + \frac{(m_5 + m_4)\cdot t_4}{2} + \frac{(m_6 + m_5)\cdot t_5}{2}$$

B.



$$AUC_I = \left(\sum_{i=1}^{n-1} \frac{(m_{(i+1)} + m_i)_t}{2}\right) - (n-1)\cdot m_1$$

Figure 9

Mean (± 95% CI) area under the curve to ground values for cortisol during the morning (M-AUCg) after unrestricted (10h) and restricted (3h) sleep. Paired T-test, *p=0.004

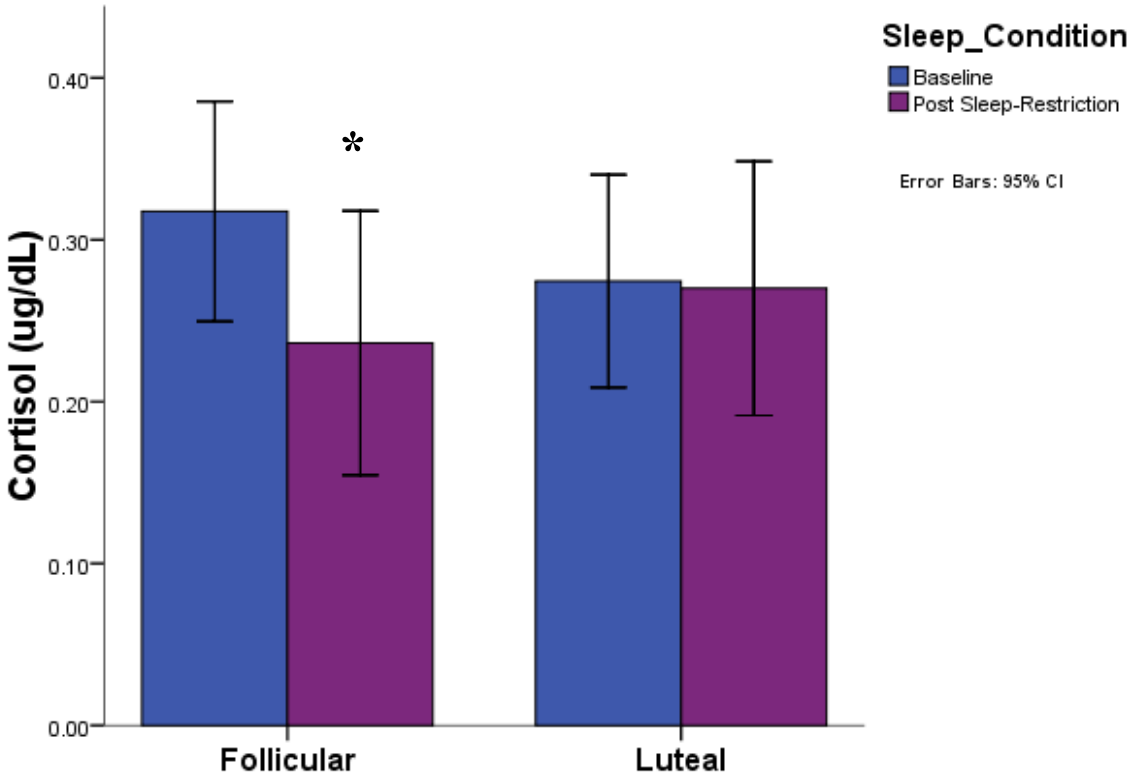


Figure 10

Mean (± 95% CI) area under the curve to ground values for cortisol during the Afternoon-Evening (E-AUCg) after unrestricted (10h) and restricted (3h) sleep during the Follicular group and the Luteal group. Paired T-test, *p=0.008

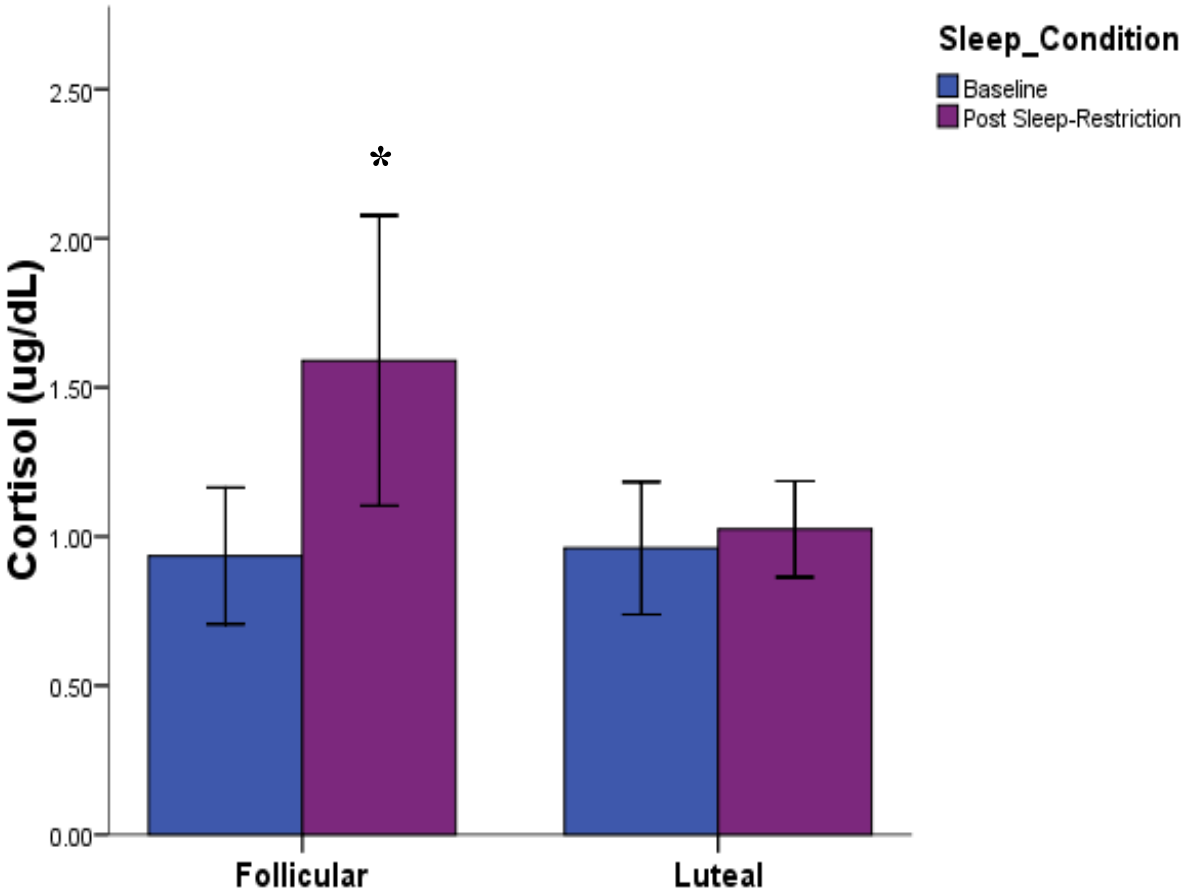


Figure 11

Mean (± 95% CI) scores for hunger after unrestricted (3h) sleep. (A) Mean scores for hunger between different menstrual phases: FP (n=9) and LP (n=9); (B) Mean scores for hunger in the combined group (n=18)

* Paired T-test, p=0.018; ** Paired T-test, p= 0.003; *** Paired T-test, p=0.000

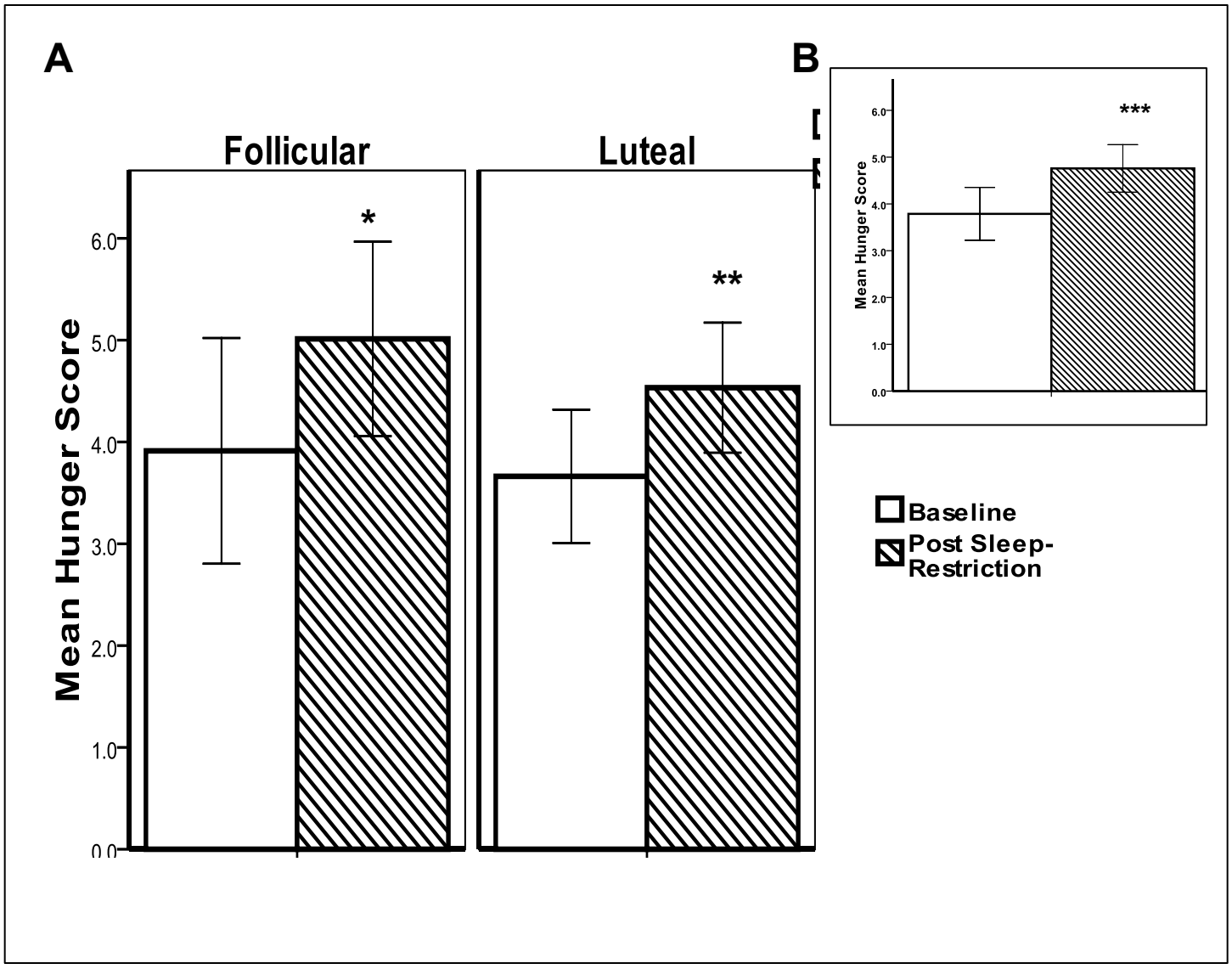


Figure 12

Mean (± 95% CI) scores for hunger after unrestricted (3h) sleep over time of day (Morning, Afternoon and Evening) for the overall group (n=18).

* Paired T-test, p=0.001

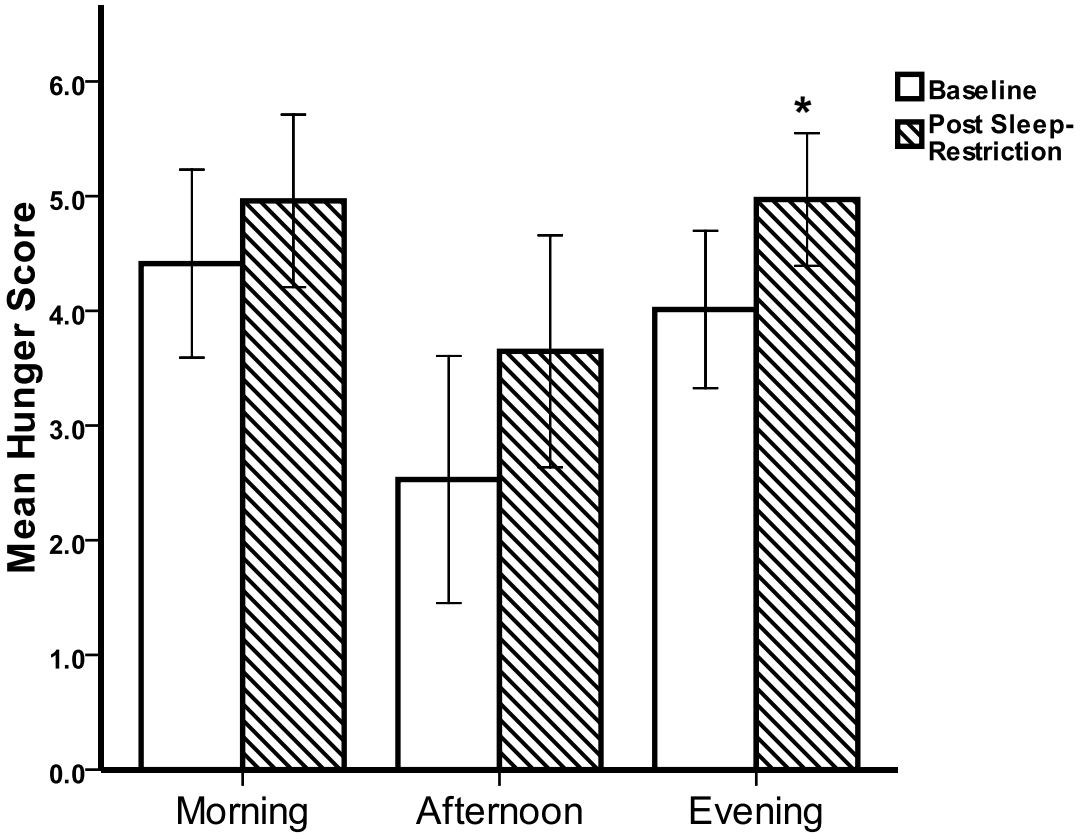
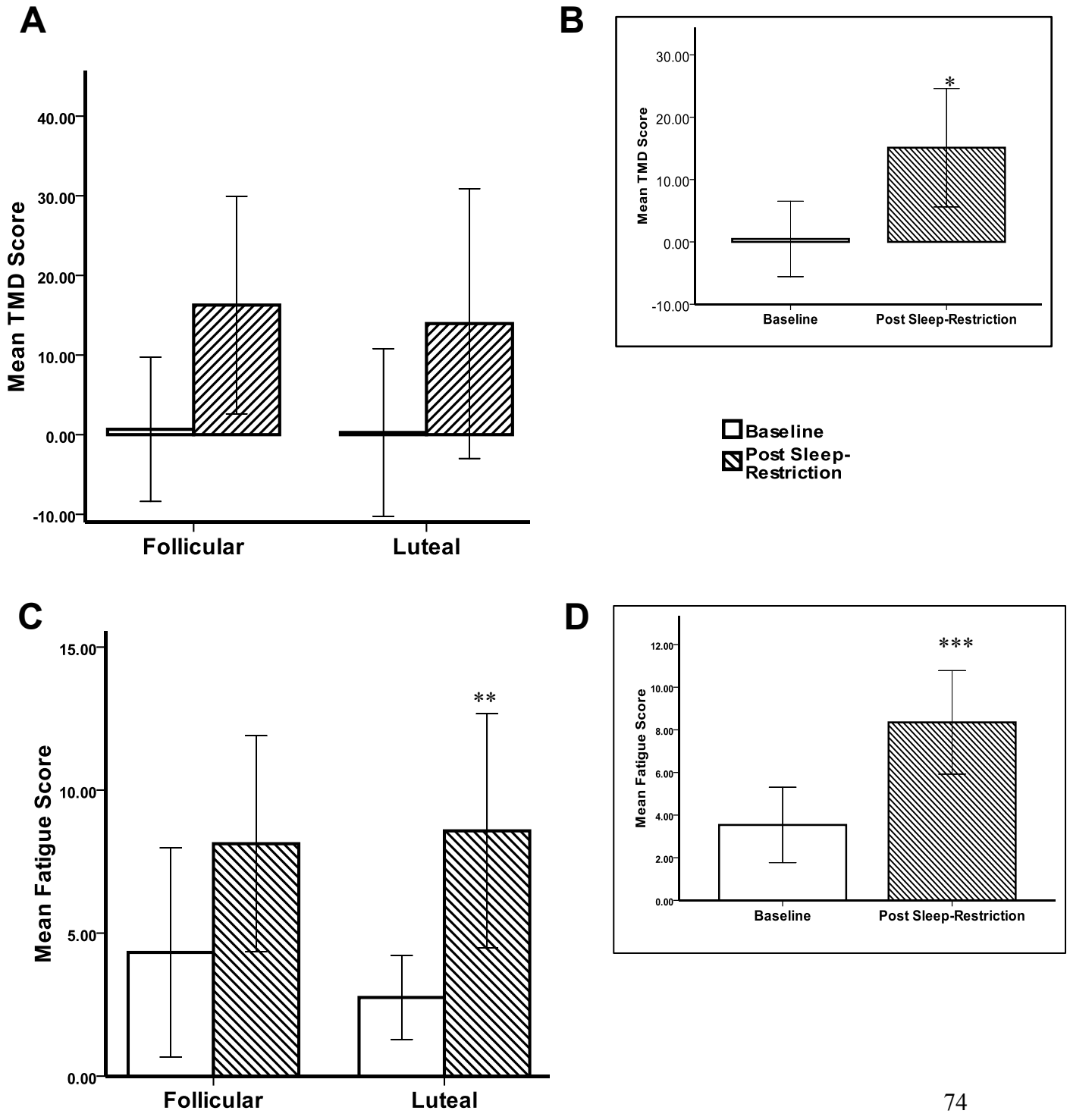


Figure 13

Mean (± 95% CI) scores for POM subscales after unrestricted (10h) and restricted (3h) sleep. **(A)**Total Mood Disturbance (TMD) mean scores between different menstrual phases: FP and LP; **(B)** TMD mean scores for overall group; **(C)** Fatigue mean scores between different menstrual phases: FP and LP; **(D)** Fatigue scores for overall group; **(E)** Vigour scores between different menstrual phases; FP and LP; and **(F)** Vigour scores for overall group.
* Paired T-test, p=0.002; ** Friedman Test, p= 0.005; ***Friedman Test, p=0.000



* Paired T-test, p=0.001; ** Paired T-test, p=0.000

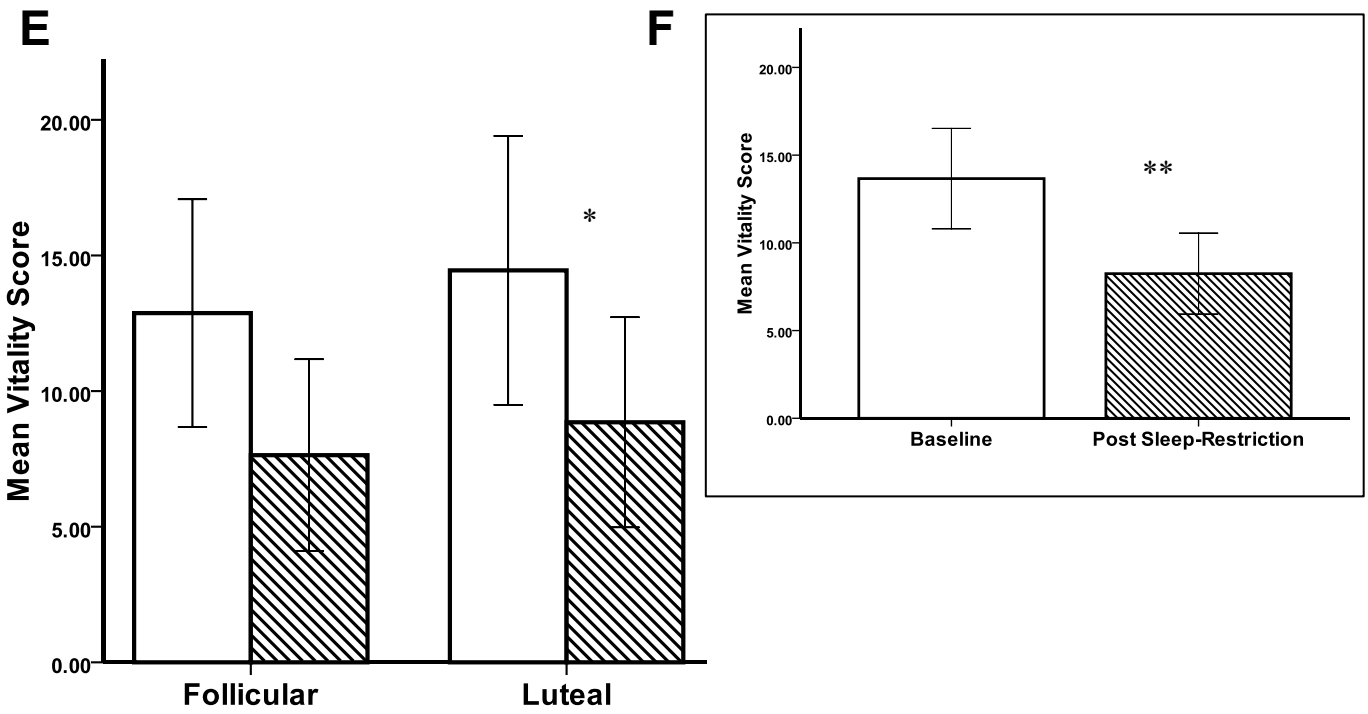


Figure 14

Positive Correlation between Difference Scores of Hunger (n=17) and Difference Scores of Fatigue (r (18)= 0.647, p=0.007)

Table 1

Descriptive characteristics of average length of menstrual cycle for each participant in the study as a function of menstrual phase group and their corresponding days of their cycle that they participated in the laboratory portion of the study.

Follicular			**Luteal**		
Participant	**Avg. Cycle (days)**	**Days studied**	**Participant**	**Avg. Cycle (days)**	**Days Studied**
1	33	7-9	10	27	19-21
2	34.5	11-13	11	26	17-19
3	28	7-9	12	28	19-21
4	28	9-11	13	28	21-23
5	33	13-15	14	34	25-27
6	27	6-8	15	32	19-21
7	30	11-13	16	29	18-20
8	31	10-12	17	30	21-23
9	29	8-10	18	34	23-25

Note. *Day 1 represents onset of menses

Table 2

One-way Analysis of variances for the descriptive characteristics (Age, BMI, MEQ, Normal Sleep, Night 2 Sleep and Night 3 Sleep) between Follicular and Luteal phase groups

	Df1	*Df2*	*F*	*p*
Age	1	16	.091	.767
BMI	1	16	.852	.370
MEQ	1	16	.003	.958
Normal Sleep	1	16	.387	.543
Night 2 Sleep	1	16	.258	.619
Night 3 Sleep	1	16	.566	.463

Note. MEQ: Morningness-Eveningness Questioniare

Table 3

One- way Analysis of Variance of cortisol values across time on the Baseline day between the Follicular (n=9) and Luteal (n=9) phase groups.

Cortisol	***Time***	***F***	***p***	***Df1***	***Df2***
	08:00	0.181	0.398	1	16
	08:30	2.906	0.108	1	16
	11:00	0.070	0.795	1	16
	14:00	0.301	0.591	1	16
	17:00	0.786	0.388	1	16
	20:00	0.787	0.388	1	16

Table 4

2x2 Mixed Analysis of variance for cortisol M-AUCg and E-AUCg values between Luteal (n=9) and Follicular (n=9) groups.

Cortisol					
	Effect	***F***	***Df1***	***Df2***	***p***
M-AUCg	Sleep	8.394	1	16	0.011*
	Phase	0.136	1	16	0.718
	Sleep*Phase	10.825	1	16	0.005**
E-AUCg	Sleep	15.315	1	16	0.001**
	Phase	3.411	1	16	0.083
	Sleep*Phase	10.241	1	16	0.005**

Note. *p<0.05, **p<0.01

Table 5

Parametric and Non-parametric repeated measure follow up tests for cortisol M-AUCg and E-AUCg values for each individual phase and overall group

Paired T-Test				
Time	***Group***	***Statistic***	***p***	***n***
M-AUCg				
	Follicular	3.984	0.004**	9
	Luteal	-0.312	0.763	9
	Overall	2.306	0.034*	18
E-AUCg				
	Follicular	-3.876	0.005**	9
	Luteal	-0.898	0.395	9
	Overall	3.150	0.006**	18
Wilcoxon Test				
Time	***Group***	***Statistic***	***p***	***n***
M-AUCg				
	Follicular	-2.666	0.008**	9
	Luteal	-0.59	0.953	9
	Overall	-2.201	0.028*	18
E-AUCg				
	Follicular	-2.668	0.008**	9
	Luteal	-0.770	0.441	9
	Overall	-2.809	0.005**	18

Note: *p<0.05, **p<0.01

Table 6

2x 2 Mixed Analysis of Variance for morning cortisol 'area under the curve with respect to increase' values (M-AUCi) between Follicular (n=9) and Luteal (n=8) phase groups

Cortisol (ug/dL)					
	Effect	***F***	***Df1***	***Df2***	***p***
M-AUCi	Sleep	2.139	1	16	0.163
	Phase	0.136	1	16	0.718
	Sleep*Phase	10.825	1	16	0.094

Table 7

One-way Analysis of Hunger ratings of the overall average score of the baseline day between Follicular phase (n=9) and Luteal phase (n=9) groups

Hunger	***Time***	***F***	***p***	***Df1***	***Df2***
	Day 1 Average	0.021	0.886	1	16

Table 8

2x2 Mixed Analysis of variance for overall average of hunger ratings between the Follicular (n=9) and Luteal (n=9) Phase groups

Hunger					
	Effect	***F***	***Df1***	***Df2***	***p***
	Sleep	22.769	1	16	0.000**
	Phase	0.343	1	16	0.022[§]
	Sleep*Phase	1.124	1	16	0.306

Note: * $p<0.05$, ** $p<0.01$

[§] This value was significant, but was categorized as a 'serendipitous finding' as it wasn't related to the original research questions.

Table 9

Follow-up paired sample t-tests for overall average hunger scores in each individual menstrual phase and overall group

Hunger				
	Group	***Statistic***	***p***	***N***
	Follicular	-3.085	0.018*	9
	Luteal	4.287	0.003**	9
	Overall	4.699	0.000**	18

Note: * $p<0.05$, ** $p<0.01$

Table 10

3x2 Repeated Measures Analysis of Variance for average overall hunger ratings between the Follicular and Luteal Phase groups

Hunger					
	Effect	***F***	***Df1***	***Df2***	***p***
	Sleep	22.769	1	16	0.000**
	Phase	0.254	1	16	0.621
	Sleep*Phase	1.124	1	16	0.306
	Time	25.610	2	15	0.000**
	Time*Phase	0.658	2	15	0.533

Note: * $p<0.05$, ** $p<0.01$

Table 11

Parametric and Non-parametric repeated measures follow-up tests for hunger ratings across time (Morning, Afternoon and Evening) for each individual phase and the overall group.

Paired T-Test				
Time	***Group***	***Statistic***	***p***	***N***
Morning	Overall	2.545	0.022	17
Afternoon	Overall	1.446	0.167	17
Evening	Overall	3.856	0.001*	17
Friedman Test				
Time	***Group***	***Statistic***	***P***	***N***
Morning	Overall	4.00	0.046	17
Afternoon	Overall	1.333	0.248	17
Evening	Overall	6.250	0.012*	17

Note: * $p<0.02$; Bonferroni corrected alpha values (0.05/3)

Table 12

7 One-way Analysis of Variance for baseline mood scores from all of the POMS subscales (Anger, Fatigue, Depression, Confusion, Tension, Vigour and TMD) between Follicular (n=8) and Luteal (n=9) Phase Groups

POMS	***Mood State***	***F***	***p***	***Df1***	***Df2***
	Anger	0.087	0.773	1	15
	Fatigue	0.892	0.351	1	15
	Depression	0.054	0.804	1	15
	Confusion	0.013	0.911	1	15
	Tension	0.799	0.401	1	15
	Vigour	0.329	0.575	1	15
	TMD	0.005	0.947	1	15

Note: TMD: Total Mood Disturbance

Table 13

2x2 Mixed Analysis of Variance for overall average mood scores from all of the POMS subscales between Follicular (n=8) and Luteal (n=9) Phase groups.

Anger					
	Effect	***F***	***Df1***	***Df2***	***p***
	Sleep	0.358	1	15	0.559
	Phase	0.782	1	15	0.845
	Sleep*Phase	0.040	1	15	0.782
Fatigue					
	Effect	***F***	***Df1***	***Df2***	***p***
	Sleep	12.06	1	15	0.004*
	Phase	0.040	1	15	0.003
	Sleep*Phase	0.163	1	15	0.782
Depression					
	Effect	***F***	***Df1***	***Df2***	***p***
	Sleep	0.358	1	15	0.559
	Phase	0.633	1	15	0.782
	Sleep*Phase	0.400	1	15	0.845
Confusion					
	Effect	***F***	***Df1***	***Df2***	***p***
	Sleep	8.495	1	15	0.011
	Phase	0.079	1	15	0.0782
	Sleep*Phase	0.084	1	15	0.776
Tension					
	Effect	***F***	***Df1***	***Df2***	***p***
	Sleep		1	15	
	Phase	0.002	1	15	0.959
	Sleep*Phase		1	15	
Vigour					
	Effect	***F***	***Df1***	***Df2***	***p***
	Sleep	13.73	1	15	0.002*
	Phase	0.030	1	15	0.854
	Sleep*Phase	0.063	1	15	0.806
TMD					
	Effect	***F***	***Df1***	***Df2***	***p***
	Sleep	13.73	1	15	0.002*
	Phase	0.007	1	15	0.933
	Sleep*Phase	0.063	1	15	0.806

Note: *p<0.008; Bonferonni corrected alpha values (0.05/6), TMD: Total Mood Disturbance

Table 14

Parametric and Non-parametric follow-up tests for 4 POMS mood variables for each individual phase and overall group

Paired Sample T-Test				
Time	***Group***	***Statistic***	***P***	***N***
Vitality	Follicular	2.144	0.069	8
	Luteal	5.272	0.001*	8
	Overall	4.658	0.000*	16
TMD	Follicular	2.276	0.057	8
	Luteal	-3.244	0.019	8
	Overall	3.827	0.002*	16
Friedman Test				
Mood Variable	***Group***	***Statistic***	***P***	***N***
Fatigue	Follicular	4.500	0.034	8
	Luteal	8.000	0.005*	8
	Overall	12.250	0.000*	16
Confusion	Follicular	4.500	0.034	8
	Luteal	0.500	0.480	8
	Overall	4.000	0.046	16

Note: *p<0.008; Bonferonni corrected alpha value (0.05/6)

Table 15

Correlations of Hunger with Cortisol (ug/dL) using baseline values and difference scores at five different time points across the day: 08:00, 11:00, 14:00, 17:00, 20:00

Baseline Correlations			
	Statistic	***P value***	***N***
08:00 Hunger vs. Cortisol	0.145[S]	0.578	17
11:00 Hunger vs. Cortisol	0.134[P]	0.608	17
14:00 Hunger vs. Cortisol	0.382[S]	0.130	17
17:00 Hunger vs. Cortisol	0.505[S]	0.039	17
20:00 Hunger vs. Cortisol	-0.313[P]	0.221	17
Difference Correlations			
	Statistic	***P Value***	***N***
08:00 Hunger vs. Cortisol	0.230[P]	0.373	17
11:00 Hunger vs. Cortisol	-0.125[P]	0.632	17
14:00 Hunger vs. Cortisol	0.656[S]	0.004	17
17:00 Hunger vs. Cortisol	-0.105[P]	0.688	17
20:00 Hunger vs. Cortisol	0.079[S]	0.764	17

Note. [P] Pearson Correlation, [S] Spearman Correlation

Table 16

Correlations of Progesterone values with Cortisol (ug/dL) using baseline values and difference scores at two different time points: Morning (M-AUCg) and Afternoon-Evening (E-AUCg).

Baseline Correlations			
	Statistic	***P value***	***N***
Progesterone vs. M-AUCg	0.169[P]	0.516	18
Progesterone vs. E-AUCg	0.150[P]	0.565	18
Difference Correlations			
	Statistic	***P Value***	***N***
Progesterone vs. M-AUCg	0.438 [P]	0.079	18
Progesterose vs. E-AUCg	-0.354[P]	0.163	18

Note: [P] Pearson Correlation, [S] Spearman Correlation

Table 17

Correlations of Hunger with Mood using baseline values and difference scores

Baseline Correlations			
	Statistic	***P value***	***N***
Hunger vs. Vigour	0.068[P]	0.802	17
Hunger vs. Fatigue	0.102[P]	0.706	17
Difference Correlations			
	Statistic	***P Value***	***N***
Hunger vs. Vigour	0.042 [P]	0.878	17
Hunger vs. Fatigue	0.647[P]	0.007*	17

Note. * $p > 0.01$; [P] Pearson Correlation, [S] Spearman Correlation

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Appendix 1

Means, Standard Deviations and Sample Sizes For Descriptive Characteristics Analyzed with ANOVAs As a Function Of Menstrual Phase

	Phase	*N*	*M*	*SD*
Age	Follicular	9	22.00	2.693
	Luteal	9	21.67	1.936
	Total	18	21.83	2.282
BMI	Follicular	9	22.033	1.8351
	Luteal	9	23.200	3.3181
	Total	18	22.617	2.6695
Progesterone	Follicular	9	77.66	8.124
	Luteal	9	245.13	9.293
	Total	18	161.40	8.468
Typical Sleep	Follicular	9	8.411	.9662
	Luteal	9	8.156	.7650
	Total	18	8.283	.8556
Night 2	Follicular	9	8.178	.8955
	Luteal	9	8.367	.6671
	Total	18	8.272	.7722
Night 3	Follicular	9	2.956	.1014
	Luteal	9	2.911	.1453
	Total	18	2.933	.1237

Note. BMI: Body Mass Index; Progesterone (pg/mL)

Appendix 2

(A) Levene's Test of Equality of Error Variances and (B) Shapiro-Wilk's Test for Normality of Distribution For Baseline Descriptive Characteristics Analyzed with ANOVAs as a Function of Menstrual Phase Group

A.

	Levene Statistic	***Df1***	***Df2***	***p***
Age	1.469	1	16	.243
BMI	2.399	1	16	.141
MEQ	.273	1	16	.609
Typical Sleep	.863	1	16	.367
Night 2	.132	1	16	.721
Night 3	.376	1	16	.548

B.

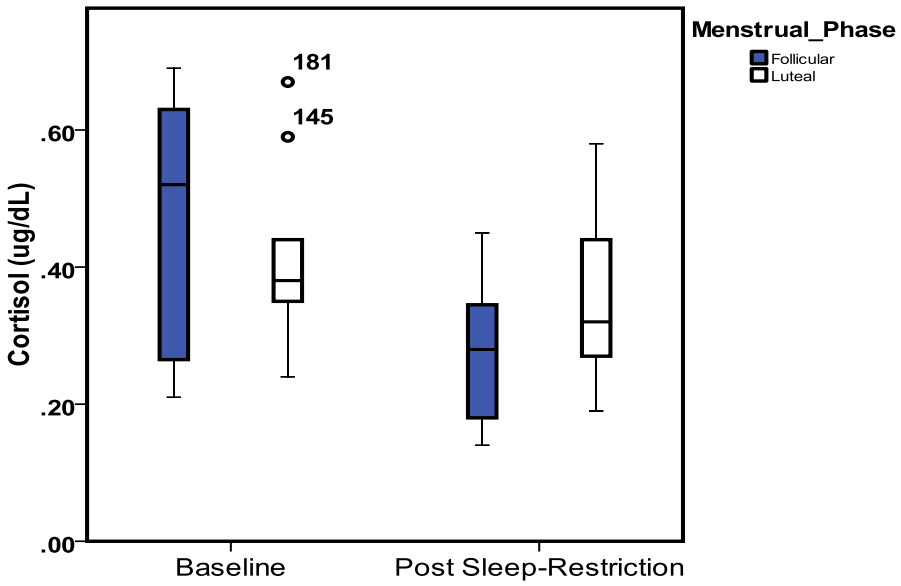
		Shapiro-Wilk		
	Phase	***Statistic***	***Df***	***p***
Age	Follicular	.906	9	.286
	Luteal	.887	9	.187
BMI	Follicular	.905	9	.285
	Luteal	.904	9	.274
MEQ	Follicular	.970	9	.892
	Luteal	.941	9	.592
Typical Sleep	Follicular	.864	9	.107
	Luteal	.933	9	.509
Night 2	Follicular	.852	9	.079
	Luteal	.932	9	.497
Night 3	Follicular	.830	9	.045*
	Luteal	.942	9	.601

Note. * p >0.05; BMI: Body Mass Index; MEQ: Morningness-Eveningness Questionnaire

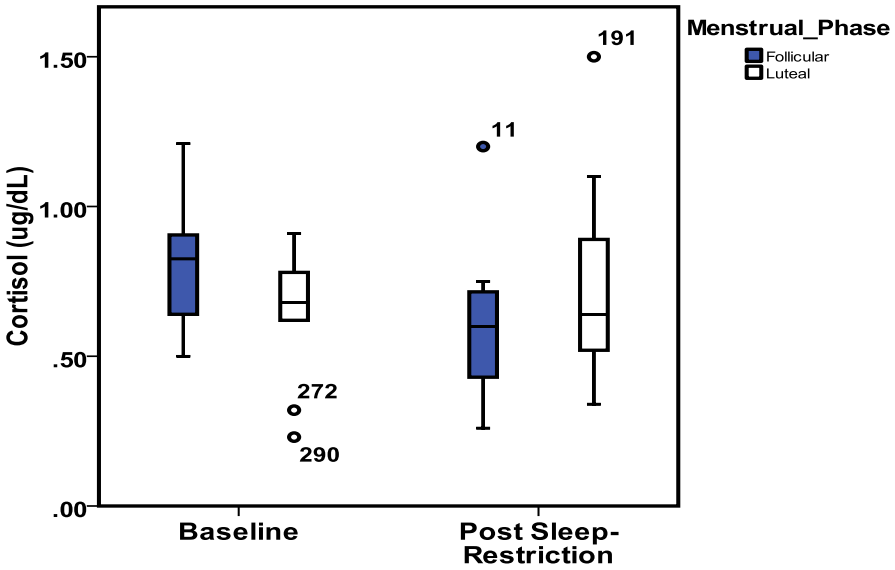
Appendix 3

Box-plots For Cortisol values Analyzed with ANOVAs and Parametric and Non-parametric Follow-up Tests As a Function of Menstrual Phase Group, Sleep Condition and Time

A. 08:00

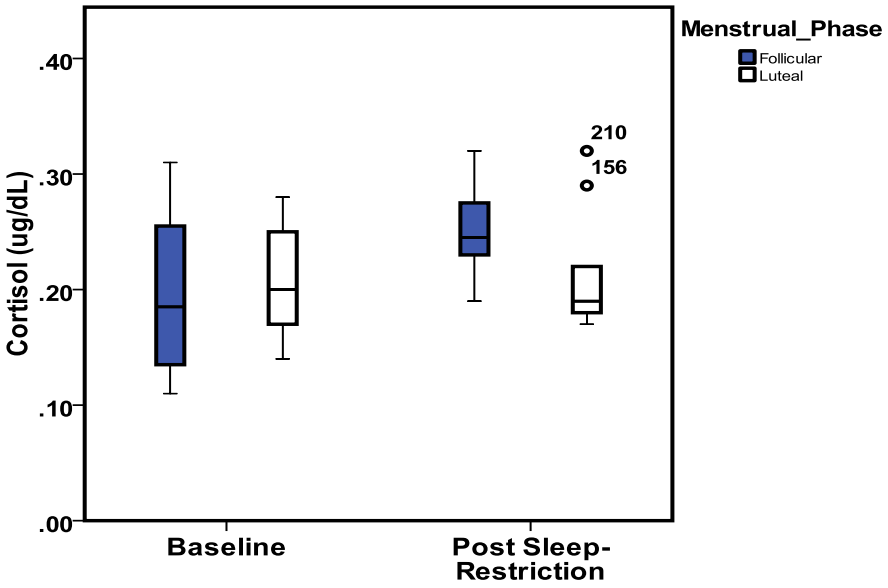


B. 08:30

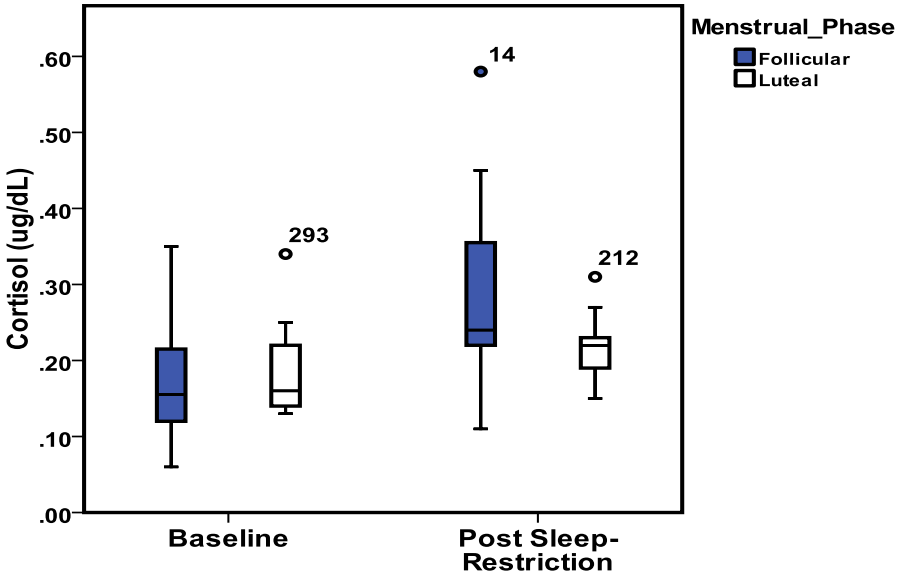


Appendix 3 continued

C. 11:00

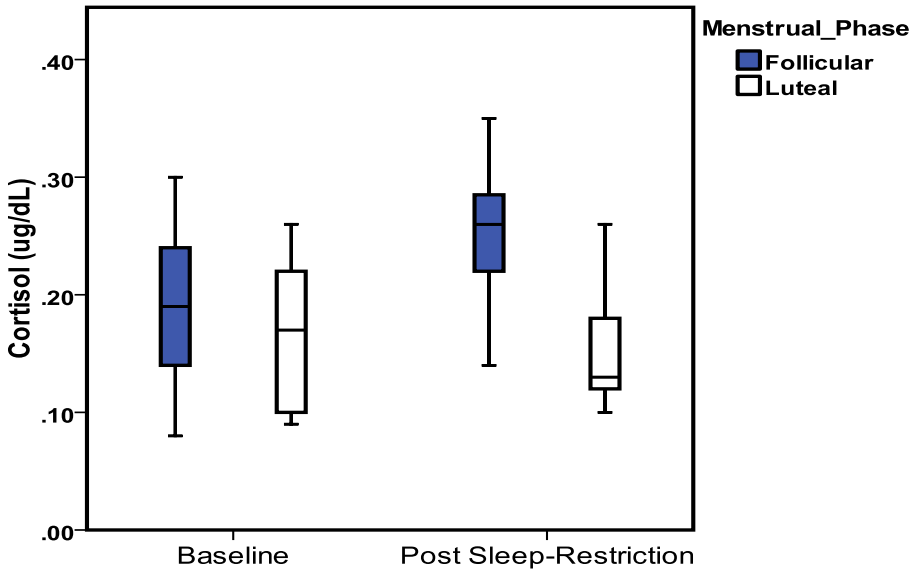


D. 14:00

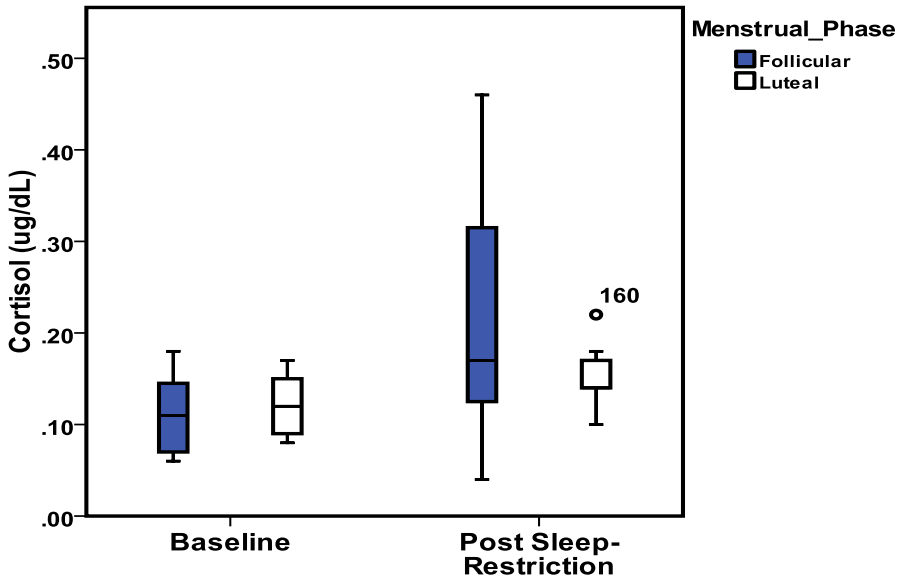


Appendix 3 continued

E. 17:00



F. 20:00



Appendix 4

Means, Standard Deviations (SD) and Sample Sizes For Cortisol Values Analyzed with ANOVAs as a Function of Menstrual Phase Group, Time and Sleep Condition

Cortisol (ug/dL)			**Follicular Group**			**Luteal Group**		
	Condition	***Time***	***M***	***SD***	***N***	***M***	***SD***	***N***
	Baseline	08:00	0.48	0.186	9	0.41	0.140	9
	Baseline	08:30	0.82	0.207	9	0.64	0.227	9
	Baseline	11:00	0.20	0.071	9	0.20	0.053	9
	Baseline	14:00	0.17	0.084	9	0.19	0.070	9
	Baseline	17:00	0.17	0.058	9	0.16	0.069	9
	Baseline	20:00	0.11	0.046	9	0.12	0.033	9

Appendix 5

(A) Shapiro-Wilk's Test for Normality of Distribution and **(B)** Levene's Test of Equality of Error Variances For Baseline Cortisol Values Analyzed with ANOVAs as a Function of Time and Menstrual Phase Group

A.

Cortisol		Shapiro-Wilk		
	Phase	***Statistic***	***df***	***p***
08:00	**Follicular**	.865	9	.108
	Luteal	.916	9	.357
08:30	**Follicular**	.910	9	.316
	Luteal	.912	9	.333
11:00	**Follicular**	.905	9	.279
	Luteal	.836	9	.052
14:00	**Follicular**	.959	9	.788
	Luteal	.858	9	.091
17:00	**Follicular**	.859	9	.093
	Luteal	.929	9	.473
20:00	**Follicular**	.956	9	.760
	Luteal	.883	9	.168

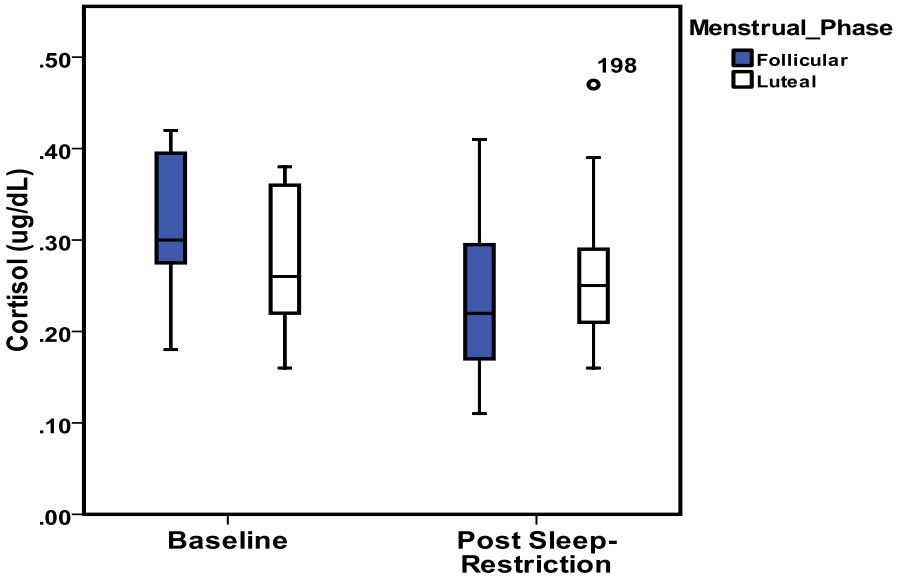
B.

Time	Levene Statistic	*df1*	*df2*	*Sig.*
08:00	.078	1	16	.784
08:30	1.852	1	16	.192
11:00	.386	1	16	.543
14:00	.096	1	16	.761
17:00	.831	1	16	.376
20:00	3.428	1	16	.083

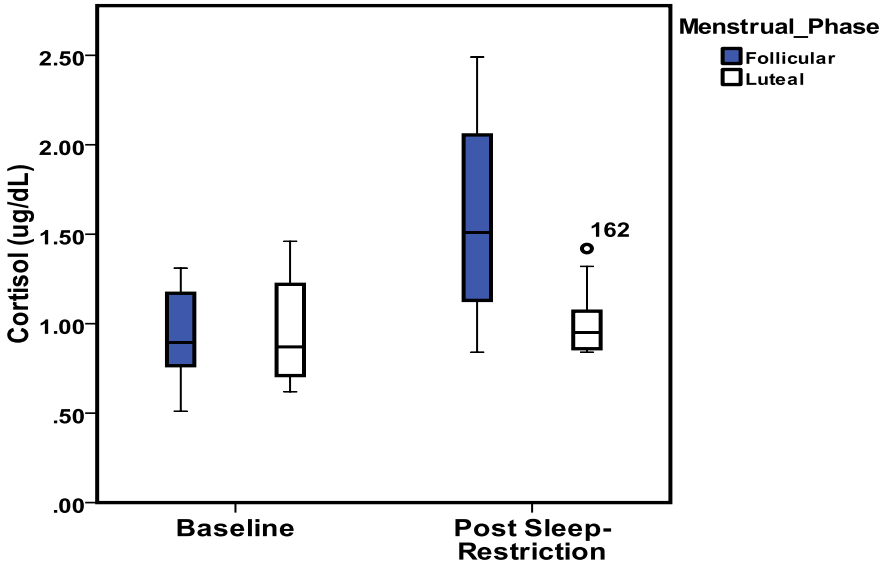
Appendix 6

Box-plots for (A) M-AUCg and (B) E-AUCg Cortisol Values Analyze with ANOVAs, and Parametric and Non-parametric Follow-up Tests As a Function of Menstrual Phase Group and Sleep Condition

A.



B. E



Appendix 7

Means, Standard Deviations (SD) and Sample Size for (A) M-AUCg and (B) E-AUCg Cortisol Values (ug/dL) Analyzed with ANOVAs and Parametric and Non-Parametric Follow-up Tests as a Function of Menstrual Phase Group and Sleep Condition

A.

Condition	Phase	*M*	*SD*	*N*
Baseline	**Follicular**	.3239	.07802	9
	Luteal	.2631	.07523	9
		.	.	
Post-sleep Restriction	**Follicular**	.2364	.09300	9
	Luteal	.2686	.10184	9
		.	.	

B.

Condition	Phase	*M*	*SD*	*N*
Baseline	**Follicular**	0.9333	0.25553	9
	Luteal	0.9583	0.28818	9
Post-sleep Restriction	**Follicular**	1.5650	0.55027	9
	Luteal	1.0217	0.21259	9

Appendix 8

Levene's Test of Equality of Error Variances For Cortisol Values Analyzed with ANOVAs and Parametric and Non-parametric Follow-up Tests As a Function of Sleep Condition

A.

Condition	*Levene Statistic*	*Df1*	*Df2*	*p*
M-AUCg	.			.
Baseline	.058	1	16	.812
Post-sleep Restriction	.059	1	16	.811

B.

Condition	*Levene Statistic*	*Df1*	*Df2*	*p*
E-AUCg				
Baseline	.355	1	16	.560
Post-sleep Restriction	7.521	1	16	.014*

Appendix 9

Shapiro-Wilk's Tests for Normality for Cortisol Values Analyzed with ANOVAs, and Parametric and Non-parametric Follow-up Tests As a Function of Menstrual Phase Group and Sleep Condition

A.

Follicular		**Shapiro-Wilk**		
	Condition	***Statistic***	***Df***	***p***
M-AUCg	Baseline	.929	8	.503
	Post-Sleep Restriction	.950	8	.708
		Shapiro-Wilk		
	Condition	***Statistic***	***Df***	***p***
E-AUCg	Baseline	.951	8	.720
	Post-sleep Restriction	.956	8	.772

B.

Luteal		**Shapiro-Wilk**		
	Condition	***Statistic***	***Df***	***p***
M-AUCg	Baseline	.911	9	.321
	Post-sleep Restriction	.903	9	.270
		Shapiro-Wilk		
	Condition	***Statistic***	***Df***	***p***
M-AUCg	Baseline	.923	9	.417
	Post-sleep Restriction	.821	9	.035*

Note. * p > 0.05

Appendix 10

Means, Standard Deviations (SD) and Sample size For Cortisol Values Analyzed with ANOVAs As a Function of Sleep Condition and Menstrual Phase Group

M-AUCi (ug/dL)				
Condition	**Phase**	**M**	**SD**	**N**
Baseline	Follicular	.0839	.05978	9
	Luteal	.0589	.05622	9
	Total	.0714	.05775	18
Post-sleep Restriction	Follicular	.0800	.05979	9
	Luteal	.0989	.06133	9
	Total	.0894	.05955	18

Appendix 11

(A) Levene's Test of Equality of Error Variances and (B) Shapiro-Wilk's Tests for Normality for M-AUCi Cortisol Valuess Analyzed with ANOVAs As a Function of Sleep Condition

A.

Condition	***Levene Statistic***	***Df1***	***Df2***	***p***
Baseline	.060	1	16	.810
Post-sleep Restriction	.052	1	16	.822

B.

Condition	**Shapiro-Wilk**		
	Statistic	***Df***	***p***
Baseline	.990	18	.999
Post-sleep Restriction	.921	18	.134

Appendix 12

Levene's Test of Equality of Error Variances For Hunger Ratings Analyzed with ANOVAs, and Parametric and Non-parametric Follow-up Tests across the Overall Day and Time of Day As a Function of Sleep Condition

A.

Condition	*Levene Statistic*	*Df1*	*Df2*	*p*
Overall Day	.			.
Baseline	410	1	16	532
Post-sleep Restriction	1.023	1	16	.328

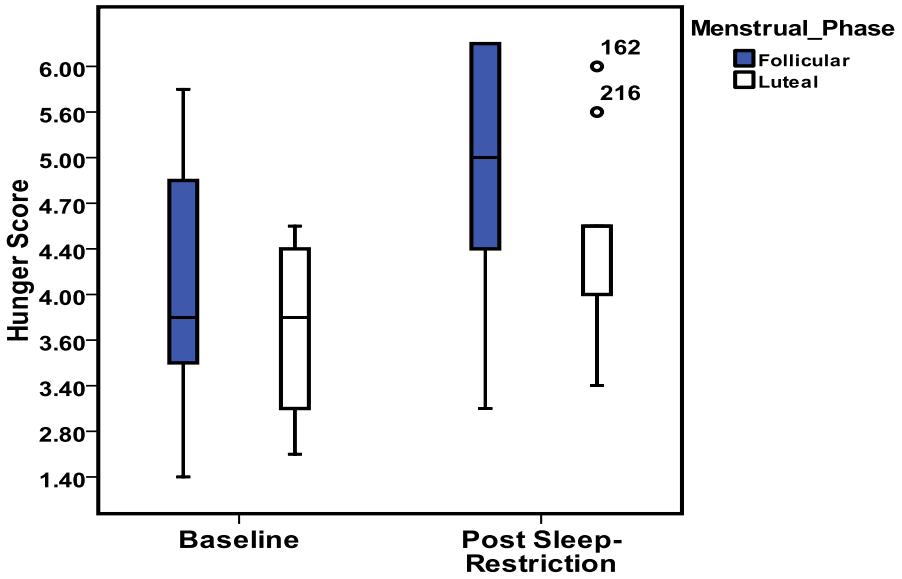
B.

Condition	*Levene Statistic*	*Df1*	*Df2*	*p*
Morning	.			
Baseline	665	1	16	.427
Post-sleep Restriction	.050	1	16	.826
	.			.
Afternoon				
Baseline	015	1	16	905
Post-sleep Restriction	9.867	1	16	.007*
Evening				
Baseline	4.040	1	16	.063
Post-sleep Restriction	17.964	1	16	.001*

Appendix 13

Box plots For Hunger Ratings Across the (A) Overall Day and Across Time (B) Morning, (C) Afternoon, and (D) Evening Analyzed with ANOVAs, and Parametric and Non-parametric Follow-up Tests As a Function of Menstrual phase group and Sleep Condition

A.



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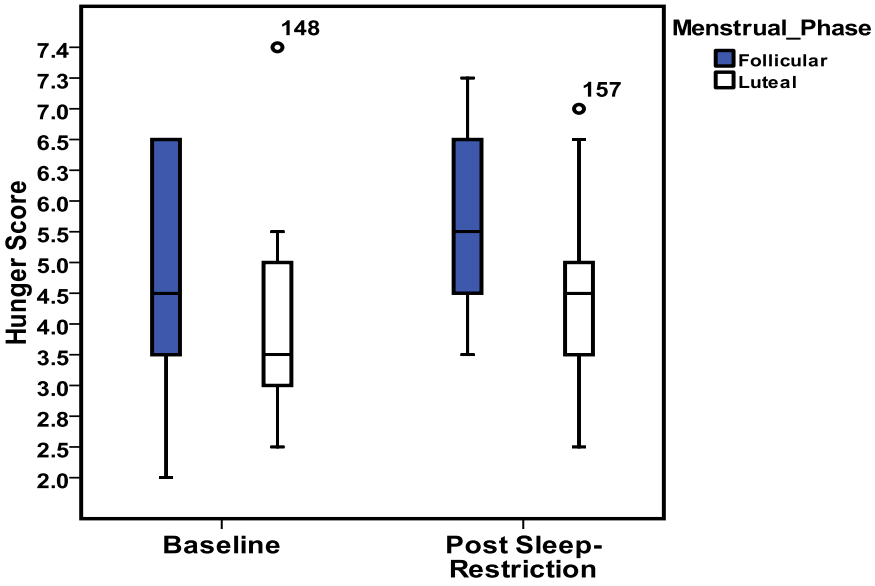
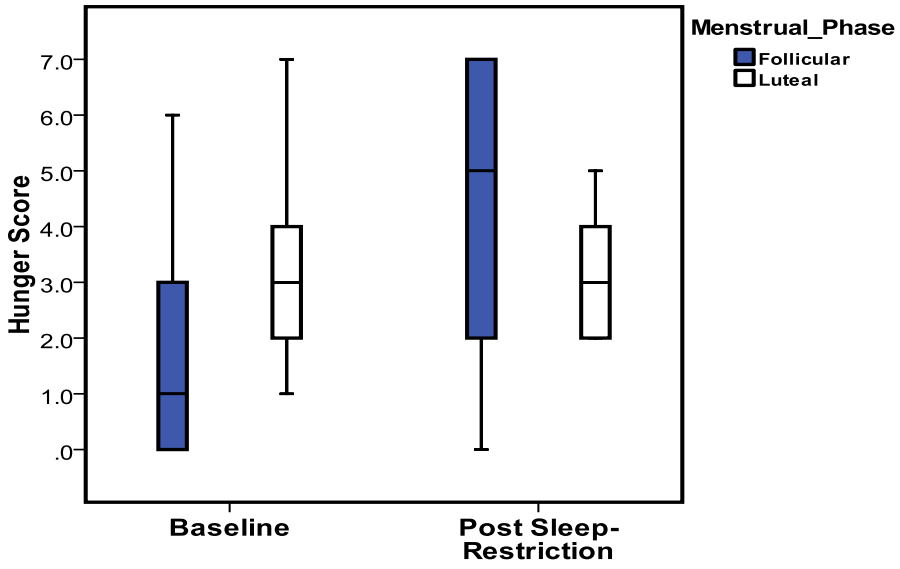


Appendix 13 continued

C.



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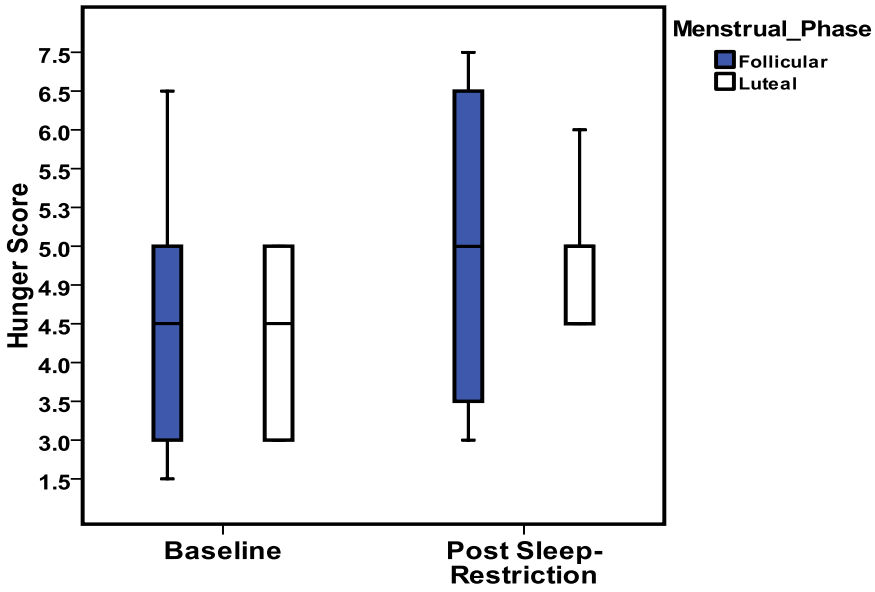


Appendix 14

Means, Standard Deviations (SD) and Sample size For Hunger Ratings Analyzed with ANOVAs, and Parametric and Non-parametric Follow-up Tests As a Function of Sleep Condition and Menstrual Phase Group

Hunger	**Phase**	***M***	***SD***	***N***
Baseline	Follicular	3.913	1.3260	9
	Luteal	3.833	.8944	9
	Total	3.871	1.0821	18
Post-sleep Restriction	Follicular	5.013	1.1420	9
	Luteal	4.533	.8307	9
	Total	4.759	.9881	18

Appendix 15

Means, Standard Deviations (SD) and Sample size for Hunger Ratings Analyzed With Parametric and Non-parametric Follow-up Tests As a Function of Time and Sleep Condition

Condition	***M***	***SD***	***N***
Morning			
Baseline	4.359	1.5009	18
Post-sleep Restriction	4.959	1.4633	18
Afternoon			
Baseline	2.647	1.9982	18
Post-sleep Restriction	3.647	1.9666	18
Evening			
Baseline	4.118	1.4634	18
Post-sleep Restriction	5.088	1.2277	18

Appendix 16

Shapiro-Wilk's Tests for Normality for Hunger Ratings Analyzed with ANOVAs, and Parametric and Non-parametric Follow-up Tests As a Function of Menstrual Phase Group

A. Overall Day

	Shapiro-Wilk		
Phase	***Statistic***	***Df***	***p***
Follicular	.946	16	.432
Luteal	.922	18	.140

B. Morning

	Shapiro-Wilk		
Phase	***Statistic***	***Df***	***p***
Follicular	.946	16	.432
Luteal	.922	18	.140

C. Afternoon

	Shapiro-Wilk		
Phase	***Statistic***	***Df***	***p***
Follicular	.862	16	.021*
Luteal	.904	18	.068

D. Evening

	Shapiro-Wilk		
Phase	***Statistic***	***Df***	***p***
Follicular	.967	16	.780
Luteal	.871	18	.018*

Appendix 17

Levene's Test of Equality of Error Variances for Mood Variables Analyzed with ANOVAs, and Parametric and Non-parametric Follow-up Tests As a Function of Sleep Condition

Anger	***LS***	***Df1***	***Df2***	***p***
Baseline	3.398	1	15	.087
Post-sleep Restriction	.379	1	15	.548
Fatigue	***LS***	***Df1***	***Df2***	***p***
Baseline	2.354	1	15	.147
Post-sleep Restriction	.003	1	15	.955

Depression	***LS***	***Df1***	***Df2***	***p***
Baseline	1.741	1	15	.208
Post-sleep Restricion	3.799	1	15	.072

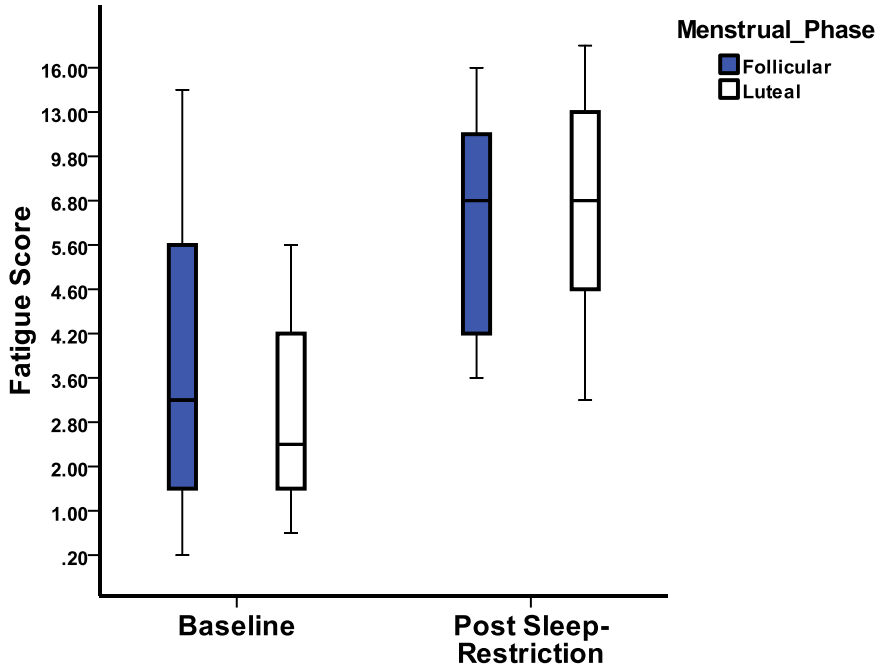
Confusion	***LS***	***Df1***	***Df2***	***p***
Baseline	1.443	1	15	.250
Post-sleep Restriction	1.747	1	15	.207
Tension	***LS***	***Df1***	***Df2***	***p***
Baseline	3.009	1	15	.105
Post-sleep Restriction	1.060	1	15	.321
Vigour	***LS***	***Df1***	***Df2***	***p***
Baseline	.900	1	15	.359
Post-sleep Restriction	.003	1	15	.959
TMD	***LS***	***Df1***	***Df2***	***p***
Baseline	.645	1	15	.435
Post-sleep Restriction	.451	1	15	.513

Note. LS: Levene Statistic

Appendix 18

Boxplots for Mood Variables Used for ANOVAs, and Parametric and Non-parametric Follow-up Tests, and Correlations as a Function of Sleep Condition and Menstrual Phase Group

A



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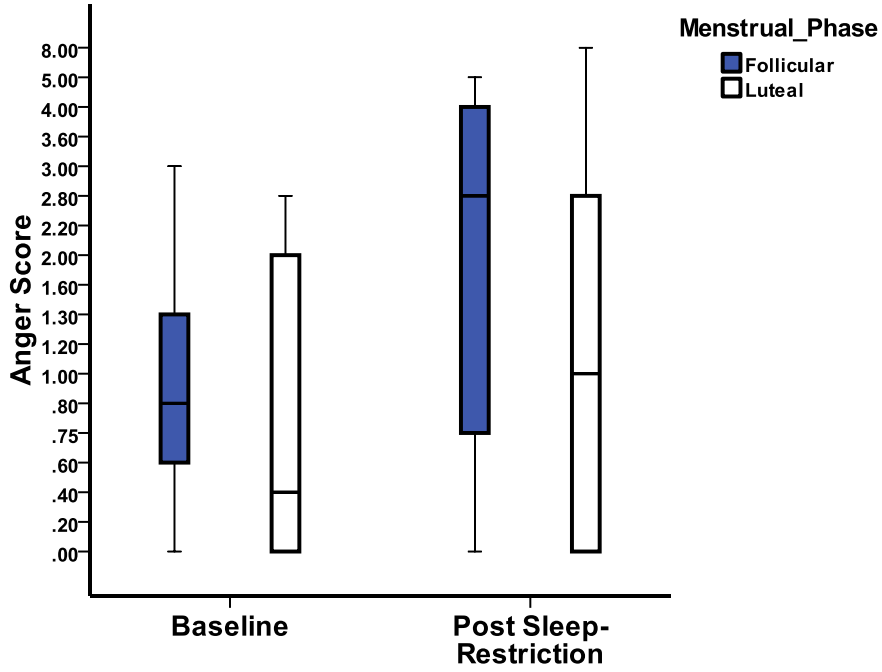
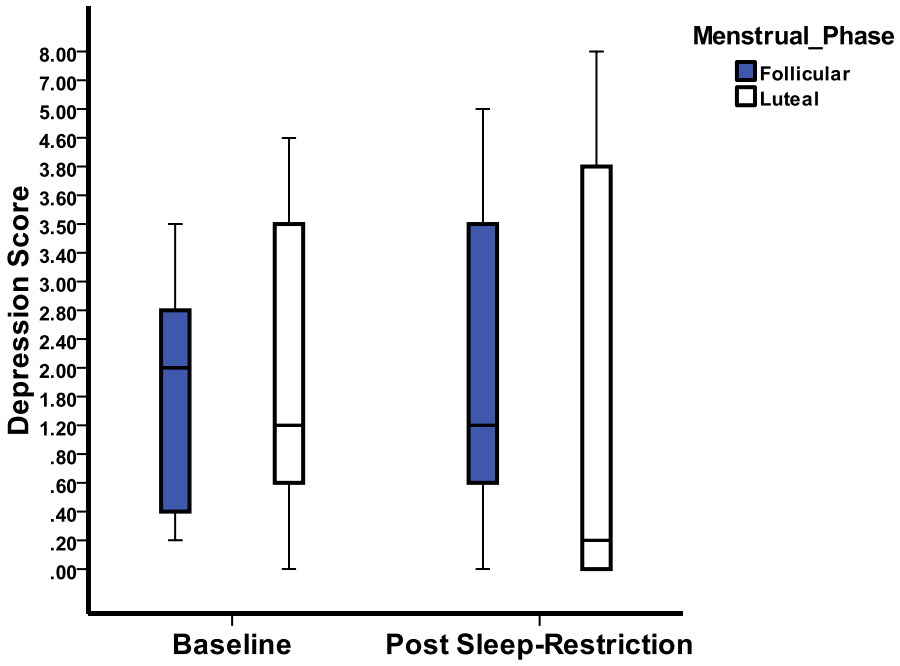


Appendix 18 continued

C



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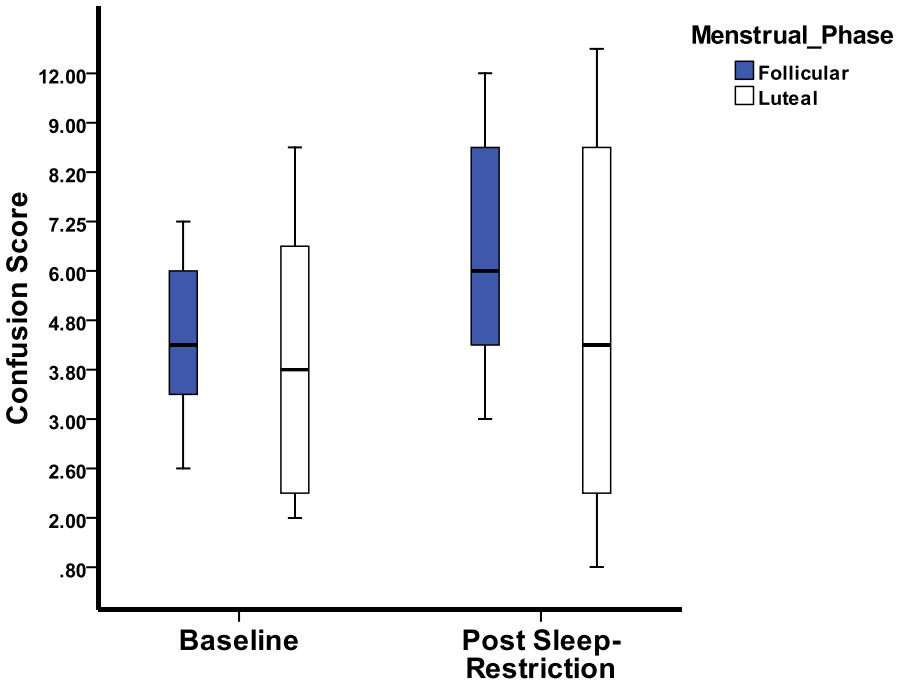
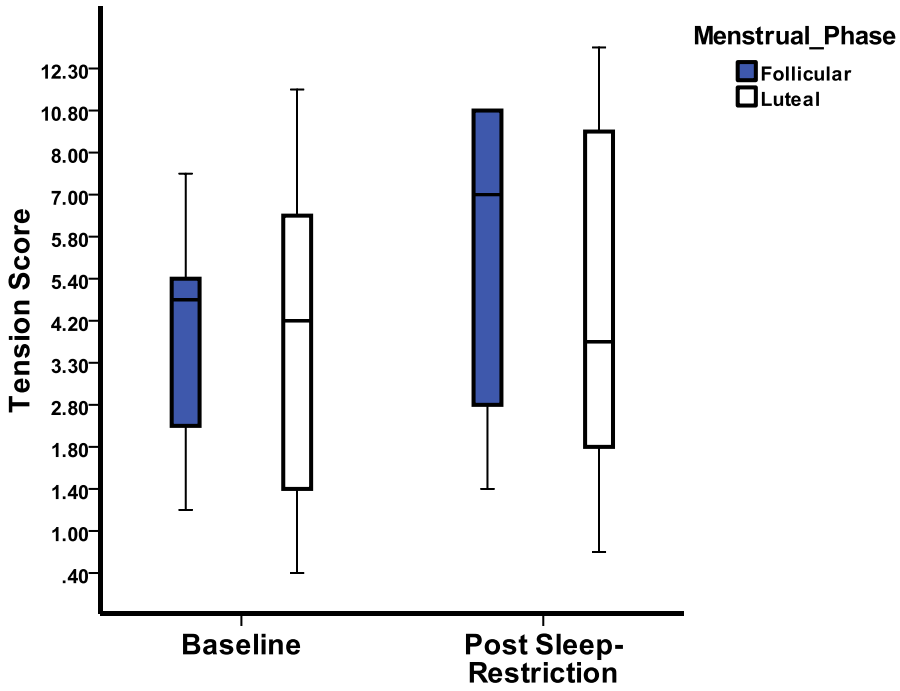


Appendix 18 continued

E



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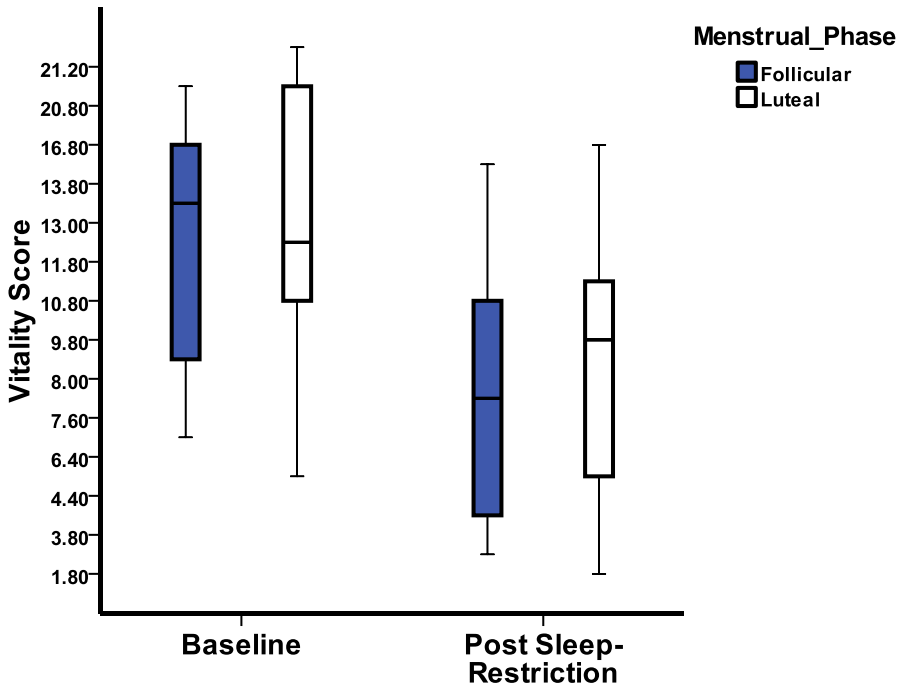
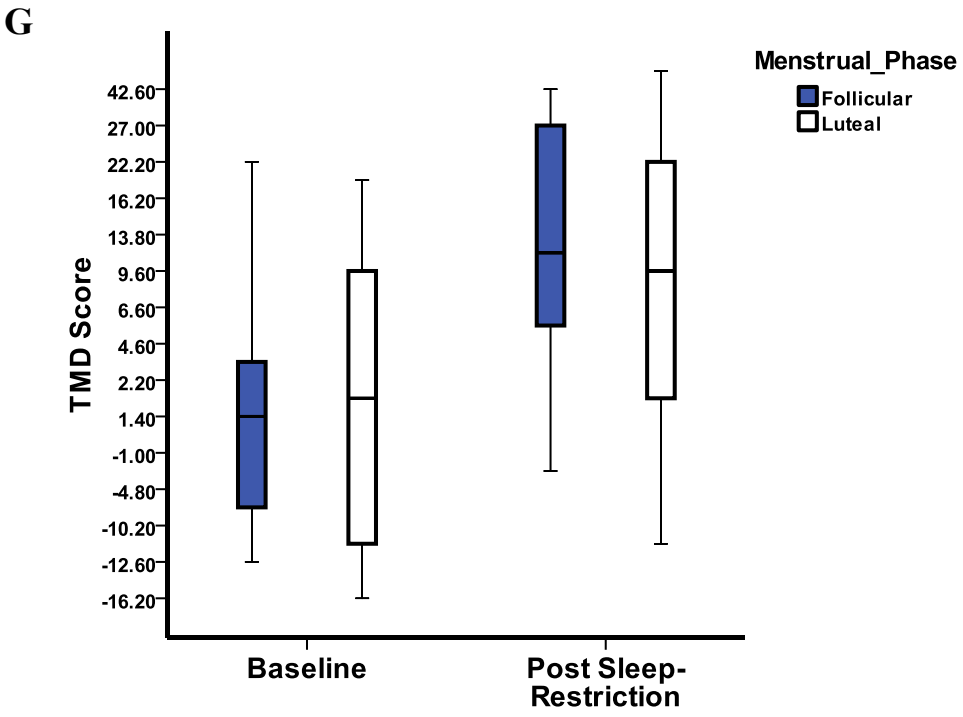


Appendix 18 continued

G



Appendix 19

Means, Standard Deviations and Sample size for Mood Variables Used for ANOVAs, and Parametric and Non-parametric Follow-up Tests, and Correlations As a Function of Sleep Condition and Menstrual Phase Group

A. Fatigue

Condition	Phase	*M*	*SD*	*N*
Baseline	Follicular	4.163	2.1540	8
	Luteal	4.750	3.9030	9
	Total	4.345	3.0511	17
Post-sleep Restriction	Follicular	6.350	3.8094	8
	Luteal	6.137	6.0719	9
	Total	6.244	4.8979	17

B. Anger

Condition	Phase	*M*	*SD*	*N*
Baseline	Follicular	1.044	.9264	8
	Luteal	1.213	1.3314	9
	Total	1.128	1.1115	17
Post-sleep Restriction	Follicular	2.375	1.8406	8
	Luteal	1.925	2.7505	9
	Total	2.150	2.2727	17

C. Depression

Condition	Phase	*M*	*SD*	*N*
Baseline	Follicular	1.044	.9264	8
	Luteal	1.213	1.3314	9
	Total	1.128	1.1115	17
Post-sleep Restriction	Follicular	2.375	1.8406	8
	Luteal	1.925	2.7505	9
	Total	2.150	2.2727	17

D. Confusion

Condition	Phase	*M*	*SD*	*N*
Baseline	Follicular	4.488	1.6066	8
	Luteal	4.363	2.6468	9
	Total	4.425	2.1161	17
Post-sleep Restrition	Follicular	6.463	2.9880	8
	Luteal	6.775	4.0376	9
	Total	6.619	3.4352	17

E. Vigour

Condition	Phase	*M*	*SD*	*N*
Baseline	Follicular	12.875	5.0188	8
	Luteal	14.450	5.9289	9
	Total	13.663	5.3684	17
Post-sleep Restriction	Follicular	9.113	4.2018	8
	Luteal	8.325	4.6438	9
	Total	8.719	4.2975	17

F. TMD

Condition	Phase	*M*	*SD*	*N*
Baseline	Follicular	.675	10.8406	8
	Luteal	.275	12.5920	9
	Total	.475	11.3525	17
Post-sleep Restriction	Follicular	13.450	12.6354	8
	Luteal	14.900	19.6326	9
	Total	14.175	15.9668	17

G. Tension

Condition	Phase	*M*	*SD*	*N*
Baseline	Follicular	4.163	2.1540	8
	Luteal	4.525	3.9030	9
	Total	4.344	3.0511	17
Post-sleep Restriction	Follicular	6.350	3.8094	8
	Luteal	6.137	6.0719	9
	Total	6.244	4.8979	17

Appendix 20

Shapiro-Wilk Test for Normality for Mood Variables Analyzed With ANOVAs, and Parametric and Non-parametric Follow-up Tests and Correlations As a Function Menstrual Cycle Phase

		Shapiro-Wilk		
	Phase	***Statistic***	***Df***	***p***
Anger	Follicular	.898	16	.075
	Luteal	.721	16	.000
Fatigue	Follicular	.912	16	.125
	Luteal	.859	16	.019
Depression	Follicular	.908	16	.107
	Luteal	.804	16	.003
Confusion	Follicular	.896	16	.070
	Luteal	.891	16	.058
Tension	Follicular	.923	16	.189
	Luteal	.842	16	.011
Vitality	Follicular	.956	16	.592
	Luteal	.933	16	.271
TMD	Follicular	.919	16	.160
	Luteal	.909	16	.114

Note. * $p > 0.05$; TMD: Total Mood Disturbance

Appendix 21

Shapiro-Wilk Test for Normality for Mood Variables Analyzed With ANOVAs, and Parametric and Non-parametric Follow-up Tests and Correlations As a Function of Sleep Condition

		Shapiro-Wilk		
	Sleep Condition	***Statistic***	***Df***	***p***
Anger	Baseline	.839	16	.009*
	Post Sleep-Restriction	.868	16	.025*
Fatigue	Baseline	.798	16	.003*
	Post Sleep-Restriction	.886	16	.049*
Depression	Baseline	.929	16	.235
	Post Sleep-Restriction	.794	16	.002*
Confusion	Baseline	.904	16	.092
	Post Sleep-Restriction	.957	16	.615
Tension	Baseline	.923	16	.191
	Post Sleep-Restriction	.902	16	.086
Vigour	Baseline	.903	16	.089
	Post Sleep-Restriction	.970	16	.838
TMD	Baseline	.958	16	.626
	Post Sleep-Restriction	.939	16	.335

Note. * $p < 0.05$

Appendix 22

Shapiro-Wilk Test for Normality for (A) Cortisol, (B) Progesterone, and (H) Hunger Baseline and Difference Values Used for Correlations

A.

Cortisol	**Shapiro-Wilk**		
	Statistic	***Df***	***p***
Baseline			
08:00	.916	18	.124
11:00	.940	18	.322
14:00	.903	18	.077*
17:00	.941	18	.330
20:00	.928	18	.199
Difference			
08:00	.908	18	.092
11:00	.964	18	.702
14:00	.801	18	.002*
17:00	.951	18	.467
20:00	.695	18	.000*

B.

Progesterone		**Shapiro-Wilk**		
	Phase	***Statistic***	***Df***	***p***
	Follicular	.958	9	.786
	Luteal	.938	9	.558

Appendix 22 continued

C.

Hunger	Shapiro-Wilk		
	Statistic	*Df*	*p*
Baseline			
08:00	.879	18	.031*
11:00	.967	18	.771
14:00	.858	18	.014
17:00	.855	18	.013*
20:00	.929	18	.209
Difference			
08:00	.917	18	.129
11:00	.964	18	.715
14:00	.954	18	.526
17:00	.905	18	.084
20:00	.905	18	.082

Note. * $p > 0.05$