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Circadian rhythm of cortisol and estradiol in healthy women

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CIRCADIAN RHYTHM OF CORTISOL AND ESTRADIOL IN HEALTHY WOMEN

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DEDICATION

This dissertation is dedicated to all those who have provided me with love, guidance and hope throughout this experience,

especially my daughters, Kiri and Eva.

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Introduction

Research is finding that human and environmental phenomena exhibit temporal variations from a steady state that are circadian in nature, rising and falling over a 24 hour period. The vast majority of processes in humans exhibit circadian rhythms, which are governed by exogenous and endogenous pacemakers. The expression of the majority of biological activities, including gene transcription, protein synthesis, and hormone secretion follows a predictable circadian variation across the day. Multiple circadian rhythms align with one another to create a synchronous system. The timing of hormone secretion influences other hormones and behaviors, which in turn impact the initial hormones in a coordinated dance of cellular, organ, tissue, and organism activity. Phase relationships among rhythms are created from timing of individual rhythms and, if in proper alignment, may contribute to the optimal functioning of the organism.

Timing of circadian rhythms is governed by central and peripheral oscillators. Oscillators work in concert with each other and environmental pacemakers to maintain optimal timing of synthesis and secretion. The result of the optimal timing of multiple oscillators is health. Temporal misalignment in oscillators may result in disruptions to health, ultimately leading to illness. Temporal misalignment may contribute to a constellation of symptoms known as sickness behaviors.

"Sickness behaviors" is a term that refers to a constellation of symptoms that result from the body's adaptive response to acute infection. The symptoms, which include alterations in affect, sleep quality, and energy level, serve an adaptive function in acute illness. When sickness behaviors become chronic, symptoms become maladaptive leading to impairment in health and

daily functioning (Jones, 2008). The symptom cluster of sickness behaviors manifests in numerous chronic conditions including depression, cancer, autoimmune disorders, and sleep disorders. The hormones, cortisol and estradiol, play a significant role in immune function. Cortisol and estradiol represent two important modulators in the human organism and exhibit distinct circadian rhythms. Cortisol and estradiol are examples of two hormones that may become desynchronized in relation to each other. Misalignment of cortisol and estradiol rhythms may contribute to sickness behaviors and immune disorders.

The phase angle difference (PAD) is a measure of the temporal relationship between two rhythms. An optimal PAD represents the temporal relationship of two rhythms that may result in health. A suboptimal PAD is one that is greater or smaller than the optimal PAD and may be associated with poorer health. A suboptimal PAD reflects a misalignment between rhythms. A suboptimal PAD between cortisol and estradiol may contribute to the chronic inflammatory reaction, which results in sickness behaviors. This study proposes that a suboptimal cortisol-estradiol PAD manifests in disturbances in health, specifically disturbed affect, poor sleep quality and low energy level. A theory of PAD misalignment will be used to examine the relationship between the rhythms of cortisol and estradiol and affect, sleep quality and energy level.

Circadian Regulation

Biological rhythms are ubiquitous in the living world. Koukkari and Sothern (2006) state, "biological rhythms are inherent to life itself… Life moves in synchrony…Rhythms are among the common strands from which the web of life itself is spun" (p. 1). A rhythm is defined as "change that is repeated with a similar pattern, probability and period" (Koukkari & Sothern, 2006, p. 20).

The circadian rhythm in humans, in theory, arises from the interactions of the central Suprachiasmatic Nucleus (SCN) oscillator with multiple peripheral clocks located in tissues and cells throughout the body. Early in circadian study, a single oscillator located in the SCN was believed to be responsible for circadian timing. Further research into the nature of the circadian system challenged the single oscillator model (Baggs, Price, DiTacchio, Panda, FitzGerald & Hogenesch, 2009; Silver & LeSauter, 2008; Vujovic, Davidson, & Menaker, 2008). Isolation studies demonstrated that individual circadian rhythms could be desynchronized with respect to other rhythms. Under conditions of constant darkness, isolation studies permitted circadian rhythms to run freely without the influence of major entrainers such as light and dark cycles. In isolation studies, participants expressed free running circadian rhythms. The free running rhythms exhibited phase lengths (tau) that varied one from another. Over time some rhythms became completely desynchronized. The differing taus reflected the activity of different circadian oscillators leading to theoretical models that involved multiple circadian oscillators. Oscillators were found to be located in peripheral organs and tissues. It is posited that peripheral oscillators are coordinated by the SCN via behavioral, neuroendocrine and autonomic pathways (Kalsbeek et al., 2006).

Within the central nervous system, multiple oscillators have been identified. Studies from animal models clarified the multioscillatory function of the SCN (Cermakian & Boivin, 2003; Inouye & Shibata, 1994; Satinoff, 1998). As the master oscillator located in the brain, the SCN consists of a circuit of tightly controlled oscillatory cells. Clusters of these SCN cells oscillate in different phases from other clusters in the network (Hastings, Oniel, & Maywood, 2007). At least four temporally differing cell clusters exist in the SCN. Each cluster is responsible for specific circadian rhythms in the organism (Kalsbeek et al., 2006).

Communication between the SCN and peripheral oscillators follows various pathways, including electrical, chemical, neural, and hormonal (Freeman, Webb, An, & Herzog, 2008). For example, the SCN outputs necessary for proper functioning of cortisol involve multiple neuronal projections. At least three main outputs are involved in endocrine function. According to Kalsbeek and colleagues (2006), the SCN affects the production of cortisol from the adrenals via intermediate neurons, endocrine neurons, and directly through connections to the adrenals via the preautonomic neurons. In addition to the direct stimulation via neural projections, circadian rhythm is further maintained through hormonal secretion.

Under natural environmental conditions, circadian rhythms are maintained to a 24-hour period by strong and weak entrainers (Aschoff, 1983; Aschoff & Wever, 1965). The most potent environmental entrainer is believed to be light. The 24-hour day/night cycle provides a strong signal that coordinates the expression of many circadian rhythms. Light acts upon the retina of the eye by stimulating the production of melanopsin. Melanopsin is a photopigment in the retinal ganglion cells of the eye that is believed to be responsible for the effect of light on the timing of the circadian clock. Numerous rhythms are believed to be regulated by the timing of the light and dark cycle (Do et al., 2009; Hannibal, 2006).

In addition to the effects of light on circadian rhythms, non-photic stimuli entrain the circadian system. Non-photic entrainers include exercise, meals, social activity, and exogenous melatonin or serotonergic activation (Halberg, Cornélissen, Otsuka, Schwartzkopff, Halberg, & Bakken, 2001; Eastman, Hoese, Youngstedt, & Liu, 1995; Mistlberger, 1991). The SCN receives non-photic input possibly through the expression of neuropeptide Y in other areas of the brain (Cermakian & Boivin, 2003). In animal studies, feeding times serve as a strong peripheral pacemaker, shifting gene expression in peripheral organs by 180 degrees while maintaining gene

expression in the SCN at the original phase (Prietner, 2003). It is believed that the SCN entrains rhythms through organizing the rest/activity clock, which in turn determines feeding schedules. Glucocorticoids may also play a role in the rate and degree of sensitivity of phase advancement related to feeding entrainment.

Integration of individual circadian rhythms may rely on the temporal relationship between photic and non-photic entrainers. Lack of coordination of photic and non-photic entrainers may contribute to misaligned phases among various rhythms. Hogenesh (2003) suggests that phase desynchrony may be related to different phase response curves for individual tissues in response to a signal or a set of different signals.

Misalignment may follow from disturbances in the central and/or peripheral nervous systems. The SCN influences endocrine function through activation of the sympathetic (SNS) and parasympathetic (PNS) nervous system. Sympathetic and parasympathetic innervations relay the temporal information from the SCN to the target organs (Kalsbeek et al., 2006). The sympathetic nervous system controls the periods of activity while the parasympathetic system dominates during periods of rest. The SNS and PSN are controlled by independent neurons in the SCN. An imbalance in timing of SNS and PSN responses may be responsible for disorganized temporal relationships among peripheral oscillators (Kalsbeek et al., 2006).

Another endogenous pathway by which the circadian rhythm is modulated involves production of genetic material. Oscillator speed is set by the rate of transcription of clock genes. Feedback loops of intracellular transcriptional-translational gene expression regulate rhythmic protein production. Mutation in genes may result in phase lengthening, shortening, and arrythmicity. The effects of the SCN are mediated indirectly by transcription factors allowing for

peripheral oscillators to oscillate with different phases (Bernard, Gonze, Cajavec, Herzel, & Kramer, 2007).

Timing of phase setting of circadian rhythms appears to be a complex activity modulated by a number of mechanisms through numerous pathways. Environmental and endogenous processes act in concert to maintain the temporal order of circadian rhythms. The multiplicity and complexity involved in the fine tuning of the circadian system points to the importance of maintaining synchronous relationships among rhythms. Healthy functioning of the organism may depend on circadian rhythm synchrony. Research has yet to explore the role that misaligned circadian rhythms may play in functioning and health. Understanding circadian rhythm function and its relationship to human health and pathology potentially holds valuable insight into etiology and treatment of health disorders, specifically sickness behaviors.

Rhythm Misalignment and Health

Healy and Williams (1988) argue that circadian organization is the product of the organism's need to predict and respond to environmental changes. Circadian rhythms assist the organism to anticipate the environmental demands necessary for survival and are an adaptive interaction of the organism and the environment. According to Wehr and Goodwin (1974), biological rhythms form the temporal anatomy of an organism, allowing the organism to adapt to a cyclically changing environment. Wehr et al. (1985) suggest disorders arising from circadian dysfunction demonstrate similar characteristics. First, rhythm disorders affect multiple systems. Second, rhythm disorders affect function as opposed to tissues and have expression in behavior changes. Third, rhythm disorders underlie diseases that to date have obscure etiologies.

Sickness behaviors, including altered affect, sleep alterations and energy disturbances, satisfy the characteristics of circadian rhythm disorders, in that they are functional disorders that

affect more than none system. In addition, sickness behaviors are common to disorders of obscure etiology such as depression, chronic fatigue, and autoimmune disorders. A fourth characteristic of rhythm disorders may be that they exhibit symptoms expressing a diurnal variation in level of severity. For example, affect demonstrates a diurnal pattern in many individuals. Melancholic symptoms peak in the morning then decrease throughout the day resulting in better moods by evening. The underlying mechanism accounting for the diurnal variation has eluded understanding (Wirz-Justice, 1995). Circadian rhythms in affect have been shown to differ in individuals based on age, gender, stress and season. Disorders, such as depression and chronic fatigue where sickness behaviors are common, also differ among groups categorized by age, gender, stress level, and season.

Theories of Circadian Rhythm and Health

A number of theories explicating a circadian rhythm and health relationship have been described. Wehr and Godwin (1974) proposed the Circadian Theory of Depression and Mania (CTDM). The CTDM is based on a multi-oscillator model. Isolation studies suggest that the circadian system is governed by at least two oscillators that under normal environmental conditions are coupled. The strong oscillator controls, inter alia, the rhythms of temperature and the HPA axis. The weak oscillator controls cycles such as the sleep/wake cycle. The weak oscillator responds to light produced by the day/night cycle. The weak oscillator entrains the strong oscillator. Stimuli from the environment maintain the coordination of the circadian oscillators to a period of 24 hours. The CTDM suggests that in individuals with depression, the endogenous, free-running circadian rhythm is longer than in non-depressed individuals. In healthy states, the circadian rhythm runs approximately 25 hours and entrains through environmental stimuli to a 24-hour cycle. Longer free-running periods entrain with late phase

positions relative to the day/night cycle. The phase of a rhythm in relation to day/night environmental phase is the individual's intrinsic period. The intrinsic period is relatively stable but can be advanced or delayed by environmental stimuli. Stimuli shown to phase advance or delay rhythms include light, drugs, hormones and estrogen (Wehr & Godwin, 1974). Stronger environmental stimuli may be needed to entrain a longer free-running period. Under experimental conditions, the strong and weak oscillators can become uncoupled. Psychological and somatic complaints have been reported in individuals where the strong and weak oscillators have become desynchronized. Kalsbeek et al. (2006) argue that a reduction in the activity of the circadian system or a misalignment of endogenous with exogenous factors may contribute to the development of disease.

Wever (1979) first proposed the Phase Shift Hypothesis (PSH) as an explanation for affective disorders. Intrinsic periods greater than 24 hours must phase shift to a greater degree than intrinsic periods that are close to the 24 hour solar cycle. Wever (1979) proposed that stronger entraining stimuli may be needed to maintain the synchronization of circadian rhythms.

Sickness Behaviors

As defined by Dantzer (2001), sickness behaviors refer to the constellation of physiological and behavioral changes that accompany the inflammatory process. Symptoms of sickness behaviors include: disturbances in affect, sleep quality, and energy level. Sickness behaviors are a normal response to an acute infection and essential for the survival of the organism. Infection instigates an adaptive response by the organism to conserve energy for the reparative process. Sickness behaviors protect the organism by blunting the physical and mental activity, and reducing production of proteins and metabolism of lipids (Jones, 2008). Behavioral changes include irritability and aggressiveness, which can lead to non-confrontation and

isolation. Additionally, Jones (2008) argues that sickness behaviors may, inter alia, be a sociological construct, protecting the community from the sick individual.

In chronic sickness behaviors, the symptoms may become debilitating and mal-adaptive for the individual. Chronic sickness behaviors manifest from the individual's acceptance as permanent features of self those behaviors characteristic of inflammatory processes. Jones (2008) proposes that the altered self becomes the basis for function. The altered self reflects a movement away from baseline stability. With chronic sickness behaviors, nonimmune stimuli may be eliciting a response or proinflammatory immune responses may be hyper-stimulated due to environmental or endogenous conditions. Peripheral inflammatory signals from the body are conducted to the brain via the vagus nerve. The areas of the brain connected to the vagus nerve include the central nucleus of the amygdala. From the amygdala, the HPA axis is activated to respond to the inflammatory stimuli resulting in the behavioral effects. Corticotropin-releasing hormone (CRH) is released in the central nervous system (CNS) and peripheral tissues in synchrony with behavioral, autonomic, and hormonal responses. Glucocorticoid signaling has been the subject of investigation in numerous studies of autoimmune disorders (Jones, 2008). Studies have focused on the difference in proinflammatory cytokines between groups of individuals exhibiting sickness behavior and healthy controls. Results from these studies have been inconsistent (Dantzer, 2001).

Prevalence of Illnesses Exhibiting Chronic Sickness Behaviors

Mood disorders have been shown to greatly affect the health of a large portion of the population world-wide (Millan, 2006), with a lifetime prevalence in women of 10% to 20% (Rowland & Odle, 2005). Depression is believed to affect 17 million people each year in the United States. Twenty-five percent of adult women report at least one episode of severe

depression over a lifetime. Women and men consistently exhibit differences in prevalence and symptoms in mood disorders. Endogenous hormones may be complicit in the gender differences between men and women. According to data from the National Comorbidity Survey (Kessler, 2000), women have higher rates of depression than men, which is unrelated to response and recall biases but may be related to sex hormones, genes, or gendered social roles. Women have a greater lifetime risk for specific mood disorders including unipolar depression, depressive subtypes of bipolar, and cyclic forms of affective disorders (rapid-cycling manic-depressive, seasonal affective disorder). Affect changes have also been associated with reproductive cycle factors, such as the use of oral contraceptives, the luteal phase of the menstrual cycle, postpartum, and menopause (Parry, 2000). Additionally, women are more likely than men to have atypical symptoms of depression (e.g., hypersomnia, hyperphagia), to have co-morbid anxiety disorders, and to attempt suicide (Gorman, 2006; Lewy et al., 1998).

The prevalence rate of sleep disruption in the general population is thought to be as high as 38%, with more than 52% of the population reporting a history of sleep problems (Manfredi, Vgontzas, & Kales, 1989). Almost 60% of the community-dwelling elderly report sleep problems (Ohayon, 2002). Sleep disorders also differ between men and women (Soares & Murray, 2006). Women are more likely to report insomnia than men in every age group (Ford & Cooper-Patrick, 2001). The ratio of women to men for insomnia is 1.4 to 1.0 (Phillips, Collop, Drake, Consens, Vgontzas, & Weaver, 2008). It has been hypothesized that sleep disturbances may be related to hormonal fluctuations in some women (Shaver, 2002; Soares & Murray, 2006). Disturbed sleep quality may result in day time sleepiness, fatigue, and depression. Menstrual factors have been shown to contribute to sleepiness, lethargy, and fatigue in women (Armitage & Hoffman, 2001; Driver & Baker, 1998).

Disturbances in energy levels differ from disturbances in sleep quality and are characterized by fatigue and sleepiness. Disturbances in energy levels are a common complaint among all age groups and characteristic of many serious disorders. One fifth of all patients presenting for health care complain of fatigue (Viner & Christie, 2005). Fatigue remains difficult to treat adequately. Only 2 percent of patients who are chronically fatigued report complete long-term resolution of symptoms (Taylor, Jason, & Curie, 2002).

Alterations in affect, sleep quality, and energy compromises the health of a significant proportion of society. The challenges to the individual and the health care system in adequately addressing sickness behaviors require substantial personal and financial resources. Lack of effective treatment modalities follows from the lack of complete understanding by science into the processes involved in disorders that manifest with the symptoms of sickness behaviors. Mechanisms by which chronic sickness behaviors occur have not been satisfactorily explored. Research into the underlying mechanisms involved in the symptoms that contribute to affect disorders, sleep disorders, and disturbances in energy levels would provide valuable insight into effective treatments. Possible causal explanations for sickness behavior may lie in the nature of individual circadian rhythms in humans.

Alterations in Affect, Sleep Quality and Energy Level in Women

Women consult health care providers most often with reports of alterations in mood and low energy levels (Redmond, 1997). Demographically, women are disproportionally affected by disorders that manifest with symptoms involving affect, sleep quality, and energy level. For example, fibromyalgia belongs to a family of autoimmune disorders and is characterized by fatigue and poor sleep. Only 10% of all reported fibromyalgia patients are men (Yunus, 2002). Studies suggest that women demonstrate higher levels of immunoreactivity when compared to

men. Greater reactivity of the immune system places women at higher risk for autoimmune disorders (Cannon & St. Pierre, 1997). Women experience more rheumatoid arthritis, systemic lupus erythematosus, and chronic fatigue syndrome than men (Allen, 2008; Greenstein, 2001; Lund & Lundenberg, 2008).

Depression affects more women than men and manifests very differently in women. The prevalence of depression in women is double that of men (Hyde, Mezulis & Abramson, 2008). Beginning in puberty, the rate of depression increases faster in girls compared to boys. During the childbearing years, women are especially susceptible to depression at times of elevated hormonal fluctuations, such as the luteal phase of the menstrual cycle, pregnancy, and the postpartum period. Postpartum depression affects approximately 13% of women (Dennis, Ross & Herxheimer, 2008). Premenstrual syndrome (PMS) is a cluster of symptoms that occur during the luteal phase of the menstrual cycle and resolve by the end of menses. Symptoms include altered mood, fatigue, and sleep disturbances. PMS places women at higher risk for developing depression later in life (Wise, Felker, & Stahl, 2008).

Given the disproportionate number of women afflicted with alterations in affect, sleep quality, and energy, understanding the gender specific factors that contribute to the development of autoimmune and depressive disorders is critical. The explanation for the increased prevalence of autoimmune and depressive disorders in women of childbearing years is complex and multi-causal. Physical, psychological, and socio-cultural factors play a role. Physiologically, a major difference between women and men lies in the products of the HPO axis, specifically estradiol in women. Much research in affect changes, sleep disturbances, and energy level in women have focused around the life cycle changes of pregnancy, menstruation, and the early postpartum period (Lee, 2001). Significant changes in reproductive hormones occur at these times. Estrogen

has been shown to be effective in treating the symptoms of altered mood, fatigue, and sleep disturbances (Dennis, Ross, & Herxheimer, 2008; Greenstein, 2001; Pannay & Studd, 1998). However, response rates of estrogen therapy have been disappointing. A large number of women do not respond to any conventional pharmacological treatment (Halbreich, 2006).

Mood disorders are characterized by disturbances in affect, sleep, and energy and have been shown to greatly affect the health of a large portion of the population worldwide (Millan, 2006). Women and men consistently exhibit differences in prevalence and symptoms in mood disorders. Endogenous hormones may be complicit in the gender differences between men and women. According to data from the National Comorbidity Survey, women have higher rates of depression than men (Kessler, 2000), which is unrelated to response and recall biases but may be related to sex hormones, genes, or gendered social roles. Women have a greater lifetime risk for specific mood disorders including unipolar depression, depressive subtypes of bipolar, and cyclic forms of affective disorders (rapid-cycling manic-depressive, seasonal affective disorder). Mood changes have also been associated with reproductive cycle factors, such as the use of oral contraceptives, the luteal phase of the menstrual cycle, postpartum, and menopause (Parry, 2000). Women are more likely than men to have atypical symptoms of depression (e,g., hypersomnia, hyperphagia), to have co-morbid anxiety disorders, and to attempt suicide. (Gorman, 2006; Lewy et al., 1998)

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Hoffman, 2001; Driver & Baker, 1998).

Cortisol

The HPA axis may play an important role in the modulation of the immune response and the development of sickness behavior. Cortisol, the end-product of the HPA axis, demonstrates anti-inflammatory properties. Cortisol is a steroid hormone produced by the adrenal glands following a signaling cascade that begins in the hypothalamus of the brain. The SCN has projections to the paraventricular nucleus (PVN), where corticotrophin-releasing hormone (CRH) is released. CRH activates the release of adrenocorticotropin hormone (ACTH) from the hypophyseal adrenocorticotrophs. ACTH stimulates the release of cortisol from the zona fasciculata of the adrenal cortex. Cortisol exerts a negative feedback directly on the pituitary and also on the synthesis and secretion of CRH.

Physiologically, cortisol has both genomic and nongenomic effects throughout the body. Glucocorticiod receptors (GRs) belong to the steroid super-family of receptors. GRs are located in all cells of the body accounting, in part, for the multi-system effects of cortisol. Cortisol exerts influence on metabolism by increasing proteolysis, gluconeogenesis, and fatty acid metabolism, and decreasing muscle protein synthesis. Cortisol exhibits anti-inflammatory effects by inhibiting prostaglandin and leukotriene production. Inflammation is reduced through inhibiting bradykinin and serotonin effects and impairing cell-mediated immunity. Cortisol increases anti-inflammatory cytokine production and decreases pro-inflammatory cytokine production. Cortisol modulates perception and emotion in the central nervous system (Molina, 2006).

Cortisol is expressed in the body in a circadian rhythm. In 90% of healthy adults, cortisol peaks within 45 minutes of awakening, declines throughout the day and begins to rise during the night hours (Minors & Waterhouse, 1981).

Estradiol

Estradiol is the most potent of the estrogen family of sex steroids. Similar to cortisol, estradiol is the end product of a cascade of hormones that begin in the hypothalamus with the release of gonadotrophin-releasing hormone (GnRH). Estradiol is the product of the activity of enzymes located in the ovarian follicle. Through positive and negative feedback mechanisms, estradiol regulates the activity of GnRH from the hypothalamus and lutenizing hormone (LH) from the pituitary. Estradiol influences the activity of many body organs including the sex organs, kidneys, intestinal mucosa, lungs, bones, brain, and endothelial cells (Molina, 2006). Estradiol contributes to the mediation of the inflammatory response. Research has linked estradiol with the regulation of cytokine genes and nitric oxide (NO) production (Nilsson, 2007).

Estradiol plays an important role in modulation of the inflammatory response in the body, demonstrating both pro-inflammatory and anti-inflammatory activity. The increased inflammatory response in women when compared with men suggests the pro-inflammatory role of estrogens. In an extensive review of the role of estrogens in inflammation by Strouse (2007), estradiol exhibits both anti-inflammatory and pro-inflammatory activity. At low levels, estradiol stimulates the inflammatory process by stimulating natural killer cells, pro-inflammatory cytokines, and antibody formation (Strouse, 2007). The pro-inflammatory stimulation of B cells by estradiol contributes to the increased incidence of infection during the late luteal and menses phases of the menstrual cycle. High levels of estradiol show anti-inflammatory effects by inhibiting proinflammatory cytokines, increasing T cell responses, and inhibiting nitric oxide release (Strouse, 2007). Higher estradiol levels during the follicular and peri-ovulatory phases contribute to resistance to infection. Estrogens act to inhibit the typical pro-inflammatory cytokines by inhibiting IL-1 and IL-6 production and suppressing tumor necrosis factor alpha

(TNF) (Strouse, 2007). Under normal non-inflammatory conditions, estradiol stimulates the HPA axis leading to increased circulating free cortisol levels. In the presence of pro-inflammatory cytokines, the activity of estradiol is opposite. Estradiol exerts an inhibitory effect on pro-inflammatory cytokines. The influence of estradiol on inflammation may be a complex interaction among estradiol, the environmental milieu and the timing of estradiol release into circulation.

Estradiol demonstrates a circadian rhythm. The normal character of the estradiol rhythm is relatively unaffected by the menstrual cycle, with the exception of the acrophase during the menstrual phase. The diurnal cycle of estradiol exhibits an early morning peak and two, three or four ultradian harmonics throughout the 24-period (Bao, Liu, van Someren, Hofman, Cao, & Zhou, 2003). During the menstrual phase, the peak in estradiol occurs later in the morning.

Chronotype

Morningness-eveningness (chronotype) is the temporal position of the diurnal activity-rest rhythm and has been shown to be important to the study of circadian rhythms. The phase of physiologic and social rhythms, including cortisol and estradiol, vary by chronotype (Kudielka, Bellingrath, & Hellhammer, 2007). The activity-rest rhythm is organized by biological, social, cultural, and environmental factors. Individuals express differences in the timing of their activity-rest rhythm with timing of activity concentrated in the morning (M-type), in the evening (E-type) or intermediate between the two extremes. M-types express a social rhythm that peaks earlier in the day than E-types, with awakening and activity times in the early morning. Approximately 24.7% of the population is M-type. E-types demonstrate activity rhythms that peak in the afternoon or early evening and represent about 26.4% of the population. E-types are 2.5 times more likely to report their general health as only poor or fair compared to morning

types (Paine et al., 2006). Evening-type is associated with mood, menstrual pain fluctuations (Takeuchi, Oishi, & Harada, 2005) and psychological disorders (Chelminski, Ferraro, Petros, & Plaud, 1999), including depression (Drennan, 1991; Honda, Suzuki, Shirota, Kaneko, & Takahashi, 1994; Shiihara et al., 1998). Eveningness is related to greater difficulty meeting familial and social demands for morning performance than morningness (Cofer et. al., 1999) and is related to poorer sleep quality (Shiihara et al., 1998; Taillard, Philip, Chastang, Diefenbach, & Bioulac, 2001). For these reasons, it has been suggested that any study on circadian rhythm take into consideration chronotype (Kerkhof, 1985).

Study Problem

In a review of the literature, Klerman (2005) suggests links between cellular rhythm disruptions and physiologic changes in circadian patterning including sleep and metabolic patterns. These "disrhythms" alter protein synthesis and metabolism. Klerman (2005) found that alterations in circadian rhythms were quantified as changes in amplitude and/or phase shifting. Research suggest a relationship between abnormalities in circadian rhythms and cardiovascular disease, respiratory disease, endocrine disorders and neurological disorders (Boivin, 2000; Brown, Varghese, & McEwen, 2004; Champaneri, Wand, Malhotra, Casagrande, & Holden, 2010; Matteucci, Caonsani, Masoni, & Giampietro, 2010).

Since early research in chronobiology, the role of temporal characteristics in circadian rhythms has been considered in health and illness. Research has focused on the temporal dimensions of drug efficacy (Hermida, Ayala, & Portaluppi, 2007; Lemmer, 2006), symptom manifestation (Berger, Farr, Kuhn, Fischer, & Agrawal, 2007; Cutolo,Villaggio, Otsa, Aakre, Sulli, & Seriolo, 2005; Murray et al., 2006; Spiegelhalder & Hornyak, 2008) and disease progression (Faber, Zehender, Baumgarten, Jeron, Furtwangler, & Just, 1995; White, 1996). The

timing of release and the circulating levels that constitute the circadian rhythm of hormones has become important in understanding health states. Disturbances in the timing of rhythmic processes have been suggested in health disorders (Berger, Farr, Kuhn, Fischer, & Agrawal, 2007; Fernandes, Stone, Andrews, Morgan, & Sharma, 2006). Particularly well studied, the cortisol acrophase has been implicated in depression and immune disorders. Estradiol has been investigated to a far lesser extent. Only one study was found that investigated both cortisol and estradiol together. The temporal properties of cortisol and estradiol were examined by Bao, Ji, van Someren, Hofman, Liu and Zhou (2004), who found a relationship between depression and phase correlations of estradiol and cortisol. In healthy women the acrophases of cortisol and estradiol were more highly correlated than in depressed women suggesting that the timing of the peak of cortisol and the peak of estradiol occur with the same interval of time between the peaks. While individual phase angle differences were not assessed, higher correlations between acrophases in controls suggest more consistent PAD in the absence of depression. A consistent PAD in healthy controls may represent an optimal PAD that contributes to health.

Research in PADs has been limited to the relationship between exogenous rhythms such as timing and intensity of light, temperature, humidity and sound and endogenous rhythms (Koukkari & Sothern, 2006). Another body of research has explored the PAD between exogenous rhythms and the sleep/wake cycle.

Little research has been found that investigates the PADs among multiple endogenous and exogenous rhythms. Specifically, few studies have been conducted on determining optimal PADs in health. Among endogenous rhythms, no research was found that examines the cortisol-estradiol PAD, nor explores the relationship of the PAD to affect, sleep quality and energy level.

Study Purpose

The purpose of this study was to identify the phase relationships between two endogenous biological rhythms and their association with health in women. This study is the first step in identifying, describing and determining the relationship between cortisol and estradiol circadian rhythms and the sickness behaviors of altered affect, poor sleep quality, and disturbed energy level. The central hypothesis for this program of research is that the temporal phase relationships of circadian rhythms, not the basal levels of a single, isolated biological rhythm, interacting with environmental rhythms contributes to the health state of an individual.

The specific aims of this study were:

Aim 1. To analyze and compare the circadian morning-eveningness rhythm and the circadian and ultradian rhythms of cortisol and estradiol in healthy premenopausal women.

Working Hypothesis 1a. Healthy premenopausal women will exhibit a circadian and ultradian rhythm in both cortisol and estradiol that can be fitted to multiple cosinor curve.

Working Hypothesis 1b. The cortisol and estradiol circadian rhythm parameters of phase, amplitude, and mesor will demonstrate independence from each other within subjects.

Aim 2. To determine the relationship between the circadian morningness-eveningness rhythm, and the circadian and ultradian cortisol and estradiol rhythms in healthy premenopausal women.

Working Hypothesis 2a. The cortisol circadian rhythm parameters of phase, amplitude, and mesor will differ between morning types and evening types. Morning types will exhibit a phase advance relative to evening types.

Working Hypothesis 2b. The estradiol circadian rhythm parameters of phase, amplitude, and mesor will differ between morning types and evening types. Morning types will exhibit a phase advance relative to evening types.

Aim 3. To determine the relationships among morningness-eveningness rhythm, cortisol and estradiol rhythms and affect, sleep quality and energy level in healthy women.

Working Hypothesis 3a. In healthy premenopausal women, the cortisol-estradiol phase angle difference will correlate nonlinearly with affect. The relationship will fit a quadratic model: $Y=B0 + B1*X +B2*X^2$, where the most positive and least negative affect is expressed at a specific phase angle difference, referred to as the optimal PAD.

Working Hypothesis 3b. In healthy premenopausal women, the cortisol-estradiol phase angle difference will correlate nonlinearly with sleep quality. The relationship will fit a quadratic model: $Y=B0 + B1*X +B2*X^2$, where the highest quality of sleep is expressed at a specific phase angle difference, referred to as the optimal PAD.

Working Hypothesis 3c. In healthy premenopausal women, the cortisol-estradiol phase angle difference will correlate nonlinearly with energy level. The relationship will resemble a quadratic equation $Y=B0 + B1*X +B2*X^2$, where the highest level of energy is expressed at a specific phase angle difference, referred to as the optimal PAD.

Working Hypothesis 3d. The specific cortisol-estradiol PAD reflecting the highest level of affect, sleep quality and energy will not differ from each other.

Working Hypothesis 3e. The specific cortisol-estradiol PAD reflecting the highest level of affect, sleep quality and energy will not differ between morning types and evening types.

Significance

Nursing science claims membership in both the discipline of nursing and the discipline of science. The function of nursing science is to describe, explore and predict the phenomena of concern to the discipline (Rogers, 1970). Nursing science examines, explores and predicts the nature of health in the human person integral with the environment (Fawcett, 2000; Donaldson & Crowley, 1978; Monti & Tingen, 1999; Newman, 1991; Rogers, 1970). The role of nursing is the "optimization of health and abilities …alleviation of suffering through the diagnosis and treatment of human response" (ANA, 2004, p.7). Humans respond in physiological, emotional, psychological and social dimensions to health and illness. The science of nursing recognizes the importance of viewing health as the interplay of all dimensions as a unified whole. While medicine focuses on disease detection and treatment, physiology focuses on function and biology focuses on the fundamental principles of organisms, nursing focuses on how humans respond to health and illness. Exploration into the defining characteristics that describe a response to health is fundamental to the science. Understanding the phenomenon of alignment and misalignment of circadian rhythms contributes to the development of an explanatory model of health. Adequate modeling of the human response to health and illness is the first step in developing interventions and applications for treatment. Health as synchronized, aligned rhythms opens avenues for nursing research yet to be explored. Basic research is directed at understanding the fundamental nature of phenomena. Basic research in nursing includes the study of the constituency of health and illness models and pathways through which human response can be predicted and altered.

Of concern in this study is the physiological response of hormone phases as a predictor of health. Health may be described as circadian rhythms in proper alignment, with optimally timed expression of peak and trough values. Optimal coordination of the circadian rhythms has the potential to determine the difference between health and illness. To this end, the study of specific

physiological and social rhythms will contribute to the understanding of the nature of the phenomenon of health and provide an explanatory model for differentiating possible etiologies in health and sickness behaviors. Exploring the synchrony between hormonal rhythms and social rhythms represents the beginning steps in understanding the contribution of rhythm synchrony in predicting health.

Explicating an accurate model of the role of circadian phases in health contributes to the development of applied and clinical research in nursing. Accurate explanatory models facilitate the development of effective, efficient, and safe nursing interventions. It is currently accepted that rhythms can be shifted and altered by environmental factors. Light acts as a strong entrainer of circadian rhythms. Through the manipulation of light exposure, alignment of circadian rhythms may be facilitated or disturbed. With the understanding of health as aligned rhythms, nursing can begin to explore interventions in the application of known entrainers, such as light, and the discovery of yet unknown environmental and endogenous entrainers.

Exploration into circadian rhythm alignment provides potential health indicators as yet unknown. New physiological parameters of health become established. Measurement of circadian expression of hormones, immune factors and neurotransmitters, inter alia, can be used as health indicators. Temporal parameters of specific health indicators take on new importance. Not only do basal levels of physiological, psychological, and social factors contribute to the definition of health but also temporal relationships between factors become significant. In addition to current health measures such as self-report surveys, blood pressure, immune function and activity, rhythm alignment may add an increased dimension of measurement. For example, the application of light therapies can be measured in terms of degree of rhythm synchrony pre

and post intervention to establish the effects of light on health. It is possible that rhythm alignment may be more sensitive and become evident sooner than self-report measures of health.

Greater understanding of rhythm alignment, as determined by the PADs among circadian rhythms, has the potential to provide understanding of optimal relationships among rhythms that define a human response to health. By identifying optimal PADs among rhythms, the differentiation between health and illness can be described, contributing significantly to basic nursing science. By identifying optimal phase angle differences among rhythms, applied science can begin to develop interventions for nursing practice. Non-invasive health modalities, such as light therapy, sound and temperature therapy can be studied and used in practice. By identifying optimal PADs among rhythms, nursing studies can measure health with greater precision.

An accurate, well-described model provides the foundation for intervention development. Phase resetting may be accomplished by a number of mechanisms, including chemical, physical and environmental manipulations. A model of rhythm alignment provides measurable variables that can evaluate efficacy in intervention. With the understanding of the role of rhythm misalignment on health, variables such as environmental entrainers, enforcement of daily routines, and timing of social activities can be studied vis-à-vis nursing interventions. Environmental entrainers of importance would include light and sound exposure, meals and social contact. Extending the period of “daytime” through artificial lighting, social activities and night eating disrupts the normal functioning of many circadian rhythms. An understanding of the impact of misaligned rhythms on health and sickness behaviors represents the first step in recognizing the importance of environmental and endogenous entrainers. The health implication of strengthening and regulating circadian rhythm entrainers follows from the discovery of the health implications conditional on aligned circadian rhythms. When the relationship of properly

aligned circadian rhythms and sickness behavior is established the value of developing phase shifting interventions can be appreciated.

Specifically, this study explores the influence of the rhythm alignment of cortisol, and estradiol on affect, sleep quality and energy level. Alterations in affect, sleep quality and energy level are classic symptoms of a multitude of varied disorders including depression, autoimmune disorders and cancer. The symptoms of changes in affect, sleep quality, and energy level are so pervasive, the term "sickness behaviors" was coined to describe the condition of low affect, poor sleep quality and low energy level (Dantzer, 2001). Chronic sickness behaviors result in loss of physical, social, and psychological function influencing all aspects of a person's life.

Studies have demonstrated that endogenous rhythms may be phase advanced and delayed through numerous environmental and social activities including application of light (Burgess, Fogg, Young, & Eastman, 2004; Elmore & Burr, 1993; Gordijn, Beersma, Korte, & van den Hoofdakker, 1999; Lewy et al., 1998), meal timing (Costa, Lievore, Ferrari, & Gaffuri, 1987), sleeping schedules (Boivin et al., 1997), grounding (Ghaly & Teplitz, 2004) and exercise (Eastman, Hoese, Youngstedt, & Liu, 1995). This study will seek to identify the optimal phase relationship between two hormone rhythms in morning-type (M-type) and evening-type (E-type) women as it relates to affect, sleep quality, and energy level. Ultimately, nursing can assist the individual and community in constructing an environment where optimal phase relationships are promoted. In addition, this study will contribute substantially to the bio-behavioral research describing the nature of health and wellness, which is central to nursing. The impact of sickness behaviors on the economic, social, and physical health of society is enormous. Development of interventions with minimal adverse effect, to alleviate this impact is imperative.

Summary

The number of people affected by disorders of affect, sleep quality, and energy levels is considerable. According to Kessler, Chiu, Demler, and Walters (2005), depression directly affects 6.7 % of the U.S. adult population. Depression represents the leading cause of disability in the U.S. for individuals between 15 and 44 years of age (WHO, 2004). Depression and autoimmune disorders afflict women in greater proportions than men, making the understanding of these disorders important women's health issues (Collop, Adkins, & Phillips, 2004; Halbreich & Kahn, 2007; Krishnan & Collop, 2006; Miaskowski, 2004; Valipour, Lothaller, Rauscher, Zwick, Burghuber, & Lavie, 2007). Sickness behaviors represent a major symptom component of depression. In addition, sickness behaviors play a major role in other debilitating disorders including, autoimmune disorders, cancer, and chronic fatigue syndrome. It is imperative that nursing research focus on explicating the underlying mechanisms by which sickness behaviors emerge in order to develop effective interventions. The scope of individuals affected and the degree of impairment underlies the necessity of urgent attention by nursing science.

Chapter 2

Theoretical Framework and Literature Review

This chapter presents the theoretical framework that underlies this study. Specific hypotheses developed to explain the relationship of misaligned rhythms and sickness behaviors will be proposed. This chapter also reviews the evidence regarding temporal characteristics of circadian rhythms underlying the physiological and behavioral functions in humans. Specifically, this chapter presents the background for a circadian regulation of the hormones cortisol and estradiol and the physiologic and psychologic processes of affect, sleep, and energy.

Cortisol-Estradiol Misalignment Hypothesis

The present study is based upon a hypothesis of rhythm alignment. Exogenous and endogenous signals coordinate in the synchronization of circadian rhythms. Light, social cues, ambient temperature, central and peripheral nervous system, and gene production, inter alia, serve to entrain rhythms to a 24-hour phase through influence on multiple oscillators. Health is the state in which the circadian rhythms of the individual are in optimal phase alignment. Sickness behaviors manifest in the absence of optimal phase alignment among circadian rhythms. Cortisol and estradiol are the end products of the HPA and HPO axes, respectively, and influence the function of the immune system. As immune modulators, the timing of cortisol and estradiol secretion may play a significant role in the function of immune factors in the body. The timing of the peak and the trough of cortisol in relation to the timing of the peak and the trough of estradiol may contribute to the performance of the immune system. Estradiol acts to both enhance and suppress the immune system while cortisol exerts suppressive immune actions. Studies that have measured estradiol levels at a single time have found immune suppressing activity at high levels and immune enhancing activity at low levels (Cushman, 2002; Kiecolt-

Glaser, McGuire, Robles, & Glaser, 2002; Sunday, Tran, Krause, & Duckles, 2006). The coupling of the estradiol and cortisol rhythms may potentially act synergistically to moderate the immune response in healthy individuals. In contrast, misaligned cortisol and estradiol rhythms may fail to adequately modulate the immune response in individuals resulting in sickness behaviors. In aligned rhythms, the stimulation of the HPA axis by estradiol is maximized contributing further to immune system modulation. The modulation of the immune system by the effects of an aligned cortisol rhythm and estradiol rhythm yields a system where the organism has the ability to respond to acute invasion by mounting an immediate and effective immune response while at the same time inhibiting over activation of the immune system. Under misaligned cortisol and estradiol rhythms the immune system may fail to down regulate following an acute exposure to a pathogen and a state of chronic pro-inflammatory activity remains. This chronic inflammatory state contributes to the expression of sickness behaviors by the organism (see figure 2.1).

Rhythm alignment may be measured by examining the phase angle difference (PAD) between two circadian rhythms. This study proposes a hypothesis of rhythm alignment that correlates a specific PAD between cortisol and estradiol with the absence of sickness behaviors. This hypothesis is based on a multiple oscillator model of circadian regulation in which both central and peripheral oscillators work together to regulate the timing of individual rhythms.

According to the Cortisol-Estradiol Misalignment Hypothesis (CEMH), environmental and endogenous entrainers function as modulators of the circadian rhythms system in humans, differentially entraining central and peripheral oscillators. The most potent environmental entrainer is light but meal-timing, social activities and exercise have also been shown to have an impact on circadian rhythms (Krusuchi, Cajochen, Werth, & Wirz-Justice, 2002; Winget,

DeRoshia, & Holley, 1985). Potential endogenous entrainers include the production and expression of proteins and hormones interacting within the internal milieu of the organism. Individual characteristics and genetic profiles, including variability in the intrinsic tau function as endogenous entrainers as well. The circadian phase positions of cortisol and estradiol is the expression of the interaction of these environmental and endogenous entrainers.

Manipulation of environmental and endogenous entrainers has been demonstrated in the literature to change the phases of various rhythms. Light pulses are able to phase advance or phase delay cortisol. In animal studies, meal timing, activity and social cues have also influenced the timing of individual rhythms. Wehr and Goodwin (1974) suggest that individuals with considerably longer or considerably shorter intrinsic periods require higher degrees of stimuli to maintain the phase positions of rhythms that are coupled appropriately with other rhythms. The lack of consistent well delineated environmental cues that indicate to the organism its temporal location in the day/night cycle lead to the loss of synchronized rhythms.

Alignment of the cortisol and estradiol circadian rhythms contributes to health in humans through the influence of cortisol and estradiol on the inflammatory process. In aligned rhythm, the anti-inflammatory effects of cortisol and the anti-inflammatory effects of estradiol work in a coordinated, synergistic manner to decrease chronic inflammation in the human organism. In the state of misaligned coupling of rhythms the actions of cortisol and estradiol are temporally uncoordinated and the anti-inflammatory activity of cortisol is not supported by the activity of estradiol. In addition, the pro-inflammatory actions of estradiol serve to work contrary to the actions of cortisol. The result of rhythm misalignment in estradiol and cortisol is an increase in chronic inflammation. Chronic inflammation is described in the literature as sickness behaviors. Sickness behaviors represent a constellation of symptoms that are debilitating and underlie many

disorders including, inter alia, depression, chronic fatigue, cancer, arthritis and fibromyalgia. Sickness behaviors are antithetical to health.

Study C-T-E Model

For purposes of this study, the theoretical inflammatory relationships between health and sickness behaviors will be assumed. This study will test the relationship between cortisol and estradiol PADs and specific sickness behaviors. The theoretical-conceptual-empirical model (Fawcett, 2000) is illustrated in figure 2.2. According to the C-T-E model, the constructs of interest in this study are Chronotype, Alignment of Rhythms and Health/Sickness Behaviors. As discussed earlier, chronotype, or morningness-eveningness represents the individual's activity/rest rhythm. Two extremes of chronotype exist; morning-type (M-type) and evening-type (E-type). M-types differ from E-types in the phase positions of numerous circadian rhythms including core body temperature, cortisol and melatonin. It is theorized that the morningness-eveningness differences in phase position may be selective to certain rhythms and therefore, not universal to all rhythms. M-types and E-types would then exhibit different PADs among specific rhythms, directly predicting the coupling of rhythms.

Alignment of rhythms predicts the quality of health. Health is a state of complete physical, mental and social wellbeing and not merely the absence of disease or infirmity (World Health Organization; WHO, 1948). Health is multi-faceted including constructs in physical, mental, emotional and spiritual human dimensions. Health includes the subjective experience of well-being and the objective expressions of physical processes and states. Well-being is defined as a state of eudaimonia, the subjective experience of feeling contented and in synchrony with the environment. Objective expressions of health include the absence of disease processes and the state of physiological functioning. Subjective expressions of health include emotions, moods,

energy levels and comfort. Sickness behaviors reflect the subjective and objective state of illness, which is the state of existence diametrically opposed to health. Sickness behaviors include alterations in affect, sleep quality and energy.

The conceptual variables in this study include the cortisol-estradiol PAD, affect, sleep quality and energy. The PAD between cortisol and estradiol represents the temporal relationship between the two rhythms. It is hypothesized that there exists a PAD in which the rhythms may be described as working in alignment with each other. All other PADs describe a cortisol-estradiol rhythm relationship that is misaligned. The optimal PAD represents the alignment of rhythms while any other PAD reflects a misalignment of rhythms. An optimal cortisol-estradiol PAD is assumed to positively impact affect, sleep quality and energy level, such that proper alignment of the cortisol and estradiol rhythms are associated with high levels of affect, better sleep quality and high energy levels.

Affect includes the emotional processes experienced by the individual which create the psychological mood disposition. Affect is measured on a bi-dimensional scale that includes positive and negative affect. Positive affect (PA) represents the degree to which an individual pleasurably engages with the environment while negative affect represents subjective distress (Crawford & Henry, 2004). PA is the degree to which an individual feels alert and excited. Negative affect (NA) is the degree to which an individual feels sad and lethargic. Affect can be closely associated with mood disorders including depression and anxiety. Mood disorders include cognitive and physiological components as well as the emotional component of affect. Depression can be characterized by low positive affect and high negative affect.

Sleep quality refers to the subjective experience of being rested after sleeping. Sleep quality reflects the restorative function of sleep and is affected by social and biological rhythms.

Energy reflects the level of vigor and vitality felt and subjectively reported by the individual. Energy exists on a continuum, anchored at one end by the term fatigue and vigor at the other end. Vigor represents sufficient energy to complete the necessary activities of living as defined by the individual. Fatigue is defined as persistent mental or physical tiredness or exhaustion (Dittner, Wessely, & Brown, 2004). A number of scales have conceptually linked energy and mood. O'Conner (2004) defines the “mood of energy” and “feelings of having the capacity to complete mental or physical activities” (p. 435). For the purpose of this study, energy represents a state of physical and mental potential and actualized ability to do work.

The operational variable of this study for the construct of chronotype is the Horne-Ostberg Morningness-eveningness Questionnaire (MEQ). The cortisol-estradiol PAD is operationalized as cortisol and estradiol immunoassays. The construct of sickness behaviors, conceptualized as affect, sleep quality and energy level are operationalized by a number of subjective measures. The operational variables for affect are positive affect and negative affect, independent subscales by the Positive and Negative Affect Schedule (PANAS) and the Profile of Moods (POMS). Two subscales of the POMS measure affect as well. The affect subscales are Depression-Dejection and Tension-Anxiety. Sleep quality is operationalized by two measures; the Pittsburgh Sleep Quality Index (PSQI) and the Subjective Sleep Quality Scale (SSQ). The PSQI provides a global measure of sleep quality. Day to day variation in sleep quality is measured using the SSQ. Energy is indexed by the POMS and an Energy Visual Analog Scale (VAS-E). The VAS-E measures the possible diurnal change in energy. The POMS is the most widely accepted and employed measure of energy levels (O'Connor, 2006).

Figure 2.1.

Cortisol-Estradiol Misalignment Hypothesis

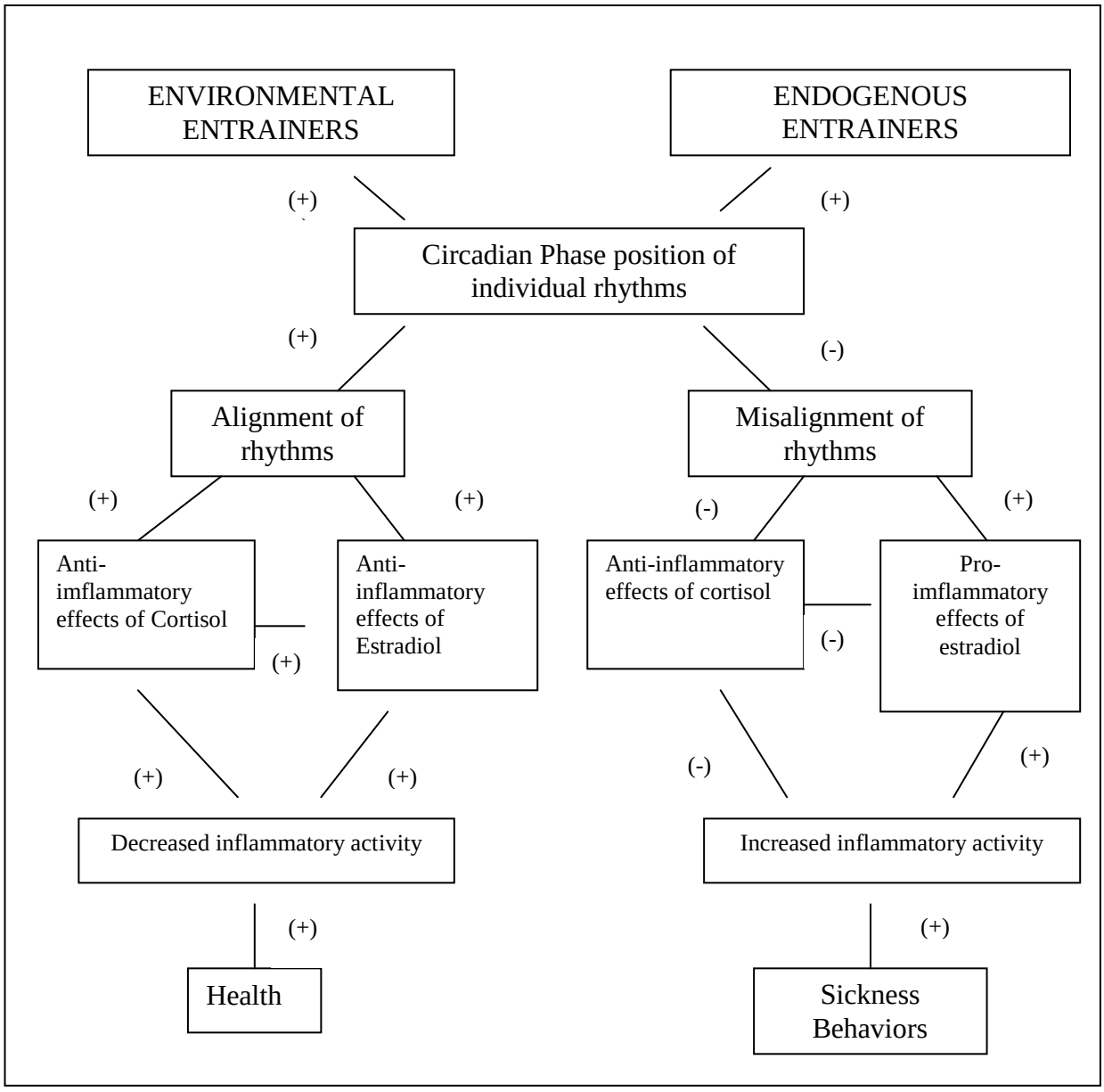
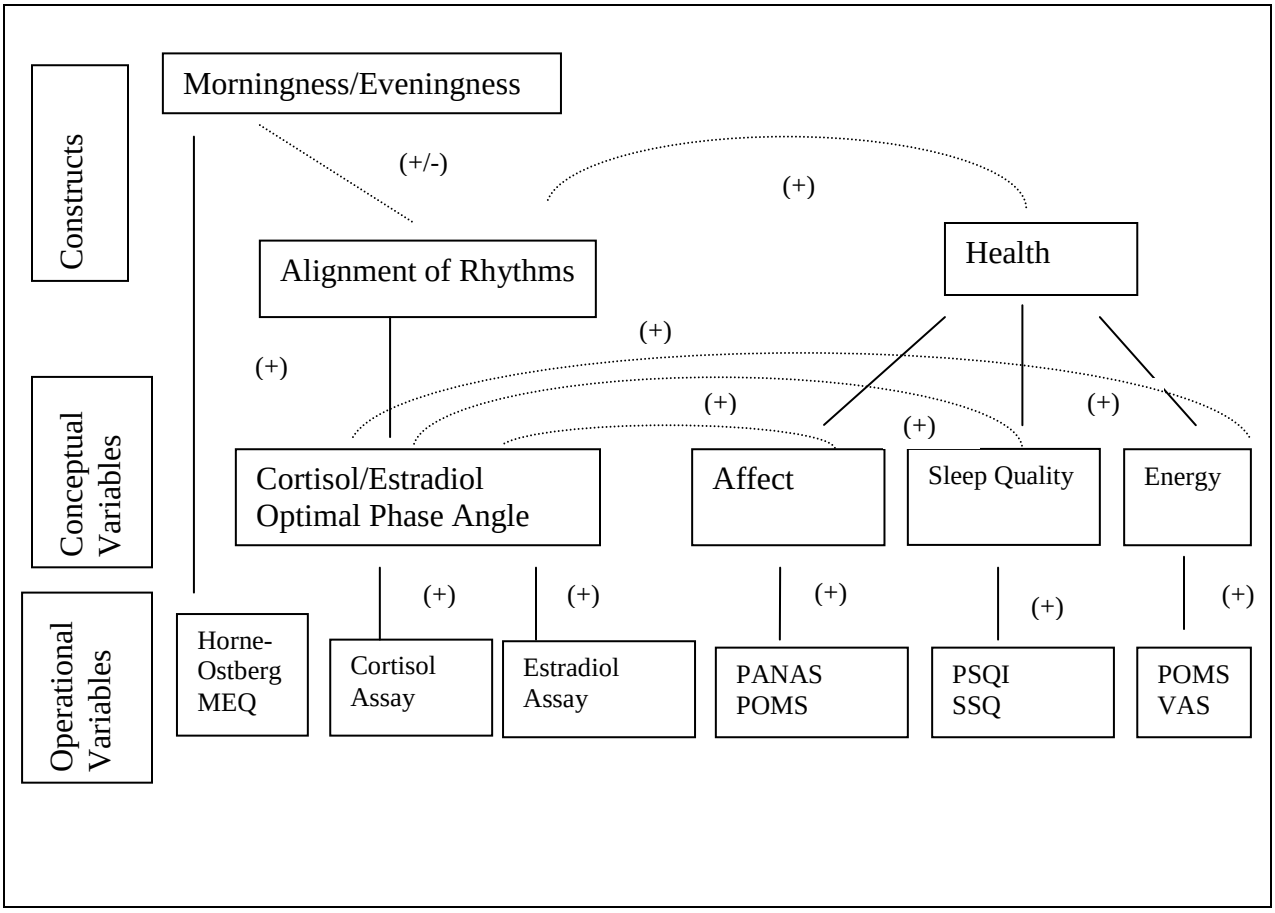


Figure 2.2
C-T-E Model



Circadian Rhythm in Humans

Evidence exists to suggest that most endogenous functions in humans follow a circadian rhythm. In addition to the secretion of hormones, production of proteins and cellular functioning, behavioral responses demonstrate endogenous circadian rhythms. Sickness behaviors, including those that involve alterations in affect, sleep/wake and energy levels show evidence that suggest circadian regulation. There are five criteria for a rhythm to be considered endogenous. They are:

a.) persistence in the absence of external cues

b.) a near 24-hour period

c.) slow change in period to an abrupt environmental change

d.) slow reversion to initial period after entrainment to a new phase

e.) drift from 24 hours after removal of synchronizers (Lavie, 2001)

Endogenous rhythms are regulated by multiple oscillators (Dijk & von Schantz, 2005). Studies suggest that affect, sleep and energy levels satisfy the criteria for being endogenous rhythms.

To accurately describe the characteristics of circadian rhythms multiple sampling over a period of time, at minimum 24-hours, is required. Much of the literature on cortisol and estradiol examine single sampling or cumulative pooling of samples, such as a 24-hour urine collection, providing information for a single point in time or averages over a given time period. Less is known about the variation in hormones across the 24-hour cycle. This literature review is limited to studies involving multiple sampling across the 24-hour day of the variables of interest in human studies. The literature review explores current understanding of the role of the circadian variations in healthy functioning. The nature of the circadian rhythm of affect, sleep quality and energy in healthy populations is discussed. The literature regarding possible influences of

chronotype on endogenous rhythms are addressed. Next, the circadian rhythms of cortisol and estradiol are discussed in healthy populations and in relationship to disturbances in affect, energy level and sleep quality. Finally, this review examines what is known regarding the phase relationships between biological rhythms. The review has been limited to human studies, as the measure of behaviors and the role of cortisol and estrogens differ significantly between human and animal studies. Only studies that examine the temporal variation in hormones over periods that can suggest the nature and characteristics of diurnal change are included in this review.

Chronotype

As noted earlier, chronotype (morningness-eveningness) reflects the phase of the activity-rest rhythm. Chronotype has been found to demonstrate relationships to other circadian rhythms, in that M-types may exhibit a phase advance vis-à-vis E-types. Studies of phase relationships in M-types and E-types support a relationship between endogenous biological rhythms and chronotype. Kudielka, Federenko, Hellhammer and Wüst (2006) report a number of circadian rhythms peak earlier in M-types including body temperature, blood pressure, catecholamine secretion and cortisol. In addition to a later acrophase in body temperature, plasma cortisol and heart rate also occur later for E-types (Bailey & Heitkemper, 1991; 2001), suggesting the possibility of a difference between chronotypes in a phase position control set point by the SCN regulating all circadian rhythms. However, when the phase advance or delay between chronotypes is measured in relation to waking time, differences among endogenous rhythms appear. The temperature rhythm difference between M-types and E-types was determined to be three hours (Waterhouse et al., 2001). A phase delay with respect to wake time indicates a reduced PAD with E-types awakening closer to the temperature minimum than M-types (Baehr, Revelle, & Eastman, 2000; Kerkhof & Van Dongen, 1996). The PAD of melatonin and wake

time, as well as, the PAD of core temperature and wake time were shorter in E-types than M-types (Duffy, Dijk, Hall, & Czeisler, 1999; Duffy Rimmer, & Czeisler, 2001; Gibertini, Graham, & Cook, 1999; Horne & Ostberg, 1977). This evidence raises the possibility that in M-types the PAD between temperature and other endogenous variables may be three hours greater than in E-types. The significance of a greater PAD has yet to be explored.

The circadian nature and the effects of chronotype on the variables significant to this study is the focus of this literature review. Studies of the temporal relationship of chronotype and mood, sleep quality and energy are evaluated. In addition, the current literature examining the nature of the circadian rhythms of cortisol and estradiol is discussed. Finally, the relationship of the physiological rhythms with the subjective experiences of mood, sleep quality and energy is reviewed.

Circadian Rhythm of Affect

Evidence suggests that affect exhibits a diurnal variation in healthy individuals. Studies have documented rhythmic changes in mood and affect over the 24-hour period. Affect is related to mood, in that affect is considered a state emotion, while mood is considered a trait emotion. It has been proposed that emotion dependent behavior is regulated through the sympathetic/parasympathetic nervous system under the influence of the SCN in the hypothalamus. Affect represents positive and negative emotional behaviors. Research has found that positive affect (PA) and negative affect (NA) are orthogonally related factors. PA is related to pleasant events and social behaviors. NA is related to negative events and perception of threat.

Many studies of affect in healthy populations involve small samples. These studies have been conducted under natural conditions and controlled environments. Controlling the environment through constant routine or temporal isolation removes the influences of masking

effects. Controlled environments give the strongest evidence of a circadian influence on daily fluctuations in mood. After five days in temporal isolation, mood exhibited a circadian variation in 18 healthy adults (five women) under normal sleep/wake conditions (Monk, Fookson, Moline, & Pollak, 1985). Mood measures were administered six times per day using the PANAS tool. The composite mood score showed a circadian rhythm that peaked earlier than the peak in core body temperature. However, one affect subscale, tense/calm, did not demonstrate a circadian rhythm. Although sample size was small, lack of rhythmic variation in one measure may suggest differences in circadian influences on some aspects of mood.

Positive and negative affect may be subject to differences in circadian influence. Murray, Allen and Trinder (2002) studied 14 healthy female university students between the ages of 18 to 24 years. Mood data were collected every three hours during waking hours under normal environmental conditions and during a 27-hour constant routine. Repeated measures ANOVAS found a significant time effect in positive affect ($p < 0.01$) but not in negative affect. PA peaked around 1300 and exhibited a nadir around 0100. In addition, the PA curve correlated to the temperature curve with a three to four hour time lag in temperature, suggesting a three to four hour PAD between PA and temperature in healthy young adult women. Lack of circadian rhythm in NA found is this study may be related to the small sample size. A larger study by Clark and Watson (1989) of 196 healthy students initially appeared to support the lack of NA rhythm. Clark and Watson (1989) assessed mood seven times every day for one week. Under natural conditions, PA demonstrated a distinct diurnal rhythm. PA rose sharply until noon and then leveled off until 2100 after which it fell. The leveling off may be attributed to significant differences in acrophases among participants. Watson, Wiese, Vaidya, and Tellegen, (1999) discuss the presence of a PA rhythm and absence of a NA rhythm by conceptualizing PA and

NA into a general systems approach. In a general systems model, PA is understood as a behavioral facilitation system (BFS) and NA reflects the behavioral inhibition system (BIS). The BFS engages the environment, seeking out pleasurable experiences. It is conditioned to the circadian organization in order to maximize the likelihood of reward and minimize the likelihood of danger. The BIS system is a reactionary system responding to threat of danger. The purpose of the BIS is to keep the organism safe and out of trouble. To this end, the system must respond immediately to the environment, regardless of circadian organization. In the absence of threat, research suggests that NA remains low (Watson, Wiese, Vaidya, & Tellegen, 1999). However, reanalysis by Cornelissen and colleagues (2005) of the data from Clark and Watson (1989) found a circadian rhythm to PA and NA after extrapolating the data over the 24-hour period. Results found an acrophase for PA at 1800 ($p < 0.001$) and an acrophase for NA at 0700 ($p = 0.031$).

Differences in both PA and NA have been noted in other studies as well. Using the Day Reconstruction Method, Stone, Schwartz, Schkade, Schwarz, Krueger, and Kahneman, (2006) studied 909 women over one working day. PA demonstrated a bimodal pattern peaking at noon and in the evening. NA peaked at mid-morning and mid-afternoon. Another study using the PANAS, found differences in the circadian rhythm of PA and NA based on whether or not sleep was disturbed for data collection. One participant, a 34 year male, collected data for 86 consecutive days at five time points during the day. For the first 44 days a sixth time point at 0300 was included. During the first 44 days, there was a significant circadian rhythm in PA ($p < 0.001$) but only a trend in significance for NA ($p = 0.096$). However, during the undisturbed sleep period, there was a significant NA circadian rhythm ($p < 0.001$) but not a significant PA circadian rhythm ($p = 0.306$). It is unclear as to why disturbed sleep would result in lack of an

NA rhythm and intact sleep would result in a lack of a PA rhythm. It is important to note that the data in this study were from a single male participant.

In a study of 40 university students (20 men, 20 women) aged 18 to 23 years, women demonstrated a two hour phase advance in both PA and NA. Women exhibited a peak in PA at 1100. For NA, the peak in sleepiness (0800 hours) and weariness (2100 hours) occurred at the same time for both men and women, however, the nadir in sleepiness and weariness occurred two hours earlier for women (Adan & Sanchez-Turet, 2001).

In summary, both PA and NA demonstrate circadian variation across the day. The PA rhythm has been consistently documented in studies, with the exception of one case study. Reported acrophases vary among studies possibly due to a bimodal rhythm or high variance in individual acrophases. Inter-individual differences may be attributable to the lack of controlling for morningness-eveningness. Acrophase in PA have been determined to occur at approximately 1100 or 1800. A bimodal acrophase may account, in part, for the study differences. Study results for NA are less convincing than for PA. A number of studies have found no diurnal rhythm to NA, while others indicate a distinct rhythm peaking at approximately 0700. Lack of NA rhythmicity may be due to methodological decisions that limit sampling to less than a 24-hour period, a dimensional difference between PA and NA or inadequate power. Positive affect and negative affect reflect different aspects of affect and follow different pathways of activation. Evidence suggests that the circadian rhythm of negative affect differs in magnitude or phase position compared to positive affect.

Chronotype and Affect

Studies on the relationship between chronotype and affect can be divided into those that investigate healthy individuals and those studies involving clinical populations. Alteration in

affect is a defining characteristic of many disorders. Clinical populations that express depressed mood as a major symptom and have been the subject of chronotype research include depression, bipolar disorders, seasonal affective disorder (SAD) and attention deficit hyperactive disorder (ADHD). Both healthy and clinical populations exhibit mood differences among chronotypes.

The majority of studies in healthy populations have found correlations between increased eveningness and depressed mood (Chelminski, Ferraro, Petros & Plaud, 1999; Hirata, Lima, de Bruin, Nobrega, Wenceslau & de Bruin., 2007; Kitamura et al., 2010). Drawn from university student populations, many of the larger studies are limited to young adults, who may share many social, economical and environmental conditions not generalizable to the broader community. In one study, 1617 college students exhibited significant negative correlations between chronotype and three depression scales; Beck Depression Inventory, Geriatric Depression Scale and the Center for Epidemiological Studies-Depression Scale (BDI r = -.174, GDS-SF r = -.182, CES-D r = -.176, all $p < .001$).In addition, E-types reported more depressive symptoms ($p < .01$) than M-types (Chelminski, Ferraro, Petros & Plaud, 1999).

Medical students represent a group of young adults who consistently exhibit disturbed and inadequate sleep patterns due to the demands of medical school. Two studies examined the relationship of chronotype and depressive symptoms in medical students. One study found a correlation between chronotype and depressive symptoms while the other study failed to find a relationship. A study of 161 medical students by Hirata and colleagues (2007) used the Beck Depression Inventory (BDI) and the Morningness Eveningness Questionnaire (MEQ) to investigate chronotype and depressive symptoms. Distribution of chronotype across the sample was inconsistent with the expected distribution, with more E-types and M-types than in the general population. The number of M-types and E-types expected in a given population is

expected to be approximately 25% in each group (Horne & Ostberg, 1977). The sample consisted of 35.4% E-types, 37.3% M-types and only 27.3% intermediate types. Eveningness was associated with depressive symptoms (OR = 0.66, 95% CI = 0.50-0.88), and this association remained significant after adjusting for the presence of familial depression and physical activity (OR = 0.71, 95% CI = 0.52-0.95; Hirata, Lima, de Bruin, Nobrega, Wenceslau, & de Bruin, 2007). The distribution toward the extremes of morningness-eveningness may reflect the nature of medical students or a property of this sample. In contrast, a Brazilian study of 342 medical students found no correlation between chronotype and psychiatric disorders. A self-reporting questionnaire (SRQ-20) was used to measure symptoms (Hidalgo, Caumo, Posser, Coccaro, Camozzato, & Chaves, 2009). Lack of significant correlations may have been due in part to the measures used. The SRQ was designed to identify psychotic disorders in addition to depressive symptoms and is limited to a dichotomous answer choice for each question. The tool was designed for use in developing countries and may not be sensitive enough to identify depressive symptoms in a sample of Brazilian medical students. The distribution of M-types and E-types may also contribute to the lack of significance. This study contained fewer M-types (14.9%) than E-types (29.8%). Fewer participants measuring as M-types in a large sample raises concerns regarding the validity of the tool in different cultures or among different populations. The complex influence of social and cultural factors on the chronotype-health relationship makes application of findings from studies conducted in other countries to an American population questionable.

Limitations related to the study of university student populations demand more investigation into the general, healthy population. In community dwelling adults, an early study by Watts and coworkers (1983) found an interaction effect for morningness and time of day in

arousal but not in stress. Affect was measured five times during the day with a stress arousal checklist The study sampled only eight E-types and nine M-types and the results for stress and chronotype trended toward significance (p = 0.07; Watts, & Robson., 1983). For the most part, community studies have used smaller sample sizes than studies involving university students.

From two studies involving larger sample sizes, a relationship between chronotype and affect has been demonstrated. Adequate sample size allows for sufficient power to detect an effect. However, both studies were conducted in non-American samples, questioning the ability to generalize the results. The impact of chronotype on health may, in part, rest in cultural influences. The negative effects of eveningness may be mitigated by cultures that are more evening oriented (Smith et al., 2002).

A Brazilian study of 200 healthy adults (118 women) 18 to 99 years was conducted using The Montgomery-Asberg Depression Rating Scale (MADRS) and the MEQ as measures of depressive symptoms and chronotype respectively. E-types had a higher chance of reporting more severe depressive symptoms compared to morning- and intermediate-chronotypes, with an odds ratio (OR) of 2.83 and 5.01, respectively. This study also found a higher incidence of depressive symptoms in women (OR = 3.36) compared to men (Hidalgo, Caumo et al. 2009). The second study was conducted with a sample of 1170 healthy Japanese adults (677 females) ages 20 to 59. Depressive symptoms were assessed using the CES-D and chronotype was assessed using the MEQ. Sleep quality was also evaluated with the Pittsburgh Sleep Quality Index (PSQI). Scores on the CES-D were higher in the E-types indicating more depressive symptoms in this group. Almost half of the extreme E-types scored above the cut-point for depression. E-types report more depressive symptoms and worse sleep but depressive symptoms associated with eveningness were not related to sleep quality (Kitamura et al., 2010).

In the studies that have focused on clinically depressed populations the chronotype-depression relationship continues. In contrast to the above large studies in healthy participants, the studies of clinically depressed individuals were conducted in the United States. Due to the possible phase shifting effects of some medications, the participants were not taking any medications at the time of the study. A small study involving 39 depressed outpatients (21 women) and 39 age and sex matched healthy controls supported the increase in eveningness among depressed participants (p = 0.014) compared to the healthy group (Drennan, Klauber, Kripke, & Goyette, 1991). In a study of 208 depressed and non-depressed participants, the BDI, HRSD, PANAS and MEQ were administered to assess the relationships among depressive symptoms, affect and chronotype. Eveningness was associated with higher depression scores as measured by the BDI but not the HRSD. Non-significant findings between the HRSD and eveningness may be associated with the fact that the scale measures other symptoms in addition to depression, suggesting that the relationship between depression and chronotype may be unique to depression and not applicable to other psychiatric disorders. Mediational and path analysis found that the Behavioral Activation System (BAS) and positive affect mediate the association between chronotype and depression severity (Hasler, Buysse, Kupfer & Germain, 2010). The study authors argue that affect may be a fully mediating factor between chronotype and depression.

Seasonal Affective Disorder (SAD) is a disorder characterized by depressed affect during the months of the year in which days are shorter and colder. In addition to chronotype studies of adults with major depression, SAD populations have also been studied in relationship to chronotype. Because of the seasonality of the disorder, SAD has been subject to considerable investigation as a chronobiological disorder. Lewy's Phase Shift Hypothesis (PSH) asserts that

seasonal affective disorder results from a phase delay in the circadian rhythm resulting from the shorter days and less exposure to light. Chronotype differences in SAD patients further support the relationship between chronotype and depressive symptoms. A large study of both Italian and Spanish participants found a small but significant difference in mood by chronotype. One thousand seven hundred and fifteen (1715) university students participated by completing the MEQ and the Seasonal Assessment Pattern Questionnaire (SAPQ). In the Italian subgroup the association between chronotype and seasonality remained but not in the Spanish subsample, illustrating possible environmental and social-cultural influences on the chronotype-health connection (Natale, Adan & Scapellato, 2005). The authors suggest that the cultural and social environment in Spain is more evening oriented providing a possible protective effect on the health of E-types.

The PSH asserts that circadian timing is responsive to light exposure, where the timing of light exposure advances or delays circadian rhythms. Light interventions have been studied in a number of SAD populations, with one study by Murray and colleagues (2005) exploring the relationship to chronotypes. This study, conducted in Australia, tested a fluoxetine and light intervention for the treatment of SAD in 61 SAD outpatients. The Hamilton Depression Scale and BDI measured depressive symptoms. Sleep was assessed by self report sleep logs and chronotype was measured using the MEQ. The study found that improvement in mood was accompanied by increased morningness after eight weeks of morning light treatment ($p > 0.001$), however, the degree of shift to morningness did not correlate with the degree of mood improvement (Murray, et al., 2005). A second study did not investigate SAD directly, but explored the seasonal shift in mood in a healthy population. In a sample of 244 healthy adults living in Australia, a change in mood and rhythm from summer to winter was examined over a

three-year period. The MEQ was used to measure phase shift and affect was measured using the Inventory of Seasonal Variation and the PANAS. A significant association was found between winter pattern seasonality of mood and within-subject phase delay in winter ($r = 0.17$, $p < 0.01$). Affect decreased during the winter months and correlated with eveningness. The decreased affect also correlated with greater shifts in the participants toward eveningness from summer to winter (Murray, Allen & Trindel, 2002).

Chronotype and light treatment have been further evaluated in other disorders with depressive symptoms, specifically, bipolar disorder and attention deficit hyperactive disorder (ADHD). Affect plays a significant role in both bipolar and ADHD. Bipolar disorders exhibit periods of depression alternating with mania and mood swings are common in ADHD. Two studies were found that included chronotype as a variable. Both studies found a relationship between chronotype and affect. In the study of 75 bipolar depressives, 81 schizoaffective/schizophrenics (SA/S) and 349 controls, a composite scale was used to measure chronotype. Chronotype differed between bipolar patients and controls and SA/S, but not between controls and SA/S. Bipolar participants reported greater eveningness than the other two groups. Eveningness was also correlated with severity and duration of depressive symptoms (Mansour et al. 2005). In the second study, three weeks of morning light treatment was given to 29 adults with ADHD. Mood was evaluated using the Hamilton Depression Rating Scale-Seasonal Affective Disorder. Phase shift was measured with the MEQ. Light therapy yielded improved affect and a shift toward morningness. In addition, the phase advance relieved the symptoms of ADHD even when the correlations with improved SAD scores were low (Rybak, McNeely, Mackenzie, Jain & Levitan, 2006).

The relationship between chronotype and affect appears to be consistent over time. Concerned with the small sample sizes and lack of random sampling for controls in prior studies, Wood and coworkers (2009) conducted a study using 190 bipolar patients and 128 controls. The Composite Scale of Morningness (CSM) measured chronotype. Bipolar participants reported greater eveningness, especially those currently experiencing depressive moods. Furthermore, the scores remained stable over two years in a subsample of 52 of the bipolar participants (Wood et al. 2009).

Lewy's research group (1998) attempted to identify the optimal phase angle difference between circadian and sleep rhythms associated with optimal mood. Fifty-one participants with SAD and 49 controls participated in a crossover design study in which AM (0600 to 0800) and PM (1900 to 2100) light was delivered for two weeks. All participants maintained a sleep schedule of 2200 to 0600 and completed a daily mood diary and the Hamilton SAD. None of the subjects used medications. Dim light melatonin onset (DLMO) was collected at seven time points to determine circadian phase. DLMO was delayed in SAD participants when compared to controls. AM light phase advanced ($t = 6.41$, $p < .001$) and decreased depression (EL 37% lower than pretreatment $t = 7.38$, $p < .001$; Lewy et al., 1998).

While sleep and affect are related, in female college students sleep problems explained 13% of variance in depressive scores (Regestein, Natarajan, Pavlova, Kawasaki, Gleason &Koff, 2010), chronotype has been shown to have an independent effect on affect. In studies involving depressed individuals, a relationship between chronotype and depressive symptoms was found to be independent of sleep quality. One hundred participants (79 females) with major depressive disorder ranging in age from 18 to 60 years were evaluated for depressive symptoms and chronotype using the HRSD and MEQ, respectively. Sleep quality was measured using the PSQI

(Gaspar-Barba, et al., 2009). As with the earlier reported study by Kitamuri and colleagues (2010), sleep quality was not found to mediate the relationship between chronotype and affect in depressed participants. While sleep quality may not mediate the relationship between chronotype and affect, sleep deprivation may represent a circadian phase regulation mechanism. By waking early or delaying sleep, circadian phase position may be altered. The effect of sleep deprivation on affect has been studied with mixed results. One study sampled 60 healthy adults, 30 E-types and 30 M-types. Half received total sleep deprivation (TSD) and half partial sleep deprivation (PSD). The POMs was used to measure mood changes. Results differed by morningness-eveningness. After sleep deprivation, there was a significant increase in depression subscale scores in M-types and a significant decrease in depression subscale scores after TSD in E-types. The changes in depression-dejection scores of E-types after TSD ($p < 0.01$) and PSD ($p < 0.01$) were significantly different from changes in M-types after TSD (Selvi, Gulec, Agargun, & Besiroglu, 2007).

In summary, evidence exists that indicate a circadian rhythm to affect in healthy and clinical populations. The evidence suggests that chronotype and affect are related. Eveningness has been associated with lower mood and greater depressive symptoms. Lack of support for a circadian rhythm and chronotype difference has been found in studies with small sample sizes. Small studies may lack sufficient power to detect differences. Choice of affect measures may account for lack of significant results in some studies. Various tools measure different dimensions of affect and depressive symptoms and may include other constructs in their measure such as anxiety, agitation or tension. Experimental studies involving light therapy further support the relationship between improved mood and increasing morningness. While chronotype is a

measure of the position of the activity-rest rhythm, sleep has not been found to mediate the relationship between mood and chronotype.

Circadian Rhythm of Sleep Quality Patterns

Chronotype and sleep are conceptually related phenomena in that chronotype is a characteristic of activity and the sleep-wake cycle represents the periods of rest and activity. Sleep quality describes the restorative function of sleep while chronotype describes the temporal nature of an individual's sleep. Sleep quality is often measured using polysomnography and subjective measures, especially the Pittsburgh Sleep Quality Index (PSQI). Sleepiness is a characteristic that represents a quality of alertness and is understood in this study as an element of energy. Sleepiness is often measured by the Epworth Sleepiness Scale (ESS). Sleepiness and chronotype will be considered in the next section on energy. Individual characteristics of the sleep/wake cycle are believed to reflect the functioning of the intrinsic circadian pacemaker governed by the SCN. Healthy individuals exhibit a strong diurnal rhythm to sleep, consolidating sleep during the night. The nature of the diurnal sleep wake cycle has been the subject of considerable study. Research over the past 50 years has led to a theory of sleep that involves two reciprocal mechanisms, the S process and the C process. Process S shuts down the arousal system, inducing feelings of sleepiness. Process C maintains wakefulness and is regulated by the circadian system. Process C is proposed to be responsible for the consolidation of sleep during the night and coordination of sleep with the environmental day/night cycle. Process C works in opposition to process S (Colton & Altevogt, 2006). Process S and process C are regulated by neurons from the SCN in the hypothalamus. Lesioning studies in animals demonstrate the regulatory effect of the SCN on sleep (Dijk & Czeisler, 1995).

Process C is responsible for the wake maintenance and wake-up zones. Sleep is inhibited during the wake maintenance zone and the wake-up zone, when the body temperature is beginning to rise. The "wake-maintenance zone" occurs approximately 6 to10 hours before the time of the core temperature minimum, and the "wake-up zone" occurs 4 to 7 hours after the minimum core temperature. In healthy individuals, sleep occurs in optimal relationship to the core body temperature rhythm. The temperature minimum occurs at the time of greatest sleepiness. Healthy individuals have a temperature minimum around 0400 and a wake maintenance zone from 1800 to 2200 (Lack & Wright, 2007a). Phase relationships between body temperature, sleep/wake and melatonin play a role in the quality of sleep. Maximum sleep quality occurs when melatonin is at its peak and core body temperature is at its nadir (Lack & Wright, 2007b). Subjective sleep quality correlates with sleep efficiency, the ratio of time asleep to total time in bed (Åkerstedt, Hume, Minors, & Waterhouse, 1994). Sleep propensity is the pressure to fall asleep and is a measure of sleep quality. Sleep propensity is objectively indexed by sleep latency, the amount of time needed to fall asleep. Sleep pressure as a measure of latency, influences the length of time it takes to fall asleep. From forced dysynchrony experiments, it was determined that sleep pressure is greatest near the nadir of core body temperature and reaches a nadir in the evening (Lavie, 2001).

A number of studies of the sleep-wake cycle have focused on the relationship between sleep and the endogenous circadian clock. Many sleep studies involve experimental conditions of forced desynchrony, constant routine and altered sleep schedules to examine the effects of time of day on sleep quality. Core body temperature is often employed to represent the circadian position of the endogenous clock. Early studies conducted in the 1960s on sleep were undertaken as isolation studies. Participants were placed in isolation and subjected to time free environments

where all cues to the time of day or night were removed. Evidence from these early studies established an endogenous rest/activity period from 24.7 hours to 25.1 hours, with large between subject variability (Lavie, 2001). Chandrashekaran, Marimuthu, and Geetha, (1997) studied the sleep/wake and core body temperatures in 11 healthy participants to examine the stability of the sleep rhythm. Participants underwent isolation conditions from 15 to 43 days. In nine participants (three women) the sleep/wake patterns and core body temperature maintained a circadian period throughout the experiment. Only two participants demonstrated misalignment between sleep/wake and core body temperatures. Sleep in these two participants demonstrated a positive correlation with the preceding wake periods. In other words, the sleep wake cycle in these two participants was regulated by the preceding wake period rather than the endogenous circadian rhythm. In this study, 'time in bed' measured sleep time. Employing polysomngraphy would have contributed valuable information in terms of sleep latency and sleep quality, allowing for a more precise measure of actual sleep.

In subsequent studies, where environments were engineered for longer or shorter day lengths, stability of an endogenous period was supported. Studies determined the sleep cycle to be approximately 24 hours and10 minutes in the majority of individuals (Lavie, 2001). Despite significant alterations to sleep schedules, the sleep cycle was maintained. In studies where sleep cycles were fragmented, the circadian rhythm in total sleep was conserved. When participants were forced to sleep on sleep schedules shorter than 3 hour "days", maximum sleep still occurred in the late morning and minimum sleep occurred in the evening (Lavie, 2001). A study by Lavie and Zvuluni (1992) investigated sleep propensity during 48 hours using a 7-min sleep/13-min wake cycle. Eight participants were asked to either attempt to fall sleep or resist sleep. Results

yielded a high within-subjects stability of the sleep cycle. Participants with short nocturnal sleep latencies and higher sleep efficiencies slept more during the day.

Timing of “bedtime” was found to influence both duration and nature of sleep. In an early study, Carskadon, and Dement (1975) studied the effects of sleep cycles consisting of 30 minutes of sleep and 60 minutes of wake. Five healthy adults (2 women) participated in the 90-minute cycles for five days. Sleep time was greatest between 0900 and 1230 and least between 2100 and 0200.

The impact of bedtime was studied by altering the time of retiring. Akerstedt, Hume, Minors, and Waterhouse (1993) studied sleep displacement over the 24-hour period and its effects on sleep quality in eight healthy participants. Sleep was fragmented into four eight-hour periods, 12 six-hour periods and 12 one-hour naps. Results showed that 46% of reduced sleep time was related to bedtimes that were close to the circadian acrophase of the core body temperature. Akerstedt, Hume, Minors and Waterhouse (1998) explored the role of circadian timing on sleep in a study of eight healthy men. Participants followed a 4-hour sleep protocol at staggered times over the 24-hour period for 13 days. Both the effects of time of day and amount of prior wakefulness were studied. Results were consistent with previous studies showing an effect of both time of day and prior wakefulness on total sleep and sleepiness. Sleep latency decreased with proximity to the core body temperature trough. The longest sleep occurred around 1000 and subjective sleepiness peaked between 0800 and 1200 hours.

In addition to studies in which participants are subjected to extremes in environmental conditions such as forced dyssyncrony or isolation, research has been conducted under more natural conditions. Akerstedt and Gillberg (1981) studied six healthy men under a modified routine where seven different “bedtimes” were imposed at weekly intervals. Time cues were

minimized and participants were isolated from daylight. The authors found that the time of day at which the participants went to sleep influenced the duration of sleep. The longest sleep occurred after evening bedtimes and the shortest sleep occurred after morning/noon bedtimes. In addition the highest probability of waking occurred around noon and the lowest between midnight and early morning. Time awake did not influence sleep duration. As time awake increased, sleep length decreased.

Numerous studies involving the phase relationship between sleep and other variables have focused on the sleep-temperature relationship. Unlike the circadian rhythm of sleep studies, phase studies often employ a naturalistic environment, with normal eating and sleeping schedules. It has been hypothesized that the temperature rhythm is in part a function of the reduction in activity during sleep. Because sleep requires the cessation of physical activity, thermogenesis attributed to large motor activity can explain the decrease in core body temperature during sleep. Studies do not support this hypothesis. Gillberg and Akerstedt (1982) investigated the relationship of sleep and temperature in a natural environment. Synchronizers were minimal within a normal eating and activity routine. Sleep schedules were altered based on four hour intervals. Six healthy males participated in a sleep schedule of seven different bedtimes. Despite different sleep schedules, body temperature remained constant despite differing bedtimes. There was no fall in temperatures at the 0700 and the 1900 bedtimes, suggesting that sleep does not stimulate the change in body temperature.

While lack of motor activity cannot completely account for the temperature rhythm, temperature affects the quality and timing of sleep. Demonstrating a distinct circadian rhythm, core body temperature influences the onset, duration and characteristics of sleep. Gilbert, van den Heuval, Ferguson and Dawson (2004) reviewed the literature on sleep and thermoregulation.

The authors report that both peripheral heat loss and core production contribute to the circadian rhythm of temperature. Studies suggest that sleep onset latency (SOL) decreases as core body temperature decreases (Lavie, 2001). Counter intuitively, passive heating and exercise in the evening has been demonstrated to decrease SOL. This may be explained by rebound effect of peripheral heating on core body temperature. Heating leads to a down regulation of the core body temperature (Gilbert, van den Heuval, Ferguson & Dawson, 2004).

The relationship of core body temperature and sleep suggest a specific phase relationship between the two rhythms. Timing of sleep propensity and temperature rhythm has been studied in healthy individuals with a high level of consistency in results across studies. Evidence suggests that the sleep rhythm and temperature rhythm maintain a consistent relationship in healthy individuals. Lack and Lushington (1996) explored the PAD between temperature and sleep in 14 healthy participants (seven women) under constant routine. Participants underwent 24 hours of constant routine followed by 24 hours of a 10/20 routine in which participants were allowed to sleep for ten minutes every half hour. Researchers found that the sleep propensity rhythm was inversely related to the temperature rhythm. As the core body temperature peaked, the propensity for sleep decreased. Sleep propensity exhibited a cosinor curve with one harmonic. A minor peak was evident at mid afternoon and a broad peak occurred at 0500. The minimum in sleep propensity occurred in the early evening. Kudo et al. (1999) studied sleep propensity in ten healthy women. A protocol of ultra-short sleep cycles was maintained for 24 hours while sleep propensity, temperature and cortisol were measured. Sleep propensity was correlated with the temperature rhythm but not the cortisol rhythm.

In a study to measure the PAD between temperature and sleep, Gradisar and Lack (2004) studied 11 healthy men and three women under a constant routine for 48 hours. Women were in

the follicular phase of the menstrual cycle. The constant routine was modified by multiple sleep latency tests to determine objective sleepiness. Data were fitted to a 24-hour cosine curve with a 12-hour harmonic. The core body temperature nadir was found to occur at 0500. The sleep onset latency peak occurred two hours after the core body temperature nadir. Data on subjective sleepiness was collected in half hour increments. Subjective sleepiness was reported to peak half an hour after the core body temperature nadir. No gender differences were noted, although only three women participated in the study, possibly accounting for lack of significant difference in gender.

Even in individuals with very long sleep/wake cycles, there is evidence to suggest a phase coupling of sleep and core body temperature. A study of 12 healthy men under isolation conditions uncovered a participant with a free-running sleep/wake cycle of 50 hours (Czeisler, Weitzman, Moore-Ede, Zimmerman, & Knauer, 1980). Analysis of the data demonstrated a consistent phase relationship between sleep and temperature even though the temperature rhythm maintained a 24-hour cycle. Core body temperature, not length of prior wakefulness, was shown to be important to sleep behaviors under isolation.

While the rhythms of sleep and temperature appear to be phase locked, lack of sleep can affect both the magnitude and phase position of the temperature rhythm. Barrett, Lack and Morris (1993) studied eight healthy adults (3 women) under both a sleep and a wake condition. Other than the variable of sleep, a constant routine was maintained for 25 hours separated by one week between experimental conditions. Rectal temperatures and sleep onset were measured. Fourier analysis was used to fit the data to a 24-hour circadian rhythm with a 12-hour harmonic. The effects of sleep were reported to significantly increase the amplitude of the temperature rhythm and to phase delay the acrophase by 29 minutes, although not statistically significantly.

The small sample size may have contributed to a lack of significance in the delay in acrophase. A phase shift was noted in an experiment in which the sleep cycle was extended from 24 hours to 27 hours. Danilenko, Cajochen, and Wirz-Justice (2003) performed two nine day protocol cross-over design experiments involving 10 healthy adults (six women). One protocol involved a fixed sleep schedule from 2330 to 0800. The other protocol involved advancing the sleep timing by 20 minutes every day until a 27-hour day was established. Both protocols maintained a constant routine of dim light and four isocaloric meals. Results demonstrated a phase drift in core body temperature over seven days of 1.62 to -2.56 hours. In the 27-hour days, the core body temperature was consistently phase advanced by 0.66 hours. These differences suggest an influence of the sleep wake cycle on core body temperature, although a relatively weak effect.

The underlying mechanism by which temperature and sleep interact is not yet well understood. One theory holds that core body temperature drives the sleep propensity rhythm and that the two rhythms are not independent endogenous rhythms. One study altered the environmental temperature as opposed to altering the sleep variable. Dewasmes, Signoret, Nicolas, Ehrhart, and Muzet (1996) examined core body temperature and sleep propensity in seven healthy men. Participants were habituated to the climate in the laboratory. Sleep was enforced from 2200 to 0700. After 48 hours, baseline core body temperature was recorded. Ambient temperature was manipulated on the following experimental day. By lowering the ambient temperature throughout the night, there occurred a phase advance of the core body temperature by 143 minutes. The phase advance in temperature was accompanied by a phase advance in sleep propensity. The evidence is even more compelling because the advances occurred in every subject in only one night.

In summary, studies indicate that the sleep wake cycle under free running conditions demonstrates approximately a 24-hour cycle in most individuals. Sleep propensity exhibits a late morning peak and a minimum in the early evening. Under naturalistic conditions the sleep wake cycle is entrained to the core body temperature, with sleep onset latency peaking around 0700, two hours after the core body temperature nadir. In contrast, under experimental conditions the sleep/wake cycle readily dissociates with the core body temperature, which maintains a consistent 24-hour rhythm under constant conditions. Some studies suggest that the sleep/wake cycle may influence the core body temperature, but that influence is weak.

Chronotype and Sleep

Sleep quality has been measured using subjective report, actigraphy and polysomnography under various conditions including laboratory, natural and shift-work. As with studies on the chronotype-affect relationship, much of the chronotype-sleep relationship has been studied in university student populations. While many studies support chronotype differences in sleep quality, other do not. Some studies have shown differences in sleep length while others have found that total sleep time does not differ between chronotypes (Gaspar-Barba et al., 2009; Floyd, 1984).

Health problems present at higher incidence rates among shift workers. Shift work has been shown to increase the likelihood of developing, inter alia, obesity, diabetes and hypertension. The relationship between health and shift work may in part be mediated by sleep quality, with numerous studies establishing correlations between poor sleep quality and shift work. Chronotype has been investigated in one study of sleep quality and shift work. In a sample of 137 healthy Chinese nurses between the ages of 21 to 58 working shift work, the relationship between chronotype and sleep was examined. Chronotype was measured using the

MEQ and sleep quality using the PSQI over one month. After controlling for age, chronotype was a stronger predictor of sleep quality than shift pattern or shift timing. E-types reported poorer sleep quality than M-types (Chung, Chang, Yang, Kuo & Hsu, 2009).

Subjective measures including reported sleep quality, sleep length and sleep needs have been used to examine the influence of chronotype on sleep quality. Studies of university students show consistent differences in sleep duration and variability between chronotypes. In a small Brazilian sample of 32 university students (8 M-types, 8 E-types and 16 intermediates), chronotype was measured using the MEQ. Sleep was monitored for a week at two time periods, once during the school week and once during vacation. Sleep was measured using wrist actigraphy. As expected, M-types went to bed earlier than E-types. In addition, M-types slept longer and maintained a more consistent sleep schedule between school days and vacation days (Korczak, Martynhak, Pedrazzoli, Brito, & Louzada,, 2008). Similar results were found in an American sample of 22 participants (11 M-types and ten E-types; determined by the MEQ). Sleep logs were completed for two weeks. E-types demonstrated greater variability between weekday and weekend sleep timing and exhibited shorter sleep duration than M-types. M-types took shorter naps, reported less physical complaints, less mental activity at night and more adequate sleep (Webb & Bonnet, 1978).

In contrast, a study of 34 medical students (19 women) did not find chronotype differences in sleep duration. Medical school participants underwent 15 days of wrist atigraphy in May and November. Chronotype was evaluated using the MEQ. Sleep parameters of duration and latency did not differ between chronotypes, however, E-types had higher sleep efficiency ($p = 0.007$; Lehnkering & Siegmund, 2007). Lack of differences in duration and latency may have been due to the chronic state of sleep deprivation common in medical school students. In another

study of university students between 17 and 24 years of age, the amount of variance in sleep quality that can be attributed to chronotype was determined to be 2% by a multiple stepwise regression. In this larger study, 1125 students completed the MEQ, POMS, PSQI, Epworth Sleepiness Scale (ESS) and the Subjective Units of Distress Scale. Poorer sleepers demonstrated greater eveningness ($p < 0.001$; Lund, Reider, Whiting & Pritchard, 2010). In an early study of 1500 healthy Japanese university students, chronotypes differed on a number of variables. Differences existed in timing and variability of wakening and retiring, sleep latency affect and sleep duration. E-types reported increased variability, longer sleep latency, shorter length and less adequate sleep than M-types (Ishihara, Miyasita, Inugami & Fukuda, 1987). Sleep characteristics were measured using the Life Habits Inventory (LHI). A male subgroup of this study (10 M-types and 11 E-types) underwent a four to six-night laboratory sleep protocol in which polysomnography was performed following the first two nights. M-types followed a 2300 to 0700 sleep schedule and E-types followed a 0100 to 0900 sleep schedule. The only chronotype difference noted was for rapid eye movement latency ($p < 0.03$). No differences were found in sleep latency, possibly due to the enforced sleep schedule, sample size or laboratory environment.

In addition to studies of university students, sleep and chronotype has been examined in community populations. Further support for chronotype differences comes from a large study conducted in a sample of healthy French adults, ranging in ages from 17 to 80 years. In 617 participants sleep schedules, sleep needs, sleep hygiene and subject daytime sleepiness were compared between chronotypes. Eveningness was associated with a greater need for sleep, less time in bed during the week and more time in bed during the weekend. E-types exhibited more irregular sleep habits and greater caffeine consumption (Taillard, Philip & Bioulac, 1999).

Objective measures are often considered higher quality evidence when compared to subjective report. In sleep research, polysomnography has been used to objectively measure the quality of sleep. Research using polysomnography has yielded inconsistent results. Studies using polysomnography usually take place under laboratory conditions. Wake and sleep timing are often controlled and most studies are limited to small sample sizes and one or a two nights of recording. In a study by Mongrain, Carter, and Dumont, (2005), participants were recorded over two nights in the laboratory with the participants determining timing of sleep, but limited to only eight hours of sleep. The laboratory nights were followed by seven days of actigraphy. Twelve (12) M-types and 12 E-types (12 women) participated. Results found differences in sleep architecture for men but not for women. In the male subgroup, M-type men showed higher percentage of stage 1 sleep and lower sleep efficiency (Mongrain, Carter & Dumont, 2005). The same authors tested whether differences in nocturnal homeostatic sleep pressure may explain differences in sleep timing by comparing slow wave activity. Twelve M-types and 12 E-types, based on melatonin onset, were compared. Initial levels and decay rates of slow wave sleep where higher in M-types compared to E-types, however, this did not hold for the extremes in chronotypes ((Mongrain, Lavoie, Selmaoui, Paquet & Dumont, 2006). Compared to intermediate types, E-types exhibited greater difficulty falling asleep and more frequent awakenings using an ambulatory skin potential measurement system (Shiihara et al. 1998). Small sample size and lack of natural environments during monitoring may contribute to the lack of conclusive evidence. Both type I and type II errors are possible under these conditions.

Several causal factors have been proposed to explain the chronotype-sleep quality link. Poorer sleep quality in E-types may be related to self-efficacy. A study involving 499 college students in Canada investigated the difference in beliefs about sleep between chronotypes. The

CSM was used to measure chronotype. While M-types and E-types demonstrated similar sleep outcome expectations, self-efficacy differed between groups with E-types exhibiting lower sleep self-efficacy scores (Digdon, 2010). Another causal factor that may explain the chronotype-sleep quality link may relate to the ability to self-awaken. M-types are more likely to self-awake than E-types, who rely heavily on environmental mechanisms to awaken in the morning. One study found that self-awakening students went to bed and awakened earlier, felt better upon awakening, and dozed less during the day than non self-awakening students (Matsuura, Hayashi & Hori, 2002). Another large study of 3978 participants conducted in Canada over the internet found increased nightmares in E-types for the subgroup of women in a study, possibly contributing to poorer sleep. Both incidence and distress severity increased with increasing eveningness (Nielsen, 2010).

In an attempt to examine the genetic contribution to sleep quality a twin study was conducted. Chronotype and sleep quality were compared in 420 monozygotic twins, 773 dizygotic twins and 329 siblings, ranging in age from 18 to 27 years. The UK study found a significant association between increased eveningness and poorer sleep quality (Barclay, Eley, Buysse, Archer, & Gregory, 2010).

Mongrain, Lavoie, Selmaoui, Parquet and Dumont (2004) explored the PAD between wake time and temperature/melatonin rhythms in relationship to sleep quality in different chronotypes. Actigraphy over seven days in a sample of 24 healthy individuals (12 women) evaluated the PAD between circadian phase measured by temperature minimum and onset of dim light melatonin and habitual wake time. Twelve participants (six women) were E-types and 12 (six women) were M-types. Participants were allowed to sleep and wake on their own schedule. Consistent with earlier studies, E-types demonstrated a shorter average PAD compared

to M-types. Furthermore, when separated into two subgroups of overlapping and non-overlapping circadian phases, non-overlapping E-type group demonstrated the shorter PAD, while overlapping E-types exhibited a longer PAD. Results suggest the possibility in two different mechanisms contributing to chronotype (Mongrain, Lavoie, Selmaoui,Parquet & Dumont. 2004).

Despite the evidence to suggest poorer sleep, hence poorer health in E-types, one study found a protective effect on health in E-types. Variability of sleep parameters has been identified as greater in E-types, possibly contributing to poorer quality of sleep. Yet, variability may suggest a greater flexibility when encountering forced changes in sleep parameters, such as sleep deprivation or shift work. Foret, Touron, Benoit and Bouard (1985) studied chronotypes and sleep polysomnography in healthy sleepers during normal sleep and after sleep deprivation. Five M-types, four E-types and ten intermediate types participated. Sleep parameters did not differ by chronotype during normal conditions and after a night and day of sleep deprivations. Sleep differed by chronotype during day sleep after a night of sleep deprivation. M-types demonstrated poorer sleep quality with a smaller percentage of REM sleep, more wakefulness, less SWS and longer latency of the first REM episode compared to E-types. Results support the hypothesis that E-types are able to adapt to changing sleep schedules more easily than M-types.

Circadian Rhythm of Energy

Sleepiness and energy level are closely related concepts. Lack of sleep may result in drowsiness. Sleep scales such as the Epworth Sleepiness Scale (ESS) and the Stanford sleepiness Scale (SSS) are often used in studies to measure subjective lack of energy. While sleepiness can be correlated with sleep latency the measures are not interchangeable (Olsen, Cole & Ambrogetti, 1998). Studies have supported the hypothesis that the subjective experience of

energy exhibits a circadian rhythm. Under diverse conditions, including natural environments, isolation and constant routines, the energy rhythm consistently expresses itself. In studies of energy, both the circadian rhythm influence and the amount of prior wake time must be considered. Studies have been designed to differentiate between the two influences. Early studies used naturalistic settings and 24-hour time frames, allowing for confounding by masking effects. Subsequent studies have employed constant routines and forced desynchrony protocols to examine the circadian rhythm of sleepiness, alertness and performance. The following reviews the literature on the circadian nature of sleepiness and alertness, as measures of perceived energy level, in healthy participants.

Under temporal isolation and normal sleep/wake cycles, a circadian rhythm has been noted to persist for at least five days (Monk, Fookson, Moline, & Pollak, 1985). The acrophase for sleepiness and weariness occurred approximately four hours after rising and the acrophase for alertness occurred seven hours after waking. In the absence of normal sleep/wake, the circadian rhythm of energy has been detected. Constant routine studies have demonstrated that sleepiness exhibits a circadian rhythm that increases during the evening and night and peaks around 0700 hours. Not surprisingly, sleepiness also shows an effect from sleep deprivation (Jaspers, Hausler, Baur, Marquart, & Hermdorfer, 2009). Dijk, Duffy, and Czeisler (1992) found that over a 40-hour constant routine protocol, 24 healthy men maintained a rhythm in alertness and performance. The rhythm followed a pattern that increased over the first three hours, leveled out for the next 13 to 14 hours and was followed by a sharp decrease at habitual bedtime. The minimum for both alertness and performance occurred shortly after the core body temperature nadir. Both alertness and performance were worse on day two suggesting an effect of wake time. Circadian rhythms in energy persist under experimental conditions of short sleep cycles and

forced desynchrony as well. Sleep cycles varied in all the studies ranging from 220 minutes to 29 hours. Results suggest that the circadian rhythm of alertness persists in the absence of a 24-hour day. A study using very short sleep/wake cycles of 220 minutes in 38 healthy men found that sleepiness peaked with the minimum in oral temperature (Moses, Lubin, Naitoh, & Johnson, 1978). This protocol also found that sleepiness was not related to amount of preceding wake time suggesting a predominance of the circadian rhythm over the amount of wake time on sleepiness.

Under shortened days the phase relationship between alertness and temperature rhythms desynchronizes and then resynchronizes with a different PAD. In addition, different measures of alertness sychronize in different relationships with body temperature. Folkard, Wever and Wildgruber (1983) studied seven healthy volunteers in isolation for 28 days. Gradually, the day was progressively shortened to 22 hours for three participants and lengthened to 29 hours for the other four. Alertness was measured every three hours. The different alertness measures demonstrated different circadian rhythms under the desynchrony experiment. Results showed that the core body temperature retained a rhythm of approximately 24 hours. Letter recognition, followed the core body temperature rhythm despite change in sleep/wake cycle. In three of the seven participants, verbal reasoning separated from the temperature rhythm, running at a 21-hour cycle. This study suggests that alertness may be regulated by circadian rhythm of temperature or the sleep/wake cycle, depending on the measure. Similarly, a forced desynchrony study conducted by Folkard, Hume, Minors, Waterhouse, and Watson (1985) found that in a 22-hour day-night cycle a normal phase relationship between alertness and temperature existed for the first nine days. During days 10 to 14 the phase relationship between alertness and temperature changed where the temperature rhythm remained entrained to the changing day length and the alertness rhythm free-ran. By the end of the protocol both alertness and temperature free-ran in

relationship to the 22-hour day but were phase locked to each other. However, the new phase relationship was approximately 7.5 hours advanced for the temperature acrophase. These results suggest that the circadian rhythms of alertness and temperature are independent of the sleep/wake cycle and each other but able to be entrained to an approximately 24-hour rhythm in the absence of external time givers, albeit in a different phase relationship.

More recently, a forced desynchrony protocol was used by Wyatt, Ritz-de Cecco, Czeisler and Dijk (1999) to examine the influence of circadian rhythm and amount of wake time on alertness. Six healthy participants (one woman) followed a 20-hour day for 24 days. Sleepiness, reaction time and cognitive tasks were measures at 30 minute intervals. Results yielded a near 24-hour circadian temperature rhythm despite the shortened day. For sleepiness there was a main effect of wake time and circadian phase. In addition, there was an interaction effect of circadian phase and length of wake time. In the reaction and cognitive performance measures there were significant main effects for both circadian rhythm and wake time but no interaction effects. Consistent with other studies, performance was worse and sleepiness greatest near the temperature nadir.

As noted earlier, different alertness measures exhibit different rhythmic properties in forced desynchrony experiments. Attention failed to demonstrate a circadian rhythm in a study of 14 healthy participants under 28-hour days (Harrison, Jones, & Waterhouse, 2007). Sleepiness scores did show a significant main effect for both wake time and circadian phase but no interaction effect. Again sleepiness was greatest with increased wake time and around the temperature minimum. In contrast, one study, in which bed times varied over a 24-hour day, did not find a relationship between sleepiness and amount of prior awake time or length of sleep (Akerstedt & Gillberg, 1981).

Masking effects are known to influence the circadian rhythm under investigation. While constant routine protocols are designed to reduce the influence of masking, constant routines provide an extremely artificial environment. In order to understand the influence of the circadian rhythm in day-to-day living, a more naturalistic environment must be studied. In relation to perceived energy levels, a number of masking influences have been identified and studied, including boredom. A study conducted under natural conditions investigated the influence of boredom and ambient temperature on the circadian rhythm of sleep. Mavjee and Horne (1994) compared subjective sleepiness at two times of the day; afternoon (1200 to 1600) and evening (1800 to 2200) under stimulating and bored and cold and warm ambient temperature conditions. While a pleasantly stimulating environment reduced sleepiness and increased alertness, it did not abolish the rhythm. Forty-eight healthy women between 19 and 32 years of age participated in eight experimental groups resulting in six participants per group. Participants arrived at either 1200 or 1800 hours. Following a meal, participants experienced either a stimulating or boring activity in either a cool or warm room. The ambient temperature did not affect sleepiness but stimulation did. The initial sleepiness ratings for the afternoon and evening subjects were very similar (afternoon = 2.6 and evening = 2.7). The results were a main effect for stimulation ($p <$ .01), with subjects in the boredom condition reporting greater levels of sleepiness. Sleepiness progressively worsened over time in both afternoon and evening but leveled off in the evening group by the third hour. Lack of difference for room temperature may be explained by the small temperature range. With only six participants in each condition, the possibility of group differences between evening and afternoon groups even with random assignment is a consideration. Another study on the masking effects of stimulation on sleepiness was conducted by Hayashi, Minami, and Hori (1998). In five healthy male university students, either an

interesting video series provided stimulation or a landscape video provided no stimulation. Each series was presented to the participants for one week every 20 minutes from 0900 to 1800. In the landscape group, reported sleepiness and fatigue were increased compared to the interesting video group. This study suggests that the ultradian rhythm may be masked by environmental conditions that are stimulating.

Another characteristic of alertness that demonstrates a circadian rhythm is sleep inertia. Inertia is the impairment of cognitive performance immediately after awakening. Scheer, Shea, Hilton and Shea (2008) studied 12 healthy adults (five women) under a forced desynchrony condition. Participants followed a 28-hour sleep/wake cycle for seven days. Sleep inertia was assessed three times during each sleep/wake period. Using core body temperature to determine circadian phase, sleep inertia demonstrated a significant circadian rhythm (p = 0.007) peaking between 2300 and 0300 and troughing between 1500 and 1900.

In summary, studies consistently demonstrate a circadian rhythm in alertness and sleepiness. Under naturalistic and experimental conditions energy, as measured by sleepiness and alertness varies across the 24-hour cycle and is strongly coupled with the core body temperature rhythm. Sleepiness reached an acrophase in the early morning around 0700, two hours after the temperature nadir at 0500 hours. Alertness and body temperature demonstrate a phase relationship that varies based on sleep-wake conditions.

Chronotype and Energy

Studies have investigated the relationship between chronotype and energy by comparing the differences in alertness and daytime sleepiness in M-types and E-types. The alertness rhythm in E-types appears to be phase delayed compared to M-types. E-types show a peak in alertness

that is 4.28 hours delayed compared to M-types, under constant routine conditions (Kerkhof & Van Dongen, 1996).

Studies on daytime sleepiness demonstrated inconsistent results regarding an association with chronotype. Two studies found no association between chronotype and daytime sleepiness under natural sleep-wake conditions. A study of 617 healthy French participants found eveningness associated with poorer sleep quality, however, daytime sleepiness did not differ between E-types and M-types. (Taillard, Philip & Bioulac, 1999). In another study, daytime sleepiness was measured by daily sleep logs and the Stanford Sleepiness Scale in 15 healthy participants over five nights. Morningness/eveningness was determined by a median cut-point on the MEQ. There was no difference noted in daytime sleepiness (Hilliker, Muehlbach, Schweitzer & Walsh, 1992). The use of a median cut-point for chronotype is unconventional and may contribute to lack of significant findings, by including intermediate types in both M-types and E-types. In contrast, a larger study involving 1165 French workers investigated the relationship between chronotype and sleepiness using a mailed questionnaire. E-types reported more morning sleepiness but overall higher energy ($p = 0.04$) than M-types (Taillard et al., 2001).

Other studies have found chronotype differences in sleepiness and alertness. In a study of 310 medical students (123 women) sleeping habits and sleepiness was examined in relationship to chronotype. Daytime sleepiness correlated with chronotype ($r = -0.18$, $p = 0.002$) with increasing sleepiness associated with greater eveningness (Hidalgo, Caumo, Posser, Coccaro, Camozzato, & Chaves, 2003).

In a study by Matchock and Mordkoff (2009), a self-report questionnaire of alertness was administered to 80 participants at four times across the day. Comparison of E-types with non E-types yielded improved performance in the morning for non E-types and improvement in

performance in the afternoon for E-types. Alertness increased for all participants during the first half of the day then decreased for non E-types in the latter part of the day (Matchock & Mordkoff, 2009).

Sleep deprivation is noted to be common among medical students and may, in part, account for the chronotype differences. As noted earlier, eveningness appears to provide a protective effect on sleep quality under conditions of sleep deprivation. This may not be true for daytime sleepiness or alertness. One study reduced sleep duration by either delaying bedtime by two hours or advancing rising in the morning by two hours, and investigated alertness and performance in 12 healthy participants, six M-types and six E-types. After two nights of altered sleep, data were compared to data from a reference night. Alertness was measured using the Activation/Deactivation Adjective Checklist every two hours after rising until 2000 hours. Alertness decreased in both conditions of sleep reduction but no differences were found in the diurnal peak of alertness. In M-types, alertness troughed at 0800 in delayed sleep and at 1200 in early rising. In contrast, the trough was the same for E-types under all three conditions (Clodoré, Benoit, Foret, Touitou, Touron, Bouard & Ayzeby, 1987). Another study of alertness as a function of chronotype in a sample of 43 healthy adults was conducted under two conditions; normal sleep routine (N = 43) and a two hour sleep reduction protocol (N = 12). E-types experienced greater sleepiness in the morning at 1000 and 1200 and reported lower alertness according to the Thayer Activation Deactivation Adjective Checklist. The rhythm for alertness flattened out for E-types but not M-types following reduced sleep (Clodoré, Fore,t & Benoit, 1986).

In summary, studies are inconsistent on a chronotype difference in energy. In some studies, the energy circadian rhythm differs in timing by chronotype, while others show no

difference. Studies with significant results often use university student populations. Non-significant results may result from methodological concerns including chonotype operational definition and cultural influences. In studies involving American samples, using conventional chronotype cut-points, timing of the energy rhythm varies between chronotypes. M-types experience sleepiness earlier than E-types and alertness was greater in the early part of the day for M-types than for E-types.

Circadian Rhythm of Affect, Sleep Quality and Energy Summary

Overall, studies examining subjective experiences of affect, sleep quality and energy have been shown to vary by chronotype. Polysomnographic measures have been less convincing. Both M-types and E-types exhibit circadian rhythms in affect, sleep, and energy under natural and experimental conditions. E-types have been found to be phase delayed compared to M-types in all three rhythms. Disorders of affect and sleep are more common among E-types and in studies of healthy participants, chronotype appears to be correlated with affect, sleep quality and energy. Increased eveningness is associated with decreased affect, poorer sleep quality and lower energy. While some studies have failed to find association, possibly due to sample size and chronotype definition, studies exist that have demonstrated an influence of chronotype. More research is needed in this area as the number of studies differentiating between chronotypes is low. Conflicting results have been found and continue to confound understanding of the role of the circadian rhythms in affect, sleep quality and energy. The nature of circadian disruptions has not been fully explicated and is under continuing investigation.

Circadian Regulation of Cortisol

Much research has focused on the role of cortisol in health, in part, due to the multiple systemic effects of cortisol and its role in the stress reaction. Cortisol plays important roles in metabolism, immune function and activation of the sympathetic nervous system. Cortisol interacts with a large number of hormones, steroids and proteins, including estradiol, leptin, and vasopression (Molina, 2006). Stress has long served as a major theoretical causal agent in illness development. Many studies on the circadian system and health focus on the theoretical assumption of illness situated within a stress model. Hypotheses are premised on the model that stress contributes to a deregulated circadian system that manifests in altered expression of cortisol secretion. Cortisol has been studied in relation to stress, mood disorders, sleep disturbances, and disease progression with conflicting and unclear results (King & Hegadoren, 2002). For example, basal cortisol levels are elevated in only 50% of depressed individuals. Gunnar and Vazquez (2001) reviewed the literature and found that in some studies, hypocortisolism has also been observed as a stress response. Discrepancies between studies may in part be due to lack of consideration of circadian phase during sampling. The majority of the human studies have focused on basal and stress response cortisol measures (Millan, 2006). Basal levels that measure cortisol at a single time point or average over a specific time period fail to account for individual differences in phase positions. Studies that measure area under the curve (AUC) and slope estimations also do not consider that differences may be attributable to individual differences in phase position of cortisol. Possibly due to the theoretical relationship between cortisol and the stress response system, few studies have examined the relationship between cortisol and other rhythms. Theoretical models of a single oscillator circadian system also contribute to the lack of study of multiple rhythms. Researchers have approached the measurement of a particular rhythm, whether that rhythm is temperature, melatonin, or cortisol,

as reflective of the status of the entire circadian system. A disruption of the circadian system would be detected in one or more of the parameters of any of the above mentioned rhythms. The development of multioscillator models made the phenomenon of uncoupling of rhythms a conceptual possibility.

It has been accepted that the cortisol circadian rhythm may be used as a marker for the phase positions of other rhythms within the individual. A large number of disorders have been examined vis-à-vis cortisol irrespective of the phases of other endogenous rhythms. The cortisol rhythm in isolation of other endogenous rhythms has been analyzed in relation to menstruation and menopause (Gudmundsson et al.,1999; Kerdelhue et al., 2002; Kerdelhue, Lenoir, Queenan, Scholler, & Jones, 2006; Parry, Javeed, Laughlin, Hauger, & Clopton, 2000; Patacchioli et al., 2006), depression and post-traumatic stress disorder (Kelle, et al., 2006; Sherman & Pfohl, 1985; Souetre, Salvati, Rix, & Pringuey, 1988; Yehuda, Golier, & Kaufman, 2005), endocrine disorders (De Martin, Giraldi, & Cavagnini, 2006; Glass, Zavaldil, Halberg, Cornelissen, & Schaaf, 1984; Reschini, D'Alberton, Catania, & Motta, 1990; Reschini & Giustina, 1978; Sasaki et al.,1983), chronic fatigue (Di Giorginio, Hudson, Jerjes, & Cleare, 2005), ulcerative colitis (Payer, Huorka, Duris, Mikulecky, Kratochvilova, & Ondrejka, 1993), cancer (Touitou, Bogdan, Levi, Benavidas, & Auzeby, 1996) and sleep (Moldosfsky, Lue, Davidson, & Gorczynski, 1989; Tomoda et al., 2003).

This section will focus on cortisol studies involving multiple sampling across the 24-hour period, irrespective of whether circadian phase was considered in the analysis. Studies in healthy and ill populations will be examined. First, studies that consider the phase of cortisol will be discussed. These studies focus group differences in the circadian cortisol rhythm. Next, studies involving the phase relationship between cortisol and other rhythms will be discussed.

Finally, results from studies that investigated flattening of the diurnal cortisol slope will be presented.

Studies examining multiple cortisol values across the 24-hour day in affect, sleep and energy. Early studies on the circadian rhythm of cortisol involved plasma levels over the 24-hour period. Since the late 1990's, salivary cortisol measures have become increasingly common. Salivary sampling offers many advantages over plasma, including increased acceptability by participants, noninvasive methods and naturalistic settings for collection. Salivary samples provide the free cortisol levels while plasma samples represent free cortisol and inactive cortisol bound to globins (CBG)s. Early studies of cortisol as a marker describing the circadian rhythm in humans have used plasma cortisol levels. The majority of studies that report the diurnal profile of cortisol over a 24-hour period have used plasma samples. Plasma cortisol measures the total cortisol in blood, both bound and unbound cortisol. Calculations can be made to correct for free (bio-available) cortisol but are often not reported. With the advent of assays to detect free cortisol in saliva, the use of salivary sampling began to replace plasma sampling. Salivary sampling offers many advantages over plasma sampling, including participant preference, naturalistic setting and measurement of free, unbound cortisol. In the late 1990's, studies have focused predominantly of the slope of the diurnal rhythm using salivary collection methods.

Altered affect is a major symptom of depression and has been measured in depression studies. Often depression studies use the affect measures of POMS and PANAS to examine improvement in health. Depression, which manifests sickness behaviors including changes in affect, sleep quality and energy, has been extensively studied in chronobiological research. In the past 35 years, 12 studies have reported on phase relationships in depression in humans. The

study results have been conflicting. Some studies have shown differences in phase position between depressed participants and healthy controls, while other studies have failed to show a difference. Many studies suffer from small sample sizes, lack of homogenous groups, and lack of appropriate or sensitive analytical methods. Comparisons of results across studies are hindered by variation in sample, design methods and statistical modeling techniques.

Of the twelve studies, seven found a difference in at least one measure of phase position between depressed and healthy participants. Four studies demonstrated no differences between groups. All studies examined cortisol under controlled settings using plasma samples. None of the studies collected data from a naturalistic setting due to the multiple sampling of plasma levels over the 24 hour period. Controlled conditions included constant routine, where conditions such as light, meals, wake state and temperature are kept constant for the duration of the experiment. Less rigid controlled conditions standardized awakening time, meals and time of retiring. Samples were collected over one or more 24-hour periods with the exception of two studies that reported on data collected only between 2000 and 0800 (Thalén, Mørkrid, Kjellman, & Wetterberg, 1997) and between 2100 and 0600 (Jarrett, Coble, & Kupfer, 1983). Sampling ranged from every 10 minutes to every hour.

Study populations consisted of different depressed groups, single depressed groups and depressed groups compared to a healthy control. Many of the studies are confounded by a sample of heterogeneity in depression diagnosis. Six studies included both uni-polar and bi-polar depressed participants. Three studies reported inclusion of participants with co-morbidities such as anxiety disorder, dysthymia, and psychotic depression. Only four studies reported controlling for type of depression in the sampling. One study examined cortisol in participants with primary depression. One study was limited to participants with endogenous depression. One study

examined women with SAD. The final study examined cortisol in a group of participants with melancholic depression. Three of the four studies with homogeneity of diagnosis reported differences between groups. Only one study of 10 melancholic depressed and 14 controls demonstrated no group difference (Wong et al., 2000).

Four studies found no phase shift between depressed and healthy controls. Of the four studies that compared depressed and healthy participants, three used cosinor analysis to describe the cortisol circadian rhythm. A study by Oren, Levendosky, Kasper, Duncan, & Rosenthal (1996) used ANOVAs and AUC to analyze the cortisol rhythm. Peak time was identified as the time at which the cortisol level was the highest. Describing the peak by the highest value may lack precision in determining the actual acrophase of the rhythm, due to timing of collection in relation to the circadian rhythm. The nadir of the rhythm was not determined. Oren, Levendosky, Kasper, Duncan, and Rosenthal (1996) studied 21 participants diagnosed with SAD and 20 healthy controls. Participants were required to remain during sampling in the research facility and were awakened at 0700. Lights were turned off at 2300. The impact of the institutional setting and standardized wake time may impact how well the sampled rhythm reflects the participant's normal rhythm. An awakening time earlier than normal may alter some participant's cortisol expression. Heterogeneity of sample may also contribute to lack of significant findings. Of the 21 SAD participants, 13 were female. Three of the 13 were postmenopausal, and five of the remaining female participants were in the follicular phase of their cycle and five were in the luteal phase. Among the 20 controls, 12 were female with five postmenopausal. The SAD participants varied on depression diagnosis. In addition to the SAD diagnosis, ten participants had a diagnosis of unipolar depression, ten had a diagnosis of bipolar II and one had a diagnosis of bipolar I.

Of the three studies that used cosinor analysis and found no differences between depressed and healthy participants, only the study by Young, Carlson, and Brown (2001) included an analysis of possible additional harmonics in the description of the cortisol rhythm. It has been suggested that in determining the acrophase and nadir of a rhythm generated by a multioscillator system, an accurate and precise value can only be obtained by including harmonic analysis (Bao, van Someren, Hofman, Cao & Zhou, 2003). Wong and colleagues (2000) used a single cosinor model to describe the cortisol circadian rhythm in ten participants with melancholic depression and 14 healthy controls. Participants were required to lie flat in bed at a research facility while cortisol samples were drawn every 30 minutes for 30 hours. No differences in phase position were noted between the depressed and the healthy groups. The authors accounted for phase of the menstrual cycle in the eight depressed and the six healthy participants. All female participants were sampled during the follicular phase of the menstrual cycle. The small sample size and type of depression studied may also account for the lack of difference in phase position. Melancholic depression is characterized by intense agitation, arousal and anxiety, suggesting increased energy. Sleep quality is characterized by insomnia and mood is universally negative. In contrast, atypical depression is marked by low energy, variable mood and hypersomnia. Given such differences in symptom presentation, atypical and melancholic depression may represent two unrelated disorders.

Posener, DeBattista, Williams, Chmura, Kalehzan, and Schatzberg (2000) used a single cosinor model to describe the cortisol rhythm in a sample of 49 unipolar depressed participants and 33 healthy controls. Of the 49 depressed participants, 11 had a co-morbid diagnosis of psychosis. Within the group of 38 non-psychotic depressed, co-morbidities included dysthymia, PTSD, panic disorders agoraphobia and obsessive-compulsive disorders. The participants with

psychotic depression were predominantly male with seven out of eleven participants. Fourteen out of 38 depressed participants without psychosis were male. Eighteen out of the 33 controls were male. In the female participants, the study did not report menstrual status or phase of the menstrual cycle. All patients were outpatients, not on medication. Plasma samples were collected in a laboratory setting every hour for 24 hours. The PMD group had a male-female ratio of 7:4. The non-PMD group had a ratio of 14:24. The control group had a ratio of 18:15. PMD subjects, non-PMD subjects and controls did not differ on acrophase (0924 hr, 0951 hr and 1004 hr, respectively) or mean levels (186.6 nmol/L, 185.9 nmol/L, 198.0 nmol/L, respectively). The non-PMD group differed from the healthy controls on amplitude (109.1 nmol/L vs. 128.6 nmol/L, p = 0.02).

While the study limited the sample to participants with a depression diagnosis of unipolar depression, co-morbidities and the inclusion of both males and females may contribute to the lack of significance. The psychotic depressed group numbered only 11 participants, allowing for a possible type II error. The use of a simple cosinor model may lack the precision to detect the actual timing of the acrophase and nadir.

The strongest support against a difference in phase position between healthy and depressed population was presented by Young, Carlson, and Brown (2001). Young, Carlson, and Brown (2001) studied 25 premenopausal women and 25 healthy controls. Controls were age-matched and matched for phase of the menstrual cycle with depressed women. Twenty-four women were in the follicular phase and 26 women were in the luteal phase of the menstrual cycle. The study was conducted in a research facility, with samples drawn every 10 minutes for 24 hours. Participants were required to remain on bedrest and meals were standardized across the day. Harmonic analysis was used to account for ultradian rhythms. No difference was found

between depressed and non depressed participants in timing of the maximum or minimum. No difference was found between groups on onset or offset of the cortisol rise. Limiting to female participants was a strength of this study; however, including women in both the follicular phase and luteal phase may contribute to the lack of significant findings. The sample size of 25 per group is larger than many studies that have been done to date on the circadian rhythm of cortisol. The depressed group was also heterogeneous on type of depression. Six participants had a diagnosis of endogenous depression. Seven participants had a diagnosis of non-endogenous depression. Seven had atypical depression, 17 recurrent unipolar depression, one bipolar depression, seven dysthymia and 9 with co-morbid anxiety disorder. The setting of the study prevented capturing the effects of living in a naturalistic setting on cortisol. The studies discussed above suggest that the group differences in the phase position of cortisol alone may not be able to account for the presence of depression. The studies suggest that further investigation of the cortisol rhythm may include the phase relationship of cortisol with other rhythms, such as estradiol.

In contrast to the above studies that found no difference between depressed and healthy individuals, the majority of studies on phase position in depression report significant phase differences. Seven out of the 11 published studies found a circadian rhythm phase shift in depression, supporting the PSH. As with the studies that report non-significance, studies reporting significant findings were conducted in research facilities with standardized diet and activity. Activity ranged from bed rest to free ambulation during day hours and sleep at prescribed hours. Meals ranged from equal caloric intake every hour to standard meals three times during the day. None of the studies provided for a naturalistic environment. All samples were collected from blood draws. Samples were heterogeneous on diagnosis, gender, and

statistical methods. Studies reported results that range from a phase advance in depression from zero to three hours. One study of SAD reported a phase delay in cortisol minimum (Avery, et al., 1997). Two studies found no difference in acrophase or nadir but report differences in onset of first secretory event and time in quiescence (Halbreich, Asnis, Shindledecker, Zumoff, & Nathan 1985; Deuschle et al., 1997). One study reported a difference in phase angle difference (PAD) between cortisol and sleep onset (Jarret, Coble, & Kupfer, 1983).

Three studies report an advance in phase position with depression. These three studies were more homogeneous in sample than the studies that report non-significant results. Where the group with depression was not homogenous, subgroups by diagnosis were analyzed revealing significant differences. Statistical analysis differed between studies, including cosinor analysis, harmonic regression and periodgram. Harmonic regression and periodgram demonstrate greater precision in determining the peak and nadir of the rhythm over cosinor analysis. The study employing cosinor analysis further explored the nadir by taking the mean of the lowest three values from samples collected every 20 minutes (Pfohl, Sherman, Schlechte, & Winokur, 1985).

In a study by Linkowski and colleagues (1985), eight unipolar depressed males, 10 bipolar depressed males and seven age-matched male controls gave blood samples every 15 minutes for 24 hours. Participants were maintained in a research facility, allowed to ambulate during the day, given standard meals, and allowed to sleep according to habit. Results were analyzed using a periodgram. In unipolar depressed males, the nadir was three hours advanced compared to controls ($p = 0.015$). Unipolar depressed participants also exhibited a shorter time in secretory activity compared to controls ($p = 0.002$). Bipolar depressed participants exhibited a 90 minute phase delay in the acrophase compared to controls ($p = 0.009$). The small sample size may contribute to a possible type I error; however the homogeneity of the groups contributes to

greater accuracy in detection of differences. The setting protocol was more relaxed than in other studies allowing for ambulation and time of retiring according to desires of the individual participants.

Pohl, Sherman, Schlechte, & Winokur (1985) controlled for type of depression by examining dexamethasone suppression test suppressors (DSTS) and nonsupressors (DSTN) as separate groups. A sample of 25 depressed participants was comprised of 17 DSTS and eight DSTN. Of the 17 DSTS, ten were female. Of the eight DSTN, five were women. The research facility setting included ambulation, standard meals throughout the day and sleeping schedule according to each participants' habits. Data was analyzed using cosinor analysis. The nadir was determined by taking the mean of the lowest three values. Results demonstrated no difference between DSTS and controls. DSTN showed a two hour phase advance in the timing of the nadir compared to DSTS (2400 verses 0227, $p < 0.01$). The difference in timing of the nadir remained after controlling for age ($p < 0.002$). There was also a trend for the acrophase of a one hour phase advance. Validity of the results is limited due to group differences. The number of participants, percentage of females, and ages differed by groups. Menstrual status and phase of menstrual cycle were not reported. The healthy controls were recruited from the hospital personnel who worked at the institution conducting the research. Some patients were receiving medications including tricyclics and lithium carbonate. However, the use of medication would more likely contribute to a lack of group differences as some medications have been shown to phase advance circadian rhythms.

A more recent study of phase position in depression was conducted by Koenigsberg and colleagues (2004). This study compares 22 participants with major depression and 20 healthy controls. Of the depressed group, 8 were female in the follicular phase of the menstrual cycle.

Seven of the healthy controls were female. The study design required participants to remain supine in the research facility for 24 hours while blood was drawn every 30 minutes. Data were analyzed using single and multioscillator models allowing for precision in determining the nadir, acrophase and ultradian components. In addition to cortisol, the study analyzed growth hormone and prolactin rhythms but failed to compare the PAD between rhythms. Results found a phase advance in the acrophase of cortisol rhythm of one hour (p = 0.00002). There was no difference in acrophase in prolactin or growth hormone allowing for the possibility of a phase angle difference between cortisol and prolactin and growth hormone. Results were significant despite a diagnostically heterogeneous group that included 69% endogenous depression, 25% psychotic depression, 38% agitated depression and 25% retarded depression. Increased precision with the use of the multioscillator model and controlling for menstrual phase may have revealed differences that the heterogeneity in diagnosis may have masked.

One study on cortisol found a difference between depressed and controls on quiescent period but not phase position. Deuschle and colleagues (1997) studied a sample of 15 depressed male inpatients and 22 healthy controls. Ages ranged from 23 – 85 years (mean = 53.1) in controls and 22 – 72 years (mean = 47.7) in depressed. Blood samples were collected every 30 minutes over a 24-hour period starting at 0800. Participants were required to remain in bed during collection with meals provided at fixed schedules. Lights went off at 2300. Napping was not allowed. Quiescent period was calculated as concentrations lower than 50% of mean in more than two consecutive samples. Depression was associated with decreased length of quiescent period in depressed patients verses controls (2:20 hour +/- 116 minutes and 5:05 hour +/- 184 minutes respectively; $p < 0.005$). The study reports that the cortisol nadir time did not differ between groups. Nadir was determined by time of lowest cortisol level during the night. Cosinor

analysis was not performed on the data. Visual inspection of figure one suggests the possibility of an earlier nadir if data were fitted to even a single cosine function. A shorter quiescent period may imply a difference in either the nadir or the acrophase between depressed participants and controls. This study is limited to males habituated to an institutional routine of fixed rising, retiring and meals timing. The influence of synchronizers such as light and meal timing that would affect circadian rhythms in naturalistic settings could not be measured in this study.

In an earlier study, Halbreich, Asnis, Shindledecker, Zumoff, and Nathan (1985) studied 32 participants with endogenous depression (ED) and 72 controls. Blood samples collected over 24 hours were analyzed using cosinor to determine nadir and acrophase. Fourier analysis was used to identify the ultradian rhythm in cortisol. Results showed a earlier onset of the first secretory event in ED participants compared to controls ($p < 0.05$) but no difference in acrophase or nadir. Fourier analysis showed significantly different ultradian rhythms in ED participants compared to controls suggesting that a multioscillator model for determining nadir might have revealed differences between groups in timing of nadir.

Studies have yielded conflicting results regarding the cortisol phase in affect. Sleep quality has also been studied in relationship to cortisol circadian rhythm, suffering from many of the same methodological and design limitations discussed with affect. A number of studies have isolated the sleep element in relationship to the circadian rhythm of cortisol for investigation. In both healthy populations and populations with disturbed sleep, the cortisol rhythm in relation to sleep has been examined. In healthy subjects, the relationship of sleep and the cortisol rhythm has been investigated by altering the normal sleep cycle and implementing constant routine protocols. Of the studies that employ multiple measures of cortisol across the experimental period, the majority fail to report the rhythm parameters, making interpretation of phase position

difficult. In healthy adults the effects of sleep on cortisol has been investigated using sleep deprivation protocols. None of the studies model the circadian rhythm or reported acrophases. Vgontzas and colleagues (1998) studied cortisol in ten healthy men after a night of total sleep deprivation. Participants were allowed to sleep on the subsequent night while plasma cortisol levels were drawn every 30 minutes for 24 hours on the night before and the night after deprivation. On the 24 hours after sleep deprivation, participants demonstrated significantly shorter sleep latencies, a higher percentage of slow wave sleep and lower total wake time. Nighttime mean plasma cortisol levels and the area under the curve levels were significantly lower compared to the pre-deprivation night, however total 24 hour levels did not differ between pre and post deprivation measures. Circadian parameters of acrophase and bathyphase were not reported. Leproult, Copinschi, Buxton and Van Cauter (1997) investigated partial and total sleep loss and cortisol**.** Plasma cortisol samples were collected from ten healthy men for 32 hours under three sleep protocols, normal sleep (2300 to 0700), partial deprivation (0400 to 0800) and total sleep deprivation. Data was analyzed using Wilcoxon signed-ranks test. The quiescent period was determined but cosinor analysis was not used. All cortisol profiles followed a normal pattern with peaks between 0700 and 0900. Results demonstrated a difference in cortisol following both partial and total sleep deprivation. Cortisol was increased from 1800 to 2300 on the second day when compared to the day before the sleep loss. The increase in cortisol was greater in the total sleep deprivation that the partial sleep deprivation (45% verses 37%). Also, the onset of the quiescent period was delayed in the both total and partial sleep loss. It is possible that the increase in cortisol and the delay in onset of quiescence may be attributable to a phase shift in the cortisol rhythm.

In another study of total sleep deprivation, Moldofsky, Lue, Davidson, and Gorczynski, (1989) studied ten healthy males under 40 hours of sleep deprivation. Plasma cortisol was drawn every 30 minutes to two hours throughout the study. Loss of a night of sleep resulted in reduced sleep latency and an increase in sleep duration on the night following. No differences in plasma cortisol were noted between the period before and the period following sleep deprivation. However, cortisol data were analyzed using mean values for four hour intervals. Such analysis may lack the sensitivity to detect cortisol differences. A cosinor rhythm was not described in this study. The studies of healthy individuals may suggest that short term sleep disturbances have little impact on the circadian cortisol rhythm. However, the lack of investigation into the cortisol rhythm cosinor parameters in these studies prohibits drawing these conclusions with any confidence.

As in the studies investigating cortisol and depression, cortisol and insomnia studies have yielded conflicting results. Only one study reported circadian parameters. Of the five recent studies involving cortisol and insomnia, three found no group differences. Two studies with non-significant findings modeled the cortisol circadian rhythm. One reported AUC results and the second reported only evening cortisol levels. Riemann and colleagues (2002) studied cortisol secretion during the night in a group of ten chronic insomniacs (six women) and ten age and gender matched controls. After two nights of acclimation to the laboratory, serum cortisol was sampled every 30 minutes from 1900 to 0900. Results demonstrated that cortisol did not differ between the insomnia group and healthy controls in area under the curve.

Varkevisser, Van Dongen, Van Amsterdam, and Kerkhof (2007) also did not find a difference in cortisol secretion in a naturalistic study of 39 insomniacs and 20 healthy controls. Well-being, including fatigue, mood and sleepiness, and cortisol were measured over the course

of a day. Insomniacs reported a decrease in well-being, however evening cortisol levels were not elevated in comparison with controls. The cortisol results are in contrast to earlier studies were insomniacs demonstrate elevated evening cortisol. Compensatory mechanisms resulting from the naturalistic setting of the experimental condition may account for the lack of elevated cortisol.

Only one study investigated the cosinor rhythm of cortisol in insomnia, describing the acrophase and amplitude of the rhythm. Varkevisser, Van Dongen, and Kerkhof (2005) conducted a study of 11 individuals diagnosed with chronic insomnia and 13 healthy controls implementing a 24-hour constant-routine protocol. Salivary cortisol was collected every three hours. Cortisol results demonstrated no differences between the insomnia group and controls in absolute values or circadian parameters of amplitude and phase. Lack of sleep under the constant routine protocol and the large interval between samples may contribute to the lack of significant group differences.

In contrast to the studies that have found no differences between controls and individuals with insomnia, two studies report significant group differences. In a study by Shaver, Johnston, Lentz and Landis (2002) sleep quality and stress were investigated in women with insomnia and healthy controls. Urine cortisol was collected over the course of the day. In the insomnia group, morning urine cortisol was elevated compared to controls ($p < 0.05$). Additionally, insomniacs reported higher levels of distress when compared to controls. No rhythm analysis was conducted to explore the influence of circadian rhythm on higher morning cortisol levels, however higher levels may reflect an earlier acrophase in insomniacs compared to controls. Rodenbeck, Huether, Rüther, and Hajak (2002) studied cortisol and sleep in seven male chronic insomniacs and seven age matched healthy men. Participants spent two nights in the laboratory for the procedure. On the second night cortisol samples were collected. Participants

went to bed at their habitual bedtime and were allowed to sleep as long as desired, rising spontaneously in the morning. Plasma cortisol was sampled every hour during the evening and night, beginning four hours before bedtime. Data was analyzed for areas under the curve for four hour intervals. The quiescent period for cortisol was also determined. Results found that cortisol levels were elevated during the evening and night in the insomnia group compared to controls. Higher levels of cortisol in the first four hours were correlated with poorer sleep quality. There was a delay in the onset of the quiescent period ($p < 0.01$) and an advance in the offset ($p < 0.01$) in insomniacs compared with controls. The shortened quiescent period could explain the elevation in cortisol levels and also suggest a circadian rhythm alteration in individuals with insomnia.

Studies examining the cortisol slope. Common to many of the studies employing salivary collection methods is a truncated sampling time frame. Most studies fail to collect cortisol over the night time frame, focusing on the period from awakening to 2200. This truncated sampling threatens accurate circadian description if essential data points are not measured. The truncated sampling may result from theoretical considerations that underlie these studies. A number of investigations on the role of cortisol in health and illness have been premised on stress activation models. Stress and emotions are thought to activate the HPA axis causing increases in cortisol. Elevated cortisol upon awaking are reflected in steeper cortisol slopes over the day time. Blunted HPA activation would result from a stress induced deregulated or exhausted HPA system leading to flattened slopes in ill individuals.

A number of disorders and diseases share a common constellation of symptoms. Individuals with cancer, fibromyalgia, depression, and trauma report similar sickness behaviors of altered mood, altered sleep quality and decreased energy. The circadian rhythm of cortisol has

been an important area of research in all these disorders. Recent studies, conducted since the late 1990's, have focused on cortisol slope to the exclusion of consideration of the cortisol phase position. Between 1997 and 2007, 19 studies have investigated the slope of cortisol in both healthy individuals and individuals experiencing alteration in mood, sleep quality and energy. Lack of analysis of phase position may reflect the theoretical assumptions that underlie the majority of the studies.

A theoretical framework proposing a phase shift model in contrast to a stress model could also explain the flattening of the cortisol slope in illness. The majority of studies that report a flattening of slope use a minimal sampling methodology. Cortisol samples are limited to a sample collection upon awakening and a few subsequent collection times over the course of the day. Few studies collected samples over a whole 24 hours. Cosinor and multioscillator analysis were not employed in the data analysis in any of the studies, although one study used multilevel growth curve analysis (Adam, Hawkley, Kudielka, & Cacioppo, 2006). Linear regression for cortisol on time was used to determine the slope of the cortisol decline throughout the day. Utilizing linear regression analysis assumes that all cortisol collection times fall on the descending arm of the sinusoidal curve that describes the circadian rhythm of cortisol. With collection times spaced every two to four hours, this assumption could easily be incorrect. The timing of first saliva collection in relation to wakening time may also play a critical role in the determined slope. Additionally, slope may be influenced by methodological considerations governing whether participants woke on their normal schedules or were awakened at a specified time. Adam, Hawkley, Kudielka, and Cacioppo (2006) studied older adults for three days at three time points; wake, 30 minutes later and at bed. Using multilevel growth curves, the study found that slope was predicted by the amount of the morning rise in cortisol. High early morning values

were associated with steeper slopes (r = - 0.553, p < 0.001). None of the studies allow for the possibility of a difference in phase position to account for the difference in slope. A sample collection occurring at the nadir of the rhythm would yield a steeper slope then when all samples occur prior to or subsequent to the rhythm's nadir. It is feasible that the reported slope difference between participants experiencing sickness behaviors and controls reflects a difference in the cortisol phase position.

Of the 19 studies, five have studied healthy participants, two have studied women with fibromyalgia, six have studied cancer, one studied trauma and one studied depression. With the exception of the studies by Catley, Kaell, Kirschbaum, and Stone (2000), which used multilevel random effects modeling, and Adam, Hawkley, Kudielka, and Cacioppo, (2006), which used multilevel growth curve modeling, all the studies used linear regression or repeated measures ANOVA to analyze the data. For the linear regressions, cortisol was regressed on time for the data that excluded the samples that reflected the cortisol awakening response (CAR). Four of the studies did not find a difference in slope between the groups. Fifteen studies found a flatter slope in the at least one group.

The majority of cortisol studies involve group differences between ill populations and healthy controls. Of five reported studies examining cortisol slope in only healthy participants, all five studies found significant differences in gender, race economic and personality factors. Outcome measures differed among all the studies. Sample sizes ranged from 80 to 781 indicating ample numbers to detect small differences in slope on each outcome.

Samples taken only at two or three time points on a single day provide the minimum data needed to determine slope. Cohen, Schwartz, Epel, Kirschbaum, Sidney, and Seeman (2006) investigated 781 healthy adults between the ages of 33 and 45 years. Saliva samples were

collected six times on a single day referenced from the time of waking of the participant until bedtime. A linear regression line was fitted to the individual's data. A statistically flatter slope was found in African Americans (r = -.26, $p < 0.0001$) even after controlling for education and income. Both low education and low income was associated with flatter slopes (r = -0.11, $p < 0.05$; r = -0.15, $p < 0.05$ respectively). Unexpectedly, poorer sleep quality was positively associated with slope (r = 0.12, $p < 0.05$) suggesting better sleep quality with flatter slope. It is important to note that sleep quality was measured with a single five point likert type question. In another study, Polk, Cohen, Doyle, Skoner, and Kirschbaum (2005) examined cortisol at 14 times over one 24-hour period in 334 healthy adults (159 men) between the ages of 18 and 54. Participants were monitored in a hotel for two days and samples collected 1830, 2230 and the following morning at 0545, 0615, 0645, and hourly between 0800 and 1600. Sleep was disturbed in order to collect samples. Results found a steeper slope in women compared to men. In men with low PA slope was flatter, however, in women with high PA the slope was flatter. The results are difficult to interpret due to methodological and sample issues. The influence of environmental and sleep disturbances could not be controlled. Sampling irrespective to variability in participants' normal wake times confound the results. In addition, 42% of the sample was comprised of smokers. Smoking has been found to impact cortisol levels.

Averaging samples over a number of days allows for the effects of daily variation contributing to a more robust profile. In a study by Sjögren, Leanderson, and Kristenson (2006), the cortisol slope of 257 adults ranging in age from 30 to 64 was investigated. Cortisol samples were obtained at wake, 30 minutes later and in the evening for three days. Averages of the three days were log transformed and linearly regressed. Flatter rhythms were associated with cynicism,

depression and exhaustion. A positive correlation was found between cortisol slope and self-esteem in women ($p = 0.02$) but not men ($p = 0.76$).

In a study of 80 healthy Chinese adults, Lai and colleagues (2005) examined the relationship between cortisol slope and positive affect finding statically significant relationships. Data from three collection times at 1200, 1700 and 2200 on two consecutive days were analyzed by ANOVA. In participants with higher affect the cortisol slope was steeper as determined by a significantly lower 2200 cortisol value compared to participants with lower positive affect ($t = 3.3$, $p < 0.001$). Negative affect did not demonstrate the same effects on the cortisol slope prompting the authors to conclude that positive resources are more predictive of cortisol activity than negative influences. The authors used the Chinese Affect scale in a sample of Chinese nationals. Validity and reliability were reported as adequate but the generalizability to an American culture of the findings is limited due to cultural differences in definition and measurement of affect between cultures. Approximately half (52%) of the sample were women. Ages of participants ranged from 19 to 55 years. No consideration was given to potentially confounding factors usually considered in circadian rhythm study including wakening time, menstrual cycle/status, and annual season. In addition to cultural, environmental and social factors unique to China limit the generalizability of this study.

A large body of literature has described the differences in cortisol slope between healthy and clinical populations. Results include both no group differences and significant group differences. Of the studies that found no difference in slope, one was conducted with participants diagnosed with fibromyalgia (FM) and one study included both FM and rheumatoid arthritis (RA) patients. The third study involved women diagnosed with metastatic breast cancer. All

studies were conducted in naturalistic settings with participants performing saliva collection at specific times during the day. All collections were performed during daytime hours.

Catley, Kaell, Kirschbaum, and Stone (2000) used multilevel random effects model to analyze salivary cortisol collected from a sample of 21 FM patients, 18 RA patients and 22 healthy controls. In the FM group, 86% were female. In the RA group, 67% were female and in the healthy control group 73% were female. Saliva samples were collected every two and a half hours between 0800 and 2100. Multilevel random effects modeling has the advantage over linear regression in not treating the participants as fixed factors. Both the participants and cortisol samples were treated as random effects. Results showed no difference in slope among the three groups. Demographic differences in the groups may account in part for the lack of significant differences in slope. The three groups differed on age with the RA group older than the other two groups. The study did not control for medication use by participants. Both the FM group and the RA group took medications including antidepressants and pain medications. The study did not report accounting for menstrual status or phase in the female participants.

The second study demonstrating no difference in slope involving FM participants was conducted by McLean and colleagues (2005). Twenty women with FM and 16 healthy controls provided saliva samples upon awakening, one hour later, five hours later and once between 1500 and 1600 for two days. Data were analyzed by repeated measures analysis of variance (ANOVA) and spearman's rank correlation. The results showed no difference in diurnal variation. This study is limited by sample size, with reported heterogeneity in the presence or absence of abuse history. The study did not report controlling for menstrual phase or menstruation status. Repeated measures ANOVA requires equally spaced fixed collection times contributing to a

24% loss of data due to non-adherence. Differences in awakening times could account for lack of statistically significant group differences.

A study of 103 women with metastatic breast cancer was conducted by Turner-Cobb, Sephton, Koopman, Blake-Mortimer, and Spiegel (2000) and examined the relationship between cortisol and social support. Saliva samples were collected at 0800, 1200, 1700 and 2100 for three days. Cortisol slope was determined by linear regression. Results showed no correlation between social support and cortisol slope. This study did not compared women with metastatic breast cancer with a healthy control group. The study did not compare symptom severity and cortisol slope. Differences in wakening time, menstrual cycle, or menstrual status were not controlled.

Fifteen out of 18 studies did report slope differences between illness and healthy groups. All of the studies compared groups where sickness behaviors typified the symptoms of the disorder or compared symptoms of sickness behaviors within one group. Six studies involving participants diagnosed with breast cancer found flatter slopes in cancer patients when compared to controls and flatter slopes in measures of fatigue, affect, anxiety, and survival. Seven studies involved healthy participants measuring hardship, affect, exhaustion, self-esteem, and trauma. One study examined depression in a population of adults with coronary artery disease (CAD). All of the studies were conducted under naturalistic conditions allowing for the entraining effects of the environment to maintain the normal rhythm of the participants. Participants collected salivary samples during the daytime hours. With the exception of one study by Bower, Ganz, Aziz, and Fahey (2005), all data were analyzed by linear regression to determine cortisol slope. Bower, Ganz, Aziz, and Fahey (2005) used multilevel modeling to describe the cortisol slope in a study of 42 breast cancer survivors and 16 controls.

Of the six studies involving breast cancer participants, one study compared the cortisol slope of breast cancer participants with healthy controls (Abercrombie, Kalin, Thurow, Rosenkranz, & Davidson, 2003). Seventeen women with metastatic breast cancer were compared to 31 controls on cortisol slope. Saliva was collected at time of waking, 1200, 1700 and 2100 on three consecutive days. Linear regression was used to determine slope and groups were compared using t-tests. The women with cancer exhibited a flatter slope ($t_{(46)} = -2.19$, $p < 0.05$) compared to controls. In controls but not cancer patients, flatter slope was significantly correlated with lower social support ($r = -0.40$, $p < 0.05$) and trended to a positive correlation with perceived stress ($r = 0.32$, $p = 0.07$).

Sephton, Sapolsky, Kraemer, and Spiegel (2000) studied 104 women with metastatic breast cancer. Saliva was collected at 0800, 1200, 1700 and 2100 for three days. Slope was determined by linear regression. The Cox proportional hazard model was used to regress the cortisol slope on survival time. The results showed that flatter slope predicted shorter survival times (*hazard ratio* = 464.9; $p = .0036$). The study further noted that only 37% of the participants exhibited normal rhythms, with a cortisol peak at 0800. Of the remaining 63%, cortisol levels peaked later in the day for 49% and earlier in 14%. These deviant rhythms exhibited lower morning and higher evening cortisol values than normal rhythms. The authors noted that participants with the flatter slopes exhibited lower morning and higher evening cortisol that was statistically significant suggesting that more participants with flat slopes may have earlier or later phase positions than normal.

The study did not report the relationship of phase position with survival time. The study was strengthened by the investigation of potential confounders. Depression pain and sleep quality were controlled. Only sleep quality demonstrated a significant relationship with slope

being higher in participants with flatter slopes. This relationship did not eliminate the significance of the relationship between slope and survival. The study did not control for menstrual status or cycle and medication use.

Using 91 women with metastatic breast cancer from the same sample as the previous study, the relationship of the cortisol slope and psychological adjustment was examined. The Weinberger Adjustment Inventory Long Form (WAI) was used to measure distress, restraint, and defensiveness. The WAI has been used as a trait measure of negative affect and depression. Participants were classified into one of four groups (non-extreme, self-assured, high anxious, and repressor). The cortisol slope was determined by linear regression and the four levels were compared using the Kruskal-Wallis test. The groups differed statistically on slope ($\chi^2 = 8.03$, $p < 0.05$) Repressors' cortisol slope was significantly flatter than self-assureds'. The pooled repressor-plus-high-anxious group's cortisol slope was significantly flatter than the self-assured group's. Repressors' cortisol slope was significantly flatter than the non-extreme group's slope. The author acknowledge that 40% of the participants demonstrated timing of peak cortisol values that was phase advanced or phase delayed when compared to the expected time for the rhythm peak.

In a second study examining the relationship between cortisol slope and repressor characteristics, 29 women with breast cancer participated in a therapy session (Giese-Davis et al. 2006). Expression of positive and negative affect was recorded by videotape and coded by researchers. The WAI was used to measure distress, restraint and defensiveness. Salivary cortisol samples were collected at 0800, 1200, 1700, and 2100. Women who demonstrated high restraint in expression and those high on negative affect exhibited flatter cortisol slopes ($B = .50$; $p = 0.03$;$B = -.47$; $p = 0.02$, respectively). Expression of positive affect was unrelated to cortisol

slope. Samples were collected at 0800 regardless of wake time. Flatter slopes would be expected in participants with earlier wakening times. This study is also limited by a small sample size.

Disturbances in affect, sleep quality and energy define, in part, depression. A number of studies examine both depression and sleep quality in a specific population. Madjirva, Tashev, Delchev, and Bakalova (1995) investigated plasma cortisol levels in a group of depressed volunteers with and without sleep disturbances. Cortisol was measured at 0800, 1600 and 2200 in 122 depressed individuals. Good sleepers comprised 60 participants (37 women) and bad sleepers comprised 113 participants (85 women). This study found higher cortisol levels at 0800 in bad sleepers compared to good sleepers on the first day of the trial. On day two, participants received oral administration of dexamethasone. Cortisol levels were higher at 2200 in the sleep-disturbed group. When compared with a control group of 65 healthy persons no difference was noted in cortisol levels between controls and depressed without sleep disturbances but in participants with disturbed sleep, cortisol levels were higher. Bower, Ganz, Dickerson, Petersen, Aziz, and Fahey (2005) studied 29 breast cancer survivors, 13 with report of fatigue and 16 non-fatigued. Saliva was collected for two days at wakening, 1200, 1700 and 2200. Slope was determined by linear regression and multilevel modeling was used to compare the groups on demographic and symptom variables. Flatter slopes were found in the fatigued group compared to the non-fatigued group. However, no differences in cortisol slope between groups were found for sleep quality and depressed mood. The authors noted that four of the 29 cortisol slopes demonstrated considerable variation from the expected phase position. In the non-fatigued women two exhibited cortisol peaks around 1200 on one of the sample days. In the fatigued women, two exhibited increased cortisol levels at the last collection time of 2200, suggesting a cortisol peak sometime during the night. This study is limited by a small sample size, which may

explain the lack of significant findings regarding the symptoms of depressed mood and sleep disturbances. Effects of menstrual status and cycle on cortisol rhythm were not considered. Approximately half the women were on the medication tamoxifen. It is unknown how the medication impacts diurnal rhythms.

Backhaus, Junghanns, and Hohagen (2004) studied cortisol and sleep quality in a group of insomniacs and healthy controls. Cortisol was measured in the morning (wakening and 15 minutes later) and evening over a period of one week in a group of insomniacs (n = 14) and healthy controls (n = 15). Results showed lower morning cortisol levels ($p < 0.05$) in the insomnia group compared to controls. The slope, measured from the initial morning sample and the evening sample was flatter for the insomnia group ($p = 0.013$). Morning cortisol was negatively correlated with subjective measures of sleep quality, in which higher scoring on sleep quality indicated poorer sleep. Results suggest that elevated morning cortisol is consistent with better sleep. The possibility of a phase shift in cortisol contributing to lower morning cortisol levels cannot be eliminated.

Another study found a flattening of the cortisol rhythm in a sample of insomniacs. Vgontzas and colleagues (2001) studied cortisol and sleep in 11 individuals with insomnia (five women) and 13 healthy controls. Controls and patients were age and body mass index matched. The participants followed a protocol of 4 consecutive nights in a sleep laboratory with scheduled bedtime between 2200 and 0600. Plasma cortisol was drawn every 30 minutes on the fourth night and modeled using cosinor analysis. Results showed a significant cortisol circadian rhythm in both groups. There were no rhythm differences between groups. Both groups peaked in the early morning and reached a nadir approximately one hour prior to sleep. Mean cortisol values were significantly higher in the insomnia group between the hours of 1200 and 1400 and lower

between 2200 and 0200. In addition the amplitude was decreased in the insomnia group compared to controls. Lack of phase difference between groups may be attributable to the three night of sleep entrainment to a 2200 to 0600 sleep schedule. The study did not determine morningness-eveningness of the participants although it did state that the participants had regular sleep schedules similar to that used in the study.

Sleep quality was studied by Palesh and colleagues (2008) in 99 women with breast cancer in relation to cortisol slope. Salivary cortisol was collected for two days at waking, thirty minutes later, at 1200, 1700 and 2100. Linear regression was used to determine the slope of the cortisol rhythm by regressing all cortisol levels on time from waking. Total number of hours in bed (TIB), sleep latency, sleep efficiency, wake episodes, and wake after sleep onset were measured using wrist actigraphy. Flatter cortisol slopes correlated with depression ($r = 0.21$; $p = 0.05$) and the average length of wake episodes ($r = 0.21$; $p = 0.04$). There were no relationships between cortisol slope and any other sleep measures. Over one third of the sample was using antidepressant medication including serotonin reuptake inhibitors (SSRIs) and tricyclics. Nineteen participants were taking medications for sleep disturbances. The use of medication may explain the lack of significant findings between cortisol slope and many of the sleep measures.

Studies examining the relationships between cortisol and other rhythms.

Studies investigating the temporal relationship between two or more biological rhythms are limited. A number of studies measured more than one biological rhythm but did not compute the phase relationship between the rhythms, rather reported phase positions of each variable individually. Some studies have found between group phase differences in one rhythm but not in another. These studies will be discussed here. A phase shift in one rhythm in the absence of a phase shift in the second rhythm suggests a possible phase angle difference between variables.

Avery and colleagues (1997) found a phase delay in cortisol for participants with SAD when compared to controls. The study by Avery and colleagues (1997) involved 12 female participants with a diagnosis of SAD and nine controls. Participants were required to maintain a routine for one week prior to sample collection in which sleep was permitted only between 2100 and 0600. Blood was collected every hour for 24 hours. A two harmonic model was used to fit the data. The cortisol minimum was delayed approximately two hours (0011 for SAD participants and 1003 for controls; $p < 0.05$) in participants with SAD. Light therapy was shown to phase advance SAD participants. Phase position for the acrophase differed between groups by approximately 30 minutes (0652 verses 0620, $p = 0.05$). Thyroid stimulating hormone (TSH) was also measured in this study. The phase position of TSH was not different between SAD participants and controls. The PAD was not reported but a two-hour phase delay in SAD participants would suggest that the PAD between depressed and control participants would be significantly different. This study is limited by a small sample size. Due to variations in menstrual cycles the authors were unable to control for menstrual cycle. Two SAD participants and one control were in the luteal phase of their cycle. In order to isolate the circadian rhythm from the effects of masking a constant routine was used during sample collection. Under constant environmental conditions and bed rest with no sleep periods, the masking effects of sleep, meals and activity are controlled. The use of constant routine and standardized awake times risks underestimating or overestimating the degree of phase shift secondary to the effects of the normal daily routine on phase placement. This study fitted the data to both a 24-hour rhythm and a 24-hour rhythm with a 12-harmonic. Differences in parameters between the two analysis techniques can be seen in the acrophases. The cosinor analysis reported a pretest acrophase of 0922 +/-1:57 hour. Under the addition of the 12 hour harmonic, the acrophase was reported as 0652 +/-0:52 hours, suggesting

greater accuracy in the 24 hour plus 12 hour harmonic model. The authors noted that two of the subjects could not be fitted to the cosinor model.

Another study in which more than one variable was measured, but the phases not compared to each other, involved cortisol and interleukin-6 (IL-6). Cortisol and plasma IL-6 levels were collected from participants in a study by Alesci and colleagues (2005). The study did not report PAD between cortisol and IL-6, but did report on the phase position of both cortisol and IL-6 in depressed and non depressed participants. Nine participants diagnosed with major depression and nine healthy controls were studied. Participants were matched on age, BMI and gender. Five of the depressed participants were female. The study was conducted in a research facility and all women were in the follicular phase of the menstrual cycle. Participants were allowed to be active during the day but required to be on bed rest between the hours of 2200 and 0700. Blood samples were collected serially between 0800 on day two until 0800 the following day. Data was fitted to both single and multiple cosinor curves. When the depressed group was compared to the control group, a phase shift was noted in IL-6, but not cortisol. In the multicomponent cosinor model, the orthophase was located at 0024 hour for controls and 1132 hour for depressed participants. The bathyphase was located at 1512 hour for controls and 0424 hour for depressed. This represents a phase shift of almost 12 hours. Cortisol did not differ significantly between depressed participants and controls. The orthophase of cortisol was located at 0652 hour for the control group and 0632 hour for the depressed group. The bathyphase was located at 0900 hour for the control group and 0804 hour or the depressed group. It is clear from this data that the group PAD significantly differed in depression. A cross-correlation between IL-6 and cortisol lag times was performed. The mean of the individual values of the correlation coefficients showed a significant lag in healthy participants but not depressed participants.

Healthy controls demonstrated a six hour lag in the orthophase of IL-6 when compared to depressed participants. Individual PADs were not reported in this study.

The study was strengthened by careful matching on gender, age, BMI and menstrual cycle phase as the authors note the significant influence of estrogens on IL-6 secretion. Homogeneity in diagnosis of major depression was increase by the exclusion of co-morbid diagnoses including anxiety, eating disorders, substance abuse and psychotic disorders. Additionally, the ability to detect with precision, the orthophases and bathyphases was improved by the use of multicomponent cosinor analysis. This study was limited by small sample and the use of antidepressant medications, which may have contributed to the lack of difference in cortisol phase position between groups. The study design that prohibited habitual rising and retiring times and the standardization of meal timing and nutrition may contribute to rhythms that were deviant from those under natural environmental conditions.

Studies have been conducted on the timing of endogenous rhythms and sleep quality parameters, such as sleep onset. One early study reported the PAD between the cortisol and the sleep rhythm was conducted by Jarrett, Coble, and Kupfer (1983). The study involved 14 participants diagnosed with primary depression and 14 controls. The controls were matched on age and gender with depressed group. Participants were controlled for menstrual cycle stage but not menstrual status. The PAD between cortisol and sleep onset was described. Participants remained ambulatory within a research facility until habitual bedtime allowing for a somewhat more naturalistic environment compared to constant routine conditions. Blood was drawn every 20 minutes. The nadir was determined by the mean of the lowest three cortisol levels. Sleep onset was determined by a three-night sleep electroencephalogram. Acutely depressed participants demonstrated a smaller PAD between cortisol nadir and sleep onset compared with

controls (188 minutes verses 239 minutes respectively; p = 0.017). The study is limited by sample size and the inclusion of participants taking medication. Five of the 14 depressed participants were taking antidepressant medications.

Circadian Rhythm of Cortisol Summary. Results of studies involving the diurnal rhythm of cortisol in health and illness have proven to be highly heterogeneous. Many studies have found significant group differences while other similarly designed studies failed to demonstrate significant differences. Lack of continuity in group characteristics may account for some of this heterogeneity in results. In studies of depressed groups, the inclusion of co-morbidities and a variety of depression types may confound results. In groups of participants with cancer and immune disorders, differences in symptom manifestation may confound results. Results suggesting a flattening of the cortisol slope have demonstrated to highest amount of consistency among studies. These studies fail to exclude to the possibility of a phase shift in cortisol as an explanation for the flattened slope. More investigation is needed to evaluate the possible influence of phase position in relation to flattened slope. Methodological differences and small sample sizes threaten confidence in results. Furthermore, the lack of investigation into the phase angle differences among rhythms may contribute to the lack of consensus among studies.

Circadian Regulation of Estradiol

No studies have been found that investigated the circadian rhythm of estradiol alone. Two studies compared estradiol and cortisol circadian rhythms. Taleb, Krause, and Goretzlehner (1993) investigated cortisol and estradiol rhythms in women with preterm labor. Results found that in preterm labor, the cortisol rhythm was phase delayed in comparison with controls. The estradiol rhythm exhibited no difference in phase position between preterm labor and term labor.

Again, while the phase angle between the two rhythms was not reported, a delay in one rhythm and not in another points to the possibility of different phase angles between rhythms. The phase shift of cortisol in the absence of a similar phase shift in estradiol suggests a possible misalignment between the rhythms.

A study by Bao and colleagues (2004) compared circadian cortisol and estradiol rhythms in 27 women, 12 with a diagnosis of major depression. Cortisol and estradiol was measured every two hours over a 24-hour period at four times during the menstrual cycle. Measures were taken on the first day of the menstrual cycle, during the late follicular phase, early luteal phase and late luteal phase. As expected, both cortisol and estradiol demonstrated clear diurnal rhythms. The best model to represent the data was found to be a peaked cosine function with one additional harmonic. For cortisol, most participants (85% of controls and 66% of depressed) exhibited an ultradian rhythm of six or eight hours. In 34% of the depressed participants a 12-hour ultradian rhythm best described the rhythm. In estradiol, the most common ultradian harmonic accounting for 56% of the controls and 54% of depressed participants was the fourth (six-hour) harmonic. Estradiol level variations were consistent across the menstrual cycle (all $p > 0.12$), with depressed exhibiting larger amplitudes than controls ($p = 0.046$). For controls, the acrophases of cortisol and estradiol were significantly correlated ($r = 0.729$, $p = 0.003$). The late luteal phase demonstrated the highest correlation. The depressed group demonstrated no correlation in acrophases during the late luteal phase, suggesting that a coupling of cortisol and estradiol is present in healthy participants but not depressed women. A decoupling of the cortisol and estradiol phases suggests a phase misalignment in depressed women but not healthy controls. This lack of correlation was evident despite the fact that a number of the depressed women were taking medications for depression, including Clonazepam, Imipramine, Fluaxatine,

Amitriptyline, and Sertraline. A documented affect of antidepressants is a phase shift in cortisol. The presence of a statistically significant difference in groups despite the potential phase shifting effects of medication may speak to a high degree of misalignment in depression or may suggest that antidepressant medication serves as decoupling agents.

While studies that examine the diurnal rhythm of estradiol are very limited, variations across the menstrual cycle of cortisol and other rhythms have been the subject of investigation. A number of studies have examined the diurnal expression of various hormones and behaviors at different times over the menstrual cycle, recognizing the difference in reproductive hormones at the different times. These studies imply that the changing environmental milieu of reproductive hormones during the various phases of the menstrual cycle influence the actions of circadian rhythms. While suggesting an influence of reproductive hormones on circadian rhythms and perhaps the SCN, these studies fall short in directly examining the potential influence of the circadian nature of the reproductive hormones themselves.

The menstrual regulation of mood has been documented since the early 1970's (May, 1976; Silbergeld, Brast, & Noble, 1971). Premenstrual syndrome and premenstrual dysphoric disorder are characterized by changes in positive and negative affect, sleep quality and energy levels occurring predominantly during the luteal phase of the menstrual cycle (Freeman, 2003; Futterman, & Rapkin, 2006); Halbreich, Borenstein, Pearlstein, & Kahn, 2003; Johnson, 2004; Reed, Levin, & Evans, 2008; Steiner et al., 2006; Wright & Badia, 1999).

Studies across the menstrual cycle have suggested that cortisol secretion varies over the cycle. Studies of mean levels show conflicting results with some reporting lower cortisol levels in the luteal phase (Symonds, Gallagher, Thompson, & Young, 2004) and some reporting elevated cortisol levels during luteal phase (Andreano, Arjomandi, & Cahill, 2008). Other studies

have found no quantitative differences in cortisol but significant differences in timing (Parry, Javeed, Laughlin, Hauger, & Clopton, 2000). In addition, temperature amplitude differences and phase delays in the luteal phase compared to follicular phase have been documented (Nakayama et al., 1997).

The limited number of studies reporting a diurnal rhythm in estradiol prohibits the formation of substantive conclusions. Adequate data does not exist to suggest an influence of the diurnal rhythm of estradiol on health. However, the evidence of health variation across the menstrual cycle suggests that the influence of estradiol requires further investigation. One characteristic of estradiol that remains unexplored is the circadian variation.

Pilot Study

A pilot study was conducted to explore the participant burden, adherence to protocol and accuracy of data collected in a study involving 24 hour salivary sampling. Fitting a nonlinear regression to data requires an adequate number of data points across an adequate range to establish an accurate representation of the rhythm. Salivary sampling requires the specific actions and attention on the part of the participant, potentially disrupting other activities. Salivary sampling demonstrates a number of advantages over plasma sampling. Salivary sampling allows for collection by the participant in a naturalistic setting, is less disruptive to routines and preferred by most participants (Hanrahan, McCarthy, Kleiber, Lutgendorf, & Tsalikian, 2006). Salivary cortisol samples possess an advantage over plasma cortisol samples in that salivary samples represent the active, free fraction of cortisol, avoiding the need to analyze the within-subject and between-subject differences in cortisol-binding globin (Gozansky, Lynn, Laudenslager, & Kohrt, 2005).

In order for mathematical models to be accurate, sampling must capture all significant information represented. Inappropriate timing of collection and failure to collect threaten accuracy. Non-adherence to collection schedules has been addressed in a number of studies. The awaking cortisol response (ACR) is often used to determine the cortisol profile upon awakening. The protocol usually includes sampling at 0, 15, 30 and 45 minutes post awakening. Studies have found that non-adherence to ARC sampling protocol results in a flatter curve (Kudielka, Broderick, & Kirschbaum, 2003; Thorn, Hucklebridge, Evans, & Clow, 2005). In studies relying on estimating non-adherence from self report, rates of non-adherence were estimated to be between 13 and 33% (Thorn, Hucklebridge, Evans, & Clow, 2005). Other studies have investigated collection adherence across the daytime hours. In electronically monitored participants, adherence to a sampling protocol of six collections during a one day period was found to be 74% (Kudielka, Broderick, & Kirschbaum, 2003). In a study of adherence to a protocol of five daily collections over a seven day period electronically monitored adherence was found to be 71% (Broderick, Arnold, Kudielka, & Kirschbaum, 2004). When collection times are not fixed, electronically monitored adherence was found to be 81% which was determined to not affect the diurnal profile of cortisol (Jacobs, Nicolson, & Derom, 2005). The diurnal cortisol profile was determine by hierarchical multiple regression techniques, therefore it remains unclear as to whether non-adherence affects the determination of the cortisol acrophase derived by nonlinear regression analysis. No study has been found to evaluate to issue of adherence on estradiol sampling. Studies have been limited to sampling protocol that encompasses only daytime hours. Night collection schedules impact on adherence has not been studied. The effect of non- adherence on curve fitting is unknown.

To assess burden a burden scale was developed. The researcher-created Participant Burden Scale is a four-item Likert scale with one additional open-ended question designed to assess the subjective experience of participation in this specific study. Item responses ranged from 1 to 4. Total possible scoring ranged from 4 to 16. A final open-ended question invited participants to add any additional thoughts regarding the study procedure.

Sample. A convenience sample of women with normal menstrual cycles was recruited from an urban university. Five women participated. University women represent a relatively homogenous group in comparison to the general population, demonstrating similar role functions, educational levels and socio-cultural status. The sample of women was selected based on responses to the Horne-Ostberg Morningness-Eveningness Questionnaire (MEQ). Women were eligible if they demonstrate eveningness (ET) or morningness (MT). Women who score from 16 to 41 are considered ET and those who score from 59 to 86 are considered MT (Horne & Ostberg, 1977). Inclusion and exclusion criteria adhered to the criteria of the small-scale study.

Procedure. Procedure for the pilot followed the procedure as described above in the small-scale study with the exception of a total collection of twelve samples from each participant. Suggestions for increasing saliva production were also not provided to participants in this pilot.

Analysis. Data was fit to the peaked cosine function with one additional harmonic: $Y=M+ACOS((t-\Phi_1)+vSIN(t-\Phi_1))+BCOS(u*t-\Phi_2)$ model (Bao et al., 2003). Due to the expected burden of waking every two hours during the night, the full set of data was fit to the model, as well as, a truncated set of data that eliminated the night-time collection.

Results. Participant burden was addressed by a researcher-developed burden scale. Total scores ranged from 8 to 11(M=9.4, SD=1.52). The highest burden was reported for sample collection during the night (M = 3.4, SD = 0.56). Lowest burden was reported for keeping the diary (M=1.4, SD = .055). Comments added by participants included, 'Difficulty generating sufficient quantity of saliva' and 'Difficulty abstaining from food/beverages prior to collection'

Three of the five participants collected all required samples within the collection window. Two participants missed samples (40%). A total of three samples were missed (5%). One participant missed one sample collection at the 2000 hour collection time. Another participant missed two collection times, one at 0600 hour and 0800 hour.

The discrepancy in acrophases between full samples and truncated samples ranged from 14 minutes to seven hours and 20 minutes (Table 2.1). The greatest discrepancy appeared in the estradiol regressions. Table 2.2 presents according to morningness-eveningness type, the results of the data obtained from the PSQI, SSQ, POMS and PANAS, the PAD between cortisol and estradiol as determined by the full sample model. The PAD for participant five differed considerably from the other four participants. In addition, participant five reported poorer sleep quality with a score greater than five on the PSQI. Scoring was considerably higher on the fatigue subscale of the POMS, lower for the vigor subscale and lower for subjective sleep quality for participant five.

Participants reported difficulty in waking during the night to collect salivary samples. Waking during the night to collect saliva samples was moderately to very difficult for all participants. Despite the reported difficulty in waking during the night to provide salivary samples, only one overnight sample was missed. Difficulty in generating sufficient saliva to adequately fill the vial was reported by participants. Techniques to increase saliva production,

including rinsing mouth with cold water, visualization and food smells may assist in saliva production.

Modeling of both cortisol and estradiol differed between full samples and truncated samples. In cortisol, acrophases for full samples and truncated samples were closer than in estradiol samples. This may be due to loss of important information for the estradiol samples. While cortisol, on average, acrophases approximately 45 minutes after awakening, estradiol acrophases during the night, at approximately 0400 (Kokkali & Southern, 2006). Failure of the truncated sample to provide similar acrophases to the full sample may be attributed to poor goodness of fit in some data sets. The small number of data points may also contribute to inaccurate regressions. In samples where the model explained greater than 70% of the variance, greater congruence between full sample acrophases and truncated sample acrophases was demonstrated. The small-scale study increased sample collection from 12 to 13 in the 24 hour period. It would possibly be of benefit to extend the collection over another 14 hours (seven collections) to increase the number of data points for analysis. Due to limited resources, extended sampling was not possible in this study but will be considered in the future.

Table 2.1.

Pilot Study: Peaked Cosine Function with One Additional Harmonic
$Y=M+ACOS((t-\Phi_1)+vSIN(t-\Phi_1))+BCOS(u*t-\Phi_2)$.
FS = Full Sample, TS = Truncated Sample with night-time values removed, CA = Cosinor Analysis

Participant	Measure	Harmonic FS	R^2 FS	Acrophase FS	Harmonic TS	R^2 TS	Acrophase TS	Acrophase CA	CA Fit
1	Cortisol	3	0.50	08:15	3	0.48	08:01	09:00	0.33
	Estradiol	4	0.66	16:25	4	0.79	09:05	17:00	0.45
2	Cortisol	3	0.74	07:52	3	0.70	08:01	09:30	0.27
	Estradiol	2	0.50	21:33	2	0.59	00:37	05:25	0.57
3	Cortisol	3	0.85	22:25	3	0.93	01:22	00:30	0.93
	Estradiol	3	0.37	08:33	3	0.19	00:58	02:20	0.72
4	Cortisol	3	0.87	14:38	3	0.89	14:08	14:00	0.10
	Estradiol	2	0.56	04:19	2	0.62	17:34	02:00	0.64
5	Cortisol	3	0.89	11:25	3	0.92	10:51	09:50	0.05
	Estradiol	2	0.84	15:05	2	0.85	14:45	13:50	0.17

Table 2.2
Pilot Study Scores on Subjective Measures.

Participant	MEQ	PSQI	POMS Vigor-Activity	POMS Fatigue-Inertia	POMS Depression-Dejection	POMS total	SSQ	PANAS Positive Affect	PANAS Negative Affect	PAD
1	MT		16	2	3.21	-11.66	4.1	37	13	8.17
2	MT	2	7	1	9.64	32.14	3.0	20	23	10.32
3	MT	4	19	9	0	-2.87	4.9	41	11	10.13
4	ET	5	9	8	11.79	28.67	4.8	29	15	10.31
5	ET	7	2	14	5.36	39.24	1.71	13	14	3.66

MEQ=Morningness-eveningness Questionnaire, PSQI=Pittsburgh Sleep Quality Index, POMS=Profile of Mood Survey, SSQ=Subjective Sleep Quality, PANAS=Positive Affect Negative Affect Scale, PAD=Phase Angle Difference, MT=morning type, ET=evening type

Chapter 3

Research Method

Design

A descriptive, comparative, correlational study design was used to explore the phase relationships among the biological rhythms of cortisol and estradiol and the social rhythm of morning-eveningness. The phase relationships were examined in relationship to altered affect, sleep quality and energy level. A sample of women with normal menstrual cycles was used in this study.

The purpose of this study was to identify phase relationships between the biological rhythms of cortisol and estradiol and describe their association with health in women. This study is the first step in identifying, describing and determining the relationship between contributing endogenous rhythms and affect, sleep quality and energy level. The specific aims of this study are:

Aim 1. To analyze and compare the circadian morning-eveningness rhythm and the circadian and ultradian rhythms of cortisol and estradiol in healthy premenopausal women.

Working Hypothesis 1a. Healthy premenopausal women will exhibit a circadian and ultradian rhythm in both cortisol and estradiol that can be fitted to multiple cosinor curve.

Working Hypothesis 1b. Healthy, premenopausal women will exhibit cortisol and estradiol circadian rhythm parameters of phase, amplitude, and mesor that demonstrate independence from each other between subjects.

Aim 2. To determine the relationship between the circadian morningness-eveningness rhythm, and the circadian and ultradian cortisol and estradiol rhythms in healthy premenopausal women.

Working Hypothesis 2a. The cortisol circadian rhythm parameters of phase, amplitude, and mesor will differ between morning types and evening types. Morning types will exhibit a phase advance relative to evening types.

Working Hypothesis 2b. The estradiol circadian rhythm parameters of phase, amplitude, and mesor will differ between morning types and evening types. Morning types will exhibit a phase advance relative to evening types.

Aim 3. To determine the relationships among morningness-eveningness rhythm, cortisol and estradiol rhythms and affect, sleep quality and energy level in healthy women.

Working Hypothesis 3a. In healthy premenopausal women, the cortisol/estradiol phase angle will correlate nonlinearly with affect. The relationship will fit a quadratic model: $Y=B0 + B1*X +B2*X^2$, where the most positive and least negative affect is expressed at a specific phase angle difference, referred to as the optimal PAD.

Working Hypothesis 3b. In healthy premenopausal women, the cortisol/estradiol phase angle will correlate nonlinearly with sleep quality. The relationship will fit a quadratic model: $Y=B0 + B1*X +B2*X^2$, where the highest quality of sleep is expressed at a specific phase angle difference, referred to as the optimal PAD.

Working Hypothesis 3c. In healthy premenopausal women, the cortisol/estradiol phase angle will correlate nonlinearly with energy level. The relationship will resemble a quadratic equation $Y=B0 + B1*X +B2*X^2$, where the highest level of energy is expressed at a specific phase angle difference, referred to as the optimal PAD.

Working Hypothesis 3d. The specific cortisol/estradiol PAD reflecting the highest level of affect, sleep quality and energy will not differ from each other.

Working Hypothesis 3e. The specific cortisol/estradiol PAD reflecting the highest level of affect, sleep quality and energy will not differ between morning types and evening types.

Human Subjects

Institutional Review Board approval was obtained from Wayne State University prior to study initiation. Individual participants were informed of the study design, procedures, participant responsibilities and compensation. Participants were informed that all information will be kept confidential and no personal identifiers were recorded with the data collected. Participants were assigned a number that was recorded on a master list, which was maintained in a separate locked file cabinet in the research office. The master list will be destroyed at the earliest possible time following completion of the study in compliance with HIC requirements. Participants were made aware that all participation is voluntary and were given the opportunity to stop the study at any time. Written consent was obtained from all participants.

Risks involved for the participants included potential loss of time, interruption of activities including sleep for a 24-hour period, possible frustration and anxiety from data collection techniques. Possible loss of time and interruption of activities resulted from the repeated collection of saliva every two hours around the clock for a total of 24 hours. Measures were taken to minimize the impact of sample collection in the design of the study. Sampling times were flexible to approximately an hour window around the two hour mark. The sample collection method has been reviewed to minimize the time and effort required. The two hour collection protocol reflects the minimum needed to insure accurate data based on the pilot study described in chapter three and conducted to determine the minimum data points needed to provide an accurate description of the participant's circadian rhythm (Butler, unpublished data,

2009). Timing of data collection was determined by the participant in order to minimize the impact of loss of sleep on required activities of the following day.

Frustration and anxiety from data collection methods include difficulty obtaining adequate saliva, awakening several times during the night and discomfort from completing questionnaires that evoke emotions. Various techniques for increasing saliva production that do not interfere with accuracy of data were offered to participants. Techniques include swishing the mouth with water, exposure to food smells, and visualization. Participants were reassured that a missed collection time during the night may be compensated for by slightly shortening the time between the remaining collections. Discomfort from survey completion was expected to be minimal. The researcher was present for most of the survey collection and available to assess the degree of distress experienced by the participant. No participants reported undue distress in completing the surveys.

There were expected individual benefits for the participants. Participants may have felt good about contributing to the understanding of circadian rhythms and the potential contribution to improving health states.

Sample

This small-scale study investigated the feasibility and potential significance to science of conducting a large-scale study. A small-scale study was warranted due to the monetary cost and subject burden of multiple cortisol and estradiol sampling. Much of the research in repeated salivary hormone sampling in humans has been limited by small sample sizes. However, even in small samples statistically significant differences have been documented. In a sample of 12 women with major depression and 15 controls, Bao and colleagues, (2004) were able to detect differences in phase correlation between depressed and nondepressed women.

Twenty-four participants were recruited for this study. One participant was dropped from analysis. Eleven morning-types (M-Types) and 12 evening-types (E-Types) were represented in the sample.

Inclusion criteria. Eligible participants had the following characteristics: premenopausal female between 25 and 35 years of age; regular menstrual cycles between 27-32 days, Caucasian, able to read and speak English, nonsmoker or willing to refrain from smoking during collection, major sleep period occurs during the night and either a morning type or evening type as determined by the MEQ.

Homogeniety of sample is important to detect the influence of the variables under investigation in a small study. Participants were selected to maximize equivalency in hormone milieu, which can vary over a range of normal, depending on phase of menstrual cycle, age and menstrual status. Hormone concentrations in women vary across the lifespan. As women approach menopause, hormone secretions loose the normal ovulatory menstrual cycle. Even within the menstrual cycle there are cyclical changes. The effect of menstrual cycle phase is not completely understood presently (Baker & Driver, 2007). Kudielka, Hellhammer, and Wust (2009) report that in the luteal phase, salivary cortisol stress response is elevated compared to the follicular phase.For reasons of reducing bias through homogenous sampling, the sample will be restricted to 25 to 35 year olds. Women in this age group are often dealing with similar social roles, such as work outside the home and rearing of school-aged or preschool-aged children. Because this is a small scale study, the sample will be limited to Caucasian women. The effects of race and ethnicity on circadian rhythm profiles have yet to be adequately explored. A few studies have suggested that differences between racial and ethnic groups do exist (DeSantis et al., 2007). The sample was limited to English-speaking women in order to control for the effects of

culture and ethnicity on circadian rhythms. Smoking and nicotine intake affects the circadian rhythm of cortisol, both short term and long term use. Kudielka, Hellhammer, and Wust (2009) report that after smoking at least two cigarettes, smokers show significant elevations of salivary cortisol levels and habitual smoking results in blunted salivary cortisol responses. The sample was further limited to women with major nocturnal sleep periods. Normal sleep patterns that occur at night are considered reflective of proper functioning of the circadian system. Abnormally early or late sleep cycles are indicative of circadian rhythm sleep disorders (Sack et al., 2007).

Exclusion criteria. Exclusion criteria included: pregnancy or lactation within the past three months, prescription drug use including oral contraceptives within the last three months, steroid use, illicit drug use, pre-existing diagnosis of any medical or psychiatric disorder, pre-existing diagnosis of an endocrine disorder, pre-existing diagnosis of sleep apnea or periodontal disease, history of oophorectomy, transmeridian travel across three or more time zones in the past month, shift work in the past three months, and unusually high stress events such as divorce, death in the family, loss of job.

Pregnancy is accompanied by significantly elevated levels of cortisol and estradiol. During lactation these levels decrease but remain different from the pre-pregnant state. Lactating women have demonstrated lower than normal cortisol levels (Kudielka, Hellhammer & Wust, 2009). Oral contraceptives suppress endogenous reproductive hormones and, therefore, prevent ovulation so that women taking these preparations do not have normal cycles. Women taking oral contraceptives demonstrate significantly lower salivary cortisol responses and a blunted salivary cortisol stress responses (Kudielka, Hellhammer, & Wust, 2009). Oral contraceptives have been demonstrated to affect sleep composition (Baker & Driver, 2007). Individuals with

endocrine and non-endocrine disorders exhibit abnormal cortisol and estradiol levels unrelated to variables in this study. Abnormal hormone levels are characteristic and defining in many disorders. This study seeks to investigate the cortisol and estradiol rhythms in healthy women.

Transmeridian travel, shift work and disrupting life events result in unusually high levels of stress. The effects of chronic and acute stress on cortisol levels are presently unknown. It has been theorized the stress causes an increased activation of the HPA axis resulting in excessive production of cortisol. Lower cortisol secretion may result from an overactive system that becomes exhausted over time and fails to produce normal levels of cortisol. Kudielka, Hellhammer, and Wust (2009) report that study results are highly heterogeneous with higher, lower and similar cortisol responses in chronic and acute stress.

Recruitment. Participants were recruited from a population of university students in an urban university. Additional participants were recruited from the community. Community dwelling ambulatory women who reside in urban and rural areas of southeast Michigan comprised the additional participants. Participants were recruited primarily through invitation by the researcher at graduate and undergraduate classes. It was emphasized the participation will not in any way affect class grades. Additional recruitment from the community was needed, as adequate sample size was not obtained through University recruitment. Additional recruitment was accomplished through flyers posted in public locations and direct approach by the investigator.

Setting. Data collection took place in the participant's home or ordinary sphere of activity. The researcher met initially with the participant at a location convenient for the participant to explain the study and obtain signed informed consent..

Major Study Variables

The major variables of interest in this study are: salivary free cortisol circadian rhythm, salivary free estradiol circadian rhythm, affect, sleep quality, and energy level.

Significant bio-markers of endocrine function include cortisol and estradiol. Cortisol reflects the functioning of the HPA axis and salivary free cortisol is equivalent to unbound cortisol in the body.

Salivary cortisol. Cortisol demonstrates a circadian and ultradian rhythm. Salivary cortisol was measured using Salimetrics' expanded range, high sensitivity, salivary cortisol enzyme immunoassay kit (catalog number 1-3002/1-3012). This assay was designed to capture the lower levels of cortisol found in saliva when compared to serum. Intra-assay coefficients of variation range from 3.35% to 3.65%. Inter-assay coefficients of variation range from 3.75% to 6.41%. Linearity of dilution tests yield recovery results from 80.1% to 97.9%. Sensitivity has been reported to be <0.003 mg/dL (Salimetrics, 2011).

Salivary estradiol. Salivary free estradiol is the biologically active form of estrogen in women of reproductive age. Estradiol has been shown to demonstrate both circadian and ultradian rhythms. Salivary estradiol was measured using Salimetrics' high sensitivity salivary estradiol enzyme immunoassay kit (catalog number 1-3702/1-3712). The intra-assay precision is determined for high, middle and low samples. Coefficients of variation are 7.0%, 6.3% and 8.1%, respectively. Inter-assay precision has been reported for high and low samples with the coefficients of variation of 6.0% and 8.9%, respectively (Salimetrics, 2011).

Affect. Affect includes the emotional processes experienced by the individual representing their psychological mood disposition. Affect was measured on a bi-dimensional scale that includes positive and negative affect. Positive affect (PA) represents the degree to which an individual pleasurably engages with the environment while negative affect represents

subjective distress (Crawford & Henry, 2004). PA is the degree to which an individual feels alert and excited. Negative affect (NA) is the degree to which an individual feels sad and lethargic. Affect can be closely associated with mood disorders including depression and anxiety. Mood disorders include cognitive and physiological components as well as the emotional component of affect. Depression can be characterized by low positive affect and high negative affect. Positive and negative affect was measured as independent subscales using the Positive and Negative Affect Schedule (PANAS). The PANAS consists of 20 mood-based adjectives (10 to measure positive affect and 10 to measure negative affect) that the participant rates on a five point likert scale. Affect is measured by the participant's subjective experience response to each of the adjectives as being (a) not a bit, (b) a little, (c) moderately, (d) quite a bit, or (e) extremely. The PANAS is scored by summing the responses related to PA and summing the responses related to NA. Adjectives reflective of PA include "active", "attentive", and "excited". NA adjectives include "hostile", "afraid" and "irritable". Higher scores on the positive affect and lower scores on the negative affect subscales are considered indicative of higher levels of affect. The PANAS has been used extensively in clinical and nonclinical populations to assess affect under varying temporal instructions ranging from "today" to "in general" (Folkman & Moskowitz, 2000; Pressman & Cohen, 2005; Steptoe & Wardle, 2005). This study used the "this week" instructions. The tool has demonstrated sound psychometric properties. Initial testing was performed on a sample of undergraduate university students. Internal consistency reliability has been reported as high, with Cronbach alphas of .88 for the PA scale and .85 for the NA scale (Watson, Clark, & Tellegen, 1988). Independence of subscales has been demonstrated in a number of studies. In a sample of 1003 nonclinical adults in the United Kingdom, Crawford and Henry (2004) tested the psychometric properties of the PANAS and found that demographic

variables had only a modest influence on PANAS scores. Crawford and Henry (2004) report reliability for the PA ranges from .86 to .90 and reliability for the NA ranges from .84 to .87. In this study, Chronbach's alpha for the positive affect subscale was .92. Chronbach's alpha for the negative affect scale in this study was .86.

Affect was also measured using the Profile of Moods (POMS) subscales Tension-Anxiety and Depression-Dejection and the POMS total score. The tension-anxiety subscale includes nine items measuring musculoskeletal tension and psychomotor agitation. The depression-dejection subscale comprises 15 items measuring personal inadequacy, hopelessness, sadness, isolation and guilt. The other subscales include Anger-hostility, Vigor-Activity, Fatigue-Inertia and Confusion-Bewilderment. Anger comprises 12 items measuring annoyance, bitterness, resentment and ill-temper. The Confusion subscale comprises seven items that assess cognitive efficiency. Fatigue-Inertia and Vigor-activity will be discussed under energy. A global estimate of mood is given by the summation of the six subscales where the Vigor-Activity subscale is weighted negatively. Internal consistency for all subscales has been reported at .90 and above. Test-retest reliability ranges from .65 for Vigor to .74 for depression (McNair, Lorr & Droppleman, 1981). In this study, Cronbach's alpha for the POMS was as follows: POMS total scale alpha was .81, Depression-Dejection subscale alpha was .90, Tension-Anxiety subscale was .79.

Sleep quality. Sleep quality refers to the subjective experience of being rested after sleeping. Sleep quality reflects the restorative function of sleep and is affected by social and biological rhythms. The Pittsburgh Sleep Quality Index (PSQI) provides a global measure of sleep quality and takes less than five minutes to complete. The PSQI consists of 19 self-rated items that generates seven component scores as well as a global score. The PSQI measures sleep quality on

a four point Likert scale ranging from 0-3. Scores range from 0 to 3 for each of the seven components. The global score ranges from 0 to 21. The seven components of the PSQI are: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication, and daytime sleep dysfunction. An overall score of five or greater indicates poor sleep. Validity and reliability was established in a sample of good and poor sleepers. Internal consistency was .83. Test-retest reliability was .85 for the global score. Validity was established by statistically significant differences in the good sleepers and the poor sleepers. (Buysee, Reynolds, Monk, & Berman, 1989).The internal consistency reliability of the scale has been reported in the literature to be .83; however, in this sample the Cronbach's alpha was computed to be inadequate at .40.

Day to day variation in sleep quality was measured using the mean score on the Subjective Sleep Quality scale (SSQ). This scale is a 14-item 6 point response Likert type scale with two subscales: quality of sleep (5 items) and effect of sleep (9 items). The scale responses range from 1 (strongly agree) to 6 (strongly disagree). Lower scores indicate poorer subjective sleep experiences. In a sample of healthy adults, the SSQ demonstrated an internal consistency of .88 and an estimated validity using a visual analog scale and the PSQI ranging from .89 to .95 and .86 to .92, respectively (Smith and Davis, 2002). This study used the SSQ total score as a measure of the sleep quality experienced the night prior to cortisol and estradiol sampling. Internal consistency reliability for the SSQ for this study sample was .91

Energy. Energy reflects the level of vigor and vitality felt and subjectively reported by the individual. Energy is the ability to do work efficiently and has been studied in the literature as alertness or lack of sleepiness. Fatigue is a complimentary concept to energy defined as persistent mental or physical tiredness or exhaustion (Dittner, Wessely, & Brown, 2004). Vigor

represents sufficient energy to complete the necessary activities of living as defined by the individual. A number of scales have conceptually linked energy and mood. O'Conner (2004) defines the “mood of energy” and “feelings of having the capacity to complete mental or physical activities” (p. 435). For the purpose of this study, energy represents a state of physical and mental potential and actualized ability to do work. Mood, on the other hand, is conceptualized as positive and negative affect. Two Visual Analog Scales (VAS), one for energy and one for fatigue, were used to measure energy. Visual analog scales are desirable measures because of the simplicity in administration and demonstrated reliability and validity(Ahearn, 1997). Visual analog scales allow for a high degree of sensitivity to change (O'Connor, 2006). The VAS-Energy is a single item measure anchored at one end by “I feel very fatigued” and “I feel very refreshed” at the other end. The VAS-Fatigue is a single item measure anchored at one end by “I feel inactive” and “full of energy” on the other end.

The POMS is the most widely accepted and employed measure of energy levels (O'Connor, 2006). POMS is a 65-item Likert type scale written at a seventh grade reading level. Participants rate themselves on the 65 adjectives along a five-point scale ranging from “not at all” to “extremely”. The POMS scale has been shown to be sensitive to changes in subjective states over days to weeks as well as sensitive to trait characteristics (McNair, Lorr, & Droppleman, 1992). The POMS factors into six subscales. Two subscales, Vigor-Activity and Fatigue-Inertia were used in this study to measure energy. Reliability has been reported by the tool developers as .87 to .89 for vigor and .93 to .94 for fatigue (McNair et al., 1992). O'Connor (2004) report reliability scores from a number of studies ranging from .87 to .92 for vigor and .90 to .94 for fatigue. The vigor and fatigue subscales have shown high correlation with other measures of fatigue and vigor (O'Connor, 2004). The vigor subscale comprises eight items

describing the positive affect of energy. The fatigue subscale comprises seven items describing low energy. The psychometric properties of the POMs were studied in a sample of 400 healthy adults, including 70 university students by Nyenhuis, Yamamoto, Luchetta, Terrien, and Parmentier (1999). Validity was determined by comparison with the Visual Analog Mood Scale, Beck depression Inventory, and the State-Trait Anxiety Inventory. Correlations ranged from .70 to .79. The cut-point between clinical and nonclinical scores of +1.5 standard deviations above the standard mean was determined in this sample to be 21.2 for tension, 30.5 for depression, 21.5 for anger, 25.1 for vigor, 20.4 for fatigue and 18.5 for confusion in female university students. These cut points are similar to the study by McNair, Lorr, and Droppleman which reported cut point scores of 25, 31.9, 20.4, 25.5, 20.9 and 20.3, respectively (1981). The POMs was chosen for this study, inter alia, because of its ability to measure both energy and mood, providing validation for both the VAS-E and the PANAS. In this study, the Cronbach's alpha for Fatigue-Inertia subscale was .84 and Vigor-Activity subscale was .82.

Psychometric testing was performed on all tools used in this study in order to evaluate the reliability and validity in the sample of normally menstruating women and reported in Tale 3.1.

Table 3.1
Cronbach's Alpha for Instruments Used with Study Sample (n=23)

Instrument	Item number	Possible Range	Cronbach's Alpha
POMS			
Total Scale Score	65	-25 - +124	0.810
Depression-Dejection Subscale Score	15	0 - 60	0.895
Tension-Anxiety Subscale	9	0 - 36	0.786
Fatigue-Inertia Subscale Score	7	0 - 28	0.840
Vigor-Activity Subscale Score	8	0 - 32	0.818
PANAS			
Positive Affect Subscale Score	10	10 - 50	0.921
Negative Affect Subscale Score	10	10 - 50	0.857
SSQ Scale Score	19	1 - 6	0.905
PSQI Scale Score	7	0 - 21	0.393

Screening variables. Morningness-eveningness expresses the social rhythms of activity and rest and reflects the integration of endogenous and external regulators. Individual circadian phase states have been measured by the Morningness-eveningness Questionnaire (MEQ; Horne, & Ostberg, 1971). The MEQ is a 19-item scale that measures the preferential likelihood of an individual being active at a given time of day. The scale consists of multiple choice questions whose sum ranges from 16 to 86. Lower scores reflect a preference for eveningness. Smith, Reilly, and Midkiff (1989) compared the validity and reliability of three morningness-eveningness scales finding the MEQ to have an overall coefficient alpha of 0.82. Most correlations between the three scales were significant. External criteria, such as rising and retiring times, alertness, and well-being, correlated strongly with the MEQ. The MEQ was further validated against oral temperature measurements, where peak activity and preferred times of the day correlated with elevated body temperature (Smith, Reilly & Midkiff, 1989).

Demographic variables. Demographic variables included height, weight, age, employment, level of education, marital status, health complaints, tobacco use, alcohol use, and caffeine use.

Extraneous study variables. Alcohol and caffeine use was treated as potential confounding variables not as exclusion criteria. Alcohol consumption in excess of 20 grams per day has been associated with higher levels of mean cortisol; however urinary cortisol was uncorrelated with amount of alcohol consumption (Thayer, Hall, Sollers & Fischer, 2006). Little is known regarding the effects of alcohol on circadian rhythms, with most research focusing on the acute stress response (Adinoff, Iranmanesh, Veldhuis, & Fisher, 1998; Bernardy, King, Parsons, & Lovello, 1996; Croissant, & Olbrich, 2004; Errico, King, Lovello, & Parsons, 2002; Wand & Dobs, 1991). Lovallo, Dickensheets, Myers, Thomas, and Nixon (2000) reported that

the diurnal patterns of cortisol did not differ between alcohol dependent participants and controls. In addition, female study participants do not exhibit the same cortisol responses as men (Croissant & Olbrich, 2004). Alcohol produces effects on menstrual cycle regularity with the severity of disturbances increasing with level of alcohol consumption. (Emanuele & Emanuele, 1997).

Studies suggest that caffeine, equivalent to two to three cups of coffee increases cortisol production. (Sung, Lovallo, Pincomb, & Wilson, 1990). Caffeine induced cortisol increases have been noted to last as long as three hours after ingestion (Lovallo, Al'absi, Blick, Whitsett, & Wilson, 1996). Few studies have included women. Lovallo, Farag, Vincent, Thomas, and Wilson (2006) found that the cortisol response to caffeine differs in women, suggesting a different mechanism in men and women. Among the pregnant women, salivary cortisol levels were significantly reduced after coffee intake (Tsubouchi, Shimoya, Hayashi, Toda, Morimoto, & Murata, 2006).

Data Collection Procedure

Potential participants were approached by the principal investigator individually and in class settings at Wayne State University. It was stressed that participation is voluntary and will have no effect on any class grade. All interested students were given the MEQ to complete. Upon completion, questionnaires were scored. Results of the MEQ were explained to all students and the principal investigator (PI) provided a contact number and e-mail. It was explained that women who scored over 70 or under 39 were potentially eligible for inclusion in the study and are encouraged to contact the investigator in person, by phone or e-mail. Participants interested in the study were provided with informed consent. After determining eligibility for participation in the study, the principal investigator (PI) determined with the participant the expected day for

the onset of menses. The investigator contacted the participant at that time. Based on the start of the menstrual cycle, an appropriate day for sampling, between day 25 and 28 of the menstrual cycle, was determined by the participant according to her work and social activity schedule. The investigator scheduled a meeting with the participant for the day prior to the sample collection day. At that time, each participant completed the demographic questionnaire and the PANAS, PSQI, and SSQ. The participant received instruction in keeping a diary and salivary sampling protocol. The diary consisted of columns with the following headings: awake time, first collection time, any food eaten 60 minutes prior, second collection time, and any food eaten 60 minutes prior. The column heading repeated for a total of thirteen collection times. In addition, the diary asked the participant to record time of sleep, caffeine intake, and alcohol intake for each collection time. Sampling protocol for cortisol and estradiol was as follows: At every collection, the participant will refrain from brushing or flossing the teeth until after the second collection of the day. Participant will not eat within the hour before collection. Immediately prior to collection the participant will rinse her mouth with cool water. After a five minute wait, the participant will expectorate through the straw provided into the sampling container provided. The participant must avoid directly touching the interior of the container or the saliva. The participant must fill the vial to the indicated 3 mL line.

After verbalizing understanding and performing a correct demonstration of the sampling procedure, the participant received the collection containers and a timer for the collection day as determined by the first day of menstruation. The morning of the day before collection, the participant completed the SSQ. On the collection day, the participant was instructed to perform the sampling protocol at time of awakening, 30 minutes later and then every two hours around the clock for the remainder of the day, for a total of 13 samples. The timer was available to be

used to help the participant to remember to take the sample on time. At night, an alarm clock was available to awaken the participant. Collection materials were kept at the bedside during the night and the participant was instructed to collect the sample in darkness while remaining in bed. Samples were stored in a cold pack at the bedside until morning and then stored in a household freezer until retrieval by the investigator on the following day. In anticipation of sample collection, the PI notified the participant by phone or email the day before to remind her to complete the SSQ and start collecting saliva on the following morning. Participants recorded collection times and any deviations from protocol in a diary. The PI arranged for collection of samples and questionnaires at the participant's convenience during the following day. The participant received $75.00 for her participation.

Data was kept in accordance with HIC guidelines in a locked file cabinet in a secured office at Wayne State University. Data was screened and compiled after each data collection period for each participant to insure that useable data was collected. Salivary collections were stored in a freezer at less than or equal to four degrees Celsius. The refrigerator was located within a locked room dedicated to research activity. A coding dictionary was developed for data entry. Demographic data, data from questionnaires and scales and salivary hormone data were coded and entered in a designated computer. Data was backed up as appropriate to prevent loss, with access to data by specified personnel approved by the Human Investigation Committee.

Data Preparation

All statistical analyses were done using the Statistical Package for the Social Sciences (SPSS) version 19.0 for PC and GraphPad Prism 5.0. Error checking routines were created as part of the database analysis application. Statistical analysis began with preparatory activities such as the treatment of missing data, identification of outliers and other such data cleaning

tasks. The distributions of all obtained measures were plotted graphically for visual inspection regarding deviation from normality, and appropriate quantitative tests such as Kolmogorov-Smirnov Goodness-of-Fit Test or The Shapiro-Wilk Test for Normality were used to evaluate these possible deviations. Visual examination of scatter plots, histograms, and other graphical summaries were employed to identify associations of interest to the aims of the project and to provide quick, but accurate, information about the data. Before proceeding with any statistical test, the data were assessed for the assumptions underlying the test.

Missing Data

A number of participants declined to answer a total of 12 questions on the subjective measures for affect, sleep quality and energy level. No reason for the missing data was given. For the affect measure of the PANAS, no questions were unanswered. The POMS scale, which measures affect and energy level, had 10 items without response for the full sample. Two participants declined to answer one item each. One participant had two missing data points, one had three missing data points and one participant declined to answer four items. Sleep quality measures resulted in one missing item on the PSQI for each of two participants. No participant declined to address any item on the SSQ. The VAS-E and the VAS-F were completed at every time point with the exception of two participants who missed one time point each. Missing data on the subjective measures were replaced by the statistical mean of the sample. Two participants had 12 of the 13 saliva collections. All other participants collected all 13 samples. Cortisol and estradiol data were curve modeled with the available data.

Salivary Cortisol and Estradiol

Salivary sampling employed a passive drool technique in which approximately 1.8 mL of saliva was collected by drooling down a straw into a collection vial according to manufacture recommended protocol. Samples were frozen to 0 degrees Fahrenheit until analysis.

Assays were run in duplicate on 310 salivary samples using Salimetrics' High Sensitivity Salivary Cortisol Enzyme Immunoassay Kits (Salimetrics, 2011) and Salimetrics' High Sensitivity Salivary Estradiol Enzyme Immunoassay Kits (Salimetrics, 2011). Salivary cortisol and estradiol levels were determined by calculating the mean of the duplicate assay results. The quantitative measurement of cortisol and estradiol was determined by using an enzyme-linked immunoabsorbent technique (ELISA) according to manufacturer's instructions (Salimetrics, 2011). The intra-assay precision for the cortisol assays was reported as 0.999 (*SD* = 0.033) μg/dL for high values and 0.097 (*SD* = 0.004) μg/dL for low values. The coefficients of variation were 3.35 and 3.65, respectively. The lower limit of sensitivity for cortisol was 0.003 μg/dL. The cortisol inter-assay precision was determined to be 1.020 (*SD* = 0.038) μg/dL for high values and 0.101 (*SD* = 0.006) μg/dL. Coefficient of variation is 3.75 and 6.41, respectively (Salimetrics, 2011). The intra-assay precision for the estradiol assay kits were reported as 20.26 (*SD* = 1.42) pg/ml for high values, 7.24 (*SD* = 0.45) pg/ml for mid-range values and 3.81 (*SD* = 0.31) pg/ml for low values. Coefficients of variation were 7.0% for high, 6.3% for mid and 8.1% for low values. Inter-assay precision was 24.62 (*SD* = 1.47) pg/ml for high values and 4.76 (*SD* = 0.42) pg/ml for low values. Coefficients of variation were 6.0% for high values and 8.9% for low values. The lower limit of sensitivity for estradiol is 1.0 pg/ml (Salimetrics, 2011).

Data Analysis

Descriptive statistics, including mean, mode, standard deviation, range and skewness were used to describe the characteristics of the sample and study variables. Statistical Package for the Social Sciences (SPSS 19.0, 2011) was used to compute the descriptive statistics.

Variables were examined to meet the assumptions of linear and nonlinear regression and correlation analysis. The assumptions of Pearson Product Moment Correlation include normal distribution, homoscedasticity and linearity. Linear correlation analysis was used to address bivariate correlations between subjective measures and chronotype. The goal of nonlinear regression is to fit the data to a model expressed as an equation that defines Y as a function of X and one or more parameters by minimizing the sum of squares. Nonlinear regression uses an iterative approach to fitting the data. Assumptions of nonlinear regressions are; normal distribution and accurate model selection. Nonlinear regression was performed using GraphPad Prism 5.0 for MacOS (2011, GraphPad Software, San Diego CA, www.graphpad.com). GraphPad Prism employs the Marquardt method of performing nonlinear regression. Automatic outlier elimination was performed using a Q value of 1%. Specific data analysis for each aim is as follows:

Aim 1 was to analyze and compare the circadian morning-eveningness rhythm and the diurnal and ultradian rhythms of cortisol and estradiol of normally menstruating women.

Working Hypothesis 1a. Normally menstruating women will exhibit a circadian and ultradian rhythm in both cortisol and estradiol that can be fitted to multiple cosinor curve.

Working Hypothesis 1b. The cortisol and estradiol circadian rhythm parameters of phase, amplitude, and mesor will demonstrate independence from each other between subjects.

Aim 1 was tested by visual examination of the raw data and curve fitting using GraphPad Prism 5.0d constrained nonlinear regression analysis. The model selected was multiple cosinor curve:

$Y=M + ACOS((t-\Phi_1) + vSIN(t-\Phi_1)) + BCOS(u*t-\Phi_2)$ where M represents the curve mean, A is the amplitude, v is a measure of peakedness and u represents the ultradian harmonic. Curves of two, three and four harmonics were compared using Akaike's Informative Criterion (AIC) and the curve harmonic with the best fit was chosen. Independence of the variables of amplitude, mesor and phase was determined using descriptive and Pearson product-moment correlation statistics.

Aim 2 was to determine the relationship between the circadian morningness-eveningness rhythm, and the diurnal and ultradian cortisol and estradiol rhythms in normally menstruating women.

Working Hypothesis 2a. The cortisol circadian rhythm parameters of amplitude, and mesor will differ between morning types and evening types.

Working Hypothesis 2b. The estradiol circadian rhythm parameters of phase, amplitude and mesor will differ between morning types and evening types. Morning types will exhibit a phase advance relative to evening types.

Aim 2 was tested by determining the acrophase as the point on the curve of the highest amplitude. Differences in phase, amplitude and mesor between the M-type group and the E-type group were determined using Independent Student T-test for normally distributed data and Mann-Whitney U for data that did not meet the assumptions of normality. Significance level was set at a p-value equal to or less than 0.05.

Aim 3 was to determine the relationships among morningness-eveningness rhythm, cortisol and estradiol rhythms and affect, sleep quality and energy level in normally menstruating.

Working Hypothesis 3a. In normally menstruating women, the cortisol-estradiol phase angle will correlate nonlinearly with affect. The relationship will resemble a quadratic model where the most positive and least negative affect is expressed at a specific phase angle difference.

Working Hypothesis 3b. In normally menstruating women, the cortisol-estradiol phase angle will correlate nonlinearly with sleep quality. The relationship will resemble a quadratic model, where the highest quality of sleep is expressed at a specific phase angle difference.

Working Hypothesis 3c. In normally menstruating women, the cortisol-estradiol phase angle will correlate nonlinearly with energy level. The relationship will resemble a quadratic model, where the highest level of energy is expressed at a specific phase angle difference.

Working Hypothesis 3d. The specific cortisol-estradiol phase angle difference reflecting the highest level of affect, sleep quality and energy will not differ from each other.

Working Hypothesis 3e. The specific cortisol-estradiol phase angle difference reflecting the highest level of affect, sleep quality and energy will not differ between morning types and evening types.

In order to address Aim 3, the PAD between cortisol and estradiol was determined by subtracting the estradiol acrophase from the cortisol acrophase. For values greater than 12 hours 24 was subtracted from the value and for values less than -12 hours 24 was added to the value to account for the circular nature of clock time. The cortisol-estradiol PAD was regressed against each of the health indicators of affect, sleep quality and energy level using the quadratic equation $Y = B0 + B1*X + B2*X^2$. The quadratic model was compared to a straight line using the Extra Sum of Squares Fit Test. A p-value of 0.05 or less was used to determine significance. For models that fit a quadratic equation significantly better than a straight line, the cortisol-estradiol

PADs were examined for equivalency in the entire sample and the subgroups of M-types and E-types.

Chapter 4: Results of Data Analysis

Introduction

The purpose of this study was to describe the circadian rhythms of cortisol and estradiol in relation to health and compare the circadian variation by chronotype. This study endeavored to describe the relationship between the cortisol-estradiol phase angle difference and health. Health was conceptualized as high levels of positive affect, low levels of negative affect, high sleep quality and high energy levels. Measures used for affect included the Profile of Moods questionnaire (POMS), and the Positive and Negative Affect Scale (PANAS). Sleep quality was measured using the Pittsburgh Sleep Quality Index (PSQI) and the Subjective Sleepiness Questionnaire (SSQ). Energy was measured by two subscales on the POMS and two visual analog scales. Phase angle differences were determined from modeling individual cortisol and estradiol circadian rhythms and calculating acrophases. The results are presented in the following sections: a) sample demographics, and b) major findings related to the study aims.

Sample Demographics

Participants were recruited from the College of Nursing at a public urban university in the Midwest and from the greater metropolitan community. Initially, 24 participants were recruited for the study, 12 M-types and 12 E-types. One participant was subsequently removed from the analysis due to the inability to dertermine an estradiol acrophase. The final sample consisted of 21 (91.3%) participants from the College of Nursing and two (8.7%) from the community. Sample characteristics are presented in Table 4.1. Eleven participants were M-types and 12 participants were E-types. The mean age of the sample was 28.7 (*SD* = 5.8) years with an inclusive range of 21 to 39 years. The mean BMI was 24.7 (*SD* = 4.5) Kg/m^2 with a range from 18.0 to 41.6 Kg/m^2. All participants were college students with 5 (21.7%) having a high school

diploma, 16 (69.6%) having a bachelors' degree, and two (8.7%) having masters degrees. Of the participants, six (26.1%) were married, 16 (69.6%) were single and one (4.3%) was divorced. Fifteen (65.2%) had no children, 3 (13.0%) had a child between one and five and 4 (17.4%) had a child over the age of five. Participants reported an average daily caffeine intake of 1.3 (*SD* = 1.1) cups per day and an average weekly alcohol intake of 2.9 (*SD* = 2.8) glasses per week. Caffeine intake ranged from zero to four cups per day and alcohol ranged from zero to 10 glasses per week.

In the M-type group, the mean age was 30.7 (*SD* = 5.4) years and the mean BMI was 23.0 (SD = 1.8) Kg/m^2. Three (27.3%) of the M-type participants were married, seven (63.6%) were single and one (9.1%) participant was divorced. Seven (63.6%) had no children, two (18.2%) had a child between one and five years of age and two (18.2%) had children between five and 12 years old. All M-type participants had a college education; two (18.2%) were working toward the Bachelor's degree, eight (72.7%) held a Bachelor's degree and one (9.1%) held a Master's degree. Daily mean caffeine intake was 1.3 (*SD* = 1.3) cups per day. Weekly mean alcohol intake for the M-type participants was 1.8 (*SD* = 1.2) glasses per week.

In the E-type women, the mean age of the participants was 25.8 (*SD* = 4.1) years. Mean BMI was 26.0 (SD =5.9) Kg/m^2. Three (25%) E-type participants were married and nine (75%) were single. Eight (22.7%) had no children, one (9.1%) participant had a child between one and five years of age and two (18.2%) had a child between the ages of five to 12. As with the M-type women, all E-type women had at least a high school degree and were working toward a college degree. Three (25%) E-type women were in the Bachelor's program, eight (66.7%) were Master's students and one (8.3%) held a Graduate degree. The E-type group consumed on

average 1.3 (*SD* = 1.2) cups of caffeinated beverages daily and 3.3 (*SD* = 2.5) glasses of alcohol weekly.

Table 4.1
Sample Characteristics

	Total Sample (n=23)			Morning-type (n=11)		Evening-type (n=12)	
Characteristic	Mean *(SD)*	Inclusive Range	Number (%)	Mean *(SD)*	Number (%)	Mean *(SD)*	Number (%)
MEQ	-	-	-	65.7(5.9)	-	34.8(5.8)	-
Age	28.7(5.8)	21-39	-	30.7(5.4)*	-	25.8(4.1)*	-
BMI	24.7(4.5)	18.0-41.6	-	23.0(1.8)	-	26.0(5.9)	-
Marital Status							
Married	-	-	6(26.1)	-	3(27.3)	-	3(25)
Single	-	-	16(69.6)	-	7(63.6)	-	9(75)
Divorced/ Separated	-	-	1(4.3)	-	1(9.1)	-	-
Number of Children (n=22)							
None	-	-	15(65.2)	-	7(63.6)	-	8(72.7)
1 to 5 years	-	-	3(13.0)	-	2(18.2)	-	1(9.1)
5 to 12 years	-	-	4(17.4)	-	2(18.2)	-	2(18.2)
Education							

High School Graduate	-	-	5(21.7)	-	2(18.2)	-	3(25)
Bachelor's Degree	-	-	16(69.6)	-	8(72.7)	-	8(66.7)
Graduate Degree	-	-	2(8.7)	-	1(9.1)	-	1(8.3)
Daily Caffeine Intake	1.3(1.1)	0-4	-	1.3(1.3)	-	1.3(1.2)	-
Weekly Alcohol Intake	2.9(2.8)	0-10	-	1.8(1.2)	-	3.3(2.5)	-

*Significant group difference at $p = 0.014$

Descriptive Statistics for major Study Variables

Descriptive results for the major study variables of affect, sleep quality and energy level are presented in Table 4.2 for the entire sample and in Table 4.3 by chronotype. Affect was measured by the POMS total scale and two subscales; Depression-Dejection and Tension-Anxiety, and by the two PANAS subscales; Positive Affect and Negative Affect. The mean score for the POMS total was 15.4 (*SD* = 24.1). Scores ranged from -24 to +86.8. For the POMS Depression-Dejection subscale, the mean score was 5.2 (*SD* = 6.9) and for the Tension-Anxiety subscale the mean score was 6.3 (*SD* = 4.2). The ranges for the subscales were 25 points and 16 points, respectively. For M-types, the POMS total averaged 9.12 (*SD* = 19.4) and for E-types the POMS total averaged 21.2 (SD =27.2). There was no significant differences between groups $t_{(21)}$= -1.22, p = 0.24). On the subscales, the means did not differ significantly between M-types and E-types. For the Depression-Dejection subscale, the mean score for M-types was 2.9 (*SD* = 3.1) and for E-types 7.3 (*SD* = 8.8). An Independent Students' T-test was -1.64 (p = 0.12). On the second affect subscale, Tension-Anxiety, M-types had a mean score of 5.9 (*SD* = 4.0) while E-types averaged 6.7 (*SD* = 4.5). Comparing the groups resulted in a T-test score of -0.44 (p = 0.67). Table 4.4 reports the results of the independent student T-test between chronotypes.

The mean scores on the two PANAS subscales measuring positive and negative affect were 33.6 (*SD* = 8.3) and 17.3 (*SD* = 5.7), respectively. Scores on the PA subscale ranged from 13 to 46 and scores on the NA subscale ranged from 10 to 30. For M-types, the PA mean was 36.5 (*SD* = 5.5) compared to E-type mean of 31.0 (*SD* = 9.8). The difference between chronotype was not statistically significant ($t_{(21)}$ = 1.62, p = 0.12). NA also did not differ between groups ($t_{(21)}$= -1.06, p = 0.30) with mean scores for M-types and E-types of 16.1 (*SD* = 4.4) and 18.6 (*SD* = 6.6) respectively.

Sleep quality was measured using the PSQI and the SSQ. The sample as a whole yielded a mean score of 3.5 (*SD* = 1.7) on the PSQI and 4.5 (*SD* = .97) on the SSQ. Scores ranged from 1 to 8 on the PSQI (higher score means lower sleep quality) and 1.7-5.9 on the SSQ (higher score means higher sleep quality). M-types and E-types did not differ from each other on either the PSQI ($t_{(21)}$= -1.44, p = 0.16) nor the SSQ ($t_{(21)}$= 0.91, p = 0.37). Mean PSQI scores for M-types was 3.0 (*SD* = 1.5) and for E-types 4.0 (*SD* = 1.8). Mean SSQ scores for M-types was 4.7 (*SD* = 0.85) and for E-types 4.3 (*SD* = 1.1).

Energy level was measured by the two POMS subscales Fatigue-Inertia and Vigor-Activity as well as two visual analog scales for energy and fatigue. The Fatigue-Inertia subscale mean score was 7.3 (*SD* = 5.4) and the mean subscale score for Vigor-Activity was 14.6 (*SD* = 5.5). The range for the Fatigue-Inertia Subscale and the Vigor-Activity Subscale were 23 and 22 points respectively. The mean scores for M-types were 6.7 (*SD* = 6.4) for the Fatigue-Inertia Subscale and 15.2 (*SD* = 6.1) for the Vigor-Activity Subscale. For E-types the means scores on the energy subscales were 7.9 (*SD* = 4.7) and 14.1 (*SD* = 5.2), respectively. Neither sub-score differed significantly between M-types and E-types, with the Student T-test for Fatigue-Inertia at -0.51 (p = 0.62) and Vigor-Activity at 0.44 (p = 0.66). Scores on the visual analog scales for energy were averaged over the 24-hour sampling for each of the energy and fatigue VAS. An average score for each participant was obtained. The sample mean score was 72.7 (*SD* = 14.8) for the energy VAS (VAS-E) with a range from 45.3 to 113.3. The overall sample mean for the fatigue VAS (VAS-F) was 73.86 (*SD* = 16.6) with a range from 45.3 to 110.5. Scores were not significantly different between chronotypes. The mean score for M-types was 69.7 (*SD* = 12.1) on the energy VAS and 69.7 (*SD* = 13.2) on the fatigue VAS. For E-types, the mean of the energy VAS was 75.7 (*SD* = 16.9) and the mean score of the fatigue VAS was 77.7 (*SD* = 19.0).

Table 4.2
Means and Inclusive Ranges for Major Study Variables (n = 23)

Variable	N	Mean(SD)	Inclusive Range
POMS			
Total Scale Score	23	15.4(24.1)	-24 - +86.8
Depression-Dejection Subscale Score	23	5.2(6.9)	0-25
Tension-Anxiety Subscale Score	23	6.3(4.2)	0-16
Fatigue-Inertia Subscale Score	23	7.3(5.4)	0-23
Vigor-Activity Subscale Score	23	14.6 (5.5)	3-25
PANAS			
Positive Affect Subscale Score	23	33.6(8.3)	13-46
Negative Affect Subscale Score	23	17.3(5.7)	10-30
SSQ Scale Score	23	4.5(.97)	1.71-5.93
PSQI Scale Score	23	3.5(1.7)	1-8
Energy VAS-Energy Scale Score	23	72.7(14.8)	45.3-113.3
Fatigue VAS-Fatigue Scale Score	23	73.86(16.6)	45.3-110.5
Cortisol Acrophase (hours)	23	9.7(3.2)	2.06-15.01
Estradiol Acrophase (hours)	23	7.9(6.4)	.19-22.19
Cortisol-Estradiol PAD (hours)	23	2.7(5.0)	-7.9-11.92

Table 4.3
Means and Inclusive Ranges for Major Study Variables by Chronotype

	Morning-type (N = 11)		Evening-type (n =12)	
Variable	Mean(SD)	Inclusive Range	Mean(SD)	Inclusive Range
POMS				
Total Scale Score	9.12(19.4)	-24-50	21.2(27.2)	-14-86.8
Depression-Dejection Subscale Score	2.9(3.1)	0-9	7.3(8.8)	0-25
Tension-Anxiety Subscale Score	5.9(4.0)	0-11	6.7(4.5)	1-16
Fatigue-Inertia Subscale Score	6.7(6.4)	0-23	7.9(4.7)	2-18
Vigor-Activity Subscale Score	15.2(6.1)	4-25	14.1(5.2)	3-22
PANAS				
Positive Affect Subscale Score	36.5(5.5)	25-44	31.0(9.8)	13-46
Negative Affect Subscale Score	16.1(4.4)	10-24	18.6(6.6)	12-30
SSQ Scale Score	4.7(0.85)	3.29-5.93	4.3(1.1)	1.71-5.29

PSQI Scale Score	3.0(1.5)	1-5	4.0(1.8)	1-8
VAS-Energy Scale Score	69.3(12.1)	45.3-84.20	75.7(16.9)	55.1-113.3
VAS-Fatigue Scale Score	69.7(13.2)	45.3-82	77.7(19.0)	52.6-110.5
Cortisol Acrophase (hours)	8.32(3.4)*	2.06-13.60	11.0(2.5)*	7.22-15.01
Estradiol Acrophase (hours)	6.24(4.7)	1.83-21.54	9.46(7.5)	.19-22.2
Cortisol-Estradiol PAD (hours)	1.5(5.1)	-3.89-6.61	3.6(6.7)	-7.90-11.92

*Difference in means between Morning-type and Evening-type $t_{(21)} = 2.16$, $p = 0.042$

Table 4.4
T-test for Significance in Health Measures Between M-types and E-types

	M-type	E-type		
Instrument	Mean(SD)	Mean(SD)	T-Test	P-Value
POMS				
Total Scale Score	9.12(19.4)	21.2(27.2)	-1.22	.24
Depression-Dejection Subscale Score	2.9(3.1)	7.3(8.8)	-1.64	.12
Tension-Anxiety Subscale Score	5.9(4.0)	6.7(4.5)	-.44	.67
Fatigue-Inertia Subscale Score	6.7(6.4)	7.9(4.7)	-.51	.62
Vigor-Activity Subscale Score	15.2(6.1)	14.1(5.2)	.442	.66
PANAS				
Positive Affect Subscale Score	36.5(5.5)	31.0(9.8)	1.62	.12
Negative Affect Subscale Score	16.1(4.4)	18.6(6.6)	-1.06	.30
SSQ Scale Score	4.7(0.85)	4.3(1.1)	.91	.37
PQSI Scale Score	3.0(1.5)	4.0(1.8)	.-1.44	.16

Reliability of the Cortisol and Estradiol Measures

Cortisol was measured using the Salimetrics High Sensitivity Cortisol Enzyme Immunoassay. The Salimetrics High Sensitivity Salivary Cortisol Enzyme Immunoassay has a sensitivity to detect 0.003 μg/dL, with serum correlation of 0.9. The intra-assay coefficients of variation for this study were 6.7 for cortisol and the inter-assay coefficients of variation were 11.9. Estradiol was measured using the Salimetrics High Sensitivity 17β-Estradiol Enzyme Immunoassay. The Salimetrics High Sensitivity Salivary 17β-Estradiol Enzyme Immunoassay has a sensitivity of 0.01 pg/mL, with serum correlation of 0.80. The intra-assay coefficients of variation for this study were 9.3 and the inter-assay coefficients of variation were 13.3. For the estradiol samples, the pH indicator in the assay diluent indicated a possible saliva pH outside of acceptable parameters. A random pH test was performed on a random 15% of the saliva samples. None of the pH values were below the acceptable value of 5. Six (19.3%) random samples were slightly higher than the acceptable upper limit of nine with values ranging from 9.03 to 9.64. Elevated pH may artificially lower the estradiol values.

Findings Related to Study Aims and Hypotheses

The purpose of this study was to explore the phase relationships between the circadian rhythms of cortisol and estradiol and the health indicators of affect, sleep quality and energy level in healthy M-type and E-type women. To address this purpose, three specific aims were developed and tested. Results are discussed in the following section.

Specific aim 1. The first specific aim of the study was to describe and compare the circadian and ultradian rhythms of cortisol and estradiol in healthy women. It was hypothesized that healthy women would exhibit a circadian rhythm in both cortisol and estradiol fitting a cosinor curve with ultradian harmonics. To address this hypothesis, levels of cortisol were

measured in μg/dL by calculating the mean of duplicate assays. Estradiol levels were measured in pg/L also by calculating the mean of duplicate assays. The cortisol and estradiol data of each participant were separately fitted to the nonlinear curve model; Y = M + A*cos(X-phaseshift) + B*cos(C*(X-d), where M is the mean of the circadian rhythm and A is the circadian rhythm amplitude. B is the amplitude of the ultradian rhythm and C is the harmonic, where the second harmonic is equal to eight hours, the third harmonic is six hours and the fourth harmonic is four hours. Finally, d is the phase position of the ultradian component.

The fit of the curve to the data points for each participant was assessed. Measuring goodness of fit in nonlinear regression differs from a linear regression. The R^2 value in a nonlinear regression determines if the curve fits the data better than a horizontal line going through the mean of all the Y values and does not necessarily constitute the main parameter in the determination of fit. Other considerations important to determining how well the curve represents the true curve include visual inspection, normality of residuals, replication tests and run tests (Motulsky & Christopoulus, 2004). Visual inspection assesses how close the points are to the curve. Normality of residuals, replication and run tests measure the extent to which the data points exhibit a normal distribution around the curve.

In this study, replication tests were not performed due to the lack of multiple values for each point. The goodness of fit results from the regression values, normality of residuals, and run tests are reported in Table 4.5. The individual cortisol and estradiol data points, overall, demonstrated good curve fit. Visual inspection of the data points yielded points close to the curve in all participants, except the estradiol curve for one participant. The estradiol data points for participant number 2 were noticeably further from the curve than in the other participants. The run test and normality of residuals were not significant for participant number 2 and no

outliers were identified. The normality of residuals test was significant for a number of cortisol curves. The curves for participants 1, 4, 6, 22, and 23 demonstrated D'Agostino normality of residual p-values less than 0.05. The estradiol curves demonstrated significance in the normality of residuals in one participant, number 4. The run tests were not significant for any of these curves. Visual inspection of the curves where normality of residuals had significant p-values confirmed the probable accuracy of the estimated acrophases. Only one run test was significant and that was in the estradiol curve for participant number 4.

Table 4.5
Goodness of Fit for Cortisol and Estradiol Curve (N=23)

Cortisol Curve					Estradiol Curve			
Participant	R2	Normality of residuals	P-Value	Run Test P-value	R2	Normality of Residuals	P-Value	Run Test P-Value
1	0.47	18.14	0.000*	0.296	0.46	0.042	0.980	0.966
2	0.55	.794	0.672	0.296	0.40	1.333	0.513	0.999
3	0.95	1.067	0.587	0.976	0.72	0.105	0.949	0.881
4	0.32	17.75	0.000*	0.576	0.68	0.340	0.843	0.043*
5	0.46	2.973	0.226	0.911	0.51	1.359	0.507	0.733
6	0.52	7.302	0.026*	0.576	0.55	0.349	0.839	0.966
7	0.88	1.612	0.447	0.533	0.56	1.987	0.371	0.954
9	0.77	7.756	0.021	0.606	0.74	0.889	0.641	0.966
10	0.63	0.229	0.892	0.347	0.54	6.045	0.049*	0.929
11	0.73	1.940	0.379	0.879	0.57	5.175	0.075	0.347
12	0.56	1.182	0.554	0.999	0.66	1.687	0.430	0.966
13	0.75	3.372	0.185	0.879	0.45	3.575	0.137	0.879
14	0.78	1.431	0.489	0.347	0.35	2.246	0.325	0.966
15	0.54	4.246	0.120	0.878	0.67	1.577	0.454	0.793
16	0.73	0.601	0.740	0.576	0.49	1.444	0.486	0.347
17	0.55	1.182	0.554	0.879	0.38	0.858	0.651	0.966
18	0.62	0.015	0.993	0.500	0.50	0.480	0.787	0.733

19	0.75	1.438	0.487	0.500	0.37	1.287	0.526	0.966
20	0.57	3.876	0.144	0.879	0.49	3.822	0.148	0.999
21	0.90	0.999	0.607	0.879	0.52	0.356	0.837	0.500
22	0.60	11.150	0.004*	0.652	0.88	5.200	0.074	0.47
23	0.69	11.850	0.003*	0.348	0.29	0.258	0.879	0.793
24	0.83	2.186	0.335	0.296	0.39	0.480	0.043*	0.296

*One-tailed significance level $p<0.05$

The second hypothesis for specific aim number one posited that the cortisol and estradiol circadian rhythm parameters of phase, amplitude and mesor would demonstrate independence from each other. The mean values for the sample are reported in Table 4.6. The cortisol acrophase mean for the sample was 9.7 (*SD* = 3.2) μg/dL. The cortisol mesor for the full sample was 0.177 (*SD* = 0.106) μg/dL and the amplitude mean was 0.161 (*SD* = 0.134) μg/dL. Estradiol mean values for acrophase, mesor and amplitude in the full sample were 7.9 (*SD* = 6.4 pg/L), 6.73 (*SD* = 3.76) pg/L and 2.92 (*SD* = 2.41) pg/L, respectively.

Test of Normality using Kolmogorov-Smirnov with Lillifors significance correction was significant at the 0.05 probability level for all cortisol and estradiol parameters. Therefore, independence of cortisol and estradiol parameters in the full sample and the subgroups M-types and E-types was determined by Spearman's Rho bivariate correlation analysis. Results can be found in Table 4.7. None of the correlations were significant at a significance level of 0.05. The *r*-value for full sample acrophases was 0.95 ($p = 0.67$). In the full sample the *r* for amplitude and mesor were -0.10 ($p = 0.64$) and -0.31 ($p = 0.15$), respectively. Similarly, M-types showed no correlations between acrophase ($r = 0.25$, $p = 0.47$), mesor ($r = -0.22$, $p = 0.52$), and amplitude ($r = -0.53$, $p = 0.09$) of cortisol and estradiol. Finally, there were no significant cortisol and estradiol correlations in the E-type subgroup on the parameters of acrophase ($r = -0.23$, $p = 0.48$), mesor ($r = -0.43$, $p = 0.16$), and amplitude ($r = 0.28$, $p = 0.38$).

Table 4.6
Curve Fit for Cortisol and Estradiol (N =23)

	Full Sample (N=23)		M-types (N=11)		E-types (N=12)	
	M(SD)	No.(%)	*M(SD)*	No.(%)	*M(SD)*	No.(%)
Cortisol						
Curve Fit						
R^2	.66(.158)	-	.64(.139)	-	.68(.177)	-
SS	.657(1.87)	-	.785(2.31)	-	.539(1.45)	-
Sy.x	.171(.236)	-	.176(.272)	-	.166(.210)	-
Harmonic						
2	-	13(56.5)	-	5(45.5)	-	8(66.7)
3	-	5(21.7)	-	3(27.3)	-	2(16.7)
4	-	5(21.7)	-	3(27.3)	-	2(16.7)
Acrophase	9.7(3.2)	-	8.32(3.4)	-	11.0(2.5)	-
Mesor	.177(.106)	-	0.18(0.11)	-	0.18(0.10)	-
Amplitude	.161(.134)	-	0.14(0.13)	-	0.17(0.14)	-
Estradiol						
Curve Fit						
R^2	.53(.14)	-	.47(.111)	-	.586(.151)	-
SS	178.5(262.6)	-	228.2(297.2)	-	132.9(230.0)	-
Sy.x	3.84(3.028)	-	4.27(3.36)	-	3.43(2.77)	-
Harmonic						

2	-	11(47.8)	-	4(36.4)	-	7(58.3)
3	-	10(43.5)	-	6(54.4)	-	4(33.3)
4	-	2(8.7)	-	1(9.1)	-	1(8.3)
Acrophase	7.9(6.4)	-	6.24(4.7)	-	9.46(7.5)	-
Mesor	6.73(3.76)	-	7.29(4.2)	-	6.21(3.4)	-
Amplitude	2.92(2.41)	-	3.00(2.3)	-	2.85(2.6)	-

Model: Y=M + A*cos(X-PhaseShift)+B*cos(C*(X-d))

Table 4.7
Cortisol and Estradiol Curve Parameters Correlations in Full Sample and by Chronotype (N = 23)

	Full Sample (N=23)		M-type (N=12)		E-types (N=11)	
	R	P-Value	*R*	P-Value	*R*	P-Value
Acrophase	0.95	0.67	0.25	0.47	-0.23	0.48
Amplitude	-0.10	0.64	-0.53	0.09	0.28	0.38
Mesor	-0.31	0.15	-0.22	0.52	-0.43	0.16

Spearman's Rho Correlations at 2-tailed significance level of 0.05

Specific aim 2. The second specific aim of this study was to determine the relationship between the circadian morningness-eveningness rhythm and the circadian rhythms of cortisol and estradiol. It was hypothesized that the circadian rhythm parameters of phase, mesor and amplitude would differ between M-types and E-types and that the phase would be advanced in M-types compared to E-types. Both the cortisol and estradiol parameters are suggested to differ between chronotypes. To test this hypothesis, Independent Sample Students' T-tests were used for the cortisol and estradiol acrophases. For nonparamentric data, Wilcoxon Mann-Whitney U two sample rank sum tests were conducted for amplitude and mesor. Based on the Kolmogorov-Smirnov tests for normality, parametric assumptions were violated in all cortisol and estradiol parameter data except cortisol and estradiol acrophases. Nonparametric tests were conducted between M-types and E-types on the parameters of mesor and amplitude in cortisol and estradiol. M-type group and E-type group are reported in Table 4.8. The cortisol acrophase mean for the subgroups of M-types and E-types were 8.32 (3.4) μg/dL and 11.0 (2.5) μg/dL, respectively. An Independent Sample Students' T-test indicated statistically significant difference in cortisol acrophase between groups ($t_{(21)}$ = 2.16, p = 0.042). The acrophase in M-types was phase advanced by 2.68 hours when compared to E-types. In addition, no group differences were found in the cortisol parameters of mesor and amplitude (see Table 4.8). The cortisol mesor for M-types was 0.18 (*SD* = .11) μg/dL and the cortisol mesor was 0.18 (*SD* = 0.10) μg/dL for E-types. The mean amplitude of cortisol curves was 0.14 (*SD* = 0.13) μg/dL for m-types and 0.17 (*SD* = 0.14) μg/dL for E-types.

The M-type estradiol acrophase was 6.24 (*SD* = 4.7) pg/L and the E-type estradiol acrophase was 9.46 (*SD* = 7.5) pg/L. For the estradiol mesor, m-types averaged 7.29 (*SD* =4.2) pg/L while the E-types averaged 6.21 (*SD* = 3.4) pg/L. The estradiol amplitude was 3.00 (*SD* =

2.28) pg/L for M-types and 2.85 (*SD* = 2.6) pg/L for E-types. In the M-type and E-type subgroups none of the estradiol parameters differed between groups at significance levels of 0.05. Analysis resulted in no significant difference between M-types and E-types in acrophase ($t_{(21)}$ = 1.23, p = 0.233), mesor (p = 0.479) or amplitude (p = 0.758).

Table 4.8
Significance test for Cortisol and Estradiol Curve Parameters between M-types and E-types

	M-types	E-types		
Variable	Mean(SD)	Mean(SD)	T-test (n_1 =11, n_2 = 12)	p-value
Cortisol				
Acrophase	8.32(3.4)	11.0(2.5)	2.16	0.042*
Mesor	0.18(0.11)	0.18(0.10)	-	.806[1]
Amplitude	0.14(0.13)	0.17(0.14)	-	.356[1]
Estradiol				
Acrophase	6.24(4.7)	9.46(7.5)	1.23	.233
Mesor	7.29(4.2)	6.21(3.4)	-	.479[1]
Amplitude	3.00(2.3)	2.85(2.6)	-	.758[1]

* Independent T-test significance at p=0.042; 2-Tailed Significance Testing
[1]Wilcoxon–Mann–Whitney two-sample rank-sum test

Specific aim 3. The third aim of this study was to determine the relationships among chronotype, cortisol and estradiol rhythms and the health indicators of affect, sleep quality and energy level. It was hypothesized that relationships exist between the cortisol-estradiol PAD and the health indicators that can be fitted to a nonlinear equation (2^{nd} degree polynomial model) Y=B0 + B1*X +B2*X^2. To test this hypothesis the cortisol-estradiol PAD was determined by subtracting the estradiol acrophase from the cortisol acrophase. Positive values up to 12 indicated that cortisol was phase delayed compared to estradiol. Negative values between -12 and zero indicated a phase advance in cortisol compared to estradiol. Because clock time is circular, positive values greater than 12 indicate a phase advance in cortisol relative to estradiol and were recalculated by subtracting 24 from the computed value. Similarly, values less than -12 indicate a phase delay in cortisol relative to estradiol and were recalculated by adding 24 to the computed value. The mean cortisol-estradiol PAD of the full sample and by chronotype is reported in Table 4.2 and Table 4.3, respectively. The cortisol-estradiol PADs and the health measures were then modeled to the equation Y=B0 + B1*X +B2*X^2. Goodness of fit was determined using the R^2 values, D'Agnostino's normality of residuals, run tests and visual inspection of the data points.

Affect. Affect was measured using PANAS and POMS scales. Two subscales of the POMS that address affect, Depression-Dejection subscale and Tension-Anxiety, were used. The curves generated from the cortisol-estradiol PAD and affect measures demonstrated data points that visually appear close to the curve in all scales (see Figure 4.1). Goodness of fit results can be seen in Table 4.9. For the full sample, correlations of the cortisol-estradiol PAD with the affect scales ranged from 0.28 for Positive Affect to 0.36 for the Depression-Dejection subscales. All the affect scales fit the quadratic model better than a straight line at a significance level of 0.05. One subscale violated the normality of residuals assumption. Significance was found for

D’Agnostino’s normality of residuals test for Positive Affect (K^2 = 7.3, p = 0.02). All run tests were nonsignificant in the affect measures.

Table 4.9
Goodness of Fit for Cortisol-Estradiol PAD and Health Indicator Measures for Full Sample (N=23)

	Goodness of Fit			Straight Line	
	R^2(DF)	Normality of Residuals (*p*)	Run Test *p*	F(DFn, DFd)	*p*
Affect					
POMS Total Score	.34(20) [1]	1.4(0.50)	0.97	5.8(1,20)	0.02 [2]
POMS Depression-Dejection Score	.36(20)	0.81 (0.66)	0.96	6.5(1,20)	0.02 [2]
POMS Tension-Anxiety Score	.30(20) [1]	5.9 (0.05)	0.90	4.4(1,20)	0.048 [2]
Positive Affect Score	.28(20) [1]	7.3(0.02) [2]	0.54	4.5(1,20)	0.047 [2]
Negative Affect Score	.30(20) [1]	1.6(0.44)	0.51	6.21(1,20)	0.02 [2]
Sleep Quality					
PSQI Score	.04(20)	1.58(0.45)	0.83	0.015(1,20)	0.90
SSQ Score	.17(20)	7.8(0.02) [2]	0.92	1.6(1,20)	0.19
Energy					
POMS Vigor-Activity Score	.13(20)	0.73(0.70)	0.70	2.8(1,20)	0.11
POMS Fatigue-Inertia Score	.06(20)	9.56(0.01)	0.98	1.3(1,20)	0.27

VAS-Energy Score	.02(20)	5.5(0.06)	0.81	0.10(1,20)	0.76
VAS-Fatigue Score	.03(20)	1.36(0.51)	0.51	0.16(1,20)	0.76

Model: Y=B0 + B1*X +B2*X^2. POMS = Profile of Moods; PSQI = Pittsburgh Sleep Quality Index; SSQ = Subjective Sleep Questionnaire; VAS = Visual Analog Scale.

[1]Correlation greater that 0.25;

[2]Significant at $p<0.05$

[3]Trend to significance at p between 0.05 and 1.00

In the M-type subgroup goodness of fit are considerably lower than for the full sample and for E-types (see Table 4.10). Examples of the M-type curve fit can be found in Figures 4.2, 4.3 and 4.4. None of the affect measures significantly fit a quadratic model better than a straight line (See table 4.10). D'Agnostino's normality of residuals were significant at the 0.05 level for POMS Tension-Anxiety ($K^2 = 6.9$, $p = 0.03$) and Positive Affect ($K^2 = 6.2$, $p = 0.046$).

In contrast, the data for the E-type group demonstrated greater goodness of fit than either the M-type or the full sample in the measures of affect. Goodness of fit results are reported in Table 4.11. Correlations for the affect scales ranged from 0.29 for Positive Affect to 0.70 for Depression-Dejection. Data visually appeared to follow the modeled curve well. None of the data violated normality of residuals or run tests. Examples of the E-type curve fit can be found in Figures 4.2, 4.3 and 4.4.

Sleep quality. Two scales measured sleep quality in this study; the PSQI and the SSQ. Upon visual inspection of the full sample, the data points for both measures did not lie close to the modeled curve. The R^2 for the PSQI and SSQ were low at 0.04 and 0.17 respectively (see Table 4.9). In addition, the curve did not statistically model the data points better than a straight line for either the PSQI ($F = 0.015$, $p = 0.90$) or the SSQ ($F = 1.6$, $p = 0.19$). Figure 4.4 illustrates the sleep quality measures for the full sample curve fit.

The PSQI and SSQ both performed in a similar manner for the chronotype subgroups. The PSQI demonstrated an R^2 of 0.18 in the M-type group and 0.03 in the E-type group. The SSQ demonstrated an R^2 of 0.23 for the M-types and 0.13 for the E-types. None of the curves for the subgroups demonstrated better fits to the data points than a straight line. Table 4.10 summarizes the goodness of Fit for M-types. Goodness of fit for the E-types can be seen in table 4.11. Figure 4.4 illustrates the curve fit for M-type and E-type.

Energy. Four measures were used to assess energy levels in the participants. Two scales were visual analog scales, one for energy and one for fatigue, in which the average of 13 time points was averaged for each participant to yield an average for the day of sample collection. Tests for normality showed a normal distribution. Scores were compared using a paired T-test. Scores on energy did not differ from fatigue ($t_{(21)}$ =1.215, p = 0.237). Scores on energy were highly correlated to fatigue scores at 0.96. Results were similar for each of the subgroups, M-types and E-types (See Table 4.13). The third and fourth energy measures are subscales of the POMS: Fatigue-Inertia and Vigor-Activity. In the full sample, none of the measures exhibited good fit to the quadratic curve. For all four measures, visual inspection of the data suggests a poor fit with the model consistent with low R^2 results (see Figure 4.3). The VAS scales fit the model with R^2 statistics of 0.01 and 0.03. Neither data set fit the model better than a straight line (see Table 4.9). Data from the Fatigue-Inertia measure also failed to fit the model curve better than straight line and demonstrated a low R^2 of 0.06. The data from the Vigor-Activity scale almost trended toward a significant fit to the model over a straight line fit (p = 0.11). R^2 goodness of fit was 0.13 for the Vigor-Activity scale. D'Angostino's normality of residuals were non significant in all measures except Fatigue-Inertia (K^2= 9.56, p = 0.01).

For the M-type subgroup, the four measures of energy gave different results as the full sample. Visual fit of the data points to the curve was poor, with the exception of the POMS Vigor-Activity score, with R^2 values ranging from 0.09 to 0.41 (see Table 4.10). None of the measures fit the curve better than a straight line (see figure 4.11), except POMS Vigor-Activity. Lowest curve values for the Vigor-Activity corresponded to a PAD of 0.67 hours. The lowest points for the VAS curves could not be determined. E-types demonstrated similar results to the full sample, with R^2 values ranging from 0.00 to 0.13 and none fitting the curve better than a

straight line. Results for the E-type group can be found on Table 4.1. No scales in the chronotype subgroups violated normality assumption.

Overall, D'Agnostino normality of residuals were significant at p-values less than 0.05 in three curve from the entire sample. The normality of residuals was significant for Positive Affect (K^2 = 7.3, p = 0.02), SSQ ($K^{2\,=}$ 7.8, p = 0.02) and POMS Fatigue-Inertia subscale (K^2 = 9.56, p = 0.01). Of the cortisol-estradiol PAD curves in the M-type women, only two were significant for normality of residuals. The POMS Tension-Anxieity D-Agostino test was significant ($K^{2\,=}$ 6.9, p = 0.03), as was the Positive Afect ($K^{2\,=}$ 6.2, p = 0.046) in M-types. No normality of residuals test in the E-type group demonstrated significance. Run tests were all nonsignificant for the cortisol-estradiol PAD and health indicator measures curve in the entire sample, the M-types and the E-types.

Table 4.10
Goodness of Fit for Cortisol-Estradiol PAD and Health Indicator Measures for M-types (n=23)

	Goodness of Fit			Straight Line	
	R^2(DF)	Normality of Residuals (*p*)	Run Test *p*	F(DFn, DFd)	*p*
Affect					
POMS Total Score	.30(8)	0.40(0.82)	0.83	2.9(1,8)	0.13
POMS Depression-Dejection Score	.20(8)	1.30(0.52)	0.83	0.15(1,8)	0.71
POMS Tension-Anxiety Score	.12(8)	6.9(0.03)	0.74	0.22(1,8)	0.65
Positive Affect Score	.07(8)	6.2(0.046)	0.52	0.82(1,8)	0.39
Negative Affect Score	.15(8)	1.8(0.41)	0.91	1.4(1,8)	0.27
Sleep Quality					
PSQI Score	.18(8)	1.13(0.57)	0.91	1.6(1,8)	0.24
SSQ Score	.23(8)	3,2(0.21)	0.99	1.5(1,8)	0.25
Energy					
POMS Vigor-Activity Score	.41(8)	1.02(0.60)	0.74	5.4(1,8)	0.049[2]

POMS Fatigue-Inertia Score	.33(8)	0.92(0.63)	0.91	1.2(1,8)	0.20
VAS-Energy Score	.09(8)	0.96(0.62)	0.52	0.64(1,8)	0.45
VAS-Fatigue Score	.21(8)	2.1(0.35)	0.33	0.72(1,8)	0.42

Model: Y=B0 + B1*X +B2*X^2. POMS = Profile of Moods; PSQI = Pittsburgh Sleep Quality Index; SSQ = Subjective Sleep Questionnaire; VAS = Visual Analog Scale.
[1]Correlation greater that 0.25
[2]Significant at $p<0.05$
[3]Trend toward significance at $p<0.1$, >/=0.05

Table 4.11
Goodness of Fit for Cortisol-Estradiol PAD and Health Indicator Measures for E-types(n=23)

	Goodness of Fit			Straight Line	
	R^2(DF)	Normality of Residuals (*p*)	Run Test *p*	F(DFn, DFd)	*p*
Affect					
POMS Total Score	.52(9) [1]	2.727(0.256)	0.825	3.972(1,9)	0.077[3]
POMS Depression-Dejection Score	.70(9) [1]	4.031(0.135)	0.854	9.640(1,9)	0.013[2]
POMS Tension-Anxiety Score	.47(9) [1]	1.727(0.422)	0.608	4.729(1,9)	0.058[3]
Positive Affect Score	.29(9) [1]	1.519(0.468)	0.392	1.578(1,9)	0.241
Negative Affect Score	.68(9) [1]	1.530(0.466)	0.652	16.18(1,9)	0.003[2]
Sleep Quality					
PSQI Score	.03(9)	1.695(0.433)	0.825	0.0239(1,9)	0.880
SSQ Score	.13(9)	5.704(0.058) [3]	0.652	0.1516(1,9)	0.706
Energy					
POMS Vigor-	.04(9)	2.259(0.323)	0.987	0.274(1,9)	0.613

Activity Score					
POMS Fatigue-Inertia Score	.13(9)	0.470(0.323)	0.652	0.656(1,9)	0.439
VAS-Energy Score	.00(9)	2.740(0.254)	0.392	0.021(1,9)	0.888
VAS-Fatigue Score	.02(9)	1.027(0.598)	0.392	0.034(1,9)	0.857

Model: Y=B0 + B1*X +B2*X^2.
POMS = Profile of Moods; PSQI = Pittsburgh Sleep Quality Index; SSQ = Subjective Sleep Questionnaire; VAS = Visual Analog Scale.
[1]Correlation greater that 0.25
[2]Significant at $p<0.05$
[3]Trend toward significance at $p<0.1$, $>/=0.05$

Table 4.12
Optimal Cortisol-Estradiol PAD Based on Curve Fit (N =23)

	Full Sample	M-type	E-type
Affect			
POMS Total Score	3.23	-	4.85
POMS Depression-Dejection Score	3.78	-	4.62
POMS Tension-Anxiety Score	3.90	-	4.00
Positive Affect Score	3.50	-	4.72
Negative Affect Score	3.57	-	2.69
Sleep Quality			
PSQI Score	-	-	-
SSQ Score	-	-	-
Energy			
POMS Vigor-Activity Score	-	0.69	-
POMS Fatigue-Inertia Score	-	-	-
VAS-Energy Score	-	-	-
VAS-Fatigue Score	-	-	-

PAD = Phase Angle Difference; POMS = Profile of Moods State; PSQI = Pittsburgh Sleep Quality Scale; SSQ = Subjective Sleep Quality Scale; VAS = Visual Analog Scale; PAD in hours
(-) unable to compute PAD

Table 4.13
Comparison of Energy and Fatigue VAS (N = 23)

	T-test (DF)	p-value	Correlation
Full Sample (N=23)	1.215 (22)	0.237	.958
M-type (n=11)	1.145 (10)	0.277	.951
E-type (n=12)	0.419 (11)	0.683	.967

Figure 4.1
*Curve Fit to Y = B0 t B1*X + B2*X^2 for Affect Measures and Cortisol-Estradiol PAD*

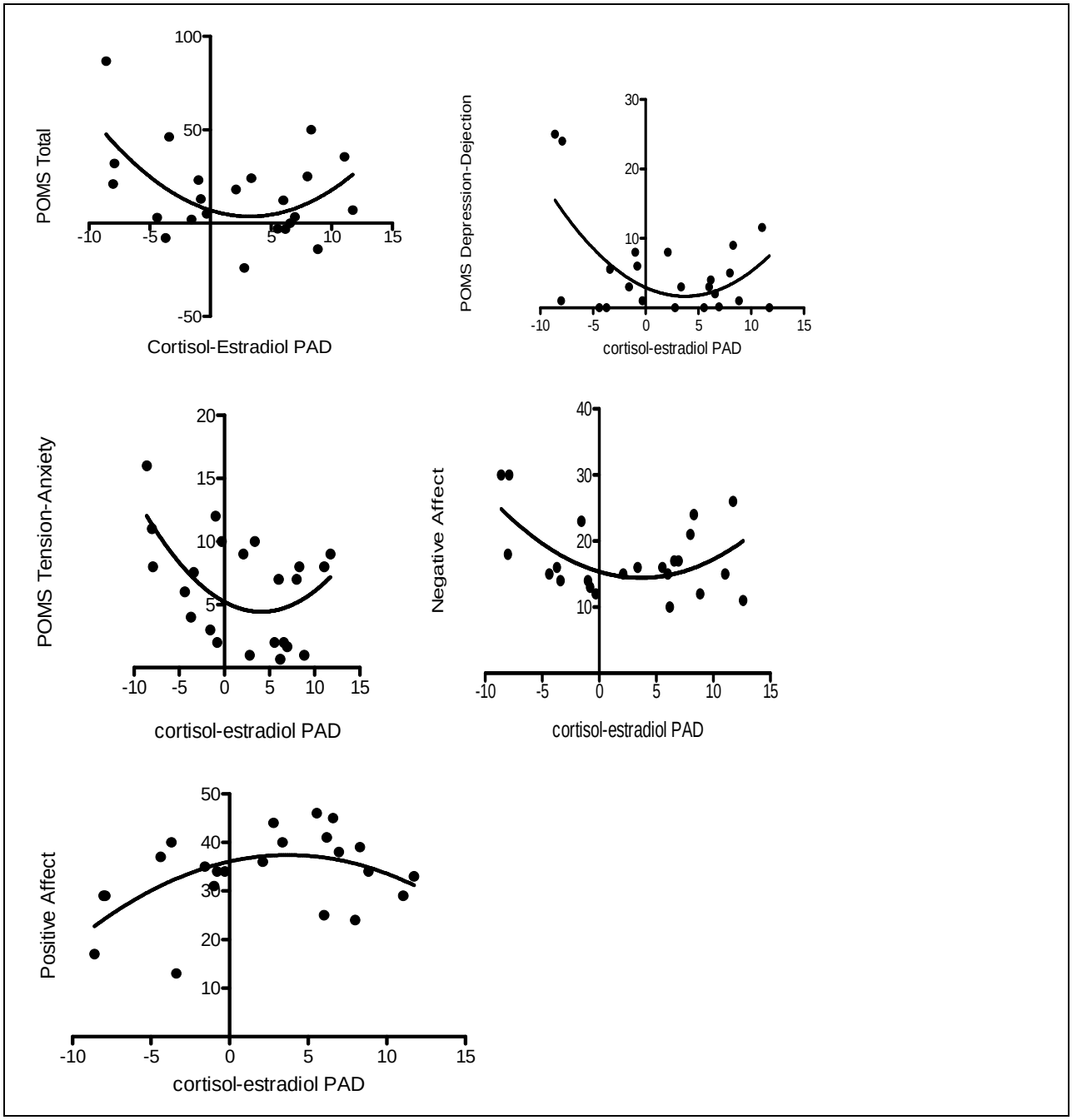


Figure 4.2

*Selected Curve Fit to Y = B0 +B1*X + B2*X^2 for Affect Measure and Cortisol-Estradiol PAD in M-types and E-types*

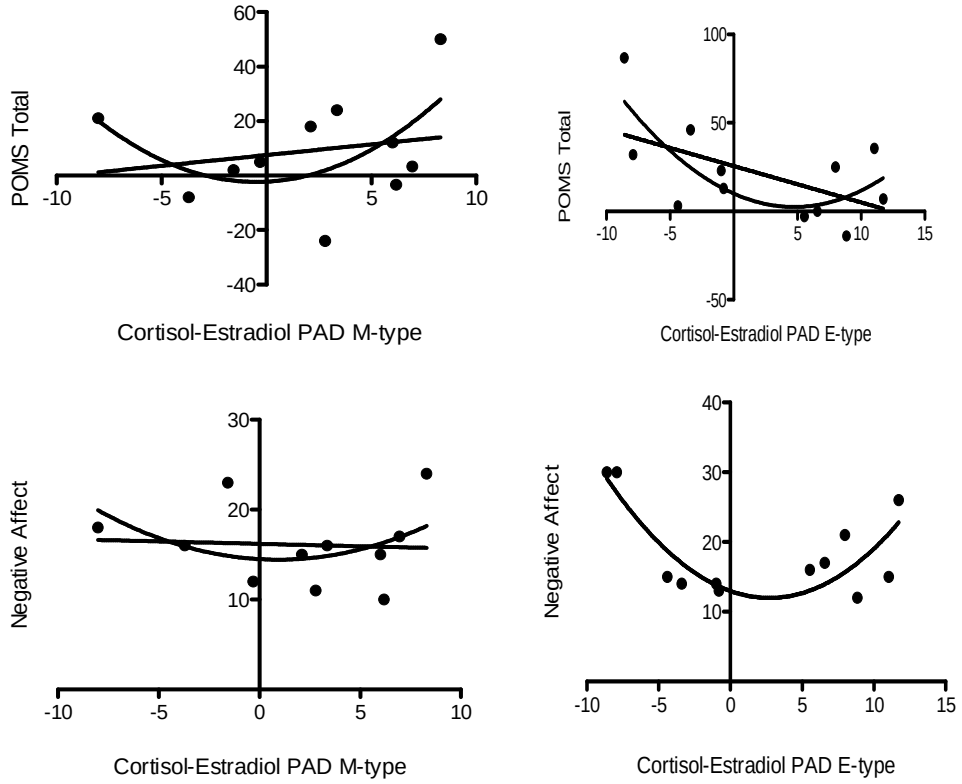


Figure 4.3

*Curve Fit to Y = B0 +B1*X + B2*X^2 for Vigor-Activity Measure and Cortisol-Estradiol PAD in Full Sample and by Chronotype*

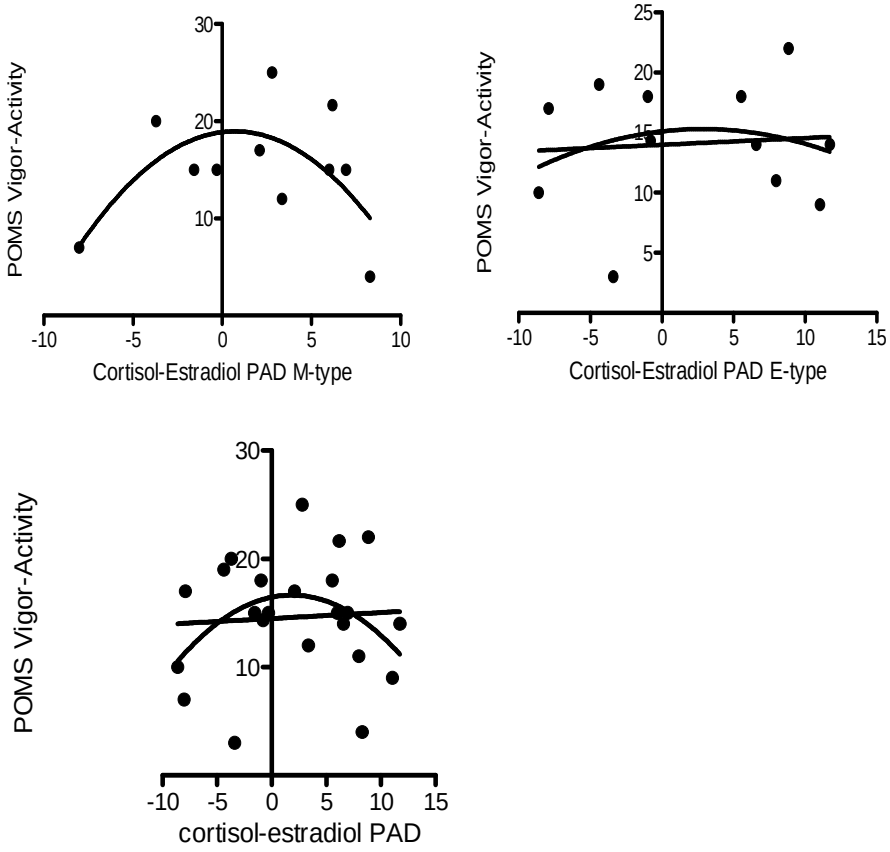
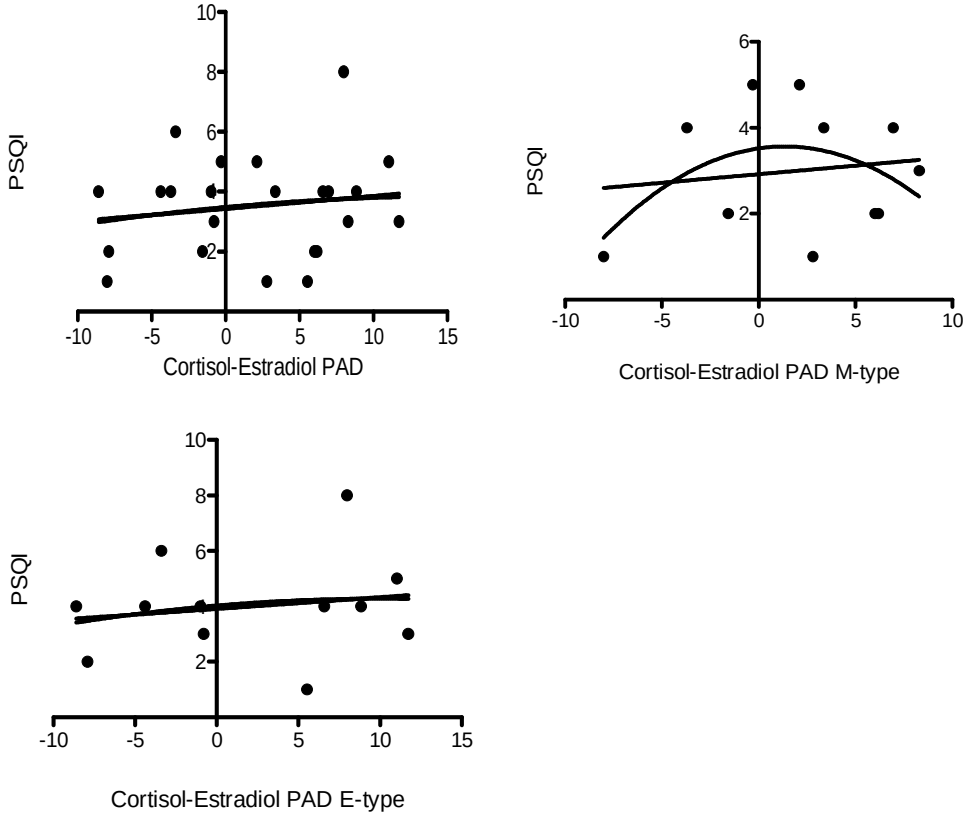


Figure 4.4

*Curve Fit to Y = B0 +B1*X + B2*X^2 for the Pittsburgh Sleep Quality Index (PSQI) and Cortisol-Estradiol PAD in Full Sample and by Chronotype*



Upon visual inspection of the cortisol and estradiol curves, it was noted that two types of curves were described by the data. The first curve type exhibited a dominant circadian rhythm with superimposed ultradian rhythms. The second curve type exhibited a dominant ultradian component with little or no circadian element (see Figure 4.5). Five (21 %) cortisol curves demonstrated an ultradian-dominant curve. Eight (33.3%) of the estradiol curves demonstrated an ultradian-dominant curve. Analyses of group differences between circadian-dominant and ultradian dominant curve types were conducted. Independent student T-tests were used for parametric distributions and Mann-U Whitney test was used for nonparamentric distributions. Table 4.14shows the results for demographic and curve parameter variables and Table 4.15 shows the results for the health variables. A significant difference was found only in the measure of the cortisol mesor between the cortisol ultradian-dominant and circadian-dominant groups. The cortisol mesor for the cortisol circadian-dominant group was .159 μg/dL (*SD* = 0.09) in the circadian-dominant group and 0.241 (*SD* = 0.143) in the ultradian-dominant group. Cortisol mesor was 0.082 μg/dL lower in the cortisol circadian-dominant group (Whitney Mann-U p = 0.05). Trends toward significance were found in the estradiol groups. The estradiol circadian-dominant group had an estradiol amplitude of 3.55 pg/ml (*SD* = 2.65) while the estradiol ultradian-dominant had an estradiol amplitude of 1.48 pg/ml (*SD* = 0.53), a difference of 2.07 pg/ml (Whitney Mann U *p* = 0.053). In addition, a trend toward significance was found in the estradiol groups on the POMS Tension-Anxiety subscale. The estradiol circadian-dominant group exhibited a mean score of 5.24 (*SD* = 4.53) and the ultradian-dominant group exhibited a mean score of 8.13 (*SD* = 2.42). An independent T-test score was -2.03 (*p* = 0.056). In the group where both the cortisol and estradiol rhythms exhibited an ultradian-dominant rhythm (n = 2), trends toward significance were found in the cortisol mesor and both the cortisol and estradiol

amplitude. In the group where both cortisol and estradiol exhibited ultradian-dominant rhythms, the cortisol mesor was 0.352 μg/dL (*SD* = 0.20), the cortisol amplitude was 0.358 μg/dL (*SD* = 0.24) and the estradiol amplitude was 2.09 pg/ml (*SD* = 0.36), verses the circadian-dominant group with measures of 0.161 μg/dL (*SD* = 0.084), 0.141μg/dL (*SD* = 0.11) and 3.00 pg/ml (*SD* = 2.51), respectively. The Whitney Mann U p-values were 0.09 for the cortisol mesor, 0.06 for the cortisol amplitude and 0.053 for the estradiol amplitude. BMI also trended toward significance in the group where both cortisol and estradiol rhythms demonstrated an ultradian-dominant pattern, with a BMI of 21.3 (*SD* = 0.81) verses a BMI of 24.7 (*SD* = 4.6). The Whitney Mann U significance level was 0.095.

Figure 4.5. *Example of A. Circadian-dominated curve type and B. Ultradian-dominated curve type*

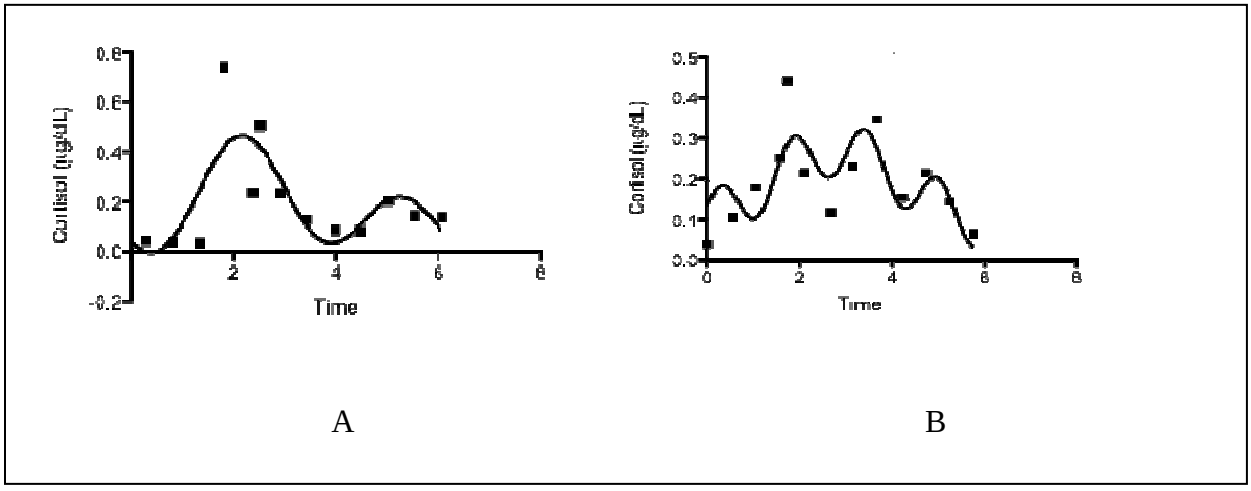


Table 4.14
Comparison of Circadian-dominant and Ultradian-dominant Curves on Demographic Variables and Rhythm Parameters

	Cortisol Circadian-Dominant (N=18) Ultradian-Dominant (N=5)		Estradiol Circadian-Dominant (N=15) Ultradian-Dominant (N=8)		Cortisol and Estradiol One or More Circadian-Dominant (N=21) Both Ultradian-Dominant (N=2)	
	T-test	Significance	T-test	Significance	T-test	Significance
Chronotype	-	0.41	-	0.58	-	0.71
Caffeine Intake	-	0.88	-	0.61	-	0.83
Alcohol Consumption	-	0.24	-	0.35	-	1.0
Age	-	0.50	-	0.86	-	0.23
BMI	-	0.11	-	0.33	-	0.095[2]
Acrophase						
Cortisol	-	0.37	-	0.58	-	1.0
Estradiol	-	0.86	-	0.81	-	0.75
Amplitude						
Cortisol	-	0.12	-	0.66	-	0.06[2]
Estradiol	-	0.71	-	0.053[2]	-	0.74
Mesor						

Cortisol	-	0.09[2]	-	0.79	-	0.05[1]
Estradiol	-	0.60	-	0.57	-	0.83
PAD	0.342	0.74	-0.153	0.88	1.06	0.39

BMI = Body Mass Index; PAD = Phase Angle Difference
[1]Significance level of 0.05;
[2] Trend toward significance at level between 0.05 and 1.0

Table 4.15
Comparison of Circadian-dominant and Ultradian-dominant Curves on Health Indicator Measures

	Cortisol		Estradiol		Cortisol and Estradiol	
	T-Test	Significance	T-test	Significance	T-test	Significance
Affect						
POMS						
Total	0.325	0.75	-0.047	0.65	-0.641	0.53
Depression-Dejection	-	0.91	-	0.69	-	0.65
Tension-Anxiety	0.520	0.61	-2.03	0.056[2]	-0.814	0.42
Positive Affect	-1.04	0.31	0.735	0.47	0.199	0.84
Negative Affect	-	0.28	-	0.48	-	0.35
Sleep Quality						
SSQ	-0.598	0.56	-0.11	0.91	-0.132	0.90
PSQI	-	0.42	-	0.43	-	0.83
Energy						
Fatigue-Inertia	-	0.50	-	0.98	-	0.96
Vigor-Activity	-0.856	0.40	0.984	0.34	-0.667	0.52
VAS-E	0.404	0.69	-0.210	0.80	-0.771	0.50
VAS-F	0.820	0.42	0.608	0.55	-0.764	0.45

Time of Wakening and Cortisol and Estradiol Parameters

Time of morning awakening was computed for each participant and compared with the cortisol and estradiol parameter of acrophase using Pearson's product moment correlations. The cortisol-wake PAD was determined by subtracting the wake time from the time of the cortisol acrophase. The estradiol-wake PAD was determined by subtracting the wake time from the time of the estradiol acrophase PAD. Pearson's product moment correlation was used to analyze the bivariate relationship between the cortisol-wake PAD, wake time, and the cortisol and estradiol acrophases. The estradiol-wake PAD did not meet the assumptions of normality using Kolmogorov-Smirov test of normality. A Spearman's Rho correlation analysis was used for determining the bivariate relationships between the estradiol-wake PAD, wake time and the cortisol and estradiol acrophases.

Significant relationships were found in the full sample and the chronotype subgroups. For the full sample, the estradiol-wake PAD correlated significantly with the estradiol acrophase ($r = 0.74$, $p < 0.001$) and the cortisol-wake PAD correlated with the cortisol acrophase ($r = 0.743$, $p < 0.001$). The cortisol acrophase also correlated significantly with wake time ($r = 0.48$, $p = 0.02$), but the estradiol acrophase did not show a significant correlation. No other significant correlations were found for the full sample (see Table 4.16).

M-types demonstrated correlations in both the cortisol-wake and the estradiol-wake PADs. The cortisol-wake PAD was significantly associated with the cortisol acrophase ($r = 0.944$, $p < 0.001$). The estradiol-wake PAD correlated with both the estradiol acrophase and wake time ($r = 0.945$, $p < 0.001$ and $r = -0.697$, $p = 0.02$, respectively). Correlations for the morning chronotype can be found in table 4.17. For the E-types, correlations were also found in the cortisol-wake and the estradiol-wake PADs. The cortisol-wake PAD correlated significantly

with the cortisol acrophase ($r = 0.704$, $p = 0.01$). The estradiol-wake PAD correlated significantly with estradiol acrophase ($r = 0.755$, $p = 0.005$). Table 4.18 presents the correlations for the E-type group. In neither M-types nor E-types did wake time correlate with either cortisol or estradiol acrophases.

Table 4.16
Correlations Between Selected Cortisol and Estradiol Variables and Time of Wakening in Full Sample (N = 23)

	Estradiol Acrophase	Wake Time	Cortisol-Wake PAD	Estradiol-Wake PAD
Cortisol Acrophase	0.16 (0.94)	0.48 (0.02)[1]	0.743 (0.00)[1]	-0.03* (0.16)
Estradiol Acrophase	-	-0.05 (0.81)	0.147 (0.50)	0.74 * (0.00)[1]
Wake Time	-	-	-0.234 (0.28)	-0.25* (0.25)
Cortisol-wake PAD	-	-	-	0.253* (0.24)

*Spearman's Rho Correlation
[1]Significance at <0.05
PAD = Phase Angle Difference

Table 4.17
Correlations Between Selected Cortisol and Estradiol Variables and Time of Wakening in Morning Chronotypes(N = 11)

	Estradiol Acrophase	Wake Time	Cortisol-Wake PAD	Estradiol-Wake PAD
Cortisol Acrophase	0.104 (0.75)	0.093 (0.79)	0.944 (0.00) [1]	0.01* (0.98)
Estradiol Acrophase	-	-0.42 (0.20)	0.380 (0.25)	0.945* (0.00) [1]
Wake Time	-	-	-0.241 (0.48)	-0.70* (0.02) [1]
Cortisol-wake PAD	-	-	-	0.445* (0.17)

*Spearman's Rho Correlation
[1]Significance at <0.05
PAD = Phase Angle Difference

Table 4.18
Correlations Between Selected Cortisol and Estradiol Variables and Time of Wakening in Evening Chronotypes (N =12)

	Estradiol Acrophase	Wake Time	Cortisol-Wake PAD	Estradiol-Wake PAD
Cortisol Acrophase	-0.23 (0.93)	0.47 (0.15)	0.704 [1] (0.01)	0.161* (0.62)
Estradiol Acrophase	-	-0.34 (0.28)	0.027 (0.93)	0.755* (0.005) [1]
Wake Time	-	-	-0.321 (0.31)	-0.270* (0.40)
Cortisol-wake PAD	-	-	-	0.308* (0.33)

*Spearman's Rho Correlation
[1]Significance at <0.05
PAD = Phase Angle Difference

Chapter 5

Discussion, Conclusions and Recommendations

Introduction

This chapter discusses the findings of this study that sought to examine the relationship between cortisol and estradiol circadian rhythms and the sickness behaviors of altered affect, sleep quality and energy level. The chapter includes discussion of the sample characteristics, study measures and the study results. Discussion of the findings is organized by study aims. Strengths and limitations of the study are addressed, as well as implications for nursing practice and research. Finally, recommendations for future research are presented.

Discussion of Sample Characteristics

The final sample consisted of 23 healthy premenopausal women. Twenty-two women were recruited from a metropolitan College of Nursing and two women were recruited from the greater community. One participant was dropped from the study due to an inability to detect an estradiol acrophase. The dropped participant did not differ significantly from the rest of the sample participants. The dropped participant was a 26 year-old M-type married woman, with no children. The participant reported drinking an average of 1.5 caffeinated drinks per day and two alcohol drinks per week. The participant used no medications, prescription, over the counter, or herbal. Usual bedtime was 2230 and on the day of salivary collection the participant went to bed at 2230 and rose at 0530. Measures on all scales, except energy, were more positive than the mean for the sample, with higher affect and better sleep quality. Score for the dropped participant on the POMS vigor-activity scale was 13, 1.6 points lower than the sample mean. The scores on the Energy VAS and Fatigue VAS were slightly lower than the mean at 72.5 and 73.6, respectively. With the exception of a slightly lower than average energy level, the dropped

participant reported less sickness behaviors than average. Based on the above data, the lack of an identifiable estradiol acrophase in this particular participant most likely a resulted of design or measurement error in the timing of collection or assaying procedures. It is also possible that the participant did not exhibit a circadian estradiol rhythm.

All women were full-time post secondary school nursing students in either the baccalaureate or Masters' programs. The mean age of the sample was 28.7 years, with ages ranging from 21 to 39 years. M-type women were older than E-type women, which was consistent with the current literature (Adan & Almirall, 1992; Chelminski, Ferraro, Petros, & Plaud, 1997; Monk, 2007; Paine, Gander, & Travier, 2006). However, in a published review, Kerkhof (1985) provides evidence that age may not differ between chronotype. All participants were either nurses or student nurses, none working the midnight shift as shift-work was an exclusion criterion. All participants were studying toward a degree in nursing at either a university or college. Participants' BMI averaged 24.7 Kg/m^2. E-type women had higher BMI than M-type women, with BMIs at 26.0 Kg/m^2 and 23.0 Kg/m^2, respectively. The chronotype differences were not statistically significant. All participants with a BMI over 25 Kg/m^2 were E-types, suggesting that lack of statistically significant differences in BMI may be due to small sample size, small effect size or sample homogeneity. Only one participant had a BMI over 30 Kg/m^2. Higher BMIs in E-types has been suggested in the literature (Soreca, Fagiolini, Frank, Goodpster, & Kupfer, 2009; Schubert & Randler, 2008).

Chronotype groups did not differ from each other on marital status or family demographics. In the sample, over half the women were single and with no children. One participant was divorced or separated. Of the six married women, half were M-type and half were E-type. Two women had children between one and five years of age, one woman in each

chronotype. Of the two women with children between five and 12 years of age, both were M-types. In the literature, one study did find that a mother's sleep wake patterns are influenced by the presence of children in which women with children exhibited an earlier chronotype (Leonhard & Randler, 2009). Lack of difference in this study may be related to sample size or student status. With only four women with children, it is impossible to draw any conclusions about motherhood and chronotype. Caffeine intake did not differ between chronotypes with an average of 1.3 cups per day in both M-types and E-types. Weekly alcohol intake was higher in the E-type women, however, this may be due to an outlier. One E-type woman reported an average weekly alcohol intake of 10 glasses per week. In the rest of the sample, no alcohol intake exceeded 3 glasses per day.

Discussion of Study Measures

This study employed five questionnaires, two visual analog scales and two biological measures. Four subscales of the POMS, and two subscales of the PANAS were also used for this study.

Affect. Affect was measured using the Profile of Moods State (POMS) total score and the subscales of Depression-Dejection and Tension-Anxiety. Average POMS scores were 15.4 for the total score, 5.2 for the Depression-Dejection subscale and 6.3 for the Tension-Anxiety subscale.

The two subscales of the Positive and Negative Affect Scales (PANAS) were used to measure affect in this study. Mean scores for this sample were 33.6 for PA and 17.3 for NA. Studies with university students found similar scores that ranged from 29 to 36 for PA and 15 to 22 for NA (Merz & Roesch, 2011; Thome & Espelage, 2004; Watson, Clark & Tellegen, 1988).

Chronotype differences for affect were not supported by this study. Independent student T-tests by chronotype were non-significant for PA ($p = 0.12$) and NA ($p = 0.30$). However, PA demonstrated greater difference between M-types and E-types than NA, with M-type women scoring 5.5 points higher (36.1 verses 31.0). It is possible with a larger sample size, the chronotype difference would be statistically significant. Current studies support higher PA in M-types than E-types, but no difference by chronotype in NA (Hasler, Allen, Sbarra, Bootzin, & Bernert, 2010; Murray, Allen, & Trinder, 2003).

Sleep quality. SSQ Scores for this sample ranged from 1.71 to 5.93 with an average of 4.5 suggesting that sleep quality was fairly high on average for the night before sample collection. Only one participant scored below 3.0 and she was an E-type, with a score of 1.71. For the PSQI, Buysse, Reynolds, Monk, Berman and Kupfer (2000) report that a cut-point score of five has a sensitivity of 84% and a specificity of 86.5% in detecting sleep disorders of patients verses controls. Five out of the 23 participants scored five or greater on the PSQI suggesting approximately a 20% incidence of disturbed sleep in this sample. This finding is consistent with the literature. Studies have reported poor sleep in the general population between 10 to 35% (Akerstedt, Fredlund, Gillberg, & Jansson, 2002, Baker & Driver, 2004) and possibly up to 60% (Lund, Reider, Whiting & Pritchard, 2010). Internal consistency for the PSQI in this sample was poor ($\alpha = 0.34$). This in part may be related to the use of the instrument with healthy women. The PSQI was designed for a clinical population and has been shown to perform better in depressed individuals than healthy controls (Buysse, et al., 1989). In contrast, the internal consistency for the SSQ was 0.91. Discrepancy between measures and poor reliability in the PSQI makes confident observations about the sleep quality of the participants in this study difficult. M-types and E-types did not differ from each other on either sleep measure; PSQI or

SSQ. The average score for the PSQI was slightly higher for E-types, 4.0 verses 3.0, but the difference was not statistically significant. This is in contrast to a number of studies that report poorer sleep quality in E-types compared to M-types (Chung, Chang,Yang, Kuo & Hsu, 2009; Ishihara, Miyasita, Inugami, Fukuda & Miyata, 1987; Webb & Bonnet, 1978). On the other hand, other studies have failed to find significant chronotype differences (Foret, Touron, Benoit & Bouard, 1985; Lehnkerig & Siegmund, 2007). Of the five participants with a score greater than or equal to the cut-point for disturbed sleep, all were E-types, supporting the evidence that suggests that E-types exhibit worse sleep quality than M-types. Also, the mean E-type scores were higher than M-types, although not statistically significant.

Energy. Energy was measured by two subscales of the POMS; Vigor-Activity and Fatigue-Inertia, and two visual analog scales; Energy VAS and Fatigue VAS. Mean score for the POMS Fatigue-Inertia subscale was 7.3. Mean score for the POMS Vigor-Activity Subscale was 14.6. The mean score on the Energy VAS was 72.7 and the Fatigue VAS was 73.9. In M-type women, the POMS Fatigue-Inertia subscale score was slightly lower and the POMS Vigor-Activity subscale score was slightly higher than in E-types, but neither score difference reached statistical significance. This is supported in the literature. Studies have found no differences between M-types and E-types on daytime sleepiness (Hilliker, Muehlbach, Schweitzer & Walsh, 1992; Taillard, Philip, & Bioulac, 1999). Of the participants scoring greater than one standard deviation above the mean on the POMS Fatigue-Inertia subscale, three out of four were E-types, suggesting a chronotype difference in energy. A few studies have also noted a difference in chronotype on energy with studies of increased daytime sleepiness reported in E-types (Matchock & Mordkoff, 2009) and alertness (Clodore, Foret, & Benoit, 1986). On the POMS

Vigor-Activity subscale, scores greater than one standard deviation below the mean were equally divided between M-types and E-types.

Salivary Measures

Salivary cortisol and estradiol were measured to determine the circadian rhythms of the biological hormones. The Salimetrics High Sensitivity Salivary Cortisol Enzyme Immunoassay had good sensitivity (0.003). The intra-assay and the inter-assay coefficients of variation were reliable at 6.7 and 11.9, respectively. Intra-assay coefficients of variation less than 10 and interassay coefficients of variation less than 15 are considered acceptable (Salimetrics, 2011).

The Salimetrics High Sensitivity Salivary Estradiol Enzyme Immunoassay intra-assay and inter-assay coefficients of variation were reliable at 9.3 and 13.3, respectively. These coefficients of variation are similar to those found in another published study (Bao et al. 2004).

In this study, pH measures for some of the estradiol samples were elevated with 19.3 % of a random sampling of 15% of the full sample with pH values above 9.0. Salivary pH values in healthy participants range from 5.67 to 7.96 across the 24-hour day, with intra-individual variation of 0.91 (Larsen, Jensen, Madsen, & Pearce, 1999) and 7.03 (*SD* = 0.54) in participants at rest (Sato, 2002). None of the cortisol samples had pH values over 9.0 and the elevated pH values showed no recognizable pattern within participant samples, suggesting possible contamination with buffer solution after testing of the samples for the cortisol assays.

The saliva samples were collected by passive drool according to the manufacturer's instructions (Salimetrics, 2011). All participants produced adequate saliva for cortisol and estradiol assaying at all collection times. The quantitative measurement of cortisol and estradiol were performed using an ELISA procedure according to the manufacturer's instructions

(Salimetrics, 2011) and processed by a skilled lab technician, trained in immunoassay techniques, at the Wayne State University, College of Nursing Biophysical Laboratory.

Discussion of the Findings Related to Study Aims

Specific Aim 1.In the first research aim it was hypothesized that cortisol and estradiol would demonstrate a circadian rhythm that could be fitted to a cosinor model and that the circadian parameters of phase, amplitude and mesor would not correlate between cortisol and estradiol. The hypotheses of the first research aim were supported in this study. In all participants, the cortisol and estradiol data converged on a cosinor model. Cortisol data demonstrated greater curve fit with lower sum of squares differences (R- values) than estradiol. Correlation coefficients for cortisol ranged from 0.32 to 0.95 with only two data sets correlating at less than 0.50. Estradiol correlation coefficients ranged from 0.29 to 0.88 with ten data sets correlating at less than 0.50. The multioscillator cosinor model has been used to model circadian rhythm in several studies (Bao et al., 2003; Koenigsberg et al., 2004; Steltman, 1997). The cortisol data fit the curve model better than the estradiol data suggesting the possibility that the circadian and ultradian profile of estradiol expression may follow a different model from that of cortisol. Gibertini, Graham and Cook (1997) suggest that the temporal expression of different circadian rhythms may follow models that are not cosinor in nature.

Five out of 24 data sets violated normality of residuals assumptions in the cortisol curve fit and two violated the normality of residuals assumptions in the estradiol curve fit at significance levels less than 0.05. In four of the five cortisol curves and one of the two estradiol curves the lack of normal distribution may be accounted for by an outlier. In each cortisol case the outlier may indicate the morning cortisol awakening response. In the estradiol curve, the outlier is the highest value and may represent the acrophase or may be due to measurement error. Violation of

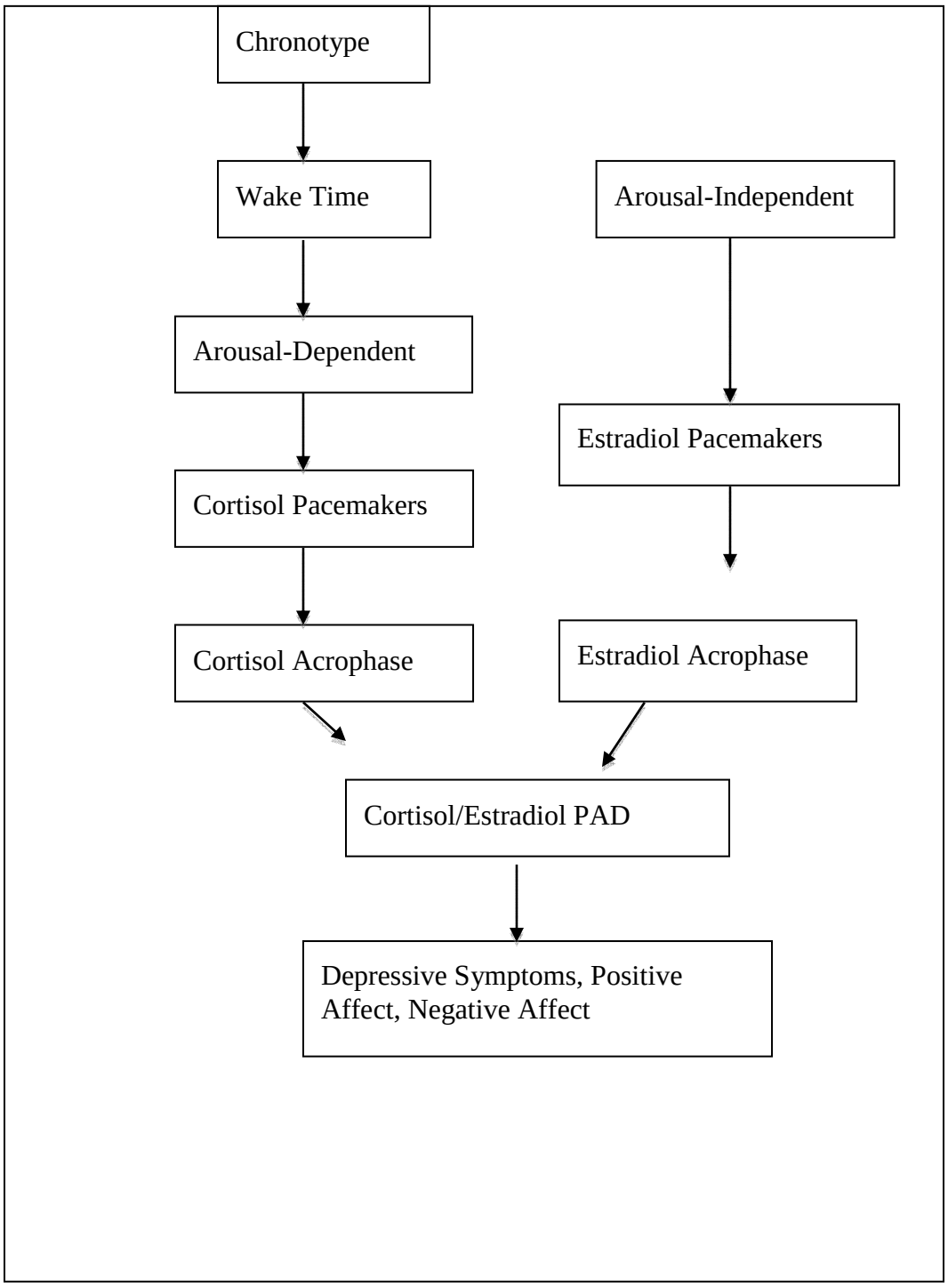
the normality assumptions suggests a systematic explanation for deviation from the chosen model. In the cortisol curves, the model may not adequately capture the cortisol awakening response. Cortisol has consistently demonstrated a robust circadian and ultradian rhythm (Baehr, Revelle, & Eastman, 2000; Elverson & Wilson, 2005; Freeman, Webb, An, & Herzog, 2008) Few studies have examined the circadian rhythm of salivary estradiol. Bao and colleagues (2003) sampled 15 women every two hours for 24 hours at four times during the menstrual cycle, fitting the estradiol data to a cosinor rhythm. The authors found the data to fit a peaked diurnal rhythm with ultradian harmonics that demonstrated a mean acrophase in the early morning. In this current study, the majority of participants' estradiol curve fit the model without violating assumptions, the correlations were lower than for cortisol. Findings of this study are consistent with Bao and coworkers (2003).

The cortisol and estradiol circadian parameters of acrophase, amplitude and mesor were uncorrelated in the full sample using Pearson's Product moment correlation for the acrophase and nonparametric Spearman's Rho rank correlation for amplitude and mesor. Correlation for the acrophase was 0.95 (p = 0.67). Lack of correlation in acrophase suggests that cortisol and estradiol are subject to different phase setting pacemakers that act independently of each other. A study by Bao and colleagues (2004) found that the cortisol and estradiol acrophases correlated only in the menstrual phase of the menses cycle. Consistent with the study by Bao and colleagues (2003), cortisol and estradiol were not correlated in the late luteal phase of this study.

It was also noted that the cortisol acrophase correlated with the wake time (r = 0.48, p = 0.02) in the full sample. The estradiol acrophase did not correlate with time of wakening, further supporting different timing of the cortisol and estradiol pacemakers. Correlation of cortisol but not estradiol with wake time raises the possibility of an arousal dependent phasing of cortisol but

not estradiol. Challet (2007) discussed the phenomenon of phasing of some rhythms in nocturnal mammals are opposite of diurnal mammals while other rhythms are phased similarly in both nocturnal and diurnal mammals. Challet (2007) refers to rhythms that are oppositely phased as arousal-dependent and rhythms that are phased the same as arousal-independent. According to Challet (2007), temperature and corticosteroid are arousal-dependent and melatonin is arousal-independent. The circadian rhythm of gene production is phased similarly and response to light is basically the same manner in nocturnal and diurnal mammals. It may be that in humans cortisol is an arousal-dependent circadian rhythm and estradiol is an arousal-independent circadian rhythm. Figure 5.1 illustrates a possible mechanism by which cortisol and estradiol may be independently entrained and phased.

Figure 5.1. *Possible pathway of cortisol and estradiol entrainment*



Specific Aim 2. The second specific aim explored the cortisol and estradiol rhythm in terms of morningness-eveningness. It was hypothesized that the cortisol rhythm and the estradiol rhythm would be phase advanced in M-type women compared to E-type women. This hypothesis was partially supported by the findings. There was a significant statistical difference in cortisol acrophase between the two groups, at a probability level of less than 0.05. However, the findings trended to significance (Mann-Whitney U $n_1 = 12$, $n_2 = 11$, $p = 0.074$), suggesting significant results may be found in a larger sample. The mean acrophase for M-types was 8.32 hours and the mean acrophase for E-types was 11.0 hours, a 2.68 hour phase advance in M-types. This finding is consistent with a number of published studies on cortisol and chronotype. Studies report a possible phase advance of cortisol in M-types compared to E-types (Bailey & Heitkemper, 1991; Kudielka, Bellingrath, & Hellhammer, 2007; Randler & Schall, 2010). The hypothesis that the mesor and amplitude would differ between chronotypes was not supported. The differences between mesor and amplitude were not statistically significant. Additionally, no chronotype differences were noted in any of the estradiol parameters. To date there are no known studies investigating the estradiol circadian rhythm parameters in M-types and E-types. Findings from this study do not support a difference by chronotype in the estradiol rhythm parameters of acrophase, amplitude and mesor.

Specific aim 3. The third specific aim investigated the relationship between chronotype, cortisol and estradiol circadian rhythms and sickness behaviors. Specifically, the sickness behaviors examined were alterations in affect, sleep quality and energy level. Specific aims 3a, b, and c hypothesized that the phase relationship between the cortisol and estradiol circadian rhythms would be nonlinearly related to affect, sleep quality and energy level and that this relationship could be fitted to a quadratic equation. For the full sample, this hypothesis was

supported in the affect measures. The hypothesis was not supported in the energy measures for the full sample. In addition, neither of the sleep measures fit a quadratic model. It is important to note that the PSQI demonstrated poor internal consistency in this sample, possibly contributing to a lack of significant findings.

Hypothesis 3d stated that the optimal PAD would not differ between affect, sleep quality and energy level. Hypothesis 3d was not supported by this study. The optimal PAD mean value was 3.60 (SD = 0.26) hours, determined by the mean of the optimal PAD of the five affect measures. All PADs for the affect measures were between 3.23 and 3.90 hours. In the M-type women, the optimal PAD as an average of the five affect measures, was 4.18 (SD = 0.89) hours. No optimal PAD was found for sleep quality or energy. Both cortisol and estradiol have been independently implicated in affect disorders. Examining a cortisol-estradiol PAD for low affect has supporting evidence. Evidence for the concept of an optimal PAD in affect has support in the literature. A number of studies identified optimal PADs between hormones and the sleep parameters of wake, midsleep and dim-light melatonin onset (DLMO) in depression. Depression severity has shown a linear association with the DLMO-wake time and the DLMO-sleep time PADs in 18 depressed women (Emens, Lewy, Kinzie, Arntz, & Rough, 2009). Depression severity demonstrated a linear relationship between temperature-midsleep PAD and the DLMO-temperature PAD, however no group differences were noted (Hasler, Buysse, Kupfer & Germain, 2010). PAD between temperature minimum and wake time in 43 SAD participants suggested a trend toward a three hour PAD associated with reduction in symptoms after light treatment that was not statistically significant (Murray, et al., 2006).

Group differences in cortisol-DLMO were found in six healthy and six depressed individuals, with approximately a two hour greater PAD in depressed participants (Buckley &

Schatzberg 2010). In addition, a six-hour optimal PAD was demonstrated between DLMO and mid-sleep in winter depression (Lewy, Lefler, Emens, & Bauer, 2006). With the exception of Buckley and Schatzberg (2010), no studies have investigated an optimal PAD between two endogenous hormones.

Hypothesis 3e explored the chronotype differences in optimal PAD postulating that there would be no difference in optimal PAD between M-type and E-type women. In affect, where an optimal PAD was found for the full sample, only the E-type women exhibited an optimal PAD. The affect, sleep quality and energy measures for M-type women did not fit a quadratic model better than a straight line. This may be due to the small sample size (n = 11) and a smaller correlation between sickness behavior and the cortisol-estradiol PAD. In E-type women the data fit a quadratic model on all five affect measures. The average PAD for affect was 4.18 (SD = 0.89), which is slightly longer than that of the full sample by about half an hour. The difference may be due to measurement error or to a difference in angle of entrainment between chronotypes. It has been observed that M-types and E-types differ in the phasing of some circadian measures and sleep parameters. E-types exhibit a temperature minimum closer to wake time than M-types (Baehr, Revelle, & Eastman, 2000). The acrophases of body temperature, plasma cortisol, and heart rate occur later in E-types (Bailey & Heitkemper 2001). Core body temperature is phase advanced by three hours in M-types, a difference that cannot be attributable to sleep or activity rhythms (Waterhouse et al., 2001). A larger PAD in E-types would suggest a greater phase delay in estradiol than in cortisol for E-type women.

The PAD between cortisol and estradiol may not be the appropriate PAD to measure in order to detect circadian misalignment in sleep and energy disturbances. This study found no significant correlations in either M-types or E-types for the sleep and energy measures with the

exception of M-type women and the POMS Vigor-Activity score. It is possible that an optimal PAD exists for sleep quality and energy level that involves other physiologic, psychological, social or behavioral circadian rhythms. For example, melatonin is a hormone secreted in a circadian nature by humans. Optimal sleep quality may be associated with a specific melatonin related PAD. Also, insulin may play a role in an optimal PAD for maximal energy levels.

Conclusions

This study is the first to investigate the PAD between cortisol and estradiol and its relationship to select sickness behaviors. The cortisol-estradiol PAD for both M-type and E-type women demonstrated variability among participants, suggesting that phase relationships are individually determined and vary from one person to another. Cortisol and estradiol acrophases demonstrated large ranges in this sample of women. The cortisol-estradiol PAD was associated with affect but not sleep quality or energy level. The cortisol-estradiol PAD correlated with affect on all five measures in the full sample and in E-type women. The cortisol-estradiol PAD did not correlate with affect measures in M-type women. None of the sickness behaviors measures differed by chronotype although with respect to affect, this may be due to the small sample size. Cortisol acrophase demonstrated a positive linear correlation with wake time but the estradiol acrophase did not correlate with wake time possibly suggesting an arousal–dependent mechanism of circadian regulation for cortisol but not estradiol.

Findings Related to Theoretical Framework

The aim of this study was to test the theoretical relationship between the cortisol-estradiol PAD and sickness behaviors. The theoretical framework directing this study proposed that the cortisol-estradiol PAD would play a significant role in the expression of sickness behaviors via an effect on the inflammatory process. This was supported only for the sickness behaviors that

manifest as alterations in affect. The framework was not supported for other sickness behaviors including alterations in sleep quality and energy. These initial findings suggest that the mechanisms by which individual sickness behaviors manifest may differ among sickness behaviors. Phase angles between hormones other than cortisol and estradiol, or between hormones and behaviors may underlie individual sickness behaviors. A singular PAD between cortisol and estradiol may not adequately explain all sickness behaviors.

The theoretical framework underling this study further asserted that exogenous and endogenous pacemakers exert differential influence over the timing of endogenous rhythms. This was supported by this study in that the cortisol rhythm and the estradiol rhythm did not correlate with participants. The cortisol rhythm did correlate with wake time suggesting an exogenous, activity related pacemaker. The estradiol rhythm was not correlated with wake time suggesting the influence of a pacemaker that is independent of the cortisol pacemaker.

Strengths and Limitations

Strengths. This study was the first to investigate the PAD between cortisol and estradiol and the relationship between the cortisol-estradiol PAD and sickness behaviors. This study has a number of strengths. The study measured cortisol and estradiol every two hours across an entire 24-hour period. The cortisol awakening response was captured by an additional saliva collection 30 minutes following wake time for a total of 13 saliva samples per participant. Multiple sampling across the 24-hour period allows for greater confidence in modeling the circadian and ultradian rhythms. Another strength was the use of multiple measures for each of the sickness behaviors; affect, sleep quality and energy level. To reduce confounding variables the sample was homogenous for race and occupation. Saliva samples were obtained at the same time in the luteal phase of the menstrual cycle for all participants, avoiding differences in circadian rhythm

characteristics due to phase of the menstrual cycle. Participants were very compliant in saliva collection and diary reporting. Very few collections were missed or insufficient. Compliance may have been high due to the study design. Participants were reminded about collecting via telephone or e-mail the night before collection. A contact phone number was given to the participant with instruction that the researcher could be reached at any time day or night with concerns. Furthermore, the curve modeling procedure did not require equal distant time points so flexibility in saliva was possible. Studies suggest that noncompliance with collection procedures affect the results (Broderick, Arnold, Kudielka, & Kirschbaum, 2004). This study was further strengthened by the natural setting in which saliva was collected. A natural setting allows for hormone expression in the body that is more consistent with the participant's daily secretion patterns. The effects of a laboratory environment on the hormone production and secretion may provide data that is inconsistent with a natural environment. This study provided insight into the relationships between circadian rhythms within the individual, as opposed to aggregate means. Understanding the temporal characteristics of rhythms and their relationship with other rhythms provides information on the functioning of peripheral oscillators. An additional strength of this study was consideration of the role of chronotype in the investigation of the cortisol and estradiol rhythms.

Limitations. This study was limited by a number of factors. First, the relationships tested must be understood as associations, not causal relationships. Generalizability is limited by the homogeneity and small size of the sample. The convenience sample was selected primarily from a cohort of graduate and undergraduate nursing students at an urban university College of Nursing. Education level, student status and race were similar across the sample. The sample consisted of 23 women; too small for adequate power to determine group differences. Non-

significant findings may be a result of type II error and significant findings may possibly be spurious due to the small sample size.

Other design limitations include sampling every two hours over a single 24-hour period, subjective measures of affect, sleep quality and energy level, and self-report compliance. It is optimal to sample salivary hormone over a number of days and take the mean values to more accurately model the circadian rhythms. Numerous studies that employ salivary sample across the day have been limited to two to six samples. Study designs that use laboratory conditions and plasma sampling have the ability to perform sampling at greater frequencies. The optimal number of salivary samples needed for both adequate curve fit and minimizing interruption to normal daily activities has not been adequately studied. Sampling every two hours has been suggested to be acceptable, however, a higher sampling rate may provide greater confidence in the rhythm parameters. This study is further limited by the use of subjective measures for the assessment of affect, sleep quality and energy level. Subjective measures have been found to inconsistently correlate with objective measures and threaten validity of results (Williamson. 2007). Lack of adequate internal consistency reliability on the PSQI measure further questions results obtained on the global sleep quality measure. Sampling in a natural environment prohibits researcher oversight of participant compliance with study procedures. Following the saliva collection protocol was the responsibility of the participant and no direct monitoring of compliance was done. Compliance was maximized by use of a diary to record exact time and conditions under which saliva as collected. Abnormally high pH values in 19% the estradiol samples represents an additional threat to assay validity.

Study Implications

This study investigated the circadian characteristics of two hormones in healthy women and their relationship to three sickness behaviors. Implications to the science and practice of nursing are discussed. Recommendations for further research are addressed.

Nursing research. One goal of nursing research is to explicate the underlying models that describe and predict health in humans. To this end, this study endeavors to explain the mechanisms by which sickness behaviors emerge from the interplay of various circadian rhythms. Understanding the PADs among rhythms in humans holds the potential to understand the development of the symptoms that are common to many disease processes. This study contributes to nursing knowledge by suggesting a possible phase relationship between cortisol and estradiol that affects affect in healthy women. This study further suggests that other sickness behaviors of disturbed sleep and energy may be the result of a different mechanism than the one identified for affect.

Evolving from the basic research that generates knowledge and understanding the models that describe processes, intervention studies may be conducted. Based on the possible relationship between the cortisol-estradiol PAD and affect, phase shifting interventions can be developed and tested to determine their effects on depression, premenstrual syndrome, and premenstrual dysphoric disorder, among others. The emerging model may suggest phase responses between cortisol and estradiol may differ based on the specific entrainer. This study suggests the possibility that cortisol represents an arousal-dependent rhythm while estradiol represents an arousal-independent rhythm. Arousal-Independent phase shifters include melatonin and γ-*Aminobutyric acid* (GABA). Arousal-dependent non-photic phase shifters include serotonin (Challet, 2007). Much nursing research is needed to understand the effects of specific entrainers on health.

Finally, explicating a model for phase-setting in human health provides a method by which to explore additional, yet unknown, phase entrainers. Measuring the effects of entrainers on an optimal cortisol-estradiol PAD can contribute to understanding the potential role of interventions in the alleviation of symptoms of illness. Potential entrainers may include such diverse phenomena as music, visual art, and physical/temporal order or disorder among many others.

Nursing practice. Initial findings from this study may contribute to nursing practice by introducing the concept of the existence of an optimal PAD in health and illness. Establishing the importance of phase relationships among endogenous circadian rhythms may lead to interventions that nurses can employ with confidence to improve care. Discovering the significance in optimizing PADs draws attention to the importance of regulating environmental factors in the acute care setting and educating clients in the home setting. The use of light and noise, temporal order and routine, may all play a significant role in the differential entrainment of circadian rhythms. When and how nursing interventions are provided may rest in the potential phase shifting effects of the intervention. Accurate and complete understanding of the models that explain the relationships among circadian rhythms are essential to the design of the timing of interventions. This study is an initial step in the development of a more complete model of an optimal cortisol-estradiol circadian rhythm in disturbed affect.

Summary

In summary, this small-scale study represents an initial step in the development of an understanding of the possible phase relationships between cortisol and estradiol and the impact of the phase relationship on sickness behaviors in women. This study suggests that an optimal cortisol-estradiol PAD may exist that reflects high levels of affect. Further study that includes a

larger, more heterogeneous sample is warranted by the findings presented here. This study suggests the possibility that chronotype differences in affect found by other studies may, in actuality reflect PAD rather than chronotype differences. Further study is warranted in order to develop an accurate and complete model of the role of phase relationships in health and to develop safe and effective nursing interventions to reduce sickness behaviors.

APPENDIX A

WAYNE STATE UNIVERSITY

HUMAN INVESTIGATION COMMITTEE
101 East Alexandrine Building
Detroit, Michigan 48201
Phone: (313) 577-1628
FAX: (313) 993-7122
http://hic.wayne.edu

Association for the Accreditation of Human Research Protection Programs, Inc. Full AAHRPP Accreditation

NOTICE OF EXPEDITED APPROVAL

To: Karyn Butler
Health Research Center
5557 Cass Ave

From: Ellen Barton, Ph.D. E Barton 1/6
Chairperson, Behavioral Institutional Review Board (B3)

Date: January 07, 2010

RE: HIC #: 117009B3E
Protocol Title: Cortisol and Estradiol Circadian Rhythms in Normally Menstruating Women
Sponsor: ° SIGMA THETA TAU INTERNATIONAL, INCORPORATED
Protocol #: 0911007749

Expiration Date: January 06, 2011

Risk Level / Category: Research not involving greater than minimal risk

The above-referenced protocol and items listed below (if applicable) were **APPROVED** following *Expedited Review* (Category 7*) by the Chairperson/designee *for* the Wayne State University Behavioral Institutional Review Board (B3) for the period of 01/07/2010 through 01/06/2011. This approval does not replace any departmental or other approvals that may be required.

- Flyer
- Contact Information Sheet
- Oral Consent Script
- Written Consent Form

° Federal regulations require that all research be reviewed at least annually. You *may* receive a *"Continuation Renewal Reminder"* approximately two months prior to the expiration date; however, it is the Principal Investigator's responsibility to obtain review and continued approval ***before*** the expiration date. Data collected during a period of lapsed approval is unapproved research and can ***never*** be reported or published as research data.

° All changes or amendments to the above-referenced protocol require review and approval by the HIC **BEFORE** implementation.

° Adverse Reactions/Unexpected Events (AR/UE) must be submitted on the appropriate form within the timeframe specified in the HIC Policy (http://www.hic.wayne.edu/hicpol.html).

NOTE:

1. Upon notification of an impending regulatory site visit, hold notification, and/or external audit the HIC office must be contacted immediately.
2. Forms should be downloaded from the HIC website at ***each*** use.

*Based on the Expedited Review List, revised November 1998

APPENDIX B

Research Informed Consent
Cortisol and Estradiol Circadian Rhythms in Normally Menstruating Women

Principal Investigator (PI): Karyn Butler
Nursing
734.231.0933

Funding Source: Sigma Theta Tau Lambda Chapter

Purpose

You are being asked to be in a research study of hormone rhythms and health because you are a woman with normal menstrual periods. This study is being conducted at Wayne State University, college of Nursing. The estimated number of study participants to be enrolled at Wayne State University is twenty four. **Please read this form and ask any questions you may have before agreeing to be in the study.**

In this research study, we are looking at the relationship between two hormones, cortisol and estradiol, as they act normally in women's bodies. We believe that how women feel, physically and emotionally is affected by the relationship between cortisol and estradiol. Information about how you feel right now, how well you sleep and how much energy you have will be studied together with your cortisol and estradiol hormones.

Study Procedures

If you agree to take part in this research study, you will be asked meet with the researcher at your home or, if you prefer, a location in Wayne State University College of Nursing. The meeting should take less than an hour. At the meeting, you will learn how to complete the study and fill out a packet of questions. On the next day, you will be asked to provide saliva (spit) samples in a tube every two hours for one day. You will be asked to wake up in the night to give saliva samples. Each time you give a saliva sample you will be asked to write, in a diary, information such as; time you gave sample, when you woke up and went to bed, how much energy you feel now, how much caffeine and alcohol you had since last writing in the diary. You will be asked to give saliva samples in a very specific way. You will be asked to give the sample before brushing or flossing your teeth. You will be asked to not eat within the 30 minutes before saliva collection. Immediately before spitting into the tube you will be asked to rinse your mouth with cool water. After a five minute wait, you will be asked to spit through the straw given to you for each tube. This should take you less than ten minutes for each saliva collection time. You will be asked to store the saliva tubes in the freezer until the next day when the researcher will pick them up. If you do not want the researcher to pick the tubes up from your house a pick-up place can be arranged at the college of nursing. All the questions that you answer and the tubes of saliva will be given a number that will identify them. Your name will not be used in order to protect your privacy. The saliva samples that you provide will be stored at -20 degrees Celsius until analysis. Upon thawing, salivary samples will be centrifuged at 3000 rpm for 15 minutes. Samples will be processed by a laboratory test called an enzyme immunoassay. We will follow the procedures developed by Salimetrics LLC, State College, PA, USA. The saliva samples you provide will be destroyed at the completion of the study.

Benefits

As a participant in this research study, there will be no direct benefit for you; however, information from this study may benefit other people now or in the future.

Risks

- There are no known risks at this time to participation in this study.

Study Costs

- Participation in this study will be of no cost to you.

Compensation

For taking part in this research study, you will be paid for your time and inconvenience with $75.00 at the time that the researcher collects the saliva tubes

Research Related Injuries

In the event that this research related activity results in an injury, treatment will be made available including first aid, emergency treatment, and follow-up care as needed. Care for such will be billed in the ordinary manner to you or your insurance company. No reimbursement, compensation, or free medical care is offered by Wayne State University. If you think that you have suffered a research related injury, contact the PI right away at (734) 231-0933.

Confidentiality

All information collected about you during the course of this study will be kept confidential to the extent permitted by law. You will be identified in the research records by a code name or number. Information that identifies you personally will not be released without your written permission. However, the study sponsor, the Human Investigation Committee (HIC) at Wayne State University, or federal agencies with appropriate regulatory oversight [e.g., Food and Drug Administration (FDA), Office for Human Research Protections (OHRP), Office of Civil Rights (OCR), etc. may review your records.

When the results of this research are published or discussed in conferences, no information will be included that would reveal your identity.

Voluntary Participation/Withdrawal

Taking part in this study is voluntary. You have the right to choose not to take part in this study. If you decide to take part in the study you can later change your mind and withdraw from the study. You are free to only answer questions that you want to answer. You are free to withdraw from participation in this study at any time. Your decisions will not change any present or future relationship with Wayne State University or its affiliates, or other services you are entitled to receive.

The PI may stop your participation in this study without your consent. The PI will make the decision and let you know if it is not possible for you to continue. The decision that is made is to protect your health and safety, or because you did not follow the instructions to take part in the study

Questions

If you have any questions about this study now or in the future, you may contact Karyn Butler, MS, CNM at the following phone number (734) 231-0933. If you have questions or concerns about your rights as a research participant, the Chair of the Human Investigation Committee can be contacted at (313) 577-1628. If you are unable to contact the research staff, or if you want to talk to someone other than the research staff, you may also call (313) 577-1628 to ask questions or voice concerns or complaints.

Consent to Participate in a Research Study

To voluntarily agree to take part in this study, you must sign on the line below. If you choose to take part in this study you may withdraw at any time. You are not giving up any of your legal rights by signing this form. Your signature below indicates that you have read, or had read to you, this entire consent form, including the risks and benefits, and have had all of your questions answered. You will be given a copy of this consent form.

Signature of participant / Legally authorized representative	Date
Printed name of participant / Legally authorized representative	Time
Signature of witness*	Date
Printed of witness*	Time
Signature of person obtaining consent	Date
Printed name of person obtaining consent	Time

*Use when participant has had this consent form read to them (i.e., illiterate, legally blind, translated into foreign language).

APPROVAL PERIOD
JAN 07 '10 JAN 06 '11
INSTITUTIONAL INVESTIGATION COMMITTEE

Signature of translator	Date
Printed name of translator	Time

APPENDIX C

Research Study Participants Needed

Healthy women between the ages of 21 to 39 are needed for a study looking at the relationship of hormones and health.

$75.00 will be provided for participation.
You will be asked to complete a packet of questionnaires and provide saliva samples every two hours for a 24 hour period.

If interested, please contact:

Karyn Butler, MS, CNM
Wayne State University, College of Nursing
5557 Cass Avenue, Detroit Michigan 48202.

Karyn 734-231-0933
Karyn 734-231-0933
Karyn 734-231-0933
Karyn 734-231-0933
Karyn 734-231-0933
Karyn 734-231-0933
Karyn 734-231-0933

APPENDIX D

Screening Questionnaire
Participant Data Sheet

Menstrual Cycle _____ Irregular _____ Regular

Number of days between periods: _____

Currently pregnant _____ Yes _____ No

Lactating within last three months _____Yes _____ No

Height: ____ Weight: _____ Calculated BMI: _____ Waist Circumference _____

Population of Place of Residence less than 2499 persons _____ Yes _____ No

Population of Place of Residence more than 49,999 persons _____ Yes _____No

Score on the Horne-Ostberg Morningness-Eveningness Questionnaire _____

Diagnosed with any of the following:

Cardiovascular Disease (hypertension, CAD, stroke) ____ Yes ____ No

Psychiatric Disorder (depression, schizophrenia) ____ Yes _____ No

Neurological Disorder (multiple sclerosis, myasthenia gravis) _____ Yes _____ No

Sleep Disorder (apnea) _____ Yes _____ No

Endocrine Disorder (Cushing's Diease, PICOs, Diabetes) _____ Yes _____No

Eating Disorder (Anorexia Nervosa, Bulimia) _____ Yes _____ No

Peridontal Disease ____ Yes _____ No

Currently taking any prescription medications ____ Yes _____ No

Name of medication _____

Currently taking any herbal or nonprescription medication daily _____ Yes _____ No

Name of herb or medication _____

Travel across three or more time zones in past month _____ Yes _____ No

Currently or within the past three months employed in shift work _____ Yes _____ No

Self-described as under unusually high levels of stress (recent divorce, death in family, loss of job, etc) _____ Yes _____ No

The information that you have provided will be used to see if you meet the criteria for participation in this study. If you are not eligible for the study, or you choose not to participate, this screening questionnaire will be immediately destroyed. If you choose to participate, the screening tool will be coded with an identifying number. The number will be recorded on a master list along with your name. The master list will be kept in a locked file cabinet separate from your information.

Horne-Ostberg Morningness-Eveningness Questionnaire

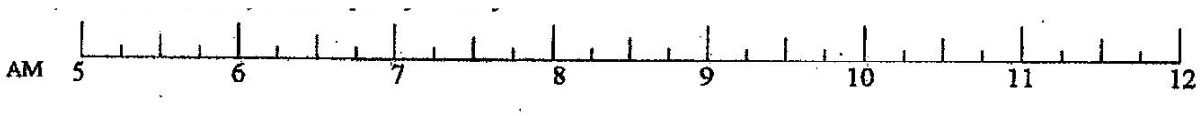
The final questionnaire

Instructions:

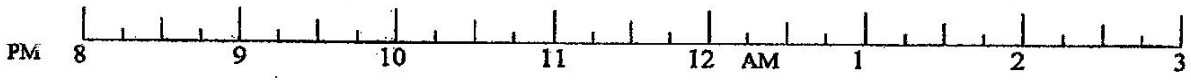
1. Please read each question very carefully before answering.
2. You are free to only answer questions that you want to answer.
3. Answer questions in numerical order.
4. Each question should be answered independently of others. Do NOT go back and check your answers.
5. All questions have a selection of answers. For each question place a cross alongside ONE answer only. Some questions have a scale instead of a selection of answers. Place a cross at the appropriate point along the scale.
6. Please answer each question as honestly as possible. Both your answers and the results will be kept, **in strict confidence**.
7. Please feel free to make any comments in the section provided below each question.

The Questionnaire with scores for each choice

1. Considering only your own "feeling best" rhythm, at what time would you get up if you were entirely free to plan your day?



2. Considering only your own "feeling best" rhythm, at what time would you go to bed if you were entirely free to plan your evening?



3. If there is a specific time at which you have to get up in the morning, to what extent are you dependent on being woken up by an alarm clock?

Not at all dependent …….
Slightly dependent …….
Fairly dependent ……..
Very dependent ……..

4. Assuming adequate environmental conditions, how easy do you find getting up in the mornings?

Not at all easy……..…….
Slightly easy ……..…….
Fairly easy……..…….
Very easy ……..……..

ID# _____
Date: _____

5. How alert do you feel during the first half hour after having woken in the mornings?

Not al all alert
…….
Slighthy
alert……..…….
Fairly
alert…….…….
Very
alert…….……..

6. How is your appetite during the first half hour after having woken in the mornings?

Very
poor……...…….
Fairly
poor…….…….
Fairly
good…….
Very
good……..

7. During the first half hour after having woken in the morning, how tired do you feel?

Very
tired…….…….
Fairly tired……...……...…….
Fairly refreshed…….
Very refreshed……..

##

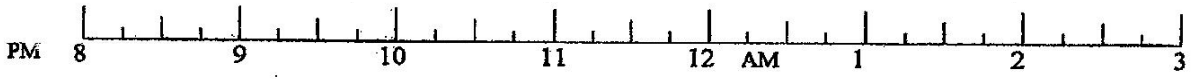
8. When you have no commitments the next day, at what time do you go to bed compared to your usual bedtime?

Seldom or never later.…….
Less than one hour later…….
1 – 2 hours later…….
More than two hours later.……..

9. You have decided to engage in some physical exercise. A friend suggests that you do this one hour twice a week and the best time for him is between 7.0 – 8.0 AM. Bearing in mind nothing else but your own "feeling best" rhythm. How do you think you would perform?

Would be on good form…….
Would be on reasonable form…….
Would find it difficult.…….
Would find it very difficult..……..

10. At what time in the evening do you feel tired and as a result in need of sleep?



11. You wish to be at your peak performance for a test which you know is going to be mentally exhausting and lasting for two hours. You are entirely free to plan your day and considering only your own "feeling best" rhythm which ONE of the four testing times would you choose?

8:00 – 10:00 AM……..…….
11:00 AM – 1:00 PM……..…….
3:00 – 5:00 PM……..…….
7:00 – 9:00 PM……...……..

ID# _____
Date: _____

12. If you went to bed at 11:00 PM at what level of tiredness would you be?

Not at all tired…….
A little tired..……...…….
Fairly tired……..…….
Very tired…….……..

13. For some reason you have gone to bed several hours later than usual, but there is no need to get up at any particular time and the next morning. Which ONE of the following events are you most likely to experience?

Will wake up at usual time and will NOT fall sleep….…….
Will wake up at usual time and will doze thereafter…….
Will wake up at usual time but will fall asleep again.…….
Will NOT wake up until later than usual……..

14. One night you have to remain awake between 4:00 – 6:00 AM in order to carry out a night watch. You have no commitments the next day. Which ONE of the following alternatives will suit you best?

Would NOT go to bed until watch was over……..…….
Would take a nap before and sleep after…….
Would take a good sleep before and nap after…...…….
Would take ALL sleep before watch…….……
…..

15. You have to do two hours of hard physical work. You are entirely free to plan your day and considering only your own "feeling best" rhythm which ONE of the following times would you choose

8:00 – 10:00 AM……..…….
11:00 AM – 1:00 PM……..…….
3:00 – 5:00 PM……..…….
7:00 – 9:00 PM……...……..

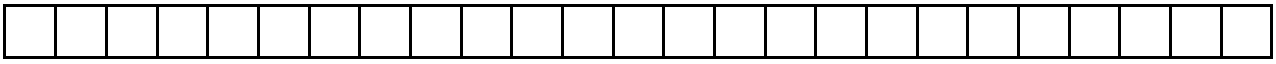
16. You have decided to engage in hard physical exercise. A friend suggests that you do this for one hour twice a week and the best time for him is between 10:00 – 11:00 PM. Bearing in mind nothing else but your own "feeling best" rhythm how well do you think you would perform?

Would be on good form…….
Would be on reasonable form…….
Would find it difficult.…….
Would find it very difficult.……..

17. Suppose that you can choose your own work hours. Assume that you work a FIVE hour day (including breaks) and that your job was interesting and paid by results. Which FIVE CONSECUTIVE HOURS would you select?

12 1 2 3 4 5 6 7 8 9 10 11 12 1 2 3 4 5 6 7 8 9 10 11 12
MIDNIGHT NOON MIDNIGHT

18. At what time of the day do you think that you reach your "feeling best" peak?



12 1 2 3 4 5 6 7 8 9 10 11 12 1 2 3 4 5 6 7 8 9 10 11 12
MIDNIGHT NOON MIDNIGHT

19. One hears about “morning” and “evening” types of people. Which ONE of these types do you consider yourself to be?	Definitely a “morning” type.…….
	Rather more a “morning” type than an “evening” type…….
	Rather more an “evening” type than a “morning” type.…….
	Definitely an “evening” type.……

Horne, J. A., & Ostberg, O. (1977). Individual differences in human circadian rhythms. *Biological Psychology*, *5*(3), 179-190. doi:10.1016/0301-0511(77)90001-1

Demographic Questionnaire
Participant Data

Today's Date _____

Date of Birth _____ Age _____

Marital Status: Married _____ Single _____

Divorced/Separated _____ Widowed _____

Other_____

Number of Children: Under 1 year of age_____

1 to 5 years old____

5 to 12 years old_____

Over 12 years old_____

Educational Level: Less than High School _____ Some High School _____

High School Grad _____ Some College _____

College Grad _____ Some Grad School _____

Master's Degree _____ Doctorate _____

Occupation: _____

On average, how many cups of coffee do you drink every day? _____ cups

On average, how many cups of caffeinated teas or colas

do you drink every day? _____ cups

On average, how much alcohol do you drink in a week? _____ drinks

What is the highest number of drinks in one sitting that you routinely drink? _____ drinks

On average, how many cigarettes do you smoke per day? _____ cigarettes

Profile of Mood States

HOW DO YOU FEEL TODAY?

Below is a list of words that describe feelings people have. Please read each one carefully. Circle the number which best describes HOW YOU FEEL RIGHT NOW. There is no right or wrong answers. Your first reaction is the best one to record.

KEY: 0 = Not at all 1 = A little 2 = Moderately 3 = Quite a bit 4 = Extremely	KEY: 0 = Not at all 1 = A little 2 = Moderately 3 = Quite a bit 4 = Extremely	KEY: 0 = Not at all 1 = A little 2 = Moderately 3 = Quite a bit 4 = Extremely
Friendly ……0 1 2 3 4 Tense ……0 1 2 3 4	Unworthy ……0 1 2 3 4 Spiteful ……0 1 2 3 4	Desperate ……0 1 2 3 4 Sluggish ……..0 1 2 3 4
Angry ……...0 1 2 3 4 Worn out ……..0 1 2 3 4	Sympathetic …….0 1 2 3 4 Uneasy …….0 1 2 3 4	Rebellious ……...0 1 2 3 4 Helpless ……...0 1 2 3 4
Unhappy ……..0 1 2 3 4 Clear-headed ……0 1 2 3 4	Restless ……0 1 2 3 4 Unable to Concentrate ……..0 1 2 3 4	Weary ……..0 1 2 3 4 Bewildered ……..0 1 2 3 4
Lively ……...0 1 2 3 4 Confused ……..0 1 2 3 4	Fatigued ……...0 1 2 3 4 Helpful …….0 1 2 3 4	Alert …….0 1 2 3 4 Deceived ……..0 1 2 3 4
Sorry for Things done ……..0 1 2 3 4 Shaky ……0 1 2 3 4	Annoyed ……..0 1 2 3 4 Discouraged …….0 1 2 3 4	Furious ……..0 1 2 3 4 Efficient ……0 1 2 3 4
Listless …… 0 1 2 3 4 Peeved ……..0 1 2 3 4	Resentful ……..0 1 2 3 4 Nervous ……0 1 2 3 4	Trusting ……0 1 2 3 4 Full of pep ……0 1 2 3 4
Considerate ……..0 1 2 3 4 Sad ……...0 1 2 3 4	Lonely ……..0 1 2 3 4 Miserable …….0 1 2 3 4	Bad-tempered ……0 1 2 3 4 Worthless ……...0 1 2 3 4
Active ……..0 1 2 3 4	Muddled ……...0 1 2 3 4 Cheerful ……0 1 2 3 4	Forgetful ……0 1 2 3 4 Carefree ……..0 1 2 3 4
Grouchy ……0 1 2 3 4 Blue ……0 1 2 3 4	Bitter ……0 1 2 3 4 Exhausted …….0 1 2 3 4	Terrified ……..0 1 2 3 4 Guilty ……..0 1 2 3 4
Energetic ……0 1 2 3 4 Panicky ……..0 1 2 3 4	Anxious …….0 1 2 3 4 Ready to Fight ……..0 1 2 3 4	Vigorous …….0 1 2 3 4 Uncertain About things ……0 1 2 3 4
Hopeless ……0 1 2 3 4 Relaxed ……. 0 1 2 3 4	Good natured ……0 1 2 3 4 Gloomy ……0 1 2 3 4	Bushed …….0 1 2 3 4 Today's date _____

PLEASE ANSWER ALL ITEMS

ID_____

ID_____

PANAS

This scale consists of a number of words that describe different feelings and emotions. Please read each item and then circle the appropriate answer next to that word. Indicate to what extent you have felt this way during the past week. You are free to only answer questions that you want to answer.

Use the following scale to record your answers.

(1) = Very slightly or not at all (2) = A little (3) = Moderately (4) = Quite a bit (5) = Extremely

	Very slightly or not at all	A little	Moderately	Quite a bit	Extremely
1. Interested	1	2	3	4	5
2. Distressed	1	2	3	4	5
3. Excited	1	2	3	4	5
4. Upset	1	2	3	4	5
5. Strong	1	2	3	4	5
6. Guilty	1	2	3	4	5
7. Scared	1	2	3	4	5
8. Hostile	1	2	3	4	5
9. Enthusiastic	1	2	3	4	5
10. Proud	1	2	3	4	5
11. Irritable	1	2	3	4	5
12. Alert	1	2	3	4	5
13. Ashamed	1	2	3	4	5
14. Inspired	1	2	3	4	5
15. Nervous	1	2	3	4	5
16. Determined	1	2	3	4	5
17. Attentive	1	2	3	4	5
18. Jittery	1	2	3	4	5

19. Active	1	2	3	4	5
20. Afraid	1	2	3	4	5

Watson, D. & Clark, L. A. (1994). *Manual for the Positive and Negative Affect Schedule - Expanded Form*. University of Iowa: Iowa, USA

Subjective Sleep Quality Scale

Directions: Your impressions of how you slept last night are important. The following 14 items pertain to just to your sleep last night. The responses range from “Strongly Agree” to “Strongly Disagree”. Please read each item and circle the appropriate response. You are free to only answer questions that you want to answer.

1. I SLEPT VERY WELL LAST NIGHT.

Strongly Agree	Moderately Agree	Slightly Agree	Slightly Disagree	Moderately Disagree	Strongly Disagree

2. I HAD A HARD TIME WAKING THIS MORNING.

Strongly Agree	Moderately Agree	Slightly Agree	Slightly Disagree	Moderately Disagree	Strongly Disagree

3. I FELT RESTED WHEN I WOKE UP.

Strongly Agree	Moderately Agree	Slightly Agree	Slightly Disagree	Moderately Disagree	Strongly Disagree

4. I HAD ENOUGH SLEEP LAST NIGHT.

Strongly Agree	Moderately Agree	Slightly Agree	Slightly Disagree	Moderately Disagree	Strongly Disagree

5. I WOKE UP TOO EARLY AND COULDN’T GET BACK TO SLEEP.

Strongly Agree	Moderately Agree	Slightly Agree	Slightly Disagree	Moderately Disagree	Strongly Disagree

6. I HAD LONG PERIODS OF WAKEFULNESS DURING THE NIGHT.

Strongly Agree	Moderately Agree	Slightly Agree	Slightly Disagree	Moderately Disagree	Strongly Disagree

7. I DIDN’T WAKE UP ALL NIGHT.

Strongly Agree	Moderately Agree	Slightly Agree	Slightly Disagree	Moderately Disagree	Strongly Disagree

8. I KEPT TOSSING AND TURNING IN BED ALL NIGHT.

Strongly Agree	Moderately Agree	Slightly Agree	Slightly Disagree	Moderately Disagree	Strongly Disagree

9. I KEPT WAKING UP DURING THE NIGHT.

Strongly Agree	Moderately Agree	Slightly Agree	Slightly Disagree	Moderately Disagree	Strongly Disagree

10. I AM PHYSICALLY VERY TIRED NOW.

Strongly Agree	Moderately Agree	Slightly Agree	Slightly Disagree	Moderately Disagree	Strongly Disagree

11. I AM MENTALLY VERY TIRED NOW.

Strongly Agree	Moderately Agree	Slightly Agree	Slightly Disagree	Moderately Disagree	Strongly Disagree

12. I AM RELAXED NOW.

Strongly Agree	Moderately Agree	Slightly Agree	Slightly Disagree	Moderately Disagree	Strongly Disagree

13. I AM VERY SLEEPY NOW.

Strongly Agree	Moderately Agree	Slightly Agree	Slightly Disagree	Moderately Disagree	Strongly Disagree

14. I AM VERY COMFORTABLE NOW.

Strongly Agree	Moderately Agree	Slightly Agree	Slightly Disagree	Moderately Disagree	Strongly Disagree

Instruments

Pittsburgh Sleep Quality Index (PSQI)

ID# _____

The following questions relate to your usual habits of sleep during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. You are free to only answer questions that you want to answer.

1. When have you usually gone to bed?
   Usual Bedtime _____
2. How long (in minutes) does it take you to fall asleep?
   Number of minutes _____
3. When do you usually get up for work?
   Getting up time _____
4. How many hours of actual sleep do you get? (May be different than when you went to bed.)
   Hours of sleep last night _____

Check the one best response. Please answer all the questions.

5. How often have you had trouble sleeping because you:

	Not during the past month	Less than once a week	1 or 2 times a week	3 or more times a week
a. Cannot get to sleep within 30 minutes	O	O	O	O
b. Wake up in the middle of the night	O	O	O	O
c. Have to get up to use the bathroom	O	O	O	O
d. Cannot breathe comfortably	O	O	O	O
e. Cough or snore loudly	O	O	O	O
f. Feel too cold	O	O	O	O
g. Feel too hot	O	O	O	O
h. Had bad dreams	O	O	O	O
i. Have pain	O	O	O	O
j. Other reasons you can't sleep	O	O	O	O

6. During the past month, how would you rate your sleep quality overall?
    a. Very good
    b. Fairly good
    c. Fairly bad
    d. Very bad

7. During the past month, how often have you taken medication to help you sleep (prescribed or over the counter?)

Not during the past Month	Less than once a week	Once or twice a week	3 or more time per week
O	O	O	O

8. During the past month, how often have you had trouble staying awake while driving?

Not during the past Month	Less than once a week	Once or twice a week	3 or more time per week
O	O	O	O

9. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?
    a. No problem at all _____
    b. Only a very slight problem_____
    c. Somewhat of a problem _____
    d. A very big problem _____

10. Do you have a bed partner or roommate?
    a. No bed partner or roommate _____
    b. Partner roommate in the other room _____
    c. Partner in same room but not same bed _____
    d. Partner in same bed _____

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ABSTRACT

CIRCADIAN RHYTHM OF CORTISOL AND ESTRADIOL IN HEALTHY WOMEN

by

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Daily variation in human processes and behaviors has been identified for centuries. Study of these circadian rhythms demonstrates their role in human health. Sickness behaviors include alterations in affect, sleep quality and energy. The study of the relationship between circadian rhythms has been limited to isolated rhythms. The role of temporal relationships among rhythms has received little attention. Sickness behaviors are prevalent in many disorders including depression, cancer, and autoimmune disorders. Two hormones that have been shown to play a role in the manifestation of sickness behaviors are cortisol and estradiol. To date the role of the relationship between cortisol and estradiol circadian rhythms and sickness behaviors remains unknown. The purpose of this study is to explore the temporal relationship between the rhythms of cortisol and estradiol and its relationship to sickness behaviors. It was hypothesized that a cortisol-estradiol phase angle difference (PAD) would exist that would correlate with optimal affect, sleep quality and energy.

A small scale, comparative, correlational design was used to test the hypothesis. A sample of twenty-three university women (11 morning-types and 12 evening-types) between the ages of twenty to thirty-five were recruited from an urban university. Salivary samples were

collected every two hours for a twenty-four hour period. Subjective measures of affect, sleep quality and energy were recorded. Salivary samples were assayed for cortisol and estradiol levels and fitted to a cosinor model with ultradian harmonics for each participant. Relationships between the cortisol-estradiol PAD and affect, sleep quality and energy measures were evaluated using a second degree polynomial equation. Results showed a significant correlation in affect measures ($p < 0.05$), but not sleep quality or energy. An optimal PAD was identified for affect at 3.6 hours.

The phase relationship between cortisol and estradiol may play a role in the development of alterations in affect which manifest in many disorders. These findings are based on a small homogeneous sample of university women. More research is needed in a larger, more heterogeneous group of women.

AUTOBIOGRAPHICAL STATEMENT

EDUCATION

2011	Doctor of Philosophy	Wayne State University	Detroit, MI
2003	Master of Science	University of Michigan	Ann Arbor, MI
1994	Bachelor of Science	Wayne State University	Detroit, MI
1987	Bachelor of Fine Art	Cleveland Institute of Art	Cleveland, OH

PROFESSIONAL APPOINTMENTS

2008 - 2009	Clinical Instructor	Michigan State University
2007 - 2008	Lecturer	University of Michigan-Flint
2007	Lecturer	University of Detroit Mercy

COMMITTEE APPOINTMENTS

2006 - 2008 Doctoral Student Forum Representative - Doctoral Program Committee

2005 - 2006 Doctoral Student Forum Representative - Search Committee

PRESENTATIONS

March 2009, *Pilot Study for Circadian Rhythm of Cortisol and Estradiol in Normally Menstruating Women,* **Butler, K.**, Refereed – Accepted as poster presentation, Midwest Nursing Research Society – 2009 Conference, Minneapolis, MN

March 2008, C*ircadian Typology and Body Composition in Postmenopausal Women,* **Butler, K.**, Davis, J., Engels, H. J., & Yarandi, H. , Refereed – Accepted as poster presentation, Midwest Nursing Research Society – 2008 Conference, Indianapolis, IN